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Myeloperoxidase-catalyzed formation of PCDD/F from chlorophenols

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Abstract

Chlorophenols (CP) are transformed in vitro to polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/F) by a biochemical-catalyzed oxidation. This is shown for 2,4,5-tri-, 2,3,4,6-tetra-and pentachlorophenol with myeloperoxidase recovered from human leucocytes in the presence of hydrogen peroxide. The yield, the reaction, and the PCDD/F-pattern found depend on the CP. The formation rates are in the μmol-per-mol range for all substrates.

The experiments confirm the suspicion that a biochemical formation of PCDD/F from precursors such as CPs can take place in the human body and that this metabolic pathway may lead to a higher inner exposure with PCDD/F than is now assumed.

1. Introduction

Several studies on humans and cows indicate a higher fecal excretion of PCDD/F, especially higher chlorinated PCDD, in relation to the dietary intake (Moser et al., 1996; Fries et al., 1996, 1997; Schrey et al., 1998). A biochemical formation of PCDD/F in the organisms from precursors such as chlorophenols (CP) would be one explanation of these findings. Biochemical formation of PCDD/F from chlorophenols have been observed in sewage sludge (Öberg et al., 1992), compost (Öberg et al., 1992, 1993) and in in vitro studies catalyzed by horseradish peroxidase (Svenson et al., 1989a, 1989b; Öberg et al., 1990; Wagner et al., 1990) or bovine lactoperoxidase (Öberg and Rappe, 1992).

Myeloperoxidase is a component of neutrophile granulocytes, a subgroup of the leucocytes in the human organism. The content of myeloperoxidase is up to $5\%_{\text{dry matter}}$ in the peripheral cells, whereas the content in growing granulocyte cells in the bone marrow can be higher.

In the present study we investigated the in vitro formation of PCDD/F from 2,4,5-trichlorophenol (2,4,5-TrCP), 2,3,4,6-tetrachlorophenol (2,3,4,6-TeCP) and pentachlorophenol (PCP) catalyzed by myeloperoxidase recovered from human leucocytes in the presence of hydrogen peroxide.

2. Material and methods

Myeloperoxidase recovered from human leucocytes and lyophylized from 0.02 M sodium acetate buffer at pH 6.0 (MPO; EC 1.11.1.7, M 6908/Lot 126H9402) was purchased from Sigma. 2,4,5-trichlorophenol (99.9% certified, C 177745/Lot 40615) and 2,3,4,6-tetrachlorophenol (97.2% certified, C 173746/Lot 59426) were obtained from Promochem (Wesel, Germany) and pentachlorophenol (certified reference material, P 16-23/Lot 127/Sn 1439) was from International Physical Laboratory. Methanolic solutions of the chlorophenols were made in concentrations of 2, 5 or 15 mg/ml.

All reactions were carried out in a potassium dihydrogen phosphate buffer (pH 5.4) with a total volume

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of 2.0 ml in 20 ml glass test tubes at 37°C. The incubation was started with the addition of hydrogen peroxide. The incubation time was 4 h in all experiments. The series were performed with two blank controls containing either the chlorophenol substrate or the myeloperoxidase while all other additions were left unchanged. The detailed list of composition is shown in Table 1.

After incubation 100 μl of a standard solution containing 17 $^{13}C_{12}$ labelled PCDD/F congeners (2.5 or 5.0 pg/μl) in toluene, 3 ml saturated ammonium sulphate solution and 3 ml ethanol were added. The samples were extracted three times with 4 ml of hexane. The dried hexane extracts were purified using standard methods including modified silicagels, alumina and activated charcoal. After addition of 2 μl of dodecane the cleaned extracts were evaporated to dryness using a nitrogen stream and 10 μl of toluene containing 2.5 pg/μl $^{13}C_{12}$ 1.2,3,4-TetraCDD were added as external standard.

The analytical instrument system consisted of a VG AutoSpec high-resolution mass spectrometer and a Hewlett Packard 5890 series II gas chromatograph equipped with a Gerstel KAS 2 vaporization system: *MS-parameters*: single ion recording mode; resolution 8000–10000 at 10%; electron impact ionization at 40 eV; perfluorokerosene lock mass check; observation of 2 ions each for native and labelled isomers; setting of 5 time windows; *GC-parameters*: column: J&W Scientific, DB-5, 60 m, 0.1 μm film thickness; temperature program: 180°C (3 min), 5°C/min, 220°C (16 min), 5°C/min, 235°C (7 min), 5°C/min, 280°C (15 min); injector program: 60°C (60 s), 12°C/s, 330°C (10 min), split off (1 min); split on (2 min); injection volume: 4 μl.

