

Differential correlation of medical/laboratory examinations with blood levels of polychlorinated biphenyls, polychlorinated quarterphenyls and 2,3,4,7,8-pentachlorodibenzofuran in Yusho patients from 2001 to 2004

Yoshiyuki Kanagawa\*,

Department of Planning Information and Management, The University of Tokyo Hospital,

7-3-1 Hongo, Bunkyo-ku Tokyo 113-8655 Japan

Phone: +81 3 5800 8716

Fax: +81 3 5800 8765

E-mail: [kanagawa-ky@umin.ac.jp](mailto:kanagawa-ky@umin.ac.jp)

Shinya Matsumoto,

Teradata Division, NCR JAPAN Ltd.

Bunichi Tajima,

Teradata Division, NCR JAPAN Ltd.

Soichi Koike,

Department of Planning Information and Management, The University of Tokyo Hospital.

Noriko Fukiwake,

Department of Dermatology, Graduate School of Medical Sciences, Kyushu University

Satoko Shibata,

Department of Dermatology, Graduate School of Medical Sciences, Kyushu University

Hiroshi Uchi,

Department of Dermatology, Graduate School of Medical Sciences, Kyushu University

Masutaka Furue,

Department of Dermatology, Graduate School of Medical Sciences, Kyushu University

Tomoaki Imamura,

Department of Public Health Policy, School of Medicine, Nara Medical University

\*Corresponding author

Department of Planning Information and Management, The University of Tokyo Hospital

7-3-1 Hongo, Bunkyo-ku Tokyo 113-8655 Japan

Phone: +81 3 5800 8716

Fax: +81 3 5800 8765

E-mail: [kanagawa-tyk@umin.ac.jp](mailto:kanagawa-tyk@umin.ac.jp)

## **Abstract**

### [Background]

The Yusho poisoning incident, which was caused by rice bran oil contaminated with polychlorinated biphenyls (PCBs), polychlorinated quarterphenyls (PCQs) and polychlorinated dibenzofurans (PCDFs) generated by heat denaturation of PCB, occurred in 1968 in western Japan. Annual physical, dermatological, dental, ophthalmological and laboratory examinations were conducted for Yusho patients after the incident. From 2001, blood levels of individual PCDF congeners were also measured. The blood levels of 2,3,4,7,8-pentachlorodibenzofuran (2,3,4,7,8-PeCDF), PCBs and PCQs in Yusho patients were found to be significantly higher than those of the general population. We investigated the relationships between blood levels of 2,3,4,7,8-PeCDF, PCBs and PCQs in Yusho patients and the items measured in the annual medical examination.

### [Subjects and methods]

Medical and laboratory examination data from 501 Yusho patients enrolled in the study from 2001 to 2004 were analyzed. The relationships between blood 2,3,4,7,8-PeCDF, PCB and PCQ levels and medical/laboratory examination data were investigated using principal components and logistic regression analyses.

### [Results]

Levels of 2,3,4,7,8-PeCDF, PCBs and PCQs in blood tended to correlate with either acneform eruptions, black comedones, cutaneous and mucosal pigmentation, and hypersecretion of meibomian glands as well as general fatigue, headaches, cough/sputum, abdominal pain, arthralgia, increased blood sugar, increased serum  $\gamma$ -GTP and decreased

total bilirubin. The majority of these signs and symptoms are included in the diagnostic criteria for Yusho.

[Conclusions]

After Yusho patients had suffered chronic exposure to these chlorinated compounds for more than 35 years, the level of 2,3,4,7,8-PeCDF in blood was significantly related to arthralgia and decreased albumin/globulin (A/G) ratio; the level of PCBs was significantly related to ophthalmologic symptoms; and the level of PCQ to increased total cholesterol.

[Keywords]

Yusho, polychlorinated biphenyls (PCBs), polychlorinated quarterphenyls (PCQs), 2,3,4,7,8-pentachlorodibenzofuran (2,3,4,7,8-PeCDF), dioxins

## **Introduction**

Yusho was a food poisoning incident that occurred in western Japan in 1968 [Masuda, 2005; Furue *et al.*, 2005; Kuratsune *et al.*, 1996; Yamaguchi *et al.*, 2002; Imamura *et al.*, 2003; Kanagawa *et al.*, 2005]. When first reported, the food poisoning incident known as Yusho was considered to be caused by polychlorinated biphenyls (PCBs). However, following a number of studies, it is now considered to be caused by complex poisoning with polychlorinated quarterphenyls (PCQs) and polychlorinated dibenzofurans (PCDFs) [Furue *et al.*, 2005; Kuratsune *et al.*, 1996; Yamaguchi *et al.*, 2002; Imamura *et al.*, 2003]. Thirty-seven years have passed since the Yusho incident occurred, and more than 1,800 patients are known to have been affected.

