Isomer-Specific Analysis of Polychlorinated Naphthalenes in Kanechlor KC 400, Yusho Rice Oil, and Adipose Tissue of a Yusho Victim

Peter Haglund[A], Eva Jakobsson[B], and Yoshito Masuda[C]

A) Institute of Environmental Chemistry, Umeå University, S- 901 87 Umeå, Sweden
B) Environmental Chemistry, Stockholm University, Wallenberg Laboratory, S- 106 91 Stockholm, Sweden.
C) Daiichi College of Pharmaceutical Sciences, 22-1 Tamagawa-cho, Minami-ku, Fukuoka 815, Japan.

1. Introduction

In February to August 1968, more than 1200 persons in south-west Japan were intoxicated by consuming a commercial rice oil (Yusho rice oil) contaminated with 1000 ppm of a technical PCB (Kanechlor KC 400). The clinical manifestation of this poisoning included chlorachne, ocular damage, diverse subjective complains associated with neurological damage, liver damage, and immunological effects [1]. Moreover, children born to affected patients showed retarded growth, abnormal tooth development, and were undersized [2]. Originally it was assumed that the toxic effects were caused solely by the PCBs in Kanechlor KC 400. Subsequent analysis revealed several toxic polychlorinated dibenzofurans (PCDFs), polychlorinated quaterphenyls (PCQs), and polychlorinated quaterphenyl ethers (PCQEs) as trace contaminants in the causal oil, and in the Kanechlor KC 400 [3]. A comparative toxicological evaluation of these chemical groups in Yusho patients adipose tissue based on the TCDD (2378-tetrachlorodibenzo-p-dioxin) toxic equivalency factor (TEF) concept indicated that 3,3',4,4',5-pentachlorobiphenyl, 2,3,4,7,8-penta-, and 1,2,3,4,7,8-hexachlorodibenzofuran accounted for the highest toxic contribution.

Polychlorinated naphthalenes (PCNs) is a closely related group of polychlorinated aromatic compounds with "dioxin-like" toxicity. Recently, several Aroclor and Clophen formulations have been analysed for PCNs [4]. All products were found to contain PCNs. The levels ranged between 2 and 900 μg/g. PCNs has also been found in Kanechlor KC 400 - but no quantification has been performed [5,6].

In the present investigation we have used a gas chromatography/ mass-spectrometry (GC/MS) technique for the isomer-specific analysis of PCNs in Kanechlor KC 400, Yusho rice oil, a Yusho victim (adipose and liver tissue), and two control persons (adipose and liver).

2. Experimental

2.1 Materials analysed

The following samples were analysed for PCNs:

- Kanechlor KC 400 (ca. 10mg).
- Yusho rice oil (ca. 10g).
- Mesentery adipose tissue and liver tissue (ca. 10g each) from a 59 year old male, diagnosed as having grade II Yusho, who died in March 1977 from respiratory problems.
- Mesentery adipose tissue and liver tissue (ca. 10g each) from two control persons from Fukuoka.

2.2 Sample extraction and clean-up

The adipose and liver tissue samples were grinded with sodium sulphate, packed into a glass column, and were extracted with 500 ml acetone: n-hexane (2.5: 1) and 500 ml n-hexane: diethyl ether (9: 1) [7,8]. The samples were fortified with an internal standard, 1,5-dibromonaphthalene (98 ng), and the solvent was carefully evaporated. The lipid content of the samples were determined gravimetrically.

Dialysis through polyethane film was used to remove the bulk of lipids [9,10]. The lipid extracts were reconstituted in an appropriate volume of cyclopentane (5-10 ml) and were quantitatively transferred to pre-rinsed polyethane tubes. The dialysis tubes were immersed into 150 ml cyclopentane. Next day the cyclopentane was removed and 150 ml fresh solvent was added. The third day the process was repeated. After completion of the dialysis process the cyclopentane phases were combined and the solvent was evaporated.

Lipid residues and other hydrolysable or polar compounds were removed from all of the samples by passing them through a multi-layer column packed with (from top to bottom): sodium sulphate, silica, potassium hydroxide/silica, silica, sulphuric acid/silica, and silica. n-Hexane was used as the mobile phase.

