suite of immune parameters including secondary immune organ weights and cellularity, T- and B-cell proliferation, NK cell function, and T-cell immunophenotyping were assessed along with developmental markers such as eye opening, ear unfolding, body weight and length, and brain weight at 8 weeks of age. Body weight was decreased while brain weight was increased following treatment with 20 µg/kg TH. NK cell function was increased after exposure to 200 µg/kg TH. Thymus weight and cellularity were not altered; however, splenic cellularity was increased by the 100 and 200 µg/kg treatments. Splenic B220 cells were increased by the 100 and 200 µg/kg treatments while CD4-/CD8+ and CD4+/CD8- cells were increased after the 200 µg/kg exposure. This study identifies that postnatal exposure to TH can modulate the developing murine immune system.

475 DERMAL EXPOSURE TO JP-8 JET FUEL DURING PREGNANCY ALTERS IMMUNOLOGICAL FUNCTION IN F1 MICE.

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Approximately 5 billion gallons of JP-8 jet fuel (JP-8) are used annually by the United States Department of Defense (DOD) making this the single largest chemical exposure to military personnel. In addition, the aviation industry extensively uses commercial jet fuel which is essentially JP-8 without certain additives for anticing, corrosion, and static dissipation. Civilian exposure can occur as well through environmentally contaminated sites where jet fuel has been reportedly spilled or leaked from supply lines or storage tanks. Many reports indicate that JP-8 alters immunological function in adult rodents. However, little is known regarding its impact on the developing immune system. Therefore, C57BL/6N pregnant dams (mated with C3H/HeJ males) were dermally exposed with 10, 25, 50, or 75 µL neat applications of JP-8 daily during gestation days 6-15. F1 offspring were evaluated for immunological alterations at 3 and 8 weeks of age. There were no treatment effects on body, liver, kidney, or thymus mass. Although slight, an increase in spleen mass and cellularity was observed at both 3 and 8 weeks. T-cell proliferation following anti-CD3 stimulation was not affected at either age. However, IgM plaque forming cell (PFC) responses were significantly suppressed in 3 week old pups exposed to 50 or 75 µL JP-8 and in all treatment groups at 8 weeks of age. Our data indicate that prenatal dermal exposure to JP-8 can affect the developing murine fetus resulting in impaired humoral immune responses detectable at adulthood.

476 PHENOTYPICALLY ALTERED MURINE BONE MARROW SUBSETS EXPRESS AND ACTIVATE THE AHR IN RESPONSE TO TCDD.

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2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD), acting through the aromatic hydrocarbon receptor (AhR), elicits numerous toxicological effects, including those to the bone marrow (BM). Phenotypic alterations to BM subsets have been demonstrated, but the mechanism for these alterations is unknown. Additionally, the presence and functionality of AhR has been shown in crude BM but not in phenotypically defined subsets. To address this, crude BM from male C57Bl6 mice was harvested, stained with either biotinylated anti-B220 or lineage specific antibodies, and enriched for B220+ cells or Lin- cells using magnetic separation. Real-time RT-PCR and Western blot indicated the presence of AhR in both subsets of BM. The presence of Arnt protein in Lin- subsets was also confirmed by Western blot. Activation of AhR was assessed using a transgenic mouse line expressing LacZ under the control of dioxin responsive elements (DREs) in the promoter region. After 10h treatment, LacZ mRNA was induced in mice treated with TCDD as compared to vehicle. Phenotypic changes in progenitor cells were assessed by dosing C57Bl6 male mice with 30 mg/kg BW TCDD in olive oil (or vehicle alone). BM was harvested at 12, 24, 48, 72 hours, and 5, 7, 9, 11, 13, and 15 days, enriched for Lin- cells by magnetic depletion and stained with a cocktail of antibodies: Sca-1 PE, cKit APC, and either CD34 FITC or TdT-FITC. When compared to vehicle, TCDD-treated cKit hi/Sca-1+ cell populations were significantly increased. The increase peaked at 250% of vehicle at 5 days, and decreased to near vehicle levels by day 15. cKit lo/Sca-1+ cell populations increased less, peaking at 150% of vehicle at 9 days. cKit/Sca-1/34+ cells showed even less of an increase, peaking at approximately 110% at 3 and 9 days post treatment. TdT+ cells decreased with TCDD treatment but recovered to vehicle levels by 13 days. The data suggests a more profound effect of TCDD treatment upon less committed cell populations, mediated by the presence of the AhR. (Funded by NIEHS Grant ES04862, Training Grant ES07026 and Center Grant ES01247)

477 REDUCTION IN THE NUMBER OF SUPERANTIGEN-SPECIFIC T CELL DIVISIONS INDUCED BY 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN RESULTS FROM INCREASED APOPTOSIS.

