Collaborative Workshop on Aquatic Models and 21st Century Toxicology

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Mitochondrial Uncoupling Disrupts Neurodevelopment in Zebrafish Embryos

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Neurons have high energy requirements, so it is not surprising that many neurological diseases are caused by defects in mitochondrial energy metabolism. However, the effects of mitochondrial dysfunction on neurodevelopment are poorly understood. 2,4-dinitrophenol (DNP) is a classic mitochondrial environmental toxicant found in wastewater and automobile exhaust. DNP disrupts the proton gradient formed across the mitochondrial inner membrane, preventing ATP production through oxidative phosphorylation. This action is referred to as uncoupling.

Here we report the resulting cellular and molecular changes in the developing embryo that result from using DNP to decrease mitochondrial ATP production in zebrafish. DNP exposure decreased mitochondrial energy production and resulted in changes in oxidative stress, which is consistent with decreased oxidative phosphorylation. Additionally, we found that these embryos could not mount a sufficient mitochondrial biogenic response to combat this dysfunction. Phenotypically, many early developmental events appeared to occur normally. However, later events such as the development of the primary motor neurons, retinal laminae and optic nerves were particularly inhibited. These observations are common characteristics of humans with mitochondrial disease.

Conclusions: We have developed a new animal model of mitochondrial disease that recapitulates many of the common neurological characteristics observed in humans. This model now provides a high-throughput platform with which to investigate mitochondrial toxicity, as well as for discovering novel therapeutics for the treatment of mitochondrial dysfunction.

All animal studies were approved by the Medical University of South Carolina Institutional Animal Care and Use Committee (AR #2850) and performed in accordance with the defined guidelines.

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Use of Medaka Fish (*Oryzias latipes*) for the Study of Environmentally Induced Transgenerational Phenotypes and Epigenetic Mechanisms

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Environmental chemicals are capable of inducing a transgenerational phenotype. An alteration in DNA methylation patterns is part of the molecular basis for germline transmission of transgenerational effects through an epigenetic mechanism. Germline cells are susceptible to environmental stressors during a critical period in development, especially when epigenetic reprogramming events are occurring. According to current models of epigenetic reprogramming in mouse primordial germ cells, all DNA methylation marks are erased and reestablished during sex determination. However, a small portion of the mouse primordial germ cell genome has been found to be resistant to global DNA demethylation, suggesting that these genomic regions might be responsible for inheritance of parental phenotype. Additionally, there is speculation that environmentally induced DNA methylation marks might also be reprogrammed during gametogenesis. Although environmentally altered epigenetic biomarkers have been identified, the functional relationship of these epigenetic marks with onset and progression of disease is currently unknown. Rodents are excellent models for epigenetic and germ cell research. However, the small aquarium fish, medaka (*Oryzias latipes*), has distinct advantages over rodents for use in transgenerational research. Medaka have well-defined genetic sex determination system (XX, XY system), the genome has been sequenced and annotated, and genetically modified transgenic lines with germ cell markers are available. Additionally, they have exclusive advantages over rodent models including external fertilization and embryo development, daily spawning, availability of large numbers of eggs and sperm, virtually unlimited number of embryos produced by the same two parents to assign to different treatment groups, a short generation time (2 months), and easy, low-cost culture. Medaka sex determination occurs between days 5 and 8 after fertilization. Germ cell reprogramming events are believed to be complementary to mouse. Here we describe some of the possibilities for medaka to serve as an alternative model to mice or rats for the study of environmental chemical-induced transgenerational phenotypes and the molecular basis of transgenerational inheritance of those phenotypes.

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Generation of a Fluorescent Transgenic Zebrafish for Detection of Environmental Estrogens

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To establish a novel *in vivo* test system for rapid detection of environmental estrogens, an ere-zvtg1: gfp transgenic zebrafish line has been generated. In this transgenic line, under control conditions, green fluorescent protein (GFP) was exclusively expressed in the liver of mature adult female fish. Male and larval transgenic fish did not express GFP but could be induced to express GFP in the liver after exposure to 17-α-ethynylestradiol (EE2). Concurrent accumulation of zvtg1 and gfp mRNAs in embryos and larvae after EE2 exposure was observed, which indicated that the expression of gfp transgene was driven by the zvtg1 promoter. Green fluorescence was first observed in the liver at 53, 74, 100 or 131 h postfertilization (hpf) after exposure to 100, 10, 1 or 0.1 ng/L EE2 from 1- to 2-cell stage, respectively. For mature male transgenic zebrafish, green fluorescence was observed after exposure to 100, 10, 1 or 0.1 ng/L EE2 for 2, 3, 4 or 7 days, respectively; for mature female, fluorescence was increased after exposure to relatively high concentrations of EE2 (10 and 100 ng/L). Green fluorescence in the liver was increased with prolonging of exposure time and was repeatedly induced after removal and re-addition of EE2. We also demonstrated that GFP expression could be induced by other estrogenic compounds, including β-estradiol (E2, 0.1µg/L), cadmium chloride (CdCl2, 10µg/L), zearalenone (50µg/L), estriol (E3, 1µg/L), diethylstilbestrol (DES, 50ng/L) and bisphenol A (BPA, 1 mg/L). These data suggest the transgenic zebrafish is sensitive and specific for detection of estrogenic compounds. Because the observed-effect concentrations are as low as those of environment and the observed-effect exposure times are very short, this transgenic fish is a promising candidate system for monitoring environmental estrogens directly, rapidly and easily.

