

Di(2-Ethylhexyl) Phthalate and its Metabolite Mono(2-Ethylhexyl) Phthalate Inhibit Steroidogenesis

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Phthalate esters are a group of synthetic plasticizers that impart flexibility to polyvinylchloride plastics and are widely used in food packaging, toys, cosmetics, and medical devices. Di(2-ethylhexyl) phthalate (DEHP) is the most commonly used plasticizer, and mono(2-ethylhexyl) phthalate (MEHP) is its active metabolite. Phthalates are known endocrine disrupting chemicals and have been shown to inhibit estradiol synthesis in previous studies, however, the mechanism of this inhibition is unknown. Estradiol synthesis requires production of progesterone, which is converted to androstenedione, then testosterone, and lastly, estradiol. Thus, the goal of this study was to identify where DEHP and MEHP disrupt estradiol production in the steroidogenic pathway. Antral follicles were isolated from CD-1 mice and exposed to vehicle, DEHP (1-100 µg/ml) or MEHP (0.1-100 µg/ml) for 96 h. After culture, media were subjected to measurements of hormone levels. The results indicate that DEHP did not affect progesterone levels compared to controls. In contrast, the highest dose of MEHP increased progesterone compared to controls ($n = 5-7$; $p \leq 0.05$). Both DEHP and MEHP decreased androstenedione and testosterone levels compared to controls ($p \leq 0.05$). Further, high DEHP doses and all doses of MEHP decreased estradiol levels compared to controls ($p \leq 0.05$). These results indicate that both DEHP and MEHP inhibit estradiol levels by targeting upstream hormones, primarily androstenedione and testosterone. Further, the results suggest that follicles are more sensitive to MEHP-induced inhibition of steroidogenesis than to DEHP-induced inhibition of steroidogenesis. Understanding the mechanism by which phthalates inhibit steroidogenesis will aid in the development of methods to prevent and treat adverse effects of phthalates.

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The Effect of Zoledronate on Chemokine Receptor Expression in Canine Osteosarcoma Cells

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Canine osteosarcoma (OS) is the most common primary bone tumor affecting dogs of large and giant breeds. OS causes aggressive localized bone destruction and metastases to distant organs, particularly the lung parenchyma. Although localized disease can be effectively managed with surgery, effective therapies for inhibiting metastatic progression remain in their infancy. Standardized palliative pain therapy for canine OS incorporates the use of zoledronate, a potent third-generation aminobisphosphonate that inhibits osteoclast activities, in combination with radiation therapy. Effective pain relief has been achieved with this specific protocol, suggesting adequate localized OS control. Interestingly, dogs treated with zoledronate and radiation subsequently die from distant metastases involving atypical anatomic sites. One potential explanation for the observed altered metastatic pattern could be zoledronate's capacity to modulate surface chemokine receptor expression, which regulate cell trafficking patterns during metastatic progression. We hypothesized that biologically achievable concentrations of zoledronate (1 uM and 5 uM) have the capacity to perturb expression of various chemokine receptors (CCR6, CCR7, CCR9, CCR10, CXCR4, and CXCR7) in canine OS cells. RNA and protein will be extracted from cultured Abrams canine OS cells. Quantitative PCR will be used to measure expression of the chemokine receptor genes; Western blotting will be used to detect the presence of chemokine receptor proteins. Results will indicate whether zoledronate enhances chemokine receptor expression, leading to abnormal metastatic lesions in canine OS.

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Molecule PAC-1 Activates Apoptotic Enzyme Caspase-3 to Induce Osteosarcoma Cancer Stem Cell Death

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Osteosarcoma cancer stem cells are resistant to apoptosis, presumably due to their ability to maintain the apoptotic enzyme caspase-3 in its inactive form, procaspase-3. A small molecule, PAC-1, has been found to directly activate procaspase-3 in a manner proportional to procaspase-3 concentrations. PAC-1 thus induces apoptosis in procaspase-3-heavy cancer cells. In this study, Abrams osteosarcoma cancer stem cells were isolated via sphere culture assay to determine procaspase-3 concentrations and to be probed with PAC-1. The effectiveness of Abrams stem cell isolation was assessed by showing equivalent expression of embryonic stem cell-associated genes Oct4 and Nanog via qualitative PCR relative to adherent

Abrams cells. Western blotting followed by densitometry testing showed that procaspase-3 levels increased five-fold in the spheres relative to adherent cells. Accordingly, the procaspase-3-heavy osteosarcoma cancer stem cell spheres should fall subject to increased apoptosis when probed with PAC-1 and therefore reduce the survival of this highly metastatic and therapy-resistant disease.