Yusho patients are known to present with various symptoms related to the skin, eyes and teeth, and have abnormal findings on physical examinations [Uenotsuchi *et al.*, 2005; Uenotsuchi *et al.*, 2005; Uenotsuchi *et al.*, 2005; Kanagawa *et al.*, 2005; Uenotsuchi *et al.*, 2005, Imamura *et al.*, 2007; Imamura *et al.*, 2007; Imamura *et al.*, 2007]. The severity of symptoms in Yusho patients has gradually improved over the past 37 years. However, a number of patients still suffer from specific Yusho symptoms [Kanagawa *et al.*, 2005; Furue *et al.*, 2005; Kuratsune *et al.*, 1996]. The initial diagnostic criteria published in 1968 were mainly: 1) proven history of ingestion of contaminated rice bran oil; 2) prominent dermatological, ophthalmological and mucosal signs; and 3) several nonspecific general signs and symptoms. Hyperglyceridemia, pulmonary disorders, intractable headache, elevated blood PCB concentrations and specific PCB patterns on gas chromatography were added to the initial diagnostic criteria in 1972 and 1976. Blood PCQ levels were added to

the criteria in 1981 [Furue *et al.*, 2005].

With recent advances in techniques for measuring individual PCDF congeners, it has become possible to precisely measure 2,3,4,7,8-pentachlorodibenzofuran (2,3,4,7,8-PeCDF) blood levels using as little as 5ml of blood, [Iida T *et al.*, 2003; Todaka I *et al.*, 2003]. Thus, measurements of 2,3,4,7,8-PeCDF blood levels have been initiated since 2001 in the routine mass screening of Yusho patients. The mean blood levels of PeCDF in these patients have been shown to be more than 10 times higher than those in normal controls [Furue *et al.*, 2005]. In 2004, the blood 2,3,4,7,8-PeCDF level was added to the present diagnostic criteria (Table 1).

In this study, we analyzed the results of medical examinations of Yusho patients whose blood 2,3,4,7,8-PeCDF levels were measured from 2001 to 2004 (33 to 37 years after the occurrence of the Yusho disaster), and investigated the relationships among the 2,3,4,7,8-PeCDF, PCB and PCQ blood levels and the clinical data from physical and laboratory examinations.

## **Methods**

### **Subjects and medical check items**

Since immediately after the incident occurred, the Yusho Study Group has conducted annual health checks of Yusho patients. Between 2001 and 2004, a total of 501 individuals (81 individuals in 2001, 371 in 2002, 343 in 2003 and 292 in 2004, including multiple health checks) underwent the Yusho mass screening. In addition to blood PeCDFs, PCBs and PCQs levels, 241 check items (52 items in a questionnaire, 55 physical and laboratory

examinations, 21 dermatological examinations, 108 dental examinations, and 5 ophthalmological examinations) were carried out (Table 2).

### Statistical analysis

The relationships between blood 2,3,4,7,8-PeCDF levels and the physical/laboratory test items were analyzed using logistic regression analysis. Since the serum half-life of 2,3,4,7,8-PeCDF is long and the present blood levels are well correlated with the amount of exposure at the time of the incident, the correlations with these was examined [Masuda et al., 2007]. Logistic regression analysis uses a formula to relate several explanatory variables to objective ones (2 values). We used the following equation which included results [y] and several factors [  $x_1, x_2, \dots, x_n$  ] affecting these results.

$$g(x) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \dots + \beta_n x_n$$

D Equation.3

### Explanatory variable

$$y = \frac{e^{g(x)}}{e^{g(x)} + 1}$$

To conduct a logistic regression analysis, we conducted a principal component analysis as an auxiliary analysis to decide the explanatory variables. Specifically, of the 241 items examined in the Yusho medical checkup, the principal component analysis was conducted on 172 items, except for those related to frequency. As a result, examination items with 1 or higher eigenvalues and high factor scores in the principal component analysis were used as representative variables. In deciding the representative variables, items with high factor

scores were not selected mechanically, but the following criteria were considered:

- (1) Items included in the criteria.
- (2) Items considered to be medically significant.
- (3) A weak factor representing an item selected by multiple factors.

Furthermore, we confirmed that items whose associations with Yusho have been indicated were not overlooked, by reference to the criteria. We extracted 49 items, including 13 questionnaire-related items, 11 physical and laboratory examination items, 10 dermatological examination items, 12 dental examination items and 3 ophthalmological examination items, as representative variables (Table 3).

Furthermore, the following patterns were set as objective variables for our logistic regression analyses:

- 2,3,4,7,8-PeCDF blood level

2 categories: [ $\geq 50$  pg/g lipids] and [ $< 50$  pg/g lipids] (Refer to the diagnostic criteria [Furue *et al.*, 2005])

- PCB blood level

2 categories: [ $\geq 2.0$  ppb] and [ $< 2.0$  ppb] (Categorized by median value)

- PCQ blood level

2 categories: [ $\geq 0.10$  ppb] and [ $< 0.10$  ppb] (Refer to the diagnostic criteria [Furue *et al.*, 2005])

- Other examination items

The 49 factors extracted by the principal component analysis were classified into normal and abnormal categories considering the characteristics of the data for each test item, from

the following viewpoints:

- (1) Factors for which the presence or absence of symptoms was confirmed by two steps in the medical checkup by a doctor were classified into two steps of presence or absence.
- (2) Factors whose measurement results had normal value standards, such as blood test results, were classified into normal or abnormal.
- (3) Items relevant to subjective symptoms, like sputum, arthralgia and general fatigue, were classified into “normal” or “abnormal” for each patient.
- (4) Items evaluated into five grades (-, ±, +, ++ and +++) of symptoms, such as severity of pigmentation, were classified into two groups, based on the criterion of “+” or above, to determine the presence of symptoms.