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Effects of Adult Dietary Seleno-L-Methionine Exposure on Embryo Development of Offspring Medaka

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Selenium is a trace element, and toxicity results when levels are too low and too high. Selenium and arsenic are major metalloids in ash from coal-fueled power plants. Due to the short interval between fertilization and adult reproduction, aquarium model fish enable analysis of transgenerational responses, up to four generations per year. For this reason, Japanese medaka (*Oryzias latipes*) was the model used to investigate how selenium affects adults and their offspring. Adult male and/or female medaka of reproductive age were fed a dry diet containing seleno-L-methionine (0.0125 mg/g) for six days. Control and treatment groups of males and females were kept isolated from each other in a series of 3 L AHAB tanks containing 1.5 L of 1% salt MilliQ water. After 6 days, untreated adults were introduced into the tanks with the exposed individuals, yielding breeding groups of 3 females and 5 males. Resultant embryos were collected for 5 days and transferred to petri dishes in a 28°C incubator until hatch. Each plate was examined daily under a stereomicroscope, observations recorded, and digital images taken at 5 and 8 days postfertilization (dpf). At 10 dpf individuals were fixed and cartilage and bone stained, and RT-PCR was performed for Collagen, Type I. 0.0125 mg/g seleno-L-methionine decreased survival rates of embryos in female exposure groups and increased the number of altered embryos. Alterations included string heart, edema, polycephaly, less pigmentation, cyclopia etc. The proportion of string hearts observed was 1.1%, 2.1%, 0.8%, 3.9%, in control male–control female, exposed female–control male, exposed male–control female, and exposed male–exposed female, respectively. Results reveal severe developmental toxicity in embryos of adults fed additional selenium. Ongoing work includes observations of changes in cartilage and related genes.

Animal care and toxicity testing protocols (A085-12-03 and A046-12-02), approved by the Duke University Institutional Animal Care and Use Committee, were followed.

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Copper Nanoparticles and or Ionic Copper (II) Causes Neurotoxicity and Cardiotoxicity in Zebrafish Embryos

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Copper oxide nanoparticles (Cu-NPs) are frequently used in medical devices, paints, or fabrics, or as antimicrobials. Their industrial applications may lead to the contamination of aquatic ecosystems. The toxicological and human health risks of NPs in the environment are hard to evaluate due to a lack of knowledge about the mechanisms by which NPs interact with biological systems. In this study, we investigated the toxicity of Cu-NPs (60nm) and ionic copper (II) in wild-type (WT) zebrafish embryos and hb9-GFP transgenic zebrafish (each *Danio rerio*, AB- strain) embryos. Here, the effects of bare Cu-NPs were compared to those seen after exposure to the mass equivalent ionic form of copper (II) ($CuCl_2$) at various concentrations (1.25-to-20 ug/ml). Toxicity was evidenced by phenotypic changes in zebrafish embryos including survival, heart rate, motor neuron development, and absorptive permeability. Both Cu-NPs and $CuCl_2$ were lethal to zebrafish embryos at 20 µg/ml (within 24 hrs) and 10 µg/ml (within 48 hrs), with $CuCl_2$ being more toxic at equivalent mass concentrations. Similarly, the heart rate was significantly reduced following exposure to either Cu-NPs or $CuCl_2$ in a concentration–time-dependent manner. Additionally, the embryo permeability studies showed that exposure to either Cu-NPs or $CuCl_2$ (5 ug/ml) for 24 hrs significantly increased the topical absorption of the fluorescent tracer 6-coumarin. Furthermore, embryos treated with either Cu-NPs or $CuCl_2$ (2.5 µg/ml for 48 hrs) showed a significant reduction (nearly 2-fold) in spinal motor neurons. These results indicate that both $CuCl_2$ and Cu-NPs can be toxic to zebrafish embryos causing significant neurotoxicity and cardiotoxicity at exposure levels that do not cause lethality.

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Effects of Seleno-L-Methionine Exposure on Embryonic Development in Medaka

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Selenium (Se) studies are becoming increasingly important with the releases of large amounts of the metalloid into streams at mountaintop removal sites and in the areas of ruptured enclosures storing coal fly-ash at adjacent energy production sites. Seleno-L-methionine (SeMet) is the chemical species of Se primarily associated with toxicity in developing organisms. Medaka (*Oryzias latipes*) embryos are especially useful in Se toxicity studies due to their transparent chorion, which enables direct observation and imaging. Embryos (developmental stage 10, 6–7 hours postfertilization) were collected, separated into six-well tissue culture plates containing 10, 20, 50, 100, 500 µM, 1mM SeMet, respectively, in a 1% salt MilliQ water (ERM) and 0.1% DMSO solution. Embryos with only DMSO in ERM acted as controls. Embryos were kept at 28°C and monitored daily. Control and affected embryos were imaged, *in vivo*, using a stereomicroscope at 5 and 8 days postfertilization (dpf). At 5 dpf, individuals were processed for Se content analysis using inductively coupled plasma mass spectrometry (ICPMS). At hatch (~10 dpf), individuals were fixed for cartilage and bone staining and processed for RT-PCR. Survival success at 10 dpf was distributed in a dose response manner with 100%, 100%, 74%, 12%, 9%, 0% in control, 10 µM, 20 µM, 50µM, 100 µM 500 µM, and 1mM, respectively. SeMet exposure inhibited swim bladder inflation, caused eye deformities, and decreased blood pigment. Additionally, at 10 µM SeMet, cartilage stains showed changes in mandibular development. This work clearly shows the strengths of the model for rapidly detecting developmental toxicity and related gene expression. Ongoing work includes observations on gene expression related to cartilage and bone formation and attempting to rescue deformities using antioxidant exposure.

