Investigation on Organochlorinated Compounds formed in Kanemi Rice Oil that Caused the "Yusho"*¹

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PCB, polychlorinated quaterphenyl (PCQ) and polychlorodibenzofuran (PCDF) were detected in Kanemi rice oil that caused the "Yusho." These chemicals could be attributable to the thermotransfer medium, which leaked out into Kanemi rice oil in the deodorizer, but the ratios of PCQ/PCB and PCDF/PCB in the oil were found to be extraordinarily high in comparison with those in the original thermotransfer media.

In this paper, the rice oil which was spiked with only PCB or PCB, PCQ and PCDF was deodorized using a laboratory deodorizer, and the changes of concentration of the chemicals were investigated.

1) PCQ and PCDF formation from PCB by deodorizing

The rice oil which was spiked with 10,000 ppm of purified Kanechlor 400, a Japanese commercial PCB preparation containing 48% chlorine, was deodorized in a laboratory deodorizer. The oil was saponified, extracted, washed and concentrated. The concentrated extract was then cleaned up by Florisil and alumina column chromatography to obtain the PCQ and PCDF fractions which were further perchlorinated in order to confirm the chemicals.

PCQ was not formed from PCB by deodorizing, but it was recognized that PCDF was formed in a small quantity, *i.e.*, the PCDF level in the oil was 3 ppb.

2) The concentration change of each component, PCB, PCQ and PCDF by deodorizing

The rice oil which was spiked with 2,375 ppm of purified Kanechlor 400, 90 ppm of PCQ and 1.35 ppm of PCDF was deodorized in a laboratory deodorizer.

The amount of PCB which retained in the oil decreased rapidly with rising of deodorizing temperature. When deodorized at 260~270°C, the remaining PCB in the oil was only 38 ppm, namely the residual ratio was 1.6%. Less-chlorinated biphenyls than penta-chlorobiphenyl evaporated more rapidly by deodorizing, and consequently the component ratio of penta- to octachlorobiphenyl in the oil increased. When deodorized in the range of 220~270°C, the PCB pattern became similar to those obtained from Kanemi rice oils that caused the "Yusho."

PCDF in the oil also decreased by deodorizing as well as PCB, but in lesser extent. At the same time, tri- and tetra-chlorodibenzofuran decreased, and the component ratio of penta- and hexa-chlorodibenzofuran in the oil increased.

On the other hand, it became clear that the composition and concentration of PCQ in the oil did not change by deodorizing. Consequently, the ratios of PCQ/PCB and PCDF/PCB in the oil increased rapidly and reached gradually to the status obtained from Kanemi rice oil.

It was concluded that PCB used as the thermotransfer medium, which contained PCQ and PCDF, leaked out into Kanemi rice oil in the deodorizer, and thereafter the ratios of PCQ/PCB and PCDF/PCB in the oil might become extraordinarily high by deodorizing.

*¹ Studies on the Compounds Related to PCB (VII)

1. Introduction

In 1975, polychlorodibenzofurans (PCDFs) were detected in Kanemi rice oil that caused the "Yusho."¹⁾ Although the actual concentration was quite low, PCDF was considered as a new causal factor of the "Yusho" in view of its strong toxicity. Meanwhile, we found that polychlorinated quaterphenyls (PCQs) were contained 0.9 to 3.5 times more than the PCB level in the oil.^{2)~5)}

Both of these chemicals were produced from Kanechlor 400 (KC-400) by heating it in an ampoule. Therefore, it was considered that they were formed during usage of PCB as thermotransfer media. However, it was recognized that the ratios of PCQ/PCB and PCDF/PCB in the "Yusho" oil were far higher than the respective ratio obtained from thermotransfer medium just before leakage.⁶⁾

In this paper, the rice oil which was spiked with PCB, PCQ or PCDF was deodorized using a laboratory deodorizer, and transformation and alteration of the ratio of every component were investigated.

