SUBCHRONIC (13-WEEK) TOXICITY OF A MIXTURE OF FOUR CHLORINATED DIBENZO-*P*-DIOXINS IN SPRAGUE-DAWLEY RATS

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1. Introduction

Chlorinated dibenzo-*p*-dioxins (CDDs) are environmental pollutants, which mostly occur as complex mixtures. It has been shown that single dose exposure to a well-defined mixture of CDDs results in additive toxicity in accordance with the relative potency of the individual components (1-3). Also a similar pattern of toxicity has been observed in subchronic studies with individual CDDs as after single dose exposure (4-7). Since human exposure to CDDs occurs mainly over prolonged periods of time it was important to extend this observation of additive toxicity to longer-term exposure settings.

The objectives of the present study were to evaluate the toxic potency and the spectrum of toxic effects of subchronic exposure to a mixture of four CDDs. The CDDs selected were four out of seven possible homologues with chlorine atoms at the critical 2,3,7,8-positions, which is a requirement for maximum toxicity. Doses were selected based on a previously conducted subchronic study with 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (HpCDD) (7). The relative potencies for the different CDDs in the mixture were derived from single dose toxicity data (1). The outcome of the study was expected to support or refute the currently used international toxicity equivalency factor (TEF) method (8, 9). Data presented here represent a preliminary report of the subchronic study with the mixture, and include only observations during and up to the end of the 13-week dosing period.

2. Materials and Methods

The test chemicals were as follows: 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (PCDD), 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin (HxCDD) and 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (HpCDD). The purities of the test chemicals were >98.5%.

Table 1. Experimental design and dose selection

Treatment		Dose (µg/kg)											
		Total Dose				Loading Dose (4 x)				Maintenance Dose (6 x)			
Group	Sex	TCDD	PCDD	HxCDD	HpCDD	TCDD	PCDD	HxCDD	HpCDD	TCDD	PCDD	HxCDD	HpCDD
1	F	0	0	0	0	0	0	0	0	0	0	0	0
Control	M	0	0	0	0	0	0	0	0	0	0	0	0
2	F	0.036	0.18	0.72	5.15	0.0027	0.017	0.082	0.68	0.0042	0.019	0.065	0.30
Mix (I)	M	0.054	0.27	1.08	7.72	0.0040	0.026	0.12	1.02	0.0063	0.028	0.098	0.40
3	F	0.43	2.16	8.64	61.7	0.033	0.21	0.99	8.15	0.050	0.22	0.78	3.65
Mix (II)	M	0.65	3.24	13.0	92.6	0.049	0.31	1.48	12.2	0.076	0.33	1.17	4.85
4	F	2.59	13.0	51.8	370	0.20	1.24	5.9	48.9	0.30	1.33	4.69	21.9
Mix (III)	M	3.89	19.4	77.8	556	0.29	1.87	8.9	73.3	0.45	2.0	7.04	29.1
5	F	7.78	38.9	155	1111	0.59	3.72	17.7	147	0.91	4.0	14.1	87.3
Mix (IV)	M	11.7	58.3	233	1667	0.88	5.58	26.7	220	1.36	6.0	21.1	131
6	F	11.7	58.3	233	1667	0.88	5.58	26.6	220	1.36	6.0	21.1	131
Mix (V)	M	17.5	87.5	350	2500	1.32	8.37	40.0	330	2.04	9.0	31.7	197
7	F	0	233	0	0	0	22.3	0	0	0	24.0	0	0
PCDD	M	0	350	0	0	0	33.5	0	0	0	36.0	0	0
8	F	0	0	933	0	0	0	107	0	0	0	84.4	0
HxCDD	M	0	0	1400	0	0	0	160	0	0	0	127	0

A total of 160 male and 160 female Sprague-Dawley rats, obtained from Sasco (Omaha, NE), were used in the study. Animals were acclimated to experimental conditions for two weeks before commencing with dosing. Rats were kept individually in stainless steel wire bottom cages (17.5 x 25.5 x 17.5 cm; Shoreline, Kansas City, MO), and given Purina 5001 rodent chow (Ralston Purina, St. Louis, MO) and deionized water *ad libitum*.

