Recent Advances in the Toxicology of Dioxin

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This session highlights some of the most recent advances in 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD or dioxin) toxicology. Several other presentations in the toxicology session could have been included, because they also provide greater insight into endpoints of dioxin toxicity and the molecular and cellular mechanisms by which they are produced. The session includes fourteen presentations: eight platform and six poster.

Tohyama (Platform # 417) provides an overview of how dioxins and related compounds became a social and political issue in Japan leading he and his colleagues to undertake a comprehensive research project on dioxin named the Core Research and Evolutionary Science and Technology (CREST) project. The CREST project is composed of three TCDD toxicity endpoint-oriented research groups: reproduction and development, brain function and behavior, and immune function. A fourth group is involved with compiling the animal data and extrapolating it to humans. The CREST project not only confirmed earlier observations on TCDD toxicity but also provided new experimental evidence on: various endpoints of toxicity caused by low dose TCDD exposure, critical windows for TCDD exposure to cause certain developmental effects, arylhydrocarbon receptor (AhR)-mediated or non-AhR-mediated toxicities, and differences in sensitivity to TCDD toxicity among various animal species and their strains. There is no doubt that experimental evidence is needed to provide the scientific basis for risk assessment of TCDD and related AhR agonists. At the Dioxin 2004 meeting seven papers supported by CREST are presented, three of these are in the present session (Platform # 161 and # 382, and Poster # 402).

Nohara *et al*. (Platform # 161) determined the effect of AhR activation on T-cell dependent immunological function. A constitutively active AhR (CA-AhR) mouse was generated that expresses CA-AhR from a T-cell specific promoter. In the absence of exposure to dioxin or other AhR agonists, CA-AhR transgenic mice express AhR and CYP1A1 in the thymus and spleen with involution of the thymus. To study the underlying mechanism, CA-AhR transfected Jurkat T cells were made, and it was found that the growth of CA-AhR positive cells was completely inhibited. The growth arrest was due to apoptosis and G1-arrest, and microarray analysis identified certain genes that may be responsible for this effect. Poellinger *et al*. (Platform # 493) also focused on a transgenic mouse line that was created to express a CA-AhR. Since the CA-AhR is continuously active at a relatively low level, beginning early in development, it resembles the human dioxin exposure scenario and may provide greater insight into the human health consequences of AhR activation. CA-AhR is expressed in all organs and increased expression of the AhR target gene CYP1A1 within the same organ as the CA-AhR demonstrates that the mutant AhR (CA-AhR) is active. Cellular localization of increased CYP1A1 protein expression as a marker of an active CA-AhR was assessed by immunohistochemistry in the lung and heart. Increased expression of

CYP1A1 in both organs was in endothelial cells of the vasculature. In CA-AhR transgenic mouse lung, increased CYP1A1 expression was in Clara cells and epithelial cells of the alveolar septum.

There are two papers in which global gene expression analysis was carried out to provide greater insight into TCDD toxicity. Ohsako *et al.* (Poster # 402) sought to determine how male prostate development was affected by *in utero* TCDD exposure. Pregnant C57BL/6J mice were administered TCDD (10 ìg/kg) on either GD 14 or GD17. Since the prostate develops from the fetal urogenital sinus (UGS), UGS tissue was collected 24 hr after each maternal dose of TCDD and assessed by microarray analysis for TCDD-induced alterations in gene expression. Genes whose expression in the UGS was the most drastically altered 24 hr after TCDD exposure on GD14 (during the critical period for prostatic bud formation) compared to 24 hr after TCDD exposure on GD17 (after prostatic bud formation occurred), included several candidate genes one of which was involucrin. The authors speculate that upregulation of involucrin in the UGS by TCDD leading to terminal differentiation of UGS epithelial cells might inhibit the outgrowth of prostatic buds from the UGS resulting in a decrease in prostate size. Qu *et al.* (Platform # 382) attempted to identify genes that modify AhR-dependent TCDD toxicity. Three mouse strains, having the same AhR sequence (BALB/c, CBA/J and C3H/He) were administered a graded single dose of TCDD (0.4, 4, and 40 ìg/kg) and sacrificed 24 hr later, followed by microarray analysis. There are 3 and 18 genes that were up- and down-regulated, respectively, only in the BALB/c strain. Glutathione-S-transferase mu6 (GSTm6) was up-regulated by TCDD exposure, but there was no change in basal GSTm6 expression in the three strains of mice. GSTm6 may be a good marker gene for detecting the existence of modifiers.

