# COMPARATIVE STUDY ON THE INDUCTIVE POTENCY OF TCDD AND TBrDD WITH THREE 2,3,7,8-MIXED-HALOGENATED DIOXINS IN LIVER MICROSOMES OF MALE RATS

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## ABSTRACT

Adult male rats were injected subcutaneously with single doses of 10 to 2000 pmoles TCDD, TBrDD or mixed-halogenated dioxins/kg body wt. The following congeners were tested: 2-bromo,3,7,8-trichloro-dibenzo-*p*-dioxin; 2,3-dibromo-7,8-dichlorodibenzo-*p*-dioxin; 2,3,7-tribromo-8-chlorodibenzo-*p*-dioxin. After 7 days the ethoxyresorufin O-deethylase was measured in the liver microsomes.

1. A similar induction of EROD was observed with TCDD as well as with equimolar doses of TBrDD.
2. The increase in enzyme activity (EROD) induced by 2-bromo,3,7,8-trichlorodibenzo-*p*-dioxin was identical to that observed after equimolar doses of 2,3,7,8-TCDD.
3. 2,3,7-Tribromo-8-chlorodibenzo-*p*-dioxin seems to possess a slightly lower inducing potency when compared with 2,3,7,8-TCDD.
4. For a final evaluation the tissue concentration/effect response will be analysed, however the prelimimary data presented here suggest a rather similar inductive potency of the five 2,3,7,8-tetrahalogenated dibenzodioxins investigated following a single injection.

## KEYWORDS

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin; 2,3,7,8-Tetrabromodibenzo-*p*-dioxin; 2-Bromo,3,7,8-trichlorodibenzo-*p*-dioxin; 2,3-dibromo-7,8-Dichlorodibenzo-*p*-dioxin; 2,3,7-Tribromo-8-chlorodibenzo-*p*-dioxin; Enzyme induction; Ethoxyresorufin O-deethylase, Wistar rats

## ABBREVIATIONS

**DMSO** = Dimethylsulfoxide; **EROD** = Ethoxyresorufin O-deethylase; **TCDD** = 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin; **TBrDD** = 2,3,7,8-Tetrabromodibenzo-*p*-dioxin; **B1C3** = 2-Bromo,3,7,8-trichlorodibenzo-*p*-dioxin; **B2C2** = 2,3-Dibromo-7,8-dichlorodibenzo-*p*-dioxin; **B3C1** = 2,3,7-Tribromo-8-chlorodibenzo-*p*-dioxin.

## INTRODUCTION

The exhaust of vehicles using leaded gasoline with organo-chlorine and -bromine scavengers contains traces of mixed chlorinated-brominated dioxins and furans (HUTZINGER et al., 1989). This exhaust is known to pollut the environment. The main toxicological concern is focused on the 2,3,7,8-substituted congeners. The aim of this study was the comparison of the potency of some 2,3,7,8-mixed, tetrahalogenated dibenzo-*p*-dioxins with the ability of TCDD and TBrDD to induce hepatic monooxygenases (EROD).

## MATERIAL AND METHODS

Animal maintenance and treatment

Male Wistar rats (Bor: Wisw/spf, TNO) weighing 350 - 450 g were purchased from Winkelmann (Borchen, FRG). During the experiment they were kept under conventional conditions at a constant day/night cycle (light from 9:00 to 21:00 h), at a temperature of 25 ± 1°C and 50% relative humidity. They received a standard pellet feed (Altromin[R] 1324) and water ad libitum.

$^{14}$C-TCDD supplied by Cambridge Isotope Laboratories (Woburn, USA) had a radiochemical purity of 97% and a specific activity of 33 mCi/mmol (according to the manufacturer). TBrDD was purchased from Promochem, Wesel, FRG (Lot Nr. MLB 13648-1), and had a chemical purity of 98% (according to the manufacturer). The synthesis of the mixed-halogenated dibenzo-*p*-dioxins has been described elsewhere (HOSSEINPOUR et al., 1989).

The substances were dissolved in a toluene/DMSO mixture (1+2; vol/vol) (ABRAHAM et al., 1989). The solutions were injected subcutaneously into the back of the animal at a volume of 0.2 ml/kg body wt using a 100 µl-Hamilton$^{R}$-syringe (Bonaduz, Switzerland). Single doses of 0.01, 0.033, 0.1, 0.333 and 2 nmoles of the above mentioned substances/kg body wt were administered. The control animals received only the vehicle.

