TOXICOLOGY 2

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Effects of TCDD, PCBs and PBDEs in Animals

Effects of halogenated aromatic hydrocarbon exposure were investigated in marmosets and rats. *Riecke et al. (8E-05)* discovered in the male marmoset treated with a single dose of 10 or 100 ng/kg of TCDD that there were morphological alterations in the heart muscle 2 and 4 weeks after dosing. The alterations were greater at 4 than at 2 weeks, and at neither time were they associated with signs of overt toxicity such as loss of body weight or increase in liver weight. In about half of the TCDD-exposed marmosets in each dosage group an increase in heart collagen was detected at the light microscopic level and this was confirmed by Western blotting which showed an increase in collagen type I in left ventricular muscle. Thus, myocardial fibrosis was caused by acute TCDD exposure at doses that did not cause overt toxicity. *Fattore et al. (8E-17)* determined the effects of oral exposure of male and female rats to a technical mixture of PBDEs (Bromkal 70-5 DE) for 28 days. This mixture contains the congeners BDE-47 and BDE-99. These are the major PBDPE congeners identified in human tissues. It was found that behavior and general health of the animals including feed consumption and body weight were not affected. Increases in total serum protein and cholesterol levels in both sexes were seen at the highest level of exposure along with dose-related increases in relative liver and kidney weights, hepatic EROD and PROD activities, and a reduction of hepatic vitamin A content. The levels of Bromkal 70-5 DE used to cause these effects were orders of magnitude higher than the levels needed to cause these same effects by TCDD. *Ishii et al. (8E-16)* reported that PCB126 suppresses carbonic anhydrase III (CAIII) mRNA and protein expression in rat liver but not skeletal muscle even though the CAIII from both tissues appears to be from the same gene. The difference in CAIII expression in response to TCDD may be due to differential constitutive regulation of the CAIII gene between liver and muscle. *Kawai et al. (8E-23)* found that rats treated with 100 ng/kg of 2,3,4,7,8-PeCDF developed signs of oxidative damage in the liver. These investigators and *Hyodo et al. (8E-09)* suggest that hepatic toxicity of PeCDF and TCDD, respectively, may be caused in part by free radical-mediated oxidative stress. *Chernyak et al. (8E-01)* studied a cohort of fire fighters from 1999-2001 who fought a fire in 1992 where exposure to dioxin-like compounds was suspected. New information on CYP1A2 activity in the firefighters 7-9 years after the fire and on blood levels PCDD and PCDF congeners is reported.

Effects of TCDD, PCBs, and PBDEs in cell cultures

Martinez et al. (8E-22) assessed gene expression profiles in TCDD treated (0.1, 1, and 10 nM) human lung cells. The normal alveolar pneumocyte cell line (HPL1A) is more sensitive to TCDD induction of CYP1A1 and CYP1B1 than a malignant alveolar pneumocyte cell line (A549). Using microarray analysis, dose-dependent effects of TCDD on gene expression in both cell lines were compared. TCDD was found to induce a pleiotropic set of genes which function in or as chemokines/cytokines, signal transduction, transcription factors, oncogenes/tumor

