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This Journal is supported by a Grant-in-Aid for Scientific Research (a Grant-in-Aid for Publication of Scientific Research Results) from Japan Society for the Promotion of Science.

Cited in: Elsevier BIOBASE/Current Awareness in Biological Sciences; EMBASE/Excerpta Medica; Index Medicus; Current Contents/Clinical Medicine; Science Citation Index; SciSearch

Journal of Dermatological Science Supplement (2005) 1, S1

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Preface

A mass poisoning involving at least 1860 individuals occurred in Kyushu, western Japan, in 1968. The incident is called Yusho oil disease because it was caused by the ingestion of rice bran oil that was contaminated with Kanechlor-400, a commercial brand of Japanese polychlorinated biphenyls (PCBs). It was later found that the rice bran oil had been contaminated not only with PCBs but also with polychlorinated dibenzofurans (PCDFs), polychlorinated quaterphenyls (PCQs), and other related compounds. Yusho is thus recognized as poisoning by a mixture of PCBs, dioxins and related compounds. For more than 35 years the patients with Yusho have suffered from various symptoms such as general malaise, headache, acneform eruption, dark-brownish nail pigmentation, increased discharge from the eyes with swelling of the eyelids, pigmentation of oral mucosa, peripheral neuropathy, irregular menstruation in women, and growth retardation in infants and children.

From the epidemiological survey of 141 Yusho patients in Fukuoka, total amounts of PCBs, PCDFs, and toxic equivalent quantity (TEQ) ingested by each patient were calculated to be 633, 3.4 and 0.62 mg, respectively. From the follow-up data of three Yu-cheng patients and five Yusho patients, fat-based concentrations of TEQ and PCBs in the Yusho patients were estimated to have decreased from 40 ppb and 75 ppm, respectively, in 1969 to 0.6 ppb and 2.3 ppm, respectively, in 1999. We recently started to measure the blood levels of dioxins in Yusho patients during the annual medical check-up. Mean blood levels of total dioxins (pg-TEQ/g lipid) and 2,3,4,7,8-PeCDF (pg/g lipid) in patients with Yusho was 3.4—4.8 and 11.6—16.8 times higher than the mean levels in normal controls, respectively. It is surprising that dioxins and PCBs remain in the tissue for such a long time. The severity of symptoms in patients with Yusho has gradually improved during the past 30 years. However, a number of patients still suffer from specific symptoms of Yusho. The levels of PCDFs are still significantly correlated with some of the specific clinical signs of Yusho such as acneform eruptions, comedones, gingival pigmentation, and elevated triglyceride levels.

In this supplement we will discuss the clinical features, laboratory findings, and blood levels of dioxins in patients with Yusho. The lessons from Yusho are important in understanding the toxicity of PCBs and dioxins in humans. I very much appreciate the contribution and participation of the patients during health examinations for the follow-up of Yusho each year. I also deeply thank all of the members of the Study Group for Yusho for their efforts to help and support patients' health and well-being.

Acknowledgement

This work was supported in part by a Grant-in-Aid for scientific research from the, Ministry of Health, Labour and Welfare, Japan.

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1574-0757/$30.00
doi:10.1016/j.descs.2005.03.001

Journal of Dermatological Science Supplement (2005) 1, S3—S10

Journal of Dermatological SCIENCE

www.intl.elsevierhealth.com/journals/jods

Overview of Yusho

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KEYWORDS
Dioxin;
Polychlorinated biphenyls (PCBs);
Polychlorinated dibenzofurans (PCDFs);
Review;
Yusho

Summary

Background: Yusho is a type of food poisoning from rice bran oil contaminated with polychlorinated biphenyls (PCBs) and various dioxins such as polychlorinated dibenzofurans (PCDFs). The victims of Yusho suffered from dermatological manifestations (acneform eruptions, comedones, etc.) in association with systemic, ophthalmological, and mucosal symptoms.
Objective: To analyze the relationship between the concentrations of dioxins/PCBs and the subjective/objective complaints of patients with Yusho.
Methods: We recently started to measure the blood levels of dioxins in the annual medical check-up of Yusho patients. In addition, we reviewed the clinical and epidemiological findings elucidated over the past 36 years by the Study Group for Yusho.
Results and conclusion: High amounts of PCBs and PCDFs are still present in a number of patients with Yusho. The majority of laboratory findings, except for triglyceride concentration, were within normal limits throughout the clinical course. However, the patients still suffered from various mucocutaneous and subjective symptoms, and these symptoms were correlated to the blood levels of polychlorinated congeners. The development of therapeutic interventions is warranted in the near future.

1. History

Yusho occurred in 1968 and involved more than 1800 people in western Japan [1,2]. The disorder was caused by the ingestion of rice bran oil contaminated with polychlorinated biphenyls (PCBs)

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1574-0757/$30.00
doi:10.1016/j.descs.2005.03.002

(commercially known as Kanechlor 400) that had been used in the refining process of the oil. A field survey showed that 166 out of 170 patients had consumed the rice bran oil produced or shipped on February 5 and 6, 1968 [3,4]. Recently, our study group found that the rice bran oil was also contaminated with various dioxins such as polychlorinated dibenzofurans (PCDFs). As a result therefore, Yusho is now recognized as mixed toxicity from PCBs and dioxins [1]. The clinical and epidemiological observations made by the Study Group for Yusho have been extensively reviewed elsewhere by Kuratsune et al. [5], Okumura in 1984 [6], and Yoshimura in 2003 [1].

2. PCBs, PCQs and dioxins in Yusho

Following the occurrence of the Yusho incident in 1968, PCBs were identified in the subcutaneous adipose tissue of patients with Yusho [7]. Analysis of whole blood PCBs started from 1973, 5 years after the Yusho incident. In 1974, the mean blood PCB levels were found to be 7 ppb in 41 patients with Yusho and 3 ppb in 37 normal controls [6]. Gas chromatograms of the PCBs in the contaminated rice bran oil showed a characteristic pattern that coincided with that of PCBs in the blood of patients [6]. The gas chromatograms of blood PCBs, therefore, have been classified into four types in the diagnostic criteria of Yusho: type A, characteristic of Yusho; type C, the pattern commonly observed in the general population; types B and BC, intermediate patterns between A and C. The majority of patients with Yusho (95%) had type A or B [6]. The average PCB levels in patients with A, B and C patterns were 9, 4 and 2 ppb, respectively [6].

In 1981, Takamatsu et al. first demonstrated that the blood levels of polychlorinated quarterphenyls (PCQs; dimers of PCBs) were elevated in patients with Yusho [8]. Iida et al. reported that the concentrations of PCQs were closely correlated with the PCB concentrations and patterns [9]. In 1986, 18 years after the incident, the PCQ levels in the adipose tissue and blood in patients were still $>$100 times higher than corresponding levels in the controls. The correlation coefficients between the concentrations of PCBs and PCQs were higher than 0.8 from 1973 to 1980 [10]. The levels of PCQs in the blood of most of the controls were lower than the detection limit of 0.02 ppb. Japanese workers who had been occupationally exposed to PCBs did not have detectable levels of PCQs in their blood although their PCB levels were as high as 33 ppb, suggesting that PCQs were rather specific congeners in Yusho [11].

Nagayama et al. first detected a significant amount of PCDFs in the contaminated rice bran oil as well as in the tissues of patients with Yusho from 1975 to 1977 [12—14]. Of the PCDF mixtures of tri- to hexachlorinated compounds in the rice bran oil, penta- (PeCDFs) and hexachlorodibenzofurans (HxCDFs) were mainly found to accumulate in the tissue. In patients with Yusho, the most abundant PCDF congener in the liver was 2,3,4,7,8-PeCDF. High concentrations of 2,3,4,7,8-PeCDF and 1,2,3,4,7,8-HxCDF of up to 25 and 72 ppb were observed in the liver and adipose tissue of Yusho patients in 1969, 1 year after the incident [3]. PCBs were found to mainly accumulate in adipose tissue; in contrast, PCDFs were present at high concentrations in the liver [14].

The formation of PCQs and PCDFs was associated with the heating process of Kanechlor 400 (PCBs) at high temperatures. The contaminated rice bran oil was shown to contain 920, 5 and 866 ppm of PCBs, PCDFs and PCQs, respectively. Therefore, the total amounts of PCBs, PCDFs and PCQs ingested by a patient were estimated to be 633, 3.4 and 596 mg, respectively, on average [15]. Polychlorinated dibenzo-*p*-dioxins (PCDDs) and coplanar PCBs were also detected in the rice bran oil and in patients' tissues [16]. The toxicities of the individual congeners of PCDDs, PCDFs and PCBs have been evaluated in comparison with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) by many organizations [17—19]. The toxic equivalent quantity (TEQ) of 2,3,7,8-TCDD is calculated for the individual congeners using the toxic equivalency factors (TEFs) of PCDDs, PCDFs and PCBs [20]. The total TEQ of the contaminated rice bran oil was calculated to be 0.98 ppm, consisting of 91% PCDFs, 8% PCBs and 1% PCDDs. 2,3,4,7,8-PeCDF was found to contribute to 69% of the total TEQ of the contaminated rice bran oil. In patients with Yusho, 89% and 76% of the total TEQ were considered to be due to the PCDFs in the adipose tissue and blood, respectively, whereas 76% and 65% of the total TEQ were attributed by a single congener of 2,3,4,7,8-PeCDF in the adipose tissue and blood, respectively [3].

3. Clinical features and diagnostic criteria

Nonspecific subjective symptoms such as general fatigue, weight loss, and anorexia were observed in the majority of patients [2,6]. In addition to these general symptoms, various characteristic objective symptoms gradually appeared in the patients, including dermatological manifestations (comedones, acneform eruptions, black spots in hair

pores, dark-brownish pigmentation of skin and nails), ophthalmological manifestations (increased cheese-like discharge from meibomian glands, pigmentation of conjunctiva, swelling of upper eyelids, temporary failure of eyesight), and oral manifestations (pigmentation of gingiva). A considerable number of patients also suffered from headache, paresthesia of extremities, abdominal pain, cough and sputa, dysmenorrhea, and growth retardation in infants and children. No patient presented with jaundice or palpable spleen [7,21,22]. Epidemiological studies showed that 13 women (10 Yusho patients, two not registered, one unspecified) in Yusho families in Fukuoka had 11 live births and two stillbirths from February to December 1968 [23,24]. Of these babies, 10 had shown characteristic grayish dark brown pigmented skin at birth (black babies). The majority of the babies were small for dates. The peculiar cutaneous pigmentation gradually disappeared, and no evidence has been obtained in regard to the possible mental and physical retardation in the black babies [23,24]. Later, it was confirmed that PCBs and PCDFs were transferred from poisoned mothers to their fetuses via placenta and breast milk [25,26].

In sharp contrast to the characteristic clinical features, the majority of laboratory findings remained within normal limits [6]. Slight anemia and leucocytosis were observed only in severe cases. No constant abnormality was encountered in liver function tests including the bromosulfophthalein test. Serum protein levels were also normal except for occasional elevation of α2-globulin fractions. Serum electrolytes, blood urea nitrogen, serum iron, and serum zinc levels were also normal. In severe cases, serum copper levels were markedly increased. Results of oral 50 g glucose tolerance tests were normal. Notable elevation of serum lipid levels, particularly those of serum triglycerides, was evident [6]. The correlation between PCB exposure and elevation of serum triglyceride levels has been a great concern since the Yusho incident. A study on workers occupationally exposed to PCBs reported a correlation between blood PCBs and serum triglyceride concentrations [8].

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 symptoms and signs (Table 1). Hyperglyceridemia, pulmonary disorders, intractable headache, elevated blood PCB concentrations, and specific PCB patterns on gas chromatograms were added to the initial criteria in 1972 and 1976 (Table 2). Blood PCQ levels were added to the criteria in 1981 (Table 2). In addition to the diagnostic criteria, therapeutic guidelines were also produced (Tables 3 and 4).

4. Annual medical check-up of Yusho patients

Healthcare services and medical examinations have been provided and carried out by the Study Group for Yusho since the incident. The annual medical check-up includes an internal examination, dermatological, ophthalmological, dental and oral, and pediatric examinations, gynecological interview, complete blood cell counts, blood chemistry, analyses of blood PCBs and PCQs, chest X-ray, electrocardiogram, and abdominal ultrasonography. Subjective symptoms were also recorded.

The severity of symptoms in patients with Yusho has gradually improved. However, a number of patients still suffer from specific symptoms of Yusho. The polychlorinated aromatic hydrocarbons have been proven to be carcinogenic in animals [27,28]. Yusho may have caused at least liver cancer in male patients [1,29].

5. Blood levels of dioxins and dioxin-related compounds in Yusho patients

Although the blood levels of dioxins and dioxin-related compounds were very low, Todaka et al. (a member of the Study Group for Yusho) recently developed a new method of determining the concentration of dioxins (PCDFs, PCDDs and coplanar PCBs) in a blood sample of just 5 g with satisfactory accuracy and reproducibility [30]. This technical advancement has allowed us to include the measurement of blood levels of dioxins in the annual medical check-up from 2001. As shown in Table 5, from 2001 to 2003 the mean blood levels of total dioxins (pg-TEQ/g lipid) and 2,3,4,7,8-PeCDF (pg/g lipid) in patients with Yusho was 3.4—4.8 and 11.6—16.8 times higher than the mean levels in normal controls, respectively. It is known that dioxins and PCBs remain in the tissue for a long time. It is indeed of note that even 36 years after the incident high concentrations of dioxins still remain in the blood of patients with Yusho.

We analyzed the relationship between blood levels of PCDFs and the clinical and laboratory findings. The majority of laboratory findings did not show specific abnormalities such as had been reported at the initial stage of Yusho, and they were not correlated with the levels of PCDFs. However, the levels of PCDFs were significantly correlated with some of the specific clinical signs such as

Table 1 Diagnostic criteria and therapeutic guidelines for Yusho (1968)

These criteria are only meant to be applied to the specific disease called Yusho, which arose in western Japan and is suspected to have been caused by the use of a specific brand of contaminated rice bran oil. Therefore, these criteria cannot be applied to other dermatological diseases that may have been caused either directly or indirectly by the use of cooking oils.

Conditions of the incident

(1) The use of a specific brand of rice bran oil.
(2) Familial occurrence is seen in most cases. When not, some enquiry must be made as to the reason.
(3) In most cases, the incident occurred after April 1968.
(4) A time interval seems to be necessary after the use of this rice oil before the appearance of symptoms.

Diagnostic criteria

Symptoms and signs: swelling of the upper eyelids, increased discharge from the eye, lack of appetite, changes in nail color, loss of hair, swelling of the limbs, nausea, vomiting, a feeling of lassitude, numbness of the limbs, arthralgia and cutaneous symptoms are presented by many victims. In particular, increased eye discharge, change in nail color, and acneform eruptions are the most representative symptoms of this disease. Furthermore, the above symptoms are often accompanied by weakened eyesight and weight loss.

The general signs of Yusho that can be observed without the use of any special tests include the following:

1. Ocular signs

Increased eye discharge (secretion by the meibomian glands), hyperemia, opacity, and pigmentation of the ocular and fornix conjunctivas, pigmentation of the corneal limbs and transient visual disturbance are observed. It is also desirable to check for secretion by Giemsa staining to differentiate the findings from other ocular diseases.

2. Cutaneous signs

With abnormal keratinization as the main lesion, the following cutaneous signs are seen in Yusho patients:

(1) Pigmentation and occasional flattening of nails are seen without any discernible deformation.
(2) Blackish fine spots seen at the follicular orifice, which is markedly enlarged and elevated.
(3) Increased perspiration on the palms.
(4) Keratotic papules developing especially in areas of active perspiration and the secretion of sebum (in the axillae, etc.).
(5) Acneform eruptions: varying from comedone to acne conglobata in severe cases.
(6) Cyst formation in the sebaceous gland (often seen in the genital region).
(7) Child cases also show the above cutaneous signs but some patients have slightly different signs, i.e. some patients present with many exfoliative erythemas (each as large as a pinhead) all over the body, in particular in the flexor aspect of the limbs, with slight itching.
(8) No itching is experienced in most patients. If there is any itching, it is only slight and no scratching is seen.
(9) The skin becomes a slightly dirty-yellowish color, but in most patients no distinct pigmentation is seen.
(10) Seborrhea sicca.
(11) Pigmentation of the oral mucosa and of the gingivae is occasionally seen.
(12) An increase of cerumen is observed.

3. General signs

(1) Anemia, hepatomegaly and splenomegaly are not seen in most patients, but fever and disturbed liver functions are occasionally seen.
(2) Patients often present with numbness of the limbs and feelings of weakness, but no distinct paralysis is observed. In addition, some patients show a weakened or indiscernible deeper reflex. Hyperalgesia is occasionally seen at the periphery of the limbs.

Most of the above signs are seen in typical cases. However, it may at times be necessary to diagnose specific patients as doubtful cases of Yusho, while comprehensively taking into account such signs as excess sweating of the palms, pigmentation of the nails, increased eye discharge, comedone formation at the malar region, and other subjective symptoms.

Table 2 Diagnostic criteria for Yusho (1976 and 1981)

The diagnostic criteria for Yusho were revised October 26, 1972. Since then, some changes in the symptoms and signs have been observed over the course of time and the following criteria are now considered appropriate:

Conditions of the incident
- Proof that Kanemi rice bran oil contaminated with polychlorinated biphenyls (PCBs) was ingested.
- There are also some cases in which PCB is transferred from mothers with Yusho to their children.
- 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 tends to suppurate.
2. Pigmentation
   Pigmentation of the face, palpebral conjunctivas and nails of both the fingers and toes (including so-called 'black babies').
3. Hypersecretion by the meibomian glands.
4. Unusual composition and concentration of PCBs in the blood.

Symptoms and signs
1. Subjective symptoms
   - A feeling of lassitude
   - A feeling of heaviness in the head or headache
   - Paresthesia of the limbs (abnormal sensation)
   - Increased eye discharge
   - Cough and sputum
   - Inconstant abdominal pain
   - Altered menstruation

2. Objective manifestations
   - Manifestations of bronchitis
   - Deformation of the nails
   - Bursitis
   - Increased neutral fat in the serum
   - Serum γ-glutamyl transpeptidase (γ-GTP)
   - Decrease of serum bilirubin
   - Neonatal small-for-date (SFD) baby
   - Growth retardation and dental abnormality (retarded eruption of permanent teeth)

Supplement to the diagnostic criteria for Yusho (1981)

Under 'Important manifestations' in the diagnostic criteria for Yusho (revised June 14, 1976), the phrase '4. Unusual composition and concentration of PCBs in the blood' is followed by a new phrase, '5. Unusual composition and concentration of polychlorinated quarterphenyls (PCQs) in the blood'.

According to the studies hitherto undertaken, the following conclusions have been made in regard to the concentration of PCQs in the blood:
(1) $\geq$0.1 ppb: an abnormally high concentration.
(2) 0.03—0.09 ppb: the boundary between high and normal concentrations.
(3) $\leq$0.02 ppb (detection limit): normal concentration.

Notes
1. With reference to the above conditions of the incident, symptoms and manifestations, and taking into account the age of the examinees and the temporal progress of their illness, a diagnosis is comprehensively made.
2. These diagnostic criteria are to be used to determine whether a patient is affected with Yusho, but they do not necessarily relate to the severity of Yusho.
3. In regard to the abnormal properties and concentration of PCBs in the blood, regional differences as well as the patient's occupation should also be considered.

Table 3 Tentative therapeutic guidelines (1968)

(1) The administration of sulfhydryl (SH) compounds.
(2) The administration of vitamin B_2, etc.
(3) The topical application of ointments or lotions containing sulfur or other keratolytic ingredients.
(4) Hexachlorophene and other similar substances are used to keep the skin clean in an attempt to prevent secondary infections and to remove unpleasant odors.
(5) If any secondary infection is noted, chemotherapy is also considered.

Table 4 Therapeutic guidelines (1972)

1. Acceleration of excretion of polychlorinated biphenyls (PCBs).
Although it is presumed that the concentration of PCBs in patients with Yusho has been substantially reduced by now, the acceleration of PCB excretion remains essential. However, no effective drugs to accelerate the excretion have yet been developed because of the special nature of PCBs.

The present conceivable methods for the acceleration of excretion of PCBs are as follows:
(1) Fasting
(2) Enzyme induction methods
(3) The oral administration of appropriate adsorbents of PCBs

The adaptation and practice of fasting or enzyme induction methods, however, require a great degree of caution.

2. Symptomatic treatments
Symptomatic treatments include the administration of various detoxication drugs (e.g. glutathione in reduced form), remedies for lipid metabolism, analgesics, vitamin B preparations for the central nervous system, and cough medicine for respiratory symptoms. Hormone therapy is also considered for endocrinological symptoms. Various symptomatic treatments for cutaneous symptoms have also been performed and plastic surgery is also undertaken in some patients.
Various ophthalmological, orthopedic and conservative dental clinics conduct their own symptomatic treatments for the symptoms presented by these patients.

3. Treatment of complications
Patients with Yusho who show nervous and endocrinological disturbances, and signs of enzyme induction often have complications that tend to become severe. Therefore, they must be treated with due caution.
Furthermore, the biotransformation of drugs is also intensified by enzyme induction so that the administration of drugs at usual doses often fails to work effectively.

acneform eruptions, comedones, gingival pigmentation, and elevation of triglyceride levels. More specific data are presented elsewhere in this supplement.

6. Treatment and future research

It has been found that highly toxic congeners such as 2,3,4,7,8-PeCDF still remain in the bodies of

Table 5 Blood concentrations of dioxins and dioxin-related congeners in patients with Yusho and normal controls

	Yusho patients at annual check-up			Normal controls (n = 52)
	2001 (n = 78)	2002 (n = 279)	2003 (n = 269)	
Blood concentration of total dioxins and dioxin-related congeners (pg-TEQ/g lipid)				
Maximum	1049.7	1126.1	1176.6	85.4
Minimum	13.9	7	5.5	8.5
Mean	179.3	136.4	125.0	37.0
S.D.	180.5	148.9	141.2	17.6
Blood concentration of 2,3,4,7,8-PeCDF (pg/g lipid)				
Maximum	1770.6	1889.7	1953.5	41.7
Minimum	6.6	301	2.6	3.5
Mean	256.1	192.0	176.2	15.2
S.D.	315.3	252.0	240.2	8.9

PeCDF: pentachlorodibenzofuran; S.D.: standard deviation; TEQ: toxic equivalent quantity.

patients with Yusho. Beneficial treatments have not yet been established. However, Yoshimura and colleagues have extensively investigated methods to accelerate the excretion of these toxic compounds in an animal model. They demonstrated that in rats the oral administration of different antidotes (8% squalene, 8% paraffin, 5% cholestyramine and 5% charcoal) increased the excretion of 2,3,4,7,8-PeCDF 2—3 times more compared with the control group [31,32]. In addition to these antidotes, it was found that dietary fiber also accelerated the excretion of the polychlorinated congeners [33]. In fact, the fecal excretion of PCBs, PCDFs and PCDDs in rats was significantly increased by treatment with a combination of rice bran fiber (10% of the diet) and cholestyramine (5% of the diet) compared with the control group [34]. Two married couples with Yusho took 10 g rice bran fiber and 4 g cholestyramine suspended in a cup of water three times a day for 2 weeks after each meal. The amounts of 2,3,4,7,8-PeCDF in their stools actually increased. Although no beneficial effects have been observed during the short period of administration, some patients stated that they noticed an improvement in their cutaneous conditions [35,36]. Similar dietary treatments (6 g rice bran fiber and 4 g cholestyramine suspended in a cup of water three times a day after meals for 2 weeks) were also administered to eight patients with Yu-cheng (toxicity from oil contaminated with PCBs and dioxins in Taiwan, similar to the Yusho incident). The fecal excretion of 2,3,4,7,8-PeCDF and PCBs were significantly promoted by this dietary supplementation [37].

Fasting (starvation) therapy has been reported to be very effective at reducing the neurological complaints and slightly effective for dermatological manifestations [38,39]. In an animal model, it was demonstrated that the concentration of polychlorinated congeners in adipose tissue rapidly decreased with starvation, while the fecal excretion markedly increased [40,41]. Fasting also translocated the PCB congener in the adipose tissue to the lung, liver, brain, blood and skeletal muscle, causing a marked increase in these tissues up to the fourth week of fasting followed by a sharp decrease [40,41]. This was also the case in patients with Yusho and Yu-cheng [42,43]. The concentration of PCBs in the blood were increased during and after fasting compared with prefasting values, indicating the mobilization of the accumulated polychlorinated congeners from the adipose tissue.

Since the successful inclusion of the measurement of dioxins in the annual medical check-up of patients with Yusho, we hope to elucidate new treatments and care for these patients in the very near future.

Acknowledgement

This work was supported in part by a Grant-in-Aid for scientific research from the Ministry of Health, Labour and Welfare, Japan.

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Journal of Dermatological Science Supplement (2005) 1, S11—S20

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Behavior and toxic effects of PCBs and PCDFs in Yusho patients for 35 years

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KEYWORDS
Half-life;
Health effect;
Polychlorinated biphenyls (PCBs);
Polychlorinated dibenzofurans (PCDFs);
Toxic equivalent quantity (TEQ);
Toxicity;
Yu-cheng;
Yusho

Summary

Background: Yusho polychlorinated biphenyl (PCB) poisoning occurred in northern Kyushu in 1968, and the patients have been suffering from various symptoms for 35 years. From the epidemiological survey of 141 Yusho patients in Fukuoka, the total amounts of PCBs, polychlorinated dibenzofurans (PCDFs) and the toxic equivalent quantity (TEQ) ingested by each patient were calculated to be 633, 3.4 and 0.62 mg, respectively.
Objectives: The purpose of this paper is to review the behavior and toxic effects of these polychlorinated congeners in Yusho patients.
Results and conclusions: From the follow-up data of three Yu-cheng patients and five Yusho patients, fat-based concentrations of TEQ and PCBs in the Yusho patients were estimated to have decreased from 40 ppb and 75 ppm, respectively, in 1969, to 0.6 ppb and 2.3 ppm, respectively, in 1999. Estimated median half-lives of three PCDFs and six PCBs were 3.0 and 4.6 years, respectively, in the first 15 years after the incident, and 5.4 and 14.6 years, respectively, in the following 15 years. Patients have recovered gradually from the typical Yusho symptoms of acneform eruptions, dermal pigmentation and increased eye discharge. However, enzyme- and/or hormone-mediated signs of high serum triglyceride, high serum thyroxine, immunoglobulin disorder, etc. remain persistent for more than 30 years.

1. Introduction

A mass poisoning, involving at least 1860 individuals, occurred in northern Kyushu in 1968. The poisoning is called Yusho, oil disease, because it was caused by ingestion of rice bran oil that was contaminated with Kanechlor-400, a commercial brand of Japanese polychlorinated biphenyls (PCBs). It was later found that the rice bran oil had been contaminated not only with PCBs but also with polychlorinated dibenzofurans (PCDFs), polychlorinated quaterphenyls (PCQs) and other related compounds. Consequently Yusho is poisoning by a mixture of PCBs, PCDFs, PCQs and related compounds [1,2]. This paper describes the behavior of PCBs and PCDFs

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1574-0757/$30.00
doi:10.1016/j.descs.2005.03.003

in Yusho patients for more than 30 years, and their toxic effects are evaluated.

2. Intake of toxic agents by Yusho patients

A survey of 141 Yusho patients who consumed the rice bran oil, which contained 920, 5 and 866 ppm of PCBs, PCDFs and PCQs, respectively [3], indicated that the average consumption of the rice bran oil was 688 ml in total, and 506 ml during the latent period before the illness became apparent. Therefore, on average, the total amounts of PCBs, PCDFs and PCQs ingested by each patient were calculated to be 633, 3.4 and 596 mg, respectively, whereas the amounts ingested during the latent period were 466, 2.5 and 439 mg, respectively. The smallest amounts ingested by a patient during the latent period were estimated to be 111, 0.6 and 105 mg, respectively. Because the concentration of dioxin toxic equivalency quantity (TEQ) in the rice bran oil was determined to be 0.98 ppm, the amount of TEQ ingested per patient was calculated to be 0.62 mg in total, and 0.456 mg during the latent period. Table 1 lists the intake of rice bran oil and TEQ by the patients with Yusho. The lowest amount ingested by a patient was also estimated (Table 1). The lowest daily intake during the latent period (28 ng/kg body weight/day) was calculated from the oil intake (235 ml) of a particular patient (56 kg) during 135 days of the latent period. The clinical severity of illness and the patient's blood PCB levels showed a close positive correlation with the total amount of oil consumed but not with the amount of oil consumed per kg body weight per day [4]. This may indicate that after exposure to highly persistent toxic agents, the level of toxic agents in the body increased up to the level at which the symptoms of Yusho developed. The total amounts of ingested toxic agents may be important in estimating the severity of illness, because these agents accumulate in the body.

3. Toxic agents in the tissue and blood of Yusho patients

3.1. PCBs

The technique for quantification of PCBs in the blood was developed after 1973, 5 years after the Yusho incident. Since that time, many blood samples of Yusho patients have been analyzed for PCB levels. The blood PCB levels of Yusho patients were always only two to three times higher than the levels in normal controls. However, the gas chromatographic patterns of PCBs in Yusho patients were different from those of the controls [5]. The chromatograms were classified into the following three types. Type A: characteristic of Yusho; type B: an intermediate pattern between types A and C; and type C: commonly observed in the general population [6]. Yusho patients with type A chromatogram patterns of PCBs usually had significantly higher PCB concentrations than controls with type C. Typical Yusho patients had type A chromatogram patterns, which were characterized by lower concentrations of 2,3′,4,4′,5-pentachlorobiphenyl (2,3′,4,4′,5-PeCB) and much higher concentrations of 2,3,3′,4,4′,5-hexachlorobiphenyl (2,3,3′,4,4′,5-HxCB) [7]. Therefore, this characteristic difference has since been adopted as one of the criteria for the diagnosis of Yusho.

The biological half-lives of PCB congeners were determined in three Taiwanese patients with PCB poisoning (Yu-cheng) who had very high blood PCB levels (156—397 ng/g blood) [8]. The half-lives of 2,3′,4,4′,5-PeCB; 2,2′,4,4′,5,5′-HxCB and 2,3,3′,4,4′,5-HxCB were determined to be 1.6, 4.2 and 5.3 years on average, respectively, from 1980 to 1995 (Table 2). The medians of half-lives of the same congeners in five Yusho patients were 17.6, 9.1 and 13.2 years, respectively, from 1982 (14 years after the incident) to 1998, which thus suggested the overall elimination to be longer than the 10-year half-life [9]. When the blood PCB concentration is as high as 100 ng/g blood, as observed in the Yu-cheng patients, the elimination of PCBs is relatively fast

Table 1 The estimated intakes of rice bran oil and 2,3,7,8-TCDD TEQ by Yusho patients

Intake	Rice bran oil	TEQ
Mean total intake per patient	688 (195—3375) ml	0.62 (0.18—3.04) mg
Mean intake during latent period	506 (121—1934) ml	0.456 (0.11—1.74) mg
Average daily intake	0.171 (0.031—0.923) ml/kg/day	154 (28—832) ng/kg/day
Lowest intake during the latent period	121 ml	0.11 mg
Lowest daily intake during the latent period	0.031 ml/kg/day	28 ng/kg/day

Data are shown as mean (range) for the 141 patients. The TEQs are calculated using 0.98 ppm in Yusho oil and 0.92 for oil density.
TCDD: tetrachlorodibenzodioxin; TEQ: toxic equivalent quantity.

Table 2 Biological half-life of PCDF and PCB congeners in Yusho and Yu-cheng patients

	Half-life (years)									
	Yu-cheng patients 0.6—15.6 years after the incident				Yusho patients 14.0—29.1 years after the incident					
	BS	SS	RK	Median	KK	TS	YUM	TH	HH	Median
2,3,4,7,8-PeCDF	2.7	3.6	2.9	2.9	14.3	7.7	6.1	5.2	11.4	7.7
1,2,3,4,7,8-HxCDF	2.7	3.6	3.5	3.5	6.5	4.5	3.9	5.1	6.9	5.1
1,2,3,4,6,7,8-HeCDF	2.6	2.5	2.2	2.5	6.6	2.6	3.5	3.5	3.4	3.5
Average	2.7	3.2	2.9	3.0	9.1	4.9	4.5	4.6	7.2	5.4
2,3′,4,4′,5-PeCB	1.6	1.9	1.5	1.6	19.5	6.9	33.7	17.6	10.4	17.6
2,2′,4,4′,5,5′-HxCB	3.4	4.2	4.2	4.2	9.1	7.4	16.0	12.9	7.4	9.1
2,2′,3,4,4′,5′-HxCB	4.4	4.5	5.5	4.5	12.8	8.9	13.7	31.0	9.5	12.8
2,3,3′,4,4′,5-HxCB	3.8	5.6	5.3	5.3	9.4	8.5	21.5	13.2	14.4	13.2
2,2′,3,3′,4,4′,5-HeCB	4.7	6.0	5.9	5.9	18.4	12.3	−237.5	13.3	443.7	18.4
2,2′,3,4,4′,5,5′-HeCB	4.3	6.0	6.0	6.0	16.7	12.2	20.4	10.3	224.6	16.7
Average	3.7	4.7	4.7	4.6	14.3	9.4	21.1 Excluding −237.5	16.4	118.3	14.6

CB: chlorinated biphenyl; CDF: chlorinated dibenzofuran; He: hepta; Hx: hexa; PCB: polychlorinated biphenyl; PCDF: polychlorinated dibenzofuran; Pe: penta.

because the half-lives are 4.6 years on average. In contrast, when the PCB concentration is as low as 3 ng/g blood, as seen in the Yusho patients 14 years after the incident, elimination is very slow because the half-lives are longer than 10 years. The shorter half-life of 2,3′,4,4′,5-PeCB in the first 10 years after exposure may thus partly cause the particular pattern as observed in Yusho patients.

Of the PCB congeners identified in Yusho patients, 2,3,3′,4,4′,5-HxCB showed strong enzyme-inducing activity in the liver and marked atrophy of the thymus in rats [10,11]. Therefore, 2,3,3′,4,4′,5-HxCB was considered to be one of the PCB congeners most causally related to the symptoms of Yusho.

Only several selected congeners of PCBs in the rice bran oil were retained in the body of the patients as described above, and most of the PCB congeners had disappeared from the body within about 1 year either by excretion or by being metabolized into hydroxy- and methylsulfone PCBs. The methylsulfone PCBs, which were probably derived from the PCBs ingested with the rice bran oil, were identified in the tissue of Yusho patients [12,13]. The fat-based concentration of methylsulfone PCBs was higher in the lung (0.67 ppm) than in adipose tissue (0.07 ppm). These values contrasted with the concentration of PCBs, which measured 0.8 and 1.3 ppm, in lung and adipose tissue, respectively [14]. Some congeners of methylsulfone PCBs either induced or changed the enzyme activity in the human body. One of the metabolites, 3′-methylsulfone-3,4,4′,5′-tetrachlorobiphenyl, was found to strongly inhibit aromatic hydrocarbon hydroxylase (AHH) activity that was either previously or simultaneously induced by 2,3,7,8-tetrachlorodibenzodioxin (TCDD) in a human lymphoblastoid cell culture [15,16]. The same methylsulfone PCB inhibited methylcholanthrene-induced AHH activity in mouse liver microsomes in aryl hydrocarbon (Ah)-responsive strains of mouse, whereas it greatly enhanced the same enzymes in Ah-non-responsive strains [17]. Some 3-methylsulfone PCBs had stronger inductive effects on aminopyrine *N*-demethylase, 7-ethoxycoumarin *O*-deethylase and benzo(a)pyrene hydroxylase than the corresponding parent PCBs did, whereas 4-methylsulfone PCBs had little effect [18]. Therefore, the health status of the patients is possibly altered by the accumulation of methylsulfone PCBs in the tissues. Human blood was found to contain hydroxylated (OH)-PCBs at concentrations of 0.2 (controls) and 0.4 (Yusho patients) ng/g blood, which corresponded to one-fifth to one-seventh of the PCB level [19].

In the umbilical cord of neonates from the coastal population, the sum of the plasma concentrations of phenolic compounds, pentachlorophenol and OH-PCBs, were negatively correlated to free thyroxine plasma levels, suggesting that these phenolic compounds can alter thyroid hormone status in newborns [20]. The phenolic compounds could inhibit thyroxin transport by competitive binding to transthyretin [21]. In animal experiments using ^{14}C-labeled 4-OH-2,3,3′,4′,5-PeCB [22], exposure of pregnant rats to this compound resulted in a drastic reduction in fetal plasma thyroid hormone concen-

tration, and in an accumulation of the compound in fetal liver, brain and plasma. A low dose of OH-PCB (0.1 nM) suppressed thyroid-hormone-induced transcriptional activation in brain-derived cell line [23]. High levels of PCB congeners in humans are probably metabolized to high levels of OH-PCBs in the blood. In Yusho patients, the metabolism of PCB congeners was accelerated by the presence of toxic PCDFs, and the level of OH-PCB would have been as high as 100 nM, suggesting that brain development would have been retarded in fetuses. Kester et al. [24] investigated the potential inhibition of human estrogen sulfotransferase by various OH-PCBs. They demonstrated that the OH-PCBs identified in human blood were extremely potent inhibitors of human estrogen sulfotransferase, suggesting that they induce estrogenic activity by increasing estradiol bioavailability in target tissues. Shevtsov et al. [25] determined the crystal structure of human estrogen sulfotransferase in the presence of the sulfuryl donor product 3′-phosphoadenosine-5′-phosphate and 4,4′-diOH-3,3′,5,5′-TCB, and the bound crystal structure gives physical evidence that certain OH-PCBs can mimic the binding of estrogenic compounds in biological systems.

3.2. PCDFs

PCDFs were first identified in the tissue of Yusho patients by Nagayama et al. [26], after the rice bran oil was proved to be contaminated with PCDFs in 1975 [27]. Of the PCDF mixtures of tri- to hexachlorinated compounds in the rice bran oil, penta- and hexachlorinated PCDFs were mainly found to persist in the tissue. The concentrations of PCDFs were determined by comparing the peak heights of gas chromatographic peaks with those of a synthesized PCDF mixture. In contrast to the PCBs, which were found to be far more abundant in the adipose tissue than in the liver, PCDFs were present at very similar levels in these two types of tissue. The accumulation of toxic PCDF congeners in the liver has also been observed in the liver of monkeys and rats [28]. Since individual PCDF congeners were separately synthesized from chlorophenols and chloronitrobenzenes [29], PCDF congeners in the tissue could be quantified by using the individual congeners as standard compounds. Although Yusho patients ingested more than 40 types of PCDF congeners in the rice bran oil [30], only several specific PCDF congeners were retained in the patients' tissues [31,32]. Most of the retained PCDF congeners had lateral (2, 3, 7 and 8) positions that were chlorinated, and all the congeners that had apparently been excreted had two vicinal hydrogenated C-atoms in at least one of the two rings. The PCDFs chlorinated at lateral positions were identified in the tissues of not only Yusho patients but also Yu-cheng patients. However, the most abundant PCDF congener in the liver was 2,3,4,7,8-pentachlorodibenzofuran (2,3,4,7,8-PeCDF) in Yusho patients and 1,2,3,4,7,8-HxCDF in Yu-cheng patients. Higher levels of PCDF congeners than in the controls continued up to 1986, when the levels of PCDF congeners were 2—73 times higher than in the controls, whereas the PCB levels in the patients were only one to five times higher than in the controls [33].

It is noteworthy to mention that the PCDF concentrations in liver were similar to those in adipose tissue, whereas the PCB concentrations were much lower in liver than in adipose tissue. This relative abundance of PCDF congeners in the liver was also noted in unaffected people [34]. The pharmacokinetics of PCDFs in humans was studied by monitoring the blood concentrations of three Yu-cheng patients from 1980 to 1995 [9,35]. The half-lives of 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF and 1,2,3,4,6,7,8-heptachlorodibenzofuran (HeCDF) were 2.9, 3.5 and 2.5 years, respectively, and the concentrations of these PCDFs had decreased from 15, 43 and 5 ppb, fat-based, respectively, at the first sampling in 1980 (Table 2). The half-lives of these PCDFs were shorter than those of very persistent PCB congeners, such as 2,2′,4,4′,5,5′-HxCB (4.2 years) and 2,3,3′,4,4′,5-HxCB (5.3 years), in the same Yu-cheng patients. Using data from five Yusho patients with PCDF analyses at 14 time points from 1982 to 1998, the median half-lives for the elimination of 2,3,4,7,8-PeCDF and 1,2,3,4,7,8-HxCDF were estimated to be 7.7 (range 5.2—14.3) and 5.1 (range 3.9—6.5) years, respectively. Changes in concentration of PCB and PCDF congeners in Yusho patients from 1968 to 1999 were estimated from the available data from Yusho and Yu-cheng patients [36]. Estimated levels of total PCBs (75 μg/g lipid) and TEQ (40 ng/g lipid) in Yusho patients just after the incident decreased to 2.3 μg/g lipid and 0.6 ng/g lipid, respectively, 30 years after the incident with half-lives during the first 15 years of 4.2 and 2.9 years, and during the following 15 years of 9.1 and 7.7 years, respectively (Fig. 1). Blood samples of 83 Yusho patients were examined in 1995 for PCB and TEQ levels, and the mean levels were 0.8 μg/g lipid (range 0.09—5.2) and 0.16 ng/g lipid (range 0.01—1.02), respectively [37]. Blood samples of 152 Fukuoka residents were examined in 1999 for determination of PCB and TEQ concentrations. Their mean levels were 0.4 μg/g (range 0.06—1.7) lipid and 28 pg/g lipid (range 9.2—100), respectively [38]. Mean values of PCBs and TEQ in Yusho patients were only two and six times higher, respectively, than those in controls in 1999.