3. Results and discussion

The results of the PCDD/F homologue groups of major interest and the sum of the PCDD/F are given in Tables 2–5 for the experiments with 2,4,5-TrCP, 2,3,4,6-TeCP, PCP and the multisubstrate experiments.

The PCDD/F concentrations in the chlorophenol free blank (No. 0) was near the detection limit for all congeners and very small amounts were detected in the myeloperoxidase free blank samples (No. 1, 4 and 6). In all other samples a significant formation of PCDD/F was observed.

Table 1
List of experiments

Experiment number	KH_2PO_4 buffer [mM]	Myeloperoxidase [U*]	Chlorophenol [mM]	H_2O_2 [mM]
0 (CP – blank)	0.375	1.0	–	0.5
2,4,5-Trichlorophenol				
1 (blank)	0.375	–	1.0 (2,4,5-TrCP)	1.0
2	0.375	1.0	1.0 (2,4,5-TrCP)	1.0
3	0.375	1.0	0.1 (2,4,5-TrCP)	0.1
2,3,4,6-Tetrachlorophenol				
4 (blank)	0.375	–	1.0 (2,3,4,6-TeCP)	1.0
5	0.375	1.0	1.0 (2,3,4,6-TeCP)	1.0
Pentachlorophenol				
6 (blank)	0.375	–	1.0 (PCP)	1.0
7	0.375	1.0	0.1 (PCP)	0.1
8	0.375	1.0	1.0 (PCP)	0.5
9	0.375	1.0	0.1 (PCP)	1.0
10	0.375	1.0	1.0 (PCP)	1.0
11	0.375	1.0	1.0 (PCP)	2.0
Equimolar multisubstrate experiments				
12	0.375	1.0	1.0 (2,4,5-TrCP) 1.0 (PCP)	1.0
13	0.375	1.0	0.1 (2,4,5-TrCP)	0.1
14	0.375	1.0	1.0 (2,3,4,6-TeCP) 1.0 (PCP)	1.0
15	0.375	1.0	0.1 (2,3,4,6-TeCP) 0.1 (PCP)	0.1
16	0.375	1.0	1.0 (2,4,5-TrCP) 1.0 (2,3,4,6-TeCP) 1.0 (PCP)	1.0

* Units as specified by the manufacturer.

Table 2
Myeloperoxidase-catalyzed formation of PCDD/F from 2,4,5-Trichlorophenol

Experiment number		PentaCDD	HexaCDD	HeptaCDD	PentaCDF	HexaCDF	PCDD/F
1 (blank)	[pg]	<0.5	9.2	17	<0.5	1.9	83.2
2	[pg]	150	140	74	89	310	782
	[μmol/mol$_{TrCP}$]*	0.21	0.17	0.07	0.13	0.41	0.99
3	[pg]	24	30	7.4	18	53	138
	[μmol/mol$_{TrCP}$]*	0.34	0.27	0.00	0.26	0.68	1.55

* Blank (experiment 1) corrected values.

Table 3
Myeloperoxidase-catalyzed formation of PCDD/F from 2,3,4,6-Tetrachlorophenol

Experiment number		HexaCDD	HeptaCDD	OctaCDD	PCDD/F
4 (blank)	[pg]	17	220	65	1,320
5	[pg]	260	2200	1100	4610
	[μmol/mol$_{TeCP}$]*	0.31	2.33	1.13	3.77

* Blank (experiment 4) corrected values.

Table 4
Myeloperoxidase-catalyzed formation of PCDD/F from Pentachlorophenol

Experiment number	OctaCDD [pg]	PCDD/F [pg]	OCDD [μmol/mol$_{PCP}$]*	PCDD/F [μmol/mol$_{PCP}$]*
6 (blank)	140	155		
7	1600	1660	15.9	16.4
8	3500	3670	3.65	3.88
9	1600	1600	15.9	16.0
10	10,000	10,400	10.7	11.1
11	6100	6350	6.48	6.80

* Blank (experiment 6) corrected values.

