dichromate, Cr(VI), on the immune system in both rats and mice following exposure from drinking water. Female mice were exposed to Cr(VI) at concentrations of 15.6, 31.3, 62.5, 125, and 250 ppm while rats were exposed to 14.3, 57.3, 172, and 516 ppm in the drinking water for 28 days. Animals were evaluated for toxicological and immune function on day 29. In mice organ weights were not affected, while decreases in body weight and erythroid parameters were observed at the high dose level. Immune parameters such as the mixed leukocyte response, anti-CD3-mediated lymphocyte proliferation, splenic phenotypes, and natural killer cell activity were not effected. While a slight stimulation was observed in the plaque assay to sRBC in the middle dose levels, the effect was not dose-responsive in nature. No effect was observed on serum IgM ELISA to the T-dependent antigens, sRBC or KLH. In female rats, body weights, organ weights, and hematological parameters were not affected. Similarly, anti-CD3-mediated lymphocyte proliferation and natural killer cell activity were not affected. A slight increase was observed in the plaque assay at the lowest dose, similar to observations in the mouse. Overall, exposure from Cr(VI) in the drinking water did not produce marked effects on the immune responses of either rats or mice. Supported by NIEHS/NTP Contract ES-05454.

461 COMPARISON BETWEEN INTRAPERITONEAL AND ASPIRATION ROUTES OF EXPOSURE TO EVALUATE THE IMMUNOTOXIC EFFECTS OF A MIXTURE OF HERBICIDES.

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The widespread use of herbicides in agriculture and the accumulation of herbicides in the environment create a need for understanding the mechanism of their toxic effects. Due to the ubiquitous nature of herbicides, exposure is frequently to a mixture. The herbicides 2, 4-D (2, 4-dichlorophenoxyacetic acid) and propanil (3, 4-dichloropropionanilide) represent two extensively used herbicides commonly sold as a mixture. Previous experiments in our lab have shown that intraperitoneal (i.p.) exposure to a mixture of these two herbicides produces synergistic immunotoxic effects on the thymus. This study utilized an aspiration model in C57Bl/6 mice to compare the immunotoxic effects on the thymus after i.p. or aspiration exposure to 2, 4-D and propanil. Mice were exposed to either a single 150 or 200 mg/kg dose of propanil or 2, 4-D, or a 150 or 200 mg/kg one to one mixture of the herbicides. Mice exposed *via* aspiration were lightly anesthetized and administered the herbicides in a DMSO and olive oil vehicle. Thymocyte populations were determined by flow cytometry 2 days post exposure. Mice exposed to a 150/150 mg/kg or 200/200 mg/kg mixture or to 200 mg/kg propanil alone had thymic atrophy following both routes of administration. CD4+CD8+ (DP) thymocytes were decreased approximately 50% compared to vehicle control. Intraperitoneal exposure to 150 mg/kg propanil resulted in thymic atrophy and a decrease in DP thymocytes. However, 150 mg/kg propanil administered *via* aspiration did not produce any significant thymic toxicity. This suggests that the mechanism inducing thymic toxicity following exposure to the mixture of herbicides is dependent on route of exposure. These data demonstrate that aspiration of the herbicides produce immunotoxic effects on the thymus. These experiments also suggest that the aspiration model is an effective method for evaluating immunotoxicity after herbicide exposure. Supported by the NIH Immunotoxicology Training Grant ES010953.

462 INDUCTION OF IFN-G CYTOKINE EXPRESSION BY CHLORPYRIFOS, CHLORPYRIFOS-OXON, AND ENDOTOXIN *IN VITRO*.

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Pesticides and their metabolites are ubiquitous in the environment as a result of widespread agricultural and domestic applications. In addition to pesticides, agricultural workers and their families are also exposed to elevated levels of allergens and endotoxin. Interactions among immunomodulatory agents have only recently become a focus of environmental health research. Few validated immunological markers are currently available to evaluate the immunotoxicity of pesticides. Cytokine profiling is a promising biomarker of the immune response since skewed Th1 and Th2 cytokine secretion is associated with autoimmune disorders, asthma, and cancer. Here, we used an *in vitro* based method to evaluate the effects of the organophosphate pesticide chlorpyrifos (CP) and its metabolite chlorpyrifos-oxon (CPO) on cytokine secretion by human lymphocytes. We also evaluated the effect of combined exposures to CP/CPO and endotoxin (LPS) in order to model the multiple exposures received by agricultural workers and their families. Human whole blood was treated with CP, CPO (both with doses of 1-10, 000mg/mL), and LPS (1.5-2.5mg/mL) either singly or in combination for 2-72 hours. At 2, 24, 48, and 72 hours supernatants were collected and assayed by ELISA for expression of interferon-g (IFN-g), the Th1 signature cytokine. Whereas no expression of IFN-g was detected in cultures treated with either CP or CPO alone, cultures treated with CP or CPO in combination with LPS expressed higher levels of IFN-g with some combinations inducing greater IFN-g expression than LPS alone ($p<0.01$). The levels of IFN-g secreted into the supernatants peaked at 48 hours and declined slightly at 72 hrs. In summary, CP and CPO alone do not induce cytokine expression, however the combinations of CP+LPS and CPO+LPS synergistically increased IFN-g expression. These results indicate a potential interaction between endotoxin and organophosphate pesticides. Finally, our results demonstrate that cytokine induction can be a sensitive marker of the immune response to combinations of environmental factors.