suppressors, cell proliferation, and metabolism. Genes identified as potential biomarkers of TCDD exposure were induced in both cell lines and at all doses of TCDD used and include DUSP1, EGR1, STK4, UROS, ALD6, ALD3, CYP1B1, CYP1A1, CTNNB1, CDH1, RRAS and F2R. Differences in the expression of specific subsets of genes between the two cell types may reflect differences in tumorigenicity and/or sensitivity to TCDD. *Hurh et al. (8E-19)* demonstrated that TCDD treatment caused the induction of CYP1A1 and CYP1B1 in a human breast epithelial cell line (MCF10A). They also observed that oxidative cell death of the MCF10A cells was caused by catechol estrogen metabolites, produced from the hydroxylation of 17β-estradiol, by these monooxygenases. Reseveratrol was found to be chemopreventive in these cells by inhibiting the induction of CYP1A1 and CYP1B1 by TCDD. *Mazina et al. (8E-07)* used MCF10A cells, which respond to insulin in the absence of serum as long as EGF is present, to show that TCDD blocks insulin signaling. The mechanism is postulated to involve the early event of insulin-induced tyrosine phosphorylation followed by dephosphorylation. The dephosphorylation process is impaired in MCF10A cells treated with TCDD. *Ha et al. (8E-08)* investigated effects of graded concentrations of TCDD on viability, proliferation, and fibronectin secretion by three types of kidney cells: glomerular mesangial cells (MMC), proximal tubular epithelial cells (LLC-PK1) and distal tubular epithelial cells (MDCK). When exposed to TCDD (1-100 nM), LDH release was increased in all cell lines in a dose- and time-dependent fashion. Cell proliferation was increased in MMC and LLC-PK1 and decreased in MDCK cells by TCDD. A sensitive effect was an increase in fibronectin secretion caused by TCDD concentrations as low as 1 nM in all three cell types. It is hypothesized that reactive oxygen species may play a role in causing these effects of TCDD on renal cells. Their finding that taurine, an antioxidant, protects against the TCDD-induced increase in fibronectin secretion supports this possibility. *Sasawatari et al. (8E14)* used LLC-PK1 cells expressing p-glycoprotein to study the effect of PCB 126 on the accumulation and p-glycoprotein-mediated transepithelial transport of vinblastine. A major finding was that PCB 126 inhibited the accumulation and transport of vinblastine by p-glycoprotein. *Chen and Bunce (8E-25)* evaluated 18 pure PBDE congeners and 3 commercial PBDE mixtures for their ability to bind AhR, activate AhR to a DRE binding form, and induce CYP1A1 protein and EROD activity in primary rat hepatocytes. Relative potencies (REPs) of individual PBDEs were calculated from EROD induction results in primary rat hepatocytes and chick embryo hepatocytes. Compared with known PCDD, PCDF, and coplanar PCB congener AhR agonists the PBDEs are at best weak AhR agonists with commercial PBDE mixtures and their principal congeners having negligible activity.

Interaction of AhR, oxidative, hypoxic, and nitric oxide signaling in TCDD action

Yoon et al. (8E-18) evaluated the role of oxidative stress and the protective effect of adult T-cell leukemia-derived factor (ADF), a thioredoxin (TRX) capable of inducing interleukin-2 receptor, on TCDD-induced hematotoxicity. TRX/ADF is a stress-inducible protein whose expression is up-regulated by viral infection and by a variety of oxidative agents. TRX/ADF is involved in the cellular defense mechanism against oxidative damage via the regulation of intracellular redox status. TCDD (20 μg/kg) decreased bone marrow cellularity, bone marrow granulocyte-macrophage progenitor cells (CFU-GM), and peripheral leukocyte numbers in wild-type mice but had no such effects in ADF transgenic mice. These findings strongly suggest that oxidative stress plays an important role in the TCDD-triggered mechanism of hematotoxicity, and suggest that TCDD may disrupt redox regulation. There was no difference in expression of AhR mRNA in bone marrow cells between wild-type and ADF transgenic mice, and TCDD did not alter AhR

