

SOCIETY OF TOXICOLOGY

41st Annual Meeting & ToxExpo™

An Official Journal of the
Society of Toxicology
Supplement



TOXICOLOGICAL SCIENCES

proteins were increased in all three HAA treated groups with DCA producing the greatest effect. These changes indicate that the HAAs perturb signal transduction by altering the phosphorylation state of phospho-tyrosine proteins. Since pharmacological protein kinase inhibitors also produce dysmorphology, HAA-induced alteration of signal transduction may be responsible for altered differentiation and development. This abstract does not present EPA policy.

1092 LOCALIZATION OF IKB AND IKK SUBUNITS IN THE SMOOTH ENDOPLASMIC RETICULUM: ACUTE EFFECTS OF TCDD IN MALE AND FEMALE RAT LIVER.

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Effects of acute 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) exposure upon protein expression in rat liver endoplasmic reticulum (ER) were determined by two-dimensional gel separation and mass spectrometry. Liver ER was prepared as microsomes from male and female Sprague-Dawley rats exposed for 72 hr to TCDD. TCDD induced cytochrome P450 enzymes, GST subunits, Hsp72 and Grp78. Mass spectrometry also revealed the presence of increased levels of IKB kinase beta (IKK- β) after dioxin treatment in males and females. This finding led us to search for other NF- κ B binding proteins or kinases in ER and possible alterations by TCDD. Western blot for IKB family members in ER showed that IKB- β and IKB- γ were highly abundant in ER while IKB- α and IKB- ϵ proteins were barely detectable. TCDD induced hyperphosphorylated forms of IKB- β in both genders, but only the 85kd form of IKB- γ was present in females while males expressed an additional IKB- γ immunoreactive form at 115 kD. Although IKK- β was in ER of both genders, IKK- α was the most abundant IKK subunit and IKK- γ was not detectable. The effect of gender and TCDD treatment upon IKB and IKK family member expression was also examined in cytosol for critical comparison to the ER. In cytosol, all IKK subunits were expressed without substantial effect by TCDD. IKB- α , IKB- β , and IKB- ϵ , but not IKB- γ , were expressed in cytosol and were unaffected by TCDD. Hyperphosphorylated forms of IKB- β were observed in cytosol only in female (not male) rats but were not increased by TCDD as was the case in ER from females. Although NF- κ B-p65 was readily found in male and female cytosol, it was not detectable in ER. IKK- α , β kinase activity, measured after immunoprecipitation from cytosol and solubilized ER, was unaffected by gender and treatment. These studies demonstrate a strength of proteomics in showing a unique distribution of NF- κ B binding proteins and their kinases in liver ER as affected by gender and TCDD treatment.

1093 T-2 AND HT-2 TOXIN INDUCED APOPTOSIS IN HL-60 HUMAN PROMYELOCYTIC LEUKEMIA CELLS.

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T-2 and HT-2 toxins belong to a group of mycotoxins, trichothecenes, which are widely encountered as natural contaminants. Exposure to such toxins may cause severe damage to the gastrointestinal mucosa and the immune system. In the present study the human promyelocytic cell line HL-60, was used to characterize the apoptotic effects of T-2 and its metabolite HT-2. Apoptotic cells were identified microscopically by chromatin condensation and nuclear fragmentation, by flow cytometric analysis and by DNA laddering. Apoptosis induced in the HL-60 cells following exposure to T-2 and HT-2 for 24 hours was concentration-dependent starting at 3.1 and 6.25 ng/ml, respectively. Little cytotoxicity (membrane damage) was observed even after exposure to concentrations (25-50 ng/ml) that induced 60-100 % of the cells to undergo apoptosis. Following exposure to 6.5 ng/ml an increased amount of apoptotic cells could be observed already after 4-6 hours. Whereas the absolute effects of toxin-induced apoptosis in the HL-60 cells seemed to vary somewhat during culturing and between different batches of cells, T-2 was more potent than HT-2. The apoptotic process could be almost completely blocked by the addition of z-VAD.fmk. In contrast, no or only minor effects were observed by the addition of DEVD.CHO, IETD.fmk and DEVD.fmk. As judged by Western blotting, no or only minor changes in bax and bcl-2 levels were observed, whereas PARP was totally degraded already 3 hours after toxin addition. Furthermore, the level of several procaspases (-3, -7, -8, -9, but not -12) were down regulated. Zn²⁺ and BAPTA-AM blocked the induction of apoptosis as judged by microscopic analysis and flowcytometry, as well as DNA fragmentation. The results suggest that T-2 and HT-2 both cause an increase in the level of Ca²⁺ thereby initiating a signal that activates several caspases resulting in chromosomal condensation, nuclear fragmentation, activation of DNA endonucleases and DNA laddering.