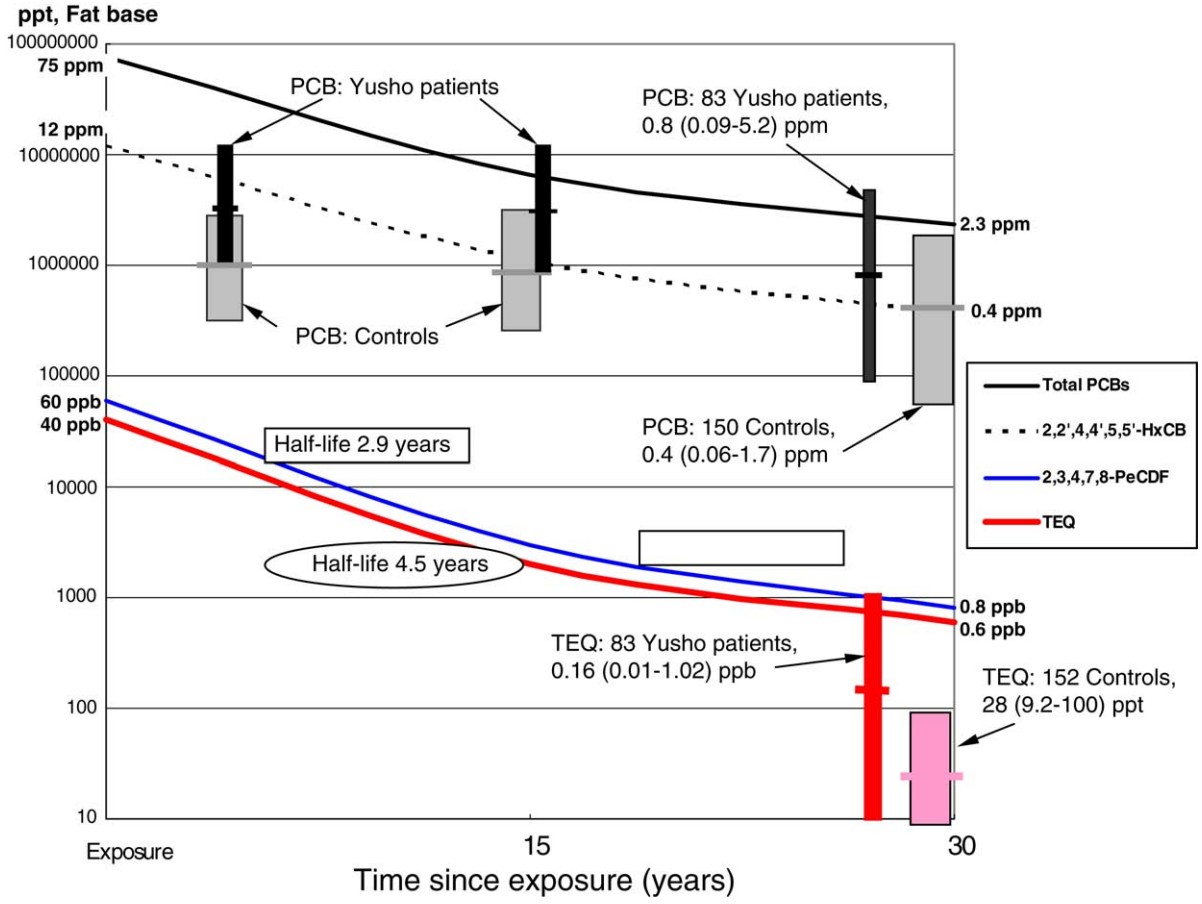

Fig. 1 Changes in PCB/TEQ concentrations in Yusho patients from 1969 to 1999. HxCB: hexaclorobiphenyl; PCB: polychlorinated biphenyl; PeCDF: pentachlorodibenzofuran; TEQ: toxic equivalent quantity.

A toxicological assessment of individual PCDF congeners was made in rats [10]. All the PCDF congeners retained in the tissue of Yusho patients exhibited a strong enzyme induction in AHH and nicotinamide adenine dinucleotide phosphate: quinone oxidoreductase (DT-diaphorase), and caused marked atrophy of the thymus and hypertrophy of the liver in rats.

PCDF congeners with at least three chlorine atoms in the ring position at 2, 3, 7 and 8 exhibited a marked increase in enzyme induction. 2,3,7,8-Tetrachlorodibenzofuran (TCDF) and 2,3,4,7,8-PeCDF significantly induced AHH and DT-diaphorase even at a single dose of 1 μg/kg body weight. Safe [39] summarized the structure of PCDFs and their toxicity in animals as follows: the AHH-inducing activities of structurally different PCDF congeners were linearly correlated with thymic atrophy, loss in body weight and immunotoxicity induced by the PCDF congeners. Of the PCDF congeners, 2,3,4,7,8-PeCDF was the most active compound regarding both enzyme induction and toxicity in animals. Therefore, 2,3,4,7,8-PeCDF is considered to be the most important etiologic agent for Yusho symptoms. The concentration of 2,3,4,7,8-PeCDF in the rice bran oil consumed by Yusho patients was 1350 ppb and the average total intake of the rice bran oil per person was 688 ml. Thus, 688 ml of rice bran oil contained 854 μg of 2,3,4,7,8-PeCDF. The total intake of this congener was calculated to be 14 μg/kg body weight, assuming the patients weighed 60 kg. This dose exceeded the enzyme-inducing dose of 1 μg/kg body weight in rats by more than 10 times. Kashimoto et al. [40] proposed that PCDFs were the major pathogenic substances in the development of Yusho, because the toxic PCDFs accumulated in the tissues and liver of Yusho patients but not in workers with occupational PCB poisoning. In a statistical study of the correlation between PCB, PCQ and PCDF concentrations in adipose tissue and clinical findings (such as headache, acneform eruptions, meibomian gland disorders, etc.) in Yusho patients, the PCDF concentration in subcutaneous adipose tissue and the total score of clinical findings had the highest correlation coefficient in female patients [41].

The relative toxicities of polychlorinated dibenzodioxin (PCDD) and PCDF congeners to 2,3,7,8-

TCDD (toxic equivalency factors, TEFs) have been estimated by many research organizations. Using international TEFs for PCDDs and PCDFs, and TEFs for PCBs from the World Health Organization (WHO) [42], the toxic contributions of the PCDDs, PCDFs and PCBs retained in Yusho patients were calculated (Table 3). Of the total TEQ concentrations, 89 and 76% were considered to be due to the PCDFs in adipose tissue and in the blood, respectively, whereas 76 and 65%, respectively, of the total toxicity were attributed to a single congener of 2,3,4,7,8-PeCDF. However, in the serum of controls,

Table 3 Concentrations of TEQ in adipose tissue and blood of Yusho patients and controls

	TEQ factor	TEQ concentration (ppt)		
		Yusho patients		Controls
		Adipose, 1977 (wet-based)	Blood, 1990—1991 (fat-based)	Serum, 1991—1992 (fat-based)
2,3,7,8-TCDD	1	0.9	2.3	3.1
1,2,3,7,8-PeCDD	1	18.0	7.2	9.2
1,2,3,4,7,8-HxCDD	0.1	0.1	0.3	0.4
1,2,3,6,7,8-HxCDD	0.1	16.0	3.6	3.9
1,2,3,7,8,9-HxCDD	0.1	0.1	0.5	0.8
1,2,3,4,6,7,8-HeCDD	0.01	0.1	0.2	0.5
OCDD	0.0001	0.0	0.1	0.1
Total PCDDs		35.1	14.1	18.0
Percentage of total TEQ		3	8	29
2,3,7,8-TCDF	0.1	4.4	0.0	0.5
2,3,4,7,8-PeCDF	0.5	850.0	120.8	8.7
1,2,3,7,8-PeCDF	0.05	1.5	0.1	0.0
1,2,3,4,7,8-HxCDF	0.1	130.0	15.3	1.2
1,2,3,6,7,8-HxCDF	0.1	14.0	3.4	0.8
2,3,4,6,7,8-HxCDF	0.1	0.1	0.0	0.0
1,2,3,7,8,9-HxCDF	0.1	nd	0.4	0.3
1,2,3,4,6,7,8-HeCDF	0.01	1.0	0.2	0.1
1,2,3,4,7,8,9-HeCDF	0.01	nd	0.0	0.0
OCDF	0.0001	nd	0.0	0.0
Total PCDFs		1000.9	140.1	11.7
Percentage of total TEQ		89	76	19
3,4,5,4′-TCB	0.0001	nd	nd	nd
3,3′,4,4′-TCB	0.0001	0.1	0.0	0.0
3,3′,4,4′,5-PeCB	0.1	72.0	4.5	14.2
3,3′,4,4′,5,5′-HxCB	0.01	3.8	1.3	0.9
Total coplanar PCBs		76.2	5.8	15.1
2,3,3′,4,4′-PeCB	0.0001	0.3	0.4	1.0
2,3,4,4′,5-PeCB	0.0005		1.6	1.4
2,3′,4,4′,5-PeCB	0.0001	0.4	1.4	4.2
2′,3,4,4′,5-PeCB	0.0001		0.0	0.1
2,3,3′,4,4′,5-HxCB	0.0005	16.9	16.7	8.0
2,3,3′,4,4′,5′-HxCB	0.0005		4.4	1.8
2,3′,4,4′,5,5′-HxCB	0.00001		0.1	0.1
2,3,3′,4,4′,5,5′-HeCB	0.0001		0.2	0.1
Total mono-ortho PCBs		17.5	24.7	16.7
Total PCBs		93.7	30.5	31.8
Percentage of total TEQ		8	16	52
Total TEQ		1130	185	61

CB: chlorinated biphenyl; CDD: chlorinated dibenzodioxin; CDF: chlorinated dibenzofuran; He: hepta; Hx: hexa; nd: not detected; OCDF: octachlorodibenzofuran; OCDD: octochlorodibenzodioxin; Pe: penta; TCB: tetrachlorobiphenyl; TCDD: tetrachlorodibenzodioxin; TCDF: tetrachlorodibenzofuran; TEQ: toxic equivalent quantity.

3,3′,4,4′,5-PeCB was considered to contribute the most to the total toxicity (23%), well surpassing the contributions by other congeners of PCDDs, PCDFs and PCBs.

4. Risk assessment of PCDD/PCDFs and PCBs from Yusho

In the general population, more than 90% of the total TEQ intake was from foods, and sources other than foods (such as air, water, soil, etc.) accounted for less than 10% of the total daily intake. The total TEQ levels (mean ± S.D.) of PCDD/PCDFs and coplanar PCBs were estimated to be 0.87 ± 0.28 ppt and 9.4 ± 7.3 ppt, respectively, for coastal fish, and 0.33 ± 0.25 ppt and 0.22 ± 0.24 ppt, respectively, for fish from the market [43]. It is noteworthy that the TEQ level of coplanar PCBs was greater than that of PCDD/PCDFs. The personal daily intakes of PCDDs, PCDFs and coplanar PCBs through foods were estimated to be 2.41, 2.16 and 51 ng, respectively, whereas the personal daily TEQ intakes from the three groups of toxic chemicals were calculated to be 40, 135 and 1100 pg, respectively [44]. The daily TEQ intakes calculated per kg body weight were 3 and 18 pg/kg body weight from PCDD/PCDFs and coplanar PCBs, respectively, assuming a typical body weight of 60 kg. The Japanese intake of TEQ from coplanar PCBs (18 pg/kg body weight/day) was much higher than that of the Dutch, whose TEQ intake from coplanar PCBs was estimated to be 1.4 and 2.5 pg/kg body weight/day as a median and a 95 percentile, respectively [45].

The WHO [46] investigated the PCDD/PCDF levels in breast milk collected from various countries, and found that TEQ level ranged from 5 to 40 ng/kg lipid. Relatively high levels of TEQ were found in the breast milk from central European countries and South Vietnam, followed by Japan, Nordic countries, Canada and the USA and then by east European countries, southeast Asian countries and New Zealand. Worldwide, babies who were fed with breast milk were estimated to consume 24—185 pg-TEQ/kg body weight/day, with a daily consumption of breast milk assumed to be 150 ml/kg body weight. Some Japanese babies were thus estimated to ingest higher levels of TEQ (100—530 pg/kg body weight/day) from PCDD/PCDFs and coplanar PCBs through breast-feeding, more than 60% of which was attributed to the TEQ of coplanar PCBs. Because the breast milk from Yusho patients was estimated to contain TEQ up to 539 pg/g lipid (of which 82% was attributable to PCDFs), a baby may consume as much as 3.3 ng-TEQ/kg/day from breast milk of a mother with Yusho, if the baby's breast milk consumption was 150 ml/kg body weight/day [47]. Current tolerable daily intake (TDI) of dioxins was established by the WHO in 1998 [42] and the Japanese Government in 1999. The values of TDI and the personal intakes of TEQ are illustrated in Fig. 2. The average and minimum daily intake of TEQ of a patient with Yusho are also shown in Fig. 2 to better understand the difference in the TEQ intake between Yusho patients and unaffected people. The Yusho limit is estimated to be 0.1 ng/kg body weight/day, because with a daily intake of 0.1 ng/kg body weight for a normal 60-year lifetime, the total intake of TEQ would eventually reach the minimum Yusho intake of 0.11 mg (Table 1). If the intake was less than that, the TEQ accumulation in a person would never exceed the minimum Yusho intake in a lifetime. When the average daily and the minimum intakes of TEQ by Yusho patients (154 and 28 ng/kg body weight/day, respectively) are compared with the average daily intakes of the general population (1—19 pg/kg body weight/day), there is a difference of more than 3 orders of magnitude. However, the periods of ingestion differ greatly for the two groups: 71 and 135 days for Yusho patients, and lifelong for the general population.

The TEQ levels of PCBs, PCDFs and PCDDs remaining in the Yusho patients were only 3—200 times higher than those in controls: 185—2000 pg-TEQ/kg lipid in the serum and adipose tissue of Yusho patients (Table 3) versus 10—60 pg-TEQ/g lipid in the breast milk of the general population [48]. In 1991, 23 years after the incident, the total TEQ level in the blood of a Yusho patient whose blood PCB chromatogram was typical of Yusho (type A) was only three times higher than that in the serum of a control, although the patient's level of PCDFs was 12 times higher than in the control (Table 3). When the intakes of nursing babies in the general population are compared with those of Yusho patients, the intakes of TEQ by babies fed with breast milk in the general population (530 pg/kg body weight/day at the highest) are 53 or more times lower than that of Yusho patients (28 ng/kg body weight/day at the lowest) (Fig. 2). Moreover, the duration for which the babies ingested toxic chemicals from breast milk are very similar to each other in the two groups: several months for babies in the general population and 1—5 months for Yusho patients. Because the intake of 28 ng-TEQ/kg body weight/day was the lowest dose to cause Yusho, the intake of 1 or 2 orders of magnitude lower than this level may be sufficient to cause mild Yusho symptoms (such as those mediated by receptor binding and enzyme induction caused by exposure) in a baby.

Besides the severe cutaneous and ocular symptoms, Yusho patients have also presented with var-

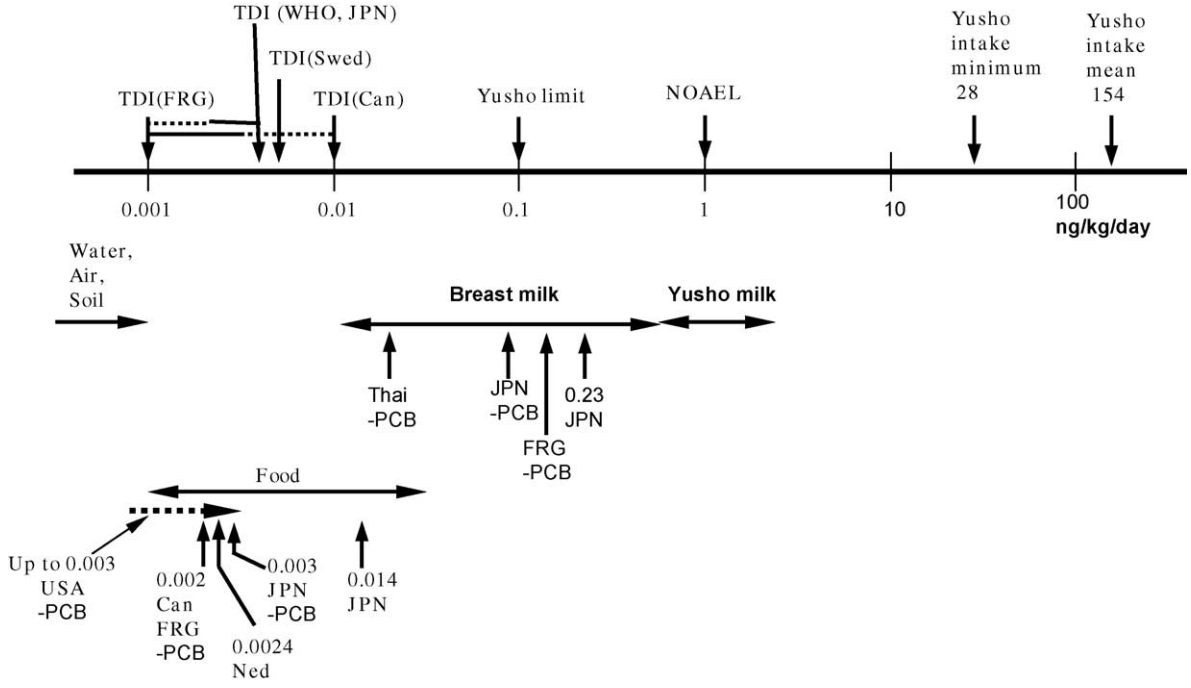

Fig. 2 Yusho intake, regulation and personal intake of TEQ. Can: Canada; FRG: Germany; JPN: Japan; Ned: Netherlands; NOAEL: no observed adverse effects level; PCB: polychlorinated biphenyls; Swed: Sweden; TDI: tolerable daily intake; TEQ: toxic equivalent quantity; Thai: Thailand; WHO: World Health Organization. —PCB: this figure does not include the TEQ from PCB.

ious other symptoms. Most symptoms were observed in the early stages of Yusho, whereas significantly elevated levels of serum thyroxines [49,50], serum triglyceride [51,52] and lymphocyte AHH [53] persisted in Yusho patients for more than 30 years after the initial exposure to PCBs and PCDFs. Pluim et al. [54] investigated the effect of PCDD/PCDFs on the concentration of thyroid hormone in humans. Thirty-eight healthy breast-fed infants were divided into two groups according to the PCDD/PCDF concentrations in the milk fat of their mothers. The total thyroxin concentrations in the blood were found to be significantly higher in the high-exposure group at birth and at ages 1 and 11 weeks. The plasma total thyroxin elevation in newborn infants is postulated to be the result of an effect of the

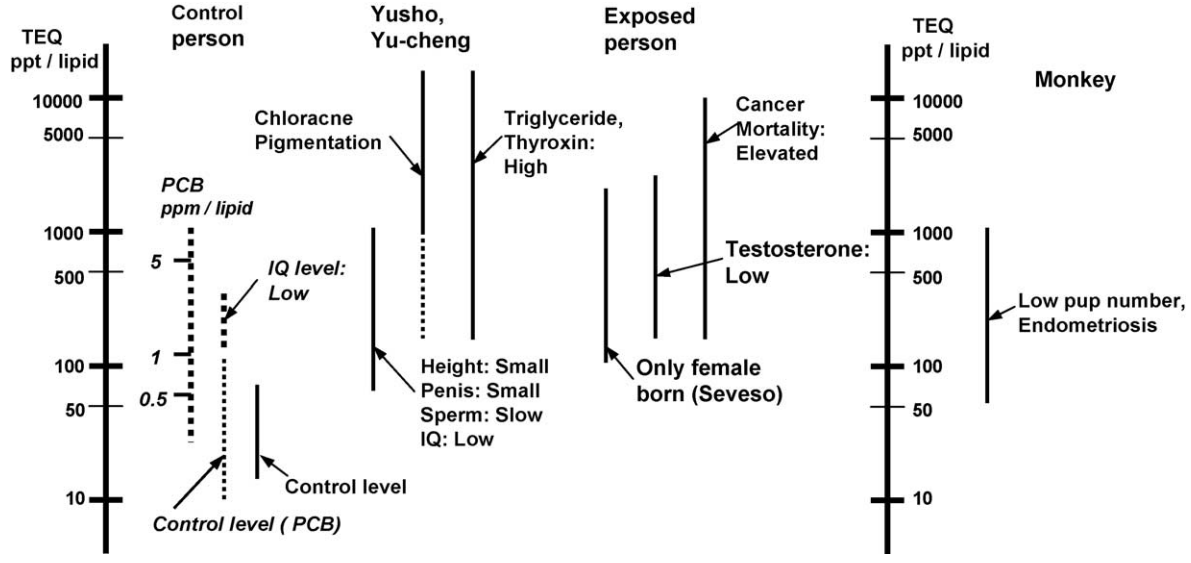

Fig. 3 TEQ and PCB levels and affected symptoms in human and monkey. IQ: intelligence quotient; PCB: polychlorinated biphenyl; TEQ: toxic equivalent quantity.

thyroxin hormone regulation system. The TEQ intake of babies in the high-exposure group was estimated from the PCDD/PCDF concentrations in the milk fat of the high exposure group to be 170 pg/kg body weight/day. This TEQ intake is smaller than the minimum intake for Yusho (28 ng/kg body weight/day) by about 2 orders of magnitude, and was actually found to induce thyroxin hormone elevation in newborn infants. Fig. 3 arranges the symptoms and disorders in Yusho and Yu-cheng patients to compare the levels of TEQ in the patients and controls [55]. TEQ levels in exposed people are also compared in Fig. 3.

Of the TCDD-exposed people in Seveso, Italy, the parents with blood TCDD levels as high as 100—2340 pg/g lipid gave birth to female babies only. Blood testosterone level was decreased in the workers exposed to high levels of TCDD. In herbicide-production workers in Germany, a significant trend was observed between cancer mortality and estimated TEQ levels. Numbers of offspring decreased in monkeys exposed to a diet containing TCDD up to 25 ppt, and the incidence of endometriosis 10 years after exposure correlated with dioxin exposure levels. Because the difference in TEQ levels between controls and exposed people with enzymatic and hormonal effects are about 1 order of magnitude, the controls with relatively high levels of TEQ may suffer from subtle effects of enzymatic and/or hormonal disorders. An epidemiological study of 212 children born to women who had eaten Lake Michigan fish proved that prenatal exposure to PCBs was associated with significantly lower intelligence quotient (IQ) scores.

Acknowledgement

This work was supported in part by a Grant-in-Aid for scientific research from the Ministry of Health, Labour and Welfare, Japan.

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Journal of Dermatological Science Supplement (2005) 1, S21—S28

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Improvement in dioxin analysis of human blood and their concentrations in blood of Yusho patients

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KEYWORDS
Blood analysis;
Non-ortho-coplanar polychlorinated biphenyls (Co-PCBs);
Polychlorinated dibenzofurans (PCDFs);
Polychlorinated dibenzo-*p*-dioxins (PCDDs);
Yusho

Summary

Background and objective: Over 35 years have passed since the Yusho incident. We have determined the concentrations of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and non-ortho-coplanar polychlorinated biphenyls (Co-PCBs) in blood samples collected from Yusho patients to establish new criteria for Yusho. Considering the fact that the concentrations of PCDDs, PCDFs and Co-PCBs in the blood samples of about 300 Yusho patients living in Japan were scheduled for measurement in 2002, it was desirable to develop more effective methods to speed up the pretreatment procedure for blood samples. In this study, we improved a method that allows many blood samples to be treated in a short period with high reproducibility in comparison with the previously described method. Using our method, we measured the concentrations of PCDDs, PCDFs and Co-PCBs in blood collected from 279 Yusho patients in 2002 and 269 Yusho patients in 2003, and compared the results with those of 52 normal controls.
Methods: The extraction procedure of PCDDs, PCDFs and Co-PCBs from the blood samples was simplified. Concentrations of the PCDDs, PCDFs and Co-PCBs were measured using high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) equipped with a solvent-cut large volume injection system.
Results and conclusion: The lipid content and the concentration of each isomer of PCDDs, PCDFs and Co-PCBs in blood determined using the improved method were almost equal to those obtained by dioxin analysis organizations that used the conventional method to analyze the same blood samples. The improved method demonstrated high reproducibility based on experiments conducted using the same serum samples. These findings indicate that the improved method is essentially equivalent to the conventional method. From the concentrations of PCDDs, PCDFs and Co-PCBs in blood samples of Yusho patients measured by the improved method, it

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1574-0757/$30.00
doi:10.1016/j.descs.2005.03.004

became clear that even now Yusho patients still have a much higher concentration of PCDFs in their blood than do unaffected people more than 35 years after the Yusho incident.

1. Introduction

The Yusho poisoning accident, which affected over 1800 people, occurred in 1968 in western Japan, and was caused by the ingestion of rice bran oil used for cooking that contained the following contaminants: polychlorinated biphenyls (PCBs), polychlorinated dibenzofurans (PCDFs) and polychlorinated dib enzo-*p*-dioxins (PCDDs) [1]. This disease was soon found to be far more difficult to manage by conventional medical treatment than initially thought, primarily due to the fact that very high levels of these compounds were persistent in the tissue of Yusho patients. Over 35 years have passed since the Yusho incident, and almost all of the typical symptoms of the Yusho patients have improved. However, some patients are still afflicted with subjective symptoms. These patients still have a much higher concentration of PCDFs in their blood than do unaffected people [2—4]. Evidence that the environment is so extensively contaminated with these chlorinated hydrocarbons as to threaten the global ecosystem has become apparent. Therefore, the study of Yusho is significant not only for those affected with the disease but also for those who are known to be potentially contaminated with PCDDs, PCDFs and non-ortho-coplanar polychlorinated biphenyls (Co-PCBs).

Extensive studies with regard to Yusho have been conducting by the Study Group for Yusho [1]. We have determined the concentrations of PCDDs, PCDFs and Co-PCBs in blood samples collected from Yusho patients to establish new criteria for Yusho [2—4]. Follow-up studies measuring the concentrations of PCDDs, PCDFs and Co-PCBs in the blood of Yusho patients are very important when considering the healthcare of these patients. With the conventional measuring method, 20—50 ml of blood is needed to exactly measure the concentrations of PCDDs, PCDFs and Co-PCBs [5]. However, because most of these patients are now over 60 years of age, collecting this amount of blood is restricted. These patients can safely supply only small volumes of blood for the measurement of PCDD, PCDF and Co-PCB concentrations. Therefore, to reduce the physical burden on patients, it was necessary to develop a highly sensitive analytic method that could accurately evaluate PCDDs, PCDFs and Co-PCBs concentrations from small (5 g) blood samples.

In addition, given that the extraction procedure of PCDDs, PCDFs and Co-PCBs from the blood by the conventional method is very complicated and time-consuming, it is not a suitable procedure for processing many samples. Recently, we developed an analytic method for measuring the concentrations of PCDDs, PCDFs and Co-PCBs in human blood samples as small as 5 g, and an efficient method for speeding up the pretreatment procedure for blood samples [6,7]. The method consists of three major steps: the extraction of lipid from human blood by an accelerated solvent extractor (ASE) system, a clean-up procedure at a scale one-quarter of that of the conventional method, and a sensitive determination method with high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) equipped with a solvent-cut large volume (SCLV) injection system used in a large volume injection technique. By using HRGC/HRMS with a SCLV injection system, the sensitivity of the GC/MS was increased to 10 times that of the classical method. Therefore, it became possible for concentrations of PCDDs, PCDFs and Co-PCBs to be measured in a blood volume of 5 g.

Using this method, we measured the concentrations of PCDDs, PCDFs and Co-PCBs in blood samples collected from 78 patients with Yusho living in Fukuoka Prefecture in 2001 [4]. Considering the fact that the concentrations of PCDDs, PCDFs and Co-PCBs in the blood samples of about 300 Yusho patients living in Japan were scheduled for measurement in 2002, it was desirable to develop more effective methods to speed up the pretreatment procedure for blood samples.

In this study, we developed a method that allows many blood samples to be treated in a short period with high reproducibility in comparison with the previously described method. We also examined efficient methods to reduce the background levels so that they do not affect the measurement of the PCDDs, PCDFs and Co-PCBs. Using these methods, we measured the concentrations of PCDDs, PCDFs and Co-PCBs in blood collected from 279 Yusho patients in 2002 and 269 Yusho patients in 2003.

2. Materials and methods

2.1. Materials

Native PCDDs, native PCDFs and native Co-PCBs, as authentic standards, were purchased from Wellington Laboratories (Ont., Canada). [$^{13}C_{12}$]-PCDDs, [$^{13}C_{12}$]-PCDFs and [$^{13}C_{12}$]-PCBs, as internal standards, were also purchased from Wellington Laboratories. An active carbon column was prepared as follows: active carbon was purchased from Nacalai Tesque (Kyoto, Japan), refluxed three times with toluene for 1 h, and dried in vacuum, after which 500 mg of the active carbon was mixed with 500 g of anhydrous sodium sulfate (Wako Pure Chemical Industries Ltd., Tokyo, Japan). A silver nitrate/silica gel was purchased from Wako Pure Chemical Industries Ltd. Distilled water used in this experiment was treated with *n*-hexane. All reagents and solvents used in this experiment were of the analytic grade of dioxin that is commercially available.

2.2. Sample preparation

The extraction of lipid from the blood samples was performed with an ASE system as previously described [6,7]. Each 5-g blood sample was loaded into the extraction cell filled with 3 g Isolute (International Sorbent Technology Ltd., Hengoed, Mid Glamorgen, UK). After 15 h of freeze-drying with a freeze dryer (VirTis Co., Inc., NY, USA), [$^{13}C_{12}$]-PCDDs, [$^{13}C_{12}$]-PCDFs and [$^{13}C_{12}$]-PCBs were added as internal standards, and lipids were extracted using an ASE system. The following programmed parameters were used for these extractions: a pressure of 2000 psi and a temperature of 150 °C, a static time of 10 min, a flushing volume of 50 ml, 180 s purging, a 60% flushing volume for two cycles, and acetone:*n*-hexane (1:3, v/v) as the extraction solvent. The extract was concentrated to near dryness, and the lipid contents were determined gravimetrically. The extracted lipid was used to carry out the clean-up procedure at a scale one-quarter of that of the conventional method. More specifically, the lipid was dissolved in *n*-hexane and treated with concentrated sulfuric acid. The separated hexane layer was applied to a column that linked a silver nitrate/silica gel column (0.5 g) and an active carbon column (0.5 g), and it was separated into two fractions. The first fraction, containing mono-ortho-coplanar polychlorinated biphenyls (mono-ortho-Co-PCBs), was eluted with 15 ml of hexane and 10 ml of 10% (v/v) dichloromethane/*n*-hexane. PCDDs, PCDFs and Co-PCBs were eluted with 25 ml of toluene as the second fraction. The eluate was concentrated to near dryness with a multiple sample concentrator (BUCHI, Labortechnik AG, Flawil, Switzerland) and transferred to a GC injection vial, and the syringe standard was added. The column packing (silver nitrate silica gel, active carbon column and anhydrous sodium sulfate) used in this experiment was washed by an ASE-200 system under the same conditions as the lipid extraction with *n*-hexane or toluene. All glassware instruments used in this experiment were treated in a high-temperature oven (ALP Co. Ltd., Tokyo, Japan) at 450 °C for 6 h.

2.3. Analysis of PCDDs, PCDFs and Co-PCBs

Concentrations of the PCDDs, PCDFs and Co-PCBs were measured using HRGC/HRMS equipped with an SCLV injection system (SGE Ltd., Victoria, Australia) [6,7]. The analytic conditions were as follows: the gas chromatograph was an HP-6890 series GC (Agilent Technologies, Inc., CA, USA) equipped with an Autospec Ultima NT (Micromass Ltd., Manchester, UK) and a solvent cut large-volume (SCLV) injection system (SGE Ltd.); the column used was a BPX-5 fused silica pre-capillary column, 0.25 mm i.d. × 5 m, 0.25 μm film thickness (SGE Ltd.); the analytic column was 0.15 mm i.d. × 30 m, 0.15 μm film thickness (SGE Ltd.); the column was heated from 160 to 300 °C at a rate of 20 °C/min, maintained at 300 °C for 8 min, cooled to 210 °C at a rate of 60 °C/min, maintained at 210 °C for 0.5 min, heated to 300 °C at a rate of 3 °C/min, and then maintained at 300 °C for 1 min. The injection temperature and ion source temperature were both maintained at 280 °C, and the carrier gas (helium) flow rate (constant flow) was 1.3 ml/min. The ionizing current, ionizing energy, accelerating voltage and trap current were 750 μA, 40 eV, 8.0 kV and 750 μA, respectively. PCDDs, PCDFs and Co-PCBs were analyzed in a single-ion record mode. The resolution was maintained at 10,000 at 5%.

For the analysis of tetrachlorodibenzo-*p*-dioxins (TCDDs), pentachlorodibenzo-*p*-dioxins (PeCDDs), hexachlorodibenzo-*p*-dioxins (HxCDDs), heptachlorodibenzo-*p*-dioxins (HeCDDs) and octachlorodibenzo-*p*-dioxin (OCDD) we used [$^{13}C_{12}$]-2,3,7,8-TCDD, [$^{13}C_{12}$]-1,2,3,7,8-PeCDD, [$^{13}C_{12}$]-1,2,3,4,7,8-HxCDD, [$^{13}C_{12}$]-1,2,3,6,7,8-HxCDD, [$^{13}C_{12}$]-1,2,3,7,8,9-HxCDD, [$^{13}C_{12}$]-1,2,3,4,6,7,8-HeCDD and [$^{13}C_{12}$]-1,2,3,4,6,7,8,9-OCDD as internal standards, respectively.

For the analysis of tetrachlorodibenzofurans (TCDFs), pentachlorodibenzofurans (PeCDFs), hexachlorodibenzofurans (HxCDFs), heptachlorodibenzofurans (HeCDFs) and octachlorodibenzofuran (OCDF) we used [$^{13}C_{12}$]-2,3,7,8-TCDF, [$^{13}C_{12}$]-1,2,3,7,8-PeCDF, [$^{13}C_{12}$]-2,3,4,7,8-PeCDF, [$^{13}C_{12}$]-1,2,3,4,7,8-HxCDF, [$^{13}C_{12}$]-1,2,3,6,7,8-HxCDF,

[$^{13}C_{12}$]-1, 2, 3, 7, 8, 9-HxCDF, [$^{13}C_{12}$]-2,3,4,6,7,8-HxCDF, [$^{13}C_{12}$]-1,2,3,4,6,7,8-HeCDF, [$^{13}C_{12}$]-1,2,3,4,7,8,9-HeCDF and [$^{13}C_{12}$]-1,2,3,4,6,7,8,9-OCDF as internal standards, respectively.

For the analysis of 3,3′,4,4′-tetrachlorobiphenyl (TCB), 3,4,4′,5-TCB, 3,3′,4,4′,5-pentachlorobiphenyl (PeCB) and 3,3′,4,4′,5,5′-hexachlorobiphenyl (HxCB) we used [$^{13}C_{12}$]-3,3′,4,4′-TCB, [$^{13}C_{12}$]-3,4,4′,5-TCB, [$^{13}C_{12}$]-3,3′,4,4′,5-PeCB and [$^{13}C_{12}$]-3,3′,4,4′,5,5′-HxCB as internal standards, respectively. [$^{13}C_{12}$]-1,2,3,4-TCDD was used as a syringe spike.

3. Results and discussion

To establish new criteria for Yusho, it was necessary to measure the concentrations of PCDDs, PCDFs and Co-PCBs in the blood of Yusho patients. We have studied the concentrations of PCDDs, PCDFs and Co-PCBs in blood samples collected from these patients [2—4]. Because most of the patients afflicted in the original incident are now over 60 years of age, they can safely supply only small volumes of blood with which to measure PCDD, PCDF and Co-PCB concentrations. Recently, we reported an analytic method for measuring the concentrations of PCDDs, PCDFs and Co-PCBs in human blood samples as small as 5 g, and an efficient method for speeding up the pretreatment procedure for blood samples [6,7]. Considering the fact that the concentrations of PCDDs, PCDFs and Co-PCBs in the blood samples of about 300 Yusho patients living in Japan were scheduled for measurement in 2002, it was desirable to develop more effective methods to speed up the pretreatment procedure for blood samples. The extraction of lipid from the blood samples was performed with an ASE system in a manner similar to that previously reported [6,7]. An ASE system that employs a new extraction procedure using organic solvents at high pressures and temperatures above the boiling point is widely used to replace Soxhlet or liquid—liquid extraction for the extraction of PCDDs, PCDFs and Co-PCBs from environmental samples [8—11]. The ASE technique has made lipid extraction from blood possible in shorter periods of time with smaller solvent volumes than those used in conventional methods. Moreover, to improve the efficiency of the ASE technique, a part of the previously reported method was improved on. Before extracting the lipid from the blood samples with an ASE system, the blood sample was freeze-dried for 16 h. This improvement made it possible to exclude the drying operation with anhydrous sodium sulfate after the extraction with the ASE system. As a result, operation time and labor were drastically reduced. Furthermore, by connecting the silver nitrate/silica column and activated carbon column, it became possible to exclude the concentration procedure of the eluate by silver nitrate/silica gel column chromatography. Simplification of the pretreatment method made it possible to process many more samples than with the previously reported method.

For high-sensitivity analysis of PCDDs, PCDFs and Co-PCBs using the 5-g blood samples, it is necessary to keep the background level as low as possible compared with the levels of PCDDs, PCDFs and Co-PCBs in the blood. We have performed the following countermeasures to effectively prevent contamination by various interfering substances that can affect the measurement of PCDDs, PCDFs and Co-PCBs: (1) a laboratory exclusively for human blood was used to prevent contamination from outside the room; (2) maintenance of the laboratory was performed to prevent contamination during the pretreatment procedure of human blood; (3) the column packing (silver nitrate silica gel, active carbon column and anhydrous sodium sulfate) was washed by an ASE system at a pressure of 2000 psi and a temperature of 150 °C with *n*-hexane or toluene; and (4) all glassware instruments used in this experiment were heated in a high-temperature oven at 450 °C for 6 h. Moreover, it became possible to exclude some processing operations by simplifying the pretreatment procedure, and to reduce the volume of the solvent used. Because the measurement of concentrations of PCDDs, PCDFs and Co-PCBs in human blood are high-sensitivity analyses of the order of picogram per gram of lipid, any PCDDs, PCDFs and Co-PCBs contaminating a reagent and solvent will affect the measurement. Therefore, the countermeasures made it possible to sufficiently reduce background levels such that they do not affect the measurement of PCDDs, PCDFs and Co-PCBs in human blood. It is necessary to investigate

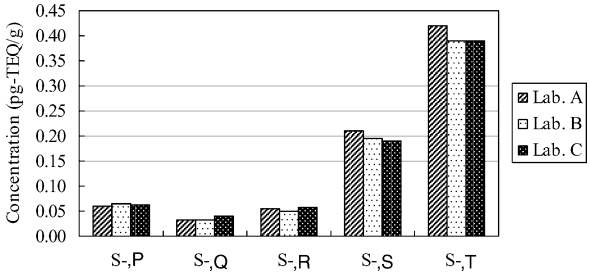

Fig. 1 Comparison of PCDDs, PCDFs and Co-PCBs concentrations in the five human control blood samples by our laboratory (Lab. A) and two different dioxin analysis organizations (Labs. B and C). Co-PCB: coplanar polychlorinated biphenyls; PCDD: polychlorinated dibenzo-*p*-dioxins; PCDF: polychlorinated dibenzofurans; TEQ: toxic equivalent quantity.

more efficient methods that can decrease the background levels as much as possible to accurately measure PCDD, PCDF and Co-PCB concentrations in the future.

