of BPA in exosomes was measured using enzyme-linked immunosorbent assay (ELISA) kit. These exosomes were labeled with PKH67 dye. To confirm their internalization, PKH67-labeled exosomes were administered to MCF-7 breast cancer cells and their uptake was observed under fluorescence microscope in a time-dependent manner. Cell Counting Kit-8 was utilized to assess viability and the proliferation of MCF-7 cells was evaluated through bromodeoxyuridine (BrdU) assay. Western blot analysis was conducted to determine the expression levels of cell cycle-related proteins. Real-time PCR was employed to quantify mRNA levels of estrogen receptor (ER) target genes. **Results:** We confirmed the ability of HepG2-derived exosomes to enter MCF-7 cells, and observed their internalization into the cells starting from 2 hours after BPA-Exo treatment. The concentration of BPA loaded within the exosomes after treating HepG2 cells with BPA was approximately 39 nM. To analyze cell proliferation and viability, we treated MCF-7 cells with the free BPA or BPA-Exo at a concentration of 2 nM. As a result of this treatment, free BPA did not significantly increase cell viability at a concentration of 2 nM. However, when MCF-7 cells were treated with 2 nM of BPA-Exo, it led to a significant increase of over 30% in the cell viability. Also, in the BrdU assay results, BPA-Exo significantly increased the cell proliferation and induced expression levels of cell cycle related-proteins. BPA is known to interact with estrogen receptors and influence estrogenic effects in the breast cancer. Interestingly, our results showed that BPA-Exo significantly upregulated the mRNA expression of pS2, an estrogen receptor (ER) target gene, by more than 11-fold, whereas the free BPA did not increase the expression of pS2. **Conclusions:** In summary, our findings indicate that liver-derived exosomes loaded with BPA significantly enhance the viability and proliferation of breast cancer cells, as well as leading to significant upregulation of ER target genes. In contrast, free BPA at the same concentration had no effect on these cellular processes. This suggests that exosome-mediated delivery of BPA may play a significant role in influencing the development of breast cancer.

PS 3916 Effects of Embryonic Atrazine Exposure on Estradiol and Dopamine Neurohormone Concentrations and Expression of Associated Gene Targets

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Background and Purpose: Atrazine is an herbicide used to control broadleaf and grassy weeds on agricultural fields in the US but this herbicide has been banned from use in the European Union since 2003, based mainly on risk of contamination of surface and groundwater. Atrazine is categorized as an endocrine disrupting chemical (EDC), altering release of luteinizing hormone from the pituitary through gonadotropin-releasing hormone in the hypothalamus. The specific mechanism that leads to this disruption is not yet clearly defined. Therefore, this study seeks to investigate the hypothesis that the neuroendocrine system is the central target for atrazine toxicity and an embryonic atrazine exposure will result in adverse effects on neurohormones and associated gene expression along the endocrine axes into adulthood. **Methods:** In this study, two neurohormones along with associated molecular targets were explored to assist in elucidating a mechanism that coincides with the observed adverse health outcomes along the endocrine axes. Using the zebrafish model, concentrations of estradiol and dopamine neurohormones were measured in atrazine exposed larvae as well as male and female adult brains using ELISA. Adult wild type zebrafish were bred to obtain embryos. Embryos were collected at 1 hour post fertilization (1 hpf) and randomly assigned to 0, 0.3, 3, or 30 ppb (µg/L) atrazine treatment to represent concentrations around the current US EPA regulatory level in drinking water of 3 ppb. Exposure was ceased at the end of embryogenesis (72 hpf) and either collected for larval evaluation or grown to different timepoints in adulthood for collection of brains [72 hpf, 6 months post fertilization (mpf), 2 years post fertilization (ypf), or 2.5 ypf]. Second, gene expression of neuroendocrine molecular targets associated with dopamine as a neurohormone (*drd2a, drd2b, slc18a2*) and estradiol (*gnrh, lh, fsh*), were examined to determine if an embryonic atrazine exposure perturbed neuroendocrine development using qPCR following the exposure from 1-72 hours post fertilization (1-72 hpf) at the same atrazine treatments. **Results:** Results indicated no significant changes ($p>0.05$) in estradiol measured in atrazine exposed larvae at 72 hpf. Estradiol concentrations measured in embryonic atrazine exposed (1-72 hpf) 6 mpf female adult brains showed a significant decrease ($p<0.05$) at 0.3 and 30 ppb while 2.5 ypf female brains showed a significant increase ($p<0.05$) at 3 ppb. Estradiol concentrations in embryonic atrazine exposed (1-72 hpf) 6 mpf and 2.5 ypf male brains both showed no significant differences ($p>0.05$). Dopamine concentrations measured in atrazine exposed larvae (1-72 hpf) displayed significant decreases ($p<0.05$) at 0.3 and 30 ppb. Dopamine concentrations measured in embryonic atrazine exposed (1-72 hpf) 2 ypf adult female brains and adult male brains both demonstrated a significant increase ($p<0.05$) at 3 and 30 ppb. No statistical significance in gene expression from the embryonic (1-72 hpf) atrazine exposure ($p>0.05$) was observed for *drd2a, drd2b, slc18a2, gnrh*, or *lh* while *fsh* exhibited a decrease in gene expression ($p<0.05$) at 0.3, 3, and 30 ppb atrazine exposure (1-72 hpf). **Conclusions:** The results of the study conclude that atrazine exposure surrounding the US EPA maximum contaminant level of 3 ppb during embryogenesis continue to show adverse effects later in adulthood along neuroendocrine pathways. Studies to further examine perturbations along the pathways associated with these biomarkers are necessary to elucidate the mechanism of atrazine toxicity.

