the immunoglobulin superfamily that amplifies the inflammatory response by triggering secretion of proinflammatory mediators, was upregulated in hepatic macrophages following administration of endotoxin to C3H/HeOuJ and C3H/HeJ mice. C3H/HeJ mice have point mutations in the toll-like-4 receptor, a critical component of endotoxin-mediated cell signaling, and are resistant to endotoxin-induced toxicity when compared to C3H/HeOuJ mice. Hepatic macrophages were isolated from livers following perfusion with collagenase, centrifugal elutriation and density gradient centrifugation. Macrophages from the livers of either C3H/HeOuJ or C3H/HeJ control mice expressed very low levels of TREM-1 mRNA as determined by semi-quantitative RT-PCR. Treatment of mice with endotoxin (3 mg/kg, ip) caused a time-dependent induction of hepatic macrophage TREM-1 mRNA expression which was maximal after 20 hr. Significantly less TREM-1 mRNA was induced in C3H/HeJ mice when compared to C3H/HeOuJ mice. In macrophages from both strains of endotoxin-treated animals, tumor necrosis factor-α (20 ng/ml) or interleukin-1β treatment (50 ng/ml, 24 hr) *in vitro* caused a further 2-10 fold increase in TREM-1 mRNA expression. Lipopolysaccharide (LPS) decreased TREM-1 mRNA expression 2-3-fold in macrophages from C3H/HeOuJ mice but did not alter its expression in cells from C3H/HeJ mice. Taken together, these data demonstrate that endotoxin is a potent inducer of TREM-1 mRNA in hepatic macrophages and that expression of the protein may be an important mechanism for amplifying the inflammatory response to hepatotoxicants. Endotoxin-resistance in C3H/HeJ mice may be due to limited expression of the TREM-1 protein. Support: NIH GM34310 and ES06897.

1599 IMMUNOMODULATION BY DIETHYLSTILBESTEROL IS DOSE AND GENDER LINKED: INFLUENCE ON THYMIC APOPTOSIS AND MITOGEN-INDUCED PROLIFERATION IN CD-1 MICE.

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It is perceived, but not shown, that the immunomodulatory influences of diethylstilbesterol (DES) may vary based on dose or gender. To address this, DES was subcutaneously administered to female and male CD-1 mice as four injections over 1 week at 0, 5, 15 and 30 μg/kg bw dose with immunological and reproductive parameters evaluated 24hr post last injection. Although female thymuses were significantly larger than male thymuses, short-term DES administration neither induced thymic atrophy nor altered relative percentages of thymic subsets. However, DES treatment did induce dose-related apoptosis in the CD4+8+, CD4+8- and CD4-8+ subsets using 7-amino-actinomycin D (7-AAD). The CD4-8- showed significant apoptosis only at the highest dose (30 μg/kg bw). Mitogen-induced proliferation of splenic lymphocytes also varied with hormonal doses and gender. In the females, splenic lymphocytes from low dose DES (5 μg/kg bw)-treated mice showed an increase in proliferative response to Con A, LPS or PMA/ionomycin compared to controls. Conversely, cultures from mice treated with the higher DES doses (15 or 30 μg/kg bw) showed suppressed proliferation, especially with Con A. In the males, DES appeared to produce minimal effects with the exception of increased proliferation to Con A in the 15μg/kg bw. Interestingly, the changes in mitogen-induced proliferation were not paralleled by similar changes in relative expression of CD90+ or CD45+ cells or ratios of anti-apoptotic Bcl-2 to apoptotic Bax proteins. Con A-activated splenocytes from DES-treated mice, specifically in the females, secreted less interferon-γ compared to controls. Collectively, these findings suggest that short-term exposure to DES generates a disparity in the immunological effects depending upon the dose of hormone and sex.

1600 THE GENERATION OF REACTIVE OXYGEN SPECIES DURING EXPOSURE OF PESTICIDE MIXTURES TO IMMUNE CELLS, *IN VITRO.*

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Recent reports suggest that pesticides pose potential health risks to the humans and animals by affecting their immune system. We have observed earlier that endosulfan and permethrin cause immune cell cytotoxicity mainly *via* apoptosis. We hypothesized that these chemicals induce immunotoxicity through Reactive Oxygen Species (ROS) formation. In an attempt to test this hypothesis, we have studied the production of ROS in splenocytes of C57Bl/6 adult male mice exposed to endosulfan and permethrin, *in vitro*. The generation of intracellular hydrogen peroxide (H_2O_2) and superoxide anion was monitored using a flow cytometer in combination with dichlorofluorescin diacetate (DCFH-DA) and hydroethidine (HE) dyes, respectively. Results of these analysis revealed that individual pesticides increased the production of both H_2O_2 and superoxide anion in a dose- and time- dependent manner. The mixtures of pesticides elicited a synergistic effect on the generation of H_2O_2. However, exposure to mixtures of pesticides had little effect on the generation of superoxide anion radicals as compared to individual pesticides. These findings suggest that the pesticide-induced immunotoxicity observed earlier may, at least in part, be associated with the generation of ROS.