Animal care and toxicity testing protocols (A085-12-03 and A046-12-02), approved by the Duke University Institutional Animal Care and Use Committee, were followed.

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Gene Expression in Triploid *Oryzias latipes*, Hints at the Genetic Needs for Triploidy

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Normal genetic events, such as X-chromosome inactivation or partial trisomy, may produce allele imbalances and lead to drastic modulation of allele specific gene expression (ASE). We have been developing methods to assess ASE within interspecies hybrids produced from mating select *Xiphophorus* parental species in an attempt to better understand gene interactions that form the basis of disease. In this diploid interspecies system we have shown substantial ASE modulation in hybrids compared to either parent. However, effects of higher levels of ploidy on ASE in vertebrates remain poorly understood because few vertebrate triploid or polyploids are available that have sufficient genetic divergence to allow detailed study. Thus, the genetic mechanisms limiting polyploidy are not well understood.

To produce a genetic model to study increased ploidy, tripoid medaka were produced via nuclear transplantation. After many hundreds of transplant trials, three triploid medaka (two females and one male) grew to mature size but were infertile. The haplomes harbored by these triploids were derived from parental strains having sufficient genetic diversity to assess ASE. Liver RNA from triploids and parental lines were subjected to Illumina based RNA-seq (100 bp, PE reads) and the data generated used to develop computational methods allowing ASE among the three haplomes. In the female triploids, the majority of transcripts examined (78%) were consistent between the two females and most of these (92% or 72% overall) had roughly equal contributions from all three alleles to total gene expression levels. However, 22% of the transcripts in the triploid females showed aberrant gene expression and 12% of the transcripts exhibited almost complete suppression of one haplome allele. The male triploid did not share these patterns with the females and exhibited a much different ASE pattern. Comparison of the three adult triploids defined genetic commonalities that may highlight alterations required for triploid development.

This research complies with the applicable EU and national German legislation governing animal experimentation, especially the German Federal Law of Animal Protection (Authorization number: 55.2-2531.01-49/08). Funding was provided partly by the NIH, ORIP, Division of Comparative Medicine grants R24-OD-011120 and R24-OD-0111

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Mining ToxCast™ Phase I and II Embryonic Zebrafish Data for Possible Endocrine Disrupting Chemicals for Transcriptomics and Predictive Modeling

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The Endocrine Disruptor Screening Program (EDSP) was established by the EPA in an effort to test chemicals that affect estrogen, androgen, or thyroid signaling across several *in vitro* and *in vivo* assays. In line with this program, ToxCast™ was formed to develop new methods for chemical testing prioritization to handle the overwhelming number of chemicals currently in use. Developmental stages are sensitive to chemical injury, therefore the embryonic zebrafish is an advantageous model to explore developmental and neurobehavioral effects of a large number of compounds. Previously, our laboratory successfully screened ~1060 ToxCast™ phase I and II chemicals using our high-throughput zebrafish assay. The aim of the current research is to begin building a predictive framework of endocrine disruption from our ToxCast™ dataset using our screening approach in tandem with whole genome transcriptomics. The first phase of this research is to determine effective concentrations of chemicals for transcriptome analysis. Mining of our screening data revealed many compounds considered priority chemicals for the EDSP and The Endocrine Disruptor Exchange (TEDX). By filtering these chemicals based on several criteria, we have compiled a list of 25 known and possible endocrine active compounds to pursue at greater depth. In brief, zebrafish were exposed at 6 hours postfertilization (hpf) and general mortality and malformations was assessed at 24 and 120 hpf. We examined the effects of exposure on photo-motor response in 24 hpf embryos using our lab-derived Photo-motor Response Assessment Tool (PRAT), and investigated effects on 120 hpf photo-induced larval locomotion using the ViewPoint Zebrabox system. Based on initial toxicity information from these 25 chemicals, we have begun studies using our screening assay to define the EC_{80} for each chemical, and a summary of the results will be discussed.

All animal studies were conducted in accordance with IACUC and AAALAC guidelines.

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High-Content Screening in Zebrafish Embryos Identifies Butafenacil as a Potent Inducer of Anemia

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Targeted high-throughput assays are needed to better predict morphologic and functional effects of chemicals on cardiovascular development. Using transgenic zebrafish (*fli1:egfp*) that stably express enhanced green fluorescent protein (eGFP) within vascular endothelial cells, we recently developed and optimized a 384-well high-content screening (HCS) assay that enables us to screen and identify chemicals affecting cardiovascular function at nonteratogenic concentrations. Within this assay, automated image acquisition procedures and custom image analysis protocols are used to quantify body length, heart rate, circulation, pericardial area, and intersegmental vessel area within individual live embryos exposed from 5-72 hours post-fertilization. After ranking acute toxicity data generated from the U.S. Environmental Protection Agency's (EPA's) zebrafish teratogenesis assay, we screened ~10% of the most potent chemicals within EPA's ToxCast™ Phase I library in concentration–response format (0.05-50 μM) using this HCS assay. Based on this screen, we identified butafenacil as a potent inducer of anemia, as exposure to 0.39–3.125 μM butafenacil completely abolished arterial circulation in the absence of effects on all other endpoints evaluated. Butafenacil is an herbicide that inhibits protoporphyrinogen oxidase (PPO) – an enzyme necessary for heme production in vertebrates. Using o-dianisidine staining, we revealed that severe butafenacil-induced anemia in zebrafish was due to a complete loss of hemoglobin following exposure during early development. Therefore, six other PPO inhibitors within the ToxCast™ Phase I library were screened to determine whether anemia represents a common adverse outcome for these herbicides. Developmental exposure to only one of these PPO inhibitors—flumioxazin—resulted in a similar phenotype as butafenacil, albeit anemia induced by flumioxazin was not as severe as butafenacil. Overall, this study highlights the utility of this assay for (1) screening chemicals for targeted effects on cardiovascular function and (2) prioritizing chemicals for future hypothesis-driven and mechanism-focused investigations within zebrafish and mammalian models.