2. Materials and Method

2.1 Materials

The rice oil was obtained from a oil manufacturer in Osaka. KC-400 preparation was distributed to our Laboratory in 1972 by the Ministry of Health and Welfare. KC-400 used for ca. 3 years at 200~220°C as heat exchanger at a rice oil manufactures factory was employed as one of the PCDF sources. Heated KC-400 means KC-400 was heated for 20 days at 360°C under the presence of water in an ampoule.⁶⁾ Kanemi rice oil used was described previously.³⁾

2.2 Reagents

PCDF (containing 46.3~56.8% chlorine), polychlorinated naphthalene (containing 50~59% chlorine) and polychlorodibenzo-*p*-dioxin (containing 54~61% chlorine) standards were obtained from Wako Pure Chemical Co. Ltd. PCQ standard was prepared as described previously.^{4), 5)} TLC plate was 20×20 cm glass plate coated with aluminium oxide 60F₂₅₄ of Merck Co. Ltd. Florisil was 60~80 mesh and activated overnight at 130°C. Alumina was obtained from Merck Co. Ltd., No. Art. 1077. Acetone, methylene dichloride, ethanol, benzene,

diethyl ether and *n*-hexane were of reagent grade for PCB analysis obtained from Wako Pure Chemical Co. Ltd. Pottassium hydroxide, anhydrous sodium sulfate, alminium chloride, sulfur monochloride, sulfuryl chloride, sulphuric acid, hydrochloric acid and sodium bicarbonate were of reagent grade obtained from Wako Pure Chemical Co. Ltd. Antimony pentachloride was of reagent grade obtained from Katayama Chemical Co. Ltd.

2.3 Method

The rice oil which was spiked with purified PCB, PCQ or PCDF was deodorized using a laboratory deodorizer as shown in Fig. 2. Purified PCB, PCQ and PCDF were described as follows.

1) Purified KC-400 (PCB)

Purification of KC-400 was made by removing the contaminant PCDF.⁶⁾ Two grams of KC-400 was dissolved with 50 ml of *n*-hexane, each of 1/10 aliquot was put on a Florisil column (20 g, 1.8 cm i.d.) and eluted successively with 130 ml of *n*-hexane and 50 ml of 2% diethyl ether in *n*-hexane. *n*-Hexane and 2% diethyl ether in *n*-hexane eluates were collected, concentrated and used as purified KC-400.

2) Purified PCQ

Two grams of heated KC-400 was dissolved with 50 ml of *n*-hexane. Each of 1/10 aliquot was chromatographed over a Florisil column (20 g, 1.8 cm i.d.). PCB was eluted with 130 ml of *n*-hexane and 50 ml of 2% diethyl ether in *n*-hexane solution. PCQ was eluted thereafter with 100 ml of 5% diethyl ether in *n*-hexane solution. The last fractions were collected, concentrated to 5 ml and rechromatographed with the same way as described above. The last fraction was concentrated and used as purified PCQ.

3) Purified PCDF

According to the method described previously,⁶⁾ PCDF contained in heated KC-400 (2 g) and KC-400 used as the thermotransfer medium (2 g) was separated by Florisil and alumina column chromatography. However, since the PCDF fractions contained small amount of PCB and polychlorinated naphthalene (PCN),⁷⁾ they were spotted on alumina TLC plated and developed with *n*-hexane. The TLC chromatogram of samples is showed in

Fig. 1. Thin layer chromatogram of standards and PCDF fractions

A, PCB (KC-400); B, polychlorinated naphthalene; C, PCDF; D, polychlorodibenzo-*p*-dioxin; E, PCQ; F, PCDF fraction from heated KC-400; G, PCDF fraction from KC-400 used as thermotransfer medium

Fig. 1. The portions of UV band corresponding to PCDF standard were scraped off, extracted and concentrated. Two concentrated extracts were combined and used as purified PCDF.