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The Influence of Environmental Conditions on the Abundance of a West Nile Virus Vector

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West Nile Virus results in significant morbidity and mortality of people, horses and birds. Epidemiologists have a need to understand *Culex* mosquito distribution, specifically *Culex pipiens* Linnaeus (Diptera: Culicidae) and *Culex restuans* Theobald, because of their ability to transmit West Nile Virus. Elevated numbers of infected vector mosquitoes increases the risk of illness, and mosquito abundance varies spatially and temporally based on variation in environmental conditions. *Culex* mosquitoes prefer to oviposit in stagnant water, making catch basins an established ovipositing site in the Chicago, Illinois area. Alternative sites for mosquito breeding include stagnant natural water sources, household containers, and discarded tires. We tested the hypothesis that adult *Culex* mosquito abundance is higher where there are more larval sites and that this relationship will vary depending on weather. We compared adult mosquito abundance recorded from 2006 to 2012 and the location of catch basins, natural standing water, and residential larval sites in a West Nile focus area in south Cook County, Illinois. The methods used included a geographical mapping of catch basins and identification of other larval habitat. Between June and August 2013, catch basins, houses, and natural water sources were sampled for larval presence. The number and species of larvae was recorded for each water source tested. A spatial statistical analysis was conducted to determine the relationship between available water sources, mosquito larvae abundance, and adult abundance. Results indicate that the abundance of mosquitoes varies from year to year, while the structure of the urban environment remains relatively constant.

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Shedding Some Light on Phthalate Activity in Circadian Rhythms

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Phthalates are ubiquitous plasticizers that act as endocrine disruptors, causing significant behavioral and health changes in rodents, domestic animals and humans. Daily biological rhythms regulate all physiological functions from body temperature to hormone production, but the effect of phthalates on rhythms has never been explored. To examine this issue we dosed mice with two model phthalates, Diethylhexyl phthalate (DEHP) or Di-n-butyl phthalate (DnBP) and analyzed behavioral rhythms and clock-controlled protein expression found in the master oscillator, the suprachiasmatic nucleus (SCN). Albumin D site-binding protein (DaBP) and peroxisome proliferator-activated receptor alpha (PPAR α) are two rhythmically expressed genes that play a role in daily behavioral rhythms and are involved in metabolism of phthalates. In the first experiment adult male CD-1 mice were gonadectomized and testosterone capsules were inserted to regulate hormone levels. The mice were then orally dosed with DEHP (750mg/kg/day) or oil daily for 30 days. In the second experiment, gonadectomized adult male and female CD-1 mice were orally dosed with DnBP (0.1 mg/kg/day) for 30 days. For both experiments the timing, amount and distribution of wheel activity was quantified. In a third experiment immunocytochemistry was used to assess numbers of cells expressing DaBP and PPAR α in the SCN of mice dosed with DEHP for 10 days. We predict that phthalate-treated mice will show an increase in overall activity as well as changes in cell numbers of rhythmic genes in the SCN. The results of this study will be the first to determine the impact of daily exposure to phthalates on the functioning of the fundamental circadian timekeeping system.

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Mitigation of Negative Effects of Corticosteroids on Biosynthetic Cartilage Matrix Production by TGF- β 1 or BMP-2

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Intra-articular corticosteroid (CS) injections are commonly used to treat equine lameness. In addition to their potent anti-inflammatory effects, CSs also suppress articular chondrocyte (AC) biosynthetic activities. We have shown that CSs significantly down-regulate expression of several BMP and TGF- β ligands by ACs. We hypothesize that co-administration of TGF- β 1 or BMP-2 to chondrocytes will mitigate the suppressive effects of CSs on collagen type II (Coll II). ACs were treated with 10^{-5} M methylprednisolone acetate (MPA) alone or with BMP-2 (100 ng/mL) or TGF- β 1 (10 ng/mL) for 5 days. Expression of collagen type II mRNAs was assessed by quantitative PCR. Collagen secretion was measured by ELISA. One-way ANOVA was used to analyze the outcomes. Administration of TGF- β or BMP-2 alone variably increased Coll II. However, neither growth factor was able to increase synthesis of these cartilage matrix components in the presence of MPA. Exogenous TGF- β 1 or BMP-2 administration did not mitigate the suppressive effects of MPA on AC matrix biosynthesis. This finding suggests that other elements in the TGF- β /BMP signaling pathway are also affected by CSs, or that factors directly required for AC matrix synthesis are negatively impacted by CS administration.

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Effect of Zoledronate on Osteogenesis of Equine Bone Marrow-Derived Mesenchymal Stems Cells

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Zoledronate (ZOL) is a bisphosphonate (BP) used to prevent osteoporosis and pathological fracture. BPs localize to mineralized matrix and, consequently, target bone-resorptive osteoclasts. Osteoprogenitor cells (OPCs) are critical for skeletal homeostasis. We hypothesize that ZOL will inhibit OPC aggregation and subsequent osteogenesis. Bone marrow-derived OPCs were collected from three healthy horses. Primary cells were seeded at 5×10^3 cells/cm² and passaged twice. After P2, cells were transferred to osteogenic medium +/- zoledronate [0.1, 0.5 and 1.0 μ M]. OPC aggregation was monitored by direct counting. After 7 and 10 days, OPC phenotype was assessed by staining for mineralized matrix (Alizarin red), alkaline phosphatase (ALP) activity and qPCR of osteogenic gene expression. Data were analyzed by ANOVA and Bonferroni's post hoc test. Over the first 7 days, ZOL did not affect cell number (DNA). However, at day 10, there was a clear reduction in OPC numbers in 1.0 μ M ZOL cultures, reflected by a 50% drop in [DNA]. ZOL significantly reduced OPC aggregation in osteogenic cultures. Osteogenic

medium induced ALP activity in OPCs. ZOL did not affect bulk ALP activity. The preliminary results of this study show that ZOL inhibits OPC aggregation and multicellular nodule formation but does not substantively affect ALP induction. Further phenotypic analyses will be required to establish the extent of ZOL's effect on OPC osteogenesis.