Finally, the PCNs were separated from the PCBs and PCDFs using high-performance gel permeation chromatography (HR-GPC) as described elsewhere [4]. With tetrahydrofuran (THF) as mobile phase PCNs are eluting a few minutes later than PCBs and PCDFs. However, the HR-GPC column (PL Gel, 8 x 600 mm, styrene-divinylbenzene copolymer, 5 μm particle size, 50 A pore size) was severely overloaded because of the high PCB levels, and the fractionation had to be repeated twice to obtain a pure PCN fraction. After evaporation and solvent exchange to iso-octane the samples were ready for analysis by GC/MS.

2.3 GC/MS analysis

The samples were analysed using a capillary gas chromatograph directly connected to an Finnigan TSQ (triple stage quadrupole) system. Injections of 2 μl sample aliquots were performed in the split-less mode and the oven was temperature programmed as follows: 80°C (2 min)- 20°C/min- 200°C- 4°C/min- 300°C, hold for 20 min. Isomer separation was achieved on a 60 m x 0.25 mm

(0.25 μm film thickness) Restec RTX-5 (5% phenyl-dimethylpolysiloxane) fused silica capillary column. Helium was used as carrier gas at a head pressure of 24 psi.

The ions source was operated under electron impact (EI) conditions at 70 eV, and the MS was tuned in the selected ion monitoring (SIM) mode. Three ions was monitored for each PCN homologue, specifically M^+, $(M+2)^+$, and $(M+4)^+$. Separate SIM descriptors and time of elution windows was used for each homologue level. The quantification was based on the sum of peak areas of the three channels, and the results were directly corrected for recoveries by using the internal standard (1,5-dibromonaphthalene).

3. Results

PCNs were only found in the Kanechlor KC 400, Yusho rice oil, and Yusho adipose tissue samples. In all other samples the levels were below the detection limit. The results of the isomer-specific analyses are summarised in Table 1. In Figure 1 the data is presented graphically as congener profiles.

The Kanechlor KC 400 sample was found to contain tetra- (TeCNs) to hepta- (HpCNs) chlorinated naphthalenes. The penta- (PeCNs) and hexachloronaphthalenes (HxCNs) accounted for the highest percentage of the total (58 % and 26 %, respectively). The total PCN concentration in the Kanechlor sample was 635 μg/g. Thus, the levels of PCNs in Kanechlor are comparable with the levels found Aroclor and Clophen formulations (2 to 900 μg/g) [4].

In the Yusho rice oil roughly the same PCN species were detected as in the Kanechlor. However, the congener profile is slightly more dominated by the highly chlorinated congeners. Once again the PeCNs and HxCNs dominates (42 % and 45 % of the total, respectively). The total PCN concentration in the rice oil sample was 2.6 μg/g. This is about one fourth the reported concentration of total PCDFs [11].

In the adipose sample from the Yusho patient a completely different pattern appears. Here, mainly TeCNs are found (45 % of the total) while only one PeCN and one HxCN peak could be detected (11 % and 43 % of the total, respectively). The latter corresponds to 12357/12467-PeCN and 123467/123567-HxCN, congeners known to be resistant to metabolism. As a result of the stability these congeners are ubiquitous in the environment and are found at high levels in fish and wildlife world-wide. Interestingly, the major TeCNs includes several congeners with vicinal hydrogens, which usually are easily metabolised [12,13]. This ambiguity is difficult to explain, but one possibility is that there are different main sources of TeCNs, and PeCNs/ HxCNs. The most probable source of the penta and hexa congeners would then be the Yusho rice oil, while the TeCNs may originate from other sources, such as: technical PCN or PCB mixtures with low chlorine content. Since the adipose tissue from the control persons did not contain any detectable quantities of PCNs it is not likely that the TeCNs appears as a result of the general back-ground pollution, but rather because of an individual exposure.