4) Laboratory deodorization apparatus

The laboratory deodorization apparatus is shown in Fig. 2. (A) was a 20 ml test tube surrounded by a water bath (B) to keep the tube at 50°C. Fifteen ml of water was put into the tube. The rate of steam inlet into the deodorization flask (D) was controlled by stopcock (C). Tape heater (TH-1) was

equipped by way of prevention of steam condensing in the steam inlet tube. The temperature of (D) was controlled by mantol heater (H). The compounds which evaporated from (D) were condensed in condensing pot (E) kept in by an ice bath. (M) was manometer. Pot (F) and (G) were equipped for removing water and chemicals which passed through pot (E).

5) PCQ and PCDF formation from PCB by deodorizing

The rice oil was spiked with 10,000 ppm of purified KC-400, and 50 ml of the oil was deodorized in the laboratory deodorizer. The conditions of deodorization were as follows: Temperature, 230~240°C; steaming time, 60 min; steaming rate, 4.2%/oil/hr; pressure, 30 mmHg.

As described previously,⁸¹ 30 ml of deodorized oil was saponified, extracted with *n*-hexane, washed and concentrated to 15 ml. Each of 1/3 aliquot was put on a Florisil column (20 g, 1.8 cm i.d.) and eluted successively with 130 ml of *n*-hexane, 50 ml of 2% diethyl ether in *n*-hexane, 100 ml of 5% diethyl ether in *n*-hexane, 250 ml of 50% diethyl ether in *n*-hexane and 250 ml of acetone. The first and second fractions of each run were collected, concentrated and analysed for PCB. Every third fractions were collected, concentrated and cleaned-up over a Florisil column according to the method as described previously.⁶¹ Every fourth and fifth fractions were collected, concentrated, dried under a stream of air and dissolved with 5 ml of 3% methylene dichloride in *n*-hexane and cleaned-up as described previously.⁶¹ Moreover, the PCQ and PCDF fraction were perchlorinated in order to confirm the

Fig. 2. Laboratorial deodorization apparatus

chemicals.

5.1) *Perchlorination of the PCQ fraction*

Perchlorination was made according to the modified method^{*1)} of Armour.⁹⁾ The PCQ fraction was poured into a 8 cm×1.5 cm i.d. pyrex glass ampoule and dried under a stream of air. One ml of antimony pentachloride was added in it. After sealing, the ampoule was heated at 200°C for 3 hr in a gas chromatograph oven. The reactant was dissolved with 10 ml of 6*N* hydrochloric acid and extracted with *n*-hexane. The extract was washed with water and 10% sodium bicarbonate solution, dried over anhydrous sodium sulfate, concentrated and analysed by ECD-GC. GC analysis was carried out on a Varian Aerograph 2100 equipped with ⁶³Ni-ECD. GC conditions: Column, 70 cm×2 mm glass column packed with 1% OV-210 on Gaschrom Q (100/120 mesh); column, detector and injector temp., 255, 320 and 250°C, respectively; carrier gas, Nitrogen (40 ml/min).

5.2) *Perchlorination of the PCDF fraction*

Perchlorination was made according to the method of Masuda *et al.*¹⁰⁾ Perchlorinated fraction was analyzed by ECD-GC (Varian Aerograph 1400). GC conditions: Column, 1.5 m×2 mm glass columns packed with 2% OV-17 and OV-1 on Gaschrom Q (100/120 mesh); column temp., 240 and 222°C, respectively; detector and injector temp., 300 and 210°C; carrier gas, nitrogen (30 ml/min).

6) *Changes of concentration and composition of PCB, PCQ and PCDF by deodorizing*

As to be similar to the proportion of the chemicals in thermotransfer medium,⁷⁾ PCB, PCQ and PCDF (2,375, 90 and 1.35 ppm, respectively) were spiked in the rice oil. Fifty ml of the oil was deodorized in the laboratory deodorizer. The conditions of deodorization were shown in Table 1.

