Toxicity of PCDD/DFs and Co-planar PCBs in Yu-Cheng Rice Oil

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1. Introduction

Yu-Cheng (rice oil disease) outbreak, involving about 2000 people, occurred in central Taiwan at 1979. This epidemic syndrome was caused by the ingestion of contaminated rice oil. The victims showed signs of chlorance, hyperpigmentation, dilatation and hypersecretion of conjunctiva glands [1]. Extensive follow-up medical investigations were carried out to clarify the cause and to evaluate the toxicity upon human [2]. Few studies about the composition of the contaminated rice oil were reported. The results were reported as total PCBs (polychlorinated biphenyls), PCDFs (polychlorinated dibenzofurans) by level of chlorination, and PCQs (polychlorinated quaterphenyls) [3-5]. This is mainly because the local court house considered the contaminated oil as important evidence and restricted its accessibility to the public soon after the outbreak. The oil was send abroad at 1992 for final disposal. Currently, the observed toxicity are mostly come from serum and tissue analyses of patients and their offspring. The contributions of the relative toxicity of PCDD/DFs and Co-planar PCBs to the observed toxicity are still unclear. This report aims to provide the needed analytical information using various analytical means to address this issue. They include: isotope-dilution GC/MS analysis of PCBs by level of chlorination and of co-planar PCBs (i.e., PCB#77, #126, and #169), isotope-dilution GC/MS analysis of total PCDDs (polychlorinated dibenzo-*p*-dioxins) and PCDFs, 2,3,7,8 substituted PCDD/DFs, and their TEQ (toxicity equivalency) values.

2. Experimental

The rice oil sample was positive for contamination and obtained from the suspected oil store. Internal standards (nine $^{13}C_{12}$-PCBs, five $^{13}C_{12}$-PCDDs) were spiked into 5 g of rice oil sample. The spiked sample was saponified in 20 ml of 0.5 N ethanolic KOH solution for 2 hr at room temperature. The saponified sample was added with 40 ml of deionized water and then extracted with 20 ml of n-hexane. The aqueous layer was extracted three times with the same amount of n-hexane. The collected n-hexane extract was concentrated to 5 ml and precleaned with an anhydrous Na_2SO_4(2 cm)|silica gel (2 g) 40% (w/w) H_2SO_4-silica gel (5 g)|silica gel(1 g)|30% (w/w) NaOH-silica gel(2 g)| 1 g silica gel|10% (w/w) $AgNO_3$-silica gel (2 g)|silanized glass wool column eluted with 100 ml n-hexane. The eluate was concentrated to 5 ml and chromatographed into two fractions with successive eluents of 30 ml of 2% dichloromethane (DCM) in n-hexane and 30 ml of 60% DCM in n-hexane on an alumina column preluted with 25 ml of n-hexane. The first eluate containing PCBs was concentrated to 5 ml. An aliquot was diluted 10 times and subjected to GC/ECD analysis to obtain the PCBs

pattern. The remaining aliquot was N_2-purged to near dryness, added with 100 µl of 5 µg/ml dibromooctafluoro biphenyl (DBOFB) as recovery standard and subjected to GC/MS analysis to determine PCBs by level of chlorination. The second eluate containing PCDD/DFs and Co-PCBs was concentrated to 5 ml. The concentrate was chromatographed into two fractions with successive eluents of 4-ml n-hexane | 1-ml 50:50 (w/w) cyclohexane/DCM | 1-ml 75:20:5 (w/w) DCM/methanol/toluene, and 20 ml toluene on an active carbon column. The toluene eluate containing PCDD/DFs was N_2-purged to near dryness, added with 50 µl of recovery standard (six $^{13}C_{12}$-PCDDs and nine $^{13}C_{12}$-PCDFs), and subjected to GC/MS analysis to determine total PCDD/DFs and 2,3,7,8-PCDD/DFs. The other eluate containing Co-PCBs was N_2-purged to near dryness, added with 100 µl of 5 µg/ml DBOFB as recovery standard and subjected to GC/MS analysis to determine PCB#77, 126, and 169.

3. Results and Discussion

The PCBs pattern in the rice oil (Fig. 1a) is similar to that in a 1:1 mixture of KC-400 and KC-500 (Fig. 1b). Therefore, traditional means of analyzing total PCBs based on the PCBs mixture could not generate correct values. The level of PCBs in this rice oil determined using GC/MS is thus more desirable and is about 176 µg/g. Co-PCBs consist mainly of PCB#77 (99.8 %) and trace amounts of PCB#126 (0.18 %) and PCB#169 (0.02%). They contribute to about 0.73 % to the total PCBs.

The concentrations of total PCBs, Co-PCBs, total PCDD/DFs, and 2,3,7,8,-substituted PCDD/DFs and the TEQs are summarized in Table 1. PCDFs is the main contaminating source as the congener profiles shown in Fig. 2. This fact indicates that PCBs is the original contaminating source, in accordance with the works of Vuceta [6], Cull [7], and Miyata[8]. Most TEQ is contributed by 2,3,7,8,-TCDF, 2,3,4,7,8-PeCDF, and 1,2,3,4,7,8-HxCDF. TEQ contributions from the PCDDs, PCDFs, and Co-PCBs are shown in Fig. 3. PCDFs contributes the most (53 %), followed by Co-PCBs (35 %), and PCDDs (12 %) to the toxicity in the contaminated rice oil.