463 ROLE OF CYTOKINE NETWORKS IN DETERMINING SUSCEPTIBILITY TO DRUG-INDUCED LIVER DISEASE.

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Using interleukin (IL)-10 deficient mice (IL-10-/-), we previously reported that IL-10 plays an important role in protecting the liver against acetaminophen (APAP)-induced liver injury. In the current work, we discovered a unique interaction that makes mice deficient in both IL-10 and IL-4 (IL-10/4-/-) highly sensitive to the hepatotoxic effects of APAP. C57BL/6 wild type (WT), IL-10-/-, IL-4-/-, or IL-10/4-/- mice were administered APAP at a dose of 120 mg/kg. Within 24 hours, 90% of the IL-10/4-/- mice died of massive hepatic damage. In contrast, all of the WT, IL-10-/-, and IL-4-/- mice survived and showed minor or no signs of liver injury. The high susceptibility of the IL-10/4-/- mice was associated with elevated levels of hepatic TNF-a mRNA, serum nitrite/nitrate, and hepatic APAP-protein adducts, which appeared to be due in part to depressed levels of hepatic glutathione but not elevated levels of CYP2E1. These findings suggest that hepatocyte homeostasis following drug-induced injury is controlled in part by the activities of both IL-4 and IL-10, which together help maintain adequate levels of glutathione and prevent overexpression of protoxicant factors including TNF-a and nitric oxide. Certain polymorphisms of IL-4 and IL-10 that lead to their under expression may have a role in determining individuals' susceptibility to drug-induced liver disease.

464 LIPOPOLYSACCHARIDE PRE-EXPOSURE SENSITIZES THE MOUSE TO DEOXYNIVALENOL -INDUCED PROINFLAMMATORY CYTOKINE EXPRESSION AND LYMPHOCYTE APOPTOSIS.

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Simultaneous exposure to lipopolysaccharide (LPS) and the trichothecene deoxynivalenol (DON, vomitoxin) synergistically induces proinflammatory cytokine gene expression *in vitro* and *in vivo*. The purpose of this study was to determine if LPS pre-exposure sensitizes the mouse to DON-induced TNF-alpha, IL-6, IL-1alpha and IL-1beta expression. To test this hypothesis, the effects LPS and/or DON treatment on production of these cytokines were assessed in male B6C3F1 mice using real time PCR and ELISA methods. Initially, mice were treated with LPS (1 mg/kg) for 8 h, orally gavaged with DON (12.5 mg/kg) and cytokine response measured beginning after 1 h. LPS-induced pro-inflammatory cytokines peaked at 2 h and were diminished within 4 to 8 h. However, subsequent DON exposure in LPS-exposed animals caused significant expression and release of proinflammatory cytokines as compared to DON alone. Expression of TNF-alpha mRNA peaked 5 folds at 1 h but the expression of IL-6, IL-1alpha and IL-1beta mRNAs was increased 5-, 4-, and 3-fold, respectively, 2 h after DON treatment compared to the mean additive responses of LPS alone and DON alone. While TNF-alpha and IL-6 were down regulated 8 h later, IL-1alpha and IL-1beta remained elevated until 24 h and was significantly higher compared to the mean additive responses of LPS alone and DON alone. Plasma levels of these cytokines were also elevated which was consistent with mRNA expression. Amplified cytokine expression was dependent on both LPS and DON dose. Mice pre-treated with LPS for 8 h and DON for 12 h exhibited a massive loss in thymocytes by apoptosis as confirmed by agarose gel electrophoresis and flow cytometry. Based on these and previous results, LPS pre-exposure appeared to sensitize mice to DON with resultant prolonged IL-1 up-regulation that might play a major role in subsequent stress-induced lymphocyte apoptosis.