mRNA expression in wild-type mice, but expression was reduced in TCDD-treated ADF transgenic mice. TRX/ADF may exert its protective effect in the AhR-mediated pathway through which TCDD induces oxidative stress. *Ma et al. (8E-10)* examined the interaction of AhR and oxidative signaling from two aspects: (1) the potential interaction between the AhR/DRE and the NfE2 related factor 2 (Nrf2)/antioxidant response element (ARE) signaling on the induction of NADP(H):quinone oxidoreductase (NQOR), and (2) activation of AhR dependent gene regulation by phenolic chemicals. Their results reveal that both the AhR/DRE and Nrf2/ARE systems require a labile factor for the induction of NQOR. Phenolic chemicals such as *tert-* butyl hydroquinone and hydroquinone induce a number of DRE-regulated AhR target genes. These results demonstrate that oxidative chemicals can activate AhR-dependent gene regulation and reveal a link between oxidative chemical exposure and AhR activation through oxidative signal transduction. *Jeong et al. (8E-20)* are interested in interactions between TCDD-induced gene expression and gene expression in response to hypoxia. They used Hepa 1c1c7 cells transfected with pm*Cyp1a1*-Luc to demonstrate that hypoxic agents such as cobalt chloride, desferrioxamine, and picolinic acid inhibit the TCDD-induced *Cyp1a1* promoter activity. *Jeong et al. (8E-21)* examined the cross-talk between hypoxia and nitric oxide on the inhibition of *Cyp1a1* induction. Hepa 1c1c7 cells transfected with pm*Cyp1a1*-Luc were treated with various inducible nitric oxide synthase (*i*NOS) inducers, *i*NOS inhibitors, or hypoxic agents. Results indicate that *i*NOS inducers can inhibit TCDD-stimulated gene expression, and that nitric oxide mediates this inhibition.

Antagonism of TCDD effects

Suh et al. (8E-15) demonstrated that di-*ortho* substituted PCB congeners inhibit AhR ligand-induced biological responses, such as modulation of CYP1A1 and LPS-induced IgM expression, in the CH12.LX murine B cell line. These studies also show that the antagonism by di-*ortho* substituted PCBs is due to the decrease of transcriptionally active AhR complex in the nucleus of AhR ligand-treated cells. These findings suggest that di-*ortho* substituted PCB antagonize the activation and nuclear translocation induced by high-affinity AhR ligands. Because di-*ortho* substituted PCBs are commonly found in PCB mixtures in the environment, the current TEF/TEQ approach may overestimate the toxicity of complex halogenated aromatic hydrocarbon mixtures. *Hyung-Chul Lee et al. (8E-13)* investigated *in vivo* effect of panax ginseng extracts on the cytochrome P450-dependent monooxygenase system in the liver of guinea pig exposed to TCDD. Ginseng extract partially inhibited the increases in cytochrome P450 specific content, NADPH-cytochrome P450 activity, ethoxycoumarin *O*-deethylase activity, and benzphetamine *N*-demethylase activity caused by TCDD. In contrast, cytochrome b_5 concentrations and cytochrome b_5 reductase activity were unaffected by all treatments.

Metabolism and disposition of PCDDs, PCDFs, and PCBs in animals

Koga et al. (8E-06) discovered major species difference in the metabolism of 2,2',3',4,4',5-hexachlorobiphenyl (PCB 138). PCB 138 was incubated *in vitro* with hepatic microsomes from rats, hamsters and guinea pigs. The order of ability to metabolize PCB 138 was guinea pig >> rat = hamster. PCB 138 metabolism was stimulated by phenobarbital-pretreatment in each species. Four mono-hydroxylated metabolites were tentatively identified. In guinea pigs, the four major metabolites were produced not only in microsomes from phenobarbital-pretreated animals but also

in uninduced microsomes. These observations suggest that PCB 138 metabolism in guinea pig liver is mainly catalyzed by a constitutive and phenobarbital-inducible cytochrome P450, namely, CYP2B18. *Shappell et al. (8E-26)* studied the effect of clenbuterol, a leanness-enhancing agent for meat producing animals previously reported to lower dioxin and furan levels in rat adipose tissue, on liver stores of these compounds. Clenbuterol tended to increase hepatic concentrations of chlorinated dioxins and furans, both in the absence of added dioxins and furans and when a mixture of both was added to the diet. It also consistently shifted chlorinated dioxins and furans from fat to liver (increases of 38-66%). Because clenbuterol decreases fat and PCDD/Fs in fat, it may have promise for use as a tool in remediation of dioxin/furan-contaminated livestock.

