MIXTURE EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN AND POLYCHLORINATED BIPHENYL CONGENERS IN RATS

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Introduction

Toxicology research on chemical mixtures is well documented, however, a review of the literature has revealed that most reports deal with acute studies employing relatively high doses of test chemicals. Results from the acute studies are considered to be of limited value for predicting human health hazards caused by environmental exposure that is typically long-term and low-level. In order to assess mixture effects, toxicology data from properly designed studies using environmentally relevant levels are required. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and polychlorinated biphenyls (PCBs) continue to be of health and environmental concern due to their toxic effects and bioaccumulation in the food chain. The current acceptable levels of human exposure to these pollutants were established based on the toxic effects of individual pollutants when they were tested alone and did not take into consideration the potential for interactive effects. Risk assessments of hazardous chemicals generally assume an additive effect that acts on target organs independently (Anonymous, 1986). This assumption may result in under- or overestimation of the toxic effects of the mixtures to which humans are exposed. The objective of the present study was therefore to investigate the mixture effects of TCDD and PCBs so that the information gained from the study may be used to assess the health impact of these pollutants. A systemic toxicology study was conducted in which biochemical, hematological and morphological effects were examined in rats dosed daily with TCDD, PCBs or in combination for 28 days in order to provide insights into the mixture effects of these pollutants.

Materials and Methods

The PCB congeners (PCB) and TCDD used in the study were purchased from Accustandard Chemical Co. (New Haven, CT). The chemical identity and purity were confirmed to be greater than 99% by GC and GC-MS. The PCB used in the present study is a reconstituted mixture similar to that reported in the milk (2), and human tissue (3). The composition and percentage (given in parentheses as w/w) of each congener in the reconstituted mixture are as follows: PCB 180 (29), 118 (25.7), 105 (15), 170 (12), 156 (8.4), 114(4.0), 167(2.5), 157(1.8), 189(0.6), 123(0.4), 169(0.2), 126(0.15), 77(0.04).

Female Sprague Dawley rats (56 ∀ 3 g), obtained from Charles River Laboratories, St. Constant, Quebec, were randomly divided into 15 groups of 5 animals in each group. Only female rats were employed because they were found to be more sensitive to the toxic effects of PCB congeners as shown in our earlier studies (4). The animals were given by gavage daily a corn oil solution(5 ml/ kg body weight) of TCDD, PCB, or a combination of both for 28 days. The dosage for each of the treatment groups is described below. Groups 1-5 received TCDD alone at 5 dose levels (group 1: control, 0 TCDD; group 2: 2.5 ng TCDD; group 3: 25 ng TCDD; group 4: 250 ng TCDD; and group

5: 1,000 ng TCDD/kg bw/day). Groups 6-10 were given various doses of TCDD in combination with the PCB mixture at 2 μg/ kg bw/day (group 6: 2 μg PCB; group 7: 2.5 ng TCDD+ 2 μg PCB; group 8: 25 ng TCDD+ 2 μg PCB; group 9: 250 ng TCDD+ 2 μg PCB; group 10: 1,000 ng TCDD+ 2 μg PCB). Groups 11-15 received TCDD and the 20 μg PCB mixture (group 11: 20 μg PCB; group 12: 2.5 ng TCDD+ 20 μg PCB; group 13: 25 ng TCDD+ 20 μg PCB; group 14: 250 ng TCDD+ 20 μg PCB; group 15: 1,000 ng TCDD+ 20 μg PCB).

At the termination of the study, the animals were anesthetized with an *ip* injection of a barbiturate-based anesthetic agent Equethesin^{T}. Blood was withdrawn from the abdominal aorta and analyzed for erythrocyte count, hematocrit value, mean corpuscular volume, mean corpuscular hemoglobin concentration, platelet count, and total and differential counts of leucocytes. Serum was prepared from the remaining blood specimens and was used for the determination of inorganic phosphate, total protein, alkaline phosphatase, aspartate aminotransferase, bilirubin, calcium, cholesterol, glucose, uric acid and lactate dehydrogenase.

Liver, heart, spleen, thymus, brain and kidney were removed and weighed. An approximate 2.0 g piece of the liver was homogenized in 0.05 M TRIS/1.15% KCl buffer. The homogenate was centrifuged to prepare 9,000 x g supernatant, and the activities of microsomal EROD (5), BROD and PROD, and MROD (6) were determined. Liver, lung and kidney (0.1-0.3 g) were hydrolyzed with ethanolic KOH and extracted with petroleum ether, and the extracts analyzed for vitamin A according to a method described previously (7). The tissues from the animals were excised and fixed in formalin. Paraffin blocks of tissues were sectioned, stained with H&E, and examined for morphological changes. Semi-quantitative scores were recorded for liver and thyroid. Data were analyzed by ANOVA and Duncan=s multiple range test to determine the groups that were significantly different ($p<0.05$).