Rats were randomized by body weight and divided into eight experimental groups of 20 males and 20 females each. Half of the rats in each group was scheduled for necropsy at the end of the 13-week dosing period and the other half after an additional 13-week off-dose period. Only results from the 13-week dosing period will be reported here. The total dose was divided into four daily loading doses and six biweekly maintenance doses, which were calculated using liver half-lives of 20 days for TCDD (10), 31 days for PCDD, 44 days for HxCDD and 60 days for HpCDD (11). This loading / maintenance dosing regimen was used, because with compounds of very long half-lives it is impossible to achieve steady state body burdens during the course of a subchronic study. Dioxins were dissolved in corn oil (Sigma, St. Louis, MO) and administered by gavage at 4 ml/kg. Experimental groups and doses are given in Table 1. The highest dose of the mixture was selected such that the toxicity would be equal to the highest dose of HpCDD (total dose of 10000 µg/kg in males) in our previous subchronic study (7), with each of the four homologues contributing one fourth of the toxic potency to the mixture. For this purpose previously in single dose studies established TEFs (1) were used. To achieve equal toxicity in males and females, 1.5 times lower doses were given to the females. Since data for subchronic toxicity of HpCDD and TCDD have been generated in the previous study (7), PCDD and HxCDD were included as positive controls in this experiment.

3. Results and Discussion

During the 13-week dosing period mortality (unscheduled sacrifices due to treatment-induced moribund condition and deaths) was observed at the two highest doses of the mixture and in the PCDD and HxCDD positive control groups (Figure 1.). At the highest dose of the mixture the first death occurred on day 25/41 (females/males) of the study, and the mortalities were 60/50%, respectively. At the second highest dose of the mixture the first deaths were observed on days 64/67 with mortality of 10/5%. In the PCDD group the first rats died on days 17/22, and mortality at the end of the 13-week dosing period