All fish were handled and treated in accordance with approved Institutional Animal Care and Use Committee protocols at the University of South Carolina–Columbia.

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Exposure to a Flame Retardant Metabolite Delays Development and Causes Persistent Neurobehavioral Alterations in Zebrafish

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Polybrominated diphenyl ethers (PBDE) are flame-retardant contaminants that are ubiquitously detected in human tissues. PBDEs are oxidatively biotransformed by cytochrome p450 enzymes into halogenated phenolic compounds (OH-BDEs) in mammals. In many cases, this hydroxylation increases structural similarity to endogenous thyroid hormones (THs). Since development is mediated by THs, dysregulation during early development could have lasting effects. In this study, overt toxicity of ten different halogenated phenols was investigated during zebrafish early development (4 hours–6 days postfertilization).

The most toxic compound tested was 6-OH-BDE-47 with a LC-value of 134 nM. The otic vesicle length (OVL), head trunk angle (HTA), and retinal pigmentation were further used as staging metrics for development and impacts of exposure were assessed. Exposure to 6-OH-BDE-47 (100 nM) resulted in a 17% decline in HTA and the OVL increased 60%. Changes in craniofacial cartilage formation were also examined in 4 dpf larvae, with deformities affecting the lower jaw. We hypothesize that these morphological changes may be mediated by disrupted TH signaling, which mediates these processes.

Exposed zebrafish are also undergoing neurobehavioral assessment as larvae and as adults to evaluate the early and persistent effects of early life exposure to 6-OH-BDE-47. All behavioral exposures were performed at doses which were found to have no gross morphological effects on development. Short-term effects in larvae will be assessed for swimming activity in response to light–dark transitions. Persistent neurotoxicity will be determined by tests of sensorimotor plasticity, predatory escape responses, and spatial learning. Preliminary testing results indicate increased larval swimming activity, decreased startle response, and increased fear response in adult animals following larval exposure (50 nM). These results could indicate that low-level exposure to OH-BDEs can alter zebrafish development and have lasting neurobehavioral effects.

Adult fish care and reproductive techniques were noninvasive and approved by the Duke University Institutional Animal Care and Use Committee.

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Vitamin D Receptor Expression in Medaka and Zebrafish Brain by *In Situ* Hybridization

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There is a growing body of evidence suggesting the role of vitamin D in brain development and function. Vitamin D has also been shown to have neuroprotective effects against toxicants, and its deficiency has been implicated as a potential risk factor for development of neurodegenerative diseases such as Parkinson's disease and multiple sclerosis in humans. Studies indicate that vitamin D receptor (VDR) is expressed in glial cells and neurons in the human and mouse brain. Using fish as model organism we studied the expression patterns of two vitamin D receptor orthologs/paralogs (alpha and beta) in adult Japanese medaka and zebrafish by *in situ* hybridization. Vitamin D receptor expression was observed predominantly in the glial cell population in the optic tectum and alongside ventricles and also in the neurons throughout the rostral–caudal axis. This study describes for the first time VDR expression in fish brain in anatomic detail.

References

1. Cui et al. 2013. The vitamin D receptor in Dopamine neuron; it presence in human substantia nigra and its ontogenesis in rat midbrain. Neuroscience 236: 77-87
2. Kesby et al. 2011. The effects of vitamin D on brain development and adult brain function. Molecular and Cellular Endocrinology 347:121-127.
3. Smolders et al. 2013. Expression of Vitamin D receptor and metabolizing enzymes in multiple sclerosis-affected brain tissue. Journal of Neuropathology and Experimental Neurology 72(2) 91-105.

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Arsenic Disrupts Vascular Development in Zebrafish

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Arsenic has been ranked number one on the hazardous substances list prioritized by the Agency for Toxic Substances and Disease Registry and U.S. Environmental Protection Agency since 1997. Several health problems have been closely linked to arsenic exposure, such as cancers of the liver, kidney, bladder and lung; skin lesions; and cardiovascular diseases. Arsenic exposure has also been described to affect vasculogenesis and angiogenesis, both regulated by key factors such as vascular endothelial growth factor and extracellular matrix proteins.[1,2] *In vitro* studies have shown that high levels of arsenic exposure can block angiogenesis,[3] while *in vivo* studies have reported conflicting results.[4] Here, we describe in detail arsenic-induced adverse effects on vascular development during specific windows of embryogenesis using zebrafish as a model. Zebrafish embryos were exposed to arsenite, an inorganic form of arsenic, at various concentrations and at different windows of development, and assessed for vascular perturbation and other developmental malformations at 72 hours postfertilization (hpf). Intersegmental vessel sprouting and growth was most perturbed by exposure to arsenite during the 24–48 hpf window, while disruption in the condensation of the caudal vein plexus was more often observed at the 48–72 hpf exposure window, reflecting when these structures develop during normal embryogenesis. The vascular growth rate was decreased by arsenite exposure, and deviated from that of control embryos at around 24–26.5 hpf. We further mapped changes in expression of key regulators of angiogenesis and vasculogenesis. In total, the expression of eight key factors involved in different aspects of vascularization was significantly altered by arsenite exposure. Our results demonstrate that arsenite is a potent vascular disruptor in the developing zebrafish embryo, providing a proof of concept that zebrafish is a robust *in vivo* model for investigating vascular toxicants.