465 INFLAMMATION AND TRAUMATIC SKELETAL MUSCLE INJURY.

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Traumatic skeletal muscle injuries result in profound histopathological changes and loss of muscle function. These injuries result in local infiltration of large numbers of mononuclear cells, degeneration of injured myofibers and phagocytic removal of

cell debris. Macrophages are critical effector cells in host defense and release a diverse array of cellular mediators including cytotoxic and proinflammatory cytokines that contribute to tissue injury. The present study evaluated the role of systemic macrophages in the injury/repair mechanisms in a traumatic skeletal muscle injury model using liposome encapsulated clodronate, a drug with well characterized monocyte/macrophage depleting qualities. C57BL/6 mice (n = 4 per group) were injected with clodronate liposomes 48 and 2 hours prior to the freeze injury of the left tibialis anterior (TA) muscle and every third day during the post-injury period. Control mice received phosphate buffered saline (PBS) liposomes. At 1, 3, 9 or 14 days post-injury, TA muscles were harvested for histology, immunohistochemistry or gene expression evaluation by quantitative real time RT-PCR. Histopathology revealed less inflammatory cell infiltration in the injured muscle of clodronate treated mice at day 3 post-injury, delayed muscle tissue recovery and impaired clearance of necrotic myofibers at day 9 post-injury; and increased fat infiltration at day 14 post-injury. Immunohistochemical evaluation of injured muscle demonstrated that Mac-1 expression was dramatically reduced in clodronate treated mice at day 3 post-injury. Clodronate treatment also significantly attenuates inflammatory (TNF-alpha, Mac-1 and MCP-1), growth (IGF-BP) factor and antioxidant (thioredoxin) gene expression in injured TA muscle compared to injured non-treated TA muscle. In conclusion, the inhibition of the inflammatory response resulted in delayed phagocytosis of the necrotic myofibers and delayed the repair process of the injured muscle tissue. Selective and time dependent modulation of the innate immune system may provide optimal resolution of skeletal muscle injury.

466 IMMUNOGENICITY OF POLYETHYLENE GLYCOL (PEG)-BASED HYDROGEL SEALANT IN LAPAROSCOPIC PORCINE PARTIAL NEPHRECTOMY.

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Our objective was to evaluate potential immunomodulatory effects of a PEG-based hydrogel sealant in a porcine model of laparoscopic partial nephrectomy. Female domestic swine underwent laparoscopic left lower pole guillotine nephrectomy. Hemostasis and tissue sealing were achieved solely by application of a biodegradable PEG-based hydrogel. Humoral and cell-mediated immunity (CMI) to the sealant were evaluated by enzyme linked immunosorbent assay (ELISA) and lymphocyte proliferation assay (LPA) at 0, 2, 6 and 12 weeks. No antibodies to the hydrogel were detected by ELISA in immunized rabbits. There were no significant differences between control and nephrectomized swine in the ELISA at 2, 6 or 12 weeks. Optical density values for all 4 groups of swine in the ELISA were near 0.2, essentially baseline. It is concluded that the presence of the hydrogel in the abdomen did not elicit IgG antibody formation. For the CMI response measured using the LPA, there were significant differences between the log of the stimulation indices for test and control swine for all three mitogens at 2 weeks post-surgery (ConA, $p \leq 0.01$; PHA, $p \leq 0.01$; PWM, $p \leq 0.05$). These differences disappeared at 6 and 12 weeks and may represent a temporal reactivity of the white cells to the surgery undergone by the test, but not the control, swine. For the cellular response to antigens in the LPA, the only significant difference between the test and control groups was a temporal response to Candida albicans at two weeks ($p \leq 0.03$). There was no significant difference between test and control pig reactivity to the hydrogel as antigen in the LPA at any time point. The PEG-based hydrogel effectively sealed the cut surface of kidneys following porcine partial nephrectomy. There were no immediate or long term (12 week) complications. There was no humoral or long term cell-mediated immune response to the hydrogel. We conclude that the hydrogel is safe and effective for abdominal use, a novel application. Supported by the UA UBRP.