Table 5
Myeloperoxidase-catalyzed formation of PCDD/F from chlorophenol mixtures

Experiment number		PentaCDD	HexaCDD	HeptaCDD	OctaCDD	HexaCDF	PCDD/F
12	[pg]	280	680	13000	1400	880	16000
	[μmol/mol$_{\Sigma CP}$]	0.20	0.43	7.64	0.76	0.59	9.70
13	[pg]	120	100	1300	140	200	1830
	[μmol/mol$_{\Sigma CP}$]	0.84	0.64	7.64	0.76	1.33	11.3
14	[pg]	12	150	3800	9700	450	14800
	[μmol/mol$_{\Sigma CP}$]	0.01	0.10	2.23	5.27	0.30	8.34
15	[pg]	0.29	7.0	84	310	29	492
	[μmol/mol$_{\Sigma CP}$]	0.00	0.04	0.49	1.69	0.19	2.78
16	[pg]	36	1100	3000	910	890	6760
	[μmol/mol$_{\Sigma CP}$]	0.02	0.47	1.18	0.33	0.40	2.70

The monosubstrate experiments with PCP showed a predominant formation of OctaCDD. The amount of OctaCDD increased with the amount of the substrate. But the highest formation rates were observed with the lower PCP concentrations (exp. 7 and 9), whereas the H_2O_2 concentration showed only influence on the PCDD/F formation with the higher dose (1 mM) (exp. 8, 10, 11) but not the lower dose (0.1 mM) (exp. 7 and 9) experiments.

The formation of PCDD/F was lower in the monosubstrate experiments with 2,4,5-TrCP and 2,3,4,6-TeCP and showed different homologue patterns. The main homologue groups were the HexaCDF, followed by HexaCDD, PentaCDD and PentaCDF (2,4,5-TrCP) or the HeptaCDD, OctaCDD and HexaCDD (2,3,4,6-TeCP). A comparison of the PCDD/F formation rates on a molar basis for the monosubstrate experiments with 1 mM concentrations (exp. 2, 5 and 10) is given in Fig. 1.

The combination of equimolar mixtures of 2,4,5-TrCP and PCP resulted in a formation of HeptaCDD as major component, whereas OctaCDD, followed by HeptaCDD were the main components in the experiments with equimolar mixtures of 2,3,4,6-TeCP and

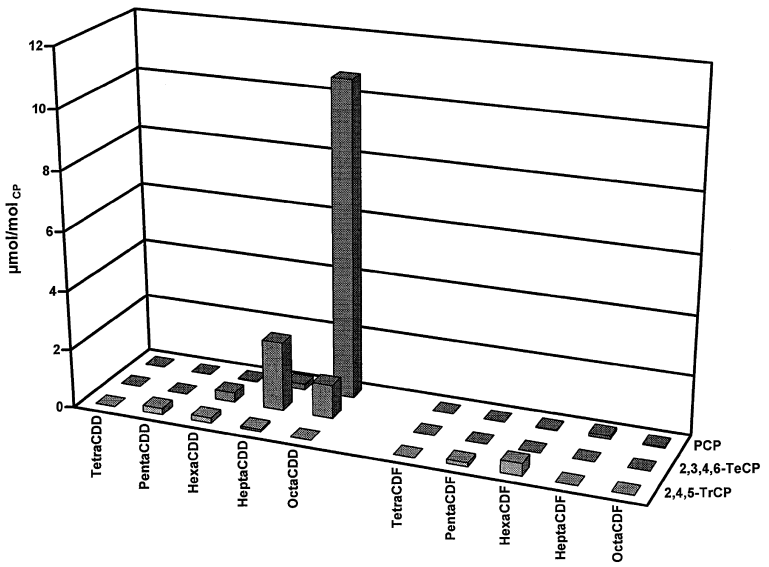

Fig. 1. PCDD/F formation rates of the 1 mM monosubstrate experiments 2, 5 and 10.

PCP. Hepta- and HexaCDD were the main components in the experiment containing all three chlorophenols. The results of the experiments 12, 14 and 16 are shown in Fig. 2.

A comparison of the experiments with studies of other authors is given in Table 6. All experiments with PCP gave OctaCDD as the main component. The formation rates in the studies with LP and HRP published by Öberg et al. (1990) and Öberg and Rappe (1992) were two to four times higher than in our own experiments with MYP showing 15.9 µmol/mol$_{PCP}$. The PCDD/F pattern found in the experiments with 2,4,5-TrCP is different between all authors. The observed differences could be an effect of the experimental conditions, especially the different peroxidase and hydrogen peroxide concentrations used. In addition the discrepancies may be attributed to different analytical procedures and to different quality grades of the CPs used.