All animal use was carried out in accordance to the standard operating protocols approved by the Institutional Animal Care and Use Committee at University of Houston (protocol nos. 12-042 and 13-028).

References

1. J. C. Chappell, S. M. Taylor, N. Ferrara, V. L. Bautch, Local guidance of emerging vessel sprouts requires soluble Flt-1. *Dev Cell* **17**, 377 (Sep, 2009).
2. N. Ferrara, H. P. Gerber, J. LeCouter, The biology of VEGF and its receptors. *Nat Med* **9**, 669 (Jun, 2003).
3. Y. H. Kao, C. L. Yu, L. W. Chang, H. S. Yu, Low concentrations of arsenic induce vascular endothelial growth factor and nitric oxide release and stimulate angiogenesis *in vitro*. *Chem Res Toxicol* **16**, 460 (Apr, 2003).
4. N. V. Soucy *et al.*, Arsenic stimulates angiogenesis and tumorigenesis *in vivo*. *Toxicol Sci* **76**, 271 (Dec, 2003).

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Toxicity Screening of the ToxCast Phase II Chemical Library Using a Zebrafish Developmental Assay

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As part of the chemical screening and prioritization research program of the U.S. Environmental Protection Agency, the ToxCast Phase II chemicals were assessed using a vertebrate screen for developmental toxicity. Zebrafish embryos (*Danio rerio*) were exposed in 96-well plates from late-blastula stage (6 hr postfertilization, pf) through day 5 pf (1-2 days post-hatch). All exposures were by immersion and renewed daily. The 685 chemicals included food additives, consumer use product ingredients, pesticides, failed pharmaceuticals, and "green" plasticizers (http://epa.gov/ncct/toxcast/chemicals.html). Intra- and inter-plate replicates were included for quality control. Developmental toxicity was initially assessed using a single nominal concentration of 80 µM: positives and a selection of negatives were confirmed by concentration–response determinations. On day 5 pf, larvae were moved from exposure solution to a control solution without chemical, and on day 6 pf were assessed for overt toxicity (*i.e.,* death, nonhatching and dysmorphology; n = 4 embryos per chemical). Dysmorphology was a combined score using both in-life observation and brightfield, high-content image analysis. Overt toxicity was noted with 46% of the chemicals tested, compared to 62% positive chemicals when the ToxCast Phase I library, consisting of mostly pesticide active ingredients, was previously tested. As with the Phase I library, the octanol–water partition coefficient ($\log_{kow}$) of the Phase II library chemicals was positively correlated with overt toxicity: there were 18% positive chemicals with $\log_{kow}$ <0; 41% positive chemicals with $\log_{kow}$ of 0 to 4; and 67% positive chemicals with a $\log_{kow}$ >4. All chemicals positive at the single concentration were further assessed for potency using a dose-response study (8-point, semi-log concentration curve: n = 3 embryos per concentration). These data demonstrate the utility of zebrafish in medium-throughput chemical testing programs for detection of adverse developmental outcomes. *This abstract may not necessarily reflect official Agency policy*.

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Investigating the Mechanisms That Mediate the Later Life Consequences of Developmental Exposure to Domoic Acid in Zebrafish

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Harmful algal blooms (HABs) have increased in incidence and geographic extent in recent years, in part due to anthropogenic activities. Such increases have potential human health consequences, as humans are regularly exposed to HAB toxins by ingesting contaminated seafood. While regulatory limits prevent harvesting seafood containing concentrations of toxins known to cause acute toxicity, there is increasing evidence that even low levels of exposure to toxic during development can have consequences for health and disease later in life. Thus, there is a great need to characterize the later life effects from developmental exposure to low, asymptomatic doses of HAB toxins. To further understand these effects, we exposed zebrafish embryos (0–5 days postfertilization [dpf]) and larvae (5–7 dpf) to varying concentrations of a harmful algal bloom toxin, domoic acid (DA) (0, 5, 10, 20, 40μM DA). Initial behavioral tests evaluating the optomotor response show that larvae treated with the highest concentration (40 μM) from 0–5 dpf showed reduced responses relative to the control at 7 dpf, but these differences dissipated by 8 and 9 dpf. The expression of candidate genes, c-fos, c-jun and olig2, which have previously been shown to be altered during DA exposure, is also currently being measured. Ongoing studies are investigating the role of DA exposure in altering cell fate and epigenetic programming and the molecular mechanisms by which these processes occur. One focus is on how DA alters oligodendrocyte precursor cell (OPC) differentiation, and how this may be linked to DA-induced oxidative stress, which has been shown in *vitro* to disrupt OPC differentiation. Our working hypothesis is that DA-induced oxidative stress alters oligodendrocyte cell fate and causes aberrant myelination, ultimately leading to prolonged deficits in learning and memory in adults.