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The precise mechanism by which 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) decreases antigen-specific T cell responsiveness is not clear. In the current study, we investigated the ability of T cells activated with staphylococcal enterotoxin A (SEA) to divide following exposure to TCDD by labeling the cells with 5, 6-carboxyfluorescein diacetate succinimidyl ester (CFSE) and analyzing them using a flow cytometer. To this end, female C57BL/6 mice were injected i.p. with 10 µg/kg bodyweight of TCDD followed by hind footpad injections of 10µg/footpad SEA. The popliteal lymph nodes (LN) were harvested on days one through four, labeled with CFSE and analyzed using a flow cytometer following 1-4 days of *in vitro* culture. In addition, the lymphocytes were analyzed for cells expressing Vβ3 or Vβ11 that become activated by SEA, as well as tested for apoptosis using TUNEL assay. Analysis using CFSE showed that TCDD-exposed lymphocytes exhibited a clear reduction in the number of cell divisions when compared to the vehicle controls. Even after reactivation *in vitro* with SEA, the lymphocytes from TCDD-treated mice demonstrated a decrease in the number of cell cycle progressions when compared to vehicle-treated mice. Also, exposure to TCDD caused a decrease in the percentage and total numbers of Vβ3+ and Vβ11+ cells. Moreover, increased levels of apoptosis were detected in the lymph nodes and in Vβ3/Vβ11 T cell subpopulations following TCDD treatment. Together, these data demonstrate that TCDD-induced apoptosis in SEA-activated T cells *in vivo* may account for decreased cell division and a consequent immunosuppression. (This work was supported in part by grants from National Institutes of Health R01ES09098, R01DA016545, R01AI053703, F31ES11562, R21DA014885, and R01HL058641)

478 EVIDENCE FOR INDUCTION OF APOPTOSIS IN T CELLS FROM MURINE FETAL THYMUS FOLLOWING PERINATAL EXPOSURE TO 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD).

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Perinatal exposure to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) causes thymic atrophy, but the precise mechanism of such toxicity remains unresolved. The current study investigated the role of apoptosis in TCDD-induced thymic involution following perinatal exposure to TCDD. To this end, C57BL/6 pregnant mice were injected intraperitoneally on gestational day (gd) 14 with a single dose of 10 µg/kg TCDD. Analysis of the thymus on gd-15, gd-16, gd-17, gd-18 and on postnatal day (PD) 1, showed a remarkable reduction in thymic cellularity 3-7 days after TCDD exposure. TCDD treatment also caused marked changes in the proportions of T-cell subsets, particularly on gd-17 and gd-18 thymocytes. *In vitro* culture of TCDD-exposed fetal or neonatal thymocytes showed increased apoptosis when compared to the controls, which peaked on gd-17. Triple-color staining involving CD4, CD8 and TUNEL showed that all four subpopulations of T cells underwent apoptosis following TCDD exposure, with the double-positive T cells undergoing the highest level. Moreover, increased cleavage of caspase-3 was seen when TCDD-exposed gd-17 thymocytes were directly tested. Furthermore, apoptosis-associated phenotypic changes were found in TCDD-treated neonatal thymocytes, which exhibited an increase in expression of CD3, αβTCR, IL-2R and CD44, but a decrease of CD4, CD8 and J11d markers. Finally, TCDD-exposed fetal and neonatal thymocytes had higher levels of Fas, TRAIL and DR5 mRNA, but the levels of Bcl-2, Bcl-xL and Bax were either unaltered or changed moderately. Taken together, these results suggest that TCDD-induced thymic atrophy following perinatal exposure may result, at least in part, from increased apoptosis mediated by death receptor pathway involving Fas, TRAIL and DR5. (This work was funded in part by grants from National Institutes of Health R01ES09098, R01 AI053703, R01 DA016545, F31ES11562, R21DA014885 and R01HL058641).

479 CONSEQUENCES OF TCDD EXPOSURE ON THE MIGRATION, PROLIFERATION, AND SURVIVAL OF ANTIGEN-SPECIFIC T CELLS.

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Previous studies have shown that TCDD causes a significant decline in the number of antigen-specific DO11.10 T cells in the spleens of mice following immunization with OVA. The loss of T cells follows what appears to be enhanced T cell activation

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