Preparation of microsomes
The animals were starved overnight and the hepatic EROD activity was measured seven days after treatment. Samples of liver tissue were homogenized in a Potter-Elvejhem homogenizer using 4 ml 0.25 M sucrose per g liver. Following the centrifugation of the homogenate (at 9.500 x g for 20 min) the supernatant was filtered through gauze and centrifuged at 100.000 x g for 60 min. The pellet was washed in 100 mM Tris HCl buffer, pH=7.4, containing 150 mM KCl and centrifuged again at 100.000 x g for 60 min. The microsomal pellet was resuspended in 100 mM potassium phosphate buffer, pH = 7.4, containing 20% glycerol and 1 mM EDTA. The measurement of the EROD activity was performed immediately after microsome preparation.

Measurement of the EROD activity
The activity of the ethoxyresorufin O-deethylase was determined spectrofluorometrically in the liver microsomes using the method of Burke (BURKE et al., 1985) modified by using a NADPH-regenerating system with a final concentration of 2.5 mM magnesium chloride, 0.25 mM NADP, 5 mM glucose-6-phosphate and 0.5 units glucose-6-phosphate dehydrogenase per ml. The substrate concentration used was 5 µM ethoxyresorufin dissolved in 10 µl DMSO. The measurement of resorufin formation was performed with a spectrofluorometer (RF 540, Shimadzu, Kyoto, Japan) at 37°C and pH 7.8 (0.1 M potassium phosphate buffer). The fluorimeter settings were: excitation and emission wavelengths 550 nm and 585 nm; exication and emission slits 5 nm, respectively. The calibration of the fluorimeter was performed with resorufin.

## RESULTS

The potency for EROD induction in the liver was very similar for the five tetra-halogenated substances investigated. No clear-cut change in enzyme activity could be observed with any of the congeners following the injection of 33 pmoles/kg body wt (corresponding to 10 ng TCDD). With all five substances tested, a dose of 100 pmoles/kg body wt (corresponding to 32 ng TCDD/kg body wt) was clearly capable of inducing hepatic EROD activity one week after the s.c. injection (see Table 1 and Figure 1). Subsequent to an injection of 333 pmoles/kg body wt (corresponding to 100 ng TCDD/kg body wt) the increase in EROD activity was:

| | 333 pmoles | 2000 pmoles |
|---|---|---|
| TCDD | 26-fold | 112-fold |
| $Br_1Cl_1DD$ | 21-fold | 138-fold |
| TBrDD | 13-fold | 152-fold |
| $Br_2Cl_1DD$ | 13-fold | 57-fold |
| $Br_2Cl_2DD$ | 10-fold | 100-fold |

Compared with the other congeners tested, 2,3,7-tribromo-8-chlorodibenzo-*p*-dioxin seems to exhibit a slightly lower EROD-inducing potency.

## DISCUSSION

The presented data for TCDD and TBrDD were in good agreement with the findings of NAGAO et al. (1990) who found a similar EROD induction for both substances on a molar basis in female Wistar rats. However, the tissue concentrations of both compounds showed some differences when the levels of TCDD and TBrDD were measured in liver and fat over a three month period after application (ABRAHAM et al., 1988; NAGAO et al., 1990).

No toxicokinetic data are available for the mixed tetra-halogenated congeners up till now. Although these preliminary results suggest a quite similar potency for EROD induction for all investigated compounds, information on the toxicokinetics of the 2,3,7,8-mixed tetra-halogenated dioxins is necessary. Without assessing the elimination half-life it is not possible to predict the extent of accumulation during repeated-dose exposure nor to compare the potency for EROD induction under these conditions. Further studies are under way in our laboratory.


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## REFERENCES


Abraham K., Krowke R., Neubert D. (1988) Pharmacokinetics and biological activity of 2,3,7,8-tetra-chlorodibenzo-*p*-dioxin. 1. Dose-dependent tissues distribution and induction of hepatic ethoxyre-sorufin O-deethylase in rats following a single injection. Arch Toxicol 62: 359-369

Abraham K., Krowke R., Neubert D. (1989) Absorption of TCDD following parenteral application in rats using various vehicles. Chemosphere 19: 893-898

Burke M.D., Thompson S., Elcombe C.R., Halpert J., Haaparanta T., Mayer R.T. (1985) Ethoxy-, pentoxy- and benzyloxyphenoxazones and homologues: a series of substrates to distinguish between different induced cytochromes P450. Biochem Pharmacol 34: 3337-3345