The total PCN concentration in the adipose tissue sample was 1.4 ng/g lipids. This is about one fifth the reported concentration of total PCDFs [14]. Furthermore, the toxic equivalency factor (EROD and AHH induction) for the PCNs tested, viz. 1,2,3,5,6,7-, 1,2,3,4,5,7-, 1,2,3,5,6,8-, 1,2,4,5,6,8-, 1,2,3,5,7,8-HxCN, and 1,2,3,4,5,6,7-HpCN, are about three orders of magnitude less than the 2,3,4,7,8-PCDF. Thus, the toxic contribution of the PCNs to the total dioxin-like toxicity is probably less than of the PCDFs and coplanar PCBs. It has to emphasised that the majority of PCN congeners have not been tested so far. Furthermore, the TEF concept only account for dioxin-like toxicity - and not any other type of toxicity. It is thus not possible today

Species	Kanechlor (μg/g)	Yusho rice oil (ng/g)	Yusho adipose, (pg/g lipid)
146		2.6	
1357		2.1	
1257/1246/1247	1.2	24	75
1467	1.4	11	
1368/ 1256	0.86	2.2	
1235/ 1358	2.5	8.8	99
1237/1234/1267	0.4	1.8	
1245	0.33	1.5	
1248	1.8	2.5	64
1258/ 1268	8.4	19	296
1458	2.8	3.0	97
1278	0.5	0.64	
12357/ 12467	67	177	165
12457	4.4	26	
12468	39	115	
12348	19	59	
12356	30	53	
12367	1.1	9.8	
12456	34	159	
12478	48	171	
12358/ 12368	52	165	
12458	61	140	
12345	8.6	19	
12378	4.2	19	
123467/ 123567	30	190	605
123457	22	176	
123568	40	287	
124568/ 124578	46	268	
123578	16	157	
123456	13	98	
1234567	33	107	
1234568	47	146	
Total PCN	**635**	2620	1401

Table 1: Levels of PCNs in Kanechlor, Yusho rice oil, and adipose tissue of a Yusho victim.

