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351 QUANTIFICATION AND LOCALIZATION OF ROS PRODUCTION BY POLYCHLORINATED BIPHENYLS AND BY 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN.

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The mechanisms by which planar polyhalogenated aromatic hydrocarbons (PHAH), such as non-ortho-substituted polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-p-dioxins, exert toxicity are not well known but may involve oxidative stress. Our prior work has shown that cytochrome P450 1A (both fish CYP1A and human CYP1A1) catalytic cycles can be uncoupled by non-ortho PCBs resulting in the release of reactive oxygen species (ROS) and in the irreversibly inactivation of the enzyme. TCDD causes a similar uncoupling, resulting in a burst of ROS release (initially 24 nmole O_2^-/min/nmole CYP1A but rapidly declining to 8 nmol O_2^-/min/nmol CYP1A), and rapid inactivation of CYP1A. An increase in TCDD-induced mitochondrial production of ROS has been demonstrated in mice. To evaluate the relative contributions of CYP1A uncoupling and mitochondrial production of reactive oxygen in response to TCDD, we have examined the cellular localization of ROS production in cultured mouse and zebrafish hepatic cell lines (Hepa-1c1c and ZF-L) using multilaser confocal microscopy. The fluorometric ROS probe dihydroethidium was used to determine the production of superoxide (O_2^-). Localization of CYP1A (or CYP1A1) within individual cells was performed by examining the production of fluorescent resorufin following the CYP1A-mediated O-deethylation of ethoxyresorufin (EROD). The release of ROS resulting from the uncoupling of CYP 1As by pHAHs represents a possible mechanism of toxicity of these aryl hydrocarbon receptor agonists. (EPA R 827102-01-0, NIH P42-ES07381, NIH 1 F32 ES012794-01).

352 COAL DUST INCREASES BAX EXPRESSION, INCREASES APOPTOSIS, AND SUPPRESSES CYP1A1 INDUCTION IN A RAT MODEL OF MIXED EXPOSURE TO POLYCYCLIC AROMATIC HYDROCARBONS AND RESPIRABLE PARTICLES.

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Apoptosis has been described in lung following exposure to inflammatory agents, such as silica. The bax gene contains 2 Ah receptor response elements, suggesting a relationship between bax-mediated apoptosis and xenobiotic metabolism. Suppression of pulmonary CYP1A1 induction by coal dust (CD) exposure occurred in the rat lung. Therefore, we hypothesized that CD exposure in rat lung causes bax-mediated apoptosis and bax expression suppresses CYP1A1 induction. To explore this relationship, male Sprague-Dawley rats were intratracheally instilled with 2.5, 10, 20, and 40 mg CD/rat or vehicle (saline). On day 11, CYP1A1 was induced by intraperitoneal (IP) injection of 50 mg/kg beta-naphthoflavone (BNF). Rats were sacrificed on day 14, and lung sections were stained by immunofluorescence and morphometrically analyzed for CYP1A1, bax and an alveolar type II (AT-II) cell marker (cytokeratins 8/18). Bax expression was increased by CD in a dose-dependent manner, and CYP1A1 expression was inversely related to bax expression in AT-II cells. Since bax is a pre-apoptotic protein, we investigated the association of apoptosis with CYP1A1 suppression. Therefore, rats were injected IP with the caspase inhibitor, Q-VD-OPH or vehicle (DMSO) on days 0, 5, 9, 10, 11, 12, and 13 post CD-exposure. CYP1A1 was induced by BNF injection on day 11. Rats were sacrificed on day 14. CD exposure significantly suppressed CYP1A1 activity, increased bax expression and increased apoptosis in pulmonary cells. The injection of Q-VD-OPH significantly suppressed bax expression and apoptosis. However, the CD-mediated suppression of CYP1A1 induction was not significantly affected. These findings suggest that CD exposure suppresses CYP1A1 induction, but this suppression is not caused by bax expression or pulmonary cell apoptosis.

353 COMPARISON OF ACUTE AND CHRONIC EXPOSURE TO NONYLPHENOL REVEALS THAT CHRONIC EXPOSURE ATTENUATES P450 INDUCTION AND RXRα LEVELS.

