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TOXICOLOGICAL EVALUATION OF URBAN WASTE INCINERATOR EMISSIONS

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INTRODUCTION

The incineration of solid urban waste raises serious problems related to the presence of highly toxic micropollutants in the emissions of incinerating plants. In particular polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) mixtures have been found in these emissions (1-5). Assessment of the toxicological risk associated with these mixtures is a major, still unsolved problem. In fact only a few of the 75 PCDD isomers and 135 PCDF isomers have been studied as regards toxicological properties (6,7), and even scarcer are the data on the toxicological effects of their mixtures, most of them artificially built by joining some individual isomers (8-13).

In a first attempt to study the potential toxicological properties of a PCDD/PCDF mixture released from an urban incinerator, we carried out an experiment to characterize its immunodepressive and enzyme-inducing properties. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has been shown to be a very potent immunosuppressant in animals (14), single doses of 1 µg/kg depressing antibody production for more than 40 days (15). Little information is available on the immunosuppressive effects of 2,3,7,8-tetrachlorodibenzofuran (TCDF) in laboratory animals (16). Very recently TCDF was compared with TCDD for its toxicity on the immune system of mice (17). It is generally believed that these two compounds induce similar toxic effects (18) when 10-30 times more TCDF is given. We reported (17) that the humoral response is more sensitive than cell-mediated reactions (Graft versus Host reaction-mitogenic responsiveness) to single TCDF exposure and that the immunosuppression induced by TCDF recovers faster than that induced by TCDD, antibody induction being totally restored by day 40.

We thus chose to investigate the effects of extracts from an incinerator on humoral antibody production, using heterologous erythrocytes as antigen. An artificial mixture containing known amounts of TCDD and TCDF was prepared as reference control, to evaluate the interaction of the two toxic agents or their possible synergism.

TCDD is among the most potent mixed-function oxidase (MFO) inducing agents yet demonstrated in mammalian liver. Low dose studies in the rat gave an ED_{50} of TCDD induction of benzo(α) pyrene-hydroxylase of 0.63 µg/kg and the smallest dose of TCDD which significantly increased enzyme activity was 0.002 µg/kg (19). In mice (C57Bl/6j) an ED_{50} of 0.29 µg/kg TCDD for hepatic AHH induction was estimated (20).

TCDF is closely related to TCDD in structure and acute toxicity (18). Its biological activity (ED_{50} for AHH induction) is almost equivalent to TCDD in the chick embryo (21). In mice (C57B1/6j) and ED_{50} of 7 µg/kg TCDF was found (20) (AHH induction).

An important observation was that there is an excellent correlation between AHH induction and the toxic potency of certain PCDD and PCDF isomers (22). Despite the large amount of information about the single compounds none is yet available on the effect of a mixture of TCDD and TCDF on these parameters (P-450 content and hepatic MFO enzyme system). It was therefore deemed interesting to investigate the inducing properties of a mixture of the two compounds and of extracts from an urban waste incinerator, containing complex mixtures of PCDD and PCDF.

MATERIALS

Animals

C57B1/6j male mice where obtaine from Charles River, Calco (Italy) and used when 10 weeks old.

Chemicals

All chemicals involved in the extraction, purification and analysis of the PCDD and PCDF micture have been reported (5). TCDF was a kind gift from Dr. C. Rappe, University of Umea, Sweden.

EXPERIMENTAL

PCDD and PCDF analysis

The PCDD and PCDF mixture was drawn from a modern municipal incinerator in Italy, equipped with electrostatic precipitators. Gas condensate collection and the methods of extraction with n-hexane and of purification were as previously described (5). An LKB 2091 GC-MS equipped with an LKB 2130 computer was used for GC-MS analysis, in the reported conditions (5).

Animal treatment

After analysis, the sample (mixture 1) was dissolved in 10 ml of acetone-corn oil (1:6 v/v) and given as a single i.p. injection to mice (7-8 per group) as such or diluted 10 times (mixture 1/10), at a dose of 10 ml/kg. TCDD (1.2 µg/kg), TCDF (10 µg/kg) and the mixture of TCDD and TCDF (respectively 1.2 µg/kg and 10 µg/kg) were dissolved and given as described for mixture 1.

Antibody production assay

$4 \cdot 10^8$ sheep red blood cells were injected i.p. 7 days after treatment with the different toxic agents and plaque forming cells (PFC) in the spleen were counted 5 days later by the Yeme's technique (23).

Statistical analysis

Results are presented as means ± s.e.; the statistical significance was evaluated by Duncan's test.