To conduct analyses on the above 3 patterns, 2,3,4,7,8-PeCDF, PCB and PCQ blood levels were added to the explanatory variables. SPSS11.5J for Windows was used for the analyses.

## **Results**

2,3,4,7,8-PeCDF blood level as an objective variable (Table 4-1)

PCB and PCQ blood levels, blood glucose level, arthralgia, gender, total bilirubin, black comedones, acneform eruption, past history of skin pigmentation and acneform eruption, increased A/G ratio, abnormal respiratory sounds, blood potassium level, and total cholesterol showed less than a 0.05 level of significance. Most of these items are considered characteristic symptoms of Yusho. Even when PCB and PCQ blood levels are excluded from the explanatory variables, older age, A/G ratio, general fatigue, arthralgia,

gender and oral pigmentation showed less than a 0.05 level of significance.

In contrast, when 49 factors were extracted by the principal component analysis (PCB and PCQ as objective variables, 2,3,4,7,8-PeCDF blood level as an explanatory variable), PCQ and PCB blood levels, arthralgia, presence or absence of previous history since 1968, A/G ratio and blood glucose level indicated a significance probability of  $\leq 0.05$  for the 2,3,4,7,8-PeCDF blood level.

PCB blood level as an objective variable (Table 4-2)

2,3,4,7,8-PeCDF blood level, sputum, age, female gender, past history of pigmentation and acneform eruption, toe nail pigmentation, hepatomegaly, headache, cheesy secretion from meibomian glands, total bilirubin, and general fatigue showed less than a 0.05 level of significance. When 2,3,4,7,8-PeCDF blood level was excluded from the explanatory variables, age, sputum, past history of pigmentation, total bilirubin, PCQ blood level, toe nail pigmentation, arthralgia, presence of a chief dental complaint, headache, and cheesy secretion from meibomian glands were significantly correlated with PCB blood levels.

In contrast, when 49 factors were extracted by the principal component analysis (2,3,4,7,8-PeCDF and PCQ as objective variables, PCB blood level as an explanatory variable), items which showed less than a 0.05 level of significance for PCB blood level (explanatory variable) included 2,3,4,7,8-PeCDF blood level and excessive eye discharge.

PCQ blood level as an objective variable (Table 4-3)

Tooth pigmentation, arthralgia,  $\gamma$ -GTP, total bilirubin, cheesy secretion from meibomian glands, general fatigue, total cholesterol, toe nail pigmentation, female gender, and oral mucosa pigmentation all showed less than a 0.05 level of significance. When

2,3,4,7,8-PeCDF blood level was excluded from the explanatory variables, past history of pigmentation, tooth pigmentation, PCB blood levels, acneform eruption, abdominal pain, pigmentation, and total cholesterol were significantly correlated with PCQ blood levels.

## **Discussion**

PCBs, PCQs and PCDFs are known as the causative agents of Yusho. The results from this study show that, the levels of 2,3,4,7,8-PeCDF, PCB and PCQ in blood were strongly related. The blood levels of 2,3,4,7,8-PeCDF, PCB and PCQ tended to correlate with older age, as adult victims were considered to have eaten greater amounts of the contaminated oil compared with child victims when the contaminated oil was available in shops in 1968. The blood levels of 2,3,4,7,8-PeCDF, PCB and PCQ also tended to correlate with female gender. This may be attributed to the fact that these chlorinated compounds are highly lipophilic and accumulate in adipose tissue [Furue *et al.*, 2005]. Females who have more adipose tissue may have thus accumulated more 2,3,4,7,8-PeCDF, PCB and PCQ.

In our study, the blood levels of 2,3,4,7,8-PeCDF, PCB and PCQ also tended to correlate with acneform eruptions, black comedones, cutaneous and mucosal pigmentation, and hypersecretion of meibomian glands, in addition to general fatigue, headaches, cough, sputum, abdominal pain, increased serum  $\gamma$ -GTP, and decreased total bilirubin. These signs and symptoms are all included in the present diagnostic criteria of Yusho (Table 1). In addition to the symptoms listed in the diagnostic criteria, arthralgia was frequently correlated to 2,3,4,7,8-PeCDF, PCB and PCQ blood levels. Using the 2,3,4,7,8-PeCDF blood level as an objective variable, cases including or not including the PCB and PCQ

levels as explanatory variables were compared. As a result, arthralgia and A/G ratio were presumably related to the 2,3,4,7,8-PeCDF blood level.

Using the PCB blood level as an objective variable, cases including or not including the 2,3,4,7,8-PeCDF level as an explanatory variable were compared. As a result, PCB blood level was presumably strongly related to ophthalmological symptoms.

The PCQ blood level was related to cutaneous, oral and ophthalmological manifestations, increased  $\gamma$ -GTP, and increased total cholesterol. When the 2,3,4,7,8-PeCDF blood level was excluded in the explanatory variables, oral pigmentation and increased total cholesterol were significantly related to PCQ blood level. The biochemical adverse effect of PCQ has been reported to include increased triacylglycerol level [Kunita *et al.*, 1982]. However, based on the results of this study, total cholesterol level, one of the markers of lipid metabolism such as triacylglycerol, was presumably related to PCQ blood level.