We prepared the five human control blood samples to determine the measurement accuracy of the improved method. Measurement of the concentrations of PCDDs, PCDFs and Co-PCBs in the five control blood samples was requested from two different dioxin analysis organizations that used the conventional method with 20-g blood samples, and their results were compared with our results (Fig. 1). It was confirmed that the improved method could produce the same lipid content as the conventional method. The concentrations of each isomer of PCDD, PCDF and Co-PCB were also nearly the same using the two methods, and the total toxic equivalent quantities (TEQs) obtained by the improved method were almost equal to those obtained by the conventional method. In addition, the improved method demonstrated high reproducibility based on experiments conducted using the same control serum sample for 10 weeks (Table 1). Moreover, recovery of the ^{13}C-labeled internal standard was 60% overall (Table 2). These findings indicate that the improved method is essentially equivalent to the conventional method in terms of the results. However, on comparison the improved method is

Table 1 Reproducibility test of the improved method conducted using the same control serum sample for 10 weeks

Congeners	Concentration (pg/g lipid)									
	Week									
	1	2	3	4	5	6	7	8	9	10
2,3,7,8-TCDD	1.9	2.0	1.7	1.6	2.0	1.9	1.7	1.9	1.8	1.9
1,2,3,7,8-PeCDD	5.8	6.2	6.7	6.8	6.1	6.4	7.1	7.4	7.3	6.7
1,2,3,4,7,8-HxCDD	7.9	7.9	7.3	7.1	8.1	6.9	8.3	8.0	7.6	6.5
1,2,3,6,7,8-HxCDD	58.4	51.9	57.6	57.9	60.9	57.5	59.5	56.2	54.1	57.0
1,2,3,7,8,9-HxCDD	10.9	9.4	9.1	8.9	10.1	8.5	9.8	9.4	8.8	9.4
1,2,3,4,6,7,8-HeCDD	152.0	151.3	144.5	155.6	142.9	142.7	136.8	132.8	124.2	134.7
OCDD	1804.8	1783.8	1817.8	1785.1	1761.9	1804.8	1852.9	1843.5	1640.5	1887.6
2,3,7,8-TCDF	ND	ND	ND	ND	ND	ND	ND	ND	1.2	ND
1,2,3,7,8-PeCDF	1.1	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3,4,7,8-PeCDF	6.0	6.1	5.6	5.1	7.1	6.1	6.1	6.6	5.7	6.2
1,2,3,4,7,8-HxCDF	7.5	7.1	7.2	7.0	7.7	6.4	7.9	7.3	7.7	7.6
1,2,3,6,7,8-HxCDF	7.5	6.6	7.4	7.9	7.7	7.2	7.9	8.2	7.5	7.5
2,3,4,6,7,8-HxCDF	2.0	ND	ND	ND	ND	ND	ND	ND	ND	2.8
1,2,3,7,8,9-HxCDF	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,3,4,6,7,8-HeCDF	15.7	16.7	16.6	17.0	18.0	18.3	18.0	15.5	17.7	18.6
1,2,3,4,7,8,9-HeCDF	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
OCDF	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,4,4′,5-TCB(81)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,3′,4,4′-TCB(77)	54.4	44.9	44.8	46.6	46.1	46.9	47.4	45.5	45.0	47.4
3,3′,4,4′,5-PeCB(126)	28.5	24.9	23.9	25.9	25.9	25.4	23.9	23.4	25.6	23.4
3,3′,4,4′,5,5′-HxCB(169)	23.6	22.4	22.5	23.5	22.9	22.4	24.5	20.2	23.3	22.8
Total PCDD	2041.7	2012.6	2044.6	2023.1	2028.6	2076.1	2059.3	1844.4	2103.9	2061.9
Total PCDF	44.3	42.5	42.8	43.0	43.7	46.2	46.1	43.0	46.8	47.4
Total PCDD/PCDF	2086.0	2055.1	2087.4	2066.1	2072.3	2122.3	2105.3	1887.4	2150.7	2109.3
Total co-planar PCB	111.5	97.3	96.2	101.1	99.9	99.7	100.9	94.1	98.9	98.6
Total	2197.5	2152.3	2183.6	2167.2	2172.2	2222.1	2206.2	1981.6	2249.6	2207.9
Total PCDDs-TEQ	17.1	16.8	17.4	17.6	17.1	18.1	18.2	17.6	17.5	16.3
Total PCDFs-TEQ	5.1	4.9	4.7	4.5	4.9	5.1	5.3	4.9	5.3	5.3
Total PCDDs/PCDFs-TEQ	22.2	21.7	22.1	22.1	22.0	23.2	23.5	22.5	22.7	21.6
Total coplanar PCBs-TEQ	3.1	2.7	2.6	2.8	2.8	2.8	2.6	2.5	2.8	2.6
Total TEQ	25.3	24.4	24.7	24.9	24.9	26.0	26.2	25.1	25.5	24.2
Lipid (%)	0.27	0.28	0.28	0.27	0.27	0.26	0.26	0.29	0.27	0.27

CB: chlorinated biphenyl; CDD: chlorinated dibenzo-*p*-dioxins; CDF: chlorinated dibenzofurans; Hx: hexa; He: hepta; ND: less than the determination limit; OCDD: octachlorodibenzo-*p*-dioxin; OCDF: octachlorodibenzofurans; PCB: polychlorinated biphenyl; PCDD: polychlorinated dibenzo-*p*-dioxin; PCDF: polychlorinated dibenzofuran; Pe: penta; TCB: tetrachlorobiphenyl; TCDD: tetrachlorodibenzo-*p*-dioxin; TCDF: tetrachlorodibenzofuran; TEQ: toxic equivalent quantity.

Table 2 Recovery of PCDDs, PCDFs and Co-PCBs at the reproducibility test

Congeners	Recovery (%)									
	Week									
	1	2	3	4	5	6	7	8	9	10
13C-2,3,7,8-TCDD	70.0	72.8	68.4	84.4	76.7	66.0	70.6	74.4	66.1	57.6
13C-1,2,3,7,8-PeCDD	81.7	92.7	85.2	96.2	85.6	72.0	79.0	86.7	80.6	68.2
13C-1,2,3,4,7,8-HxCDD	100.0	114.2	101.1	111.5	95.6	74.2	91.0	108.0	93.1	81.6
13C-1,2,3,6,7,8-HxCDD	89.6	104.1	89.3	100.0	85.2	67.1	84.7	96.4	87.1	73.9
13C-1,2,3,7,8,9-HxCDD	91.9	102.8	93.9	98.3	89.0	73.5	79.9	92.6	82.1	68.6
13C-1,2,3,4,6,7,8-HeCDD	100.6	111.6	93.3	102.8	84.5	74.7	88.9	108.7	88.7	79.4
13C-OCDD	92.2	101.5	91.7	97.6	81.4	73.5	88.6	106.4	80.9	84.5
13C-2,3,7,8-TCDF	88.2	92.4	83.6	101.6	97.9	79.2	89.8	84.3	87.6	76.0
13C-1,2,3,7,8-PeCDF	72.3	78.4	70.6	80.5	73.6	60.9	69.1	66.6	67.0	56.9
13C-2,3,4,7,8-PeCDF	77.7	82.0	82.1	96.2	84.1	72.6	80.0	83.9	81.5	69.2
13C-1,2,3,4,7,8-HxCDF	86.0	96.7	85.0	95.1	85.5	69.3	82.1	90.4	82.8	72.3
13C-1,2,3,6,7,8-HxCDF	81.4	94.6	81.1	92.8	81.1	67.9	78.8	88.7	77.1	69.8
13C-2,3,4,6,7,8-HxCDF	108.2	113.3	100.9	115.5	96.9	80.2	91.2	98.6	92.7	82.3
13C-1,2,3,7,8,9-HxCDF	104.9	112.1	94.6	100.1	85.5	72.8	86.7	85.1	90.1	75.1
13C-1,2,3,4,6,7,8-HeCDF	91.1	97.1	85.6	93.6	80.1	68.2	80.8	84.1	74.6	74.0
13C-1,2,3,4,7,8,9-HeCDF	96.3	102.1	92.2	98.3	83.5	70.9	89.0	102.7	85.8	79.3
13C-OCDF	83.3	96.5	87.4	95.5	77.2	71.1	85.7	101.7	79.5	80.0
13C-3,4,4′,5-TCB(81)	62.2	77.6	70.0	90.6	88.1	64.0	59.1	77.3	62.1	58.2
13C-3,3′,4,4′-TCB(77)	64.3	76.3	67.2	89.9	85.0	64.4	63.3	75.8	62.0	57.9
13C-3,3′,4,4′,5-PeCB(126)	68.0	79.0	71.1	88.1	78.9	64.6	71.4	75.9	70.5	58.2
13C-3,3′,4,4′,5,5′-HxCB(169)	85.2	90.7	87.5	93.6	87.3	71.6	76.5	86.3	76.2	67.9

CB: chlorinated biphenyl; CDD: chlorinated dibenzo-*p*-dioxins; CDF: chlorinated dibenzofurans; Hx: hexa; He: hepta; OCDD: octachlorodibenzo-*p*-dioxin; OCDF: octachlorodibenzofurans; PCB: polychlorinated biphenyl; PCDD: polychlorinated dibenzo-*p*-dioxin; PCDF: polychlorinated dibenzofuran; Pe: penta; TCB: tetrachlorobiphenyl; TCDD: tetrachlorodibenzo-*p*-dioxin; TCDF: tetrachlorodibenzofuran.

more effective for treating many samples within a short period of time with high reproducibility. Using this method, we measured the concentrations of PCDDs, PCDFs and Co-PCBs in blood collected from 279 Yusho patients in 2002 and 269 Yusho patients in 2003. These results, including the data in 2001, are presented in Table 3. The mean total TEQ concentrations of PCDDs, PCDFs and Co-PCBs in the blood of Yusho patients in 2001—2003 were 179.3, 136.4 and 125.0 pg-TEQ/g lipid, for each year respectively, and the concentrations were 4.9, 3.7 and 3.4 times higher than those in normal controls, respectively. Although the concentrations of PCDDs and Co-PCBs in the Yusho patients and normal controls were nearly the same, the PCDF levels in Yusho patients were significantly higher than those in normal controls. The PCDF concentrations in the Yusho patients in 2001—2003 were 13.8, 10.3 and 9.5 times higher than those in normal controls, for each year respectively. Moreover, of the PCDF isomers for Yusho patients in 2001—2003, the concentration of 2,3,4,7,8-PeCDF was about 16.8, 12.6 and 11.6 times higher than those in normal controls, for each year respectively. These results indicate that even now Yusho patients still have a much higher concentration of 2,3,4,7,8-PeCDF in their blood than do unaffected people more than 35 years after the Yusho incident.

When HeCDD and OCDD in blood samples are extracted by an ASE system, they show high concentrations compared with those obtained by the conventional method. Although the cause of this phenomenon is unclear, it is possible that these compounds are not fully extracted from the blood by the conventional method. However, because the toxic equivalency factor (TEF) values of HeCDD and OCDD are small, at 0.01 and 0.0001, respectively, they do not significantly influence the lipid-based total TEQ values. Nevertheless, the details of this discrepancy need to be clarified. Another remaining problem is that the level of sensitivity of GC/MS must be increased two to three times to accurately measure the concentrations of PCDDs, PCDFs and Co-PCBs in a blood volume of less than 5 g. Because the concentrations of PCDDs, PCDFs and Co-PCBs in human blood are in the order of picogram per gram of lipid, most of the analytic work must be carried out by a highly sensitive system. The response of the analytic system can be increased with the introduction of a large volume of the final extract into the

Table 3 Concentrations of PCDDs, PCDFs and Co-PCBs in the blood of Yusho patients collected in 2001—2003

Congeners	Concentration (pg/g lipid)															
	Yusho patients												Normal controls			
	2001 (n = 78)				2002 (n = 279)				2003 (n = 269)				1997 (n = 54)			
	Mean	S.D.	Minimum	Maximum	Mean	S.D.	Minimum	Maximum	Mean	S.D.	Minimum	Maximum	Mean	S.D.	Minimum	Maximum
2,3,7,8-TCDD	1.8	1.1	0.5	4.1	1.7	0.8	0.5	4.4	1.7	0.8	0.5	5.6	2.1	1.4	0.3	8.5
1,2,3,7,8-PeCDD	20.4	11.0	3.3	53.5	11.1	5.9	1.5	46.8	9.7	5.4	0.5	45.2	8.8	4.4	1.1	23.5
1,2,3,4,7,8-HxCDD	2.4	1.8	1.0	7.7	2.9	1.8	1.0	10.8	2.6	1.6	1.0	8.5	2.9	1.9	0.0	10.1
1,2,3,6,7,8-HxCDD	56.7	43.4	4.4	230.1	53.0	41.7	6.0	290.7	50.4	42.6	3.8	348.5	30.9	14.6	5.9	75.5
1,2,3,7,8,9-HxCDD	4.6	2.3	1.0	11.0	5.1	3.8	1.0	41.0	3.9	2.7	1.0	17.9	4.9	2.8	0.0	14.2
1,2,3,4,6,7,8-HeCDD	26.6	20.7	5.4	143.6	63.4	53.7	10.8	556.3	38.6	22.9	8.5	167.2	33.6	16.7	9.0	85.0
OCDD	667.7	750.5	137.5	6226.3	877.2	728.2	172.5	9158.6	763.3	438.9	147.6	3706.1	627.6	557.9	123.8	3060.3
2,3,7,8-TCDF	1.8	2.5	0.5	14.4	1.4	0.9	0.5	6.3	1.2	0.7	0.5	4.9	2.0	1.3	0.4	8.2
1,2,3,7,8-PeCDF	1.1	0.9	0.5	4.2	0.9	0.8	0.5	6.3	0.8	0.7	0.5	5.6	1.7	1.0	0.0	4.4
2,3,4,7,8-PeCDF	256.1	315.3	6.7	1770.6	192.0	252.1	3.1	1889.7	176.2	240.2	2.6	1953.5	15.2	8.9	3.5	41.7
1,2,3,4,7,8-HxCDF	82.7	117.2	2.0	632.3	59.0	99.6	1.0	769.9	52.0	87.2	1.0	737.7	8.1	4.9	2.5	22.4
1,2,3,6,7,8-HxCDF	29.7	34.4	1.0	176.1	22.4	29.1	1.0	210.0	20.4	27.0	1.0	231.8	7.4	3.5	1.7	16.3
2,3,4,6,7,8-HxCDF	ND				ND				ND				2.0	1.5	0.0	5.8
1,2,3,7,8,9-HxCDF	ND				ND				ND				4.0	3.3	0.0	13.7
1,2,3,4,6,7,8-HeCDF	3.9	2.3	1.0	10.8	3.2	4.0	1.0	39.8	2.8	2.6	1.0	22.8	7.0	2.7	3.2	13.4
1,2,3,4,7,8,9-HeCDF	ND				ND				ND				ND			
OCDF	2.0	0.0	2.0	2.0	2.0	0.4	2.0	9.1	2.0	0.2	2.0	5.6	4.6	9.1	0.0	52.6
3,4,4′,5-TCB(81)	5.4	2.3	5.0	20.6	5.6	3.1	5.0	41.0	5.3	1.8	5.0	21.8				
3,3′,4′,4′-TCB(77)	7.8	4.7	5.0	28.5	11.0	7.2	5.0	46.1	8.6	6.4	5.0	71.8	20.7	14.2	5.0	101.1
3,3′,4,4′,5-PeCB(126)	84.4	58.5	17.8	319.5	103.1	71.7	5.0	560.9	98.1	65.3	11.2	531.7	110.5	79.9	11.0	432.9
3,3′,4,4′,5,5′-HxCB(169)	207.1	166.0	31.0	964.0	200.0	154.5	12.7	1131.4	183.8	139.2	12.7	1115.6	57.1	32.9	10.6	135.6
Total PCDD	780	766	177	6423	1014	782	212	9802	870	470	181	3924	706	586	40	3219
Total PCDF	381	461	18	2594	284	375	13	2744	259	352	12	2938	58	45	24	334
Total PCDD/PCDF	1161	840	308	6493	1299	866	232	9886	1129	602	242	3991	758	599	117	3334
Total coplanar PCBs	305	168	66	1006	320	186	28	1220	296	168	37	1218	187	115	42	594
Total	1466	921	373	6822	1618	948	313	10294	1425	703	293	4933	946	665	3621	174
PCDDs-TEQ	28.9	14.0	5.7	70.2	19.5	10.4	3.3	78.5	17.6	9.9	2.2	82.5	15.2	6.3	2.4	35.3
PCDFs-TEQ	139.8	171.8	4.1	966.7	104.6	137.9	2.1	1029.4	95.8	131.1	1.8	1074.4	10.1	5.2	2.7	25.8
PCDDs/PCDFs-TEQ	168.7	180.4	11.7	1036.9	124.1	146.7	5.4	1107.9	113.3	139.7	3.9	1156.9	25.3	11.2	5.1	61.1
Coplanar PCBs-TEQ	10.5	5.8	2.2	32.6	12.3	7.7	0.6	59.4	11.7	6.9	1.4	56.0	11.6	8.2	1.3	44.6
Total TEQ	179.3	180.5	13.9	1049.7	136.4	148.3	7.0	1126.1	125.0	141.2	5.5	1176.6	36.9	17.6	8.5	85.4
Lipid (%)	0.3	0.1	0.2	0.6	0.3	0.1	0.2	0.6	0.4	0.1	0.2	0.6	0.3	0.1	0.2	0.5
Age (years)	65.3	11.2	33.0	84.0	63.6	12.6	30.0	88.0	65.7	11.7	32.0	89.0	40.6	16.9	18.0	81.0

The data from normal contols are cited in the report by Matsueda et al. (7th Symposium on Environmental Chemistry, 4—5 June 1998, Kyoto, Japan). CB: chlorinated biphenyl; CDD: chlorinated dibenzo-*p*-dioxins; CDF: chlorinated dibenzofurans; Hx: hexa; He: hepta; ND: less than the determination limit; OCDD: octachlorodibenzo-*p*-dioxin; OCDF: octachlorodibenzofurans; PCB: polychlorinated biphenyl; PCDD: polychlorinated dibenzo-*p*-dioxin; PCDF: polychlorinated dibenzofuran; Pe: penta; TCB: tetrachlorobiphenyl; S.D.: standard deviation; TCDD: tetrachlorodibenzo-*p*-dioxin; TCDF: tetrachlorodibenzofuran; TEQ: toxic equivalent quantity.

GC/MS. Although several large-volume injection techniques have been proposed for increasing the sensitivity of GC/MS, the technique using a SCLV injection system has thus far been reported to be the most useful for analysis of PCDDs, PCDFs and Co-PCBs in human blood [12]. By using HRGC/HRMS equipped with a SCLV injection system, the sensitivity can be increased to 10 times that of the classic method. Large-volume injection using SCLV injectors is based on the selective separation of the solvent from the pre-column, and venting only to the solvent through a solvent-cut valve, while the analytic compounds are focused and condensed in a cold trap component and are separated by the analytic column. By this system, in contrast to the usual splitless system, expansion of a sample band can be depressed. Moreover, because most interfering matrices in an injected sample can be removed by the pre-column of a SCLV injection system, the analytic column used can be a narrow bore (0.1 mm) with a thin film thickness (0.1 μm). Narrow-bore columns emerged to answer the need for faster analytic times, narrower peaks and more efficient separations. By using a large-volume injection system combined with narrow-bore capillary column GC, it appears possible that extremely narrow peaks can be obtained and the mass sensitivity increased. The new technique is currently being tested for the determination of PCDDs, PCDFs and Co-PCBs in human blood samples.

Acknowledgements

Dr. Takashi Todaka is a resident researcher funded by the Japan Food Hygiene Association. This work was supported in part by a Grant-in-Aid for scientific research from the Ministry of Health, Labour and Welfare, Japan.

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Journal of Dermatological Science Supplement (2005) 1, S29—S31

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Blood chemistry, alpha-fetoprotein and hepatitis B surface antigen in Yusho

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KEYWORDS
Alpha-fetoprotein;
Blood chemistry;
Hepatitis B surface antigen (HBsAg);
Polychlorinated dibenzofurans (PCDFs);
Yusho

Summary

Background and Objective: An incident of accidental human exposure to polychlorinated biphenyls (PCBs) occurred in the western part of Japan in 1968. The disease is known as Yusho, because its cause was the ingestion of rice bran oil that was contaminated with PCBs. The various symptoms such as acneform skin eruptions were observed in the early stage in Yusho patients. An important fact is that polychlorinated dibenzofurans (PCDFs) were detected in the contaminated rice oil. PCDFs have a much higher toxicity than do PCBs. Analysis of blood concentration of PCDFs was performed throughout Japan in 2002. There have been no reports on the relationship between blood concentration of PCDFs and blood chemistry, alpha-fetoprotein or hepatitis B surface antigen (HBsAg) in Yusho. This is the first study to report on the relationship between blood concentration of PCDFs and blood chemistry, alpha-fetoprotein or HBsAg in Yusho.
Methods: We analyzed blood chemistry by measuring the following 20 items—total protein, serum albumin, alanine and aspartate aminotransferase, lactate dehydrogenase, alkaline phosphatase, leucine aminopeptidase, gamma-glutamyl transferase (GGT), total bilirubin, conjugated bilirubin, cholinesterase, total cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride, glucose, amylase, creatine kinase, urea nitrogen, creatinine and uric acid. Alpha-fetoprotein and HBsAg were also measured. We studied the relationship between blood concentrations of total PCDFs and the items of the blood chemistry analysis, alpha-fetoprotein and HBsAg.
Results: Of the 20 items of blood chemistry, alpha-fetoprotein and HBsAg, we found three items (GGT, HDL cholesterol and creatinine) were significantly related to the total PCDF level using three-way analysis of variance (ANOVA).
Conclusion: A significant relationship between three items of the blood chemistry analysis (GGTP, HDL cholesterol and creatinine) and total PCDF levels in the blood was observed in 2002. The blood concentrations of total PCBs and PCDFs have now decreased; however, the PCDFs in patients with Yusho still affect blood chemistry.

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1574-0757/$30.00 © 2004 Published by Elsevier Ireland Ltd on behalf of Japanese Society for Investigative Dermatology.
doi:10.1016/j.descs.2005.03.005

1. Introduction

In 1968, an incident of accidental human exposure to polychlorinated biphenyls (PCBs) occurred in the western part of Japan [1]. The disease is known today as Yusho, oil disease, because its cause was the ingestion of rice bran oil that was contaminated with PCBs used as a coolant in the manufacturing process. The various symptoms such as acneform skin eruptions and many constitutional symptoms observed in the early stage in Yusho patients have gradually improved since the incident, although blood PCB levels are still higher in these patients compared with the general population. An important fact is that polychlorinated dibenzofurans (PCDFs) were detected in the contaminated rice oil [2]. PCDFs have a much higher toxicity than do PCBs [3]. However, there have been no reports describing the effect of PCDFs on blood chemistry, alpha-fetoprotein or hepatitis B surface antigen (HBsAg). In the present study, we examined the blood chemistry, alpha-fetoprotein and HBsAg in patients with Yusho, and we studied the relation with blood concentration of PCDFs using statistical analyses.

2. Materials and methods

2.1. Participants and investigation items

In 2002, the blood concentrations of PCDFs were studied in 279 patients with Yusho. A fasting blood sample was taken for the analyses. The following 20 items of blood chemistry were measured using an autoanalyzer (Hitachi 7150, Hitachi Ltd., Tokyo, Japan): total protein, serum albumin, alanine and aspartate aminotransferase (ALT and AST), lactate dehydrogenase (LDH), alkaline phosphatase, leucine aminopeptidase (LAP), gamma-glutamyl transferase (GGT), total bilirubin, conjugated bilirubin, cholinesterase, total cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride, glucose, amylase, creatine kinase (CK), urea nitrogen, creatinine and uric acid. The tumor marker alpha-fetoprotein was measured by radioimmunoassay techniques (Eiken Chemical Co., Japan). The presence or absence of HBsAg was determined by standard radioimmunoassay techniques (Dinabbott, Tokyo, Japan).

2.2. Investigation and statistical methods

The concentrations of PCDFs in the blood were determined with a high-resolution gas chromatograph and a high-resolution mass spectrometer equipped with a solvent-cut large volume injection system [4]. Blood samples were analyzed for the 10 following furans: 2,3,7,8-tetrachlorodibenzofuran, 1,2,3,7,8-pentachlorodibenzofuran, 2,3,4,7,8-pentachlorodibenzofuran, 1,2,3,4,7,8-hexachlorodibenzofuran, 1,2,3,6,7,8-hexachlorodibenzofuran, 2,3,4,6,7,8-hexachlorodibenzofuran, 1,2,3,7,8,9-hexachlorodibenzofuran, 1,2,3,4,6,7,8-heptachlorodibenzofuran, 1,2,3,4,7,8,9-heptachlorodibenzofuran, and octachlorodibenzofuran. Total PCDF values and the toxic equivalent quantity (TEQ) were calculated.

The data were analyzed using a three-way analysis of variance (ANOVA) model. The total PCDF value proved to be most suitable for this analysis. The dependent variable was the logarithmic value of the blood concentration of total PCDFs. Sex and age were independent variables. A *P*-value less than 0.10 was considered statistically significant when the item had no interaction with sex and/or age.

3. Results

The relationships between blood concentrations of total PCDFs and each of the 20 items of blood

Table 1 The 20 items of the blood chemistry analysis, alpha-fetoprotein and HBsAg and the statistical relevance to blood concentrations of PCDF

	P
Total protein	0.129
Albumin	0.887
ALT	0.349
AST	0.129
LDH	0.382
Alkaline phosphatase	0.308
LAP	0.262
GGT	0.068[a]
Total bilirubin	0.029[a,b]
Conjugated bilirubin	0.150
Cholinesterase	0.401
Total cholesterol	0.944
HDL cholesterol	0.077[a]
Triglyceride	0.105
Glucose	0.569
Amylase	0.558
CK	0.309
Urea nitrogen	0.169
Creatinine	0.081[a]
Uric acid	0.161
Alpha-fetoprotein	0.945
HBsAg	0.874

ALT: alanine aminotransferase; AST: aspartate aminotransferase; LDH: lactate dehydrogenase; LAP: leucine aminopeptidase; GGT: gamma-glutamyl transferase; HDL cholesterol: high-density lipoprotein cholesterol; CK: creatine kinase; HBsAg: hepatitis B surface antigen.

[a] $P < 0.10$.

[b] The item had interaction with sex.

chemistry, including total protein, serum albumin, ALT, AST, LDH, alkaline phosphatase, LAP, GGT, total bilirubin, conjugated bilirubin, cholinesterase, total cholesterol, HDL cholesterol, triglyceride, glucose, amylase, CK, urea nitrogen, creatinine and uric acid; and alpha-fetoprotein; and HBsAg are shown in Table 1. The following three items were significantly related to total PCDF level: GGT ($P = 0.068$), HDL cholesterol ($P = 0.077$) and creatinine ($P = 0.081$). The P-value of total bilirubin was 0.029; however, the item had interaction with sex.

4. Discussion

It has been reported that blood PCB concentration is associated with blood chemistry, including triglyceride, total cholesterol, GGT, total bilirubin and conjugated bilirubin, in patients with Yusho [5—8]. However, there have been no reports describing the effect of PCDFs on blood chemistry, alpha-fetoprotein or HBsAg. In this study, the relationship between the blood PCDF concentration and blood chemistry, alpha-fetoprotein or HBsAg was investigated for the first time using three-way ANOVA. Of the 20 items of the blood chemistry analysis, total PCDF value was significantly related to three items (GGT, HDL cholesterol and creatinine). This is the first report of a statistical analysis of the relationship between PCDFs and blood chemistry, alpha-fetoprotein and HBsAg in Yusho patients. Further study is necessary to assess the exact relationship between the effects of PCDFs on blood chemistry, alpha-fetoprotein and HBsAg.

Acknowledgement

This work was supported in part by a Grant-in-Aid for scientific research from the Ministry of Health, Labour and Welfare, Japan.

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Journal of Dermatological Science Supplement (2005) 1, S33—S38

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Cardiac, pulmonary and renal function in Yusho patients

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KEYWORDS
Cardiac system;
Polychlorinated biphenyls (PCBs);
Polychlorinated dibenzofurans (PCDFs);
Renal system;
Respiratory system

Summary

Background: Thirty-five years after the Yusho incident, some symptoms, signs and laboratory abnormalities are still found in Yusho patients.
Objective: To describe the cardiovascular, respiratory and renal abnormalities caused by Yusho, especially in relation to blood polychlorinated dibenzofuran concentration.
Methods: A total of 358 officially registered patients with Yusho participated in this study. Medical records of the patients obtained from the annual nationwide health examinations held from 2001 to 2003 were used in the study. The symptoms, signs and laboratory findings in cardiac, respiratory and renal systems were compared with blood concentrations of polychlorinated biphenyls (PCBs) and 2,3,4,7,8-pentachlorodibenzofuran (PeCDF).
Results: Airway symptoms such as cough and sputum were frequently seen in Yusho patients, whereas other symptoms, signs and laboratory abnormalities were not remarkable. There were marginal relationships between cough and blood concentration of PCBs, and between sputum and 2,3,4,7,8-PeCDF.
Conclusion: Organs of the respiratory system remain affected by Yusho 35 years after the incident, whereas little effect on cardiac and renal systems is observed.

1. Introduction

Various studies have proven that the major causative agents of Yusho are not polychlorinated biphenyls (PCBs) but polychlorinated dibenzofurans (PCDFs) [1]. However, the relationship between blood PCDF concentration and both clinical

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1574-0757/$30.00
doi:10.1016/j.descs.2005.03.006

manifestations and laboratory findings remains unclear, because an accurate technique for measuring blood PCDF concentration had not yet been established.

The major physical complaints associated with Yusho include various subjective symptoms such as general fatigue, headache and weakness, as well as numbness of the lower extremities [2]. Marked findings from laboratory analyses are liver dysfunction and abnormalities in lipid metabolism. In contrast to this, symptoms, signs or laboratory findings in cardiovascular systems have not been noted so far. Those in respiratory systems have also not been emphasized from the onset of Yusho, even though the organs of the respiratory system are one of the major targets of toxicity by PCBs and their related compounds [3,4]. There have been reports that airway symptoms such as cough and sputum are common, and that PCB concentration in sputum is elevated in patients with Yusho [5]. Several basic and epidemiological studies have suggested the risk of lung carcinogenesis [6,7], although this has not been firmly established. Only limited data have been reported regarding the influence of PCBs (and related compounds) on renal function.

In this paper, cardiovascular, respiratory and renal abnormalities caused by Yusho are described, especially in relation to blood PCDF concentration, data of which have become available due to recent developments in measurement technology.

2. Patients and methods

2.1. Patients

The participants for the analyses were officially registered Yusho patients who voluntarily provided blood samples for the measurement of dioxin levels at the nationwide health examinations of Yusho patients from 2001 to 2003. The health examinations have been conducted annually since 1986 to promote the health of the patients and to determine the health status of chronic Yusho patients [4,8].

2.2. Symptoms, signs and laboratory findings

Original data of the patients were acquired from medical records obtained at the Yusho health examinations [4]. The standardized examination includes questions on cough and sputum, and records of both heart sounds and breath sounds. The examination also includes electrocardiogram (ECG), chest radiograph and urinalysis, as well as blood urea nitrogen (BUN) and creatinine. In this study, the prevalence of symptoms, signs and laboratory findings were divided into three categories: cardiac (heart murmur, hypertension and ECG), respiratory (cough, sputum, breath sounds and chest X-ray), and renal (urinalysis, blood BUN and creatinine). Of the items of the urinalysis, urinary sugar was excluded from the analysis, because information on food intake was not clear from the records.

The technical issues regarding the measurement of chemicals such as PCBs and 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) are described elsewhere in this supplement [18]. The concentrations of these chemicals were compared with symptoms, signs and laboratory findings.

2.3. Statistical analyses

The associations between these chemicals and clinical findings such as symptoms, signs and laboratory data were analyzed using a multiple logistic model. Taking into consideration the repeated participation of the patients in the health examinations, a robust estimator of variance was used to estimate the confidence interval for the odds ratio assuming that the observations are independent across the individuals, but not within repeated observations of individuals. Blood levels of total PCB and PCDF were log-transformed with base of 10 because of their highly skewed distribution. The explanatory variables, the symptoms and signs, were included in the model as binary variables adjusted for dummy variables of age (categorized into ranges: 30—59, 60—69 and 70—89 years), sex as a binary variable, smoking status (either non-smoker or current smoker), and drinking habit (either non-drinker or current drinker).

All tests were two-tailed, and P values <0.05 were considered statistically significant. All statistical analyses were performed with Stata Statistical Software: release 8.2 (Stata Corporation, 2003; College Station, TX, USA).

3. Results

3.1. Demographic data of the patients

The number, sex and age of the patients is shown in Table 1. The total number of patients was 358, and 48% were males. The mean age in 2003 was 64.6 years (range 31—89 years). The number of times the patients participated in the Yusho health examinations during the study period were one, two and three for 141, 166 and 51 patients, respectively. In 2001, 2002 and 2003, there were 78, 279 and 269 patients who participated in the Yusho health examination, respectively.

Table 1 Demographic data of the patients

Year of participation in Yusho health examination	Year			Number of patients by times of participation
	2001	2002	2003	
2001 only	9 (6)[a]			
2002 only		71 (37)		141
2003 only			61 (29)	
2001 and 2002	9 (5)	9		
2001 and 2003	9 (3)		9	166
2002 and 2003		148 (75)	148	
2001, 2002 and 2003	51 (18)	51	51	51
Total	78 (32)	279 (135)	269 (125)	358 (173)
Mean age (range), years	65.3 (33–85)	63.6 (30–88)	65.7 (32–89)	64.6 (31–89)[b]

[a] Number of patients who participated in that year (number of males).
[b] Age in 2003.

3.2. Cardiac problems in Yusho patients

Although heart murmur was audible in 2.2–2.7% of the patients (Table 2), there was no relationship with either the concentration of PCBs or of PCDFs (Tables 3 and 4).

In the health examinations during the study period, hypertension with systolic blood pressure ≥160 mmHg and/or diastolic blood pressure ≥95 mmHg was found in 0–3.5% of the patients older than 40 years (Table 2). The frequency of hypertension in the present study was less than that observed by Akagi et al. [9], who reported that hypertension was seen in 10 out of 59 (16.9%) Yusho patients older than 40 years. However, there was no relationship between hypertension and the concentrations of PCBs and PCDFs (Tables 3 and 4). ECG abnormalities were detected in 23.6–26.9% of the Yusho patients. Although the proportion of ECG abnormalities was similar over the 3-year period,

Table 2 Prevalence of the symptoms and signs, and laboratory data

Symptoms, signs and laboratory data	Year of health examination for Yusho					
	2001		2002		2003	
	Number positives/ total number	Percentage	Number positives/ total number	Percentage	Number positives/ total number	Percentage
Cardiac						
Heart murmur	2/73	2.7	6/271	2.2	6/265	2.3
Hypertension[a]	0/75	0.0	8/260	3.1	9/259	3.5
ECG	17/72	23.6	46/185	24.9	47/175	26.9
Respiratory						
Cough	42/76	55.3	139/273	50.9	123/266	46.2
Sputum	43/76	56.6	138/272	50.7	129/266	48.5
Breath sounds	3/73	4.1	3/271	1.1	4/265	1.5
Chest X-ray	19/73	26.0	31/153	20.3	37/145	25.5
Renal						
Urinary protein	3/78	3.8	25/278	9.0	23/266	8.6
Urinary blood	12/78	15.4	38/278	13.7	43/266	16.2
Urobilinogen	2/78	2.6	11/278	4.0	10/266	3.8
BUN[b]	7/78	9.0	33/278	11.9	31/269	11.5
Creatinine[c]	7/78	9.0	23/278	8.3	8/269	3.0

BUN: blood urea nitrogen; ECG: electrocardiogram.
[a] Confined to patients 40 years or older with systolic blood pressure ≥160 mmHg and diastolic blood pressure ≥95 mmHg.
[b] BUN is defined as positive if more than 20 mg/dl.
[c] Creatinine is defined as positive if more than 1.2 mg/dl.

Table 3 Association of symptoms with blood PCB concentration

Symptoms, signs and laboratory data	Odds ratio[a] (95% confidence interval)	*P*
Cardiac		
Heart murmur	2.05 (0.18—23.61)	0.57
Hypertension[b]	0.65 (0.12—3.54)	0.62
ECG	1.20 (0.45—3.16)	0.72
Respiratory		
Cough	1.89 (0.92—3.89)	0.08
Sputum	1.71 (0.79—3.71)	0.17
Breath sounds	1.75 (0.29—10.62)	0.54
Chest X-ray	0.54 (0.15—1.86)	0.33
Renal		
Urinary protein	3.01 (0.87—10.44)	0.08
Urinary blood	0.61 (0.25—1.46)	0.27
Urobilinogen	0.30 (0.06—1.48)	0.14
BUN[c]	1.81 (0.60—5.51)	0.29
Creatinine[d]	1.47 (0.33—6.58)	0.61

BUN: blood urea nitrogen; ECG: electrocardiogram.
[a] Odds ratio for 10-fold increase in blood PCB level in ppb (whole blood).
[b] Confined to patients 40 years or older with systolic blood pressure ≥160 mmHg and diastolic blood pressure ≥95 mmHg.
[c] BUN is defined as positive if more than 20 mg/dl.
[d] Creatinine is defined as positive if more than 1.2 mg/dl.

Table 4 Association of symptoms with blood PCDF concentration

Symptom, signs and laboratory data	Odds ratio[a] (95% confidence interval)	*P*
Cardiac		
Heart murmur	0.88 (0.34—2.29)	0.79
Hypertension[b]	0.77 (0.32—1.87)	0.57
ECG	0.71 (0.44—1.15)	0.17
Respiratory		
Cough	1.22 (0.83—1.79)	0.31
Sputum	1.46 (0.98—2.17)	0.06
Breath sounds	0.95 (0.26—3.52)	0.94
Chest X-ray	0.69 (0.42—1.11)	0.12
Renal		
Urinary protein	1.26 (0.65—2.42)	0.49
Urinary blood	0.81 (0.52—1.26)	0.35
Urobilinogen	0.59 (0.29—1.21)	0.15
BUN[c]	1.36 (0.81—2.28)	0.24
Creatinine[d]	1.56 (0.70—3.47)	0.28

BUN: blood urea nitrogen; ECG: electrocardiogram.
[a] Odds ratio for 10-fold increase in blood PCB level in ppb (whole blood).
[b] Confined to patients 40 years or older with systolic blood pressure ≥160 mmHg and diastolic blood pressure ≥95 mmHg.
[c] BUN is defined as positive if more than 20 mg/dl.
[d] Creatinine is defined as positive if more than 1.2 mg/dl.

details of the ECG abnormalities were not available. There was no relationship between ECG abnormalities and blood PCB concentration or blood PCDF concentration. To date, no specific relation between cardiovascular abnormalities and blood concentrations of PCBs/PCDFs has been detected.

3.3. Respiratory problems in Yusho patients

At the health examinations carried out from 2001 to 2003, nearly 33—35 years after the Yusho incident, cough was seen in 42 out of 76 (55.3%), 139 out of 273 (50.9%), and 123 out of 266 (46.2%) patients, in each year, respectively. There was a marginal relationship between cough and blood PCB concentration ($P = 0.08$), whereas there was no relationship between cough and PCDF concentration. In addition, sputum was seen in 43 out of 76 (56.6%), 138 out of 272 (50.7%), and 129 out of 266 (48.5%) patients, in each year, respectively. There was a marginal relationship between sputum and blood PCDF concentration ($P = 0.06$), but not PCB concentration. As described previously, smoking status was adjusted for in the analysis in this study. However, to confirm whether respiratory symptoms occur independently of smoking, the relationships between airway symptoms and the concentrations of PCBs and PCDFs were analyzed only in non-smokers. Sputum was still marginally related to the concentration of PCDF ($P = 0.10$).

Although wheezing was not rare at the onset of Yusho and was audible in 2.7% of Yusho patients 20 years after its onset, few patients had abnormal lung sounds at the examinations carried out from 2001 to 2003. Chest X-ray abnormalities were found in 20.3—26.0% of the patients. However, specific changes found at the onset of Yusho have not been detected in these 15 years. There was no relationship between chest X-ray abnormalities and blood concentrations of PCBs and PCDFs. Organs of the respiratory system are one of the major targets of PCBs and PCDFs, and airway symptoms and signs have not improved 35 years after the onset of Yusho.