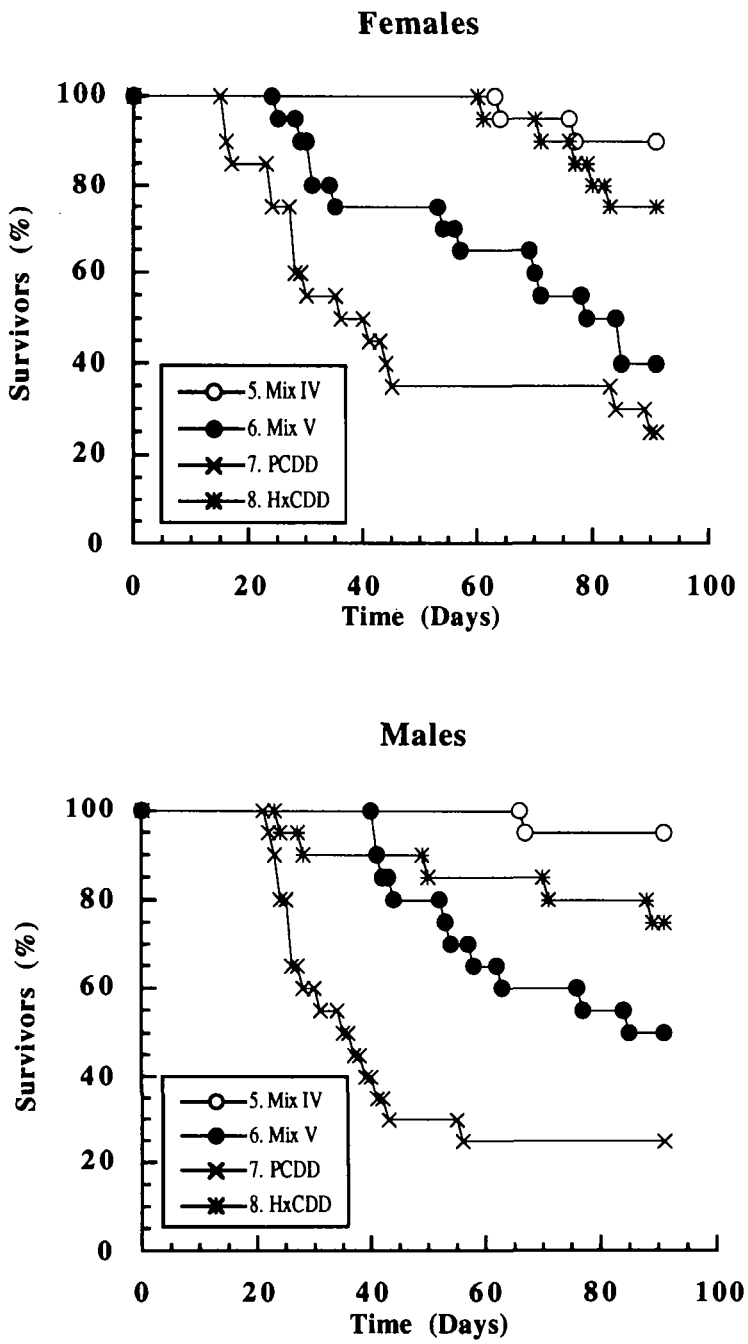

Figure 1. Time-course of mortality for female (upper panel) and male (lower panel) rats.

was 75% in both genders. HxCDD-treated rats started dying on day 61/24 with mortality of 25% in both genders at the end of the dosing period. Main causes of death were either wasting syndrome or hemorrhage mainly in the gastrointestinal tract without major body weight loss (or a combination thereof). In general, mortality caused by wasting alone occurred earlier, whereas hemorrhage caused deaths at later time points.

Mortality in the two highest dose groups of the mixture was exactly the same as in our subchronic study with HpCDD (7), strongly supporting the concept of additive toxicity. This was also the case for HxCDD, when compared to the potency of TCDD in the previous subchronic study and using a TEF of 0.05, derived from the single dose toxicity data (1). However, PCDD caused higher mortality than predicted by the previous subchronic and single dose studies, probably due to slight variability in the very steep portion of the dose-response curve for mortality.

Body weight gain was decreased at the two highest doses and in the two positive control groups beginning in the first week of the study (Figure 2). At week 13 rats in the highest and the second highest dosage groups of the mixture were 10.1/27.4% (females/males) and 7.0/21.2% below controls, respectively. The PCDD dosage group was 17.5/26.8%, and the HxCDD 6.6/23.9% below controls. Changes in body weight gain correlated well with mortality as generally observed in previous studies with CDDs.

Macroscopical changes observed at necropsies after treatment with the mixture were qualitatively the same as after treatment with single CDDs.

These preliminary data strongly support the validity of the TEF approach, which is currently used to estimate toxic potency of complex mixtures containing CDDs and other dioxin-like compounds (cf.8).

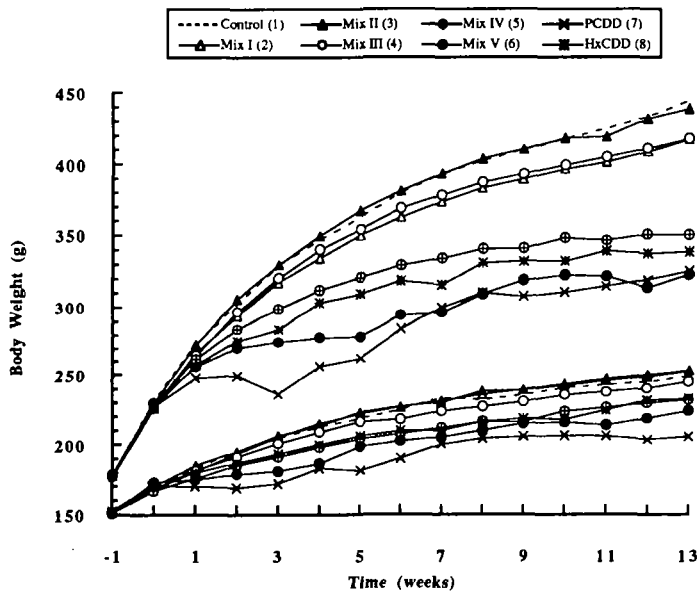

Figure 2. Mean body weights of male (upper set of curves) and female (lower set of curves) rats (n = 20-mortality) during the dosing period.

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