1199 A RELIABLE SCORING SYSTEM TO ASSESS DEVELOPMENTAL TOXICITY OF ENVIRONMENTAL CONTAMINANTS USING ZEBRAFISH EMBRYOS.

C. Willett, T. Fremgen, P. McGrath and C. Zhang. *Phylonix Pharmaceuticals, Inc., Cambridge, MA.* Sponsor: P. Mayeux.

Biological testing has become an increasingly major component of ecological risk assessment and monitoring. A rapid, reproducible and inexpensive predictive vertebrate model for hazard identification would greatly contribute to ecological risk assessment. We are developing such a model using the zebrafish embryo, which has several inherent advantages: the free-living zebrafish embryo is completely transparent, facilitating experimentation and analysis, its entire body plan is established by 24 hours post-fertilization (hpf), and by 5 days post-fertilization (dpf), virtually all organs are functioning, including the liver. We have developed a simple, rapid and reliable scoring system to detect and quantify developmental toxicity. Endpoints include mortality, hatching frequency, heart rate, circulatory abnormalities and other morphological parameters. Embryos were treated by semi-static immersion in compounds diluted in fish culture medium from 24 hours post-fertilization (hpf) to 120 hpf; fresh compound was added daily. Endpoints were assessed every 12 hours until 72 hpf, and then every 24 hr until 120 hpf. Using this scoring system, we have analyzed five compounds representing different classes of environmental toxins: 2, 3, 7, 8-tetracholorodibnezo-p-dioxin (TCDD), benzene, hexachlorobutadiene (HCBD), ethanol and 2, 4-dinitrotoluene (DNT). Our results indicate that the scoring system is predictive of toxicity in other vertebrates, and that the zebrafish is a promising model for assessing environmental toxicity.

1200 DEVELOPMENTAL TOXICITY OF MIXTURES OF DI- AND TETRACHLOROETHANE AND DICHLOROPROPANE IN EMBRYO CULTURE.

J. Andrews, H. Nichols and E. Hunter. *RTD, NHEERL, ORD, USEPA, Research Triangle Park, NC.*

Drinking water chlorination results in numerous chlorinated byproducts. We evaluated the developmetal and embryo toxicity of 1, 3-dichloropropane (1, 3DP), 2, 2-dichloropropane (2, 2DP), 1, 1-dichloroethane (1, 1DE) and 1, 1, 2, 2-tetrachloroethane (TCE) in rat whole embryo culture (WEC) due to their presence in chlorinated drinking water, their structural similarities, and a lack of developmental toxicity data for these compounds. Humans could be exposed to these four chlorinated propanes and ethanes (CPEs) simultaneously in drinking water and hence we evaluated them alone and in combination. Toxicity profiles were generated by exposing gestational day (GD) 9.5 rat embryos in WEC to the CPEs for 48 hours. The individual CPEs were all dysmorphogenic in WEC and embryonic exposure resulted primarily in rotation and heart defects. The embryonic effects from exposure were compared based on developmental score (DEVSC), death and dysmorphology as the parameters of comparison. Concentrations of individual CPEs chosen for the mixture studies were predicted to produce DEVSCs 25% below control values. These equipotent mM concentrations (14.5 1, 1DE, 1.5 TCE, 16 2, 2DP, 5.5 1, 3DP) were then used to determine the toxicity of all possible combinations, based on a dose-additivity model, of the four CPEs. Eight of mixture combinations gave experimental DEVSCs which were not significantly different from the predicted scores while three of the mixtures (1, 3DP/2, 2DP;TCE/1, 3DP/2, 2DP;TCE/1, 3DP/2, 2DP/1, 1DE) gave scores which were significantly lower than predicted. Embryo mortality was additive in ten of the eleven treatment groups, with one mixture significantly more embryo toxic (27% mortality) than predicted. Dysmorphology was significantly elevated in all treatment groups compared to controls and was neither significantly different between the groups nor different from dysmorphology seen in embryos following exposure to the individual compounds. These data suggest that the developmental toxicity of these halogenated propanes and ethanes is additive. This abstract does not necessarily reflect EPA Policy.

1201 ZEBRAFISH BIOASSAYS FOR ASSESSING SUBSTANCE ABUSE.

C. Parng, N. Anderson and P. McGrath. *Phylonix Pharmaceuticals, Inc., Cambridge, MA.* Sponsor: J. McCullough.

Substance abuse often leads to clinical impairment or distress resulting profound social, occupational and medical impact. New approaches to reduce the harm caused by the use of alcohol, tobacco, prescriptive and illicit drugs are urgently needed. Due to inherent attributes including, optical clarity, rapid development, a well-defined nervous system and predictable behavior, we used the zebrafish embryo as an animal model to study substance abuse. We observed growth retardation, abnormal morphology in the heart, brain, trunk, craniofacial structure and nerve system, as well as, induced apoptosis in response to continuous administration of alcohol. These results suggest ethanol-treated zebrafish embryos exhibit clinical manifestations similar to those of human fetal alcohol syndrome and it is therefore a model for studying the impact of alcohol and other substance abuse on fetus. In addition, using subtractive hybridization, we showed that gene expression is regulated in response to alcohol treatment.