According to the method described previously,⁸⁾ 5 ml of the deodorized oil was saponified, extracted with *n*-hexane, washed and concentrated to 5 ml. The concentrated extract was chromatographed on a Florisil column (20 g, 1.8 cm i.d.) according to the same method as described in (2.3.5). *n*-Hexane and 2%

diethyl ether in *n*-hexane fraction were combined, concentrated and analyzed for PCB by ECD-GC. The determination of PCB was made by the method described previously.¹¹⁾ Five percentage of diethyl ether in *n*-hexane fraction was concentrated and analyzed for PCQ by ECD-GC. The determination of PCQ was carried out by measuring the total peak areas. Fifty percentage of diethyl ether in *n*-hexane and acetone fraction were combined, concentrated and dried under a stream of air. The residue was dissolved in 5 ml of 3% methylene dichloride in *n*-hexane, cleaned-up on a alumina column⁶⁾ and analysed for PCDF by ECD-GC. The determination of PCDF was carried out by measuring the peak high.

GC conditions for PCB analysis showed as follows

a) For quantitative analysis: GC, Varian Aerograph 1400 equipped with ⁶³Ni-ECD; column, 1.8 m×2 mm glass column packed with 2% OV-1 on Gaschrom Q (100/120 mesh); column, detector and injector temp., 178, 295 and 235°C, respectively; carrier gas, nitrogen (30 ml/min).

b) For qualitative analysis: GC, Varian Aerograph 2100 equipped with ⁶³Ni-ECD; column, 5 m×2 mm glass column packed 2% Apiezon L on Gaschrom Q (100/120 mesh); column, detector and injector temp., 245, 300 and 230°C, respectively; carrier gas, nitrogen (10 ml/min).

GC condition for PCQ analysis: GC, Varian Aerograph 2100 equipped with ⁶³Ni-ECD; column, 1 m×2 mm glass column packed with 2% OV-210 on Gaschrom Q (100/120 mesh); column, detector and injector temp., 235, 310 and 208°C, respectively; carrier gas, nitrogen (30 ml/min).

GC condition for PCDF analysis: GC, Varian Aerograph 2100 equipped with ⁶³Ni-ECD; Column, 1.5 m×2 mm glass column packed with 2% OV-17 on Gaschrom Q (100/120 mesh); column, detector and injector temp., 215, 300 and 240°C, respectively; carrier gas, nitrogen (30 ml/min).

3. Result and Discussion

As a rule, the deodorization conditions for rice oil were as follows: The temperature of deodorization, 230~270°C; steaming time, 60

^{*1} Maeda, K., Miyata, H., Murakami, Y., Kashimoto, T.: Abstract of papers, 35th Meeting of Food Hyg. Soc. Japan, May, p. 44, (1978).

min; steaming rate, 3%/oil/hr; pressure, 2~3 mmHg. In this experiment, at the beginning only a vacuum of the deodorizer could reach to the condition. However, since PCQ and PCDF were formed from PCB at high temperature and high pressure,⁷⁾ it was considered then that the lower vacuum would not be a serious problem. It was supposed that rather the deodorizing temperature and steaming rate than vacuum might have more effect on the reaction.

3.1 PCQ and PCDF formation from PCB by deodorizing

Over 90% of PCB evaporated from the oil by deodorizing, and consequently the PCB concentration in the oil decreased from 10,000 to 867 ppm. The PCB pattern was similar to that obtained from Kanemi rice oil that caused the "Yusho."

1) PCQ formation from PCB

Although perchlorinated sample was concentrated as little as possible and analysed by ECD-GC, octadeca-chlorinated quaterphenyl ($C_{24}Cl_{18}$) was not detected. Under this GC conditions, the limit of quantitation was 2 ppb of PCQ in the oil. Therefore, it was considered

that PCQ was formed from PCB by deodorizing.