Hosseinpour J., Schwind K.H., Thoma H. (1989) Brominated/chlorinated dibenzo-*p*-dioxins and furans: Synthesis of standards and detection in fly ash from a municipal waste incinerator. Chemosphere 19, 109-114

Hutzinger O., Essers U., Hagenmaier H. (1989) Untersuchungen zur Emission halogenierter Dibenzodioxine und Dibenzofurane aus Verbrennungsmotoren beim Betrieb mit handelsüblichen Kraftstoffen. FVV Informationstagung Motoren, Heft R 454

Nagao T., Neubert D., Löser E. (1990) Comparative studies on the induction of ethoxyresorufin O-deethylase by 2,3,7,8-TCDD and 2,3,7,8-TBrDD. Chemosphere: in press


**Table 1: Induction of EROD by Mixed-Halogenated Dioxins in Hepatic Microsomes of Male Rats**

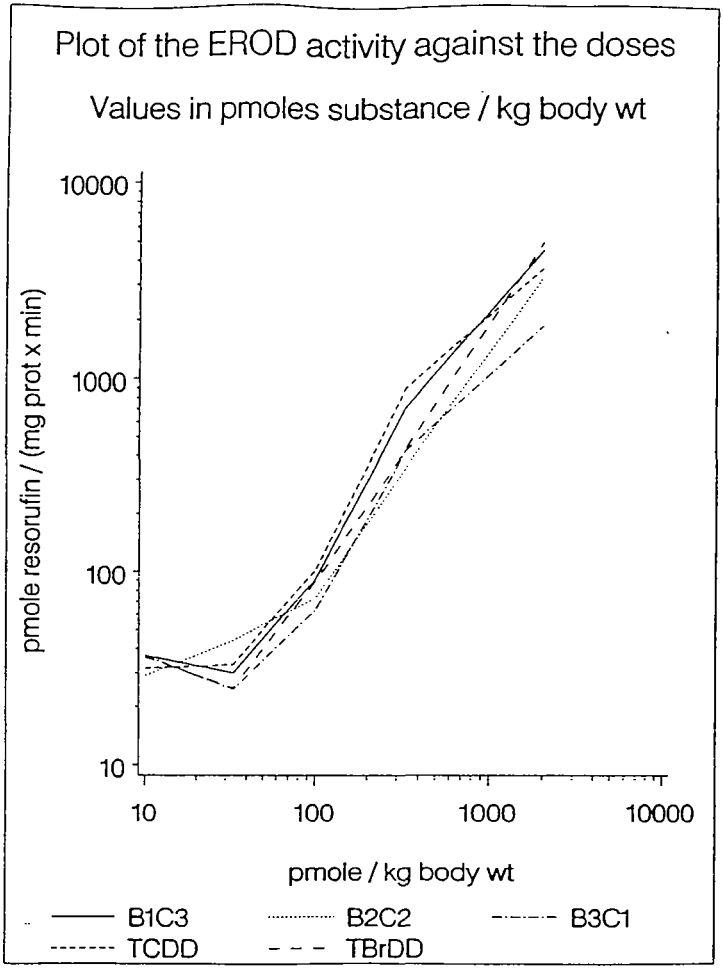
Control (n = 14) 33 ± 10

| | Inductor | | | | |
|---|---|---|---|---|---|
| Dose | TCDD | $Br_1Cl_3$ | $Br_2Cl_2$ | $Br_3Cl_1$ | TBrDD |
| 10 | 32 ± 6 | 37 ± 8 | 29 ± 9 | 37 ± 7 | 36 ± 7 |
| 33 | 33 ± 9 | 30 ± 3 | 44 ± 2 | 25 ± 3 | 25 ± 8 |
| 100 | 102 ± 39 | 91 ± 24 | 73 ± 7 | 63 ± 14 | 89 ± 11 |
| 333 | 887 ± 340 | 703 ± 41 | 343 ± 69 | 419 ± 91 | 428 ± 204 |
| 2000 | 3700 ± 860 | 4560 ± 221 | 3310 ± 534 | 1880 ± 633 | 5000 ± 1790 |

Dose in pmoles/kg body wt

EROD values (Mean ± SD; n = 3) in pmoles resorufin/(mg protein x min)

Figure 1:


Plot of the EROD activity against the doses
Values in pmoles substance / kg body wt
pmole resorufin / (mg prot x min)
10000
1000
100
10
10
100
1000
10000
pmole / kg body wt
B1C3
B2C2
B3C1
TCDD
TBrDD