PS 3917 Identification of environmental chemicals that stimulate the human GnRHR and KISS1R provides insight into possible stimuli for early puberty

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Background and Purpose: In the last decade, there has been an increased prevalence of early breast development in girls, suggesting the possible influence of environmental factors. Given that the re-activation of the reproductive system during puberty is driven by the hypothalamic neuropeptides kisspeptin and gonadotropin-releasing hormone (GnRH), we conducted a study to see if an ambient chemical may activate the kisspeptin or GnRH receptor. **Methods:** We utilized HEK293 cell lines genetically-engineered to over-express the GnRH receptor (GnRHR) or kisspeptin receptor (KISS1R) to identify receptor agonists within the Tox21 10K compound library, a collection of authorized pharmaceuticals and environmental chemicals. Agonists were identified utilizing a Ca^{2+} flux detection assay as the primary screening method. This assay involved testing at 15 different concentrations ranging from 0.7 nM to 57.5 µM using a 1536-well plate format. Active compounds were confirmed using a p-ERK detection assay and by measuring the expression of genes (by qRT-PCR) that are transcribed following receptor activation in mouse hypothalamic or gonadotrope cell lines. Molecular docking modeling was employed to forecast the likely binding configuration between small compounds and receptors. **Results:** Fifty-eight compounds were chosen from the primary Ca^{2+} flux screening as possible agonists of GnRHR and/or KISS1R. Thirty GnRHR agonist candidates demonstrated p-ERK activity in HEK293-GnRHR cells, including two known GnRHR agonists (fertirelin and gonadorelin). A group of cholinergic agonists with structures similar to methacholine were also discovered to be GnRHR agonists. When administered to mouse gonadotrope cells which express *Gnrhr* (LβT2 cells), these agonists enhanced the expression of genes known to act downstream of the Gnrhr, such as *Fos*, *Jun*, and/or *Egr1*. Molecular docking revealed a potential interaction between the GnRHR and five agonists; Asn305 was the most conserved binding site for GnRHR. Six KISS1R agonist candidates exhibited p-ERK activity in HEK293-KISS1R cells. One of these compounds was musk ambrette. Treatment of murine hypothalamic cells that express *Kiss1r* (mHypoA-GnRH/GFP cells) with musk ambrette led to increased expression of *Gnrh1*, and molecular docking studies demonstrated that musk ambrette establishes contacts with the His181, His309, and Gln122 residues of the KISS1R. **Conclusions:** In conclusion, through the utilization of the Tox21 10K compound library and a quantitative high-throughput screening technique followed by confirmatory testing in pertinent cell types (specifically, mHypoA-GnRH/GFP and LβT2), we have confirmed known agonists and identified novel agonists for the GnRHR and KISS1R. Further studies are necessary to investigate whether or not these agonists play a role in the increasing prevalence of early puberty in girls.