1094 ALTERATIONS IN EXPRESSION OF CYTOKINE NETWORK AND APOPTOSIS SIGNALING GENES IN MOUSE LIVER AFTER SUBCUTANEOUS EXPOSURE TO FUMONISIN B₁.

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Fumonisin B₁ (FB₁) is a naturally occurring mycotoxin produced primarily by *Fusarium verticillioides* and related fungi, which are common contaminants of corn throughout the world. FB₁ is a carcinogen and causative agent of several lethal animal diseases. Liver is the primary target organ in mice. Our previous studies showed altered expression of cytokines in mouse liver after FB₁ treatment. To further investigate the genes involved in the cytokine network and apoptosis signaling, male and female BALB/c mice (5/group) were injected subcutaneously with either saline or 2.25 mg/kg/day of FB₁ for 5 days. FB₁ treatment caused increased expression of tumor necrosis factor α (TNF α), interleukin (IL)-1 α , IL-1 β , IL-1 receptor antagonist (IL-1Ra), IL-6, IL-10, IL-12 p40, IL-18 and interferon γ (IFN γ) in male liver, with a similar increase in females except that IL-1 β and IL-18 were unaltered. Control females showed higher basal levels of IL-1 α , IL-1Ra, IL-10, IL-12 p40 and IFN γ as compared to males. Expression of TNF receptor 55 and TNF receptor associated death domain (TRADD) was increased, with no changes in Fas signaling molecules, Fas, Fas ligand, Fas associated death domain (FADD) and Fas-associated protein factor (FAP). Expression of c-Myc, B-Myc, Max and Mad oncogene transcription factors and apoptotic genes, namely Bcl-2, Bax, Bad and caspase 3 was also increased after FB₁ treatment. FB₁ caused an activation of cytokine network in liver along with TNF α signaling pathways. FB₁-induced expression of TNF α , IL-1 α , IL-1 β , IL-6 and IFN γ could play a role in the observed liver toxicity, whereas increased expression of IL-1Ra and oncogenes could be responsible for the cancer promoting properties of FB₁.

1095 IMMUNOTOXIC EFFECTS OF ENDOSULFAN AND PERMETHRIN VIA THYMOCYTE APOPTOSIS.

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Altered immune responses have been observed following occupational, inadvertent, or therapeutic exposure to xenobiotics. Many pesticides are known to cause immunotoxicity. Exposure to mixtures of pesticides, either concurrently or sequentially, may result in potentiating this effect mainly because one can effect the metabolism of the other. The objective of this study was to determine the effect the insecticides endosulfan, permethrin and their mixtures on C57BL/6 male mice thymocytes *in vitro*. Permethrin, is a broad-spectrum synthetic pyrethroid, is a widely used insecticide in agriculture and public health. Endosulfan is a highly toxic chlorinated hydrocarbon insecticide used worldwide. We examined the immunotoxic potential of these pesticides using a flow cytometric technique in combination with 7-Amino Actinomycin D (7AAD) to distinguish live, early apoptotic, and late apoptotic/necrotic cells. DNA ladder assay, hallmark of apoptosis, was also used to determine the occurrence of apoptosis. Both endosulfan and permethrin were found to cause significant apoptotic death of thymocytes in a dose- and time-dependent manner. Thus, permethrin at 50, 100 and 300 μ M was found to cause 5.49, 11.49 and 26.11% early apoptotic cell death, respectively. Endosulfan at 25, 50 and 250 μ M was found to cause 11.91, 15.72 and 68.01% early apoptotic cell death, respectively. For the mixture study, concentrations of 100 μ M permethrin and 50 μ M endosulfan was selected and that found to cause 27.09% apoptosis. Thus, these pesticides in mixture have an additive immunotoxic effect. No significant late apoptotic cells were found at these concentrations for either pesticide when exposed for 12 hours. DNA ladder assay confirmed the presence of DNA fragments. The results of this study suggest that the mixtures of endosulfan and permethrin have additive immunotoxic effects on C57BL/6 mice thymocytes.

1096 OXIDIZED PHOSPHATIDYL SERINE STIMULATES PHAGOCYTOSIS OF APOPTOTIC CELLS.

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A critical event during programmed cell death or apoptosis is the acquisition of plasma membrane changes that allows phagocytes to recognize and engulf cells before they rupture. Externalization of phosphatidylserine (PS) has been considered a hallmark of apoptosis. Oxidative stress is an inherent part of the apoptotic program with an unknown specific function. We have previously found that apoptosis induced by oxidants and non-oxidants such as phorbol myristate acetate (PMA) and anti-Fas, was accompanied with PS oxidation and externalization in apoptotic cells. We hypothesize that PS oxidation plays important role in recognition and phagocytosis of apoptotic cells by macrophages. To test this hypothesis, 1-palmitoyl