# Combined Effects of 2,3,7,8-tetrachlorodibenzo-p-doxin and polychlorinated biphenyls congeners in rats

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## Introduction

There has been considerable interest in conducting toxicity studies on mixtures since this approach represents realistic human exposure and would provide a better model to predict the health impacts of environmental chemicals. However, risk assessment of the chemicals is largely based on the toxicity data of individual compounds by assuming simple additive effects of these compounds [1]. This practice has been accepted by regulatory agencies provided that the concentrations of chemicals are extremely low, and there are no interactions. The existence of interactions among the chemicals co-administered to test animals may under or over estimate the effects of a mixture if the simple additive rule is applied. Previously, we demonstrated an antagonistic effect in rats when tetrachlorodibenzo-*p*-dioxin (TCDD) was co-administered with polychlorinated biphenyls congeners (PCBs)[2]. The hepatic microsomal EROD, MROD and UDPGT activities of TCDD were decreased when co-administered with PCB congeners. To further explore the combined effects of these pollutants, we examined and report results on tissue residue levels of TCDD and histopathological changes in target organs of rats exposed to TCDD, PCBs and mixtures of both.

## Materials and Methods

The experimental procedures and reagents were as previously described [2]. Briefly, groups of 5 female rats were given corn oil solution (5 ml/kg bw) daily containing PCB congeners, TCDD or a combination of both for 28 days as described in the table below ( Table 2a,b). The composition of the PCB congener mixture expressed by % in weight shown in the parenthesis is as follows: PCB 180

( 29%), 118 ( 25.7), 105( 15 ), 170 ( 12.1), 156 (8.4), 114 (4.0), 167 (2.51), 157(1.83) 189 (0.62), 123 (0.4), 169 (0.18), 126 (0.15) 77 ( 0.04). At the termination of the study the rats were anesthetized with an *ip* injection of Equithesin™, and tissues and blood were taken for clinical, biochemical, hematological and histopathological analysis as previously described[2]. Clinical, biochemical and hematological results had been reported elsewhere [2] . This communication describes results on liver residue analysis and histopathological examination.

**Liver Residue Analysis:** Liver samples were processed in batches of eight. Each batch consisted of one laboratory blank, 6 samples and another QA/QC sample such as chicken liver (background sample), NRC carp (reference material), duplicate sample or native spiked duplicate sample. The samples (ca. 0.1-0.4 g) were weighed into screw-capped centrifuge tubes and spiked with 40 pg of the $^{13}C_{12}$ – labelled surrogate. The samples were digested in sulfuric acid (5.0 ml) at room temperature for one hour and then extracted 4 times with hexanes at 3 ml each . The extracts were combined and one-half was cleaned up using a multilayer silica column (0.5 g neutral silica, 2 g sulfuric acid on 0.5 g silica and 1.0 g of NaOH on 0.5 g silica). The column was pre-eluted with 10 ml hexanes, and after the sample was added, it was eluted with 30 ml of hexane again. The final extract was evaporated, and internal standards (100 pg of each of $^{13}C_{12}$-1234-TCDD and $^{13}C_{12}$-123789-HxCDD) were added prior to analysis. Analysis of the 2,3,7,8-TCDD was performed on a high resolution gas chromatograph/high resolution mass spectrometer (HRGC/HRMS) using a Hewlett Packard 5890 Series II GC interfaced to a VG 70SE HRMS. The HRMS was operated in the EI/SIR mode at 10000 resolution. The GC operating conditions were as follows: The injection port and initial oven temperature were respectively at 300$^{o}$ C and 150$^{o}$C. The oven temperature was programmed to increase in 3 stages: (1) the oven stayed at 150$^{o}$ C 1 min then increased at 12$^{o}$ C/min to 200 $^{o}$ C; (2) increased to 230$^{o}$ C at 3$^{o}$ C/min and stayed at this temperature for 10 min, and (3) then increased to 310 $^{o}$ C at 8$^{o}$ C/min and held at this temperature for 10-15 min. A 60 m DB5 column (J&W) was used. The internal diameter was 0.25 mm and the column film thickness was 0.25 µm. A splitless injection was used. Two µl injections were made. A series of calibration standards containing native and $^{13}C_{12}$-labelled surrogate was used. TCDD analysis was only performed in the two highest dose groups (250 and 1,000 ng groups) where interactive biochemical effects were observed [2].

**Histopathology examination:** The tissues and organs were excised and fixed in 10% buffered formalin (pH 7.4) and dehydrated with graded alcohol. Tissues were paraffin blocked and sectioned to ca. 5 μm thickness and stained with hematoxylin and eosin for light microscopic examination. The tissues examined were brain, pituitary, thyroid, parathyroid, bronchi, trachea, thoracic aorta, salivary glands, thymus, lung, skeletal muscle, skin, eye, optical nerve, heart, liver, kidney, adrenal glands, spleen, pancreas, oesophagus, gastric cardia, fundus and pylorus, duodenum, ileum, jejunum, colon, urinary bladder, bone marrow, mesenteric lymph nodes, mammary glands and reproductive organs.