PS 3918 Effects of polycarbonate emissions generated by 3-dimensional printing on circulating steroids and anterior pituitary hormones

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Background and Purpose: 3-dimensional (3D) printing is commonly used to produce a wide range of plastic consumer products in manufacturing settings, schools and homes. The heating of plastic feedstock during the printing process generates emissions containing particulate matter and potentially toxic chemicals that can be inhaled by people near the printers during the printing process. Manufacturers may have engineering controls such as shields and exhaust vents in place to prevent workers from inhaling particulate and fumes. However, less expensive printers found in schools and homes often don't have these devices to protect people from exposure. Exposure to emissions generated during printing is associated with dysfunction of a number of physiological systems. For example, we previously demonstrated that inhalation of polycarbonate (PC) emissions generated during 3D printing results in deposition of bisphenol A (BPA) in the respiratory system. BPA exposure disrupts both reproductive and metabolic function. Based on these data we hypothesized that endocrine disruption in rats exposed to 3D printer emissions (3DE) would be associated with inhalation of BPA in the PC stock. **Methods:** Male Sprague Dawley rats (n = 48) were exposed to filtered air or particulate and fumes generated using black PC filament with 5 desktop-3D-printer nozzles (exposure 4 h/day (d)). The 4 h average particle concentration delivered to the breathing space was 2.15mg/m^3. Animals were exposed for 1d or 4 d/week, or until they had been exposed for 1, 8, 15 or 30 d. The after the last exposure animals were euthanized, and serum was isolated. Using BPA measured during a single exposure, a deposition model estimated that BPA deposition in the respiratory system was 0.115, 0.917, 1.72 and 3.44 µg after 1, 8, 15 and 30d of exposure. **Results:** Testosterone concentrations of 3DE and air control animals were higher after 8 and 15 d of exposure than after 1d of exposure. This difference was more pronounced in the control animals. Progesterone was reduced after 1, 15 and 30d of 3DE, as were thyroid stimulating hormone (TSH) levels after 1, 15 and 30d of 3DE, and 8 and 30d of air control exposure. Increases in follicle stimulating hormone (FSH) levels

were observed after 1, 8 and 30d of 3DE, and in circulating estradiol levels after 15 and 30d of exposure of 3DE. **Conclusions:** Based on these results, inhalation of 3DE results in changes in circulating concentrations of hormones associated with the regulation of reproduction and metabolism, and these effects are, at least in part, due to BPA in the PC stock. Changes in hormone levels may potentially have significant effects on reproductive and endocrine function in workers and children. These findings are consistent with studies looking at the ingestion of BPA. Disclaimer. *"The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention.*

