COMPARING MIXTURES OF DIOXIN-LIKE AND NON DIOXIN-LIKE PCBs TO TCDD

Deborah E. Burgin[1], Janet J. Diliberto[2], Linda S. Birnbaum[2]

[1]UNC Toxicology/USEPA, MD 74, 86 T.W. Alexander Dr, RTP, NC USA
[2]US EPA, NHEERL, ETD, MD 66, 86 T.W. Alexander Dr, Research Triangle Park, NC USA

Introduction

Environmental exposures to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) always occur as part of a complex mixture. In order to assess the potential risk associated with these exposures, the Toxic Equivalents are estimated which is the weighted sum of the relative potencies of each dioxin-like chemical in the mixture[1]. While this method adequately accounts for the effects of mixtures of TCDD or dioxin-like (DL) chemicals, there are almost always non-dioxin-like (NDL) chemicals present, in particular, the NDL polychlorinated biphenyls (PCBs).

To further examine the interactions of dioxins with NDL PCBs, the present study compared the effects on multiple responses of different laboratory-defined mixtures, based on mass ratios found in food, of dioxin and NDL PCBs in both wildtype and CYP1A2 knockout male and female C57BL/6J mice. These chemical groups are: 1) TCDD alone; 2) DL Mix A containing TCDD, 1,2,3,7,8 Pentachlorodibenzo-p-dioxin (PeCDD), 2,3,4,7,8 Pentachloro-dibenzofuran (4-PeCDF), 3,3',4,4',5-Pentachlorobiphenyl (PCB 126); 3) NDL Mix B containing 2,3',4,4',5-pentachlorobiphenyl (PCB 118), 2,2',3,4,4',5'-hexachlorobiphenyl (PCB 138), 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153), 2,2',3,4,4',5,5'-heptachlorobiphenyl (PCB 180); and 4) Mix C - a combination of Mixes A and B.

Materials and Methods

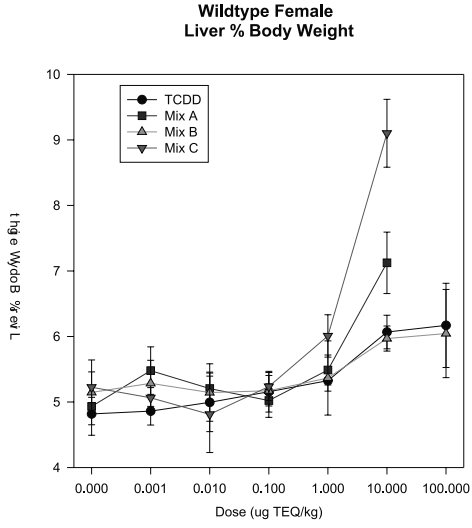
Chemicals

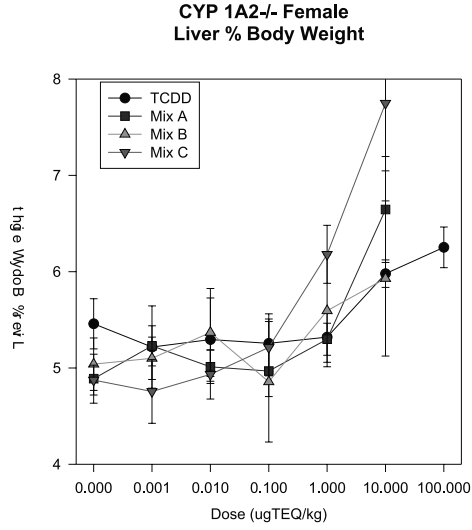
TCDD, PeCDD and 4-PeCDF were purchased from Radian Corporation (Austin, TX; purity > 98 %). PCB 118, PCB 126, PCB 138, PCB 153 and PCB 180 were obtained from Accustandard (New Haven, CT) and were greater than 98 % pure. All other chemicals were obtained from Sigma Chemical Co. (St Louis, MO) and were of the highest grade commercially available.

Animals and Treatment

Female and male wild type C57BL/6J mice (10 weeks old) were obtained from Jackson Laboratory (Bar Harbor ME, USA) and allowed to acclimate for seven days. CYP1A2 null mice were originally obtained from Dr Frank Gonzalez at the National Cancer Institute (NIH, USA). Mice from our colony received a single exposure to TCDD alone, Mixtures A, B, or C or corn oil by gavage at a dosing volume of 10 ml/kg. The dose levels used were 0.0, 0.001, 0.01, 0.1, 1.0, and 10.0 mg TEQ/kg body weight for Mix A, B and C, and the same plus 100.0 mg /kg for TCDD. The mass ratio of PCBs in Mix B were chosen based on the levels found in food and the environment, and the WHO TEF for PCB 118 was used to compare that mixture to TCDD[1].

