tion revealed no test article-related effects. The no observable adverse effect level (NOAEL) for immunotoxicity endpoints was 75 mg/kg/day. The data demonstrate that there were no GSK1349572-mediated alterations in T and B cell number or the diversity of the T cell repertoire and no effect on immune responsiveness in a TDAR, and therefore suggest no unusual compound-specific risk of developmental immunotoxicity in pediatric clinical studies.

PS 663 THE EFFECTS OF TRIBUTYLTIN (TBT) ON SPLENOCYTES IN F1 RATS EXPOSED TO TBT *VIA* PLACENTA, THEIR DAM'S MILK, AND/OR FOOD.

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Immunotoxicity is one of the major toxic effects of tributyltin (TBT) as well as neurotoxicity. Immunotoxicity is stronger on F1 rats exposed to TBT via placenta and dam's milk than adults. In our previous study, neurotoxic effects of TBT on F1 rats was observed and remained after cessation of exposure. Whether or not the immunotoxicity of TBT remains after cessation remains to be clarified. In this study, TBT immunotoxicity in F1 rats at 9 wk was evaluated by determining TNFα in the supernatant of the splenocytes. Pregnant Wistar rats were exposed to TBT at 0 and 125 ppm in their food. The F1 rats were exposed to TBT via the placenta and their dam's milk. After weaning, exposure to TBT in the F1 rats was stopped. At 6 wks of age, the rats were randomly assigned to either the control or the group fed a diet containing 125 ppm of TBT. The F1 rats were divided into the control-control (CC) group; the TBT-control (TC) group, exposed to TBT via placenta and dam's milk; the control-TBT group, exposed to TBT via food; and the TBT-TBT (TT) group, exposed to TBT via placenta, dam's milk, and food. At 9 wks, spleens were excised and cell suspensions of splenocytes were prepared. Concentrations of TNFα in the supernatant of activated splenic macrophages, T cells and B cells, were determined by ELISA. Mean body weights in the TC and TT groups were significantly lower than that in the CC group. Mean values of TNFα in the supernatant of B cells were higher in the TC and TT groups. There were no significant differences for TNFα in the supernatant of macrophages or the T cells. The effects of TBT on the splenocytes in the F1 rats exposed to TBT via the placenta and their dams' milk may continue after cessation.

PS 664 USE OF TDAR (SRBC) IMMUNOTOXICITY MONITORING IN AN EXTENDED ONE GENERATION REPRODUCTIVE TOXICITY TEST WITH LEAD ACETATE.

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The "Extended one-generation reproduction study" in rats as proposed by draft OECD guideline and the Agricultural Chemical Safety Assessment (ACSA) Technical Committee of the ILSI Health and Environmental Sciences Institute (HESI), requires evaluation of immunotoxicity in a functional antibody response assay. In the main study, Lead Acetate was provided in drinking water at 0, 100, 800 and 1700 ppm to F0 adult Wistar rats throughout pre-mating, mating, gestation, and lactation, and to the F1 generation to adulthood. A TDAR, sheep RBC (sRBC) assay was performed in the F1 generation subset designated for immunotoxicity assessment following a single iv injection of standardised sRBC (2x10^8/rat) between PND63-67. These animals were sacrificed 6 days later and serum prepared. Samples were analysed in a commercial ELISA kit for rat anti-sRBC IgM levels (Life Diagnostics Inc., PA, USA). The procedure was shown to be sensitive for the detection of immunosuppressive effects in a validation study and in a concurrent positive control group in the main study. Age-matched rats of each sex were treated with cyclophosphamide at 30 mg/kg bw/day for 5 consecutive days, then serum prepared on the 6th day. The positive control response was strongly and consistently reduced. There was no clear statistically significant difference between negative control and treated groups, although there was an indication of a trend to a lower response in male rats treated with Lead Acetate. This outcome showed that although Lead is considered to exhibit immunosuppression in some experimental models, the TDAR sRBC assay in the adult F1 rats in this protocol did not show a strong immunosuppressive response. However, it was shown that it is practicable to include a valid TDAR assay into this integrated developmental neurotoxicity, immunotoxicity and reprotoxicity protocol.