Enzyme induction assay

Mice were killed by cervical dislocation and livers were immediately removed, rinsed in saline and blotted dry. The tissues were homogenized with an Ultra-Turrax apparatus in 0.05M phosphate buffer pH 7.4 (1:4 v/v) and centrifuged at 9000xg for 20 min in a refrigerated centrifuge. The supernatant fractions were stored at -70°C until used for cytochrome P-450, 7-ECD and protein assay. Cytochrome P-450 was determined according to Omura and Sato (24). 7-Ethoxycoumarin O-deethylase activity was assayed according to Greenlee and Poland (25). Proteins were measured according to Lowry et al. (26).

RESULTS

Analysis of incinerator extract

The amounts of individual classes of PCDD and PCDF employed for the experiment are listed in Table 1. They correspond approximately to the amount of micropollutants released in about 0.5 seconds or the amount obtained from about 0.5 kg of waste (27).

Table 1 - PCDD-PCDF dose administered (ng/kg), corresponding to mixture 1

	Tetra	Penta	Hexa	Hepta	Octa	Total
PCDD	3688 (17.5)[a]	4816 (22.9)	7152 (34.0)	4332 (20.6)	1064 (5.0)	21052
PCDF	8800 (35.3)	7696 (30.9)	4540 (18.2)	3004 (12.1)	880 (3.5)	24920

[a]In brackets the percentage of the individual classes of isomers.

Effect on antibody production

The effect of an artificial mixture of TCDD and TCDF at a ratio of about 1:8 was first investigated. As shown in Fig. 1, TCDD inhibited antibody production by 80% while TCDF at the dose used was not depressive. However, when the two toxic agents were administered together in the same solution, the degree of inhibition (50%) was significantly lower than that induced by TCDD alone (80%) when results are expressed either as PFC/10^6 splenocytes or as PFC/spleen. Thus, the presence of TCDF can modify the immunosuppressive capacity of TCDD. The simultaneous administration of 3.688 µg/kg tetrachlorodibenzodioxin and 8.8 µg/kg tetrachlorodibenzofuran (mixture 1) or of a dose 10 times lower (mix 1/10) did not significantly modify the immune response, antibody production being only 15% inhibited.

EFFECT OF SIMULTANEOUS ADMINISTRATION OF TCDD AND TCDF AND OF MIXTURES FROM INCINERATOR ON ANTIBODY PRODUCTION.