3.4. Renal problems in Yusho patients

As shown in Table 2, urinalysis data from the examinations carried out from 2001 to 2003 were as follows: urinary protein 3.8—9.0%, urinary blood 13.7—16.2%, and positive urobilinogen 2.6—4.0%. Blood levels of BUN and creatinine were elevated in 9.0—11.9% and 3.0—9.0% patients, respectively. There was no relationship between these parameters of renal dysfunction and the concentrations of PCBs or PCDFs.

4. Discussion

Okumura and Katsuki investigated the health condition of 27 patients with Yusho immediately after the onset [10]. Of these patients, they reported symptoms, signs and laboratory findings in 18 patients older than 15 years (median 34, range 15—60). According to their report, fatigue and skin eruption were the major initial symptoms. Although face edema was seen in 3 of the 18 (17%) patients, they reported that it was not due to a cardiovascular disorder such as congestive heart failure. In 4 of the 18 (22%) patients, heart murmur was audible, and all of the murmurs were judged to be functional murmur. Blood pressure and ECG were normal in all 18 patients. Since then, few reports have described the symptoms, signs and laboratory abnormalities in the cardiovascular system in Yusho. In 1981, Kreiss et al. reported the positive relationship between blood pressure and blood PCB concentration in the study in Triana, Alabama, USA [11]. Sequentially, Akagi et al. investigated the relationship between blood pressure and blood PCB concentration in 59 patients with Yusho over the age of 40 years [9]. Hypertension with systolic blood pressure $\geq$160 mmHg and/or diastolic blood pressure $\geq$95 mmHg was found in 16.9% of the patients. However, there were no significant differences from the general population at the same age. Finally the investigators concluded that blood PCB concentration is not related to hypertension in Yusho patients. In 1988, Hirota et al. reported symptoms and signs in 285 Yusho patients 20 years after the onset of Yusho [4]. In the report, there was no description of heart sounds even though it was included as a routine checking item, suggesting that the investigators did not pay much attention to cardiovascular signs.

The respiratory system, including bronchiolar Clara cells, is one of the major targets of poisoning by PCBs and related compounds [3]. These chemicals have been detected in the respiratory system of Yusho patients [5]. Administration of these chemicals to experimental animals induced both pathological and functional changes [12—14]. With regard to the effects on the respiratory system as well as other target organs, several investigators have shown that the major causative agents of Yusho are PCDFs rather than PCBs [12,15].

The most marked respiratory symptoms at the onset of Yusho were cough, sputum and wheezing. The emergence of cough and sputum were almost simultaneous with cutaneous symptoms, followed by the emergence of wheezing [5]. Shigematsu et al. reported that 77 out of 203 (38%) Yusho patients suffered from cough and sputum similar to chronic bronchitis, and that concurrent respiratory infection was not rare [5]. Chronic bronchitis-like symptoms were seen not only in smokers but also in non-smokers. High concentrations of PCBs were detected in the sputum of Yusho patients. These airway symptoms improved gradually for 10 years from the time of onset, and no marked change has since been seen. At the health examination carried out 20 years after the onset of Yusho, airway symptoms such as cough and sputum were reported in 51.0% and 52.0% of patients, respectively. [16]. These symptoms came after the most frequent symptoms such as fatigue, headache and numbness of the lower extremities. At the examinations carried out from 2001 to 2003, cough and sputum were found in nearly one-half of the Yusho patients, indicating that airway symptoms persist over a long period of time in cases of poisoning by PCBs and PCDFs.

Hirota et al. reported that there was no relationship between blood PCB concentration and airway symptoms, but that there was a marginal relationship between blood PCB concentration and occurrence of abnormal lung sounds [16]. In the present study, a marginal relationship was seen between cough and PCB concentration, between sputum and PCDF concentration, but not between abnormal breath sounds and PCB/PCDF concentration. These discrepancies may be due to the analysis method, i.e. the effect of smoking, which has the most serious effect on airway symptoms, was adjusted for in the present analysis. Because a marginal relationship was found between sputum and blood PCDF concentration in the present study, it indicates that respiratory symptoms remain one of the major clinical manifestations of Yusho, and that sputum expectoration may be one of the main excretion systems of these chemicals.

At the onset of Yusho, reticulonodular densities in the chest X-ray examination were seen in 35% of patients, and acinar, patchy opacity and atelectatic shadow superimposed in 10% of patients. However, these abnormal shadows were not detected at the health examination 20 years later. Although lung function tests suggested an existence of small airway disease in Yusho patients, the details are unclear because of the small scale of the study.

Kuratsune et al. performed a cohort study in Yusho patients. In the study, 120 of the 1761 patients had died, and lung cancer mortality in men was suggested to be high [17]. In an animal experiment system, administration of PCB promoted the incidence and proliferation of lung cancer [7]. However, a high incidence of lung cancer in Yusho patients has not been proven. Careful follow-up is warranted regarding lung carcinogenesis in Yusho patients.

There are few reports concerning renal dysfunction in Yusho patients. At the onset of Yusho, Oku-

mura et al. described that kidney was not palpable and renal dysfunction was not seen in any of the 18 patients examined. Urinalysis was normal in 17 of the 18 patients, and in one patient urobilinogen was 1+ [10]. Both PCBs and PCDFs are predominantly metabolized in the liver, and may not have much influence on the renal system.

In summary, nearly 35 years after the onset, cough and sputum are still seen in many Yusho patients in both smokers and non-smokers. The data obtained from 2001 to 2003 showed that there is still a marginal relationship between sputum and blood PCDF concentration, even if adjusted for smoking status. Recent studies have shown that PCDFs are the major causative agent of Yusho. These results suggest that the respiratory system is still one of the major targets of Yusho, and that the respiratory tract may be one of the major excretion systems of PCBs and PCDFs. Cardiovascular and renal systems have not been recognized as major targets of Yusho. Although some Yusho patients currently have abnormalities in these systems, these abnormalities do not appear to be direct effects of PCBs or PCDFs.

Acknowledgement

This work was supported in part by a Grant-in-Aid for scientific research from the Ministry of Health, Labour and Welfare, Japan.

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Journal of Dermatological Science Supplement (2005) 1, S39—S44

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Neurological signs and symptoms in patients with chronic PCB poisoning (Yusho accident) for more than 36 years

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KEYWORDS
Dioxin;
Polyneuropathy;
Polychlorinated biphenyls (PCBs);
Sensory neuropathy;
Yusho accident

Summary

Background: The existence of peripheral neuropathy after chronic exposure to polychlorinated biphenyls (PCBs) is still controversial because studies concerning the effects of PCBs on the peripheral nervous system are rare.
Objective: The purpose of this study was to determine the correlation between neurological signs and symptoms and the concentration of serum PCBs.
Materials and methods: Neurological data collected from the results of a nation-wide health examination of 450 male and 557 female Yusho victims (chronic PCB poisoning) exposed more than 36 years ago were compared with recent measurements of the serum PCB concentration and patterns.
Results: The frequency of sensory disturbance detected by neurological examination was significantly higher in the group of officially acknowledged victims (male, $P = 0.014$; female, $P = 0.001$) than in age-matched controls. Significant differences were not observed between the serum PCB patterns and the neurological findings, but the serum PCB concentration was significantly higher in the group with decreased tendon reflex in officially and non-officially acknowledged female Yusho victims (male, $P = 0.994$; female, $P = 0.014$).
Conclusion: These results suggest that the long half-life of PCBs and their accumulation in fatty tissue can lead to persistent mild impairment of the peripheral nervous system even long after exposure.

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1574-0757/$30.00
doi:10.1016/j.descs.2005.03.007

1. Introduction

Although more than 36 years have passed since the contamination of rice bran oil with polychlorinated biphenyls (PCBs) occurred (the Yusho accident), the blood concentration of polychlorinated dibenzofurans (PCDFs), a derivative of PCB, in the blood of patients with Yusho is still higher than that in normal controls [1]. In general, it is considered that central and peripheral nerve involvement in Yusho victims is not common, but it is also well known that many symptoms such as numbness, sensory loss or paresthesia are often observed in Yusho patients. Further, a high incidence of distal symmetric sensory neuropathy in a group of dioxin-exposed workers has also been reported [2], which suggests the existence of subclinical involvement of the peripheral nervous system [3]. Therefore, we reviewed the correlation between the objective neurological findings and serum PCB concentration and PCB pattern using nationwide health examination data of Yusho victims 36 years after the first exposure.

2. Materials and methods

We determined the blood concentration of dioxin-like isomers from 1986 to 2002 in blood samples collected in a nationwide health examination of Yusho victims using a high-resolution gas chromatograph/high-resolution mass spectrometer (HRGC/HRMS) equipped with a solvent-cut large volume (SCLV) injection system. The accelerated solvent extraction (ASE) method was employed for the treatment of blood samples [1]. We collected the most recent data on serum PCB concentration, PCB pattern and subjective/objective neurological data from these patients, who included 1007 officially (OAY) and non-officially acknowledged (NOAY) Yusho accident victims (450 males, mean age 58.2 ± 17.6 years; 557 females, mean age 58.9 ± 16.9 years).

Data on subjective neurological symptoms were obtained by means of questionnaires and a clinical examination performed by a neurologist. An age-matched control group, which included 71 males (mean age 55.8 ± 19.9 years) and 66 females (mean age 64.3 ± 15.0 years), was selected from normal volunteers who visited the Brain Dock section of Omuta Rosai Hospital, National Chikugo Hospital and the Department of Neurology, Kyushu University Hospital.

Data obtained from patients and normal controls were analyzed using the χ^2 goodness of fit test and one-way analysis of variance (ANOVA). A P value < 0.05 was considered significant.

Table 1 Neurological symptoms observed in the officially and non-officially acknowledged (OAY and NOAY, respectively) victims of Yusho and the age-matched control group

Neurological complaint	Negative, *n* (%)	Positive, *n* (%)	Total	*P*-value
Headache				
OAY				
Male	191 (55.0)	156 (45.0)	347	<0.0001[a]
Female	121 (32.5)	251 (67.5)	372	<0.0001[a]
NOAY				
Male	55 (55.0)	45 (45.0)	100	<0.0001[a]
Female	53 (29.8)	125 (70.2)	178	<0.0001[a]
Control				
Male	61 (85.9)	10 (14.1)	71	–
Female	52 (78.8)	14 (21.2)	66	–
Numbness (subjective sensory disturbance)				
OAY				
Male	152 (43.8)	195 (56.2)	347	<0.0001[a]
Female	140 (37.6)	232 (62.4)	372	<0.0001[a]
NOAY				
Male	58 (58.0)	42 (42.0)	100	<0.0001[a]
Female	83 (46.6)	95 (53.4)	178	<0.0001[a]
Control				
Male	66 (93.0)	5 (7.0)	71	–
Female	60 (90.9)	6 (9.1)	66	–

Data were analyzed with the χ^2 test for independence.
[a] $P < 0.05$.

3. Results

The results of the analyses are shown in the Tables. The frequencies of subjective neurological complaints such as headache, paresthesia in the extremities and numbness observed in Yusho patients and age-matched controls were compared, and the subjective complaints were found to be significantly higher in the group of Yusho patients (Table 1). Of the objective neurological signs such as decreased tendon reflex or sensory impairment in extremities, the latter was observed more frequently in the OAY group compared with the age-matched controls (males, $P = 0.014$; females, $P = 0.001$) (Table 2).

Next, we evaluated the correlation between serum PCB pattern and PCB concentration and objective neurological signs. A correlation between the serum PCB pattern and objective neurological signs was not observed (Table 3). When we evaluated the correlation between serum PCB concentration and objective neurological signs, we found that the higher the serum PCB concentration, the higher the frequency of decreased tendon reflex in the female OAY and NOAY patients (female, $P = 0.014$) (Table 4). In addition, a mild correlation between the serum PCB concentration and sensory impairment in the group of male NOAY patients was observed ($P = 0.030$) (Table 4).

4. Discussion

In this paper, we reported on neurological studies of patients with chronic PCB poisoning for more than 36 years and the correlation with PCB concentration and PCB pattern. Although the group of OAY patients showed a significantly high frequency of sensory impairment, there was no relationship between the serum PCB concentration or PCB pattern in patients with sensory impairment or decreased tendon reflex and those without.

A report of a neurological examination after serious poisoning by PCB-contaminated cooking oil indicated the existence of peripheral neuropathy in 54% of 28 patients [4]. We analyzed a much large number of OAY and NOAY patients 36 years after exposure to PCBs and found a significantly higher rate of objective sensory disturbance in OAY patients compared with age-matched controls (Table 2; 17% in males, 16.5% in females). This is the first report of mass data from neurological examination of a group with chronic PCB poisoning.

Neurological signs and symptoms are not common in acute or chronic PCB poisoning. However, PCBs are readily soluble in oil, and traces of PCBs continue to be detected in OAY patients since they were first contaminated by the rice bran oil in the original incident more than 36 years ago [1]. These findings

Table 2 Neurological signs observed in the officially and non-officially acknowledged (OAY and NOAY, respectively) victims of Yusho and the age-matched control group

Neurological complaint	Negative, *n* (%)	Positive, *n* (%)	Total	*P*-value
Decreased/absent tendon reflex				
OAY				
Male	284 (81.8)	63 (18.2)	347	0.088
Female	310 (83.3)	62 (16.7)	372	0.547
NOAY				
Male	85 (87.6)	12 (12.4)	97	0.258
Female	156 (88.1)	21 (11.9)	177	0.117
CNT				
Male	64 (90.1)	7 (9.9)	71	—
Female	53 (80.3)	13 (19.7)	66	—
Objective sensory disturbance				
OAY				
Male	288 (83.0)	59 (17.0)	347	0.014[a]
Female	309 (83.5)	61 (16.5)	370	0.001[a]
NOAY				
Male	91 (91.0)	9 (9.0)	100	0.413
Female	166 (93.8)	11 (6.2)	177	0.134
CNT				
Male	67 (94.4)	4 (5.6)	71	—
Female	64 (97.0)	2 (3.0)	66	—

Data were analyzed with the χ^2 test for independence.
[a] $P < 0.05$.

Table 3 Correlation between neurological signs and serum PCB pattern

Neurological sign	PCB pattern				Total	*P*-value
	C, *n* (%)	BC, *n* (%)	B, *n* (%)	A, *n* (%)		
Tendon reflex						
Male						
OAY						
Normal	42 (17.6)	33 (13.9)	77 (32.4)	86 (36.1)	238	0.146
Decreased	13 (28.3)	2 (4.3)	13 (28.3)	18 (39.1)	46	
NOAY						
Normal	27 (39.1)	9 (13.0)	17 (24.6)	16 (23.2)	69	0.319
Decreased	5 (31.3)	1 (6.3)	7 (43.8)	3 (18.8)	16	
Total						
Normal	69 (22.5)	42 (13.7)	94 (30.6)	102 (33.2)	307	0.231
Decreased	18 (29.1)	3 (4.8)	20 (32.2)	21 (33.9)	62	
Female						
OAY						
Normal	57 (22.8)	13 (5.2)	59 (23.6)	121 (48.4)	250	0.799
Decreased	12 (26.1)	2 (4.3)	8 (17.4)	24 (52.2)	46	
NOAY						
Normal	43 (37.4)	16 (13.9)	30 (26.1)	26 (22.6)	115	0.278
Decreased	5 (45.5)	3 (27.3)	3 (27.3)	0 (0)	11	
Total						
Normal	100 (27.3)	29 (7.9)	89 (24.4)	147 (40.3)	365	0.948
Decreased	17 (29.8)	5 (8.8)	11 (19.3)	24 (42.1)	57	
Sensory disturbance						
Male						
OAY						
Normal	43 (18.1)	30 (12.6)	76 (31.9)	89 (37.4)	238	0.432
Impaired	13 (24.5)	5 (9.4)	20 (37.7)	15 (28.3)	53	
NOAY						
Normal	27 (38.0)	10 (14.1)	17 (23.9)	17 (23.9)	71	0.600
Impaired	4 (57.1)	0 (0)	1 (14.3)	2 (28.6)	7	
Total						
Normal	70 (22.6)	40 (12.9)	93 (30.1)	106 (34.3)	309	0.477
Impaired	17 (28.3)	5 (8.3)	21 (35.0)	17 (28.3)	60	
Female						
OAY						
Normal	58 (22.7)	13 (5.1)	52 (20.3)	133 (52.0)	256	0.072
Impaired	14 (26.9)	2 (3.8)	18 (34.6)	18 (34.6)	52	
NOAY						
Normal	45 (37.2)	18 (14.9)	32 (26.4)	26 (21.5)	121	0.854
Impaired	4 (44.4)	1 (11.1)	3 (33.3)	1 (11.1)	9	
Total						
Normal	103 (27.3)	31 (8.2)	84 (22.3)	159 (42.2)	377	0.131
Impaired	18 (29.5)	3 (4.9)	21 (34.4)	19 (31.1)	61	

Data were analyzed with the χ^2 goodness of fit test.

and the reported clinical results may suggest that the long half-life of PCBs and their accumulation in adipose tissues or possibly in myelin sheaths of peripheral nerves can lead to persistence of peripheral nervous system impairment long after the period of exposure [5,6]. In general, a decreased or absent tendon reflex indicates demyelination or axonal damage of large afferent and/or efferent myelinated peripheral nerves, whereas sensory impairment such as numbness, paresthesia and anesthesia are caused by disorders of small myelinated and/or unmyelinated fibers.

Table 4 Correlation between neurological signs and serum PCB concentration

Neurological sign	No. of patients	PCB concentration, mean ± S.D. (pg/g lipid)	*P*-value
Tendon reflex			
Male			
OAY			
Normal	279	3.66 ± 3.35	0.880
Decreased	56	3.59 ± 2.51	
NOAY			
Normal	80	2.67 ± 1.93	0.222
Decreased	7	3.67 ± 3.29	
Total			
Normal	359	3.44 ± 3.11	0.994
Decreased	63	3.44 ± 2.50	
Female			
OAY			
Normal	293	3.36 ± 2.92	0.140
Decreased	49	4.02 ± 2.51	
NOAY			
Normal	152	2.61 ± 2.30	0.434
Decreased	14	3.10 ± 2.10	
Total			
Normal	445	3.10 ± 2.74	0.014[a]
Decreased	63	3.95 ± 2.48	
Sensory disturbance			
Male			
OAY			
Normal	283	3.56 ± 3.23	0.426
Impaired	58	3.59 ± 2.51	
NOAY			
Normal	84	2.62 ± 1.96	0.030[a]
Impaired	7	4.38 ± 2.76	
Total			
Normal	367	3.34 ± 3.01	0.120
Impaired	65	3.98 ± 3.02	
Female			
OAY			
Normal	294	3.41 ± 2.78	0.462
Impaired	59	3.59 ± 2.51	
NOAY			
Normal	161	2.62 ± 2.33	0.380
Impaired	10	3.28 ± 1.49	
Total			
Normal	455	3.19 ± 2.79	0.801
Impaired	69	3.28 ± 2.18	

Data were analyzed with one-way analysis of variance (ANOVA).
[a] $P < 0.05$.

Unfortunately, this nationwide health examination of Yusho victims does not include neurophysiological, neuroradiological or detailed neurological data on whether these neurological symptoms come from peripheral neuropathy or cervical/lumbar radiculopathy. However, several reports have shown chronic peripheral neuropathy long after treatment of lung tuberculosis [7]. These data suggest the possibility that acute PCB poisoning or continuous release of a very low dose of PCBs from adipose tissue causes a very mild dysfunction or fragility of peripheral nerves, which causes the high frequency of peripheral neuropathy or radiculopathy compared with the age-matched control [8,9].

Although this study did not reveal the mechanisms or pathogenesis of mild neuropathy observed in Yusho patients, electrophysiological or neuroradiological examination, in addition to the detailed neurological examination, will be needed for health examinations of Yusho victims in the future because clinical peripheral neuropathy was found in this group of Yusho patients.

In conclusion, we analyzed the data from a nationwide health examination of victims of Yusho more than 36 years after the first exposure and found the existence of sensory disturbance in OAY patients, and a correlation between decreased tendon reflex in female patients and elevated serum PCB concentration. These results suggest that the long half-life of PCBs and their accumulation in fatty tissue may lead to persistent mild peripheral nerve system impairment, especially in the sensory nerve system, even long after the period of exposure.

Acknowledgement

This work was supported in part by a grant-in-aid for scientific research from the Ministry of Health, Labour and Welfare, Japan.

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Journal of Dermatological Science Supplement (2005) 1, S45—S55

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Complete blood cell counts and blood chemistry in Yusho

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KEYWORDS
Blood;
Chemistry;
Creatine phosphokinase;
Polychlorinated biphenyls (PCBs);
Yusho

Summary

Background: Because of their lipophilic nature, polychlorinated biphenyls (PCBs) bioaccumulate in the food chain and their residues have been detected in foods. Consequently, they accumulate readily in the human body. Reports suggest that PCB blood levels remain constant or increase. Little, however, is known about the long-term hazardous effects of PCBs and dioxins on human health. Yusho is a type of food poisoning caused by PCBs and dioxins that contaminated rice bran oil. We analyzed blood samples of the Yusho patients from 1986 to 2002, and studied changes in blood cell counts, blood chemistry and tumor markers.
Participants and methods: A population of 1041 patients was divided into patient and control groups based on the diagnostic criteria established for Yusho and participant's blood polychlorinated quarterphenyl (PCQ) levels. In total, 1666 blood and 1652 urine samples from 374 patients in the patient group (PCQ levels = 0.1 ppb), and 373 blood and 302 urine samples from 151 people in the control group (PCQ levels $<$ 0.02 ppb) were analyzed. Blood levels of PCBs, PCQs, polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) were determined, and we analyzed their correlation with the data of complete blood cell counts, blood chemistry and urinalysis.
Results and conclusion: Blood analyses, blood chemistry and urine values in Yusho patients were not significantly different from those in the control group 34 years after the Yusho incident. PCBs, PCQs or PCDFs may, however, affect hematogenesis, serum potassium, serum phosphorus, protein metabolism and creatine kinase metabolism because these parameters had slight but significant correlations with the levels of PCBs, PCQs or PCDFs. Exposure to PCBs and the related organochlorine compounds should be avoided.

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1574-0757/$30.00 © 2004 Published by Elsevier Ireland Ltd on behalf of Japanese Society for Investigative Dermatology.
doi:10.1016/j.descs.2005.03.008

1. Introduction

Polychlorinated biphenyls (PCBs) are industrial compounds, or their byproducts, which have diverse commercial applications and are widespread throughout the environment and in chemical waste dump sites. Although their production has been banned since 1973 in Japan, exposure to PCBs is still possible. Because of their lipophilic nature and their chemical stability, PCBs bioaccumulate in the food chain, and their residues have been detected in foods. Consequently, they accumulate readily in the human body [1]. Reports suggest that PCB levels in the blood remain constant or increase [2], but little is known about the long-term hazardous effects of PCBs and their related compounds, dioxins, on human health [3,4].

In 1968, Yusho was reported widely in western Japan as a type of food poisoning caused by PCBs and dioxins that contaminated rice bran oil. PCB contamination occurred in the production of cooking oil, during which commercial PCB preparations were used for heat exchange [5]. Pyrolysis of PCBs and chlorinated benzenes at high temperatures produced polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-*p*-dioxins (PCDDs). The rice bran oil contained not only PCBs, including coplanar PCBs, but also polychlorinated quaterphenyls (PCQs), PCDFs, PCDDs, as well as other related substances.

We examined Yusho sufferers annually from 1968, and we analyzed their blood samples from 1986 to 2002. Here we report on changes in blood cell counts, blood chemistry, urinalysis and tumor markers in patients with Yusho.

2. Participants and methods

A total of 5584 blood samples were obtained annually from 1041 patients from 1986 to 2002. There were 474 male patients (mean age 50.3 years, range 1—91) and 567 female patients (mean age 51.7 years, range 1—87). Of these 1041 patients, we selected two groups: the patient group comprised 374 patients (1666 blood and 1652 urine samples) who satisfied the diagnostic criteria for Yusho with high blood levels of PCQs (=0.1 ppb), and the control group consisted of 151 individuals (373 blood and 302 urine samples) who did not satisfy the criteria for Yusho with normal blood levels of PCQs (<0.02 ppb) (Table 1) [6]. PCBs, PCQs, PCDDs and PCDFs in blood were determined by gas chromatography—mass spectrometry at Nagasaki or Fukuoka Laboratory of Public Health. Blood analyses, serum chemistries and urinalysis were done at Nishinippon SRL Co. Standard assay kits were used. The examined items included standard complete blood cell counts (i.e. red blood cells [RBC], hemoglobin [Hb], hematocrit [Hct], platelets [Plt] and white blood cells [WBC]); serum chemistries (i.e. sodium, potassium, calcium, phosphorous, total protein [TP], albumin, blood urea nitrogen [BUN], creatinine, creatine kinase [CK], amylase, alpha-fetoprotein [AFP], hepatitis B virus surface [HBs] antigen); erythrocyte sedimentation rate (ESR), and urinalysis (pH, protein, sugar, urobilinogen).

2.1. Statistical analysis

Data were analyzed with Stat View software. The annual mean blood levels of PCBs and PCQs were plotted to calibrate respective regression lines in the two groups, and the half-lives of excretion for these congeners were determined. Standard methods were used to obtain summary statistics, such as means and Student's *t*-tests or Mann—Whitney tests. All tests were two-tailed. With regards to blood cell counts, blood chemistries, CK and urinary pH, the correlations between each index and the blood levels of PCBs, PCQs, PCDDs, PCDFs or toxic equivalent quantity (TEQ) were analyzed in the patient group (with high blood PCQ levels). A 2×2 table was made in order to obtain the χ^2 distribution for patients with high or low levels of CK. A similar analysis was conducted on HBs antigen. A multiple regression analysis was performed to examine the influence of PCBs, PCQs, PCDDs, PCDFs and TEQ on the values of TP, CK and urinary pH in the patient group. A P value <0.05 was regarded as statistically

Table 1 Demographic data

Sex	Patient group (PCQ $\geq$ 0.10 ppb)			Control group (PCQ $<$ 0.02 ppb)		
	n	Number of samples	Age, mean $\pm$ S.D. (years)	*n*	Number of samples	Age, mean $\pm$ S.D. (years)
Male	181 (48.4%)	802 (48.1%)	58.9 $\pm$ 14.5	53(35.1%)	101 (27.1%)	56.1 $\pm$ 15.4
Female	193 (51.6%)	864 (27.9%)	61.8 $\pm$ 10.7	98 (64.9%)	272 (72.9%)	58.6 $\pm$ 14.5
Total	374 (100%)	1666 (100%)	60.9 $\pm$ 12.7	151 (100%)	373 (100%)	58.7 $\pm$ 14.8

PCQ: polychlorinated quarterphenyl.

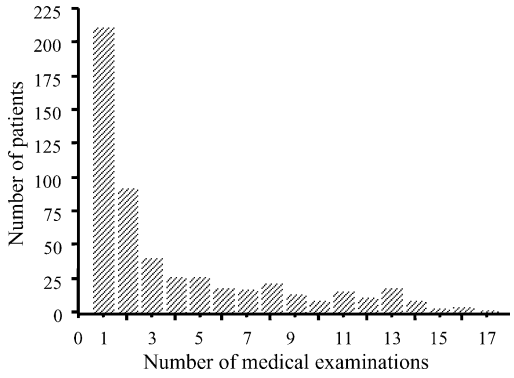

Fig. 1 Histogram of number of medical examinations.

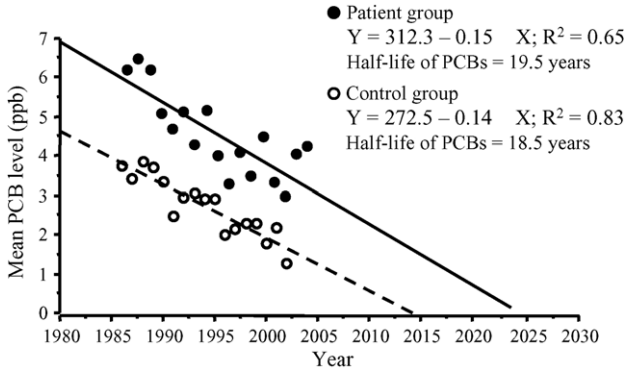

Fig. 2 Change in PCB level over time. PCB: polychlorinated biphenyl.

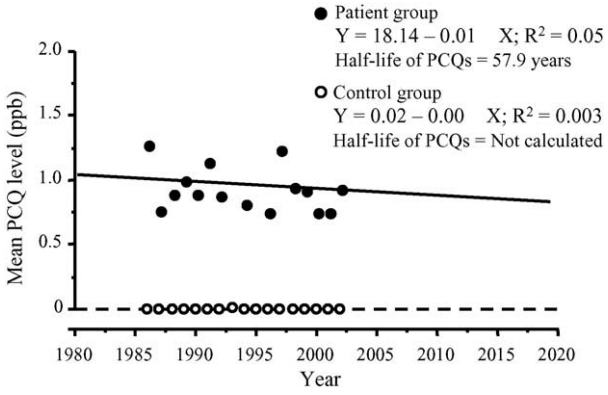

Fig. 3 Change in PCQ level over time. PCQ: polychlorinated quarterphenyl.

significant in all analyses except for the correlation analysis, for which a P value <0.01 was considered significant.

3. Results

The patients were examined up to 17 times between 1986 and 2002. The histogram in Fig. 1 shows the number of medical examinations. The blood levels of PCBs, PCQs, PCDFs and TEQ were significantly higher in the patient group than in the control group (Table 2). The chronological changes in mean levels of PCBs and PCQs between 1986 and 2002 are shown in Figs. 2 and 3. The median excretion half-life for total PCBs was roughly 19.5 years in the patient group (Fig. 2). PCQ blood levels decreased very gradually (Fig. 3). All values of complete blood cell counts (RBC, Hb, Hct, Plt and WBC) were within normal ranges both in the patient and control groups (Table 3). However, RBC count was slightly but significantly higher in the males of the patient group, but lower in the females of the patient group (Table 3). A significant negative correlation was observed with RBC versus PCBs, and Hb versus PCBs. A slight but significant correlation was demonstrated with RBC versus PCQs, Hb versus PCQs, Hct versus PCQs, and Plt versus PCQs in the males

Table 2 Blood levels of PCBs, PCQs, PCDDs, PCDFs and TEQ

Sex	Index	Patient group			Control group		
		n	Mean ± S.D.	Maximum – minimum	n	Mean ± S.D.	Maximum – minimum
Male	PCBs (ppb)[a]	802	4.8 ± 3.7	31.0 – 0.0	101	2.9 ± 2.0	11.0 – 0.0
	PCQs (ppb)[a]	802	0.9 ± 10.8	12.0 – 0.1	101	0.0 ± 0.001	0.01 – 0.0
	PCDDs (pg/g lipid)	28	1085.4 ± 934.0	5037.8 – 289.7	21	731.6 ± 414.9	1897.5 – 305.7
	PCDFs (pg/g lipid)[a]	28	134.0 ± 167.5	804.5 – 25.0	21	29.7 ± 18.9	68.7 – 14.4
	TEQ (pg/g lipid)[a]	328	78.7 ± 77.9	372.8 – 19.4	21	23.6 ± 13.8	62.6 – 8.5
Female	PCBs (ppb)[a]	864	4.6 ± 3.4	32.0 – 0.0	272	2.8 ± 2.2	12.0 – 0.0
	PCQs (ppb)[a]	864	1.8 ± 1.2	9.4 – 0.1	272	0.0 ± 0.001	0.01 – 0.0
	PCDDs (pg/g lipid)	25	879.7 ± 306.8	1741.5 – 365.9	23	1186.2 ± 661.5	3121.6 – 390.1
	PCDFs (pg/g lipid)[a]	25	492.2 ± 392.7	1365.3 – 63.9	23	32.4 ± 14.5	71.0 – 11.2
	TEQ (pg/g lipid)[a]	25	221.7 ± 158.5	595.9 – 48.4	23	31.7 ± 17.9	72.8 – 6.7

PCB: polychlorianted biphenyl; PCDD: polychlorinated dibenzodioxin; PCDF: polychlorinated dibenzofuran; PCQ: polychlorinated, quarterphenyl; S.D.: standard deviation; TEQ: toxic equivalent quantity.

[a] $P < 0.05$ between control and patient groups (t-test).

Table 3 Blood cell count data

Sex	Index	Patient group			Control group		
		n	Mean ± S.D.	Maximum – minimum	*n*	Mean ± S.D.	Maximum – minimum
Male	RBC ($\times 10^3$/mm^3)[a]	795	461.7 ± 45.4	601.0 – 287.0	74	447.8 ± 57.4	571.0 – 285.0
	Hb (g/dl)[a]	795	14.7 ± 1.4	19.0 – 8.0	74	13.3 ± 1.7	17.8 – 9.4
	Hct (%)	795	44.1 ± 3.8	59.1 – 29.7	74	43.2 ± 5.1	55.1 – 29.8
	Plt ($\times 10^4$/mm^3)	795	22.1 ± 6.0	41.0 – 1.4	74	21.5 ± 4.7	35.7 – 12.3
	WBC ($\times 10^3$/mm^3)	795	6.3 ± 1.5	12.4 – 2.9	74	6.1 ± 1.6	10.0 – 3.1
	MCV (μm^3)	795	95.7 ± 5.9	119.0 – 60.0	74	96.6 ± 5.1	108.0 – 86.0
	MCH (pg)	795	32.0 ± 2.1	37.6 – 15.5	74	32.0 ± 1.6	36.5 – 28.4
	MCHC (%)	795	33.4 ± 1.2	36.9 – 25.9	74	33.2 ± 1.0	35.3 – 30.9
Female	RBC ($\times 10^3$/mm^3)[a]	854	417.8 ± 34.8	514.0 – 209.0	198	423.7 ± 38.4	545.0 – 333.0
	Hb (g/dl)[a]	854	12.8 ± 1.2	16.0 – 6.7	198	13.0 ± 1.4	16.7 – 9.4
	Hct (%)[a]	854	39.3 ± 3.3	52.7 – 22.7	198	40.2 ± 3.8	50.7 – 29.9
	Plt ($\times 10^4$/mm^3)	854	21.9 ± 8.0	46.4 – 4.4	198	21.7 ± 5.8	41.3 – 4.2
	WBC ($\times 10^3$/mm^3)[a]	854	5.2 ± 1.3	10.4 – 2.4	198	5.8 ± 1.6	10.1 – 3.0
	MCV (μm^3)	854	94.3 ± 5.4	113.0 – 66.0	198	94.9 ± 5.0	110.0 – 76.0
	MCH (pg)	854	30.7 ± 1.9	37.8 – 18.7	198	30.8 ± 1.7	35.0 – 24.5
	MCHC (%)	854	32.6 ± 1.3	36.1 – 27.5	198	32.4 ± 1.2	34.9 – 28.1

Hb: hemoglobin; Hct: hematocrit; MCH: mean cell hemoglobin; MCHC: mean corpuscular hemoglobin concentration; MCV: mean corpuscular volume; Plt: platelet; RBC: red blood cell; S.D.: standard deviation; WBC: white blood cell.
[a] $P < 0.05$ between control and patient groups (*t*-test).

of the patient group (Table 4). WBC also showed a slight negative correlation to PCBs in the males of the patient group (Table 4).

Serum electrolytes (Na, K, Ca and P) were also within normal limits both in the patient and control groups. Serum potassium levels were slightly higher in the patient group compared with the control group. Serum levels of phosphorus in the females of the patient group were lower than those in the control group. Serum levels of TP, albumin, BUN and creatinine were also within the normal range both in the patient and control groups (Table 5). A signifi-

Table 4 Correlation of PCBs, PCQs, PCDDs, PCDFs and TEQ with blood cell count

Sex	Index	Correlation coefficient (*n*)				
		vs. PCBs	vs. PCQs	vs. PCDDs	vs. PCDFs	vs. TEQ
Male	RBC	−0.22 (795)[a]	−0.14 (795)[a]	0.12 (28)	0.25 (28)	0.24 (28)
	Hb	−0.13 (795)[a]	−0.11 (795)[a]	−0.05 (28)	0.00 (28)	0.00 (28)
	Hct	−0.06 (795)	−0.13 (795)[a]	0.02 (28)	0.01 (28)	0.02 (28)
	Plt	−0.18 (789)[a]	0.10 (789)[a]	0.15 (28)	−0.11 (28)	−0.13 (28)
	WBC	−0.13 (795)[a]	−0.05 (795)	0.16 (28)	0.01 (28)	−0.03 (28)
	MCV	0.28 (795)[a]	0.05 (795)	−0.14 (28)	−0.36 (28)	−0.34 (28)
	MCH	0.16 (795)[a]	0.06 (795)	−0.20 (28)	−0.32 (28)	−0.31 (28)
	MCHC	−0.18 (795)[a]	0.04 (795)	−0.18 (28)	−0.02 (28)	−0.05 (28)
Female	RBC	−0.14 (854)[a]	0.03 (854)	−0.40 (25)	0.02 (25)	0.00 (25)
	Hb	−0.09 (854)[a]	0.03 (854)	0.17 (25)	0.15 (25)	0.15 (25)
	Hct	−0.06 (854)	0.00 (854)	0.12 (25)	0.13 (25)	0.13 (25)
	Plt	−0.03 (854)	0.09 (844)	−0.31 (24)	−0.14 (24)	−0.15 (24)
	WBC	−0.02 (854)	−0.01 (854)	−0.10 (25)	−0.14 (25)	−0.13 (25)
	MCV	0.11 (854)[a]	−0.05 (854)	0.48 (25)	0.16 (25)	0.17 (25)
	MCH	0.06 (854)	0.00 (854)	0.50 (25)[a]	0.18 (25)	0.39 (25)
	MCHC	−0.08 (854)	0.07 (854)	0.29 (25)	0.16 (25)	0.45 (25)

Hb: hemoglobin; Hct: hematocrit; MCH: mean cell hemoglobin; MCHC: mean corpuscular hemoglobin concentration; MCV: mean corpuscular volume; PCB: polychlorinated biphenyl; PCDD: polychlorinated dibenzodioxin; PCDF: polychlorinated dibenzofuran; PCQ: polychlorinated quarterphenyl; Plt: platelet; RBC: red blood cell; TEQ: toxic equivalent quantity; WBC: white blood cell.
[a] $P < 0.01$ (significant correlation).

Table 5 Serum chemistry data

Sex	Index	Patient group			Control group		
		n	Mean ± S.D.	Maximum − minimum	*n*	Mean ± S.D.	Maximum − minimum
Male	Na (mEq/l)	802	141.0 ± 2.4	149.0 − 127.0	75	141.1 ± 2.0	145.0 − 138.0
	K (mEq/l)[a]	802	4.2 ± 0.4	8.9 − 3.1	75	4.1 ± 0.5	6.4 − 3.3
	Ca (mg/dl)	638	9.2 ± 0.4	10.7 − 7.8	75	9.2 ± 0.4	10.1 − 8.1
	P (mg/dl)	801	3.1 ± 0.5	5.0 − 0.8	75	3.1 ± 0.5	4.8 − 1.7
	TP (g/dl)[a]	800	7.2 ± 0.4	8.7 − 5.1	75	7.4 ± 0.5	8.9 − 6.3
	Albumin (g/dl)	802	4.4 ± 0.3	5.6 − 3.3	75	4.4 ± 0.3	5.0 − 3.0
	BUN (mg/dl)	802	16.8 ± 4.8	40.0 − 2.0	75	16.8 ± 5.7	42.0 − 7.0
	Creatinine (mg/dl)	801	1.1 ± 0.3	2.9 − 0.5	75	1.1 ± 0.3	2.1 − 0.6
Female	Na (mEq/l)	861	141.5 ± 1.9	147.0 − 134.0	203	141.6 ± 1.8	147.0 − 135.0
	K (mEq/l)[a]	861	4.3 ± 0.4	6.0 − 3.1	203	4.2 ± 0.4	5.6 − 3.2
	Ca (mg/dl)	828	9.3 ± 0.4	10.8 − 8.2	203	9.3 ± 0.4	10.7 − 7.6
	P (mg/dl)[a]	861	3.4 ± 0.0	5.5 − 1.1	203	3.5 ± 0.5	6.1 − 2.2
	TP (g/dl)	862	7.4 ± 0.4	8.9 − 6.3	203	7.4 ± 0.5	9.0 − 6.2
	Albumin (g/dl)	862	4.4 ± 0.2	5.4 − 3.3	203	4.4 ± 0.3	5.4 − 3.7
	BUN (mg/dl)	863	16.0 ± 5.6	90.0 − 5.0	203	15.4 ± 4.7	32.0 − 7.0
	Creatinine (mg/dl)	861	0.9 ± 0.3	9.0 − 0.4	203	0.9 ± 0.2	1.6 − 0.3

BUN: blood urea nitrogen; TP: total protein.
[a] $P < 0.05$ between control and patient groups (*t*-test).

cant correlation was observed with Na versus PCQs, creatinine versus PCQs (male), P versus PCQs (female), TP versus PCQs (female), and albumin versus PCQs (female) in the patient group (Table 6). Serum levels of TP were slightly lower compared with the control group and the correlation between PCBs and TP was significant in the male patients (Tables 5 and 6). Levels of BUN were significantly correlated with the levels of PCBs in the male patients. TP exhibited a significant correlation with BUN in the male, female and total patients ($P < 0.05$) (correlation coefficient: male, −0.11 [$n = 800$]; female, −0.12 [$n = 862$]; total, −0.13 [$n = 1662$]).