PS 665 LONG-TERM IMMUNOTOXIC EFFECTS OF COMBINED PRENATAL AND NEONATAL ATRAZINE EXPOSURE IN BALB/C MICE.

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Atrazine & its metabolites are present at high levels in a high percentage of water supplies in agro-intensive areas. Given that humans in these areas use water from these contaminated sources, we investigated the long-term effects of prenatal & neonatal exposure to atrazine on the immune system. To determine these effects, pregnant Balb/C dams were exposed to 700 μg atrazine per day from day 10 post-coitus to day 10 postpartum. All offspring were allowed to nurse their natural mother & were weaned at 25 days of age. Offspring were randomly segregated by sex & exposure & aged to approximately 1 year of age at which time their spleens were removed for analysis. Phenotypic analysis was performed using flow cytometry & cytokine production was measured by cytometric bead array after anti-CD3/anti-CD28 stimulation of the spleen cells.. The spleen cells were stained to determine total T cells, CD8+ T cells, CD4+ T cells, B cells, granulocytes, macrophages, NK cells, nTreg & LAG-3+-Treg cells. Cytokines measured include interleukin (IL)-2, IL-4, IL-6, interferon-γ, tumor necrosis factor-α, IL-17A, IL-10 & TGF-β1 which measures the functional ability of the cells to respond to stimulation as well as allows for further classification of the T cells into TH1, TH2, TH17 cells. One year old female offspring had significant decreases in the percentage of CD8+ T cells & significant increases in granulocytes & NK cells. There was a trend (same type of change; 1 of 2 runs significant) towards an increase in LAG-3+ Treg cells. Male offspring showed significant decreases in the percentage of CD8+ T cells & significant increases in CD4+ as well as a trend towards a significant increase in NK cells. This is the first time the long term changes in immune cell phenotype have been documented after a prenatal & neonatal exposure to atrazine & it demonstrates that these early life exposures can result in permanent changes to the immune system. Supported by NIH grants ES014698, RR016440 & RR020866

PS 666 EFFECTS OF CIGARETTE SMOKE ON INFECTIOUS BURDEN, RESPIRATORY INFECTION AND INFLAMMATORY RESPONSE WITH CHLAMYDIA PNEUMONIAE IN A MOUSE MODEL.

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Chlamydia pneumoniae (Cpn) is a ubiquitous pathogen that causes acute respiratory disease, particularly in children. No data currently exist on the effect of smoking on the course of Cpn respiratory infection. Given the alterations in the immune status of children following exposure to mainstream cigarette smoke (MCS) and the increased incidence of lower respiratory tract infection (LRTI) following CS exposure in young children, we hypothesized that exposure of juvenile C57BL/6J mice to MCS would increase the severity and duration of Cpn infection and alter airway responsiveness. Mice were exposed to MCS for either 1 or 2 weeks, followed by intratracheal instillation with Cpn (1x10^5 IFU/ml). Animals were then sacrificed at days 13-14 and 25-28 post-infection (PI). Cpn burdens in the lungs were determined using rtPCR and found to be similar in both air and MCS-exposed mice in both sets of experiments. Chronic pulmonary inflammation was noted in all Cpn-infected mice at both PI time points, but was most prominent at 25-28 days PI in both treatment groups. In those mice exposed for 2 weeks to MCS, significant differences between the groups were seen in serum cytokine levels (IL-4 and IFN-g),but only at the later timepoint (day 25 PI). There was also a shift in the ratio of IFN-g/IL-4 over time towards a Th2 bias in both experiments, which could be associated with the increased pulmonary inflammation seen here at day 25 PI. The worsening Cpn-induced inflammation that followed infection in the absence of increased bacterial burdens, and the shift to a Th2 bias in the MCS-exposed animals suggests that cigarette smoke (at least in part) may contribute to the exacerbation of injury; alterations of the T-cell response important in the resolution of some adverse lung lesions may be responsible for such effects. Supported by a Sub-contract with SUNY Downstate Med Ctr.