Myeloperoxidase-catalyzed formation of PCDD/F from chlorophenols

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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 [1-5]. A biochemical formation of PCDD/F in the organisms from precursors such as chlorophenols (CP) would be one explanation of these findings. Biochemical formations of PCDD/F from chlorophenols have been observed in sewage sludge [6], compost [6, 7] and in *in vitro* studies catalyzed by horseradish peroxidase [8-11] or bovine lactoperoxidase [10].

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) catalzed by myeloperoxidase recovered from human leucocytes in the presence of hydrogen peroxide.

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 (C 177745 / Lot 40615) and 2,3,4,6-tetrachlorophenol (C 173746 / Lot 59426) were obtained from Promochem (Wesel, Germany) and pentachlorophenol (P 16-23 / Lot 127 / Sn 1439) was from International Physical Laboratory. Methanolic solutions of the chlorophenols were made with 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 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 hours 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 $^{13}C_{12}$-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 8,000 - 10,000 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}.

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 (PCP)	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.

Results and discussion

The results of the PCDD/F homologue groups of major interest and the sum of the PCDD/F are given in the 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 to 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.

The monosubstrate experiments with PCP showed a predominant formation of OctaCDD. The amount of OctaCDD built 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 an influence on the PCDD/F formation in the higher dosed (1 mM) (exp. 8, 10, 11) but not in the lower dosed (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 figure 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 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 figure 2.

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 25 µg/l [12]. 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.

So the observed higher excretion rates of higher chlorinated PCDD/F in relation to the dietary intake found in the mass balances studies [1, 2] might be explained by peroxidase-catalyzed metabolic transformations of chlorophenols.

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	2,200	1,100	4,610
	[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	1,600	1,660	15.9	16.4
8	3,500	3,670	3.65	3.88
9	1,600	1,600	15.9	16.0
10	10,000	10,400	10.7	11.1
11	6,100	6,350	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	13,000	1,400	880	16,000
	[μmol/mol $_{\Sigma CP}$]	0.20	0.43	7.64	0.76	0.59	9.70
13	[pg]	120	100	1,300	140	200	1,830
	[μmol/mol $_{\Sigma CP}$]	0.84	0.64	7.64	0.76	1.33	11.3
14	[pg]	12	150	3,800	9,700	450	14,800
	[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	1,100	3,000	910	890	6,760
	[μmol/mol $_{\Sigma CP}$]	0.02	0.47	1.18	0.33	0.40	2.70

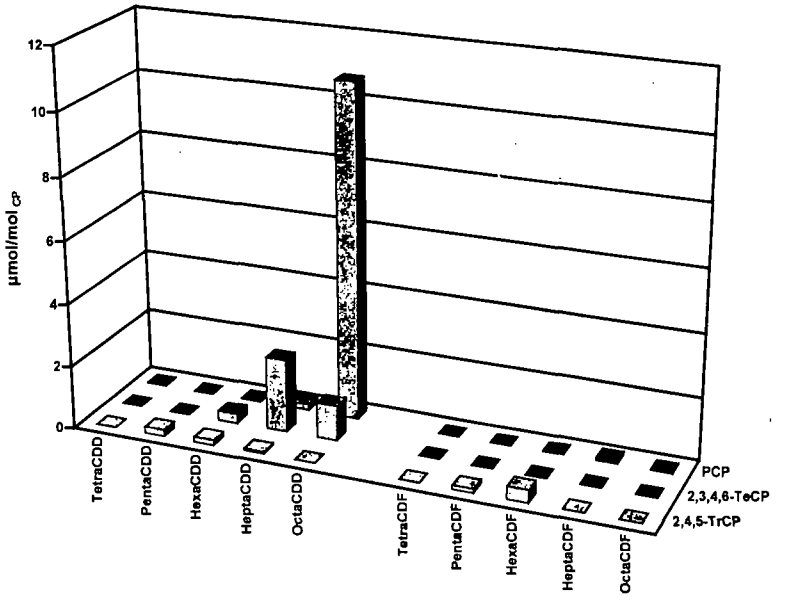

Figure 1: PCDD/F formation rates of the 1 mM monosubstrate experiments 2, 5 and 10