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Nonylphenol (NP) is an environmental estrogen that also binds the pregnane X-receptor (PXR). Different laboratory feeding studies have shown both up-regulation and down-regulation of P450s by nonylphenol. The inconsistencies between laboratories for the differential effects of nonylphenol may be due to length of treatment. FVB/NJ mice were treated for 7 days with 0, 25, 50 and 75 mg/kg/day NP, and FVB/N-TgN(MMTVneu)Mul202 mice were treated for 32 weeks with 0, 30 and 45 mg/kg/day NP. The transgenic mice treated for 32 weeks at 45mg/kg/day demonstrated higher incidence of mammary cancer, indicating that the dose of NP provided was sufficient to cause estrogenic effects. After exposure, mice were euthanized, livers excised, and steroid hydroxylase assays and Western blots were performed for CYP 3A, CYP2B6 and CYP 2B10. Western blots revealed that CYP3A was not significantly induced by nonylphenol, but CYP 2B6 and CYP2B10 were induced 1.6X and 5X, respectively after the 7 day exposure. Estradiol and testosterone hydroxylation assays demonstrated an increase in the production of estriol and 16β-hydroxytestosterone, respectively, further demonstrating the induction of CYP2B enzymes. However, 32-week treatment showed no significant increase in testosterone or estradiol hydroxylation, nor significant induction of P450s. Clontech cDNA Atlas® arrays were performed to determine a potential mechanism for the lack of induction in mice treated for 32 weeks. Interestingly, RXRα, PXR's heterodimerization partner was reduced 1.9X. Q-PCR was performed to confirm down-regulation and indicated a 2.5X decrease in transcript levels in mice treated for 32 weeks with NP. Mice treated for 7 days showed no change in RXRα levels. Furthermore, Q-PCR of PXR, CAR and ER were not altered in the livers of mice from either NP treatment group. This data suggests that long-term exposure to nonylphenol can reduce RXRα levels, and attenuate P450 induction. This may ultimately reduce an organism's ability to adapt to toxicant exposure.

354 A NOVEL CLASS OF CYTOCHROME P_{450} REDUCTASE REDOX CYCLERS: CATIONIC MANGANOPORPHYRINS.

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Manganoporphyrins are potent antioxidants and are currently being developed as novel therapeutic agents. The objective of this study was to investigate whether manganoporphyrins inhibit drug metabolism. *In vitro* studies were done to examine this issue. Microsomal cytochrome P_{450} activity was assessed using two different substrates, benzyloxyresorufin (BR) and methoxyresurofin (MR). The cationic manganoporphyrins AEOL 10113 and 10123 were found to be potent inhibitors of cytochrome P_{450} metabolism in both rat and human liver microsomes with IC_{50s} of 0.665 μM and 0.607 μM respectively. Since manganoporphyrins are redox active, we test the hypothesis that the mechanism of cytochrome P_{450} inhibition was due to redox cycling with cytochrome P_{450} reductase. We tested this hypothesis by examining their ability to stimulate NADPH and oxygen consumption in presence of purified human cytochrome P_{450} reductase. AEOL 10113 had Km = 4.3 μM, indicating that it was a potent redox cylcer. The manganoporphyrins AEOL 10113 and 10123 were nearly 2 orders of magnitude more potent than corresponding AEOL 10150 and 10201 in inhibiting cytochrome P_{450} metabolism and as redox cyclers with cytochrome P450 reductase. Structure activity relationships suggested that substitution of longer alkyl chains on the meso porphyrin position and decreased charged lessened the manganoporphyrin's ability to redox cycle with cytochrome P_{450} reductase and inhibit cytochrome P_{450} metabolism. To further examine the possible mechanism of cytochrome P_{450} inhibition, oxygen consumption studies were performed in the presence of a flavin domain inhibitor, diphenyleneiodinium (DPI). DPI blocked the consumption of oxygen in a concentration-dependent manner. These data are consistent with the hypothesis that manganoporphyrins redox cycle with the flavin domain of cytochrome P_{450} reductase and compete for electrons with cytochrome P_{450s}.

355 SF-1 FUNCTIONS SYNERGISTICALLY WITH CREB TO MEDIATE CAMP STIMULATION OF CYP1B1 VIA A FAR UPSTREAM ENHANCER (FUER).

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Cyp1B1 plays a key role in the activation of polycyclic aromatic hydrocarbons in tissues like the adrenal ovary and testis that are hormonally regulated *via* cAMP. In primary adrenal cells Cyp1B1 is substantially induced by cAMP. Previously we used Cyp1B1-luciferase reporters to characterize a cAMP stimulation mechanism in Y-1 adrenal cell line, which depends on a far upstream enhancer region (FUER, -5298 to -5110). SF-1 and CREB commonly mediate cAMP responses. Here we show that SF-1 and CREB are equally essential for the cAMP activation of FUER. Four SF-1-binding sites in FUER can participate relatively equally and additively in support of an essential CREB interaction at the 3' end of the FUER. DAX-1, a suppressor of SF-1, also blocked FUER activity. An AP-1 binding site in proximal promoter region regulates basal activity of Cyp1B1 which is enhanced by FUER in Y-1 cells. The role of FUER has been tested by chromatin immunoprecipitation assay with anti-acetyl histone H3 anti-body in primary rat adrenal cells. Stimulation by

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