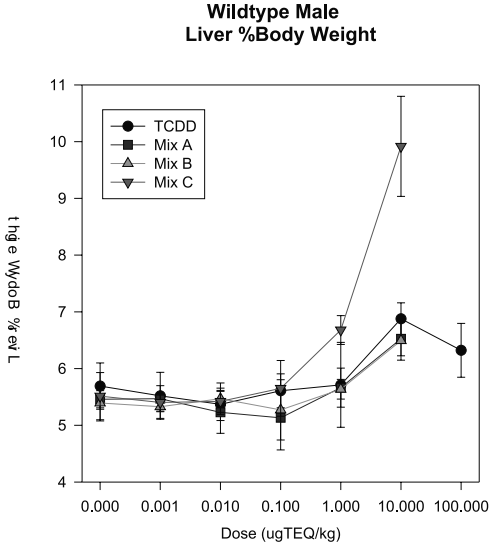
Seven days after initial exposure, the study was terminated and the mice were killed by an overdose of CO_2. Livers, brains, kidneys, spleen, lungs, and serum were collected and frozen in liquid nitrogen for future analysis. Determination of ascorbic acid, uric acid, total glutathione (GSH), total thyroxine (TT4) and total triiodothyronine (TT3) concentrations were performed as described previously[2].

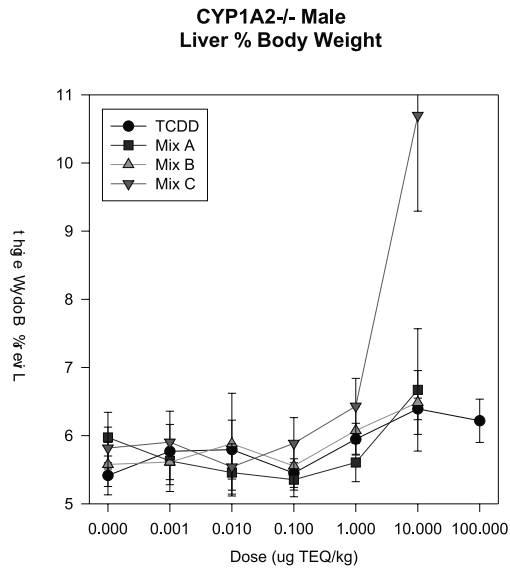




Results

No effects were seen in body weight or other tissues, in male or female wildtype or knockout mice. When exposed to Mix C, liver weight increased significantly, and to a much higher level than the other mixtures in both sexes and both strains (see graphs). Male wildtype and knockout mice showed a greater decrease in TT3 when exposed to TCDD than their female counterparts. Mix A and Mix B did not appear to have an impact on TT3 results, but with Mix C both strains and sexes showed a decrease





over controls in TT3 at the highest dose. The highest dose levels of Mix A and Mix C showed a significant decrease in TT4 over controls. The highest dose levels of Mix B showed a decrease in wildtype (male and female) TT4 levels. Ascorbate and urate levels increased at the highest dose for both female wildtype and knockout when exposed to Mix A. No clear changes were observed for GSH.

Conclusion

Although we expected an increase in liver weights in Mix B and Mix C, liver weight was increased only in Mix C in both male and female, wildtype and knockout. The effect appears to be greater than additive. The decrease in TT3 seen in the male mice expose to TCDD indicates a possible endogenous estrogenic component to maintenance of triiodothyronine. Endogenous estrogen levels may also play a role in ascorbic acid cycling when impacted by dioxin-like compounds (Mix A) that act as exogenous estrogens.

No effects were observed in levels of glutathione, a marker of oxidative stress, in either wildtype or knockout, although this is not surprising due to the large pools of glutathione in the body. Based on these findings, it appears that these mixtures have a synergistic effect greater than the sum of the individual congeners, or TCDD alone.

Previous studies conducted in our lab on oxidative stress indicators demonstrated that CYP1A2 does not appear to affect the response following TCDD administration[3]. This study was conducted in male knockouts. There does not appear to be a strain difference in response to chemical exposure, but there does appear to be a gender difference.

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