Serum CK levels in the male patients were significantly higher than those in the control males (Table 7A). When the percentage abnormality of serum CK levels was compared between the patient

Table 6 Correlation of PCBs, PCQs, PCDDs, PCDFs and TEQ with serum chemistry in the patient group

Sex	Index	Correlation coefficient (*n*)				
		vs. PCBs	vs. PCQs	vs. PCDDs	vs. PCDFs	vs. TEQ
Male	Na	−0.08 (802)	0.13 (802)[a]	−0.09 (28)	0.17 (28)	0.14 (28)
	K	−0.02 (802)	0.01 (802)	0.15 (28)	−0.11 (28)	−0.14 (28)
	Ca	0.01 (638)	0.02 (638)	−0.35 (21)	−0.48 (21)	−0.51 (21)
	P	0.04 (801)	0.08 (801)	0.00 (28)	0.24 (28)	0.20 (28)
	TP	−0.10 (800)[a]	0.00 (800)	0.15 (28)	0.15 (28)	0.18 (28)
	Albumin	−0.26 (802)[a]	0.00 (802)	0.31 (28)	−0.32 (28)	−0.34 (28)
	BUN	0.16 (802)[a]	0.01 (802)	−0.15 (28)	0.06 (28)	0.33 (28)
	Creatinine	0.00 (801)	−0.10(801)[a]	0.10 (28)	0.30 (28)	0.30 (28)
Female	Na	0.02 (861)	−0.04 (861)	−0.23 (25)	−0.30 (25)	−0.27 (25)
	K	0.06 (861)	0.05 (861)	0.08 (25)	0.06 (25)	0.04 (25)
	Ca	−0.01 (828)	0.06 (828)	−0.06 (23)	0.51 (23)	0.47 (23)
	P	0.06 (861)	0.10 (861)[a]	−0.09 (25)	0.08 (25)	0.08 (25)
	TP	0.08 (862)	0.20 (862)[a]	0.01 (24)	0.26 (24)	0.23 (24)
	Albumin	−0.05 (862)	0.10 (862)[a]	−0.15 (24)	0.23 (24)	0.23 (24)
	BUN	0.08 (863)	−0.06 (863)	0.42 (25)	0.46 (25)	0.44 (25)
	Creatinine	0.17 (861)[a]	0.01 (861)	0.09 (25)	0.17 (25)	0.12 (25)

BUN: blood urea nitrogen; PCB: polychlorinated biphenyl; PCDD: polychlorinated dibenzodioxin; PCDF: polychlorinated dibenzofuran; PCQ: polychlorinated quarterphenyl; TEQ: toxic equivalent quantity; TP: total protein.
[a] $P < 0.01$ (significant correlation).

Table 7

Sex	Patient group			Control group		
	n	CK, mean ± S.D. (UI/l)	Maximum − minimum	*n*	CK, mean ± S.D. (UI/l)	Maximum − minimum
A: Serum CK value						
Male[a]	541	143.5 ± 90.7	942.0 − 28.0	46	113.6 ± 47.7	278.0 − 13.0
Female	580	125.8 ± 71.5	827.0 − 31.0	124	112.5 ± 54.0	329.0 − 15.0

Sex		Patient group		Control group	
		n (%)	CK, mean ± S.D. (UI/l)	*n* (%)	CK, mean ± S.D. (UI/l)
B: Rates of high serum CK					
Male[b]	High (CK > 197 UI/l)	99 (8.8%)	288.1 ± 117.8	2 (1.2%)	258.5 ± 27.6
	Normal (CK ≤ 197 UI/l)	442 (39.4%)	111.1 ± 35.4	43 (25.4%)	106.8 ± 36.3
Female	High (CK > 181 UI/l)	84 (7.5%)	253.3 ± 100.3	18 (10.7%)	218.1 ± 35.5
	Normal (CK ≤ 181 UI/l)	496 (44.2%)	104.2 ± 32.5	106 (62.7%)	88.1 ± 31.6
Total[b]	High	183 (16.3%)	272.1 ± 111.2	20 (11.8%)	242.6 ± 102.4
	Normal	938 (83.7%)	107.4 ± 34.1	149 (88.2%)	94.4 ± 35.3

CK: creatine kinase; S.D.: standard deviation.
[a] $P < 0.05$ between control and patient groups (*t*-test).
[b] $P < 0.05$ between control and patient groups (χ^2-test).

and control groups, the percentage abnormality of CK was significantly higher in the male patients (Table 7A and B). There was a significant correlation between serum CK and PCBs in the male patients (Table 8). A multiple regression analysis elucidated significant influence of PCB, TP, albumin and BUN on CK (Table 9).

No significant difference was found between patient and control groups regarding ESR, serum amylase in the male patients and AFP. Serum amylase in the female patients was slightly higher compared with the females in the control group. The proportion of those who were positive for HBs antigen was significantly higher in the patient group than in the control group (Tables 10 and 11). Results of the urinalysis were also within the normal range; however, urinary pH was significantly lower in the patient group than in the control group (Table 12). Urinary pH was correlated with PCB levels in the patient group (Table 13). A multiple regression analysis revealed that PCBs, TP, BUN, PCDDs and CK significantly influenced urinary pH (Table 14).

4. Discussion

In the present study we analyzed the laboratory findings and blood levels of PCBs and dioxins in two selected populations: a patient group with high levels of PCQs (=0.1 ppb), and a control group with normal blood levels of PCQs (<0.02 ppb). It is known that large amounts of PCQs contaminated the rice bran oil in the Yusho incident. The blood levels of PCQs are an important factor for the diagnosis of Yusho because high levels of blood PCQs still persist in the majority of patients. It has been reported that blood levels of PCQs are minimal in other situations such as occupational exposure to PCBs [7], suggesting that the high blood levels of PCQs are a pivotal marker for Yusho. The blood levels of PCBs gradually decreased with the years; however, the excretion of PCQs was extremely slow as shown in this study.

All values of complete blood cell counts were not affected as much in the patient group compared with the control group. The values of RBC, Hb and

Table 8 Correlation of CK with PCBs, PCQs, PCDDs, PCDFs and TEQ in the patient group

Sex	Correlation coefficient (*n*)				
	vs. PCBs	vs. PCQs	vs. PCDDs	vs. PCDFs	vs. TEQ
Male	0.14 (541)[a]	0.06 (541)	0.13 (28)	−0.27 (28)	−0.23 (28)
Female	0.08 (580)	−0.01 (580)	0.08 (25)	0.0 (25)	−0.01 (25)

CK: creatine kinase; PCB: polychlorinated biphenyl; PCDD: polychlorinated dibenzodioxin; PCDF: polychlorinated dibenzofuran; PCQ: polychlorinated quarterphenyl; TEQ: toxic equivalent quantity.
[a] $P < 0.01$ (significant correlation).

Table 9 Multiple regression analysis of CK in the patient group

A: With PCBs			B: With PCQs			C: With PCDDs		
Parameter	Standard regression coefficient	*t* value	Parameter	Standard regression coefficient	*t* value	Parameter	Standard regression coefficient	*t* value
PCBs	0.12	3.88[a]	PCQs	0.03	0.89	PCDDs	0.30	1.72
Total protein	−0.15	−4.01[a]	Total protein	−0.14	−3.72[a]	Total protein	−0.17	0.17
Albumin	0.13	3.13[a]	Albumin	0.11	2.77[a]	Albumin	0.05	0.23
BUN	0.19	4.79[a]	BUN	0.20	5.15[a]	BUN	0.26	1.23
Creatinine	0.07	1.73	Creatinine	0.06	1.54	Creatinine	0.07	0.31
Hct	−0.08	−2.38	Hct	−0.08	−2.28	Hct	0.18	1.05
Ca	−0.04	−1.16	Ca	−0.05	−1.33	Ca	0.12	0.55
Urine pH	0.02	0.68	Urine pH	0.01	0.22	Urine pH	0.53	2.89[a]
Intercept	227.78	3.00[a]	Intercept	262.80	3.46[a]	Intercept	−820.94	−1.60
$n = 941$; $R^2 = 0.09$			$n = 941$; $R^2 = 0.08$			$n = 43$; $R^2 = 0.24$		

D: With PCDFs			E: With TEQ		
Parameter	Standard regression coefficient	*t* value	Index	Standard regression coefficient	*t* value
PCDFs	0.10	0.51	TEQ	0.09	0.49
Total protein	−0.002	0.01	Total protein	0.01	0.003
Albumin	0.10	0.43	Albumin	0.10	0.43
BUN	0.24	1.08	BUN	0.24	1.10
Creatinine	0.06	0.27	Creatinine	0.06	0.27
Hct	0.21	1.18	Hct	0.21	1.17
Ca	−0.03	−0.13	Ca	−0.20	−0.10
Urine pH	0.43	2.37	Urine pH	0.44	2.37
Intercept	−433.61	−0.87	Intercept	−452.28	0.91
$n = 43$; $R^2 = 0.18$			$n = 43$; $R^2 = 0.18$		

BUN: blood urea nitrogen; CK: creatine kinase; Hct: hematocrit; PCB: polychlorinated biphenyl; PCDD: polychlorinated dibenzo-dioxin; PCDF: polychlorinated dibenzofuran; PCQ: polychlorinated quarterphenyl; TEQ: toxic equivalent quantity.
[a] $P < 0.01$.

Hct were very slightly lower in the female patients than in those of the control group. The values of RBC and Hb in the female patients showed a negative correlation with the levels of PCBs and PCQs. In the Yu-cheng cohort study in Taiwan, a substantial proportion of the exposed women are diagnosed with anemia, requiring treatment for anemia two to three times more frequently compared with controls. Some PCBs have weak estrogenic activity; therefore the anemia limited to women may be

Table 10 Other blood data

Sex	Index	Patient group			Control group		
		n	Mean ± S.D.	Maximum − minimum	*n*	Mean ± S.D.	Maximum − Minimum
Male	ESR 1-hour (mm)	764	8.5 ± 9.3	100.0 − 0.5	84	10.7 ± 13.8	105.0 − 0.5
	ESR 2-hour (mm)	753	19.0 ± 16.4	134.0 − 0.5	81	22.7 ± 23.0	153.0 − 1.5
	Amylase (IU/l)	801	108.1 ± 53.3	593.0 − 4.4	75	107.5 ± 49.2	346.0 − 42.0
	AFP (ng/ml)	794	9.8 ± 4.4	102.2 − 0.1	45	3.9 ± 2.6	10.1 − 0.5
Female	ESR 1-hour (mm)	838	13.3 ± 11.0	85.0 − 0.5	211	14.0 ± 12.3	111.0 − 0.5
	ESR 2-hour (mm)	830	28.0 ± 19.3	128.0 − 0.5	211	29.4 ± 21.0	129.0 − 1.0
	Amylase (IU/l)[a]	858	108.2 ± 38.8	367.0 − 8.9	202	97.1 ± 35.9	276.0 − 49.0
	AFP (ng/ml)	851	3.4 ± 2.6	47.5 − 0.1	139	4.0 ± 4.7	48.5 − 0.3

AFP: alpha-fetoprotein; ESR: erythrocyte sedimentation rate; S.D.: standard deviation.
[a] $P < 0.05$ between control and patient groups (*t*-test).

Table 11 HBs antibody positive rates

Sex	Patient group, *n* (%)			Control group, *n* (%)		
	(−)	(±)	(+)	(−)	(±)	(+)
Male	709 (43.2%)	2 (0.1%)	77 (4.7%)	47 (24.6%)	0 (0%)	4(2.1%)
Female[a]	805 (49.1%)	4 (0.2%)	44 (2.7%)	139 (72.8%)	0 (0%)	1 (0.5%)
Total[a]	1514 (92.3%)	6 (0.4%)	121 (7.4%)	186 (97.4%)	0 (0%)	5 (2.6%)

HBs: hepatitis B virus surface.
[a] $P < 0.05$ between control and patient groups (χ^2-test).

Table 12 Urinalysis

Sex	Index	Patient group				Control group			
		n	Mean ± S.D.	Maximum − minimum	Median	*n*	Mean ± S.D.	Maximum − minimum	Median
Male	Urinary protein (rank 1−5)	735	1.2 ± 0.5	4 − 1	1	85	1.4 ± 0.8	5 − 1	1
	Urinary glucose (rank 1−5)	794	1.1 ± 0.4	5 − 1	1	85	1.1 ± 0.4	5 − 1	1
	Occult blood (rank 1−5)	795	1.2 ± 0.7	5 − 1	1	85	1.3 ± 0.7	3 − 1	1
	Urinary urobilinogen (rank 1−5)	789	2.1 ± 0.8	4 − 2	2	85	2.1 ± 0.3	3 − 2	2
	Urinary pH	778	5.8 ± 0.8	8 − 5	6	83	6.0 ± 0.9	8 − 5	6
Female	Urinary protein (rank 1−5)	857	1.1 ± 0.5	5 − 1	1	217	1.1 ± 0.4	4 − 1	1
	Urinary glucose (rank 1−5)	857	1.0 ± 0.3	5 − 1	1	217	1.1 ± 0.4	4 − 1	1
	Occult blood (rank 1−5)	857	1.5 ± 0.9	5 − 1	1	217	1.4 ± 0.9	5 − 1	1
	Urinary urobilinogen (rank 1−5)	854	2.1 ± 0.2	4 − 2	2	216	2.1 ± 0.3	4 − 2	2
	Urinary pH[a]	853	5.8 ± 0.8	8 − 5	6	216	6.0 ± 0.9	8 − 5	6

Urinary protein, glucose, occult blood and urobilinogen ranks: 1 (−), 2 (±), 3 (+), 4 (++), 5 (+++).
[a] $P < 0.05$ between control and patient groups (Mann−Whitney test).

related to menstrual blood loss [8]. However, most of the female Yusho patients were postmenopausal with a mean age of 61.8 years. In contrast, the values of RBC and Hb were very slightly higher in the male patients compared with the controls. The values of RBC, Hb and Hct in the male patients exhibited a negative correlation with the levels of PCBs and PCQs. The results of a study in which rats were administered a very high dose of TCDD suggest the presence of mild hypochromic microcytosis and that hematological changes are not affected by co-administration of PCB [9]. In addition, there is no abnormality in human hematology findings for workers occupationally exposed to PCBs [10,11]. Decreased Hb, Hct, RBC and eosinophils were found in rats treated with a very high dose of PCB 105 (2,3,3′,4,4′-pentachlorobiphenyl), and the females were affected more than the males [12]. This suggests that PCBs and PCQs may differentially affect hematogenesis according to gender.

In our study, the WBC counts showed a slight but significant decrease only in the female patients, although neither PCBs, PCQs, PCDDs, PCDFs nor TEQ significantly correlated with WBC counts. A negative correlation was detected between WBC counts and PCBs in the male patients. It is worthy of note that New York City fire fighters exposed to PCBs and PCDFs presented normal WBC counts [10].

Table 13 Correlation of urinary pH with PCBs, PCQs, PCDDs, PCDFs and TEQ in the patient group

Sex	Correlation coefficient (*n*)				
	vs. PCBs	vs. PCQs	vs. PCDDs	vs. PCDFs	vs. TEQ
Male	−0.13 (778)[a]	0.05 (778)	−0.06 (25)	−0.35 (25)	−0.41 (25)
Female	−0.10 (853)	−0.04 (853)	−0.45 (28)	−0.32 (28)	−0.30 (28)
Total	−0.10 (1631)[a]	0.00 (1631)	−0.16 (53)	−0.18 (53)	−0.19 (53)

PCB: polychlorinated biphenyl; PCDD: polychlorinated dibenzodioxin; PCDF: polychlorinated dibenzofuran; PCQ: polychlorinated quarterphenyl; TEQ: toxic equivalent quantity.
[a] $P < 0.01$ (significant correlation).

Table 14 Multiple regression analysis of urinary pH in the patient group

A: With PCBs

Parameter	Standard regression coefficient	*t* value
PCBs	−0.14	−4.23[a]
CK	0.02	0.68
Total protein	−0.10	−0.10[a]
Albumin	0.10	0.10
BUN	−0.16	−0.16[a]
Creatinine	−0.04	−0.04
Hct	−0.05	−0.05
Ca	−0.01	−0.01
Intercept	6.92	9.68[a]

$n = 941$; $R^2 = 0.06$

B: With PCQs

Parameter	Standard regression coefficient	*t* value
PCQs	−0.08	−2.34
CK	0.01	0.22
Total protein	−0.11	−3.00[a]
Albumin	0.12	2.97[a]
BUN	−0.18	−4.60[a]
Creatinine	−0.03	−0.75
Hct	−0.06	−1.76
Ca	0.001	0.03
Intercept	6.68	9.29[a]

$n = 941$; $R^2 = 0.08$

C: With PCDDs

Parameter	Standard regression coefficient	*t* value
PCDDs	−0.41	−2.97[a]
CK	0.37	2.89[a]
Total protein	−0.14	−0.82
Albumin	0.15	0.83
BUN	−0.15	−0.83
Creatinine	−0.17	0.92
Hct	−0.31	−2.34
Ca	−0.23	−1.37
Intercept	15.56	3.75[a]

$n = 43$; $R^2 = 0.18$

D: With PCDFs

Parameter	Standard regression coefficient	*t* value
PCDFs	−0.28	−1.78
CK	0.33	2.37
Total protein	−0.05	−0.27
Albumin	0.11	0.56
BUN	−0.08	−0.41
Creatinine	−0.20	−1.02
Hct	−0.43	−3.03[a]
Ca	−0.03	−0.20
Intercept	10.06	2.22

$n = 43$; $R^2 = 0.16$

E: With TEQ

Index	Standard regression coefficient	*t* value
TEQ	−0.29	−1.86
CK	0.33	2.37
Total protein	−0.04	−0.21
Albumin	0.10	0.54
BUN	−0.09	−0.45
Creatinine	−0.20	−1.03
Hct	−0.42	−3.02[a]
Ca	−0.05	−0.32
Intercept	10.52	2.78

$n = 43$; $R^2 = 0.16$

BUN: blood urea nitrogen; CK: creatine kinase; Hct: hematocrit; PCB: polychlorinated biphenyl; PCDD: polychlorinated dibenzo-dioxin; PCDF: polychlorinated dibenzofuran; PCQ: polychlorinated quarterphenyl; TEQ: toxic equivalent quantity.

[a] $P < 0.01$.

Total WBC counts in animal models exposed to certain organochlorine compounds vary [13,14]. Total WBC counts from nonbreeding kestrels were significantly increased in PCB-exposed males but not females [15]. Long-term exposure to PCBs may induce impairment of granulocyte function due to inappropriate activation. PCBs elevate intracellular Ca^{2+} in human granulocytes [16], which affects their function of killing invading microorganisms by ingestion into phagocytic vacuoles and bombarding them with oxidants and the contents of intracellular granules [17]. Coplanar PCBs have been shown to impair immune function by decreasing in vivo cytotoxic T lymphocyte activity and antibody production in exposed animals [18,19]. PCB 126 (3,3′,4,4′,5-pentachlorobiphenyl) binds to the aryl hydrocarbon (Ah) receptor in a similar way as do 2,3,7,8-dioxins [20]. The Ah receptor-independent mechanism of immunotoxicity also demonstrates a lack of inhibition of antibody response on exposure to TCDD or PCBs [21,22].

All serum electrolytes were generally not affected in the patient group. Potassium was slightly higher than in the control group. The levels of potassium did not correlate with PCBs, PCQs, PCDDs, PCDFs or TEQ. Serum potassium values are reported to decrease in penned white pelicans exposed to PCBs [23]. In 1988, serum concentrations of potassium showed no abnormality in 102 Yusho patients [24]. Serum inorganic phosphorus values in the female patients were slightly lower compared with the controls. Serum phosphorus levels showed a positive correlation to PCQs. Urinary excretion of phosphorus is significantly higher in Yusho patients than in the controls in 1988 [24]. Values of serum inorganic phosphorus were not significantly changed by the treatment with PCBs in penned white pelicans [23]; in addition, serum PCB levels do not correlate with the urinary excretion of phosphorus [24]. In contrast, the phosphorus concentration in chick serum decreased when PCBs were administered [25].

The levels of TP were lower in the male patients compared with the males of the control group, and showed a slight negative correlation with PCBs levels. The TP levels showed a small positive correlation with the levels of BUN in the male patients but not in the control group. Decreased TP and albumin plasma concentrations are demonstrated in pigeons administered with Arochlor 1254, which may be due to a reduced hepatic protein synthesis [26]. The overall increase in protein breakdown may cause the increased plasma concentration of urea as found in our data [26].

In Yusho, the percentage abnormality of serum CK in the patients was higher than in the controls. Arochlor 1254 dose-dependently inhibits both the fusion of myoblasts into multinucleated myotubes and CK activity without effect on cell density [27]. Dehydration, hyperexercise and high levels of PCBs affect elevation of serum CK [28]. The reason for its elevation is not obvious but PCBs may have a role.

Large-scale studies have reported increased mortality from cancer, lymphoma and sarcoma, brain tumor, non-Hodgkin's lymphoma, malignant melanoma and sarcoma [29—34]. In addition, PCBs have been shown to induce liver cancer in rats and are classified as probable human carcinogens [35,36]. However, liver tumor is not associated with exposure to PCBs [33]. Malignant neoplasm mortality in Yucheng does not differ significantly from that in the general Taiwanese population [37]. As to markers for malignant neoplasms, only AFP was analyzed in this study, with no elevation being found in Yusho patients. The proportion of patients who were positive for the HBs antigen was 7.4%. The HB virus and hepatitis C virus are also carcinogenic. Areas where Yusho occurred are high-risk areas for hepatoma [38,39].

Urinary pH in Yusho patients was more acidic than that in controls, and it correlated with PCB levels. Exposure to PCBs induces an increase in urinary ascorbic acid in rats [40—42]. Therefore, ascorbic acid or some other metabolites may be involved in the urinary acidosis.

In general, complete blood cell counts, blood chemistry and urinalysis are not so affected in Yusho. However, PCBs, PCQs and PCDFs may affect hematogenesis, serum potassium, serum phosphorus, protein metabolism and CK metabolism. Exposure to PCBs and related organochlorine compounds should be avoided.

Acknowledgement

This work was supported in part by a Grant-in-Aid for scientific research from the Ministry of Health, Labour and Welfare, Japan.

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Journal of Dermatological Science Supplement (2005) 1, S57—S63

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Ophthalmic findings in Yusho

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KEYWORDS
Dioxin;
Polychlorinated biphenyls (PCBs);
Polychlorinated dibenzofurans (PCDFs)

Summary

Background: The ocular signs in Yusho include hypersecretion by the meibomian glands, abnormal pigmentation of the bulbar conjunctiva, unusual pigmentation of the limbal conjunctiva, pigmentation of the tarsal conjunctiva and edema of the eyelid.
Participants and methods: The ocular symptoms in Yusho patients were analyzed to investigate their relationship with the concentration of dioxins in the blood. The participants were patients with Yusho who underwent examinations including measurement of blood dioxin levels and ocular symptoms in 2002.
Results and conclusion: The significant relation between the increase in ocular discharge and the level of polychlorinated dibenzofurans (PCDFs) in the blood is currently considered strong. No significant relationship with blood PCDF levels was found with any of the other four ocular symptoms. Although the blood levels of polychlorinated biphenyls (PCBs) and dioxins are now decreasing in Yusho patients, they still cause abnormal discharge from the eye.

1. Introduction

Systematic epidemiological studies based on the observation of initial case series have clarified that ocular symptoms in Yusho manifest as an increase in conjunctival discharge, edema of the upper eyelid, visual disturbances and ocular pain [1]. The ocular signs of the disease within 1 year after accumulated ingestion include hypersecretion by the meibomian glands, abnormal pigmentation of the bulbar conjunctiva, unusual pigmentation of the limbal conjunctiva, pigmentation of the tarsal conjunctiva and edema of the eyelid [2]. These ocular symptoms are closely related to the concentration of polychlorinated biphenyls (PCBs) and the gas-chromatographic patterns of PCBs in the patient's blood [3]. Although PCBs used as a heating medium for rice bran oil were initially believed to be the causal agent of Yusho oil disease, subsequently the effects of polychlorinated dibenzofurans

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1574-0757/$30.00 © 2004 Published by Elsevier Ireland Ltd on behalf of Japanese Society for Investigative Dermatology.
doi:10.1016/j.descs.2005.03.009

(PCDFs; dioxin-related compounds created from the degeneration of PCBs by heat) on health was suspected (reviewed in [4]). Further investigation showed that the most important causal agents of Yusho were PCDFs, rather than PCBs [1,5].

Recently, the ocular manifestations, which were extremely prominent at the time of onset, have remarkably subsided but many patients still suffer from them, particularly abnormal discharge from the eyes. Because of recent technical developments in the measurement of the group of dioxins such as polychlorinated dibenzodioxins (PCDDs) and PCDFs, the measurement of blood levels of PCDFs and related compounds has become possible with the small amounts of blood collected at general health examinations of the patients with Yusho. Consequently, determination of blood concentrations of these compounds in patients with Yusho was started from the annual health examinations in 2001.

About 40 PCDF congeners were identified in the rice bran oil that was ingested by Yusho patients [6]. Of the various PCB derivatives, 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) was known to predominantly persist in the tissues of patients with Yusho and Yu-Cheng [7]. An animal study revealed that PCDFs cause severe atrophy of the thymus and significant hypertrophy of the liver in rats [8]. In this paper, we analyze the relationship between blood PCDF levels and ocular symptoms in Yusho patients.

2. Methods

2.1. Participants and examination items

The participants were 259 patients with oil poisoning (128 males, 131 females) for whom blood PCDF levels were measured and all five ocular examinations were carried out in 2002. The age of the patients ranged from 30 to 88 years. The results of ocular findings were linked to blood PCDF levels for relevant analysis, and the relationship between ocular symptoms or examination results and blood PCDF levels was investigated. The ophthalmic examination items were increased discharge from the eye, edema of the eyelids, pigmentation of the eyelids, hypersecretion by the meibomian glands and follicular formation in the conjunctiva. These items were graded according to severity as follows: (1) no symptoms; (2) very mild symptoms; (3) moderate symptoms; (4) heavy symptoms; and (5) severe symptoms. The blood samples were investigated for 2,3,4,7,8-PeCDF, total PCDFs, total toxic equivalent quantity (TEQ) and total PCDF—TEQ.

2.2. Investigation and statistical methods

Detailed analyses were conducted with the data from 259 patients with Yusho for whom levels of PCDFs or related compounds were measured in 2002. The data underwent a multiple regression analysis (general linear model) in which the logarithmic values of the total PCDF levels, 2,3,4,7,8-PeCDF levels, total PCDF—TEQ and total TEQ were the dependent variables, and the severity of each examination item was the fixed factor. When the dependent variables (logarithmic levels of the total PCDF levels, 2,3,4,7,8-PeCDF levels, total PCDF—TEQ and total TEQ) were compared with the levels actually measured, the distribution of the logarithmic levels was found to be closer to a normal distribution. Thus, the logarithmic levels were used. The association of the severity of each examination item with the logarithmic values of the total PCDF levels, 2,3,4,7,8-PeCDF levels, total PCDF—TEQ and total TEQ was assessed using multiple linear regression analysis. The SAS software package (SAS Institute, Cary, NC, USA) was used to perform all statistical analyses. A two-tailed P-value <0.05 was considered statistically significant.

3. Results

The ocular findings in the 259 patients were as follows: abnormal discharge from the eye (139/

Table 1 Relationship between blood PCDF levels and ocular symptoms in Yusho patients

	2,3,4,7,8-PeCDF	Total PCDF	Total PCDF—TEQ	Total TEQ
Discharge	0.045	0.024	0.024	0.039
Meibomian gland	0.607	0.392	0.69	0.38
Pigmentation	0.731	0.708	0.82	0.645
Follicle	0.326	0.654	0.364	0.558
Edema	0.873	0.799	0.815	0.857

Logarithmic value of blood PCDF levels were analyzed for the relationship with ocular symptoms in Yusho patients. Data shown are two-tailed P-values. Discharge: increased discharge from the eye; meibomian gland: hypersecretion by the meibomian gland; pigmentation: pigmentation of the eyelids; follicle: follicular formation in the conjunctiva; edema: swelling of the eyelids. PCDF: polychlorinated dibenzofuran; PeCDF: pentachlorodibenzofuran; TEQ: toxic equivalent quantity.

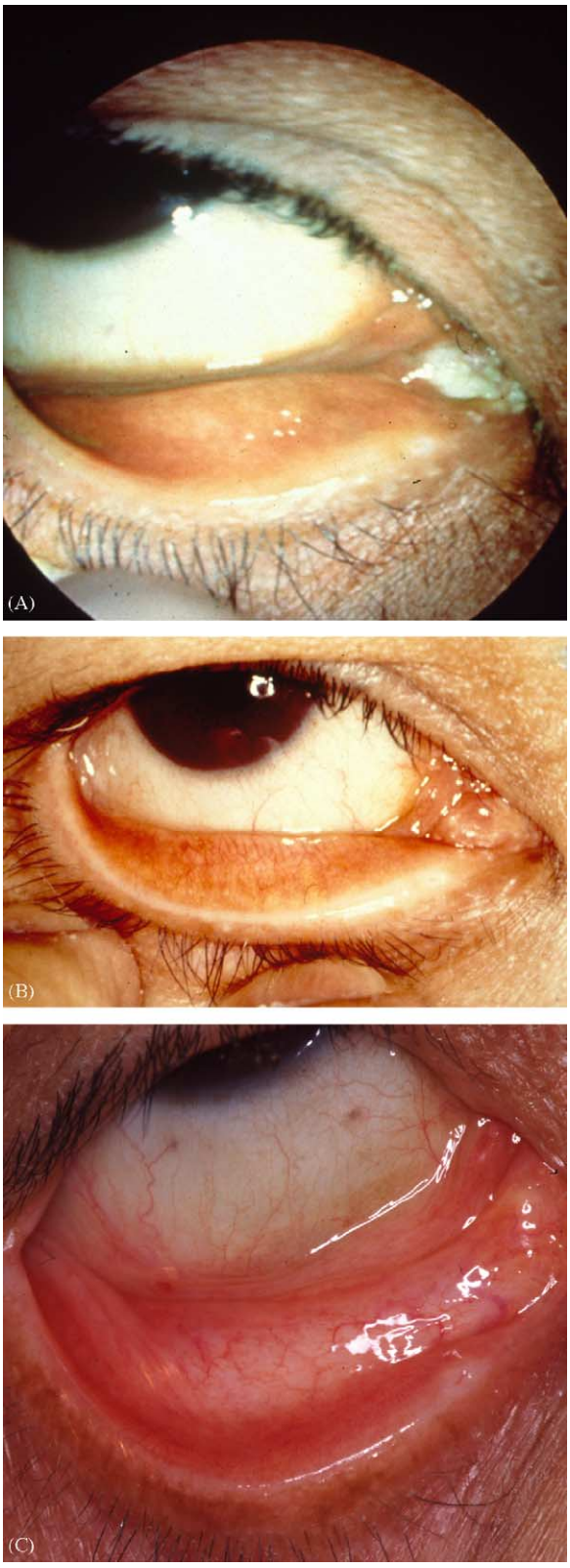

Fig. 1 Change in hyperpigmentation of palpebral conjunctiva of a woman (33 years at the time of the incident) after Yusho poisoning. (A) Within 1 year after the incident (33 years of age). (B) Thirteen years after the incident (46 years of age). (C) Twenty-nine years after the incident (62 years of age). Hyperpigmentation of palpebral conjunctiva after the Yusho incident was initially severe with abnormal discharge (A), but gradually improved (B), and finally no pigmentation was found (C).

259 = 53.7%); swelling of the eyelid (27/259 = 10.4%); abnormal pigmentation of the palpebral conjunctiva (51/259 = 19.7%); follicular formation in the conjunctiva (43/259 = 16.6%); and hypersecretion by the meibomian glands (28/259 = 10.8%).

The relationship between the concentration of dioxins in the blood and ocular symptoms was analyzed. We found that the concentrations of 2,3,4,7,8-PeCDF, total PCDFs, total PCDF—TEQ and total TEQ were significantly related to abnormal discharge from the eye after adjusting for sex and age ($P < 0.05$). The remaining ocular symptoms had no significant relationships with the analyzed blood dioxins (Table 1).

4. Discussion

Yusho is food poisoning by rice bran oil contaminated with PCBs and their heat-degraded by-products such as PCDDs and PCDFs. The Yusho incident occurred mainly in the Kyushu area and widely in western Japan in 1968. The strange disease was characterized by acneform eruptions, pigmentation of the skin and eye discharge. Eleven years later, a similar incident occurred in Yu-Cheng, Taiwan. Similar to Yusho patients, the common ophthalmologic complaints of 117 Yu-Cheng patients examined within 1 year of the incident were increased eye discharge (81%), edematous swelling of the eyelids (59%), conjunctival pigmentation (67%), and hypersecretion by the meibomian glands (70%). As with Yusho patients, the frequency and severity of these findings were also closely related to PCB concentrations in the blood [9]. In Yusho, the prevalence of ocular symptoms within 1 year of the incident was as follows: hypersecretion by the meibomian glands (52.6%), abnormal pigmentation of limbal conjunctiva (45.2%), abnormal pigmentation of tarsal conjunctiva (29.4%), and swelling of the eyelids (15.8%). To date, more than 35 years have passed since the Yusho poisoning incident. The objective ophthalmic symptoms are disappearing (Fig. 1), but many Yusho patients still suffer with these symptoms. In this study, it was found that many patients still have abnormal discharge from the eye (53.7%), and 10—20% of patients still have either hypersecretion by the meibomian glands, swelling of the eyelids, follicular formation in the tarsal glands or abnormal pigmentation of the palpebral conjunctiva.

Clinical manifestations and the course of Yusho are disproportionately severe and persistent relative to the observed blood levels of PCBs, whereas patients occupationally exposed to pure PCBs have a characteristically mild and benign clinical course despite blood PCB levels that are often much higher than those noted in Yusho patients [5]. In 1975, 1976 and 1977, Nagayama et al. [10—12] detected a significant amount of PCDFs in the contaminated rice bran oil consumed by Yusho patients as well as in the tissues of Yusho patients. Further investigation showed that the most important causal agents for Yusho were PCDFs, rather than PCBs [1].

PCB poisoning and ocular symptoms have been investigated in experimental animal studies. Swelling of the eyelids and a purulent discharge from the eyes of monkeys administrated with PCBs have been reported [13]. The histological changes in the tarsal glands induced by PCBs in experimental animals, and autopsy cases of Yusho patients showed that within several months after oral administration of PCBs, with or without PCDFs, typical swelling of tarsal glands and edema of eyelids was induced.

A white, cheese-like secretion issuing from the orifice of the duct of the meibomian gland when the eyelid was squeezed was one sign of poisoning in Yusho patients (Fig. 2). In the rhesus monkey, abnormal hyperkeratosis of the ductal epithelium was observed histopathologically [14]. One month after the ingestion of PCBs, the monkeys began to spontaneously secrete a discharge from the eyes and, when pressure was applied to the eyelids, white cheese-like matter was excreted. Histological studies showed the meibomian glands to be compressed by a keratin cyst while they had atrophied [1]. KOH treatment and Sudan III staining of meibomian glands in experimental PCB-poisoned monkeys showed the partial disappearance of the glands and enlargements of the ducts (Fig. 3) [15]. Pathological changes in the meibomian gland were also observed clinically in chronic blepharitis. A dysfunction of the meibomian gland was diagnosed based on meibomian gland expression, meibography, tear osmolarity and Schirmer's test [16].

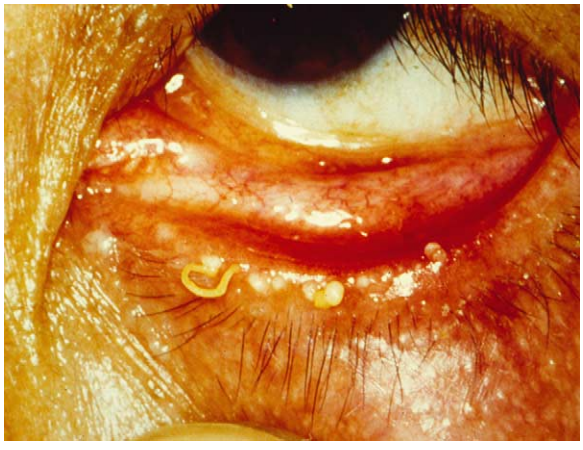

Fig. 2 Abnormal cheese-like secretion from the meibomian glands in a Yusho patient.

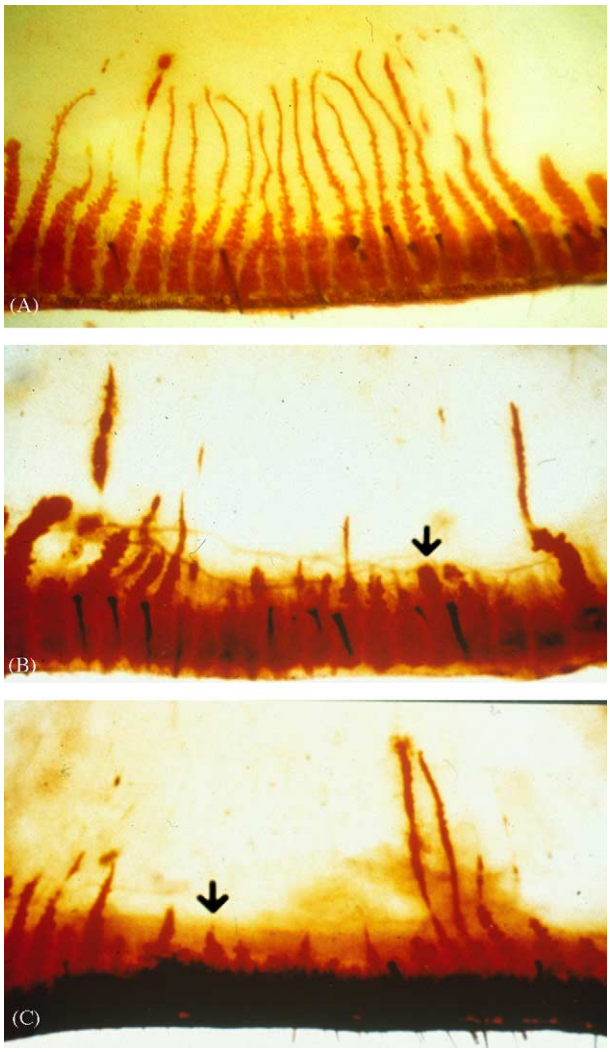

Fig. 3 Meibomian glands in experimental PCB poisoning. (A) Meibomian glands of right upper eyelid in naïve rhesus monkey. (B) Meibomian glands of right upper eyelid in PCB-poisoned rhesus monkey. Partial disappearance of the glands and enlargement of the ducts. (C) Meibomian glands of right upper eyelid in PCB-poisoned crab-eating monkey. Almost all meibomian glands have disappeared in the center of the eyelid.