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Cytokine Expression in the Presence of Lactoferrin and Probiotic Supplementation in a Systemic *Staphylococcus aureus* Piglet Model

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Sepsis due to *Staphylococcus* species is a significant problem in preterm neonates, particularly those that are formula-fed rather than breastfed. The aim of this study was to examine the effect of supplementing infant formula with the probiotic *Bifidobacterium longum* subsp. *infantis* (*B. infantis*) and/or bovine lactoferrin (bLf) on cytokine expression in a piglet model of systemic *Staphylococcus aureus* (*S. aureus*) infection. Piglets are an especially good model for the human infant, due to similarities in gastrointestinal physiology, immune response and anatomy. The hypothesis was that the combination of *B. infantis* and bLf would promote a synergistic effect on the immune system, allowing the piglets to mount an appropriate immune response during the course of infection. It was also hypothesized bLf would promote *B. infantis* colonization in the intestine thereby increasing the beneficial effects of *B. infantis* on the immune system. Female, colostrum-deprived newborn piglets (n = 15) were individually housed and randomized to receive formula supplemented with one of the four treatments: bovine whey (4 g/L) (control), whey (4 g/L) + *B. infantis* (10⁹ CFU/mL), bLf (4 g/L), or combined bLf (4 g/L) + *B. infantis* (10⁹ CFU/mL). Piglets were infected with *S. aureus* (10⁵ CFU/mL at 1 mL/kg body weight) on day 7 and tissues were collected at euthanasia on day 12. Cytokine expression (IFN- γ , IL-6, and IL-10) was analyzed in spleen, lung, and liver tissues using quantitative real-time polymerase chain reaction. The aim of this research is to reduce neonatal morbidity due to systemic *S. aureus* infections. Formula supplemented with *B. infantis* and bLf has the potential to improve the neonatal immune response to systemic infections.

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Analysis of Streptococcus equi M-Protein: Comparisons Between Wild-Type, covS Mutant and Pinnacle IN Strains

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Streptococcus equi subsp. equi (*S. equi*) is the causative agent of strangles, a highly contagious disease of horses that causes inflammation and swelling of the nasopharyngeal lymph nodes. Vaccination against strangles is recommended for young horses. The commercially available modified live vaccine, Pinnacle IN, causes clinical symptoms of strangles in 20-25% of weanlings. Pinnacle IN has a covS single nucleotide mutation that may contribute to its attenuation, but this site has the ability to revert causing adverse vaccine reactions. CovS is a sensor of virulence and produces a signaling protein when it detects there are appropriate conditions for host infection. CovS then signals covR, which produces a protein that can up- or down-regulate several virulence genes, including SeM that encodes M protein. A covS deletion mutant was created to remove the gene, thereby eliminating the chance for reversion and producing a strangles vaccine. Testing of the deletion mutation in horses demonstrated partial protection of the animals from a *S. equi* challenge. We hypothesize that reduced expression of surface immunogens in the deletion strain, such as M protein, reduce the efficacy of the vaccine. Our work will compare the DNA sequences of SeM in the various strains, use quantitative PCR to assess alterations in SeM expression, and use Western blotting to examine differences in abundance of M protein in the various isolates. These data will indicate whether there is a connection between covS deletion, M protein abundance, and the efficacy of the putative new vaccine strain.

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The Influence of Urban Stormwater Management Practices on Mosquito Blood Feeding Behavior and the Potential for West Nile Virus Enzootic Amplification

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West Nile Virus (WNV) transmission intensity and exposure risk are not homogeneous across an urban landscape. Some of this variability in transmission intensity can be attributed to differences in host utilization patterns of the primary mosquito vectors, which determine the proportion of vector-host interactions that result in virus transmission. This project is designed to determine whether the species of birds that mosquitoes (*Culex* sp.) feed upon differs between detention basins and the surrounding residential land use, as well as the influence of the presence of invasive plants or communal bird roosts in these basins. We extracted host DNA from mosquito blood meals using the QIAamp DNA Micro Kit. Vertebrate host DNA was amplified using avian- and mammalian-specific primer pairs. These PCR products were purified, sequenced, and then analyzed using the National Center for Biotechnology Information database. This study will help identify habitat factors that facilitate the amplification of WNV in suburban environments by identifying how stormwater detention basins and their flora influence which bird species are fed on. This information could assist in the development of surveillance and control protocols for monitoring and reducing risk of WNV exposure for humans, domestic animals, and wildlife.

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