PS 3919 Control Performance of Medaka Extended One Generation Test Designs

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Background and Purpose: Assessment of endocrine activity and disruption in humans and wildlife involves conduct of specific assays to evaluate relevant pathways and adverse effects. Multiple *in vivo* test guidelines (TGs) have been validated for mammals, amphibians, and fish, focusing on the estrogen, androgen, thyroid, and steroidogenesis pathways. They are now being conducted widely to satisfy regulatory requirements to identify chemicals that have potential to interact with endocrine pathways. However, these assays often require the use of a substantial number of laboratory animals, and their outcome can lead to significant regulatory actions. It is therefore critical that the assays are sufficiently reliable and robust. The Medaka Extended One Generation Test (MEOGRT; OECD TG 240/EPA OCSPP 890.2200) is an *in vivo* assay designed to provide comprehensive data on adverse effects and endocrine-relevant endpoints for key aspects of the fish life cycle. Potential adverse effects on population-relevant parameters are assessed including survival, growth, development, sex ratio (phenotypic vs genetic) and reproduction (fecundity/fertility). In addition, providing mechanistic information and linkage between results from other studies where there is evidence for a chemical having the potential to interact with endocrine pathways, liver vitellogenin, secondary sexual characteristics, and gonad histopathology are assessed. There is currently no set mechanism to review established TGs and assess their utility or performance, although the OECD TG 240 states that "it is anticipated that when a sufficient number of studies is available to ascertain the impact of this new study design, the TG will be reviewed and if necessary revised in light of experience gained". The current work aims to use historical control data to better understand the test performance and endpoint relevance, facilitate understanding of variability within the method, and aid interpretation of study data. **Methods:** Control data were collated for 25 control groups from 24 independent studies conducted following the MEOGRT TG (TG 240/OCSPP 890.2200) or providing similar data as the MEOGRT design, including 14 Medaka Multigeneration Tests (MMT) / MMT-like tests conducted prior to adoption of TG 240. The data were assessed across 17 biological validity criteria within the OECD/EPA TGs for: a) the number of control groups for which the relevant data were reported; b) the number of control groups meeting the relevant validity criterion; and c) the percentage of control groups meeting each validity criterion, where reported. These assessments were conducted for all 25 control groups as well as a comparison of the 14 MMT/MMT-like study control groups vs. 11 MEOGRT control groups. **Results:** Overall, for 9/17 criteria assessed, the relevant validity criterion was not achieved in at least 90% of the 25 control groups, including 4/5 criteria relating to reproduction. There was a mean of 2 validity criteria failures per control group, and 2 criteria not reported per control group. The proportion of MEOGRT control groups meeting the relevant validity criterion was higher vs. MMT/MMT-like control groups for almost all criteria. For the MEOGRT control groups, 4/17 criteria were not achieved in at least 90% of the control groups including the two criteria related to F1 fecundity. There were fewer validity criteria failures per control group in the MEOGRT control groups (mean of 2 failures) vs. MMT/MMT-like (mean of 1 failure). The MEOGRT control groups had a higher rate of reported validity criteria (mean of 2 not reported) vs. the MMT/MMT-like studies (mean of 3 not reported). **Conclusions:** The results of the first stage of this retrospective data analysis indicate that the studies following the MEOGRT study design had enhanced compliance to biological control validity criteria, possibly due to the increased replication in the MEOGRT compared with the MMT/MMT-like studies. There is however still a high likelihood of one or more validity criteria failures in the MEOGRT, particularly those related to reproduction endpoints which increases the potential for studies to be repeated. This is not ideal considering the high animal use of the studies, and current lead times to have them placed within the few experienced laboratories offering the test. Reliable historical control data ranges will now be developed for the core study endpoints and the associated validity and performance criteria. Further analysis will be conducted including descriptive statistics for each endpoint, assessment of inter and intra-laboratory variation, and variance component analysis. Such analyses will provide data-driven evidence to assist with a realistic interpretation of validity and performance criteria and identify areas for test facility and TG improvement where appropriate. **This abstract does not necessarily represent US EPA policy.*

PS 3920 Effects of Developmental Exposure to Microplastic Leachates on Development and Behavior of Larval Zebrafish (*Danio rerio*)