## Results and Discussion

Residue analysis showed a dose-dependent accumulation of TCDD in the liver (Table 1). As shown in the table, the levels of TCDD increased from 1.49 ppb to 24 ppb as the dosage increased from 250 to 1,000 ng/kg bw. When 250 ng TCDD was co-administered with 2 μg and 20 μg PCBs, accumulation in the liver decreased from 1.49 to 0.84 and 0.79 ppb respectively. As the TCDD dosage increased to 1000 ng, no reduction in liver TCDD due to co-exposure with PCBs was observed (24, 31 and 24 ppb in the TCDD control, and TCDD with 2 μg and 20 μg PCBs, respectively, Table 1). The observed levels of TCDD in the liver correlated very well with the hepatic microsomal enzyme activities. In the 250 ng TCDD dose groups where the liver TCDD level decreased, antagonistic effects on EROD, MROD and UDPGT activities were also observed. Antagonism of TCDD effects by PCB mixtures and individual congeners have been reported by a number of investigators. It was reported that Aroclor 1254 partially inhibited EROD induction and immunosuppressive effects of TCDD in C57BL/6J (Bannister et al., 1987), and B3C3F1 (Harper et al., 1995) mice. The antagonism occurred at the low ( 0.01-0.015 μmol/kg) but not at the high dose of TCDD (0.03-0.05 μmol/kg) ( Bannister et al., 1987). As shown in Figure 1a-c, there is an apparent suppression of EROD, MROD and UDPGT activities by 2 μg and 20 μg PCBs when TCDD dose is at 250 ng. When the TCDD dosage increased to 1,000 ng, the inhibitory effect of PCBs was no longer observable. This observation was also consistent with the liver residue data of the 1,000 ng TCDD groups which showed no decreases by co-exposure with PCBs. However, it remains to be determined as to why at the higher dose of TCDD (1,000 ng)+ 20 μg PCBs, the inhibitory effect of PCBs was not observed.

Examination of tissues revealed that liver, thyroid and thymus were the target organs. Both TCDD and PCBs elicited similar histopathological effects in thyroid, thymus and liver with the former being more potent. No treatment related lesions were found in other tissues.

Table 1: Levels of TCDD in the liver of rats treated with TCDD alone or in combination with PCBs.

| Group | Treatment TCDD, ng/kg | PCB, μg/kg | Levels of TCDD in liver (ppb) |
|---|---|---|---|
| Control | 0 | 0 | ND |
| 4 | 250 | 0 | 1.49 ±0. 32 |
| 5 | 250 | 2 | 0.84 ± 0. 11 |
| 9 | 250 | 20 | 0.79 ± 0.11 |
| 10 | 1,000 | 0 | 24.14 ± 10.26 |
| 14 | 1,000 | 2 | 30.8 ± 2.45 |
| 15 | 1,000 | 20 | 24.16 ± 2.24 |

examined. Thyroid changes were dose-dependent, and characterized by angular collapse of follicles, decreased colloid density and thickening of follicular epithelium (Table 2a). Alterations in the thymus consisted of reduced thymic cortex and increased medullary volume. TCDD produced minimal to moderate changes depending on the dosage while the effect of PCBs were minimal to mild in nature (Table 2a). Treatment related changes in the liver were characterized by accentuated hepatic zones, anisokaryosis of hepatocytes, increased cytoplasmic density and vacuolation (Table 2 b). Histological changes in the liver appeared to be extremely sensitive to PCB exposure. In contrast, biochemical measurements indicative of liver effects such as microsomal enzyme activities showed no alterations at these doses of PCBs. Histological effects of co-exposure to PCBs and TCDD varied and appeared to be organ and tissue specific. For instance, the severity of anisokaryosis, vesiculation and vacuolation as produced by 1,000 ng TCDD showed no changes due to co-exposure of PCBs (Table 2 b) . On the other hand, an additive effect in the thyroid follicles, colloid and epithelial height was observed, particularly in the 20 μg PCBs + 25 ng TCDD, and 20 μg PCBs + 250 ng TCDD groups (Table 2a). There appeared to be a synergistic effect in the medullary volume of the thymus where the increased volume was more than mathematical summation of the groups receiving the highest dose of PCBs or TCDD alone.

In summary, the present study demonstrated both additive (thyroid and thymus) and antagonistic effects (EROD, MROD and UDPGT ) of PCBs and TCDD. The antagonistic effect of PCBs on microsomal enzyme activities could be explained by reduced accumulation of TCDD in the liver in the presence of PCBs. The above observations indicate that the mixture effects are complex that are organs and tissue specific, and risk assessment of chemical mixtures requires an understanding of mechanisms of effects.

**Acknowledgement**

The authors are indebted to Ms Colleen Tashiro of Wellington Laboratories, Guelph, Ontario, Canada for performing the HRGC-MS analysis, and Dr. Jamie Nakai for useful discussions.