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The Antirheumatic Drug, Leflunomide, Interferes with the Dopamine Synthesis Pathway

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Leflunomide is a drug most commonly used to treat rheumatoid arthritis. Leflunomide's metabolite, teriflunomide, produces a therapeutic effect by inhibiting dihydroorotate dehydrogenase (DHODH), an enzyme involved in *de novo* pyrimidine biosynthesis. Leflunomide has also been shown to activate the aryl hydrocarbon receptor (AhR), a receptor that alters gene expression of proteins involved in drug detoxification. Studies have shown that when exposed to leflunomide, zebrafish lack normal levels of melanocyte-derived pigment. Melanocytes, as well as certain neuronal cells, are derivatives of neural crest cells and produce a pigment that is synthesized from tyrosine. Dopamine, an important neurotransmitter, is also derived from tyrosine. With this knowledge, we hypothesized that leflunomide also targets dopamine biosynthesis. Zebrafish were exposed to 2.5 μM and the gene expression levels of enzymes involved in the dopamine synthesis pathway were measured. In addition, a transgenic line of zebrafish (*Tg(dat:gfp)*) expressing green fluorescent protein (GFP) under the control of the dopamine transporter promoter was used to analyze the fluorescence of embryos exposed to leflunomide compared to controls. Tyrosine hydroxylase, the enzyme catalyzing the initial step of dopamine synthesis, was downregulated after exposure to leflunomide, and dopamine decarboxylase (ddc), the enzyme catalyzing the second step, was upregulated. The upregulation of ddc could possibly be explained by a feedback loop acting to compensate for the lack of substrate needed to make dopamine. Tyrosinase, an enzyme that links the melanin and dopamine biosynthesis pathways, was also downregulated. In addition to alterations in gene expression levels, leflunomide exposure caused a decrease in GFP fluorescence in the *Tg(dat:gfp)* zebrafish line. These data offer evidence that leflunomide affects dopamine synthesis and signaling. Further work will be done to investigate leflunomide's mechanism of action (AhR or DHODH inhibition). Zebrafish are an excellent model for looking at environment–cell interactions, and the discoveries can be directly translated to human health.

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Zebrafish as a Biomonitoring Tool to Evaluate the Ogeechee River Water System

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Natural ecosystems are a complex web of interactions between the organisms, their physical environment, and the influences they have on one another. Freshwater ecosystems are constantly subjected to environmental, natural, or anthropogenic stressors that can have broad and lasting implications on the aquatic community, including mass kills and abnormal developmental effects. The ability to rapidly assess the health of a freshwater ecosystem using biomonitoring tools provides an early-stage opportunity to properly manage a potentially detrimental stressor. Recently, a major fish and organism kill caused by various environmental stressors occurred in a local freshwater system, the Ogeechee River. This study evaluates the effects of stressors on Ogeechee River streams with acceptable and impaired standards set by Georgia Environment Protection Division. We have identified four streams within the Ogeechee River that fall under category 1–4 representing acceptable levels, excess fecal coliforms counts, excess mercury levels and impaired biological integrity (BioF) respectively. Our study uses qualitative and quantitative zebrafish development bioassays on water samples from each of the categories to provide assessment of the effect of stressors on the aquatic community. Our results indicate BioF water has the most impairing effect on zebrafish embryo hatching and survival. Further, molecular analysis indicates upregulation of catalase and metallothionein RNA expression, suggesting a potential oxidative and metal-based toxicity. This early detection of stressors and understanding the subsequent effects will contribute to a growing knowledge base in making appropriate system management decisions.

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High-Content Screening Assay for Identification of Chemicals Impacting Spontaneous Activity in Zebrafish Embryos

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Although cell-based assays exist, rapid and cost-efficient high-content screening assays within intact organisms are needed to support prioritization for developmental neurotoxicity testing in rodents. During zebrafish embryogenesis, spontaneous tail contractions occur from late-segmentation (~19 hours postfertilization, hpf) through early pharyngula (~29 hpf) and represent the first sign of locomotion. Using transgenic zebrafish (fli1:egfp) that stably express eGFP beginning at ~14 hpf, we have developed and optimized a 384-well-based HCS assay that quantifies spontaneous activity within single zebrafish embryos after exposure to test chemicals in a concentration–response format. Following static exposure of one embryo per well from 5 to 25 hpf, automated image acquisition procedures and custom analysis protocols were used to quantify total body area and spontaneous activity in live embryos. Survival and imaging success rates across control plates ranged from 87.5–100% and 93.3–100%, respectively. Using our optimized procedures, we screened 16 chemicals within the U.S. Environmental Protection Agency's ToxCast™ Phase I library, and found that exposure to abamectin and emamectin benzoate—both potent avermectins—abolished spontaneous activity in the absence of gross malformations. Overall, compared to existing locomotion-based zebrafish assays conducted later in development, this method provides a simpler discovery platform for identifying potential developmental neurotoxicants.

All fish were handled and treated in accordance with approved Institutional Animal Care and Use Committee protocols at the University of South Carolina–Columbia.