Fritz *et al*. (Platform # 432) demonstrate that poorly differentiated prostate tumors develop relatively infrequently in *Ahr* $^{+/+}$ C57BL/6J TRAMP mice, but their incidence is increased significantly in *Ahr* $^{-/-}$ and *Ahr* $^{+/-}$ TRAMP mice. The prostates of TRAMP mice of all AhR genotypes develop PIN and well-differentiated adenocarcinoma lesions typical of the TRAMP model. However, what is different is that *Ahr* $^{-/-}$ and *Ahr* $^{+/-}$ TRAMP mice progress beyond these lesions to develop large poorly differentiated prostate tumors at a significantly higher incidence than *Ahr* $^{+/+}$ TRAMP mice. This suggests that the AhR may be a tumor suppressor in the prostate. The next paper (Platform # 266) is an extension of an earlier finding that TCDD releases rat liver epithelial cells from contact inhibition by upregulating cyclin A expression and cyclin A/cdk2 activity. The authors show that flavonoid compounds that are agonists or antagonists/partial agonists for the AhR (ANF, BNF, and 3'M4"NF) stimulate cell proliferation in confluent WB-F344 cells in a concentration-dependent manner. In contrast, non-AhR agonists (PCB 153 and fluorene) have no effect on proliferation of these growth-inhibited liver cells.

Bohonowych and Denison (Platform # 660) assessed the difference in susceptibility to TCDD toxicity among four laboratory animal species by using a binding assay for [^{3}H]-TCDD in liver cytosol fractions of guinea pig, mouse (C57BL/6), hamster, and rat (Sprague-Dawley). Binding of [^{3}H]-TCDD decreased more rapidly from unoccupied rat hepatic cytosol than occupied cytosol. Dissociation of [^{3}H]-TCDD in liver cytosol fractions differed between the four species and was in decreasing order: guinea pig > mouse and rat > hamster. This rank order is generally similar to that for TCDD-induced lethality with guinea pig being the most sensitive and hamster the least sensitive.

Yonemoto *et al*. (Poster # 153) describe a non-invasive AhR agonist biomarker for humans using cells sloughed off into breast milk after delivery. Breast milk cells consist of a spherical suspension of cells, including oil droplets and a few adhesive epithelial keratinized cells. In

response to *in vitro* exposure to TCDD, the level of CYP1A1mRNA expressed varied for over a 10^4 range. The expressed CYP1A1mRNA level was higher than that in leukocytes. In a human volunteer study, mothers provided milk specimens for the first week after delivery. In non-smoking mothers, the levels of PCDDs, PCDFs and coplanar PCBs in breast milk were significantly correlated with an induced level of CYP1A, suggesting the possibility of using this endpoint as a marker for dioxin exposure.

Three papers on TCDD effects in the rhesus monkey, from the same Japanese research group, are included in the session (Posters: # 568, # 504, and # 468). Pregnant rhesus monkeys were subcutaneously dosed with 30 or 300 ng TCDD/kg on gestation day 20, followed by maintenance doses given every 30 days until postnatal day 90. All three papers appear to have used this TCDD dosing regimen that is described in only one of the three papers. Yasuda *et al*. (Poster # 568) examined effects of TCDD on teeth development in rhesus monkey offspring. In rats, *in utero* and lactational TCDD exposure affects molar development, and in children exposed to high levels of TCDD-like compounds tooth abnormalities were reported. In TCDD exposed rhesus monkey offspring that were stillborn or died as infants abnormal development of the teeth occurred. The effect was dose-dependent, being observed at a maternal dose of 300 ng/kg, but not at 30 ng/kg of TCDD. Korenaga *et al*. (Poster # 504) found histopathological effects in the liver of rhesus monkeys administrated TCDD. Liver sinusoidal endothelial cell injury and impairment in intrasinusoidal microcirculation may have been caused by TCDD, because infarction, focal fatty change, and microthrombi formation, rarely found in the liver of control rhesus monkeys, were observed in those treated with TCDD. AhR was detected by immunoblot in control, but not TCDD-exposed, monkey liver. However, Ohta *et al*. (Poster # 468) found that TCDD did not affect AhR expression in the liver. The latter group also determined that some genes related to apoptosis and other signaling pathways were affected by TCDD.

Effects of graded doses of TCDD on human $CD34^+$ progenitor cells from normal human donors stimulated with G-CSF, as well as in human myeloid leukemic cell lines HL60 (promyelocytic leukemia) and K562 (chronic myelogenous leukemia) are presented in the next paper (Platform # 128). The major finding was that a TCDD concentration that inhibited colony growth caused genes involved in the processes of proliferation, differentiation, and transformation to be affected. In the final paper, Ikeda *et al.* (Poster # 470) developed a simplified method for TCDD assay in chicken eggs. The method can detect TCDD-contaminated eggs.

Acknowledgement: This session was organized with support from the Center of Environmental Information Science (CEIS) in cooperation with the Ministry of the Environment, Japan. A special thank you is directed to Dr. Rie Masho at the CEIS for making this scientific session possible.