1787 Evaluation of the Toxicity of Welding Fume Particles on Murine Macrophage (RAW 264.7) and Human Placental (HTR-8/SVneo) Cell Lines

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Occupational exposure to welding fumes is associated with a decline in pulmonary function. Because men have traditionally comprised the bulk of the construction workforce, the majority of research performed on welding fume exposure in relation to reproductive toxicology has mainly focused on the adverse effects of exposure on sperm. According to the US Department of Labor Women's Bureau, the number of female welders in the United States has increased over the past decade. With the trend of more women entering the welding workforce, it is imperative that the adverse effects of welding fume exposure on female reproductive organs also be explored. In this study, murine macrophage cells (RAW 264.7) and human placental trophoblast cells from the first trimester (HTR-8/SVneo) were used to better understand the mechanisms of toxicity associated with stainless steel (SS) and mild steel (MS) welding rods. Fumes generated from MS are mainly comprised of iron and manganese, while SS welding fumes also contain hexavalent chromium and nickel. We hypothesized that the presence of these metals would play a role in the pro-inflammatory responses and cytotoxicities observed. Compared to MS, SS caused a significantly greater decrease in cellular viability, measured via MTT assay, along with greater damage to the nuclear DNA (comet assay) and damage to the cellular membrane LDH release, especially at the 24 h time point. Measured via electron paramagnetic resonance, exposure of cells to SS also produced greater hydroxyl radicals as compared to MS, while both SS and MS generated significant amounts of intracellular ROS. Using ELISA, production of the pro-inflammatory mediator Endothelin-1 (ET-1) was also measured. Upregulated in the setting of pulmonary hypertension and necessary for fetal formation, ET-1 levels in HTR-8/SVneo cells were increased more than 10-fold of normal circulating levels when exposed to MS and SS. Our data shows that both MS and SS are toxic to murine macrophage and human placental cells, though SS appeared to have more damaging effects. With such little data available on the effects of welding fume exposure on the female reproductive system, our results shed some light on this very important matter.

1788 *In Utero* TCDD or TCDF Effects on Pituitary and Testicular Steroidogenic Pathways

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In utero exposure to TCDD can reduce epididymal sperm counts in adult rats. A mode-of-action (MOA) involving AhR-induced suppression of fetal leutenizing hormone (Lh) and testis steroidogenic genes has been proposed. Experiments were performed to evaluate fetal AhR activation on pituitary hormone and testicular steroidogenic genes expression and to examine the potency of 2,3,7,8-tetrachlorodibenzofuran (TCDF) relative to TCDD. Pregnant Sprague Dawley rats (n = 5/group) were gavaged with 30, 300, 1000, 3000, 6000, or 10000 ng/kg of TCDD or TCDF on GD8. The GD8 loading dose was followed by GD9-20 daily maintenance doses of 0.3, 3, 22, 66, 132, 220 ng/kg/day for TCDD or 3, 30, 220, 660, 1230, and 2200 ng/kg/day for TCDF. We also examined the response to a single GD15 dose of 10,000 ng/kg for both congeners. In both study designs, maternal and fetal endpoints were examined on GD20. TCDD tissue concentrations in dam liver and adipose and the whole fetus were higher than TCDF (e.g., up to approximately 4-, 3- and 6-fold, respectively). One to two orders of magnitude higher maternal TCDD and TCDF concentrations were observed in maternal tissue relative to the whole fetus. AhR activation was observed in all tissues examined (including fetal pituitary and testis) as evidenced by *Cyp1a1* induction. Dose dependent decreases in *Lhb* and *Fshb* gene expression were observed in fetal pituitary after TCDD but not with TCDF. TCDD, but not TCDF, produced a dose-dependent decrease in fetal testis steroidogenic genes (*Star*, *Cyp11a1*, *Cyp17a1*, and *Scarb1*). While the evidence suggests a potential mode-of-action for reduced epididymal sperm counts, relatively high doses of TCDD were required. The lack of an effect of TCDF on fetal pituitary gonadotropin and testis steroidogenic gene expression could be secondary to limited tissue concentration accumulation caused by its self-induced metabolic clearance. TCDF's absent response and unique kinetics should be uncertainty considerations in the development of its relative potency and toxic equivalency factor.