The change in meibomian glands were precisely observed in PCB- and PCDF-poisoned monkeys (Fig. 4) [17]. In that study, the changes in the meibomian glands were divided into four stages according to the histological examinations. In the initial or early stage, the gland presented with hyperplasia and hyperkeratosis of the ductal epithelium with slightly dilated lumen, but no apparent

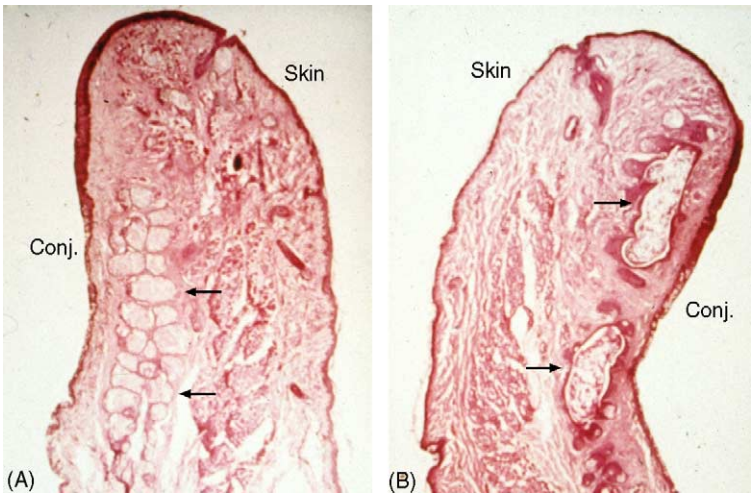

Fig. 4 Sagittal plane of lower eyelid in monkey. (A) Normal control: normal glands and ducts. (B) 32 days after PCB administration: ducts are dilated, glands are atrophic, and the walls of the glands are slightly hyperkeratinated. Conj.: conjunctiva.

changes of the surrounding alveoli were found. The second stage was characterized by accumulated keratin in the ductal lumen and atrophic alveoli showing a decrease of sebaceous cells with squamous metaplasia. Following the second stage the gland revealed the most characteristic histology of the lesion: keratinous cyst, which exhibited abundant keratin plugs in the remarkably dilated lumen and thin wall of the duct with disappearance of the surrounding alveolar project. This stage was classified as the third or keratinous cyst stage. In the last or collapse stage, only the atrophic wall of the cyst remained, probably due to a discharge of the contents spontaneously or by squeezing. Keratinous cyst of the duct with atrophy or disappearance of the alveoli of the tarsal glands were the fundamental changes, so the hypersecretion by the tarsal gland did not seem to be an enhanced activity of the gland but discharge of keratinous plugs. Dogs administered with experimental pentachlorobiphenyl and squalane also showed these characteristic changes of meibomian glands: dilation of the duct and squamous metaplasia of the alveolar cells [18]. The meibomian gland taken from the eyelid of a 25-year-old man who had demonstrated symptoms of Yusho for 1 year showed a dilation of the duct with an accumulation of keratin and atrophy of the alveoli with squamous metaplasia [1]. Pathological changes of the meibomian glands in Yusho patients were regarded as a process of keratinous cyst formation of the duct [1].

A study in Yu-Cheng patients showed similar symptoms and blood dioxins to Yusho patients, except higher concentrations of 2,2′,4,4′,5,5′-hexachlorobiphenyl (HxCB; 2.2 times higher in Yu-Cheng patients compared with Yusho patients), and lower concentrations of 2,3,4,7,8-PeCDF (3.8 times lower in Yu-Cheng patients compared with Yusho patients) [19]. It is interesting to note the relationship between ocular symptoms and blood levels of 2,3,4,7,8-PeCDF and other dioxin-related compounds in Yu-Cheng patients, because the blood concentration of 2,3,4,7,8-PeCDF in Yusho patients with abnormal discharge from the eye was less than two times higher than that in Yusho patients without abnormal discharge from the eye (data not shown).

PCBs, PCDFs and PCDDs accumulate in the body through contaminated food such as meat and fish [20,21]. Because these chemicals persist for a long time in humans [22], they affect the offspring via the placenta [23,24] and via breast milk [25]. In animal studies, prenatal exposure to PCBs and tetrachlorodibenzodioxin (TCDD) caused significant developmental toxicity including poor visual recognition [26]. In humans, children exposed to dioxins transplacentally and via contaminated breast milk suffer from severe adverse effects [27,28]. Patients with pre- and/or postnatal exposure to PCBs and/or their derivatives have a lower birth size and age [29], developmental [30,31], immunological [32—34], neurological [35,36], cognitive [37,38], musculoskeletal [39] and behavioral [35] abnormalities and lower intelligence [37,40,41], although to date no detailed study on ophthalmic abnormalities has been reported. In this study, 11 patients (six males, five females) who had possibly been exposed to PCBs and their derivatives through placenta and/or breast milk, and three patients (two males, one female) displayed abnormal discharge from the eye, although their PCDF levels, total PCDF levels, total PCDF—TEQ and total TEQ were not high (data not

shown). The reason behind this remains to be elucidated.

In this study, we have shown the relationship between PCDF levels and ocular symptoms in Yusho patients. Previous studies had revealed the relationship between concentration of PCBs and ocular symptoms, and it was thus suspected that the levels of dioxins are related to the severity of ocular symptoms. However, only one of the five ocular symptoms studied, abnormal discharge from the eye, was currently found to be related to blood PCDF level. The reason for this result (i.e., no relationship between blood PCDF levels and the remaining four ocular symptoms) may be that the meibomian glands have completely undergone atrophic change, and all the keratinous plugs in the glands may have been evacuated so that no more hypersecretion was seen in many patients.

More than 35 years have passed since the Yusho incident, and the decrease in TEQ may reduce the severity of the symptoms, because TEQ concentration just after the Yusho incident was calculated to be 40 ppb and it decreased to 600 ppt in 1995 [19]. In addition to the weakened activity of the disease as a result of decreasing concentrations of dioxins in the body, the healing process in the individual patient may have worked to absorb abnormal pigmentation.

In addition to the report that PCBs themselves do not cause severe symptoms as observed in the Yusho patients, there are reports describing people exposed to high levels of PCDFs did not suffer from the symptoms as much as Yusho patients. However, it should be noted that symptoms of Yusho are not caused only by PCDFs, but also by various toxins such as PCBs, coplanar PCBs and PCDDs that were present in the contaminated rice bran oil.

Acknowledgement

This work was supported in part by a Grant-in-Aid for scientific research from the Ministry of Health, Labour and Welfare, Japan.

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Journal of Dermatological Science Supplement (2005) 1, S65—S72

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Oral mucosa and dental findings in Yusho

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KEYWORDS
Oral pigmentation;
Polychlorinated biphenyls (PCBs);
Polychlorinated dibenzofurans (PCDFs).

Summary

Background: Yusho is a disease caused by the ingestion of rice bran oil contaminated with polychlorinated biphenyls (PCBs), polychlorinated dibenzofurans (PCDFs) and related compounds. Oral lesions such as oral pigmentation, anomalies of the dental root shape and deficiency of tooth germs have been observed in Yusho patients.
Objective: The purpose of this study was to determine the prevalence of oral lesions, especially oral pigmentation, in Yusho patients, and also to describe the relationship between oral lesions and PCBs or PCDFs.
Methods: Visual and radiographic examinations were performed on Yusho patients who visited the dentist during the annual health examination in Fukuoka prefecture. The data obtained from 1968 to 2003 were analyzed.
Results: Gingival pigmentation was the most common of all types of oral pigmentation seen in Yusho patients. Of all the examined Yusho patients, the proportion who had gingival pigmentation was more than 60% during the early phase after the Yusho incident, but this value had decreased to below 30% in 1993. However, it subsequently increased again to about 50% in 2003. Yusho patients with a blood PCB pattern typical of Yusho showed the highest incidence of oral pigmentation 5 years after PCB poisoning. As time passed, however, there was no specific difference in the prevalence of oral pigmentation between any type of PCB pattern, either specific to Yusho or commonly observed in the general population. Analysis of data by three-way analysis of variance (ANOVA) in 2001 and 2002 showed that there was a close relationship between the presence of upper gingival pigmentation and blood PCDF levels.
Conclusions: The prevalence of oral pigmentation still remains high even after 35 years, and PCDFs may be responsible for the presence of oral pigmentation.

1. Introduction

Almost 35 years have passed since the first case of Yusho, caused by an incident of massive poisoning by polychlorinated biphenyls (PCBs) and related compounds, was reported. In addition to clinical findings

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1574-0757/$30.00 © 2004 Published by Elsevier Ireland Ltd on behalf of Japanese Society for Investigative Dermatology.
doi:10.1016/j.descs.2005.03.010

such as general malaise, loss of appetite, increased discharge from the meibomian glands, etc., various skin symptoms such as acneform eruption and pigmentation were striking features characteristic of Yusho in the initial stage of the outbreak [1]. Dental examination revealed that oral pigmentation, anomalies of the dental root shape and retarded eruptions of permanent teeth were observed during the early phase after the Yusho incident [2]. As time passed, oral pigmentation faded very slowly but persisted in about half of the Yusho patients [3]. In this article, we describe the change in oral lesions mentioned above, and the relationship between oral lesions and peripheral blood PCB and/or polychlorinated dibenzofuran (PCDF) concentration.

2. Materials and methods

Oral examination was performed on Yusho patients who visited the outpatient clinic at Kyushu University Dental Hospital or the dentist during the annual health examination in Fukuoka prefecture. After asking about chief complaints, a visual examination was carried out to determine the presence or absence of the oral lesions listed in Table 1. Oral pigmentation was subdivided into four groups: gingival pigmentation, pigmentation of buccal mucosa, palatal pigmentation, and pigmentation of lips. The severity of oral pigmentation was classified into five groups: −, none; ±, very slight; +, slight; ++, moderate; and +++, severe (Figs. 1–4). Panoramic radiographs were also taken to observe the condition of marginal bone resorption, anomalies of dental root shape and any deficiency of permanent tooth germs.

Table 1 Items of the oral examination

Gingivitis
Periodontitis
Anomalies of tooth eruption
Discoloration of teeth
Malocclusion
Hypoplasia of dental hard tissue
Oral pigmentation

3. Results

3.1. Oral pigmentation

Oral pigmentation was the most prominent feature of the oral lesions seen in Yusho patients. During the early phase after the Yusho incident, the prevalence of oral pigmentation was about seven times higher in Yusho patients than in clinically healthy people (Table 2). Oral pigmentation tended to be mostly brownish or dark brownish in color and was pleomorphic in shape exhibiting a diffuse band-like form, a rounded form, etc. (Figs. 1–4). However, no specific pattern of pigmentation characteristic to Yusho has yet been observed. In addition, no definitive difference between sexes has been observed. As for the localization of oral pigmentation, gingival pigmentation was the most common, followed by pigmentation of lips and pigmentation of buccal mucosa (Fig. 5). Pigmentation of the palate or tongue was only rarely observed.

The examination to determine the prevalence of oral pigmentation showed that more than about 60% of the Yusho patients suffered from gingival pigmentation from 1972 to 1982. As time passed, the prevalence of gingival pigmentation decreased

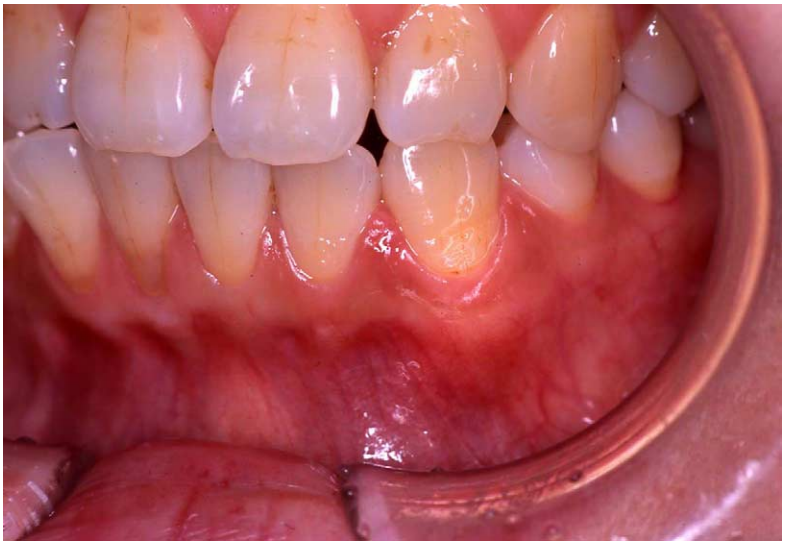

Fig. 1 Very slight gingival pigmentation (±).

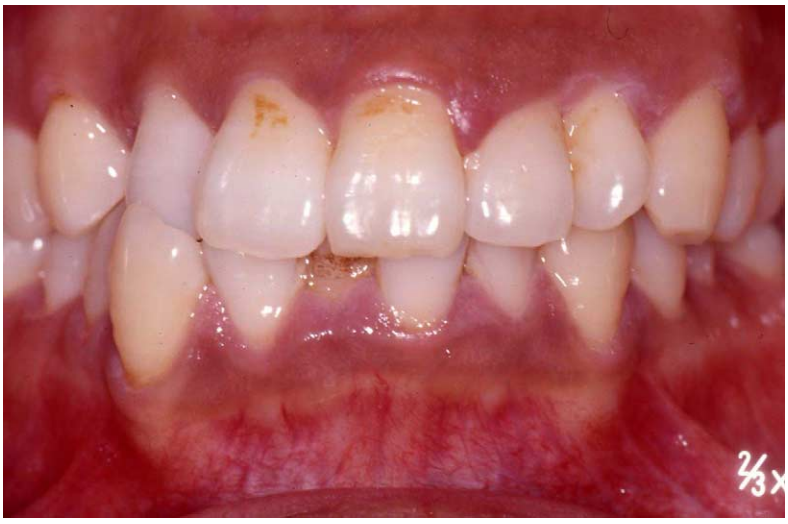

Fig. 2 Slight gingival pigmentation (+).

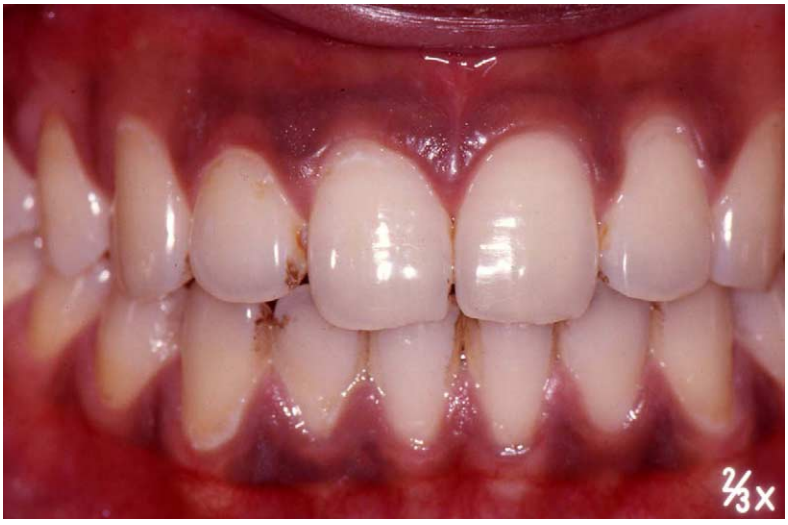

Fig. 3 Moderate gingival pigmentation (++).

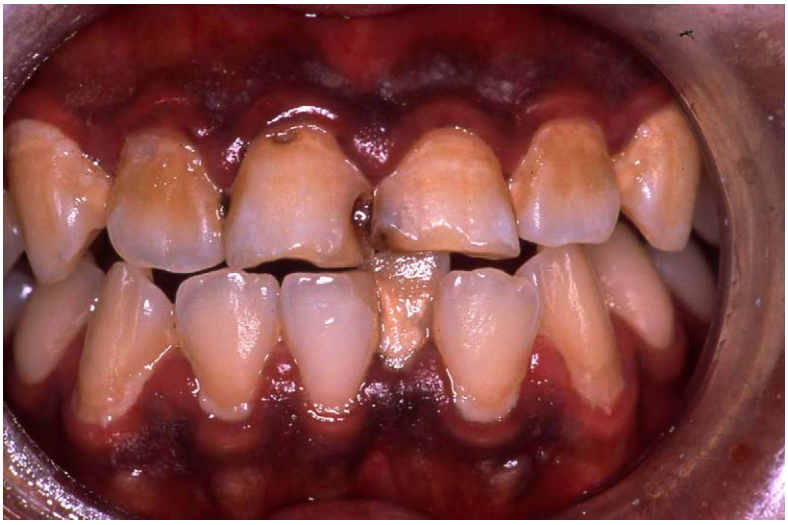

Fig. 4 Severe gingival pigmentation (+++).

Table 2 Prevalence of oral pigmentation by age group

	Age group (years)							Total
	0–10	11–20	21–30	31–40	41–50	51–60	>61	
Yusho patients								
+	10	5	12	10	4	2	1	44
–	6	5	5	5	2	3	0	26
%	62.5	50.0	70.6	66.7	66.7	40.0	100.0	62.9
Healthy controls								
+	2	2	3	4	4	3	2	20
–	26	26	40	42	26	20	14	194
%	7.1	7.1	7.0	8.7	13.3	13.0	12.5	9.3

(+) With oral pigmentation, (–) without oral pigmentation, (%) proportion of those with oral pigmentation relative to total number of patients or healthy persons in each age group (reproduced with permission from Aono and Okada [6]).

somewhat from 1987 to 1993, but increased gradually thereafter (Fig. 5). With regard to pigmentation of the lips and buccal mucosa, a gradual decrease in prevalence was observed from 1982, and less than 5% of Yusho patients suffered from such pigmentation in 2003. Soon after the PCB poisoning, moderate (++) or severe (+++) oral pigmentation was commonly observed in Yusho patients. Although oral pigmentation faded only very slowly, Yusho patients with moderate or severe oral pigmentation were rarely observed recently. Table 3 shows the relationship between gas chromatographic patterns of blood PCBs and the prevalence of oral pigmentation in 1973, 1982 and 2002. In 1973, Yusho patients with type A blood PCB pattern, which was specific to Yusho, showed the highest incidence of oral pigmentation. In contrast, oral pigmentation in Yusho patients with type C blood PCB pattern, which was commonly observed in the general population, was less common than in Yusho patients with type A or B blood PCB pattern. In 1982 and 2002, however, there was no specific difference in the prevalence of oral pigmentation between any type of blood PCB pattern. Fig. 6 shows the prevalence of oral pigmentation and the mean value of blood PCDF concentration according to age group in 2002. The prevalence of oral pigmentation tended to be higher in younger patients than in elder patients. In contrast, the mean value of blood PCDF concentration was lowest in Yusho patients in their 30s, and increased to its peak value in patients in their 60s, but decreased thereafter. However, analysis of data by three-way analysis of variance (ANOVA) showed a close relationship between the presence of upper gingival pigmentation and blood PCDF concentration (see [4]).

3.2. Other lesions

Follow-up examination revealed that retarded eruption of permanent teeth, deficiency of tooth germs and anomalies of dental root shape (Fig. 7) were observed in affected children, but oral lesions such as malocclusion, discoloration of teeth and hypoplasia of dental hard tissue were rarely observed. Retarded eruption, deficiency of tooth germs and anomalies of dental root shape were observed in nine of 51 examined children (17.6%), in four of 43

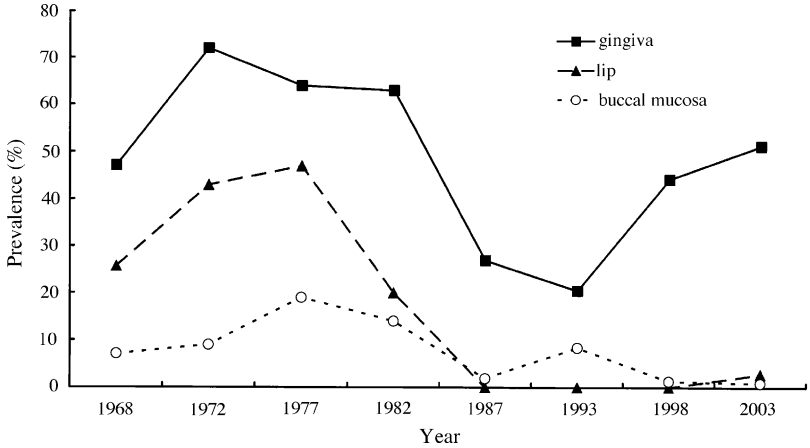

Fig. 5 Change in prevalence of oral pigmentation from 1968 to 2003.

Table 3 Change in prevalence of oral pigmentation by type of PCB pattern[a]

Type of PCB pattern	1973			1982			2002		
	+	−	%	+	−	%	+	−	%
A	23	9	71.9	30	9	76.9	24	14	63.2
B	15	7	68.2	10	4	71.4	16	14	53.3
BC	7	8	46.7	1	0	100.0	1	0	100.0
C	20	14	58.8	19	5	79.2	28	15	65.1
Total	65	38	63.1	60	18	76.9	69	43	61.6

(+) Yusho patients with oral pigmentation, (−) Yusho patients without oral pigmentation, (%) proportion of Yusho patients with oral pigmentation relative to total number of patients with each type of PCB pattern.

[a] Gas chromatographic pattern of blood PCBs: type A is specific to Yusho patients; type B is an intermediate pattern between types A and C; type C is commonly observed in the general population; and type BC is indistinguishable from type B or C.

examined children (9.3%), and in 36 of 47 examined children (76.6%), respectively, from 1969 to 1976 (Tables 4—6). However, lesions such as anomalies of tooth eruption were not observed in affected children who recently visited the dentist during the annual health examination.

4. Discussion

Fukuyama et al. reported that oral pigmentation, parakeratosis of the gingiva, anomalies of dental root shape, retarded eruptions of permanent teeth and deficiency of tooth germs were observed in Yusho patients [2,5]. Of all these lesions, oral pigmentation was the most prominent feature in Yusho patients. Soon after the Yusho incident, the prevalence of oral pigmentation was about seve times higher in Yusho patients than in clinically healthy controls [6]. The precise mechanism for the presence of oral pigmentation after PCB poisoning has not been sufficiently clarified because no oral pigmentation similar to that seen in Yusho patients was observed in animals with experimental PCB poisoning [7,8]. Histological examination of the biopsied gingiva with pigmentation from Yusho patients revealed that numerous melanosomes and melanosome complexes were present in the keratinocytes from the basal cell layer to the suprabasal cell layer [9]. Similar findings were observed in the conjunctiva of Yusho patients [10]. Both melanocyte-stimulating hormone (MSH) and adrenocorticotropic hormone (ACTH) have been known to stimulate melanocytes directly [11]. PCBs have been reported to suppress the secretion of adrenocortical hormones, which are thought to inhibit the secretion of MSH and ACTH [12]. Taking these findings into consideration, it is probable that PCBs indirectly stimulate melanocytes to produce melanosomes, resulting in an overproduction of melanosomes in oral mucosa.

In 1973 (5 years after the Yusho incident), the prevalence of oral pigmentation was higher in Yusho patients with type A blood PCB pattern compared with Yusho patients with type C blood PCB pattern, but there were no specific differences in the pre-

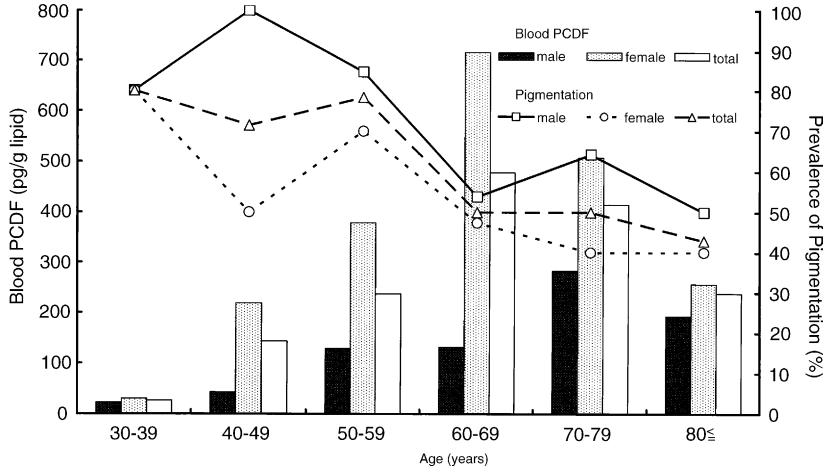

Fig. 6 Mean concentration of blood PCDF and prevalence of oral pigmentation by age in 2002. PCDF: polychlorinated dibenzofuran.

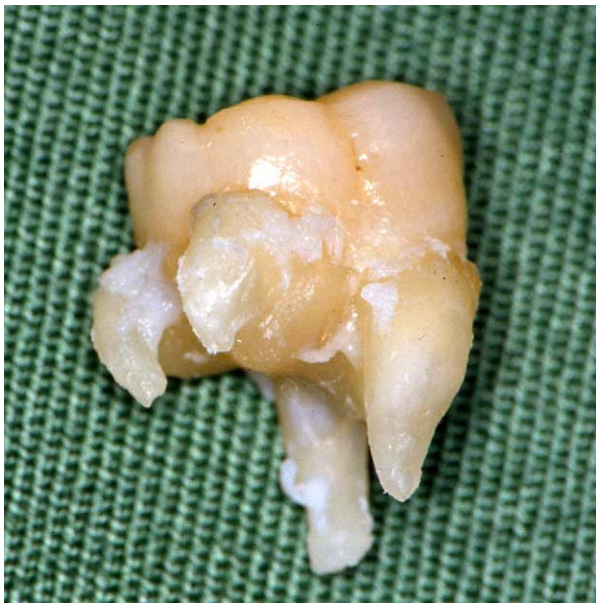

Fig. 7 A lateral view of the lower right second molar extracted from a Yusho patient.

valence of oral pigmentation between any type of blood PCB pattern in 1982 and 2002 (14 and 34 years after the Yusho incident, respectively). As time passed, blood PCB concentrations were found to decrease across all PCB patterns, but the PCB pattern of any individual hardly changed from A to B or C [13]. These conflicting findings may contribute to the discrepancy mentioned above. In accordance with the gradual decrease of blood PCB concentration after PCB exposure, it has been reported that the prevalence of pigmentation of both skin and conjunctiva have decreased over the years [14,15]. It is potentially noteworthy that the prevalence of gingival pigmentation has increased from 1992. Similar findings were observed in two female Yusho patients for whom surgical elimination of gingival pigmentation with a sharp curette was performed for esthetic reasons [2]. Within 1 year, oral pigmentation similar to that seen before surgery reappeared at the operated area in both patients. The reasons for these phenomena are not yet understood. Okumura et al. reported that PCB and PCQ concentrations were found to be 36 and 91 times higher, respectively, in oral mucosa than in blood [16]. Higher accumulation of PCBs in oral mucosa may be responsible for the reappearance of gingival pigmentation. Alternatively, the decrease in number of Yusho patients who visited the dentist during the annual health examination in Fukuoka Prefecture may contribute to the increase in prevalence of gingival pigmentation. It is probable that Yusho patients without severe symptoms, including gingival pigmentation, may not have attended the annual examination. If this were the case, the proportion of Yusho patients with oral pigmentation would increase, thus resulting in the high prevalence of oral pigmentation.

It has recently been demonstrated that the most important causal agents for Yusho are PCDFs [17]. An inverse correlation seemed to be found between the

Table 4 Number of affected children with retarded eruption of permanent tooth

Age at poisoning (years)	+	−	%
Fetus	2	4	33.3
0–2	0	10	0.0
3–5	3	10	23.1
6–8	3	11	21.4
9–11	1	4	20.0
12–14	0	3	0.0
Total	9	42	17.6

(+) Yusho patients with retarded eruption, (−) Yusho patients without retarded eruption, (%) proportion of Yusho patients with retarded eruption relative to total number of patients in each age group (reproduced with permission from Fukuyama et al. [2]).

Table 5 Number of affected children with deficiency of tooth germs

Age at poisoning (years)	+	−	%
Fetus	1	5	16.7
0–2	0	10	0.0
3–5	1	12	7.7
6–8	2	12	14.3
Total	4	39	9.3

(+) Yusho patients with deficiency of tooth germs, (−) Yusho patients without deficiency of tooth germs, (%) proportion of Yusho patients with deficiency of tooth germs relative to total number of patients in each age group (reproduced with permission from Fukuyama et al. [2]).

Table 6 Number of affected children with anomalies of dental root shape

Age at poisoning (years)	+	−	%
Fetus	0	4	0.0
0–2	6	3	66.7
3–5	9	3	75.0
6–8	13	1	92.9
9–11	5	0	100.0
12–14	3	0	100.0
Total	36	11	76.6

(+) Yusho patients with anomalies of dental root shape, (−) Yusho patients without anomalies of dental root shape, (%) proportion of patients with anomalies of dental root shape relative to total number of patients in each age group (reproduced with permission from Fukuyama et al. [2]).

prevalence of oral pigmentation and mean PCDF concentration in 2002. However, it is particularly noteworthy that analysis by three-way ANOVA showed a close relationship between the presence of upper gingival pigmentation and blood PCDF concentration. It is possible that PCDFs may be involved in the development of oral pigmentation. Because no close relationship has been found between blood PCDF concentration and the presence of lower gingival pigmentation, however, further studies are needed to clarify the role of PCDFs in the development and perpetuation of oral pigmentation.

Although oral lesions such as malocclusion, discoloration of teeth and hypoplasia of dental hard tissue were rarely observed, the prevalence both of anomalies of tooth eruption and dilaceration of tooth roots seemed high. It has been generally accepted that poor nutrition and ectopic localization of tooth germs are involved in the deficiency of tooth germs and retarded eruptions of permanent teeth, respectively. In addition, dilaceration of dental roots is commonly observed in clinically healthy persons because tooth roots physiologically tend to curve in the distal direction. These findings suggest that PCBs may not be necessary to cause oral lesions such as anomalies of dental root shape and tooth eruption. Fukuyama et al. reported that such lesions were observed at a much higher frequency in affected children with high blood PCB concentrations than in those with low blood PCB concentrations [2]; however, it is probable that PCBs may contribute to the presence of such lesions. In crab-eating monkeys with experimental PCB and PCDF poisoning, epithelial diseases such as dyskeratosis and keratocysts were observed but no severe changes were observed in the connective tissues [7]. Similarly, cyst-like formations of various sizes and configurations were observed between secretory ameloblasts, but no severe morphological changes were observed in cementoblasts and odontoblasts in Wistar King A (WKA) rats intraperitoneally injected with PCB and PCDF [8]. These experimental findings may indicate that PCBs and PCDFs have cytotoxic effects on the cells derived from the oral ectoderm but not on the cells derived from the neural crest. The dental lamina and Hertwig's epithelial root sheath, both of which are derived from the oral ectoderm, have been generally understood to play a major part in the development of tooth germs and dental roots [18]. PCBs and PCDFs may be involved in anomalies both of dental root shape and tooth eruption through direct or indirect cytotoxic effects on the cells derived from the oral ectoderm. In addition, it has been demonstrated that metabolic disorders of vitamin D and calcium, which occur following PCB exposure, cause anomalies of bone ossification and arrest development in childhood [19,20]. Yagi et al. reported that calcium concentration increased in the kidneys and decreased in the bones. A disordered calcification in the alveolar bone may also cause such lesions [21]. Further studies are needed to better understand the mechanism of such lesions caused by PCB and PCDF poisoning.

Acknowledgement

This work was supported in part by a grant-in-aid for scientific research from the Ministry of Health, Labour and Welfare, Japan.

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Journal of Dermatological Science Supplement (2005) 1, S73—S80

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Dermatological manifestations in Yusho: correlation between skin symptoms and blood levels of dioxins, such as polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs)

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KEYWORDS
Dioxin;
Polychlorinated biphenyls (PCBs);
Polychlorinated dibenzofurans (PCDFs);
Skin symptoms;
Yusho

Summary

Background and objective: Yusho occurred in western Japan in 1968 and was caused by ingestion of rice bran oil that was contaminated with polychlorinated biphenyls (PCBs) and dioxins such as polychlorinated dibenzofurans (PCDFs). At that time, the skin symptoms presented by patients with Yusho were at their most prominent and worst severity. Analysis of blood to determine the concentration of dioxins started in 2001 in Fukuoka prefecture, and in 2002 the examination was performed throughout Japan. There have been no reports on the relationship between blood concentration of dioxins and skin symptoms in Yusho. This is the first report to examine the relationship between blood concentration of dioxins and skin symptoms in Yusho, using statistical analyses.
Methods: Using the global skin severity grade, we analyzed the change in skin symptoms, which were examined at the annual medical check-up of patients with Yusho. We also investigated the relationship between the items of the annual dermatological examination and blood concentrations of total PCDFs and total PCBs.
Results: The severity of skin symptoms improved significantly in the first 20 years; nowadays, however, further improvement can hardly be observed. Using three-way analysis of variance (ANOVA), we found that of the 21 items of the dermatological examination, nine were significantly related to total PCDFs, and five were related to total PCBs. Only one item was significantly related both to total PCDFs and total PCBs.

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1574-0757/$30.00
doi:10.1016/j.descs.2005.03.015

Conclusion: More than 36 years have passed since the Yusho incident, and about 60% of the patients currently present with no skin symptoms. In contrast, in about 40% of the patients, characteristic skin symptoms of Yusho, such as pigmentation of skin, black comedones and acneform eruptions, could still be observed. Our analysis of the relationship between skin symptoms and blood concentrations of total PCDFs and total PCBs proves that not only PCBs but also PCDFs have an important role in the skin symptoms of Yusho.

1. Introduction

Yusho occurred in more than 1800 people in western Japan in 1968. At first, the disorder was thought to be caused by ingestion of rice bran oil contaminated by polychlorinated biphenyls (PCBs), which had been used in heat-conducting materials. Later, our study group found that the rice bran oil had also been contaminated with dioxin-related compounds such as polychlorinated dibenzofurans (PCDFs). As a result therefore, Yusho is now recognized as mixed toxicity from PCBs and dioxin-related compounds [1].

In 1973, 5 years after the outbreak of Yusho, the concentration of PCBs in the blood of Yusho patients was analyzed. The pattern of chromatograms of blood PCBs were classified into four types: type A, pattern characteristic of Yusho; type C, pattern commonly observed in the general population; types B and BC: intermediate patterns between types A and C [2]. Since that time, the relationship between clinical symptoms and the level and pattern of PCBs has been investigated. However, because of technical limitations at that time, it was very difficult to determine the precise blood concentrations of PCDFs.

Nowadays, however, techniques are markedly improved, and the blood concentration of PCDFs can be examined precisely and the results can be reproduced consistently. Of the various PCDFs, 2,3,4,7,8-pentachlorodibenzofuran (2,3,4,7,8-PeCDF) was found to be responsible for about 70% of the dioxin toxicity, and the most important causative agent in Yusho [3]. The accumulated PCB- and dioxin-related compounds were estimated at levels as high as 75 μg/g lipid and 40 ng/g lipid, respectively, in one patient with severe symptoms in 1968 [4,5]. These accumulated compounds are gradually excreted from the body via feces, urine, sputum, cutaneous sebum, sweat, etc. Therefore, 30 years after the outbreak of Yusho, the blood concentrations of these compounds have now decreased to 2.3 μg/g lipid and 0.6 ng/g lipid, respectively, but they do remain at levels higher than those in normal controls [4,5].

The analysis of blood concentrations of dioxins began in 2001 in Fukuoka prefecture, and since 2002 the analysis was performed throughout Japan. This allowed us to investigate the relationship between the clinical manifestations of Yusho (including skin symptoms) and laboratory data and levels of PCBs/dioxins.

In patients with Yusho, following the onset of nonspecific symptoms such as general malaise, loss of appetite and headache, several characteristic symptoms of Yusho gradually appeared, including acneform eruption (Fig. 1A—D), dark-brownish nail pigmentation (Fig. 1E and F), increased discharge from the eyes with swelling of eyelids, pigmentation of oral mucosa (Fig. 2), peripheral neuropathy, irregular menstruation in women, and growth retardation in infants and children [6—8].

In fact, these symptoms of the skin and mucous membranes are major and important factors in the diagnostic criteria of Yusho. Other skin symptoms such as xerosis, hyperhidrosis, nail deformity, hyperkeratosis and hair loss were also observed. Almost all symptoms of Yusho have improved spontaneously [6—8]. The affected patients have become older; and thus age-related senile clinical symptoms have become more obvious than the above-mentioned symptoms of Yusho. However, some patients still complain about dermatological and other subjective symptoms of Yusho.

The purpose of this study is to review the clinical course of skin symptoms of Yusho since its outbreak, and to analyze the relationship between the blood levels of PCBs/dioxins and skin symptoms.

2. Materials and methods

2.1. Annual medical check-up

Since 1968, dermatological examination was performed at the annual medical check-up of patients with Yusho. In 2002, 279 patients agreed to having their blood analyzed for levels of PCBs and dioxins. Table 1 shows the dermatological examination sheet used in the annual medical check-up of Yusho patients.

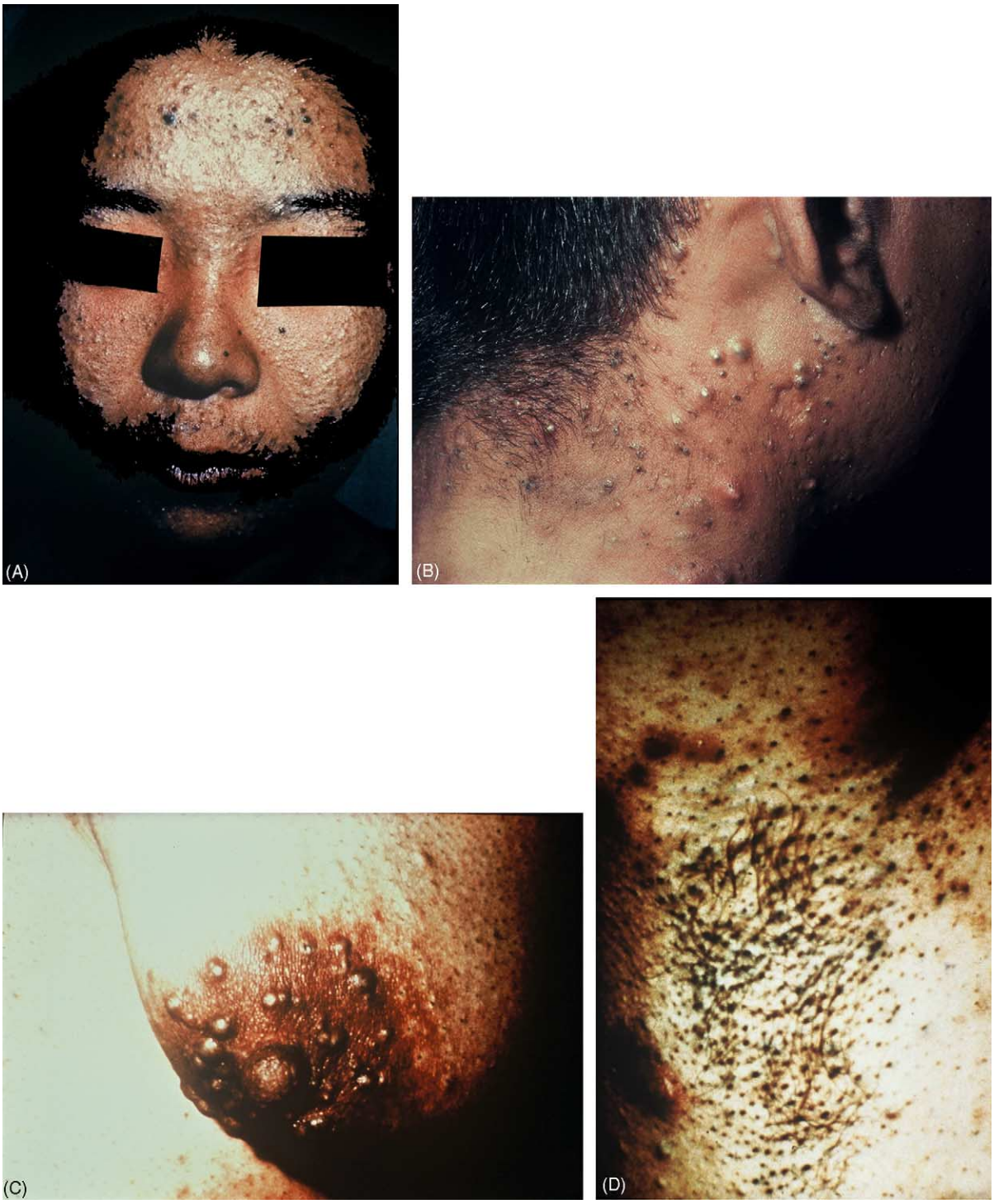

Fig. 1 (A) Clinical appearance of the face of a young female at the time of the outbreak. Typical skin symptoms of Yusho, such as diffuse pigmentation, many black comedones, severe acneform eruptions and formation of scars could be observed. (B) Clinical appearance of the neck of a young male at the time of the outbreak. Black comedones, cysts and acneform eruptions could be observed. (C) Clinical appearance of a nipple at the time of the outbreak. Black comedones and cysts could be observed. (D) Clinical appearance of an armpit at the time of the outbreak. Multiple black comedones and cysts could be observed. (E) Clinical appearance of hand nails at the time of the outbreak. Diffuse pigmentation could be observed. (F): Clinical appearance of toenails at the time of the outbreak. Diffuse pigmentation could be observed.