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Table 2a: Severity of histological changes in the thyroid and thymus of rats treated with TCDD, PCBs and combination of both.

| Group | Treatment | Thyroid | | | Thymus | |
|---|---|---|---|---|---|---|
| | PCB µg+ TCDD, ng | Reduced follicles | Reduced colloid density | Incr'd epithial height | Reduced cortical volume | Incr'd medullary volume |
| 1 | 0+ 0 | 0.13 | 0 | 0.38 | 0 | 0 |
| 2 | 0 + 2.5 | 0 | 0 | 0.3 | 0.75 | 0.75 |
| 3 | 0 + 25 | 0.14 | 0.3 | 0.38 | 0.24 | 0 |
| 4 | 0 + 250 | 1.55 | 0.9 | 0.6 | 0.4 | 0 |
| 5 | 0 + 1,000 | 1.9 | 1.4 | 1.4 | 3 | 0.7 |
| 6 | 2 + 0 | 0.5 | 0 | 0.4 | 0.1 | 0.1 |
| 7 | 2 + 2.5 | 0.38 | 0.31 | 1 | 0.13 | 0.25 |
| 8 | 2 + 25 | 1.5 | 0.6 | 2 | 0 | 0.2 |
| 9 | 2 + 250 | 2 | 1.6 | 1.9 | 0.15 | 0.3 |
| 10 | 2 + 1,000 | 2.4 | 2.1 | 2.5 | 3 | 1.1 |
| 11 | 20 + 0 | 1.5 | 1.6 | 1.6 | 0.55 | 0.3 |
| 12 | 20 + 2.5 | 0.8 | 1.35 | 1.4 | 0 | 0.5 |
| 13 | 20 + 25 | 2.3 | 2.1 | 2.1 | 0.3 | 0.35 |
| 14 | 20 + 250 | 2.3 | 2.2 | 2.2 | 0.1 | 0.4 |
| 15 | 20 + 1,000 | 1.9 | 2.3 | 2.3 | 2.8 | 3 |

Data are mean value of animals. The severity of histology grading is as follows: 0, normal, 1, minimal; 2, mild; 3, moderate; 4, severe. For tissue changes that are focal, locally extensive and multi focal, a score of less than an integer is assigned. The scores are: minimal focal, 0.25; minimal locally extensive, 0.50; minimal multi focal, 0.75; mild focal, 1.25; mild locally extensive, 1.50; mild multi focal, 1.75, etc.

Table 2b: Severity of histological changes in the liver of rats exposed to TCDD, PCBs and combination of both.

| Group | Treatment | Liver | | | | |
|---|---|---|---|---|---|---|
| | PCB µg+ TCDD, ng | Accentuated zonation | Anisokaryosis | Vesiculation | Periportal vacuolation | Incr'd perivenous homogeneity |
| 1 | 0+ 0 | 0.3 | 0 | 1 | 0.5 | 0.1 |
| 2 | 0 + 2.5 | 0.2 | 0.6 | 1.2 | 1.4 | 0.5 |
| 3 | 0 + 25 | 1.25 | 1.25 | 1.5 | 0 | 0.63 |
| 4 | 0 + 250 | 1.1 | 1.4 | 1.4 | 0.8 | 0.9 |
| 5 | 0 + 1,000 | 1.9 | 2.4 | 2 | 2.6 | 1.2 |
| 6 | 2 + 0 | 0.8 | 0.5 | 1 | 0.2 | 0.3 |
| 7 | 2 + 2.5 | 1 | 1 | 0.5 | 0 | 0.38 |
| 8 | 2 + 25 | 0.9 | 2.5 | 1.2 | 0 | 0.8 |
| 9 | 2 + 250 | 1.4 | 2.2 | 2 | 0 | 0.8 |
| 10 | 2 + 1,000 | 2 | 2.4 | 1.6 | 2.1 | 1.8 |
| 11 | 20 + 0 | 0.7 | 2.2 | 0.4 | 0.5 | 1 |
| 12 | 20 + 2.5 | 0.7 | 2.1 | 1.2 | 1.7 | 1.5 |
| 13 | 20 + 25 | 1.5 | 2.2 | 1.6 | 0.6 | 1.4 |
| 14 | 20 + 250 | 1.8 | 1.9 | 0.6 | 0.5 | 1.9 |
| 15 | 20 + 1,000 | 2.1 | 2 | 1.4 | 2.4 | 1.6 |

Data are mean value of animals. The severity of histology grading is as follows: 0, normal, 1, minimal; 2, mild; 3, moderate; 4, severe. For tissue changes that are focal, locally extensive and multi focal, a score of less than an integer is assigned. The scores are: minimal focal, 0.25; minimal locally extensive, 0.50; minimal multi focal, 0.75; mild focal, 1.25; mild locally extensive, 1.50; mild multi focal, 1.75, etc.

Figure 1. Liver microsomal enzyme activities of rats exposed to TCDD, PCBs and combination of both (a denotes groups that are significantly different from vehicle control ( $p \leq 0.05$) , b- significantly different from the 250 ng TCDD group, and c- significantly different from the 1,000 ng TCDD group).