1789 Prenatal Exposure to Di(2-Ethylhexyl) Phthalate Causes Long-Term Transgenerational Effects on Female Reproduction in Mice

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Di(2-ethylhexyl) phthalate (DEHP) is a plasticizer ubiquitously used in materials such as medical tubing, building products, and children's toys. Humans are exposed to DEHP daily by ingestion, inhalation, and dermal contact. DEHP is a known endocrine disruptor that affects female reproduction. However, it is not known if DEHP exposure can cause long-term effects on female reproduction, and if these effects can happen in multiple generations. Thus, this study tested the hypothesis that prenatal DEHP exposure affects follicle numbers, estrous cyclicity, and alters hormone levels in multiple generations of aged female mice. Pregnant CD-1 mice were orally dosed with corn oil (vehicle control) or DEHP (20 and 200 µg/kg/day, and 500 and 750 mg/kg/day) from gestation day 10.5 until birth. The F1 females were mated with untreated males to create the F2 generation, and the F2 females were mated with untreated males to create the F3 generation. At 1 year, ovaries, hormones, and estrous cycles were analyzed in each generation. Prenatal DEHP exposure altered estrous cyclicity, increased the presence of ovarian cysts, and decreased total follicle numbers in the F1 generation. It also decreased anogenital distance and altered follicle numbers in the F2 generation, and it altered estrous cyclicity and decreased folliculogenesis in the F3 generation. Further, prenatal DEHP exposure affected sex steroid, gonadotropin, and peptide hormone levels in all three generations. Collectively, these data show that prenatal exposure to DEHP has multi- and transgenerational effects on female reproduction, and it may accelerate reproductive aging. *Supported by NIH P01 ES022848, US EPA RD-83459301, and T32 ES007326.*

1790 Effects of Trichloroethylene Metabolite S-1,2-Dichlorovinyl-l-Cysteine on Placental Cell Invasion

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Trichloroethylene (TCE), an industrial solvent and c, is a widespread environmental contaminant that poses an ongoing threat to human health. Trichloroethylene exerts its deleterious effects through its metabolites including S-(1, 2-dichlorovinyl)-L-cysteine (DCVC). There is considerable evidence connecting TCE exposure to increased risk of pregnancy complications. Because insufficient trophoblast invasion is a defining pathological feature of pregnancy morbidities characterized by poor placentation, we evaluated the effect of DCVC exposure on invasion and migration capacity, proliferation and secretion of matrix metalloproteinases (MMPs) and cytokines. First-trimester human extravillous trophoblast cells, HTR-8/SVneo, were exposed *in vitro* to 10-100 µM DCVC for 24 h. Invasion capacity was measured using transwell chamber inserts coated with Matrigel, whereas migration capacity was measured using non-coated transwell inserts. Cell proliferation was measured CyQUANT® fluorescent nucleic acid stain quantified by plate reader. Cytokines and MMPs were measured using ELISA. Following 24 h of treatment with 20 µM or 100 µM DCVC, invasion capacity decreased by 18.0% (not significant) and 45.3% ($P<0.05$), respectively, compared to control, whereas migration capacity was reduced by 30.3% and 53.0% ($P<0.05$). In addition, after 24 h treatment, only 20 µM DCVC significantly reduced cellular proliferation (by 37.0% compared to control), with non-significant decreases of 30.20% and 32.0% at 10 and 50 µM, respectively. Extracellular levels of MMP-2 and 9 were not significantly changed with 20 µM DCVC treatment: however, 100 µM DCVC significantly reduced MMP-2 and 9 levels by 53.0% and 30%, respectively, compared to non-treated controls. Cytokine IL-8 significantly decreased by 46.0% and 38.5%, respectively, whereas levels of TGF-beta were unchanged. The TCE metabolite DCVC substantially suppressed invasion and migration capacity and decreased proliferation, accompanied by a decrease in extracellular MMP-2 and 9 and a decrease in IL-8. Future research will evaluate the effects of DCVC on epithelial-mesenchymal transition associated with an invasive cellular phenotype.