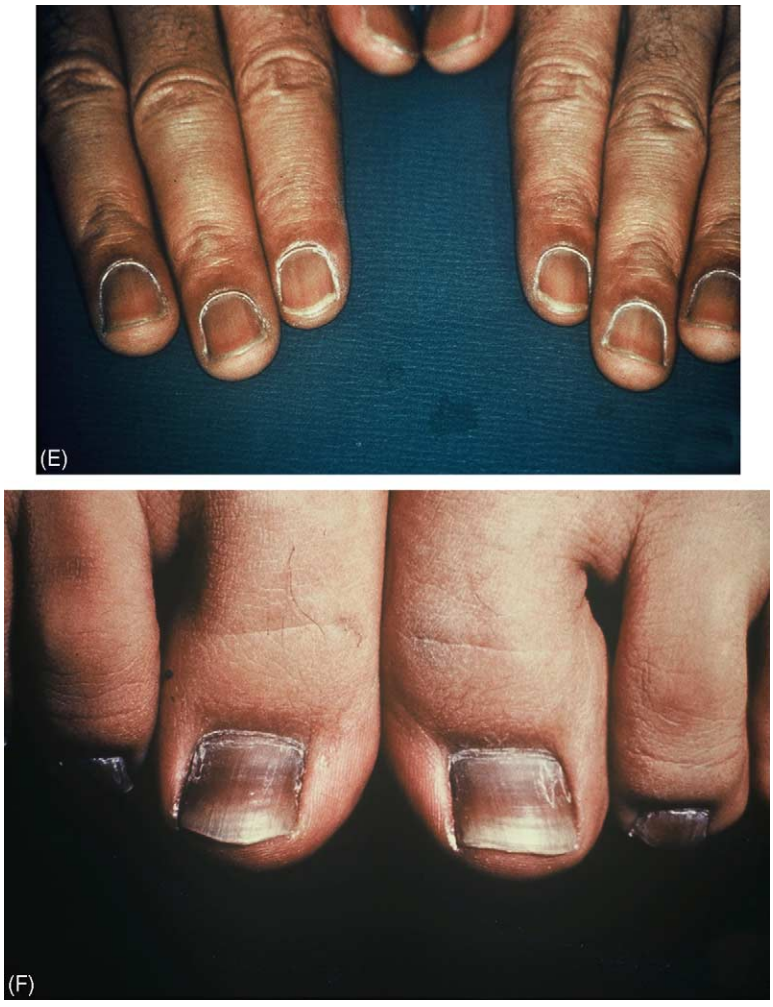

Fig. 1. (*Continued*).

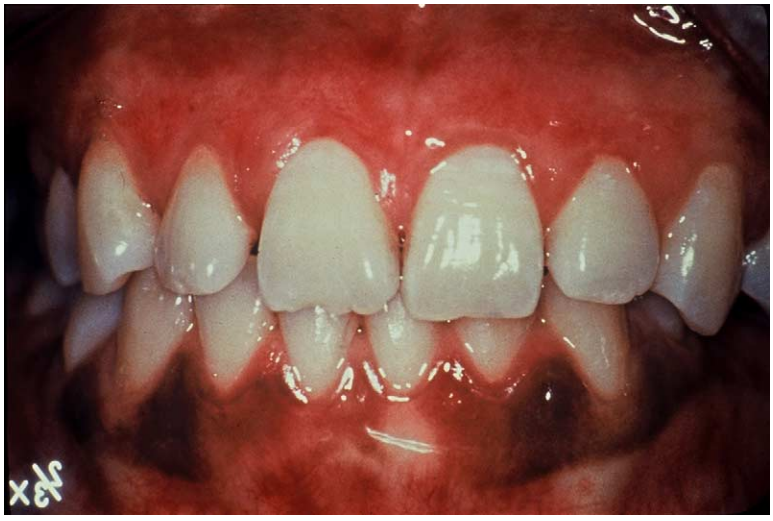

Fig. 2 Clinical appearance of gingivae at the time of the outbreak. Diffuse pigmentation could be observed.

Table 1 The dermatological examination sheet used in the annual medical check-up of patients with Yusho

Interview			
Recent purulence of skin eruptions		Yes	No
Recent recurrence of cystic lesions		Yes	No
Past history of acneform eruptions		Yes	No
Past history of pigmentation		Yes	No
Physical examination			
Symptoms	Sites	Grade	
Black comedones	Face	− ± + ++ +++	
	Ear	− ± + ++ +++	
	Torso	− ± + ++ +++	
	Other sites	− ± + ++ +++	
Acneform eruptions	Face	− ± + ++ +++	
	Genital area	− ± + ++ +++	
	Buttocks	− ± + ++ +++	
	Torso	− ± + ++ +++	
	Other sites	− ± + ++ +++	
Scar formation	Face	− ± + ++ +++	
	Torso	− ± + ++ +++	
	Other sites	− ± + ++ +++	
Pigmentation	Face	− ± + ++ +++	
	Nails	− ± + ++ +++	
	Toenails	− ± + ++ +++	
	Other sites	− ± + ++ +++	
Nail deformity		− ± + ++ +++	

2.2. Global skin severity grade

To evaluate skin symptoms, the following criteria 'the global skin severity grades' were arbitrarily proposed: grade 0: no skin eruptions; grade I: circumscribed pigmentation; grade II: black comedones; grade III: acneform eruptions; grade IV: extensive distribution of the acneform eruptions [6,7]. This system of evaluation was established in 1969 in Fukuoka prefecture and it has been used ever since. In 2002 in Fukuoka prefecture, 112 patients participated both in the dermatological examination and in the analysis of the blood concentrations of dioxins and PCBs.

2.3. Measurement of PCBs and dioxins

The concentrations of dioxins and PCBs in the blood were determined with a high-resolution gas chromatograph and a high-resolution mass spectrometer equipped with a solvent-cut large volume injection system. An accelerated solvent extraction method was used for the treatment of the blood samples. The technique requires a blood sample of only 5 g [9].

Blood samples were analyzed for the 7 following dioxins: 2,3,7,8-tetrachlorodibenzodioxin (2,3,7,8-TCDD), 1,2,3,7,8-pentachlorodibenzodioxin (1,2,3,7,8-PeCDD), 1,2,3,4,7,8-hexachlorodibenzodioxin (1,2,3,4,7,8-HxCDD), 1,2,3,6,7,8-hexachlorodibenzodioxin (1,2,3,6,7,8-HxCDD), 1,2,3,7,8,9-hexachlorodibenzodioxin (1,2,3,7,8,9-HxCDD), 1,2,3,4,6,7,8-heptachlorodibenzodioxin (1,2,3,4,6,7,8-HpCDD) and octachlorodibenzodioxin (OCDD). In addition, blood samples were analyzed for the 10 following furans: 2,3,7,8- tetrachlorodibenzofuran (2,3,7,8-TCDF), 1,2,3,7,8-pentachlorodibenzofuran (1,2,3,7,8-PeCDF), 2,3,4,7,8-pentachlorodibenzofuran (2,3,4,7,8-PeCDF), 1,2,3,4,7,8-hexachlorodibenzofuran (1,2,3,4,7,8-HxCDF), 1,2,3,6,7,8-hexachlorodibenzofuran (1,2,3,6,7,8-HxCDF), 2,3,4,6,7,8-hexachlorodibenzofuran (2,3,4,6,7,8-HxCDF), 1,2,3,7,8,9-hexachlorodibenzofuran (1,2,3,7,8,9-HxCDF), 1,2,3,4,6,7,8-heptachlorodibenzofuran (1,2,3,4,6,7,8-HpCDF), 1,2,3,4,7,8,9-heptachlorodibenzofuran (1,2,3,4,7,8,9-HpCDF) and octachlorodibenzofuran. The following coplanar PCBs were assessed: 3,4,4′,5-tetrachlorobiphenyls (3,4,4′,5-TCB), 3,3′,4,4′- tetrachlorobiphenyl (3,3′,4,4′-TCB), 3,3′,4,4′,5-pentachlorobiphenyl (3,3′,4,4′,5-PeCB), 3,3′,4,4′,5,5′-hexachlorobiphenyl (3,3′,4,4′,5,5′-HxCB). Subsequently, total PCDF values and the toxic equivalent quantity (TEQ) were calculated.

2.4. Statistical analysis of the relationship between skin symptoms and blood concentrations of dioxins and PCBs

The data were analyzed using a three-way analysis of variance (ANOVA) model. The total PCDF value proved to be most suitable for this analysis (data not shown). The dependent variable was the logarithmic value of the blood concentration of total PCDFs. Sex and age were independent variables. Using the same method, a statistical analysis of the relationship between skin symptoms and PCB concentration in the blood was also performed.

3. Results

3.1. Global skin severity grade

Table 2 shows the change in global skin severity grade in Fukuoka prefecture since 1969. It shows that the skin symptoms improved remarkably in the first 20 years (Fig. 3A and B), after which skin symptoms improved very slowly. Nowadays, any additional change in patients' skin is so minor that further improvement is barely noticeable. About 60% of the patients have grade 0: in these patients, the characteristic skin symptoms of Yusho cannot be observed. However, about 40% of the patients have

Table 2 The change in the global skin severity grade since 1969

Severity	Year				
	1969 (%)	1979 (%)	1989 (%)	1999 (%)	2002 (%)
0	11.6	42.7	54.6	61.6	59.5
I	24.7	15.5	13.0	9.6	4.3
II	25.6	22.7	18.5	17.8	16.4
III	24.7	17.3	13.0	11.0	16.4
IV	13.4	1.8	0.9	0	3.4

Data are expressed as the proportion of patients with a specific grade in that year.

grade I, II, III or IV, and in these patients the characteristic skin symptoms of Yusho can still be observed.

3.2. Blood concentrations of dioxins and PCBs

In 2002, the concentration (mean $\pm$ S.D.) of dioxins in the blood was 136.4 $\pm$ 148.3 pg-TEQ/g lipid (range 7—1126.1 pg-TEQ/g lipid). The concentration of 2,3,4,7,8-PeCDF was 192.0 $\pm$ 252.1 pg-TEQ/g lipid (range 3.1—1889.7 pg/g lipid). The concentration of PCBs in the blood was 3.383 $\pm$ 2.765 ppb (range 0.25—25.1 ppb). In 52 normal volunteers, the concentration of dioxins in the blood was 37.0 $\pm$ 17.6 pg-TEQ/g lipid (range 8.5—85.4 pg-TEQ/g lipid), and the concentration of 2,3,4,7,8-PeCDF was 15.2 $\pm$ 8.9 pg/g lipid (range 3.5—41.7 pg/g lipid).

3.3. Relationship between blood concentrations of the dioxins and items of the dermatological examination

Table 3 shows the 21 items of the dermatological examination and *P* values indicating the statistical significance of the relationship between each of the items and total PCDF level. The items that had interaction with sex, age or both were excluded. Of the 21 items of the dermatological examination, nine items were significantly related to the total PCDF level ($P < 0.05$ was considered significant).

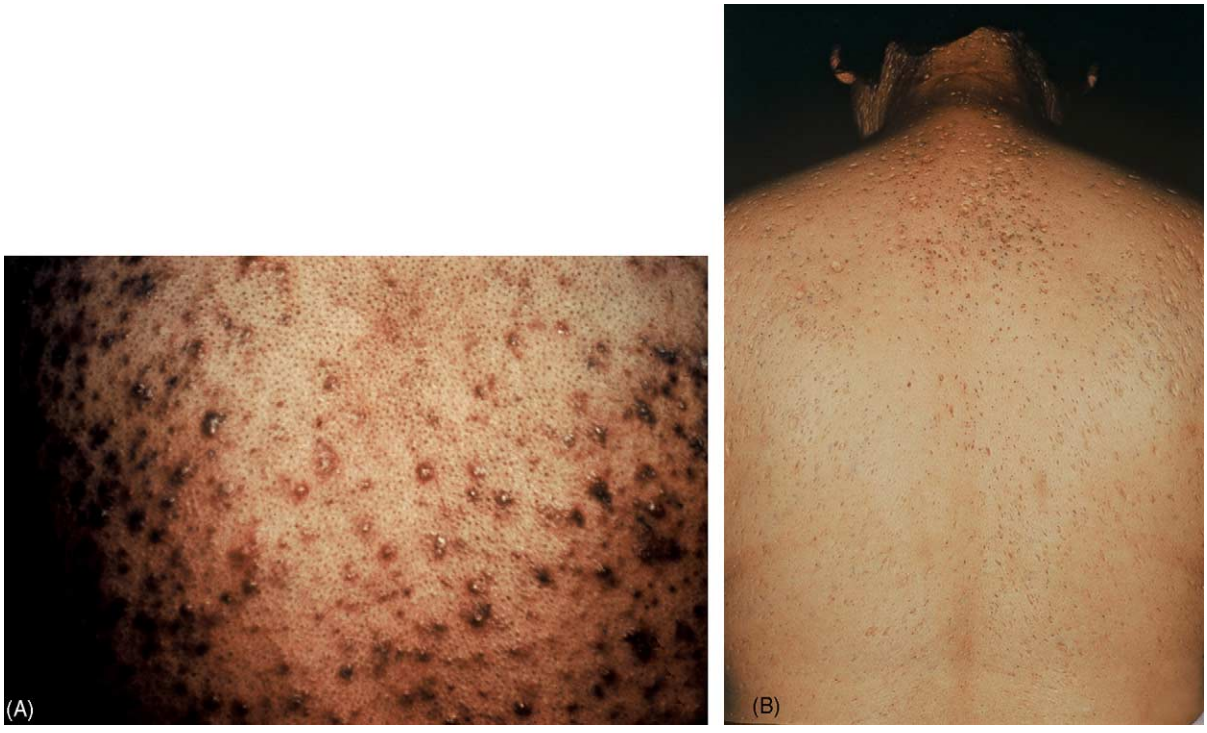

Fig. 3 (A) Clinical appearance of the back of a middle-aged female at the time of the outbreak. Diffuse and severe acneform eruptions, black comedones, and diffuse pigmentation could be observed. (B) Clinical appearance of the back of the same female 25 years after the outbreak. The skin eruptions had improved remarkably; however, follicles and black comedones were still striking.

Table 3 The 21 items of the dermatological examination and their statistical relevance to blood concentrations of PCDF and PCB

		P	
		PCDF	PCB
Interview			
Recent purulence of skin eruptions		0.006[a]	0.503
Recent recurrence of cystic lesions		0.000[c]	0.197[c]
Past history of acneform eruptions		0.025[a]	0.596
Past history of pigmentation		0.008[a]	0.230
Physical examination			
Symptoms	Sites		
Black comedones	Face	0.013[c]	0.002[a]
	Ear	0.006[a]	0.000[c]
	Torso	0.155	0.002[a]
	Other sites	0.006[c]	0.022[a]
Acneform eruptions	Face	0.025[a]	0.458[c]
	Genital area	0.053[c]	0.682[c]
	Buttocks	0.002[a]	0.087[c]
	Torso	0.000[c]	0.462
	Other sites	0.002[a]	0.603
Scar formation	Face	0.000[c]	0.008[a]
	Torso	0.037[a]	0.001[a]
	Other sites	0.062[b]	0.009[c]
Pigmentation	Face	0.193	0.022[c]
	Nails	0.594	0.892
	Toenails	0.786	0.999
	Other sites	0.460	0.000[c]
Nail deformity		0.038[a]	0.001[c]

[a] $P < 0.05$.
[b] $P < 0.10$.
[c] The item had interaction with sex, age or both.

We divided the patients into two groups according to global skin severity grade: grade 0 group and grade I—IV group. We compared the blood dioxins and 2,3,4,7,8-PeCDF levels in both groups. The concentration of dioxins in the blood in the grade I—IV group was 202.279 ± 227.492 pg-TEQ/g lipid, and in the grade 0 group it was 126.691 ± 138.985 pg-TEQ/g lipid. Using Student's *t*-test, we found that the blood levels of dioxins in the two groups were significantly different ($P = 0.0316$). The concentration of 2,3,4,7,8-PeCDF in the blood in the grade I—IV group (304.498 ± 382.392 pg/g lipid) was significantly different from that in the grade 0 group (179.414 ± 241.132 pg-TEQ/g lipid; $P = 0.0363$, Student's *t*-test).

3.4. Relationship between blood concentration of PCBs and items of the dermatological examination

Table 3 also shows the relationship between each of the items of the dermatological examination and total PCB value. The items that had interaction with sex, age, or both were excluded. Of the 21 items of the dermatological examination, five items were significantly related to total PCB level. One of the items, 'Scar formation on body', was significantly related to both total PCDF level and total PCB level.

In addition, we compared the blood level of PCB in the grade 0 group with that in the grade I—IV group. The blood level of PCB in the grade I—IV group was 3.357 ± 2.268 ppb, and in the grade 0 group it was 2.238 ± 1.395 ppb. Using Student's *t*-test, we found a significant difference in the blood level of PCBs between the two groups ($P = 0.001$).

4. Discussion

Previous reports have pointed out that the severity of skin lesions in patients with Yusho has gradually improved over the years [5,6]. As shown with the global skin severity grade, this was true for the first 20 years. After this time, however, barely any

further improvement could be observed. Nowadays, the skin symptoms are almost fixed, and there is a tendency of polarization: no skin symptoms or only slight symptoms could be observed in 60% of the patients and, in contrast, characteristic skin symptoms of Yusho, such as black comedones and acneform eruptions, could be observed in 40% of the patients.

Using three-way ANOVA, the relationship between the blood concentration of dioxins and skin symptoms was investigated. Of the 21 items of the dermatological examination, nine items were significantly related to total PCDF value, and five items were significantly related to total PCB value. Only one item was significantly related to both total PCDF level and total PCB level. These results suggest that both dioxins, such as PCDFs, and PCBs contributed to the formation of the characteristic skin symptoms of Yusho such as pigmentation, black comedones and acneform eruptions.

It is already known that dioxins and PCBs remain in the tissue for a long time. It is indeed noteworthy that even 36 years after the outbreak high concentrations of dioxins are still present in the blood of patients with Yusho.

This is the first report of a statistical analysis of the relationship between dioxins and skin symptoms in Yusho patients. However, the analysis has just begun and further investigation is necessary to acquire more conclusive data.

Acknowledgement

This work was supported in part by a grant-in-aid for scientific research from the Ministry of Health, Labour and Welfare, Japan.

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Journal of Dermatological Science Supplement (2005) 1, S81—S83

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Sex ratio in the children of Yusho patients

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KEYWORDS
Dioxin;
Polychlorinated biphenyls (PCBs);
Polychlorinated dibenzofurans (PCDFs);
Sex ratio;
Yusho

Summary In some cases of intoxication with dioxins and dioxin-related compounds such as Yu-cheng, Seveso, Russian and Austrian chloracne cohorts, there was a significant reduction in the male-to-female sex ratio in children born to men who had been exposed to dioxins and dioxin-related compounds before age 20 years or in their early 20s. The aim of this study was to ascertain whether parental exposure to dioxin-related compounds at a particular age affected the sex of subsequent offspring in Yusho. Maternal exposure had no significant effect on the sex ratio of the offspring. The sex ratio of children born to men exposed before age 20, men exposed after age 20, or to parents who were both exposed were not significantly different from the expected sex ratio of 0.514.

Public concern is increasing about the hazardous effects of dioxin and dioxin-related compounds, in particular regarding reproductive outcomes. Yoshimura et al. reported that there was no significant difference in the sex ratio in 85 live births from 1968 to 1977 of offspring born to parents with Yusho compared with the expected ratio in the general population [1]. However, whether the parents' sex and age at exposure influence the sex ratio of the offspring remains to be determined.

Yusho oil disease occurred in western Japan in 1968. Yusho was caused by ingestion of rice cooking oil that was contaminated with polychlorinated biphenyls (PCBs) and other dioxin-related compounds, such as polychlorinated dibenzofurans (PCDFs). More than 1800 people presented with several clinical symptoms, such as acneform eruptions, pigmentation of the skin, nails and conjunctivae, increased discharge from the eyes and numbness of the skin [2,3]. Another incident of ingestion of contaminated rice cooking oil occurred in Yu-cheng, Taiwan. The causal agents were quite similar to those of Yusho, namely PCBs and PCDFs [4].

Exposure to dioxins and dioxin-related compounds also occurred in Seveso, Italy, and other countries [5]. In the Yu-cheng, Seveso, Russian and Austrian chloracne cohorts, there was a significant reduction in the male-to-female sex ratio in children born to men who had been exposed to dioxin or dioxin-related compounds before age 20 years or in their early 20s [5—8]. In contrast, in the

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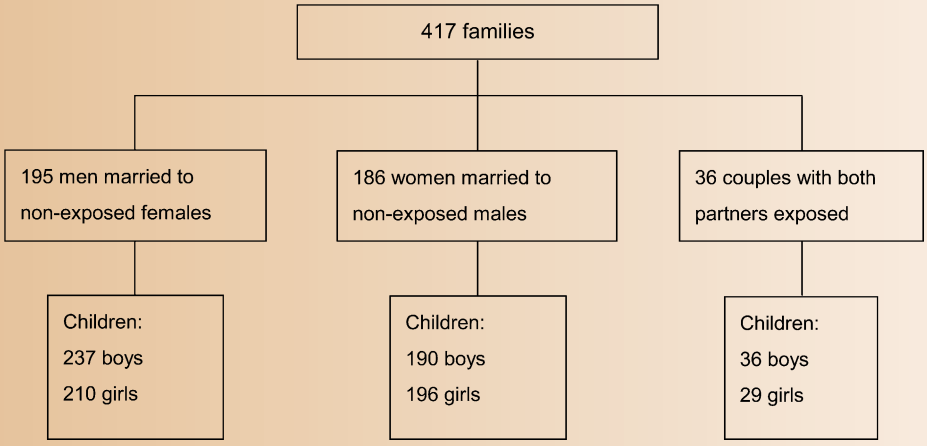
1574-0757/$30.00
doi:10.1016/j.descs.2005.03.014

Table 1 Characteristics of the Yusho study population



'Both partners exposed' includes one couple in which the mother was 18 years of age at exposure.

Table 2 Number and sex ratio of children born from 1969 to 2002 to individuals exposed to dioxins

Exposure status for dioxin-related compounds	Children		Sex ratio	*P*-value
	Male	Female		
Only father exposed, <20 years	194	174	0.527	0.613
Only father exposed, ≥20 years	43	36	0.544	0.590
Only mother exposed, <20 years	168	181	0.481	0.223
Only mother exposed, ≥20 years	22	15	0.595	0.327
Both partners exposed	36	29	0.554	0.520

'Both partners exposed' includes one couple in which the mother was 18 years of age at exposure.

Yu-cheng and Seveso cohorts, maternal exposure had no influence on the male-to-female sex ratio of offspring [4,9].

The aim of our study was to ascertain whether parental exposure to dioxin-related compounds at a particular age affected the sex of subsequent offspring.

Live births from 1969 to 2002 were documented for cases in which at least one of the parents were Yusho patients. Data on birth and sex of all live-born children were obtained by face-to-face interview or by telephone with each patient or a close relative. We were able to make contact with 417 couples: in 195 couples, only the husbands were affected; in 186 couples, only the wives were affected; and in 36 couples, both parents were Yusho patients (Table 1).

The sex ratio is the number of males (M) divided by the sum of male and female (F) children, i.e., M/(M + F). The observed sex ratio in offspring of Yusho parents was compared with the expected sex ratio of 0.514 using a χ^2 test. Table 2 shows the proportion of boys born to men and women who had been exposed to dioxin-related compounds at age <20 years and ≥20 years. Analysis of the data shows that maternal exposure had no significant effect on the sex of the offspring. The sex ratios of children born to men exposed before age 20, men exposed after age 20, or to parents who were both exposed were not significantly different from the expected sex ratio of 0.514.

In the Seveso and Austrian chloracne cohorts, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) was the primary causal agent [5,8]. In two cohorts in Russia, 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (PeCDD) was the primary causal agent [7]. In Yu-cheng, PCDFs and PCBs were the primary causal agents [4]. The mechanism of how dioxins influence the sex ratio has not yet been proven. The reason why no significant difference was observed in the sex ratio in offspring in Yusho cohort remains to be elucidated.

Acknowledgement

This work was supported in part by a Grant-in-Aid for scientific research from the Ministry of Health, Labour and Welfare, Japan.

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Journal of Dermatological Science Supplement (2005) 1, S85—S93

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Relationship of clinical symptoms and laboratory findings with blood levels of PCDFs in patients with Yusho

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KEYWORDS
Clinical symptoms;
Laboratory findings;
Polychlorinated dibenzofuran (PCDF) levels;
Yusho

Summary

Background and objective: Since the Kanemi Yusho poisoning incident, patients with Yusho have been followed up for 35 years in annual health examinations for Yusho symptoms by a national Study Group for Yusho. Because of recent advances in the technology for the measurement of dioxins, the determination of blood polychlorinated dibenzofuran (PCDF) levels has become possible with high accuracy. Thus, the purpose of this study was to investigate the relationship between clinical symptoms and dioxins, one of the causal agents, in patients with Kanemi Yusho oil poisoning disease.

Methods: The participants were patients with oil poisoning disease who had undergone general examinations including measurement of PCDF levels, internal medicine, examination sheet (biochemistry, hematology), and dermatological, dental and ophthalmological examinations in 2001 and 2002. We investigated the presence or absence of symptoms in these examinations and the relationship with PCDF levels by methods such as three-way analysis of variance (ANOVA).

Results: Large differences were found between the examination results in 2001 and those in 2002. Items for which the relationship between the symptoms or the results and PCDF levels was currently considered strong were polychlorinated biphenyl (PCB)-related items, and items of a gingival nature and gingival sites.

1. Introduction

Yusho is a food poisoning by rice bran oil (Kanemi oil) that occurred mainly in western Japan in 1968 [1]. Polychlorinated biphenyls (PCBs), used as a heat conductor during the refining process of rice bran oil, were initially believed to be the causal agent.

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1574-0757/$30.00
doi:10.1016/j.descs.2005.03.013

Subsequently, dioxins such as polychlorinated dibenzofurans (PCDFs), produced from PCBs under high temperature, were suspected to be involved in the pathogenesis of Yusho [1–6]. At present, Yusho is considered to be a combined poisoning by PCBs, dioxins and their related congeners.

In accordance with the recent technical advances in the measurement of dioxin concentration, the Study Group for Yusho started to assess the blood levels of dioxins in the annual medical check-up from 2001.

In this study, based on data from the medical examinations, we investigated the correlation of blood concentrations of PCDFs with the clinical symptoms and laboratory findings in patients with Yusho.

Table 1 The laboratory examination sheet of the annual medical check-up of Yusho patients

Blood concentration of PCBs and PCB-related compounds	
Total PCB	ppb
Peak 1 (2,4,5,3',4'-pentachlorobiphye	ppb
Peak 2 (2,4,5,2',4',5'-hexachlorobiph	ppb
Peak 3 (2,3,4,5,3',4'-hexachlorobiphy	ppb
PCB pattern	A B BC C
CB ratio	
Total PCQ	ppb

Urinalysis					
Protein	-	±	+	++	+++
Sugar	-	±	+	++	+++
Occult blood	-	±	+	++	+++
Urobilinogen	-	±	+	++	+++
pH					

Hematological examination	
Erythrocyte sedimentation rate (ESR)	mm
Erythrocyte sedimentation rate (ESR)	mm
White blood cell count (WBC)	$\times 10^3/\mu l$
Red blood cell count (RBC)	$\times 10^4/\mu l$
Hemoglobin	g/dl
Hematocrit	%
Mean corpuscular volume (MCV)	μm^3
Mean corpuscular hemoglobin (MCH)	pg
Mean corpuscular hemoglobin concentration (MCH	$\times 10^4/\mu l$
Platelet cell count	

Blood chemistry	
Total bilirubin	mg/dl
Direct bilirubin	mg/dl
Glutamic-oxaloacetic transaminase (GO	U/l
Glutamic-pyruvic transaminase (GPT)	U/l
Total protein	g/dl
Albumin	g/dl
A/G	
Zinc sulfate turbidity test	K.U.
Thymol turbidity test	K.U.
Alkaline phosphatase (ALP)	U/l
Leucine aminopeptidase	U/l
γ-glutamyl transpeptidase (γ-GTP)	U/l
Cholinesterase (ChE)	U/l
Lactate dehydrogenase (LDH)	U/l
Creatine phosphokinase (CPK)	U/l
Total cholesterol	mg/dl
High-density lipoprotein (HDL) choles	mg/dl
Triacylglycerol	mg/dl
β-lipoprotein	mg/dl
Blood urea nitrogen (BUN)	mg/dl
Creatinine	mg/dl
Sodium (Na)	mEq/l
Potassium (K)	mEq/l
Calcium (Ca)	mg/dl
Inorganic phosphorus (P)	mg/dl
Amylase	U/l
Blood sugar	mg

Immunological examination			
HBs antigen	-	±	+
α-fetoprotein			ng/ml

CB ratio: concentration ratio of 2,3,3′,4,4′,5-hexachlorobiphenyl/2,3,3′,4,4′,5-pentachlorobiphenyl; A/G: albumin/globulin ratio; HBs: hepatitis B surface; PCB: polychlorinated biphenyl; PCQ: polychlorinated quarterphenyl.

2. Methods

2.1. Participants and investigation items

The participants were 78 patients with Yusho for whom measurement of PCDF levels and other clinical/laboratory examinations were completed in 2001, and 279 patients with Yusho for whom measurement of PCDF levels and other clinical/laboratory examinations were completed in 2002.

Using the data from each year, we analyzed the correlation of levels of PCDFs with the clinical/laboratory findings, including physical examinations for internal medicine, blood chemistry, complete blood cell counts, and dermatological, oral and dental, and ophthalmological examinations.

2.2. Analytical methods

Using data from 78 patients with Yusho examined in 2001, detailed analyses were conducted by Dr. Kaneko, Section head, System Development and Knowledge Discovery Section, National Cancer Center Research Institute [7]. Based on the results, it was found that: (1) the levels of total PCDFs were the most suitable values to represent the toxicity of dioxins in the blood of patients with Yusho, and (2) regarding the factors affecting the total levels of PCDFs, there was a significant contribution from sex and age.

Based on these analyses, the logarithmic values of the total levels of PCDFs were set up as variables, and sex and age were dealt with as fixed factors. In addition, presence or absence of each symptom of the examination items was dealt with as another fixed factor. The three-way analysis of variance (ANOVA) was considered to be the most suitable model to confirm significant differences. Statistical Package for the Social Sciences (SPSS) was used for the statistical analysis.

2.3. Examination items

The annual medical check-up included 241 items of clinical and laboratory examinations as follows (see also Tables 1–5):

Table 2 The interview and physical examination sheet of the annual medical check-up of Yusho patients

Life history		
Alcohol	No	ake, <180 ml/da Sake 180– 540 ml/day ake, >540 ml/da
Smoking	No	Have ever smoked Yes

Chief complaint	No	Yes	( )	( )

Past history				
Before the incident	No	Yes	( )	( )
After the incident	No	Yes	( )	( )

Subjective symptoms					
Items	Grade			Frequency	
General fatigue	–	±	+	Sometimes	Often
Headache	–	±	+	Sometimes	Often
Cough	–	±	+	Sometimes	Often
Sputum	–	±	+	Sometimes	Often
Abdominal pain	–	±	+	Sometimes	Often
Diarrhea	–	±	+	Sometimes	Often
Constipation	–	±	+	Sometimes	Often
Numbness	–	±	+	Sometimes	Often
Arthralgia	–	±	+	Sometimes	Often
Troubles with menstr	–	±	+	Sometimes	Often

Physical examination			
Height		cm	
Body weight		kg	
Pulse rate		bpm	
Blood pressure	/	mmHg	(max / min)
Physique (nutrition)	normal	fat	thin
Heart sounds	normal	abnormal	
Respiratory sounds	normal	abnormal	
Hepatomegaly	–	+	Size ( )
Splenomegaly	–	+	Size ( )
Edema	–	+	Sites ( )
Lymphadenopathy	–	+	Sites ( )
Tendon reflex	normal	decline	hyper
Sensing	normal	abnormal	
Chest radiograph	normal	abnormal	(lung, mediastinum, lung + m
Electrocardiogram	normal	abnormal	
Abdominal ultrasonograph	normal	abnormal	

Table 3 The dermatological examination sheet of the annual medical check-up of Yusho patients

Interview		
Recent purulence of skin eruptions	Yes	No
Recent recurrence of cystic lesions	Yes	No
Past history of acneform eruptions	Yes	No
Past history of pigmentation	Yes	No

Physical examination

Symptoms	Sites	Grade				
Black comedones	Face	−	±	+	++	+++
	Ear	−	±	+	++	+++
	Trunk	−	±	+	++	+++
	Other sites	−	±	+	++	+++
Acneform eruptions	Face	−	±	+	++	+++
	Genital are	−	±	+	++	+++
	Buttocks	−	±	+	++	+++
	Trunk	−	±	+	++	+++
	Other sites	−	±	+	++	+++
Scar formation	Face	−	±	+	++	+++
	Trunk	−	±	+	++	+++
	Other sites	−	±	+	++	+++
Pigmentation	Face	−	±	+	++	+++
	Fingernails	−	±	+	++	+++
	Toenails	−	±	+	++	+++
	Other sites	−	±	+	++	+++
Nail deformity		−	±	+	++	+++

Table 4 The odontal and periodontal examination sheet of the annual medical check-up of Yusho patients

Chief complaint	Toothache Gingival bleeding Pus discharge Gingival swelling Feeling of tooth extrusion

Items for oral examinati			Sites		
Gingivitis	No	Yes	7-4 7-4	3-3 3-3	4-7 4-7
Marginal periodontit	No	Yes	7-4 7-4	3-3 3-3	4-7 4-7
Retarded eruptions of permanent teeth	No	Yes	7-4 7-4	3-3 3-3	4-7 4-7
Tooth pigmentation	No	Yes	7-4 7-4	3-3 3-3	4-7 4-7
Odontogenesis imperfecta	No	Yes	7-4 7-4	3-3 3-3	4-7 4-7
Abnormal occlusion	No	Yes			
Other findings	No	Yes	(		)

Mucous pigmentation									*Pattern	**Color
Upper gingivae	−	±	+	++	+++	7-4	3-3	4-7		
Lower gingivae	−	±	+	++	+++	7-4	3-3	4-7		
Right buccal mucosa	−	±	+	++	+++					
Left 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.

Table 5 The ophthalmological examination sheet of the annual medical check-up of Yusho patients

Subjective symptom					
Abnormal discharge from	−	±	+	++	+++
Objective symptoms					
Edema of the eyel	−	±	+	++	+++
Conjunctival pigmentation	−	±	+	++	+++
Cysts of meibomian glands	−	±	+	++	+++
Cheesy secretions from meibomian glands	−	±	+	++	+++

Examination	Number of items
Examination sheet	52
Internal medicine	55
Dermatology	21
Dentistry	108
Ophthalmology	5
Total	241

3. Results

3.1. Clinical and laboratory items with a significant relationship with blood levels of PCDFs

In Table 6, we have listed the items that had a relationship with blood levels of PCDFs with a significant probability (P-value <0.1 and <0.05). The results of the analyses showed that many items interacted with sex and age.

3.1.1. Items showing a significant relationship with blood levels of PCDFs both in 2001 and 2002 without any interaction with age and sex

The three items that showed a significant relationship with blood PCDF concentration ($P < 0.05$) were increased peak 3 (2,3,4,5,3′,4′-hexachlorobiphyenyl [HxCB]) of the PCB pattern, pigmentation of upper gingivae and pigmentation of upper gingivae (diffuse). Black comedones (other sites) and pigmentation of upper gingivae (site 3—3) were also added when we included items with a P-value <0.1 (Tables 6 and 7).

3.1.2. Items showing a significant relationship with blood levels of PCDFs either in 2001 or 2002 without any interaction with age and sex

Items that showed a significant relationship ($P < 0.05$) with blood levels of PCDFs either in 2001 or 2002 without any interaction with age and sex were increased total PCB concentration, increased peak 2 (2,4,5,2′,4′,5′-HxCB) of the PCB pattern, increased peak 3 (2,3,4,5,3′,4′-HxCB) of the PCB pattern, increased CB ratio (concentration ratio of 2,3,3′,4,4′,5-HxCB:2,3,3′,4,4′,5-pentachlorobiphenyl [PeCB]), increased total polychlorinated quarterphenyl (PCQ) concentration, decreased direct bilirubin, increased triglyceride (TG), increased β lipoprotein, duration (years) of smoking, abnormal heart sounds, abnormal respiratory sounds, hepatomegaly, lymphadenopathy, recent purulence of skin eruption, past history of acneform eruptions, past history of pigmentation, black comedones (ear), black comedones (trunk), black comedones (other sites), acneform eruptions (buttocks), acneform eruptions (other sites), scar formation (trunk), nail deformity, gingivitis (lower, site 3—3), pigmentation of upper gingivae (site 7—4), pigmentation of upper gingivae (site 4—7), pigmentation of lower gingivae (site 4—7), pigmentation of lower gingivae (spotted), pigmentation of lower gingivae (scattered), pigmentation of lower gingivae (brownish), pigmentation of left buccal mucosa (faint), and pigmentation of palate (spotted) (Tables 6 and 8).

4. Discussion

In 1968, immediately after the Yusho incident, the Study Group for Yusho clarified the causal agent and treatments for patients. Recent advances in the measurement of blood levels of dioxins have enabled us to include the measurement of dioxins in the annual check-up of patients with Yusho [8,9]. With the measurement of dioxins, it has become possible to analyze the relationship between various clinical symptoms and levels of dioxins in Yusho

Table 6 Items found to have a significant relationship with levels of total PCDFs ($P < 0.1$) in 2001 or 2002

		2001			2002			Both in 2001 and 2002
		P-value	Interaction		*P*-value	Interaction		
			Sex	Age		Sex	Age	
Items of the laboratory examination								
1	Total PCB concentration ↑	0.000**	○		0.000**			c
2	Peak 2 (2,4,5,2′,4′,5′-hexachlorobiphyenyl) ↑	0.005**	○		0.000**			c
3	Peak 3 (2,3,4,5,3′,4′-hexachlorobiphyenyl) ↑	0.000**			0.000**			c
4	PCB pattern	0.000**			0.000**	○		c
5	CB ratio ↑	0.000**			0.000**	○		c
6	Total PCQ concentration ↑	—			0.000**			
7	Urinary protein ↑	0.064			0.940			
8	While blood cell count ↑	0.122			0.090*	○		
9	Total bilirubin ↓	0.158			0.029**	○		
10	Direct bilirubin ↓	0.007**			0.15			
11	γ-GTP ↑	0.657			0.068*	○		
12	High-density lipoprotein cholesterol ↓	0.148			0.077*			
13	Triglyceride ↑	0.016**			0.105			
14	β lipoprotein ↑	0.016**			0.418			
15	Creatinine ↑	0.237			0.081*			
Items of internal medicine examination								
1	Presence of alcohol drinking	0.877			0.055*			
2	Presence of smoking	0.590			0.003**		○	
3	The number of cigarette smoked ↑	0.895			0.001**		○	
4	Duration (years) of smoking ↑	0.134			0.011**			
5	Diarrhea ↑	0.192			0.038**	○		
6	Numbness	0.073*			0.464			
7	Frequency of numbness	0.061*			0.671			
8	Body weight ↓	0.596			0.098*			
9	Nutrition ↓	0.940			0.065*			
10	Abnormal heart sounds	0.940			0.026**			
11	Abnormal respiratory sounds	0.708			0.023**			
12	Presence of hepatomegaly	0.880			0.013**			
13	Hepatomegaly (3 fingerbreadths)	—			0.039**			
14	Presence of splenomegaly	0.880			0.058*			
15	Edema	0.292			0.015**		○	
16	Lymphadenopathy	0.948			0.042**			
17	Abnormal abdominal ultrasonograph	0.105			0.062*			
Items of dermatological examination								
1	Recent purulence of skin eruption	0.378			0.006**			
2	Recent recurrence of cystic lesions	0.061*			0.000**	○		a
3	Past history of acneform eruptions	0.471			0.025**			
4	Past history of pigmentation	0.623			0.008**			
5	Black comedones (face)	0.514			0.013**	○		
6	Black comedones (ear)	0.441			0.009**			
7	Black comedones (trunk)	0.426			0.000**			
8	Black comedones (other sites)	0.062*			0.006**			b
9	Acneform eruptions (genital area)	0.332			0.006**	○		
10	Acneform eruptions (buttocks)	0.516			0.025**			
11	Acneform eruptions (trunk)	0.957			0.053*	○		
12	Acneform eruptions (other sites)	0.500			0.002**			
13	Scar formation (face)	0.014**			0.000**	○		a
14	Scar formation (trunk)	0.217			0.037**			
15	Scar formation (other sites)	0.575			0.062*			
16	Nail deformity	0.648			0.038**			
Items of dentistry examination								
1	Gingivitis (lower, site 3—3)	0.048**			0.609			

Table 6 (*Continued*)

		2001			2002			Both in 2001 and 2002
		P-value	Interaction		*P*-value	Interaction		
			Sex	Age		Sex	Age	
2	Other	0.959			0.067*			
3	Pigmentation of upper gingivae	0.029**			0.036**			a
4	Pigmentation of upper gingivae (site 7—4)	0.171			0.095*			
5	Pigmentation of upper gingivae (site 3—3)	0.035**			0.061*			b
6	Pigmentation of upper gingivae (site 4—7)	0.167			0.062*			
7	Pigmentation of upper gingivae (diffuse)	0.011**			0.008**			a
8	Pigmentation of lower gingivae (site 4—7)	0.077*			0.012**		○	a
9	Pigmentation of lower gingivae (diffuse)	0.985			0.068*		○	
10	Pigmentation of lower gingivae (spotted)	—			0.039**			
11	Pigmentation of lower gingivae (scattered)	0.059*			0.373			
12	Pigmentation of lower gingivae (brownish)	0.008**			0.914			
13	Pigmentation of left buccal mucosa (diffuse)	—			0.075*		○	
14	Pigmentation of left buccal mucosa (faint)	—			0.097*			
15	Pigmentation of palate (spotted)	—			0.038**			
Items of ophthalmology examination								
		—		—	—	—	—	—

○: Interaction present; a: items with $P < 0.05$ both in 2001 and 2002; b: items with $P < 0.1$ both in 2001 and 2002; c: items with $P < 0.1$ both in 2001 and 2002, but showing interactions with sex and/or age. CB ratio: concentration ratio of 2,3,3′,4,4′,5-hexachlorobiphenyl/2,3,3′,4,4′,5-pentachlorobiphenyl gas chromatographic PCB pattern: type A, characteristic of Yusho; type C, commonly observed in the general population; types B and BC, intermediate patterns between types A and C. GTP: glutamyl transpeptidase; PCB: polychlorinated biphenyl; PCDF: polychlorinated dibenzofuran; PCQ: polychlorinated quarterphenyl.