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Characterization of a Full Length Pregnane X Receptor, *In Vivo* Expression, and Identification of PXR Alleles in Zebrafish

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The pregnane X receptor (PXR) (nuclear receptor NR1I2) is a ligand activated transcription factor, mediating responses to diverse xenobiotic and endogenous chemicals. The properties of PXR in fish are not fully understood. Here we report on characterization of full-length zebrafish PXR, *Danio rerio*, and *pxr* expression *in vivo*. Sequence variation among clones in the initial effort prompted sequencing of multiple clones from a single fish. There were two prominent variants, one sequence with S183, Y218, and H383 and the other with I183, C218, and N383, which we designate as alleles *pxr*1* (*nr1i2*1)* and *pxr*2 (nr1i2*2)*, respectively. In COS-7 cells cotransfected with a PXR-responsive reporter gene, the full-length Pxr*1 (the more common variant) was activated by known PXR agonists clotrimazole and pregnenolone 16α-carbonitrile but to a lesser extent than the full-length human PXR. Activation of full-length Pxr*1 was only 10% of that with the Pxr*1 LBD. qPCR analysis showed prominent expression of *pxr* in liver and eye, as well as brain and intestine of adult zebrafish. *Pxr* was expressed in adult heart and kidney at levels similar to that in intestine. The expression of *pxr* in liver was weakly induced by ligands for mammalian PXR or CAR (NR1I3). *Pxr* expression was also investigated in embryonic zebrafish throughout development. PCB153 does induce *pxr* expression in zebrafish at 72 hours postfertilization. The results establish a foundation for PXR studies in this vertebrate model. PXR allelic variation and the differences between the full-length PXR and the LBD in reporter assays have implications for assessing the action of PXR ligands in zebrafish.

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Low Levels of Arsenic Affect the Innate Immune Response in Embryonic Zebrafish

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Inorganic arsenic (iAs) in drinking water has become an international concern, and recently there has been a focus on how low-level exposures affect human health. Arsenic exposure is associated with diabetes, hypertension, stroke, immune deficiencies, and many types of cancers. Although arsenic is known to affect the immune system, there has been little progress in identifying underlying molecular mechanisms. The innate immune system, which is essentially the first line of defense against viruses, bacteria, and other pathogens, has now become a target of investigation based on previous studies linking low-level arsenic exposures to changes in pathways that regulate development of the innate immune system as well as deficiencies in macrophage and neutrophil function. To better understand how arsenic alters innate immunity, zebrafish embryos were exposed to 10 and 100 ppb concentrations of iAs at 5 hours postfertilization (hpf). Two transgenic zebrafish lines were used to measure the effect of iAs on the innate immune response, Tg(mpeg1:EGFP) for macrophage (MP) studies and Tg(mpo:EGFP) for neutrophil (NP) studies in which MPs and NPs are tagged with green fluorescent protein (GFP). At 4 days postfertilization (dpf) *Edwardsiella tarda* (*E. tarda*) were microinjected into the otic vesicle in order to visualize MP or NP migration to the infection site, after which various parameters (migration, filopodia extension, phagocytosis) were measured. Consistent with previous studies, iAs-exposed larvae failed to mount an innate immune response. We have also grown *E. tarda* to 10 and 100 ppb, injected it into the otic vesicle of 4 dpf larvae that were not exposed to iAs and observed a robust immune response, suggesting that iAs is affecting the function of MP and NP rather than masking the bacterium from detection by the innate immune system. Additional studies are underway to understand the molecular and functional effects of iAs on MPs and NPs. Specifically, embryos will be disaggregated into single cell suspensions and sorted by fluorescence-activated cell sorting to isolate GFP+ MPs or NPs. Isolated MPs and NPs will then used in phagocytic and respiratory burst assays to measure changes in their ability to mount a proper immune response, and will be subjected to transcriptomic analysis to determine alterations in gene function resulting from iAs exposure.

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Differential Binding of Land-Applied Wastewater to the Three Estrogen Receptors—Era, Erba, and Erbb—of the Atlantic Croaker (*Micropogonias undulatus*)

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The binding of estrogens to estrogen receptors (ERs) in target tissues plays a key role in the regulation of many important physiological processes. Xenoestrogens are natural and synthetic chemicals that are released into the environment and can bind to and activate ERs. These endocrine-disrupting compounds (EDCs) can make their way into aquatic ecosystems, which may lead to the disruption of normal endocrine function in fish and other species. Due to the prevalence of EDCs in the environment and their potential to harm wildlife, it is important to develop an effective mode-of-action screening assay that encompasses a broad range of possible estrogenic compounds. Although vertebrates possess multiple ER subtypes with different ligand binding and transactivation properties, most screening is done using a single ER subtype, the human ERa. This approach may result in an underreporting of estrogenic contamination, particularly for nonmammalian species. Teleost fish express three ER subtypes (ERa, ERba and ERbb), with ligand binding profiles distinct from each other and mammalian ERs (ERa and ERb). We used bacterially expressed teleost ERs in competitive binding assays to investigate the ability of groundwater samples from a municipal site that uses tree spraying for wastewater treatment to bind ERs. Groundwater collected from Well 3 at the site was concentrated by vacuum centrifugation (SpeedVac) and reconstituted at 10X concentration. Well 3 water concentrate had a higher relative binding affinity (RBA) to the ERs than did controls at 1X, 3X, and 6X concentrations. However, the RBAs were not dose dependent, suggesting assay inhibition at higher concentrations.