467 IMMUNOTOXICITY OF A COPLANAR AND NONCOPLANAR POLYCHLORINATED BIPHENYL (PCB) CONGENER IN A FISH MODEL.

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Polychlorinated biphenyls (PCBs) are a well-studied class of environmental contaminants known to be immunotoxic for both mammals and fish. Depending upon the particular structure of a PCB congener (i.e., coplanar or noncoplanar), different degrees and/or types of immunotoxic effects have been observed in exposed species. For this study, the effects of a single intraperitoneal (i.p.) exposure of bluegill sunfish (*Lepomis macrochirus*) to either a coplanar (PCB 126) or noncoplanar (PCB 153) PCB congener on host immunocompetence was determined by examining non-specific and cell-mediated immune parameters. Specifically, phagocyte-mediated intracellular and extracellular superoxide ($O_2^{\cdot-}$) production as well as mitogen-stimulated T- and B-lymphocyte proliferation were examined using kidney or spleen cells recovered from fish on 7, 14 or 21 d post-exposure. Both PCB congeners acted to depress innate and cell-mediated immune functions at all post-exposure timepoints (compared to controls) with the exception of extracellular $O_2^{\cdot-}$ production which was unaltered following PCB exposure. This study demonstrates that structurally different PCB congeners, thought to act by different mechanisms to produce toxicological effects in exposed species, may actually impact the immune response in a similar manner. This work was supported by a Hudson River Foundation Graduate Fellowship and USACEHR contract no. DAMD 17-99-9011.

468 IMPACT OF MATERNAL TOXICITY ON POSTNATAL IMMUNE SYSTEM-RELATED PARAMETERS IN RATS.

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Maternal toxicity during the entire gestation and lactation period might affect the pre-and postnatal development of the immune system of the offspring. As in the absence of a toxicological active substance maternal toxicity can also be mimicked by changes in body weight induced by food restriction, a postnatal developmental study was performed in which next to a control group two groups of pregnant rats were food restricted (approx. 85 and 70% of ad libitum) and two groups were treated with 20-or 100 µg dexamethasone/kg. In order to study the potential immunotoxic effects in these different groups enhanced immunopathology (paying attention to thymus, spleen, various lymph nodes and bone marrow), hematology and lymphocyte subset analysis in blood by flow cytometry was performed in the offspring on various post natal days (PND1, PND 21 and PND 70). Moreover, immune function was studied in offspring on PND 21 and PND 70 by means of a T cell-dependent antibody response to both SRBC (as measured by Plaque Forming Cell assay in spleen cells) and to KLH (as measured by ELISA). During gestation and lactation maternal body weight was affected on almost all days in the food restricted and dexamethasone-treated animals. Pup weights showed some non-significant differences at day 1-4 between the various groups, which became significant in all groups from day 7, except the low dexamethasone-treated group. Subset analysis of blood lymphocytes by flow cytometry revealed that at PND 4 lymphocyte subsets could hardly be determined, and at PND 21 they almost resembled those of adult rats. Mainly in the highest dexamethasone group lymphocyte subset numbers were affected. Immunopathology also revealed the immaturity of lymphoid organs at PND 4 which clearly was improved from PND 21. The further evaluation of the effects in the different treatment groups on immunopathology and the T cell-dependent antibody response on PND 21 and PND 70 (as measured by the SRBC-PFC assay and by KLH-ELISA) will be presented and discussed.

469 DEXAMETHASONE-INDUCED IMMUNOTOXICITY FOLLOWING FETAL VS. ADULT EXPOSURE.

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Immunotoxicity data using adult exposure to toxicants may not reflect perinatal vulnerability. As a result, direct life stage comparisons are needed to establish dose-response relationships, target organ sensitivities, and expected immunotoxic changes. This study compared gestational vs. adult life stages for suceptibility to dexamethasone-induced immunotoxicity including selected developmental-reproductive endpoints. Dexamethasone-21 phosphate was administered (s.c.) to pregnant CD rats at days 6-21 of gestation (0, 0.0625, 0.125, 0.25, 0.5 mg/kg/day) with identical exposure of non-pregnant adult females. Some reproductive (anogenital distance) and growth (body weight) measures of pups were altered. In the juvenile (5 wk.), the delayed type hypersensitivity response to KLH was significantly reduced at all doses examined and this pattern continued into adulthood (13 wk.). In contrast, the DTH response of adults exposed to DEX was unaltered even at the highest dose. Few DEX-induced changes were seen in offspring or adult blood parameters or in splenocytes analyzed for cell surface makers (by flow cytometry). The thymus of both exposed pups (both ages) and adults showed a marked reduction in the medulla/lobe area beginning with the 0.125 mg/kg/day DEX exposure level. Macrophage production of TNF and NO was only marginally affected as was splenocyte production of IL-4 and IFN gamma. In contrast, pups assessed as juveniles were significantly depressed in splenic IL-2 and IL-10 production. DEX exposure altered serum antibody levels across age groups with an increase of KLH-specific IgG (beginning with the 0.0125 mg/kg/day dose) while total IgE was reduced. These results suggest that while DEX exposure produces some common alterations following *in utero* vs. adult exposure, fetal exposure (even at the lowest doses tested) produces marked and persistent functional loss (DTH) not evident in exposed

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