First in vivo studies with rats fed with 0.1 mg/d of purified PCP over 14 days showed no higher PCDD/F

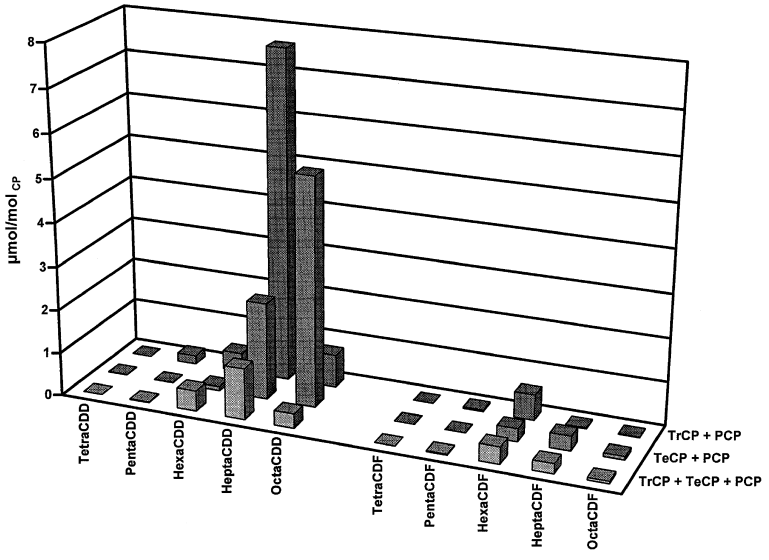

Fig. 2. PCDD/F formation rates of the multisubstrate experiments 12, 14 and 16.

Table 6
Comparison with data from literature[a]

Parameter		Öberg et al. (1990); (1992) LP	Öberg and Rappe HRP	Svenson et al. (1989b) MYP	This study HRP
Experimental conditions					
2,4,5-TrCP	[mM]	0.1	0.1	0.1	0.1
PCP	[mM]	0.1	0.1	–	0.1
Peroxidase	[U/ml]	1.3–1.7	1.3–1.7	1.3–1.7	0.5
H_2O_2	[mM]	0.2	0.2	9.0	0.1
*Formation of PCDD/F from 2,4,5-TriCP**					
PentaCDD	[μmol/mol_{TrCP}]	3.60	1.27	3.37	0.34
HexaCDD	[μmol/mol_{TrCP}]	1.01	0.86	5.88	0.27
PentaCDF	[μmol/mol_{TrCP}]	0.37	0.06	0.37	0.26
HexaCDF	[μmol/mol_{TrCP}]	0.04	0.03	0.75	0.68
Σ PCDD/F	[μmol/mol_{TrCP}]	5.73	2.43	10.5	1.55
Formation of PCDD/F from PCP					
OctaCDD	[μmol/mol_{PCP}]	34.8	57.9	–	15.9

[a] LP = lactoperoxidase, HRP = horseradish peroxidase, MYP = myeloperoxidase
* Wagner et al. (1990) found a formation of 5,7 $\mu g_{\Sigma PCDD/F}/g_{2,4,5\text{-}TrCP}$. The formation rate on a molar basis cannot be calculated due to missing rates for the several homologue groups.

levels in the liver in comparison to a control group. Administration of reagent grade PCP and technical grade PCP showed a formation of higher chlorinated PCDD (Feil and Tiernan, 1997). The main component was OctaCDD with levels 2.6 or 1042 times higher in comparison to the control group. In other experiments a formation of OctaCDD from nonachloro-2-phenoxyphenol, which is a known contaminant of technical PCP, was observed in rats (Huwe et al., 1998). Both studies confirm a formation of PCDD/F in vivo from PCP impurities.

All observed formation rates are in the μmol-per-mol range and so – at first glance – they seem to be of minor importance. But the estimated intake of pentachlorophenol of unexposed persons in Germany is in the range of 1–2 μg/d. Chlorophenols can also be significant metabolites of other chlorinated substances such as chlorobenzenes and chlorocyclohexanes. Typical serum concentrations of pentachlorophenol of unexposed persons from Germany are in the range of up to 20 μg/l and occupationally exposed individuals show CP concentrations 10 to 1000 times higher (Butte and Heinzow, 1995; Kommission Human-Biomonitoring des Umweltbundesamtes, 1997). On this background and under consideration of the observed formation rates in the in vitro experiments additional PCDD/F formations in the pg/d range would be expected.

It must be mentioned that the CPs used in the present study were of high purity and the influence of minor impurities of the CPs on the formation rate of PCDD/F was not examined. It can not be excluded that specific impurities are in particular responsible for the observed formations of PCDD/F.

4. Conclusion

The in vitro studies confirm the suspicion that a biochemical formation of PCDD/F from precursors such as chlorophenols can take place in the human body and that this metabolic pathway may lead to a higher inner exposure with PCDD/F as up to now estimated by food analyses or duplicate studies. Thus, part of the observed higher excretion rates of higher chlorinated PCDD/F in relation to the dietary intake found in the mass balances studies (Schrey et al., 1998, Moser et al., 1996) might be explained by peroxidase-catalyzed metabolic transformations of chlorophenols.

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