1202 TEST RESULTS WITH EIGHT CHEMICALS IN A DROSOPHILA-BASED DEVELOPMENTAL TOXICITY PRESCREEN.

D. W. Lynch. *DART, BHAB, NIOSH, Cincinnati, OH.*

To further characterize the Drosophila-based prescreen to detect developmental toxicants, the following 8 chemicals were evaluated - methyl mercury chloride (MMC), methotrexate (MTX), L-phenylalanine (LPA), sodium arsenate heptahydrate (SAH), cadmium chloride (CC), vinblastine sulfate (VBS), aminopterin (APN) and mitomycin C (MC). All of the test agents are mammalian developmental toxicants and/or teratogens. One-to-three experiments, each employing multiple concentrations and including a concurrent control, were conducted with each chemical using our published protocol (Teratogenesis, Carcinogenesis, and Mutagenesis 11:147-173, 1991). Drosophila were exposed throughout development (egg through third instar larva) in culture vials to medium containing the test chemical. A mated, untreated, Oregon-R wild-type female (Mid-American Drosophila Stock Center, BGSU, Ohio) was added to each vial and allowed to oviposit for 20 hours, then removed. Emerging offspring were collected over 10 days, and examined microscopically (25x) for bent humeral bristles and wing blade notches, morphological defects shown to occur with an increased incidence in flies exposed to developmental toxicants. In each experiment, the incidence of the two defects at each concentration was compared to the controls using chi-square. In cases where replicate data were available at a given concentration, incidence data were also pooled and compared to the pooled controls. The incidence of bent humeral bristles was statistically increased ($p<0.05$) in flies exposed to MMC, LPA, SAH, CC, VBS, and MC. VBS also statistically increased ($p<0.05$) the incidence of eye defects. The incidence of wing blade defects was statistically increased ($p<0.05$) in flies exposed to MTX and APN. These results with 8 diverse chemicals provide additional support for increased utilization of this assay as a prescreen for the detection of developmental toxicants.

1203 ZEBRAFISH AS A PREDICTIVE MODEL FOR ASSESSING TOXICITY OF CHEMOTHERAPEUTICS.

P. McGrath, T. Fremgen, C. Zhang and C. Willett. *Phylonix Pharmaceuticals, Inc., Cambridge, MA.* Sponsor: P. Mayeux.

The efficacy of many chemotherapeutics is mitigated by high toxicity. A reproducible and inexpensive predictive method for screening novel therapeutics for general toxicity would be extremely valuable. We previously demonstrated the feasibility of using zebrafish embryos to study toxicity by assessing the LC50 of 20 commercially available compounds and found good correlation between zebrafish and other mammalian models. We have further tested the model by assessing the general toxicity, including the LC50, MTD, and target organ morphology, of 15 chemotherapeutic compounds, including paclitaxel, tamoxifen, dexamethasone and actinomycin D. Embryos were treated by semi-static immersion in compounds diluted in fish culture medium from 24 hours post-fertilization (hpf) to 120 hpf. Mortality was assessed and fresh compound was added daily. MTD and organ morphology were assessed at 120 hpf. Our data correlates well with mammalian data and supports zebrafish as an alternative animal model for general toxicity testing of pharmaceutical compounds.

1204 THE TERATOGENIC EFFECTS OF ENVIRONMENTAL ETHANOL EXPOSURE.

E. J. Loucks[1,2], T. Rubenstein[2] and M. J. Carvan[1,2]. *[1]Biological Sciences, University of Milwaukee-Wisconsin, Milwaukee, WI and [2]Great Lakes WATER Institution, Milwaukee, WI.*

The long-range goal of this project is to elucidate the molecular mechanism by which ethanol perturbs embryonic and fetal development, and to identify genes that plays a role in the sensitivity to ethanol-induced teratogenesis. Maternal exposure to ethanol from alcoholic beverages and many consumer products has been linked to developmental abnormalities in human and laboratory animals. Fetal exposure to alcohol is primarily dictated by voluntary maternal behavior and societal influences. Generally, consumption of alcohol induces alterations in facial features, major organs, and bone structures and will trigger damaging effects on the brain, which will lead to learning disabilities and behavioral problems. This can have a tremendous toll on the affected individuals, their families, and society as a whole. Data from twin studies and animal models argue strongly for a robust genetic component to ethanol-induced teratogenesis. In order to take advantage of the powerful genetic capabilities of the zebrafish to identify genes that influence sensitivity,