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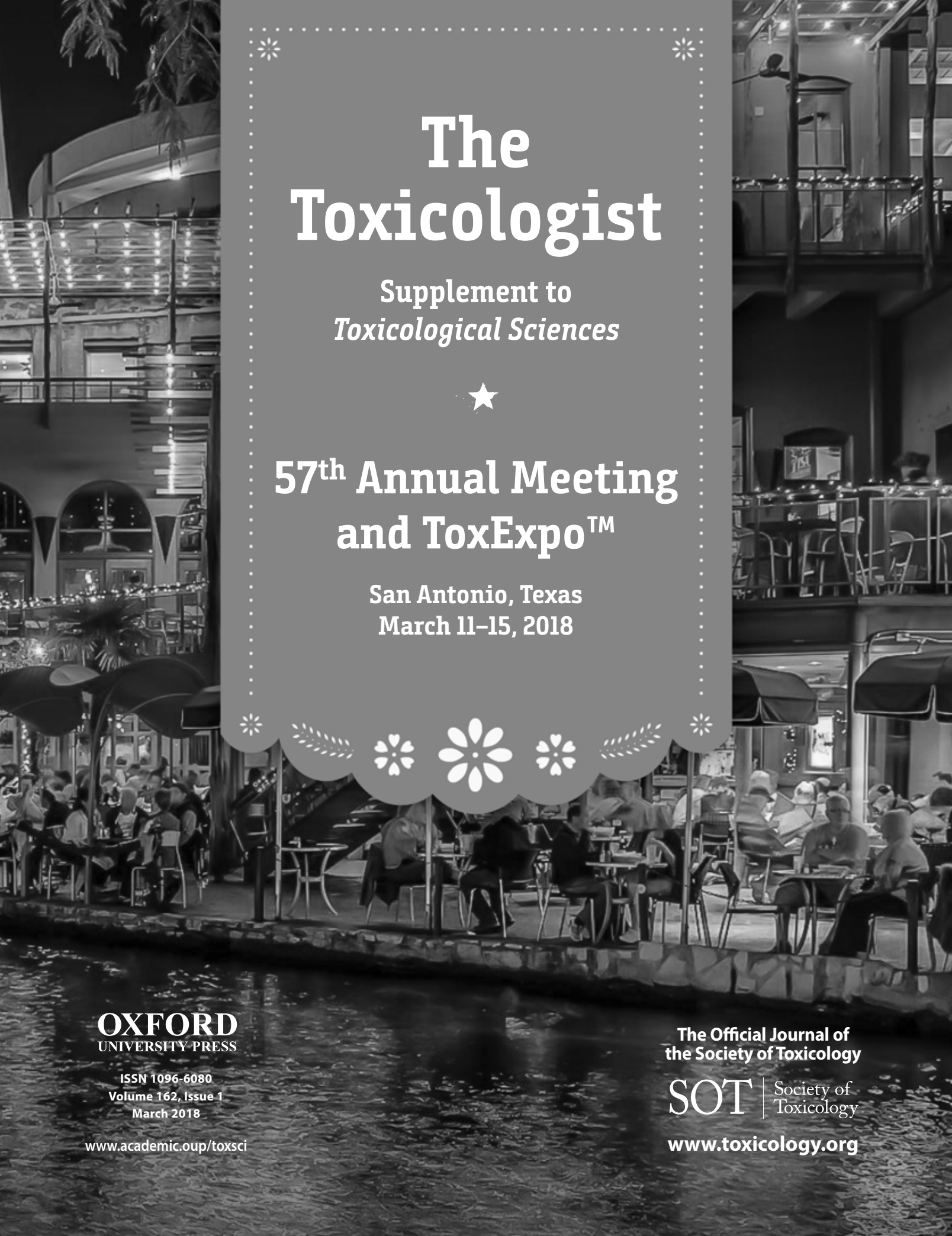

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