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Background and Purpose: Microplastics (MPs) are widespread environmental contaminants that have garnered increasing concern. These minute plastic particles contain additives, such as heavy metals, bisphenols, and disinfection byproducts, which have the potential to leach into the surrounding environment. However, the risk posed by these leachates, especially during critical stages of development, remains poorly understood. This study investigates the impacts of early developmental exposure to leachates from real MPs on the development and behavior investigated in the zebrafish model. **Methods:** Plastics, acrylonitrile butadiene styrene (ABS), polyethylene terephthalate (PET), polyamide 66 (PA66), polystyrene (PS), and reinforced glass fiber (MIX), were sourced from a recycling facility, washed, then frozen in liquid nitrogen and crushed to diameters below 500 µm to form MPs. Leachates were collected by suspending 2 g MPs in 50 mL of zebrafish-rearing media (E3) for 24 hours, while exposing them to UV-C light and agitation to simulate environmental weathering conditions. Zebrafish embryos were exposed directly to the leachates as well as four serial dilutions from each leachate (20-fold dilution factor) between 6- and 120-hours post-fertilization (hpf). At 120-hpf, effects on larval survival, overall development, prevalence of developmental defects, background activity, and behavioral responses to acute stressors and peripheral irritants were evaluated. Fluorescence and UV spectroscopy were used to identify the dissolved organic material (DOM) content in MP leachates. **Results:** Our initial findings demonstrate that exposure to MPs leachates did not alter survival, overall development, prevalence of developmental defects, or background activity of larval zebrafish. However, larvae exposed to PA66 leachates showed exaggerated behavioral responses at the lowest concentration ranges and depression in behavioral responses at the highest concentration ranges. Exposure to ABS leachates showed depression in behavioral responses at various exposure concentrations. Spectroscopic investigations indicate the presence of DOM in the collected leachates. **Conclusions:** This study provides valuable insights with significant translational relevance into the potential adverse effects of early developmental exposure to MPs leachates on behavior. Our findings highlight the importance of understanding the effects of exposure to these widely prevalent environmental contaminants on early development.

PS 3921 A WIDESPREAD APPROACH TO ASSESS TERATOGENICITY, THYROID DISRUPTION, AND DEVELOPMENTAL NEUROTOXICITY CAUSED BY ORGANIC CHEMICALS IN ZEBRAFISH EMBRYOS

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Background and Purpose: Chemicals pose an increasing risk to human and environmental health. Significant causes for concern are Teratogenicity, Neurotoxicity, and Endocrine-Disrupting Chemicals (EDCs). Using the zebrafish larvae as a New Alternative Methodology (NAM), we showcase a high-content strategy to distinguish the embryotoxic potential of reference substances as well as their thyroid disruptor potential and developmental neurotoxicity. **Methods:** Embryos of the transgenic fish line Tg(*tg*:mCherry) were exposed to a set of chemicals -Potassium perchlorate (PP), Benzophenone-2 (BP2), Propylthiouracil (PU), Acetaminophen (APA)- to evaluate the fluorescence of the reporter in the thyroid gland. By fluorescence measurements of the reporter for the thyroglobulin (tg), the compounds PP, BP2, and PU were properly classified as having a goitrogenic outcome for concentrations < EC10 of the systemic toxicity. To determine the potential downstream effects of the impaired endocrine system, T4 and T3 levels can be assessed by a newly developed LCMS technique for whole-embryo homogenates, which allowed for the detection of a T4 decline for PP, BP2, and PU-treated embryos from ca. 0.5 $\mu g_{T4}/L_{extract}$ to around 0.1 $\mu g_{T4}/L_{extract}$. Furthermore, gene expression analyses of thyroid genes (*tsh*β, *tpo,* and/or *tg*) were assessed using RT-qPCR. **Results:** A dose-dependent induction of *tg* mRNA for PP, BP2, and PU-treated zebrafish embryos was identified. However, this induction could not rescue the low T4 level-phenotype as pointed by the LCMS analyses. This could potentially be related to one of the chemicals' frequent modes of action to inhibit the thyroid peroxidase, which might have suppressed the iodide oxidation needed for functional T4. The results were complemented with the developmental toxicity (malformations; mortality) and DNT (with the Light/Dark transition Assay) assays in zebrafish to emphasize the applicability of thyroid disrupting assay, especially for EDCs, as its indirect effect related to them. **Conclusions:** In summary, APA resulted teratogenic for zebrafish embryos but having not goitrogenic effect, while BP2 was classified as toxic but likely not teratogenic and thyroid inhibitor under the testing conditions. PP and PU were classified as not toxic for zebrafish embryos but thyroid inhibitors, reinforcing the importance of studying the potential endocrine-disrupting effect of chemicals.

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