INTERACTIONS OF POLYCHLORINATED DIOXINS, DIBENZOFURANS AND BIPHENYLS IN WILD TYPE C57BL/6J AND CYP1A2 NULL MICE.

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Introduction

Environmental exposures to 2,3,7,8-tetrachlorodibenzo-p-dioxin always occur as part of a complex mixture[1,2]. In order to assess the potential human and ecological risk associated with these exposures, the Toxic Equivalency Factor (TEF) method was developed[3,4]. While this method adequately accounts for the toxic effects of mixtures of TCDD or dioxin-like chemicals, there are almost always non-dioxin-like chemicals present, in particular, the non-dioxin-like PCBs. Recent studies in this laboratory have examined the effects of different Aroclor 1254 mixtures that differ slightly in composition resulting in significant differences in TEQs. Our initial studies indicate that for induction of CYP1A1 the TEF method adequately predicts the response of the two mixtures. However, these studies also indicate that for some responses, such as decreases in serum thyroxin, the TEF methodology underpredicts the responses of these mixtures. To further examine the interactions of dioxins with non-dioxin-like PCBs, in the present study we have tested and compared the effects of different laboratory defined mixtures of dioxins and non-dioxin-like PCBs in both wild type C57BL/6J and CYP1A2 null mice.

Materials and Methods

Chemicals:

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 1,2,3,7,8-Pentachlorodibenzo-p-dioxin (PeCDD) and 2,3,4,7,8-pentachlorodibenzofuran (4-PeCDF) were purchased from Radian Corporation (Austin, TX) (purity > 98%). 3,3',4,4',5-Pentachlorobiphenyl (126), 2,2',3,4,4',5'-hexachlorobiphenyl (138) 2,3',4,4'-,5-pentachlorobiphenyl (118), and 2,2',4,4',5,5'-hexachlorobiphenyl (153) and 2,2',3,4,4',5,5'-heptachlorobiphenyl (180) were obtained from all was obtained from Accustandard (New Haven CT) and was greater than 98% pure. All other chemicals were obtained from Sigma Chemical Co. (St Louis MO) and were of the highest grade available.

The dioxins, dibenzofurans and PCBs were initially dissolved in acetone and then diluted with corn oil (Sigma Chemical Co.). The acetone was removed from the corn oil solution by evaporation in a Speed Vac SVC100 (Savant Instruments Inc., Farmingdale NY). Dosing solutions were prepared by diluting the acetone-free stock corn oil solutions with additional corn oil. Three mixtures were prepared and the compositions of these mixtures are listed in Table 1.

Mixture A contained TCDD, PeCDD, 4-PeCDF and 126. Mixture B contained PCBs 118, 138, 153 and 180. Mixture C contained all of the test chemicals.

Animals and Treatment:
All procedures employed in these studies were reviewed and approved by the Institutional Animal Care and Use Committee of the National Health & Environmental Effects Research Laboratory at the U.S. Environmental Protection Agency prior to the initiation of the experiments. 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 Frar' Gonzalez at the National Cancer Institute (USA). Mice were randomly assigned to treatment groups, group housed in plastic cages with hardwood shavings and allowed free access to food (Purina rodent chow) and tap water. The room was maintained on a 12-hr light:dark cycle at 22 ± 2° C and 50 ± 5% relative humidities. Mice received a single exposure to either TCDD alone, Mixture A, B, or C or corn oil alone by gavage at a dosing volume of 10 ml/kg. The TCDD dose ranged from 0.001 to 100 ug/kg. Mixture A was administered at doses ranging from 0.001 to 100 ug TEQ/kg. Mixture B was administered at doses of 0.001 to 100 ug TEQ/kg based on the WHO_{98} TEF for PCB 118. Mixture C was administered based on the TEQ dose and was administered at doses ranging from 0.001 to 10 ug TEQ/kg.

Seven days after the initial exposure, the study was terminated and the mice were killed by an overdose of CO_2. Livers, brains, kidneys, spleen and lungs were collected and weighed. These organs were then frozen in liquid nitrogen for future analysis of markers of oxidative stress. Serum was collected for the determination of thyroxin concentrations.

Results and Discussion
The administration of either TCDD or the mixtures did not result in any treatment-related mortality in any of the strains or gender of mice examined. Liver/body weight ratios were increased in the mice treated at the two highest doses of the TCDD and Mixture A, B and C. Mixture C increased liver/body weight ratios to a greater extent than did TCDD or mixtures A and B (Figure 1). Lung/body weight ratios were increased at the highest dose of TCDD examined but not with any of the mixtures. Spleen, kidney and brain weights were not altered at the doses and time examined in the present study.

The present study examines the interactions between mixtures of dioxins, dibenzofurans and PCBs. These initial studies suggest that combinations of dioxins, dibenzofurans and PCBs can produce greater responses on liver weight than when these mixtures are administered alone. Future analysis of this data will aid in determining if these interactions are additive or synergistic. It should be noted that both the dioxins and the non dioxin-like PCBs examined in this study are liver tumor promoters. The increased liver weight in the mice treated with Mixture C suggests potential interactions of these chemicals as tumor promoters and as possible carcinogens.

References
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Table 1. Ratio's of chemicals in Mixtures A, B and C

Chemical	Mixture A	Mixture B	Mixture C
TCDD	1	-	1
PeCDD	1	-	1
4-PeCDF	2	-	2
PCB 126	45	-	45
PCB 118	-	10,000	10,000
PCB 138	-	20,000	20,000
PCB 153	-	30,000	30,000
PCB 180	-	20,000	20,000

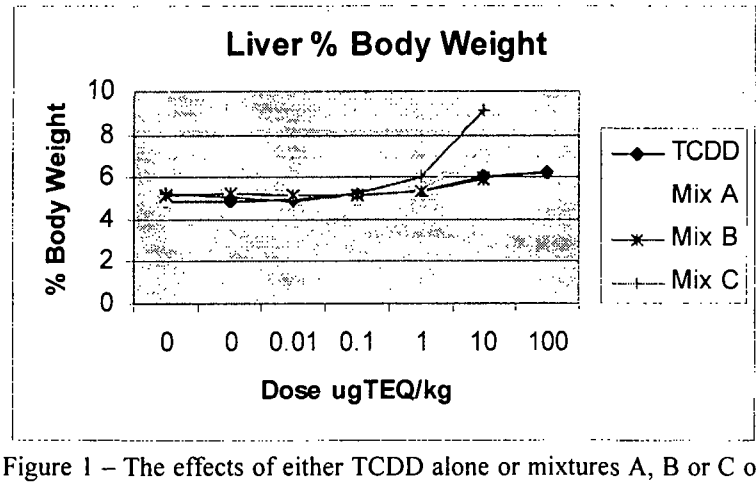

Figure 1 – The effects of either TCDD alone or mixtures A, B or C on liver/body weight ratios in female C57Bl/6J mice