Like Kanemi Yusho, Taiwan Yucheng, a health hazard caused by PCB or PCDFs, has been reported to have a high incidence of symptoms of chloracne, goiter, arthritis, and anemia [Guo YL *et al.* 1999]. Chloracne and arthritis are considered e symptoms common to Yusho and Taiwan Yusho. Health hazards caused by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the Italy Seveso event have also been studied. In a death survey, conducted 20-25 years after the Seveso event, a high incidence of deaths due to cancer, circulatory disease, chronic obstructive, pulmonary disease (COPD), and diabetes mellitus was reported [Bertazzi PA, *et al.* 2001] [Consonni D, *et al.* 2008]. It thus seems necessary to examine the presence of a relationship between Yusho and COPD in the future, since cough, sputum, and bursitis, included in the Yusho criteria, are also symptoms seen in COPD.

Cutaneous, mucosal and ophthalmological manifestations, related to the blood levels of 2,3,4,7,8-PeCDF, PCB and PCQ in this study, were considered characteristic of Yusho and were included in the diagnostic criteria.

## **Conclusions**

Although 35 years have passed since the occurrence of Yusho, the 2,3,4,7,8-PeCDF blood level appeared related to the PCQ and PCB blood levels, arthralgia and A/G ratio; PCB blood level was strongly related to ophthalmological symptoms; while PCQ blood level was related to total cholesterol.

## References

- Bertazzi PA, Consonni D, Bachetti S, Rubagotti M, Baccarelli A, Zocchetti C, Pesatori AC: **Health effects of dioxin exposure: a 20-year mortality study.** *Am J Epidemiol.* 2001, **153**(11):1031-1044.
- Consonni D, Pesatori AC, Zocchetti C, Sindaco R, D'Oro LC, Rubagotti M, Bertazzi PA: **Mortality in a population exposed to dioxin after the Seveso, Italy, accident in 1976: 25 years of follow-up.** *Am J Epidemiol* 2008, **167**(7):847-858.
- Furue M, Uenotsuchi T: **Steps for establishment of the diagnostic standard in Yusho patients.** *Fukuoka Igaku Zasshi* 2005, **96**:124-134. (In Japanese)
- Furue M, Uenotsuchi T, Urabe K, Ishikawa T, Kuwabara M; **Overview of Yusho.** *J Dermatol Sci* 2005, Suppl **1**:S3-S10.
- Guo YL, Yu ML, Hsu CC, Rogan WJ: **Chloracne, goiter, arthritis, and anemia after polychlorinated biphenyl poisoning: 14-year follow-Up of the Taiwan Yucheng cohort.** *Environ Health Perspect* 1999, **107**(9):715-719
- Guo YL, Lambert GH, Hsu CC, Hsu MM **Yucheng: health effects of prenatal exposure to polychlorinated biphenyls and dibenzofurans.** *Int Arch Occup Environ Health* 2004, **77**(3):153-158.
- Iida T, Todaka T, Hirakawa H, Tobiishi K, Matsueda T, Hori T, Nakagawa R, Furue M.: **Follow-up survey of dioxins in the blood of Yusho (in 2001).** *Fukuoka Igaku Zasshi* 2003, **94**:126-135.
- Imamura T, Kanagawa Y: **A study on correlations between blood serum levels of PCDFs**

**and clinical symptoms in patients with Yusho (78 patients for 2001, 279 patients for 2002).** *Health and Labour Sciences Research, Summarized and allotted study report 2003.* (In Japanese).

Imamura T, Matsumoto S, Kanagawa Y, Tajima B, Matsuya S, Furue M, Oyama H.A: **Technique for identifying three diagnostic findings using association analysis.** *Med Biol Eng Comput* 2007, **45**(1):51-59.

Imamura T, Kanagawa Y, Matsumoto S, Tajima B, Uenotsuchi T, Shibata S, Furue M: **Relationship between clinical features and blood levels of pentachlorodibenzofuran in patients with Yusho.** *Environmental Toxicology* 2007, **22**(2):221-237.

Imamura T, Kanagawa Y, Matsumoto S, Tajima B, Uenotsuchi T, Shibata S, Furue M: **Epidemiological Aspects of Yusho; Clinical features and blood levels of Pentachlorodibenzofuran in Yusho Patients.** *DIOXIN 2007 27th International Symposium. Organohalogen Compounds* 2007, **69**:87-90

Kanagawa Y, Imamura T: **Relationship between blood PCDFs level and symptoms in Yusho patients.** *Fukuoka Igaku Zasshi* 2005, **96**:169-179. (In Japanese).

Kanagawa Y, Imamura T: **Relationship of clinical symptoms and laboratory findings with the blood serum levels of PCDFs in patients with Yusho.** *J Dermatol Sci* 2005, Suppl **1**:S85-S93.

Kunita N, Kashimoto T: **Biological effects of PCB-related substances.** *The Saishin-Igaku* 1982, **57**:378-383. (In Japanese).