Effects of endocrine disruptors that are not AhR agonists

Kubota et al. (8E-11) investigated the effects of *p*-nonylphenol, an estrogenic alkylphenol degradation product, on proteinase secretion by human promyelocytic leukemia U937 cells. *In vitro* exposure to *p*-nonylphenol (0.05 - 300 μM) dose-dependently suppressed 92 kDa gelatinase secretion. Co-incubation with antiestrogens generally did not protect against the inhibition of 92 kDa gelatinase secretion, and 17ß-estradiol itself had no significant effects over a wide range of concentrations. These results suggest that with regard to 92 kDa gelatinase regulation, *p*-nonylphenol interacts with the estrogen receptor and transduces signals in a manner distinct from that of estradiol. In contrast to the inhibition of 92 kDa gelatinase secretion, *p*-nonylphenol strongly induced the secretion of several casein-degrading proteinases (MW 40-80 kDa) in a dose-dependent manner. Consequently, *p*-nonylphenol differentially affects the secretion of different proteinases by U937 cells. This appears to be the first report that *p*-nonylphenol modulates different proteinase secretion by human cells differentially. *Kubota et al. (8E-12)* determined if bisphenol A can induce apoptosis and/or stimulate caspase activities in human breast cancer cells under conditions in which it causes time-dependent cell death (1-24 h). Bisphenol A (100 μM) induced DNA ladder formation in both estrogen receptor-positive MCF-7 cells and estrogen receptor-negative MDA-231 cells. These results suggest that bisphenol A induced apoptosis in both cell types, and that this apoptosis in breast cancer cells is independent of estrogen receptors. Caspase-1, -8, and -9 activities were unaffected in each cell type whereas caspase-3 and -6 activities were induced in both. This is the first demonstration that bisphenol A can induce human mammary carcinoma cell apoptosis independent of estrogen receptors. This study suggests that apoptosis occurs via caspase-3 and -6 signaling pathways. *Agletdinov et al. (8E-02)* examined effects of 2,4-D dimethylamine salt (2,4-DMA) treatment on protein synthesis and content in rat liver in the presence and absence of exogenous thyroxin. 2,4-DMA is a herbicide manufactured and widely used in Russia. Rats drank 2,4-DMA-containing water for 4 weeks at total doses equivalent to the LD_{50} and 10% of the LD_{50}. Mitochondrial protein content was reduced by the high dose, unaffected by thyroxin, and unaffected in response to both together. Cytosolic protein content was increased in response to the high dose of 2,4-DMA, and even more so in response to thyroxin alone and 2,4-DMA plus thyroxin. Hepatic protein synthesis was greatly reduced by the high dose of 2,4-DMA. Thyroxin alone reduced protein synthesis, but protein synthesis was increased when thyroxin and the high dose of 2,4-DMA were given in combination. *Gilmanov et al. (8E-03)* examined effects of 2,4-DMA on thyroid-dependent metabolic reactions. Thyroxin alone significantly decreased α-oxoglutarate, succinate, and pyruvate metabolism by hepatic mitochondria, while 4 weeks exposure to 2,4-DMA at 10% the LD_{50} had no significant effect on metabolism of these substrates. The combination, however, increased α-oxoglutarate and

succinate metabolism. Treatment with the LD_{50} dose of 2,4-DMA significantly decreased α-oxoglutarate and pyruvate metabolism by hepatic mitochondria, while thyroxin protected against these decreases. Comparable experiments were conducted using liver slices, but the only significant effects observed were reductions in pyruvate metabolism in response to both doses of 2,4-DMA, an increase in α-oxoglutarate metabolism in response to the low dose of 2,4-DMA plus thyroxin, and a decrease in α-oxoglutarate metabolism in response to the high dose of 2,4-DMA. *Galimov et al. (8E-04)* investigated effects of 2,4-DMA treatment on glutathione concentrations and the activity of some enzymes of its metabolism in rat testes. Sexually mature male rats were given 2,4-DMA solution in LD_{50} and 0.1 LD_{50} total doses for 1 and 3 months. No significant effects were seen after 1 month. After 3 months, however, glutathione concentrations, glutathione reductase activity, and γ-glutamyltransferase activity were increased.