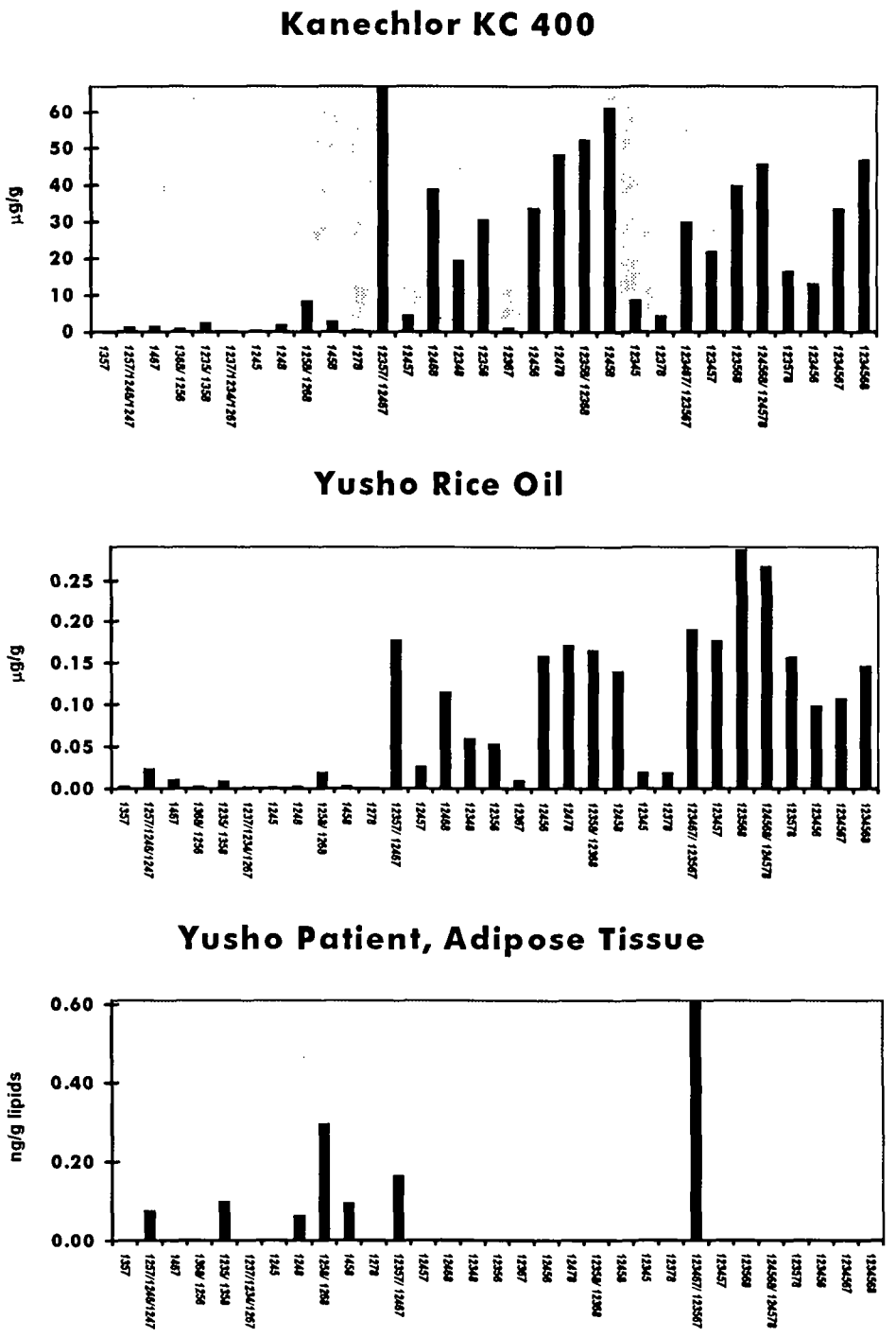

Figure 1: PCN congener profiles for Kanechlor KC 400, Yusho rice oil, and Yusho patient adipose.

to come to any definite conclusion regarding the toxic impact of the PCNs on the Yusho victims. It is however clear that PCNs are present as trace contaminant in the Yusho rice oil and adipose tissue from the Yusho patient, at levels comparable to those of PCDFs.

4. Conclusions

Analysis of Kanechlor KC 400, Yusho rice oil, and adipose and liver tissue from a Yusho victim and two control persons has shown that PCNs are present as contaminants in both the Kanechlor and the causal rice oil. The PCNs have also been taken up by the Yusho victim and are found in the adipose tissue at levels comparable with those of the PCDFs. The isomer-specific results indicate that the Yusho victim has been able to metabolise the majority of the PCN congeners. Only a few highly chlorinated PCNs have been resistant to metabolism and are found in the adipose tissue, viz. 1,2,3,5,7- and/or 1,2,4,6,7- PeCN and 1,2,3,4,6,7- and/or 1,2,3,5,6,7-HxCN. These congeners are also the most stable in fish and wildlife.

5. References

1) Kuratsune M., Yoshimura T., Matsuzaka J., and Yamaguchi A. (1972): Environ. Health Perspect. 1, 119.

2) Kuratsune M. (1980), In: Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins, and Related Products, ed. Kimborough R.D., Elsevier/ North- Holland, Amsterdam, pp. 287-302.

3) Rappe C. and Buser H-R. (1980), In: Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins, and Related Products, ed. Kimborough R.D., Elsevier/ North-Holland, Amsterdam, pp. 287-302.

4) Haglund P., Jakobsson E., Asplund L., Athanasiadou M., and Bergman Å. (1993): J. Chromatogr. 634, 79-86.

5) Miyata H., Kashimoto T., and Kunita N. (1977): J. Food Hyg. Soc. 18, 260-265.

6) Buser H.R., Rappe C., and Garå A. (1978): Chemosphere 5, 439-449.

7) Jensen S., Reutergårdh L., and Jansson B. (1983): FAO Fisf Tech. Pap. 212, 21.

8) Smith L.H., Stalling D.L., and Johnson J.L. (1984): Anal. Chem. 56, 1830-1842.

9) Huckins J.N., Tubergen M.W., Lebo J.A. Gale R.W., and Schwartz T.R. (1990): J. Assoc. Off. Anal. Chem. 73, 290-293.

10) Bergqvist P.-A., Strandberg B., and Rappe C. (1993): Anal. Proc. 30, 404-405..

11) Tanabe S., Kannan N., Wakimoto T., Tatsukawa R., Okamoto T., and Masuda Y. (1989): Toxicol. Environ. Chem. 24, 215-231.

12) Asplund L., Jansson B., Sundström G., Brandt I., and Brinkman U.A.Th. (1986): Chemosphere 15, 619-628.

13) Järnberg U., Asplund L., de Wit C., Grafström A.-K., Haglund P., Jansson B., Lexen K., Strandell M., Olsson M., and Jonsson B. (1993): Environ. Sci. Technol. 27, 1364-1374.

14) Tanabe S., Kannan N., Wakimoto T., Tatsukawa R., Okamoto T., and Masuda Y. (1989): Toxicol. Environ. Chem. 24, 215-231.