Kuratsune M, Yoshimura H, Hori Y, Okumura M, Matsuda Y: **Yusho - A human disaster caused by PCB and related compounds** 1996, Kyushu University Press, Fukuoka.

Masuda Y: **Behavior and toxic effects of PCBs and PCDFs in Yusho patients for 35 years.** *J Dermatol Sci* 2005, Suppl 1:S11-S20.

Masuda Y, Yoshimura T, Kajiwara J: **Changes in PCBs and PCDFs blood levels in patients for 38 years since the occurrence of Yusho.** *Fukuoka Igaku Zasshi* 2007, **98**(5):182-195.

Todaka T, Hirakawa H, Tobiishi K, Iida T: **New protocol of dioxins analysis in human blood.** *Fukuoka Igaku Zasshi* 2003, **94**:148-157.

Uenotsuchi T, Furue M, Nakayama J, Asahi M, Kanagawa Y, Imamura T: **Evaluation of dermatological symptoms of Yusho patients in the annual examinations of 2003-2004.** *Fukuoka Igaku Zasshi* 2005, **96**:216-219. (In Japanese).

Uenotsuchi T, Inoo Y, Tadakuma S, Haratsuka R, Kanagawa Y, Imamura T, Furue M: **Sex ratio of newborn infants from parents with Yusho.** *Fukuoka Igaku Zasshi* 2005, **96**:183-184. (In Japanese).

Uenotsuchi T, Lio Y, Tadakuma S, Haraduka R, Kanagawa Y, Imamura T, Furue M: **Sex ratio in the children of Yusho patients.** *J Dermatol Sci* 2005, Suppl 1:S81-S83.

Uenotsuchi T, Nakayama J, Asahi M, Kohro O, Akimoto T, Muto M, Shimizu K, Katayama I, Kanzaki Y, Kanagawa Y, Imamura T, Furue M: **Dermatological manifestations in Yusho: correlation between skin symptoms and blood levels of dioxins, such as polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs).** *J Dermatol Sci* 2005, Suppl 1: S73-S80.

Uenotsuchi T, Nakayama K, Asahi S, Takamichi O, Akimoto T, Muto M, Kiyomizu K, Katayama I, Kanzaki Y, Kanagawa Y, Imamura T, Furue M.: **Skin symptoms in Yusho**

**patients related to blood dioxin level.** *Fukuoka Igaku Zasshi* 2005, **96**:164-168. (In Japanese).

Yamaguchi N, Kaneko S: **A study on evaluation of carcinogenesis in patients with Yusho and A study on health evaluation in Yusho. 2001, 2002.** *Health and Labour Sciences Research. (integrated study report, summarized and allotted study report)* 2002.

#### Glossary

1) Black comedones (other sites):

black comedones appearing on body parts other than the face, auricle, and trunk

2) Palatal findings

Palatal lesions known to occur in Yusho patients include pigmentation and parakeratosis in the gingiva, dental root dysplasia, and odontatrophia.

Palatal findings from examinations in Yusho patients are recorded as follows:

(1) The upper teeth and lower teeth are each divided into 3 sites:

site 1 = 7-4, site 2 = 3-3, and site 3 = 4-7.

(2) Pigmentation patterns are divided into the following patterns recognized in Yusho

patients:

Condition 1 = diffuse, Condition 2 = punctate, Condition 3 = linear,

Condition 4 = zonal, Condition 5 = cloudy, Condition 6 = island shaped

**Table 1. Diagnostic criteria for Yusho (as presently supplemented)**

The diagnostic criteria for Yusho were revised on October 26, 1972; supplemented on June 14, 1976; and an item related to blood polychlorinated quarterphenyl (PCQ) level was added on June 16, 1981. The study group of Yusho started to measure blood levels of dioxins in annual medical check-ups from 2001. It was considered appropriate to add an item corresponding to the blood 2,3,4,7,8-PeCDF level; therefore the criteria were supplemented and further revised on September 29, 2004.

**Conditions of the incident**

- 1) Proof that Kanemi rice bran oil contaminated with polychlorinated biphenyls (PCBs) was ingested.
- 2) There are also some cases in which PCB is transferred from mothers with Yusho to their children.
- 3) Familial occurrence is also seen in many cases.

**Important manifestations**

1. Acneform eruptions  
Black comedones seen on the face, buttocks and other intertriginous sites; comedones with inflammatory manifestations; and subcutaneous cysts with atheroma-like contents that tended to suppurate.
2. Pigmentation  
Pigmentation of the face, palpebral conjunctivae, gingivae, and nails etc. (including so-called 'black babies').
3. Hypersecretion of the meibomian glands.
4. Unusual composition and concentration of PCBs in the blood.
5. Abnormal level of blood PCQ
  - 1)  $\geq 0.1$  ppb: an abnormally high concentration.
  - 2) 0.03 to 0.09 ppb: the boundary between high and normal concentrations.
  - 3)  $\leq 0.02$  ppb (detection limit): normal concentration.
6. Abnormal level of blood PeCDF
  - 1)  $\geq 50$  pg/g lipids: an abnormally high concentration.
  - 2) 30 to 50 pg/g lipids: a relatively high concentration.
  - 3)  $< 30$  pg/g lipids: normal concentration.