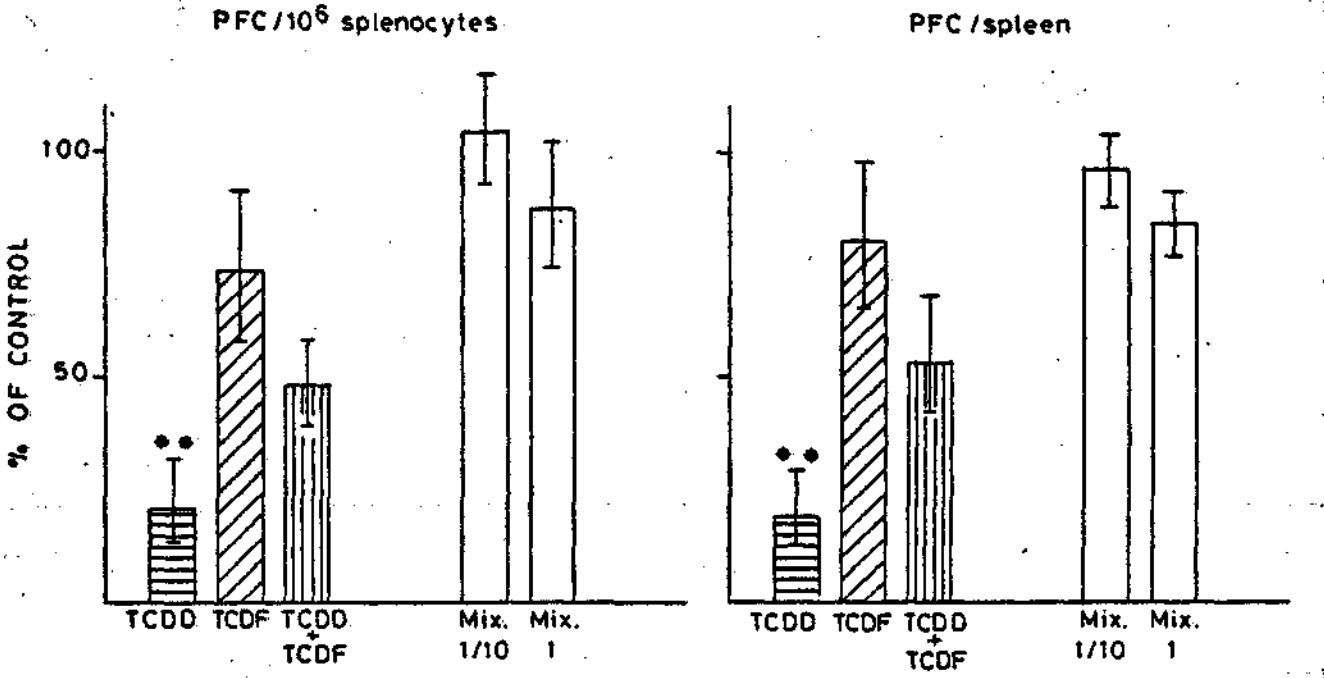

FIG. 1

TCDD (1.2 µg/kg), TCDF (10 µg/kg) and extracts from an incinerator were dissolved in acetone:corn oil (1:6 v/v) and given i.p. on day -7. $4 \cdot 10^8$ SRBC were given i.p. on day 0 and the test was done 5 days later.
Mix 1 = extract containing 3.7 and 8.8 µg/kg of tetrachloro-dibenzodioxin and -dibenzofuran respectively; see Table 1.
Mix 1/10 = dose one-tenth Mix 1

Effect on cytochrome P-450 content and on 7-etoxycoumarin-O-deethylase activity

Twelve days after a single dose of 1.2 µg/kg TCDD, cytochrome P-450 content was still about 3 times higher than in control mice. As expected, there was a shift of 2 nm in the peak absorption maximum (from 450 nm to 448 nm; see Table 2). This shift was also observed when TCDD was administered simultaneously with TCDF. No effect on maximum peak absorption was observed with TCDF alone. In this respect, no clear-cut response was seen when incinerator extracts were given.

Parallel to cytochrome P-450 induction, a ten-fold increase was observed in the activity of 7-etoxycoumarin-O-deethylase. TCDF at 10 µg/kg had no inducing effect.

When the same doses of chlorinated compounds were given in combination, an induction pattern similar to that caused by TCDD alone was observed; however the content of cytochrome P450 was only doubled in comparison to controls and was significantly lower ($p \leq 0.05$) than in mice treated with TCDD alone.

In the same treatment groups the activity of 7-ECD was 8 times higher than in control mice, but somewhat lower (1179 pmol/min/mg prot. versus 1315) than in TCDD treated animals.

The effect of an extract from urban incinerator smoke containing 3.3 and 8.8 µg/kg of tetrachloro-dibenzodioxins and dibenzofurans was then evaluated: cytochrome P-450 content

significantly raised and this inductive effect was more evident on 7-ECD activity. A ten times lower dose of incinerator extract had no effect on cytochrome P-450 or 7-ECD.

Table 2 - Effect of simultaneous administration of TCDD, TCDF and incinerator mixtures on cytochrome P-450 levels and 7-ethoxycoumarin-O-deethylase activity.

Treatment	Cytochrome P-450 or P-448[a] (nmol/mg prot)	7-Ethoxycoumarin-O-deethylase (pmol/min/mg prot)
Vehicle	0.63 ± 0.028[b] (6)[c]	143 ± 6 (5)
2,3,7,8 TCDD	1.87 ± 0.07** (6)	1315 ± 62 (6)**
2,3,7,8 TCDF	0.75 ± 0.04 (6)	173 ± 15 (6)
TCDD + TCDF	1.57 ± 0.18**+ (5)	1179 ± 98 (5)**
Mix 1	0.97 ± 0.06* (5)	352 ± 40 (6)**
Mix 1/10	0.82 ± 0.017 (6)	234 ± 16 (6)

** $p \leq 0.01$
* $p \leq 0.05$ from control group (Duncan's test)
+ $p \leq 0.05$ from mice treated with TCDD alone (Duncan's test)

[a] Cytochrome P-450 indicates the microsomal co-binding pigment found in control mice.
[b] Mean ± S.E.
[c] In parentheses the number of observations.
For treatment schedule refer to Fig. 1

CONCLUSIONS

Our preliminary results indicate that microsomal enzyme induction is a prompt, sensitive biological parameter of exposure to PCDD and PCDF, even when these compounds are in mixtures whose composition is not completely known.

The observation that the administration of a dose of TCDF inactive *per se* simultaneously with an active dose of TCDD reduced the immunosuppressive and enzyme inducing capacity of TCDD suggest a competitive effect at the receptor level. From this finding with a given TCDD:TCDF ratio, however, it is impossible to extrapolate the effects of different ratios or of higher active doses of TCDF. These unexpected biological effects illustrate the complexity of problems to be tackled in order to improve the prediction of toxic effects of mixtures of chemicals released simultaneously in the environment. For the time being, statements on the harmlessness to humans of urban waste incinerator emissions appear untimely (28).

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