1601 DEOXYNIVALENOL-INDUCED APOPTOSIS MEDIATED BY P38 MAPK-DEPENDENT P53 GENE INDUCTION IN RAW 264.7 MACROPHAGES.

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Deoxynivalenol (DON, vomitoxin) and other trichothecene mycotoxins cause immunosuppression by inducing leukocyte apoptosis. Upstream signaling transduction mechanisms contributing to DON-mediated apoptosis were investigated in RAW 264.7 cells, a murine macrophage model. PP1, a Src-family-tyrosine kinase inhibitor selective for Hck, and 2-AP (2-aminopurine), the chemical inhibitor of dsRNA-dependent protein kinase (PKR), additively inhibited DON-induced caspase-3 activity and apoptosis as well as phosphorylation of the mitogen activated protein kinases p38, ERK and JNK. PP1 and 2-AP also inhibited DON-induced p53 binding activity and subsequent phosphorylation of its substrate p21. Pretreatment with PFTα, an inhibitor of p53, abrogated DON-induced caspase-3 and apoptosis. The p38 inhibitor, SB 203580, abrogated DON-induced p21 phosphorylation as well as reduced DON-induced p53 binding activity, whereas ERK and JNK inhibitors were partially inhibitory. Finally, p38 inhibition blocked DON-induced apoptosis, ERK inhibition promoted DON-induced apoptosis, and JNK inhibition had no effect. The results suggest that the principal pathway for DON-induced apoptosis in the macrophage involves the sequential activation of Hck/PKR, p38, p53, caspase-3. (Supported by NIEH Grants ES-09521 and ES-03358).

1602 JP-8 JET FUEL DOES NOT ALTER SERUM CYTOKINE LEVELS IN B6C3F1 MICE FOLLOWING 7-DAY ORAL OR DERMAL EXPOSURE.

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The US Air Force uses approximately 2.5 billion gallons of jet propellant-8 fuel per year. As a result, a considerable number of military personnel are exposed to JP-8 fuel during refueling and servicing of aircrafts, and maintenance of fuel storage tanks. Those exposed have increased incidence of headaches, blocked nasal passages, ear infections, skin irritation, or fatigue. In rodent studies, we have previously reported that 7-day dermal or oral exposure to JP-8 suppresses humoral immunity and decreases thymus weight and cellularity. Additionally, other published studies have demonstrated that a single, dermal exposure to JP-8 modulated serum cytokine levels in mice, thereby accounting for a possible mechanism of immunosuppression. To determine if alterations in serum cytokines occurred after 7 days of exposure to JP-8, the following study was performed. B6C3F1 female mice aged 7-10 weeks of age were exposed to JP-8 dermally (50 uL applied to the clipped dorsal thorax of mice with an average weight of 20 g) or orally (2000 mg/kg/day) for a duration of 7 days. Serum was collected 24 hours after the last exposure to JP-8. Using ELISA and Cytometric Bead Array methods, it was determined that levels of IL-2, IL-4, IL-5, IL-6, IL-10, and TNF-alpha were not significantly altered after exposure to JP-8 *via* the oral or dermal route. It was also learned that, when used as a negative control in the dermal study, acetone as compared to olive oil induced a suppressive effect on IL-4 and IL-6 serum cytokine levels. Consequently, interpretation of immunological dermal studies utilizing acetone should be made with caution. Overall, these findings indicate that serum cytokine levels were not elevated after a 7-day exposure to JP-8, regardless of the route of administration. These observations indicate that serum cytokine levels are not sustained above normal levels following repeat exposure to JP-8.

1603 HEPATIC PHASE I AND II ENZYME PROFILES AFTER 7-DAY DERMAL OR ORAL EXPOSURE TO JP-8 JET FUEL.

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Many military and industrial personnel are exposed to JP-8 jet fuel during service and refueling of aircrafts, and maintenance of storage tanks. In toxicological studies using mice, it has been demonstrated that JP-8 can induce immunosuppression fol-