**Standard symptoms and findings**

1. Subjective symptoms
  - 1) General fatigue
  - 2) Headaches, dull headaches
  - 3) Paresthesia of the extremities (abnormal sensation)
  - 4) Increased eye discharge
  - 5) Cough and sputum
  - 6) Inconstant abdominal pain
  - 7) Altered menstruation
2. Objective findings
  - 1) Manifestation of bronchitis
  - 2) Deformation of nails
  - 3) Bursitis
  - 4) Increased neutral fat in the serum
  - 5) Increased serum  $\gamma$ -glutamyl transpeptidase ( $\gamma$ -GTP)
  - 6) Decrease in serum bilirubin
  - 7) Small-for-date baby
  - 8) Growth retardation and dental abnormality (retarded eruption of permanent teeth)

**Table 2. Annual medical check examination sheet of Yusho patients**

---

**(1). Laboratory examination**

**Blood concentrations of PCB- and dioxin-related compounds**

Total PCB, Peak 1, Peak 2, Peak 3, PCB pattern, CB ratio, Total PCQ, Dioxin-related compounds  
Urinalysis (Protein, Sugar, Occult blood, Urobilinogen, pH)

**Hematological examination**

ESR (1-hour), ESR (2-hour), WBC, RBC, Hb, Ht, MCV, MCH, MCHC, PLT

**Blood chemistry**

T-Bil, D-Bil, GOT, GPT, TP, Alb, albumin/globulin(A/G) ratio, ZTT, TTT, ALP, LAP,  $\gamma$ -GTP, ChE, LDH,  
CPK, TC, HDL-cholesterol, TG,  $\beta$ -lip, BUN, Cre, Na, K, Ca, P, Amy, blood sugar level

**Immunological examination** (HBs antigen,  $\alpha$ -fetoprotein)

**(2) . Interview and physical examination**

**Life history** (Alcohol, Smoking)

**Chief complaint**

**Past history**(Before the incident, After the incident)

**Subjective symptoms**

General fatigue, Headache, Cough, Sputum, Abdominal pain, Diarrhea, Constipation  
Numbness, Arthralgia, menstruation disorders

**Physical examination**

Body height, Body weight, Heart rate, Blood pressure, Nutrition, Heart sounds, Respiratory sounds,  
Chest radiography, ECG, Abdominal ultrasonography,  
Hepatomegaly, Splenomegaly, Edema, Lymphadenopathy, Tendon reflex, Sensory examination,

**(3) . Dermatological examination**

**Interview**

Recent tendency to purulent skin eruptions, Recent recurrence of cystic lesions,  
Past history of acneform eruptions, Past history of pigmentation,

**Physical examination** (severity and sites)

Black comedones, Acneform eruptions, Scar formation, Pigmentation, Nail deformity,

**(4) . Dental examination**

**Chief complaint**

Toothache, Gingival bleeding, Pus discharge, Gingival swelling, Feeling of tooth extrusion, Pigmentation

**Items for oral examination** (No/Yes, site)

Gingivitis, Marginal periodontitis, Retarded eruptions of permanent teeth,  
Tooth pigmentation, Odontogenesis imperfecta, Abnormal occlusion, Other findings,

**Mucosal pigmentation** (severity, site, \*pattern, \*\*color)

Upper gingivae, Lower gingivae, Rt. buccal mucosa, Lt. buccal mucosa, Palate,  
Upper lip, Lower lip

**Teeth radiograph** (No/Yes)

\*Selection items for pattern (Diffuse, Spotted, Band-like, Linear, Faint, Scattered)

\*\*Selection items for color (Black, Brownish, Dark-brownish)

**(5) . Ophthalmological examination**

**Subjective symptoms** (Abnormal discharge from the eyes)

**Objective symptoms**

Edema of the eyelid, Conjunctival pigmentation, Cysts of meibomian glands,  
Cheesy secretion from meibomian glands,