Results and Discussion

Administration of TCDD to rats up 1,000 ng daily for 28 days resulted in a broad spectrum of effects including growth suppression, hepatomegaly, thymic atrophy, liver microsomal induction, decreased liver and lung vitamin A, and biochemical, hematological and morphological changes in thyroid, thymus and liver. Most of these effects were observed in the 250 and 1,000 ng groups. Treatment with 2 and 20 μg PCB generally had no effects on the above endpoints, but produced morphological changes in the liver, thyroid and thymus.

Increased microsomal EROD and MROD activities were observed in the 250 and 1,000 ng TCDD groups. Co-administration with PCB resulted in a decrease in the activities of EROD and MROD (Figure 1). The decreased TCDD-induced MFO activities, which were more pronounced at 250 ng than at 1,000 ng, are probably a result of antagonistic effects on CYP 1A1 and 1A2 isozymes by non-ortho substituted PCB congeners that are structurally similar to TCDD.

Morphological alterations in the thyroid consisted of reduced colloid density, collapsed follicles and epithelial thickening. The effects on the thyroid were minimal even at the highest doses of TCDD and PCB, and no mixture effects were noted. Histopathological changes in the thymus were TCDD related, comprising of cortical and medullary atrophy. The severity of the thymic changes, rated as moderate, was the highest in the group receiving a combination of 1,000 ng TCDD + 20 μg PCB. Co-administration of PCB and TCDD appeared to show an additive effect on the thymus. The severity of histological scores was given the following ratings: 1: minimal, 2: mild, 3: moderate, and 4: severe.

Changes in the liver consisted of cytoplasmic vacuolation, nuclear hyperchromicity,

anisokaryosis, and an increased homogenous appearance of cytoplasm in the midzonal and perivenous areas. The homogenous appearance in the functional lobules probably reflects proliferation of the smooth endoplasmic reticulum. These changes, observed in both TCDD and PCB treated groups, were more severe in TCDD treated rats. A dose-dependent increase in anisokaryosis was seen in the groups receiving TCDD alone, which was reduced in combination with 2, and 20 μg PCB (Figure1). Similarly, cytoplasmic vacuolation was also less severe in the TCDD-PCB combined groups (Figure 1). Considering the biochemical and histological effects on the liver, it seems that co-administration with PCB had an antagonistic effect on the TCDD induced changes.

PCB 153 (2,2',4,4',5,5'-hexachlorobiphenyl) was reported to be a TCDD antagonist in C57BL/6 mouse hepatic Ah cytosolic receptors (8). It was also reported that co-administration of PCB 153 and TCDD (up to 5,000 nmol/kg) resulted in a significant synergistic effect (9). Previous work from our laboratory demonstrated additive biochemical and histological effects on the liver of rats given mirex, kepone, or photomirex alone or in combination with Aroclor 1254 and polybrominated biphenyls (10).

Serum biochemistry was generally not affected by TCDD, PCB or co-administration of both, with the exception of serum cholesterol and protein, which were elevated in the highest TCDD group. Co-administration of TCDD and PCB appeared to have an additive effect on serum cholesterol. Hemoglobin and hematocrit were reduced by 1,000 ng TCDD. The hematological effects of TCDD were augmented by the low dose PCB (2 μg /kg), but were partially antagonized by the high dose PCB (Figure 1). The biphasic nature of the PCB effect on TCDD induced hematological changes suggests that the mixture effect is also dose-dependent. In most cases, co-administration with PCB did not alter TCDD induced hematological changes.

In summary, the effects of mixtures are very complex; they may be additive, antagonistic or synergistic depending on dose levels, endpoints measured and mode of administration. Only through increased understanding of mechanisms of action and toxicokinetic behaviors of the interacting chemicals can the combined effects of a mixture be predicted.