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Ciliopathy Proteins Regulate Proteasomal Degradation of Signaling Mediators, Revealing Potential Treatment Design for Ciliopathies

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The ciliopathies are a group of disorders caused by structural or functional defects in cilia and their anchoring structures, the basal bodies, with concomitant defects in broad paracrine signaling pathways that include Sonic Hedgehog, Wnt and Notch. To understand the mechanism(s) by which ciliopathy proteins regulate signaling transduction, we first examined the function of three ciliopathy proteins that localize to the basal body in Notch signaling. We show that loss of each protein leads to persistent Notch signaling in zebrafish embryos and cells, resulting from the stabilization of the Notch receptor intracellular domain (NICD) and JAG1 ligand. Similar accumulation of Sonic hedgehog components GLI2/3FL and SUFU was observed in neurons from mutant mice. These findings mirror the accumulation of β-catenin in ciliopathies (Gerdes et al. 2007), leading us to posit a broad proteasome-dependent role for these proteins. Therefore, we then tested whether activation of proteasome activity can rescue the defects caused by depletion of ciliary proteins. We were able to ameliorate BBS-established Notch and Wnt signaling defects, either by overexpression of proteasome activator proteins, or by treating cells and zebrafish embryos with the chemical proteasome agonist sulforaphane. Taken together, our data indicate that basal body-proteasome regulation is a common mechanism governing the regulation of paracrine signaling, and suggest that activation of the proteasome might be of clinical benefit to some ciliopathy patients.

Reference

Gerdes, J.M., Liu, Y., Zaghloul, N.A., Leitch, C.C., Lawson, S.S., Kato, M., Beachy, P.A., Beales, P.L., DeMartino, G.N., Fisher, S.*, et al.* (2007). Disruption of the basal body compromises proteasomal function and perturbs intracellular Wnt response. Nat Genet *39*, 1350-1360.

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Sublethal Dioxin Exposure Impacts Axial Bone Development in Japanese Medaka (*Oryzias latipes*)

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Recent studies from mammalian, fish, and *in vitro* models have identified bone and cartilage development as sensitive targets for dioxin (TCDD) and other aryl hydrocarbon receptor ligands. In this study, we assess how low-level embryonic TCDD exposure impacts axial osteogenesis in Japanese medaka (*Oryzias latipes*), a vertebrate fish model. Embryos from three transgenic medaka lines (*twist:EGFP, osx:mCherry, col10a1:nlGFP*) were exposed to a range of TCDD concentrations and reared to 10 days postfertilization (dpf) (hatchling stage) and 20 dpf (larval stage). Individuals were stained for mineralized bone matrix and imaged *in vivo* to assess skeletal alterations within medial and rostral vertebrae in relation to qualitative spatio-temporal analysis of osteoblast and osteoblast precursor cell populations. TCDD exposure impacted axial bone development through an overall attenuation of skeletal mineralization resulting in truncated centra, increased intervertebral ligament area, and reduced neural and hemal arch length. At later stages of development (20 dpf) this effect was more pronounced. Effects on mineralization were consistent with modifications in cell number and cell localization of transgene-labeled osteoblast and osteoblast progenitor cells. Through targeted RT-PCR, we confirmed altered transgene expression (*twist, osx,* and *col10a1),* as well as a significant reduction in *osc* expression, a marker of differentiated osteoblasts. Our results indicate that sublethal TCDD exposure impacts either proliferation and/or differentiation of osteoblasts during critical periods of bone growth and mineralization in medaka. Further work is underway to anchor our observed phenotypes with a global analysis of altered gene expression using RNA-Seq, and to extrapolate the effects to human health using mammalian *in vitro* models.

All animals in this study were used in compliance with protocols approved by the North Carolina State University Institutional Animal Care and Use Committee.

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Adverse Outcomes in Zebrafish Following a Parental Benzo[a]pyrene Exposure

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Multigenerational impacts of polycyclic aromatic hydrocarbon exposure are suggested by human cohort studies. To begin to develop adverse outcome pathways associated with parental dietary benzo[a]pyrene (BaP) exposure, zebrafish were used. Only F0 adult zebrafish were fed 0, 10, 114, or 1012 µg BaP/g diet at a feed rate of 1% body weight twice/day (equivalent to 0, 0.21, 2.3 and 20 µg BaP/g fish) for 21 days. Parental gonads were sectioned and evaluated for previtellogenic, vitellogenic, mature, and atretic oocytes in females and spermatogonia, spermatocytes, spermatids, and spermatozoa in males. Ovarian atresia was significantly decreased following high dose BaP exposure. The number of fertilized eggs was significantly decreased in F0 fish exposed to 20 BaP/g fish, but total egg production was not significantly affected by BaP in the F0, F1, and F2 generations. F1 (but not F2 – F4) mortality was significantly increased in larvae whose parents were exposed to 2.3 and 20 µg BaP/g fish by 48 and 56 hours postfertilization, respectively. Time to hatch in the higher doses significantly decreased in only the F1 and F4 generations. Multigenerational phenotypic impacts were detected in three generations (F1, F2, and F3) following the F0 exposure. Body morphology deformities (e.g. shape of body, tail and pectoral fins) were most extreme in the F1 generation although still present in the F2 generation. Craniofacial structures (e.g. length of brain regions and size of optic and otic vesicles), although not significantly affected in the F1 generation, emerged as significant deformities in the F2 generation. Swim bladder impacts in the F1 generation corresponded to BaP treatment, but in the F3 generation fish in all treatments had affected swim bladders. Molecular analysis is now being used to elucidate mechanisms that are associated with the phenotypic deformities detected across generations.

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