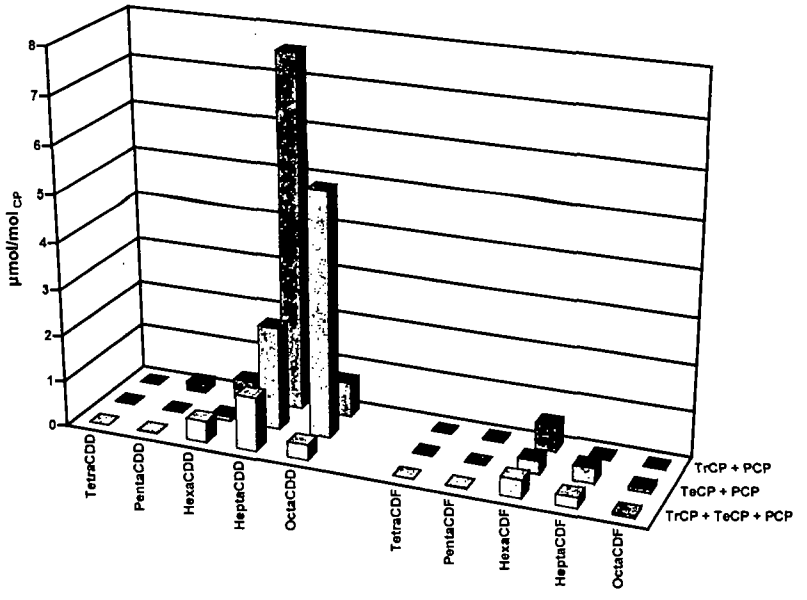

Figure 2: PCDD/F formation rates of the multisubstrate experiments 12, 14 and 16

Conclusion

A myeloperoxidase-catalyzed formation of PCDD/F in the presence of hydrogen peroxide in a µmol-per-mol range was observed for 2,4,5-trichlorophenol, 2,3,4,6-tetrachlorophenol and pentachlorophenol and different equimolar mixtures of these chlorophenols. The PCDD/F formed showed different homologue patterns and the formation rates increased with the grade of chlorination of the examinded chlorophenols.

These *in vitro* studies confirm the suspicion that a biochemical formation of PCDD/F from precursurs 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.

References

[1] Schrey, P.; Wittsiepe, J.; Mackrodt, P.; Selenka, F., Human fecal PCDD/F-excretion exceeds the dietary intake, Organohalogen Compounds, 30, 51-56 (1996)

[2] Schrey, P.; Wittsiepe, J.; Mackrodt, P.; Selenka, F., Human fecal PCDD/F-excretion exceeds the dietary intake, Chemosphere, (1998 (in press))

[3] Moser, G. A.; Schlummer, M.; McLachlan, M. S., Human absorption of PCDDs, PCDFs, and PCBs from food, Organohalogen Compounds, 29, 385-388 (1996)

[4] Fries, G. F.; Paustenbach, D. J.; Wenning, R. J.; Mathur, D. B.; Luksemburg, W. J., Transport of chlorinated dioxin and furan contaminants in pentachlorophenol-treated wood to milk and adipose tissue of dairy cattle, Organohalogen Compounds, 29, 447-452 (1996)

[5] Fries, G. F.; Dawson, T. E.; Paustenbach, D. J.; Mathur, D. B.; Luksemburg, W. J., Biosynthesis of hepta- and octa-chlorodioxins in cattle and evidence for lack of involvement by rumen Microorganisms, Organohalogen Compounds, 33, 296-301 (1997)

[6] Öberg, L. G.; Andersson, R.; Rappe, C., De novo formation of hepta- and octachlorodibenzo-p-dioxins from pentachlorophenol in municipal sewage sludge, Organohalogen Compounds, 9, 351-354 (1992)

[7] Öberg, L. G.; Wagman, N.; Andersson, R.; Rappe, C., De novo formation of PCDD/Fs in compost and sewage sludge - a status report, Organohalogen Compounds, 11, 297-302 (1993)

[8] Svenson, A.; Kjeller, L.-O.; Rappe, C., Enzymatic chlorophenol oxidation as a means of chlorinated dioxin and dibenzofuran formation, Chemosphere, 19, (1-6), 585-588 (1989)

[9] Svenson, A.; Kjeller, L.-O.; Rappe, C., Enzyme-mediated formation of 2,3,7,8-tetrasubstituted chlorinated dibenzodioxins and dibenzofurans, Environ. Sci. Technol., 23, (7), 900-902 (1989)

[10] Öberg, L. G.; Glas, B.; Swanson, S. E.; Rappe, C.; Paul, K. G., Peroxidase-catalyzed oxidation of chlorophenols to polychlorinated dibenzo-p-dioxins and dibenzofurans, Arch. Environ. Contam. Toxicol., 19, 930-938 (1990)

[11] Wagner, H. C.; Schramm, K. W.; Hutzinger, O., Biogenic polychlorinated dioxin and furan from trichlorophenol, Organohalogen Compounds, 3, 453-456 (1990)

[12] Kommission Human-Biomonitoring des Umweltbundesamtes, Stoffmonographie Pentachlorphenol - Referenz- und Human-Biomonitoring-Werte (HBM), Bundesgesundhbl., 6, 212-222 (1997)