---

**Table 3. Variables selected for principal components analysis**

No.	Variables	Factor Score	Examination classification
1	Pigmentation of lower gingivae	0.735	Dental examination
2	Blood sugar (increase)	0.443	Laboratory examination
3	Abdominal pain	0.408	Questionnaire
4	Past history of pigmentation	0.498	Dermatological examination
5	Arthralgia	0.437	Questionnaire
6	Pigmentation of the upper lip (diffuse)	0.401	Dental examination
7	Total glycerides (increase)	0.361	Laboratory examination
8	Sputum	0.307	Questionnaire
9	Mean corpuscular volume (increase)	0.419	Laboratory examination
10	$\gamma$ -GTP (increase)	0.367	Laboratory examination
11	Pigmentation of the upper lip (band-like)	0.581	Dental examination
12	albumin/globulin(A/G) ratio (decrease)	-0.471	Laboratory examination
13	General fatigue	-0.327	Questionnaire
14	Pigmentation (toe nails)	0.447	Dermatological examination
15	Tooth pigmentation	0.336	Dental examination
16	Pigmentation of the palatal mucosa	0.204	Dental examination
17	Pigmentation of the right buccal mucosa (band-like)	0.339	Dental examination
18	Past history (after the incident)	0.241	Questionnaire
19	Dental chief complaint	0.389	Dental examination
20	Nail deformity	-0.272	Dermatological examination
21	Numbness	0.208	Questionnaire
22	Pigmentation (face)	0.280	Dermatological examination
23	Abnormal discharge from the eyes	-0.297	Ophthalmological examination
24	Abnormal respiratory sounds	0.295	Physical examination
25	Pigmentation (left. buccal mucosa)	0.266	Dental examination
26	Total cholesterol (increase)	0.246	Laboratory examination
27	Cough	0.192	Questionnaire
28	Past history (Before the incident)	0.246	Questionnaire
29	Acneform eruptions (other sites)	0.297	Dermatological examination
30	Mucosal pigmentation of upper gingivae (linear)	0.363	Dental examination
31	Cheesy secretion from meibomian glands	-0.260	Ophthalmological examination
32	Presence of hepatomegaly	-0.317	Questionnaire
33	Direct-bilirubin (increase)	0.225	Laboratory examination
34	Abnormal heart sounds	0.239	Questionnaire
35	potassium level	0.286	Laboratory examination
36	Acneform eruptions (trunk)	0.216	Dermatological examination
37	Pigmentation (fingernails)	0.231	Dermatological examination
38	Malocclusion	0.274	Dental examination
39	Black comedones (other sites)	-0.323	Dermatological examination
40	Urinalysis protein	0.363	Laboratory examination
41	Systolic blood pressure (low)	-0.294	Questionnaire
42	Toothache	0.311	Dental examination
43	Edema of the eyelids	0.305	Ophthalmological examination
44	Headache	0.188	Questionnaire
45	Chief complaint	-0.224	Questionnaire
46	Pigmentation of the mucosa of the upper lip (spotted)	0.292	Dental examination
47	Black comedones (face)	0.240	Dermatological examination
48	Total bilirubin (increase)	0.225	Laboratory examination
49	Past history of acneform eruptions	0.248	Dermatological examination

**Table 4. Results of logistic regression analysis****4-1. 2,3,4,7,8-PeCDF blood level**

List of explanatory variables which showed less than 0.10 level of significance when 2,3,4,7,8-PeCDF blood level (2 categories) and the factors selected by the principal components analysis were used as objective variables and explanatory variables, respectively

Explanatory variables	$\beta$	Standard deviation	Wald	Significance probability	Exp (B)
1 PCB blood level	1.641	0.355	21.350	0.00000**	5.159
2 PCQ blood level	8.235	1.844	19.949	0.00001**	3771.361
3 Blood sugar (increase)	0.038	0.011	11.310	0.00077**	1.038
4 Arthralgia	3.734	1.159	10.382	0.00127**	41.857
5 Gender (female)	3.456	1.115	9.605	0.00194**	31.679
6 T-bilirubin (increase)	-3.310	1.194	7.681	0.00558**	0.037
7 Black comedones (face)	-2.216	0.836	7.021	0.00806**	0.109
8 Past history of skin pigmentation	3.576	1.435	6.209	0.01271**	35.735
9 A/G ratio (decrease)	1.978	0.825	5.748	0.01651**	7.225
10 Acneform eruptions (trunk)	3.809	1.650	5.331	0.02095**	45.088
11 Respiratory sounds (abnormal)	6.036	2.780	4.714	0.02991**	418.145
12 Acneform eruptions (other sites)	-5.514	2.721	4.107	0.04270**	0.004
13 Potassium level (increase)	-1.849	0.917	4.071	0.04361**	0.157
14 Past history of acneform eruptions	-2.630	1.304	4.065	0.04378**	0.072
15 Total cholesterol (increase)	-0.023	0.011	3.910	0.04799**	0.978
16 Heart sound (abnormal)	13.341	7.892	2.857	0.09096*	621883.792

\*  $P < 0.10$ , \*\*  $P < 0.05$

List of explanatory variables which showed less than 0.10 level of significance when PCB and PCQ blood levels were excluded from the explanatory variables in the above analysis

Explanatory variables	$\beta$	Standard deviation	Wald	Significance probability	Exp (B)
1 Past history of pigmentation	1.719	0.445	14.943	0.00011**	5.582
2 Age (old)	0.053	0.014	13.637	0.00022**	1.055
3 A/G ratio (decrease)	0.723	0.336	4.646	0.03113**	2.061
4 General fatigue	-0.652	0.304	4.593	0.03211**	0.521
5 Arthralgia	0.633	0.299	4.478	0.03433**	1.884
6 Gender (female)	0.615	0.334	3.390	0.06558*	1.850
7 Pigmentation of the right buccal mucosa (band-like)	2.596	1.422	3.332	0.06793*	13.416

\*  $P < 0.10$ , \*\*  $P < 0.05$

#### 4-2. PCB blood level

Explanatory variables that showed less than 0.10 level of significance when the PCB blood level (2 categories) and factors selected by the principal components analysis were used as objective variables and explanatory variables, respectively