4. Conclusions

The contributions of the relative toxicity of PCDD/DFs and Co-planar PCBs to the observed toxicity in Yu-Cheng rice oil are determined using various analytical means. The contaminating source appears to be a 1:1 mixture of KC-400 and KC-500. Total PCBs is 176 µg/g. TEQ contributions from the PCDDs, PCDFs, and Co-PCBs are 53 %, 35 %, and 12 %, respectively. The link between the level of these toxic chemicals in the contaminated rice oil and to those in the victims is under further investigation.

5. Acknowledgments

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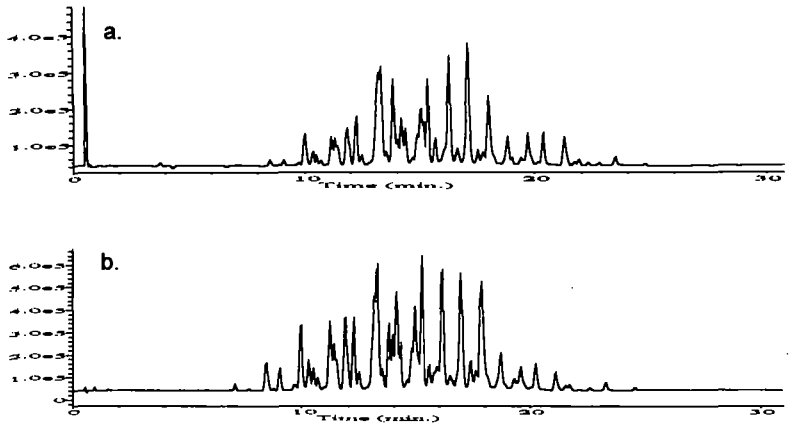

Figure 1. PCBs Patterns in (a) rice oil, and (b) mixture of 1:1 KC-400/KC-500

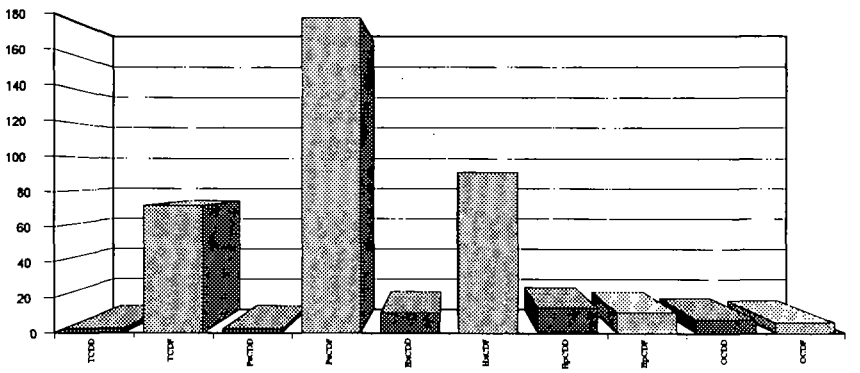

Figure 2. Congener profile of PCDD/DFs

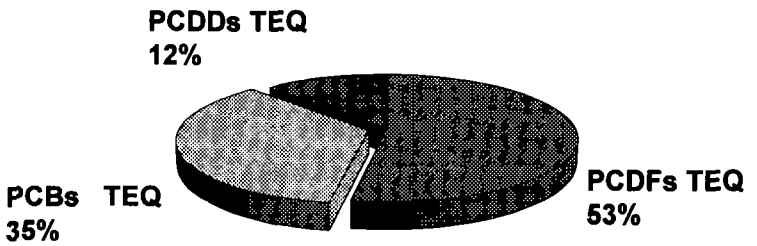

Figure 3. Relative TEQ contributions from PCDD, PCDFs and PCBs

Table 1. Concentrations (ng/g,) of PCDD/DFs, PCBs, Co-PCBs, as well as the TEQ in rice oil.

TCDD	2.608	2,3,7,8-TCDD	2.608
PeCDD	2.513	1,2,3,7,8-PeCDD	2.151
HxCDD	11.678	1,2,3,4,7,8-HxCDD	5.756
HpCDD	14.587	1,2,3,6,7,8-HxCDD	0.766
OCDD	7.796	1,2,3,7,8,9-HxCDD	0.403
TCDF	72.095	1,2,3,4,6,7,8-HpCDD	8.890
PeCDF	176.880	OCDD	7.796
HxCDF	90.725	PCDDs TEQ	4.472
HpCDF	11.767		
OCDF	6.218	2,3,7,8-TCDF	30.326
		1,2,3,7,8-PeCDF	2.649
2 Cl PCB	184	2,3,4,7,8-PeCDF	16.288
3 Cl PCB	9055	1,2,3,4,7,8-HxCDF	77.027
4 Cl PCB	101051	1,2,3,6,7,8-HxCDF	1.604
5 Cl PCB	47945	2,3,4,6,7,8-HxCDF	1.465
6 Cl PCB	16328	1,2,3,7,8,9-HxCDF	1.449
7 Cl PCB	1104	1,2,3,4,6,7,8-HpCDF	9.4
8 Cl PCB	108	1,2,3,4,7,8,9-HpCDF	0.376
Total PCB (μg/g)	176	OCDF	6.218
		PCDFs TEQ	19.568
		PCB77	1290
		PCB126	2.35
		PCB169	0.38
		PCBs TEQ	13.154

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