References

1 Anonymous (1986). Guidelines for chemical mixtures **1986**. *Federal Register*, September 25. 51FR 34014-34029.

2. Mes, J. Davies, J.D., Doucet J., Weber D. and McMullen E. *Environ. Technology* **1993**, 14, 555-565.

3 Kannan, N., Tanabe, S., and Tatsukawa., R., *Arch Environ. Health,* **1988,** 43**,** 11-14.

4. Chu, I, Villeneuve, D.C., Yagminas, A. et.al., *Fund. Appl. Toxicol.,* **1995,** 26, 282-292.

5. Lubet, R.A., Nims, R.W., Mayer, R.T. et.al., *Mutat. Res*., **1985**, 142, 127-131.

6. Burke, M.D., Thompson, S., Elcombe, C.R., et.al., *Biochem. Pharmacol*., **1985,** 34, 3337-3345.

7. Håkansson, H. Waern F. and Ahlborg, *U.G. J. Nutr*. **,1987,** 117, 580-586.

8. Bigel, L. Harris, M. Davis, D. et.al, *Toxicol. Appl. Pharmacol.,* **1989,** 97**,** 561-571.

9. Bannister, R. and S. Safe, S. *Toxicology,* **1987,**44, 159-169.

10. Chu, I., Villeneuve, D.C., Becking, G.C. et.,al., *J. Toxicol. Environ. Health,* **1980,** 6, 421-432.

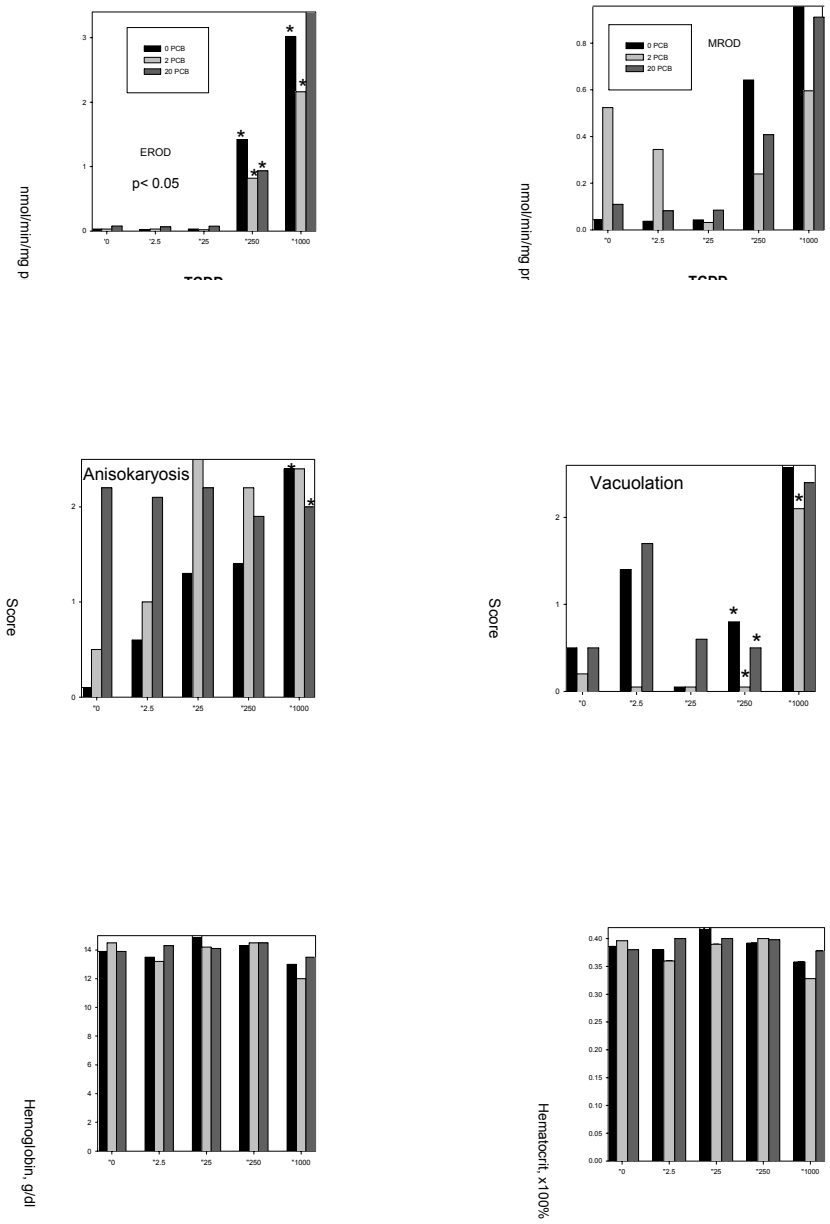
0 PCB
2 PCB
20 PCB
EROD
p< 0.05
nmol/min/mg p
MROD
nmol/min/mg pr
Anisokaryosis
Score
Vacuolation
Score
Hemoglobin, g/dl
Hematocrit, x100%

Figure 1