2) PCDF formation from PCB

Perchlorinated sample was analysed by ECD-GC, and a peak with retention time corresponding to octachlorodibenzofuran ($C_{12}Cl_8O$) was confirmed on 2% OV-17 and OV-1 column as shown in Fig. 3. Moreover, since the PCDF concentrations were equal level on two different column, the peak was confirmed as octachlorodibenzofuran. The PCDF concentration in the oil was 3 ppb.

From the results, it became clear that small amount of PCDF was formed from PCB by deodorizing.

However, it was considered that the PCDF formation didn't actually affect the ratio of PCDF/PCB in the oil because the PCDF level in the deodorized oil was below 1/1,000 of the level in Kanemi rice oil that caused the "Yusho."⁸⁾

3.2 Changes of the concentration and composition of PCB, PCQ and PCDF in the rice oil by deodorizing

1) PCB

With rising of deodorizing temperature (room temp.→270°C), PCB in the oil evaporated rapidly from the deodorization flask (D), and consequently, the amount of PCB which remained in the oil decreased rapidly from 2,375 to 38 ppm as shown in Table 1 and Fig. 4.

It was observed that over 90% of PCB evaporated from the oil when deodorized at higher temperature than 220°C (Table 1).

Figure 5 shows the gas chromatograms of PCB in KC-400, deodorized oil and Kanemi rice oil on 2% Apiezon L column. As obvious from the figure, the component of PCB which evaporated from the oil by deodorizing was mainly peak No. b~17, less chlorinated biphenyl than pentachlorobiphenyl,¹²⁾ and consequently, the content of penta- to octa-chlorobiphenyl which were retained in the oil increased.

It was considered that the phenomenon might result from the difference of vapor pressure among each component of PCB.¹³⁾

When deodorized at 220~230°C, the PCB pattern was similar to that obtained from Kanemi rice oil (Feb. 5th), and at 260~270°C, it was similar to that obtained from Kanemi rice oil (Feb. 10th) as shown in Fig. 5.

However, the PCB pattern from the deo-

Fig. 3. Gas chromatograms of octachlorodibenzofuran and perchlorinated PCDF fraction

Table 1. Retention of PCB, PCQ and PCDF in Deodorized Rice Oils Spicked with 2,375 ppm of KC-400, 90 ppm of PCQ and 1.35 ppm of PCDF

Conditions of deodorization				PCB		PCQ			PCDF		
Temperature (°C)	Steaming time (min)	Steaming rate (%) ^{a)}	Pressure (mmHg)	Retention (ppm)	Residual ratio (%)	Retention (ppm)	Residual ratio (%)	PCQ/PCB (g/g)	Retention (ppm)	Residual ratio (%)	PCDF/PCB (μg/g)
Room temp.	0	0.0	760	2,375	100.0	90.0	100.0	0.04	1.35	100.0	568
180~190	60	7.2	36~39	1,664	70.1	95.3	106.1	0.06	1.13	83.6	678
200~210	60	7.6	38~42	745	31.4	87.8	97.6	0.12	0.90	66.4	1,204
220~230	60	3.8	37	230	9.7	90.0	100.0	0.39	0.63	46.5	2,730
240~250	60	3.1	37~40	65	2.7	90.4	100.5	1.39	0.37	27.6	5,739
260~270	60	4.9	30~38	38	1.6	90.9	101.0	2.39	0.14	10.1	3,553

^{a)} Steam (g)/sample (g)/steaming time (hr) × 100

Fig. 4. Concentrations of PCB, PCQ and PCDF in deodorized oils

deodorized oil didn't completely agreed with that from Kanemi rice oil (Fig. 5). The cause might be explained that in the case of Kanemi rice oil thermotransfer medium had continuously leaked out into the oil during the deodorizing period, whereas in this experiment thermotransfer medium was put into the rice oil before deodorizing.