	Explanatory variables	$\beta$	Standard deviation	Wald	Significance probability	Exp (B)
1	2,3,4,7,8-PeCDF blood level	0.012	0.003	15.412	0.00009**	1.012
2	Sputum	-2.818	0.855	10.876	0.00097**	0.060
3	Age (old)	0.129	0.039	10.834	0.00100**	1.137
4	Past history of pigmentation	-3.832	1.167	10.783	0.00102**	0.022
5	Gender (female)	-1.986	0.693	8.220	0.00414**	0.137
6	Past history of acneform eruptions	2.785	1.023	7.409	0.00649**	16.202
7	Pigmentation (toe nails)	-1.907	0.750	6.472	0.01096**	0.149
8	Hepatomegaly	-7.627	3.134	5.920	0.01497**	0.000
9	Headache	-1.554	0.704	4.871	0.02732**	0.211
10	Cheesy secretion from meibomian glands	2.623	1.248	4.414	0.03564**	13.773
11	Total bilirubin (increase)	-1.611	0.770	4.372	0.03654**	0.200
12	General fatigue	1.374	0.676	4.138	0.04194**	3.951

\*  $P < 0.10$ , \*\*  $P < 0.05$

Explanatory variables which showed less than 0.10 level of significance when 2,3,4,7,8-PeCDF blood level was excluded from the explanatory variables in the above analysis

	Explanatory variables	$\beta$	Standard deviation	Wald	Significance probability	Exp (B)
1	Age (old)	0.115	0.033	11.920	0.00056**	1.121
2	Sputum	-2.040	0.663	9.473	0.00208**	0.130
3	Past history of pigmentation	-2.432	0.958	6.440	0.01116**	0.088
4	Total bilirubin (decrease)	-1.701	0.686	6.145	0.01318**	0.182
5	PCQ blood level	1.142	0.480	5.654	0.01742**	3.134
6	Pigmentation (toe nails)	-1.418	0.621	5.213	0.02242**	0.242
7	Arthralgia	-4.886	2.357	4.299	0.03814**	0.008
8	Presence of a chief dental complaint	1.544	0.748	4.263	0.03896**	4.682
9	Headache	-1.276	0.624	4.179	0.04093**	0.279
10	Cheesy secretion from meibomian glands	2.616	1.291	4.110	0.04264**	13.682
11	Past history of acneform eruptions	1.650	0.853	3.743	0.05304*	5.205
12	Black comedones	-3.569	2.086	2.926	0.08716*	0.028
13	Mean corpuscular volume (increase)	-0.080	0.047	2.847	0.09154*	0.923
14	Urinalysis protein (increase)	-0.619	0.372	2.771	0.09600*	0.539

\*  $P < 0.10$ , \*\*  $P < 0.05$

### 4-3. PCQ blood level

Explanatory variables which showed less than 0.10 level of significance when PCQ blood level (2 categories) and the factors selected by the principal components analysis were used as objective variables and explanatory variables, respectively.

	Explanatory variables	$\beta$	Standard deviation	Wald	Significance probability	Exp (B)
1	2,3,4,7,8-PeCDF blood level	0.040	0.010	16.276	0.00005**	1.041
2	Tooth pigmentation	6.737	2.073	10.564	0.00115**	843.324
3	Arthralgia	-3.082	1.138	7.337	0.00675**	0.046
4	$\gamma$ -GTP (increase)	-0.065	0.024	7.132	0.00757**	0.937
5	Total bilirubin (decrease)	3.282	1.384	5.626	0.01769**	26.621
6	Cheesy secretion from meibomian glands	-7.612	3.230	5.554	0.01844**	0.000
7	General fatigue	2.983	1.273	5.497	0.01905**	19.756
8	Total cholesterol (increase)	0.042	0.018	5.260	0.02182**	1.043
9	Pigmentation (toe nails)	-3.974	1.906	4.348	0.03705**	0.019
10	Gender (female)	-2.227	1.111	4.015	0.04509**	0.108
11	Pigmentation of the right buccal mucosa (band-like)	-7.584	3.846	3.889	0.04860**	0.001
12	Acneform eruptions (other sites)	8.124	4.436	3.354	0.06705*	3372.941
13	Headache	-1.901	1.116	2.903	0.08842*	0.149

\*  $P < 0.10$ , \*\*  $P < 0.05$

Explanatory variables which showed less than 0.10 level of significance when 2,3,4,7,8-PeCDF blood level was excluded from the explanatory variables in the above analysis

	Explanatory variables	$\beta$	Standard deviation	Wald	Significance probability	Exp (B)
1	Past history of pigmentation	3.117	0.989	9.932	0.00162**	22.588
2	Tooth pigmentation	3.929	1.260	9.727	0.00182**	50.869
3	PCB blood level	0.437	0.156	7.842	0.00510**	1.547
4	Acneform eruptions (trunk)	-3.260	1.297	6.318	0.01195**	0.038
5	Abdominal pain	-1.779	0.781	5.184	0.02280**	0.169
6	Pigmentation (face)	4.678	2.105	4.937	0.02629**	107.516
7	Total cholesterol (increase)	0.021	0.010	4.310	0.03790**	1.021
8	Acneform eruptions (other sites)	3.613	2.003	3.252	0.07132*	37.059
9	Pigmentation of the upper lip (patchy)	3.161	1.830	2.984	0.08407*	23.590
10	$\gamma$ -GTP (increase)	-0.021	0.013	2.721	0.09901*	0.980

\*  $P < 0.10$ , \*\*  $P < 0.05$