In addition, peak No. (47, 48) was higher

than peak No. 49 in the PCB pattern from Kanemi rice oil (Fig. 5). However, it was observed that the ratio of these peaks was reversed in the PCB pattern from deodorized oil. Since the ratio scarcely changed by deodorizing as shown in Fig. 5, it was considered that the ratio of these two peaks in thermotransfer medium which had leaked out into Kanemi rice oil might be different originally

Fig. 5. Gas chromatograms of PCB in deodorized rice oils and Kanemi rice oils on 2% Apiezon L column

from that in the KC-400 employed for this experiment.

2) PCQ

As shown in Table 1, the level of PCQ which was retained in the oil didn't change by deodorizing. Figure 6 showed gas chromatograms of PCQ in non-deodorized oil and oils deodorized at 200~210°C and 260~270°C, and no change was observed in PCQ pattern.

Therefore, the component ratio of PCQ in Kanemi rice oil must be identical to that of PCQ in the thermotransfer medium which had leaked out into the oil.

3) PCDF

The amount of PCDF in the oil decreased rapidly from 1.35 to 0.14 ppm with rising of deodorizing temperature as well as PCB as shown in Table 1. However, the evaporation

rate of PCDF was much lower than that of PCB, *i.e.*, heating at 260~270°C, caused 6.3 times higher residue of PCDF than that of PCB (Table 1). Figure 7 showed gas chromatograms of PCDF on deodorized oils and Kanemi rice oil. Peak No. 1~3, 5~9, 10~14 and 15~16 in Fig. 7 were tri-, tetra-, penta- and hexa-chlorodibenzofuran, respectively.

The composition of PCDF also changed by deodorizing as well as PCB as shown in Fig. 7, *i.e.*, the level of tri- and tetra-chlorodibenzofuran decreased, and penta- and hexa-chlorodibenzofuran increased with rising of deodorizing temperature.

4) The values of PCQ/PCB and PCDF/PCB

As described above, the rates of PCB, PCQ and PCDF evaporation from the by deodorizing were very different. Consequently, the ratios of PCQ/PCB and PCDF/PCB in the oil increased rapidly with rising of deodorizing temperature (Table 1, Figs. 4 and 8). The tendency was clearly observed at the ratio of PCQ/PCB. Deodorization at 260~270°C ele-

Fig. 6. Gas chromatograms of PCQ in deodorized oils

Fig. 7. Gas chromatograms of PCDF in deodorized oils and Kanemi rice oil

vated the ratio of PCQ/PCB in the oil by 60 times. Also the ratio of PCDF/PCB became a order higher than those of nondeodorized oil.

The relative concentrations of PCQ and PCDF to PCB in the deodorized oil and Kanemi rice oil were shown in Fig. 8. As shown in the figure, it became clear that the proportion of three chemicals in the oil reached gradually to that obtained from practical Kanemi rice oil^(4),5) with rising of deodorizing temperature. However, the ratio of PCDF/PCB in the oil was fairly lower than that in Kanemi rice oil. The difference might be attributed to original PCDF level in the thermotransfer medium.

From the results above mentioned, it was concluded that PCB used as the thermotransfer medium, in which PCQ and PCDF had generated, leaked out in to Kanemi rice oil in the deodorizer, and thereafter the ratios of PCQ/PCB and PCDF/PCB in the oil might become extraordinarily high by deodorizing.

Fig. 8. Relative concentration of PCQ and PCDF to PCB (=1) in deodorized rice oils and Kanemi rice oils

A, nondeodorized rice oil; B, rice oil deodorized at 200~210°C; C, Kanemi rice oil manufactured on Feb. 10th in 1968; D, rice oil deodorized at 240~250°C; E, rice oil deodorized at 260~270°C; F, Kanemi rice oil manufactured on Feb. 9th in 1968; G, Kanemi rice oil manufactured on Feb. 10th in 1968

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