* $P < 0.10$.

** $P < 0.05$.

patients. A significant relationship was found with many PCB-related items ($P < 0.05$). The relationship between PCB pattern/CB ratio and levels of PCDFs was already demonstrated by Yamaguchi and Kaneko [7]. They also reported that blood levels of PCDFs in patients with PCB pattern type A or B exceeded the maximum blood levels in normal controls. In addition, CB ratio was related to blood PCDF levels in Yusho patients. The present study confirms the clear relationship between PCB-related items and blood PCDF levels. The observed significant relationship between PCBs and PCDFs was not surprising because Yusho is a food poisoning by rice bran oil that was contaminated with PCBs and related congeners such as PCDFs; however, it should be pointed out that high concentrations of these poly-

Table 7 The items showing a significant relationship with levels of total PCDFs ($P < 0.1$) without any interaction with sex and age

Items	*P*-value		Significant both in 2001 and 2002
	2001	2002	
Items of the laboratory examination			
Peak 3 (2,3,4,5,3′,4′-hexachlorobiphyenyl) ↑	0.000**	0.000**	a
Items of internal medicine examination			
Items of dermatological examination			
Black comedones (other sites)	0.062*	0.006**	b
Items of dental and oral examinations			
Pigmentation of upper gingivae	0.029**	0.036**	a
Pigmentation of upper gingivae (site 3—3)	0.035**	0.061*	b
Pigmentation of upper gingivae (diffuse)	0.011**	0.008**	a

a: Items with $P < 0.05$ both in 2001 and 2002; b: items with $P < 0.1$ both in 2001 and 2002. PCDF: polychlorinated dibenzofuran.

* $P < 0.10$.

** $P < 0.05$.

Table 8 The number of items for which a significant relationship ($P < 0.1$) was seen with blood levels of total PCDFs in 2001 and 2002 without any interaction with both sex and age

	2001		2002		Either in 2001 or 2002		Both in 2001and 2002	
P-value	<0.1	<0.05	<0.1	<0.05	<0.1	<0.05	<0.1	<0.05
Laboratory examination	7	6	6	4	12	9	1	1
Internal medicine	2	0	11	6	13	6	0	0
Dermatology	3	1	11	10	13	11	1	0
Dentistry	7	5	9	5	13	7	3	2
Ophthalmology	0	0	0	0	0	0	0	0
Total	19	12	37	25	51	33	5	3

PCDF: polychlorinated dibenzofuran.

chlorinated compounds still remain in the body even 34 years after the poisoning incident.

Patients initially suffered from various subjective and objective symptoms as presented in the 'Diagnostic Criteria and Therapeutic Guidelines for Yusho (1968)' (see [17]). The most striking symptoms were oral and dermatological manifestations as follows: (1) pigmentation and occasional flattening of nails are seen without any discernible deformation; (2) blackish fine spots seen at the follicular orifice, which is markedly enlarged and elevated; (3) increased perspiration on the palm; (4) keratotic papules developing especially in areas where active perspiration and secretion of sebum (in the axillae, etc.) are observed; (5) acneform eruptions, varying from comedone to acne conglobata in severe cases; (6) cyst formation in the sebaceous gland (often seen in the genital region); (7) child cases also show the above cutaneous signs but some are slightly different, i.e. there are some cases in which many exfoliative erythemas as big as a pinhead can be seen all over the body, and, in particular, in the flexor aspect of the limbs, with slight itching; (8) no itching is experienced in most cases or, if any, it is only slight and no scratching is seen; (9) the skin becomes a slightly dirty-yellowish color, but in most cases no distinct pigmentation is seen; (10) seborrhea sicca; (11) pigmentation of the oral mucosa and of the gingivae is occasionally seen; and (12) an increase of cerumen.

The ophthalmological symptoms were also distinguished: (1) increased eye discharge (hypersecretion by the meibomian glands); (2) hyperemia, opacity, and pigmentation of the ocular and fornix conjunctivae; (3) pigmentation of the corneal limbs and transient visual disturbance; and (4) swelling of the upper eyelids.

Regarding subjective symptoms, patients often presented with numbness of the limbs and feelings of weakness, but no distinct paralysis is observed. In addition, some patients show a weakened or indiscernible deeper reflex. Hyperalgesia or arthralgia was occasionally seen at the periphery of the limbs. The characteristic symptoms gradually subsided over the years. The diagnostic criteria were therefore revised in 1972, 1976, and 1981. In the revised 'Diagnostic Criteria for Yusho (1976 & 1981)' (see [17]), the important manifestations were designated as follows: (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 tends to suppurate; (2) pigmentation (pigmentation of the face, palpebral conjunctivae, and nails of both the fingers and toes, including so-called 'black babies'); (3) hypersecretion by the meibomian glands.

Of the general criteria for Yusho, the following subjective/objective items were included as reference symptoms and signs: (1) a feeling of lassitude; (2) a feeling of heaviness in the head or headache; (3) paresthesia of the limbs (abnormal sensation); (4) increased eye discharge; (5) cough and sputum; (6) inconstant abdominal pain; (7) altered menstruation; (8) manifestations of bronchitis; (9) deformations of the nails; (10) bursitis; (11) increased neutral fat in the serum; (12) serum γ-glutamyl transpeptidase; (13) decrease in serum bilirubin; (14) neonatal small-for-dates baby; and (15) growth retardation and dental abnormality (retarded eruption of permanent teeth).

Although the specific clinical symptoms and signs disappeared gradually, in our present investigation we found that a substantial number of symptoms such as diarrhea, abnormal heart sounds, abnormal respiratory sounds, hepatomegaly, edema, lymphadenopathy, acneform eruptions, comedones, nail deformity, and gingivopalatal pigmentation still exhibited significant relationships with blood levels of PCDFs either in 2001 or 2002. Of these symptoms, gingival pigmentation and black comedones had a relationship with blood levels of PCDFs both in 2001 and 2002. In sharp contrast to the characteristic clinical features, the majority of laboratory findings remained within normal limits even at the onset of Yusho symptoms [10]. Slight anemia and leucocyto-

sis were observed only in severe cases. No constant abnormality was encountered in liver function tests including the bromosulfophthalein test. Serum protein levels were also normal except for occasional elevation of α2-globulin fractions. Serum electrolytes, blood urea nitrogen, serum iron, and serum zinc levels were also normal. Remarkable elevation of serum lipids, particularly those of serum TG, was evident. The elevation proved to be due to increased pre-β-fraction on the agarose gel electrophoresis [10]. In the present study, it was still evident that blood levels of PCDFs had a significant relationship with decreased direct bilirubin, increased TG and increased β lipoprotein either in 2001 or 2002, indicating a prolonged influence of polychlorinated congeners on lipid metabolism. Interestingly, a study on workers occupationally exposed to PCBs reported a positive correlation between blood PCBs and serum TG [11], suggesting the possibility that increased levels of TG may be attributable to the toxicity of PCBs rather than that of PCDFs [12—16].

The analysis of blood dioxins in the annual medical check-up of patients with Yusho enabled us to understand the clinical and laboratory manifestations of patients in association with blood levels of PCBs and dioxins. Further studies are necessary to more precisely disclose the chronic effects of the polychlorinated congeners on the human body.

Acknowledgement

This work was supported in part by a Grant-in-Aid for scientific research from the Ministry of Health, Labour and Welfare, Japan.

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Journal of Dermatological Science Supplement (2005) 1, S95—S104

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The concepts of the new criteria for Yusho poisoning

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KEYWORDS
Diagnostic criteria;
Human blood;
Multiplicative model;
Polychlorinated dibenzofurans (PCDFs);
2,3,4,7,8-Pentachlorinated dibenzofuran;
Yusho

Summary

Background: The current diagnostic criteria for Yusho poisoning do not include dioxin levels, although one of the subgroups of dioxins, polychlorinated dibenzofurans, was shown to contribute the most to the total toxic equivalent quantity in the blood of Yusho patients.
Objective: To propose new diagnostic criteria for Yusho using blood dioxin levels.
Subjects and methods: Participants of the nationwide health examination for Yusho in 2001 and 2002, and randomly selected residents of Fukuoka City, Japan, were included in this study. A multiplicative model was applied to blood 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) level with age and sex as explanatory variables. A logistic regression model including 2,3,4,7,8-PeCDF level, age and sex was also used.
Results: Three criteria are proposed based on different approaches: 2,3,4,7,8-PeCDF level adjusted for age and sex (criterion 1), its one-tailed upper prediction limit (criterion 2), and the estimated probability of being a Yusho patient (criterion 3). By applying these three criteria to potential victims who had not been diagnosed as having Yusho according to the current diagnostic criteria, the same people were identified as Yusho sufferers. Criterion 1 with an upper 99 percentile of age- and sex-adjusted 2,3,4,7,8-PeCDF level of controls as a cut-off was determined, from a practical perspective, to be superior to the other criteria.

1. Introduction

Yusho patients are diagnosed according to the 'Diagnostic Criteria for Yusho'. To date, more than 1800 people have been officially registered as Yusho patients based on these criteria [1]. The initial

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1574-0757/$30.00
doi:10.1016/j.descs.2005.03.012

criteria published in 1968 referred to the symptoms and signs of Yusho. After revisions were made in 1972, 1976 and 1981, the compositions and concentrations of blood polychlorinated biphenyls (PCBs) and polychlorinated quarterphenyls (PCQs) were included in the criteria [2]. The concentrations of blood dioxins, however, have not been included in the diagnostic criteria because of the practical difficulty in their measurement, although polychlorinated dibenzofurans (PCDFs) were shown to contribute the most to the total toxic equivalent quantity in the blood of Yusho patients [3,4].

Recently an improved method of measuring the concentration of dioxins in a blood sample of as little as 5 g with high validity and reproducibility was developed [5]. The new method makes it possible to routinely measure blood dioxin levels in the blood samples obtained from participants of the nationwide health examination for Yusho. In this paper, we propose new diagnostic criteria for Yusho using blood dioxin levels developed from statistical analyses of blood dioxin levels in the participants of the nationwide health examination for Yusho and in the general population. This is the first attempt to identify Yusho patients on the basis of blood dioxin levels.

2. Subjects and methods

2.1. Participants of the nationwide health examination for Yusho

The nationwide health examination for Yusho has been conducted annually since 1986 to promote the health of the patients and to determine the health status of the chronic Yusho patients [6]. The examination is open not only to officially registered Yusho patients, but also to those who regard themselves as potential victims. Participation in the examination is voluntary. Blood samples of the participants who were willing to have their dioxin levels measured were collected and analyzed. The number, sex, age and place of residence of the subjects are summarized in Table 1. The total number of blood samples obtained was 452 (provided by 391 people). In 2001, 78 officially registered Yusho patients and 3 people who regarded themselves as potential victims provided blood samples. In 2002, blood samples were collected from 279 officially registered Yusho patients and 92 potential victims. Sixty officially registered Yusho patients and one person who regarded herself as a potential victim were repeatedly measured (i.e. in both 2001 and 2002). In total, the participants were 297 officially registered Yusho patients and 94 potential victims. The method of measurement of dioxin levels in the blood of the participants of the nationwide health examination for Yusho has been described by Iida and Todaka [5].

2.2. Controls

The data of the controls were provided by the courtesy of Professor Kono, Kyushu University. The subjects and the method of analysis have been described by Masuda et al. [7]. The study was conducted in 1999 on randomly selected residents in an area of Fukuoka City, Japan. The age and sex of the controls are summarized in Table 1. The number of controls was 152 (75 men and 77 women), and their mean age was 36.5 years (range 20—60).

2.3. Reproducibility of the measurements

In this study, reproducibly was defined as the agreement between measurements for the same person collected in different years. Sixty officially registered Yusho patients and one person who regarded herself as a potential victim were repeatedly measured (i.e. in both 2001 and 2002). The mean age in

Table 1 Age, sex and place of residence of the study subjects

	Year of measurement	Number (men/women)	Age[a] (years)		Residence (%)		
			Mean (S.D.)	Minimum—maximum	Fukuoka	Nagasaki	Other
Officially registered Yusho patients[b]	2001	78 (32/46)	65.3 (11.2)	33—84	100	0	0
	2002	279 (135/144)	63.6 (12.6)	30—88	40	28	32
People who regard themselves as potential victims[b]	2001	3 (1/2)	57.0 (3.5)	53—59	100	0	0
	2002	92 (38/54)	54.1 (17.4)	5—81	26	39	35
Controls	1999	152 (75/77)	36.5 (11.8)	20—60	100	0	0

[a] Age in the year of measurement.
[b] Sixty officially registered Yusho patients and one person who regarded herself as a potential victim were repeatedly measured (i.e. in both 2001 and 2002).

2002 of these 61 participants (23 men and 38 women) was 65.5 years (range 34—85).

The mean difference between the two measurements for the same person was used as an index of the bias. Because the measurement values were log-transformed, the mean difference was calculated as the geometric mean of the ratio of the values measured in 2001 and 2002. The agreement between the two values was expressed with the intraclass correlation coefficient, r_i [8]. The strength of the agreement was assessed according to the grades proposed by Landis and Koch [9]. Although this grading system was originally used to describe the relative strength of the agreement associated with Kappa statistics, this scale was applied here to the intraclass correlation coefficient because of the equivalence of Kappa statistics and the intraclass correlation coefficient [10].

2.4. Statistical analyses

Because of the skewed distribution of blood dioxin level, we used logarithms of the data with base of 10 in the statistical analyses of the dioxin congener measurements. When the result of the measurement was under the detection limit, the concentration of the congener was assumed to be one-half of the detection limit. Association of 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) level with age and sex was analyzed using a multiplicative model with explanatory variables of age as an continuous variable and sex as a dummy variable with men and women coded as 0 and 1, respectively. Interaction between age and sex was tested by including the product of age and sex terms in the model. The Shapiro—Wilk *W*-test was used to test the normality of the distribution. A logistic regression analysis was conducted to estimate the probability of being a Yusho patient. The difference between Yusho patients and controls was included in the logistic regression model as a dependent variable. Log-transformed 2,3,4,7,8-PeCDF level, and log-transformed age and sex were included as explanatory variables. All tests were two-tailed, and *P* values less than 0.05 were considered statistically significant. All statistical analyses were performed with Stata Statistical Software: release 8.2 (Stata Corporation, 2003; College Station, TX, USA).

3. Results

3.1. The characteristics of the subjects

Table 1 shows data on the age, sex and place of residence of the subjects according to their group (officially recognized patients, potential victims, controls) and the year of measurement. A difference in age distribution and residency was found between the participants of the nationwide health examination and controls. The mean age as of 2002 for the 297 officially registered Yusho patients was 63.9 years (range 30—88) and for the 94 people who regarded themselves as potential victims it was 54.2 years (range 5—81). Controls were younger than the participants in the health examination; mean age at measurement was 36.5 years (range 20—60). All controls lived in Fukuoka prefecture, whereas only 40% of the participants of the health examination lived in Fukuoka prefecture. The remaining participants lived in Nagasaki and other prefectures mainly in western Japan. Women made up 51.5%, 56.5% and 50.7% of the officially registered Yusho patients, potential victims and controls, respectively.

3.2. Blood dioxin levels

The blood dioxin levels are shown in Table 2 by subject group and year of measurement. The data from potential victims measured in 2001 are not shown in Table 2, because there were only three potential victims. Concentrations of certain dioxin congeners show marked differences between Yusho patients and controls. The geometric mean blood levels of 1,2,3,4,6,7,8-heptachlorodibenzodioxin (HpCDD), octachlorodibenzodioxin (OCDD), 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-hexachlorodibenzofuran (HxCDF), 1,2,3,6,7,8-HxCDF and 3,3′,4,4′,5,5′-hexachlorobiphenyl (HxCB) (PCB 169) in Yusho patients measured in 2002 were more than three times as high as those in controls. The greatest difference was found in 2,3,4,7,8-PeCDF levels. Comparison between blood levels of 2,3,4,7,8-PeCDF in officially registered Yusho patients measured in 2002 and controls showed that the geometric mean for Yusho patients was 11 times that for controls, and the maximum value for Yusho patients in 2002 reached 73 times that for controls. Because of this difference, only 22% and 28% of the Yusho patients measured in 2001 and 2002, respectively, were within the range of 2,3,4,7,8-PeCDF level observed in the controls.

The difference in congener concentrations was less evident between people who regarded themselves as potential victims and controls. Blood levels of 2,3,4,7,8-PeCDF in potential victims remained only 2.4 times higher than that in controls. The difference in concentrations of other congeners was less than two-fold, except for that of 1,2,3,4,6,7,8-HpCDD and OCDD between potential victims and controls. The overlap in the distribution of

Table 2 Basic statistical data on dioxin levels (pg/g lipid) in blood

Congeners	DL	Officially registered Yusho patients measured in 2001 (n = 78)				Officially registered Yusho patients measured in 2002 (n = 279)				Potential victims[a] measured in 2002 (n = 92)				Controls (n = 152)			
		n[b]	Geometric mean[c]	Minimum[c]	Maximum	n[b]	Geometric mean[c]	Minimum[c]	Maximum	n[b]	Geomet ric mean[c]	Minimum[c]	Maximum	n[b]	Geometric mean[c]	Minimum[c]	Maximum
2,3,7,8-TCDD	1	56	1.43	0.5	4.1	230	1.43	0.5	4.4	63	1.17	0.5	5.0	123	1.38	0.5	5.0
1,2,3,7,8-PeCDD	1	78	17.5	3.3	53.5	279	9.71	1.5	46.8	92	6.84	1.2	22.1	151	5.18	0.5	15.0
1,2,3,4,7,8-HxCDD	2	34	1.80	1.0	7.7	197	2.36	1.0	10.8	68	2.55	1.0	11.4	97	2.26	1.0	9.3
1,2,3,6,7,8-HxCDD	2	78	44.1	4.4	230	279	40.8	6.0	291	92	24.0	5.9	106	152	18.3	4.4	45.0
1,2,3,7,8,9-HxCDD	2	70	3.92	1.0	11.0	250	4.11	1.0	41.0	80	4.18	1.0	17.7	115	2.68	1.0	10.0
1,2,3,4,6,7,8-HpCDD	2	78	21.6	5.4	144	279	51.2	10.8	556	92	62.1	14.5	288	152	16.6	5.1	96.0
OCDD	4	78	514	137	6226	279	739	172	9159	92	776	229	2836	151	231	2.0	4200
2,3,7,8-TCDF	1	41	1.12	0.5	14.4	189	1.12	0.5	6.3	40	0.82	0.5	6.2	21	0.60	0.5	5.7
1,2,3,7,8-PeCDF	1	32	0.84	0.5	4.2	95	0.75	0.5	6.3	20	0.64	0.5	2.8	20	0.57	0.5	8.8
2,3,4,7,8-PeCDF	1	78	105.9	6.7	1771	279	80.6	3.1	1890	92	17.4	2.2	263	152	7.25	2.2	26.0
1,2,3,4,7,8-HxCDF	2	78	30.6	2.0	632	273	20.3	1.0	770	85	5.11	1.0	112	146	4.41	1.0	29.0
1,2,3,6,7,8-HxCDF	2	77	16.8	1.0	176	278	12.7	1.0	210	88	5.39	1.0	26.0	149	3.93	1.0	18.0
2,3,4,6,7,8-HxCDF	2	28	1.45	1.0	6.4	67	1.27	1.0	10.3	25	1.33	1.0	10.0	69	1.63	1.0	14.0
1,2,3,7,8,9-HxCDF	2	3	1.03	1.0	2.3	2	1.01	1.0	5.8	0	—	—	—	0	—	—	—
1,2,3,4,6,7,8-HpCDF	2	65	3.21	1.0	10.8	186	2.31	1.0	39.8	59	2.30	1.0	27.3	60	1.82	1.0	39.0
1,2,3,4,7,8,9-HpCDF	2	0	—	—	—	2	1.01	1.0	3.5	0	—	—	—	1	1.01	1.0	3.5
OCDF	4	0	—	—	—	1	2.01	2.0	9.1	1	2.03	2.0	7.5	2	2.07	2.0	42.0
3,4,4′,5-TCB(#81)	10	2	5.18	5.0	20.6	14	5.27	5.0	41.0	5	5.23	5.0	15.5	6	5.24	5.0	22.0
3,3′,4,4′-TCB(#77)	10	27	6.88	5.0	28.5	154	9.17	5.0	46.1	50	8.94	5.0	44.6	1	5.06	5.0	30.0
3,3′,4,4′,5-PeCB(#126)	10	78	70.0	17.8	320	277	83.4	5.0	561	91	63.2	5.0	387	131	41.6	5.0	430
3,3′,4,4′,5,5′-HxCB(#169)	10	78	158	31.0	964	279	153	12.7	1131	91	61.8	5.0	318	151	37.1	5.0	160

CB: chlorobiphenyl; CDD: chlorodibenzodioxin; CDF: chlorodibenzofuran; DL: detection limit; Hp: hepta; Hx: hexa; Pe: penta; OCDD: octachlorodibenzodioxin; OCDF: octachlorodibenzofuran; TCB: tetrachlorobiphenyl; TCDD: tetrachlorodibenzodioxin; TCDF: tetrachlorodibenzofuran.

[a] People who regarded themselves as potential victims.

[b] Number of samples with the concentration higher than or equal to the detection limit.

[c] Values under the detection limit were substituted by one-half of the detection limit.

Table 3 Reproducibility of the measurements in 2001 and 2002

Congeners	Mean increase[a] (%)	95% confidence interval of the mean increase	*P*	r_i
2,3,7,8-TCDD	−10.1	(−26.2 to 9.5)	0.29	0.33
1,2,3,7,8-PeCDD	−39.1	(−48.0 to −28.8)	<0.001	0.31
1,2,3,4,7,8-HxCDD	55.1	(30.5 to 84.2)	<0.001	0.43
1,2,3,6,7,8-HxCDD	13.4	(6.3 to 21.0)	<0.001	0.93
1,2,3,7,8,9-HxCDD	23.7	(11.4 to 37.4)	<0.001	0.75
1,2,3,4,6,7,8-HpCDD	206.0	(165.0 to 253.4)	<0.001	0.29
OCDD	51.3	(38.7 to 65.1)	<0.001	0.74
2,3,7,8-TCDF	−3.8	(−24.9 to 23.2)	0.75	0.32
1,2,3,7,8-PeCDF	−6.4	(−24.1 to 15.4)	0.53	0.31
2,3,4,7,8-PeCDF	1.1	(−6.8 to 9.7)	0.78	0.98
1,2,3,4,7,8-HxCDF	−2.1	(−11.7 to 8.5)	0.68	0.97
1,2,3,6,7,8-HxCDF	−5.0	(−12.9 to 3.5)	0.24	0.95
2,3,4,6,7,8-HxCDF[b]				
1,2,3,7,8,9-HxCDF[b]				
1,2,3,4,6,7,8-HpCDF	−27.5	(−36.9 to −16.7)	<0.001	0.63
1,2,3,4,7,8,9-HpCDF[b]				
OCDF[b]				
3,4,4′,5-TCB(#81)[b]				
3,3′,4,4′-TCB(#77)	39.8	(13.0 to 72.8)	<0.01	−0.14
3,3′,4,4′,5-PeCB(#126)	12.2	(4.1 to 20.9)	<0.01	0.85
3,3′,4,4′,5,5′-HxCB(#169)	6.8	(−0.3 to 14.4)	0.06	0.94

CB: chlorobiphenyl; CDD: chlorodibenzodioxin; CDF: chlorodibenzofuran; Hp: hepta; Hx: hexa; Pe: penta; OCDD: octachlorodibenzodioxin; OCDF: octachlorodibenzofuran; r_i: intraclass correlation coefficient; TCB: tetrachlorobiphenyl; TCDD: tetrachlorodibenzodioxin; TCDF: tetrachlorodibenzofuran.

[a] The geometric mean of the ratio of the measurement in 2002 to the measurement in 2001, shown by the percentage increase, because the measurement values were log-transformed.

[b] The reproducibility of the congener was not analyzed because measurements in both 2001 and 2002 were below the detection limit for more than one-half of the subjects.

2,3,4,7,8-PeCDF level among people who regarded themselves as potential victims and controls was greater than that between Yusho patients and controls. In 74% of the potential victims, the 2,3,4,7,8-PeCDF level was within the range observed in the controls.

3.3. Reproducibility of the measurements

The mean difference and the intraclass correlation coefficients, r_i, of the measurements in the years 2001 and 2002 for the same people are shown in Table 3. The difference in mean (an index of the bias) was not statistically significant for blood concentrations of 2,3,7,8-TCDD, 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF and 3,3′,4,4′,5,5′-HxCB (PCB 169). Of these congeners, 2,3,4,7,8-PeCDF showed the smallest bias in the measurements of the 2 years. The mean difference was only 1.1% (95% confidence interval [CI], −6.8 to 9.7).

Certain congeners showed strong agreement between the measurements in 2001 and 2002. There was almost perfect agreement (i.e. intraclass correlation coefficient >0.8) for 1,2,3,6,7,8-HxCDD, 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, 3,3′,4,4′,5-PeCB (PCB 126) and 3,3′,4,4′,5,5′-HxCB (PCB 169). For 1,2,3,7,8,9-HxCDD and OCDD, agreement between the measurements was substantial. The highest intraclass correlation coefficient for the measurements was observed in 2,3,4,7,8-PeCDF. The agreement between its measurement values in 2001 and 2002 is shown in Fig. 1.

3.4. Association of 2,3,4,7,8-PeCDF level with age and sex

A multiplicative model was used to analyze the association of blood 2,3,4,7,8-PeCDF level with age and sex for controls. Because the interaction term of age and sex was not statistically significant ($P = 0.89$), it was omitted from the model. Age and sex terms were statistically significant. Because sex was included in the model as a dummy variable coded as 0 for men and 1 for women, a multiplicative model was obtained: concentration of 2,3,4,7,8-PeCDF in blood = $A\ \text{age}^B\ C^{\text{sex}}$. Estimated coefficients A, B and C were 0.293 (95% CI, 0.139—0.618), 0.884 (95% CI, 0.678—1.090) and 1.152 (95% CI, 1.006—1.319), respectively. The

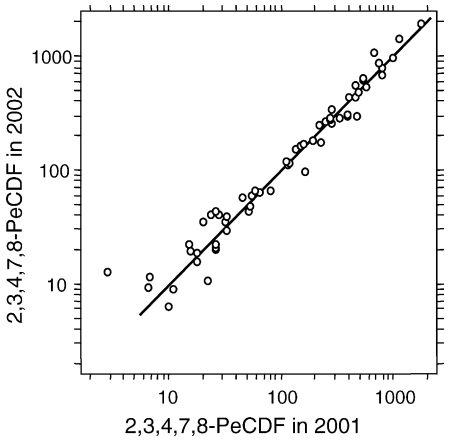

Fig. 1 The reproducibility of the measurement of blood 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) level (pg/g lipid). The measurement in 2002 is plotted against the measurement in 2001 for the same person on logarithmic scales.

blood 2,3,4,7,8-PeCDF level in controls, its regression curve and its two-tailed 95% prediction limits are plotted against age in Fig. 2.

3.5. Comparison of blood 2,3,4,7,8-PeCDF level among subject groups

The age and sex of the subjects should be taken into account for the comparison between groups with different age and sex composition, because blood 2,3,4,7,8-PeCDF level is dependent on age and sex, as indicated above. In applying the multiplicative model to obtain blood 2,3,4,7,8-PeCDF level adjusted for age and sex, men and age 63.9 years (the mean age of the officially registered Yusho patients) were used as baseline values for sex and age. Fig. 3 shows the distribution of adjusted 2,3,4,7,8-PeCDF level in the controls and those who regarded themselves as potential victims. The distribution of crude 2,3,4,7,8-PeCDF level in officially registered Yusho patients is also shown. Adjustment for age and sex was not applied to the Yusho patients, because most 2,3,4,7,8-PeCDF in the blood of Yusho patients is considered to be derived from short-term exposure to the contaminated rice oil, whereas it is widely accepted that environmental exposure, mainly via food, attributes to long-term, age-dependent accumulation of PCDFs in the general population [11]. In cases where a participant was analyzed twice, a mean value for the 2 years is shown.

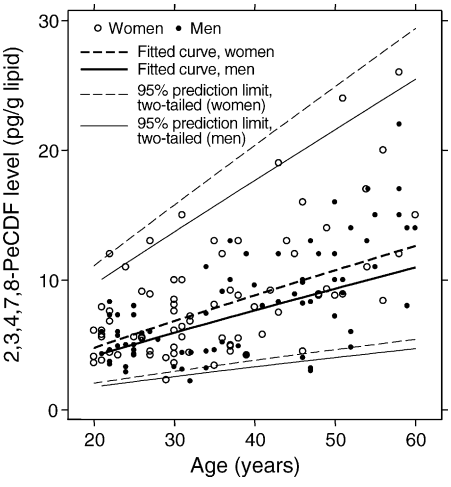

Fig. 2 Blood 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) level (pg/g lipid) in controls plotted against age. The fitted curve of the multiplicative model and its two-tailed 95% prediction limits are shown separately for men and women.

The age- and sex-adjusted 2,3,4,7,8-PeCDF level in the controls ranged from 3.94 to 26.75 pg/g lipid, and the geometric mean was 11.60 pg/g lipid. The distribution of its log-transformed value approximated the normal distribution. The deviation of the observed distribution from the normal distribution was not statistically significant ($P = 0.08$). The mean and standard deviation of the adjusted log-transformed level of 2,3,4,7,8-PeCDF in pg/g lipid were 1.064 (95% CI, 1.035—1.093) and 0.181 (95% CI, 0.163—0.204), respectively. The distribution of age- and sex-adjusted 2,3,4,7,8-PeCDF level for the potential victims skewed to the right with the minimum value similar to that in the controls (4.71 pg/g lipid) and the maximum value 8.5 times higher than that in the controls (227.3 pg/g lipid).

3.6. Blood PCQ level and gas chromatographic patterns of blood PCB

Table 4 shows the blood PCQ level and the type of gas chromatographic pattern of blood PCB for the officially registered Yusho patients and people who regarded themselves as potential victims. If a person was repeatedly measured (i.e. in both 2001 and 2002), the higher PCQ level and the type of PCB pattern more typical of Yusho patients from the 2 years were adopted as the representative values for that person. High blood PCQ level ($\geq$0.03 ppb) and PCB chromatographic pattern of types A, B or BC were considered to represent exposure to the contaminated rice bran oil responsible for the Yusho incident. Both blood PCQ level and PCB pattern were measured in 63 officially registered Yusho patients and 52 potential victims. Among these participants, 49 Yusho patients (78%) and 13 potential victims (25%) showed blood PCQ levels $\geq$0.03 ppb and PCB patterns of type A, B and BC. The distribution of blood PCQ level and type of PCB

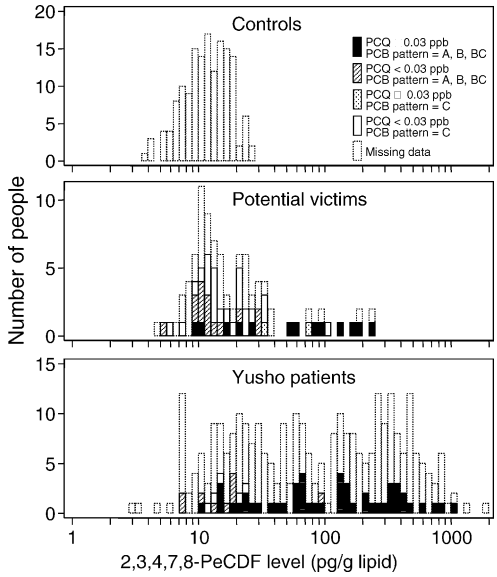

Fig. 3 The distribution of blood 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) level (pg/g lipid) by subject group. The values of controls and those who regarded themselves as potential victims were adjusted for age and sex using the mean age of Yusho patients and male sex as baseline in the multiplicative model. The values of officially registered Yusho patients were not adjusted for age and sex. If a person was repeatedly measured (i.e. in both 2001 and 2002), the mean value of the two measurements is shown.

pattern in relation to blood 2,3,4,7,8-PeCDF level is shown in Fig. 3.

4. Proposal of the new criteria for Yusho

Here we propose three new diagnostic criteria for Yusho based on different approaches. In the first approach, the blood 2,3,4,7,8-PeCDF level in the subject to be diagnosed was compared with that in the general population. The second approach uses a regression model to estimate the prediction limit of the congener level in the general population. The third approach uses a logistic regression analysis to estimate the probability of being a Yusho patient so that subjects could be diagnosed by congener level and their demographic characteristics. Of the dioxin congeners, 2,3,4,7,8-PeCDF was selected for comparison because out of all the congeners (1) the difference in the distribution of blood level between Yusho patients and the general population was greatest, and (2) reproducibility of the values measured in 2 years was highest with the smallest bias and largest intraclass correlation coefficient. The proposed diagnostic criteria were applied to the 13 potential victims who were likely to have been exposed to the causal agent of Yusho because of their high blood PCQ levels and the gas chromatographic patterns of blood PCBs.

4.1. Criterion 1

Subjects were classified as Yusho patients if their log-transformed, age- and sex-adjusted blood 2,3,4,7,8-PeCDF level (with the baseline of 63.9-year-old men) was higher than the one-tailed upper 99 percentile (mean + 2.33 S.D.) of the controls. This criterion can be translated to an age- and sex-adjusted blood 2,3,4,7,8-PeCDF level higher than 30.6 pg/g lipid. With this criterion, 8 of the 13 potential victims were classified as Yusho patients. These eight people were 57—81 years of age, and their crude 2,3,4,7,8-PeCDF level ranged from 58 to 237 pg/g lipid.

4.2. Criterion 2

Subjects were classified as Yusho patients if their blood 2,3,4,7,8-PeCDF level was higher than the

Table 4 Blood PCQ level and type of PCB chromatographic pattern in the participants of the nationwide health examination for Yusho in 2001 and 2002

PCQ level (ppb)	Officially registered Yusho patients						People who regarded themselves as potential victims					
	Type of PCB pattern[a]					Total	Type of PCB pattern[a]					Total
	A	B	BC	C	NA		A	B	BC	C	NA	
>0.1	26[b]	15[b]	7[b]	0	4	52	3[b]	5[b]	0[b]	1	2	11
0.03—0.09	0[b]	1[b]	0[b]	0	0	1	2[b]	3[b]	0[b]	1	1	7
<0.03	0	9	0	5	2	16	2	11	1	23	10	47
NA	82	66	9	47	24	228	9	1	0	2	17	29
Total	108	91	16	52	30	297	16	20	1	27	30	94

NA: not assessed; PCB: polychlorinated biphenyl; PCQ: polychlorinated quarterphenyl.

[a] The gas chromatographic patterns of PCBs in the blood: type A, peculiar to Yusho; type B, resembling type A; type C, common or similar to the PCB gas chromatogram of unaffected people; type BC, intermediate pattern between types B and C.

[b] Exposure to the contaminated rice bran oil, the causal agent of Yusho, is highly probable.

one-tailed upper 99% prediction limit of the regression curve fitted to the multiplicative model of blood 2,3,4,7,8-PeCDF level with age and sex as explanatory variables. With this criterion, the same eight people who were diagnosed as Yusho patients according to criterion 1 were diagnosed as Yusho patients.

4.3. Criterion 3

The logistic model used to estimate the probability of being a Yusho patient was:

$$\log\left(\frac{p}{1-p}\right) = \alpha + \beta_1 \log_{10}[2,3,4,7,8\text{-PeCDF level (pg/g lipid)}] + \beta_2 \log_{10}(\text{age}) + \beta_3 \,\text{sex},$$

where sex is a dummy variable with men and women coded as 0 and 1, respectively. The regression coefficients α, β_1, β_2 and β_3, were -15.07 (95% CI, -19.70 to -10.44), 4.70 (95% CI, 3.18–6.22), 6.62 (95% CI, 3.69–9.55) and -0.78 (95% CI, -1.48 to -0.07), respectively. Subjects who had a probability of 0.99 or higher were diagnosed as Yusho patients. With this criterion, eight potential victims were classified as Yusho patients. The result was identical as the classification based on criteria 1 and 2.

5. Discussion

This study found marked differences in distribution of blood dioxin levels between Yusho patients and controls randomly selected from the general population. Of the 21 congeners examined, the difference in blood level was most distinctive for 2,3,4,7,8-PeCDF. Its blood level (geometric mean) in Yusho patients was 11 times higher than that in controls. The association of blood 2,3,4,7,8-PeCDF level with age and sex was statistically significant. The blood level of this congener increased with age, and female subjects tended to show higher levels.

The difference in blood 2,3,4,7,8-PeCDF level between the Yusho patients and controls observed in this study was astonishing considering that more than 30 years has passed since the ingestion of the contaminated rice bran oil by the Yusho patients. This is mainly due to the persistent nature of 2,3,4,7,8-PeCDF in the human body. The biological half-life of 2,3,4,7,8-PeCDF in Yusho patients was estimated to be 7.7 years [12]. Using the difference in blood 2,3,4,7,8-PeCDF level, we proposed three diagnostic criteria to identify Yusho patients by the blood concentration of this congener. It may be reasonable, from a biological perspective, to include the blood 2,3,4,7,8-PeCDF level in the criteria because PCDFs, which contain this congener as a component, are estimated to account for most of the toxicity in Yusho poisoning [3,4].

The three proposed diagnostic criteria were applied to the potential victims who had high concentrations of PCQs and a PCB chromatographic pattern typical of Yusho patients. The diagnosis by the three proposed criteria identified the same eight people as possible Yusho patients, even though the criteria adopted different approaches. Of the three criteria, criterion 1 is conceptually simplest and easiest to interpret. The result of the diagnosis was equivalent to the diagnosis by the other proposed criteria. From a practical perspective, therefore, it may be concluded that criterion 1 is superior to the other proposed diagnostic criteria based on blood 2,3,4,7,8-PeCDF level.

A limitation of this study is the comparability of the controls and participants of the health examination for Yusho. The blood dioxin levels of these two populations were measured in different laboratories (the Fukuoka Institute of Health and Environmental Sciences, Fukuoka, and ERGO Laboratory, Hamburg, Germany). Both laboratories, however, have a good reputation worldwide for their measurement of dioxin congeners. Quantification of the methods adopted by the two laboratories has been examined [4,5,7,13]. Even if a difference exists between the 2,3,4,7,8-PeCDF levels measured by the two laboratories, it would be too small to be an obstacle in the comparison.

Another possible problem in comparability of blood 2,3,4,7,8-PeCDF level is the difference in the characteristics of the participants of the Yusho health examination and the controls. If the baseline values of 2,3,4,7,8-PeCDF level were influenced by the different characteristics of the two populations, the cut-off values determined from the controls may not be applicable to the participants of the health examination. There was a geographical difference in the place of residence of the study subjects; all controls lived in Fukuoka prefecture, whereas 60% of the participants of the annual health examination for Yusho lived in other prefectures of Japan. Although, to date, no detailed report has been published on the geographical difference in blood 2,3,4,7,8-PeCDF level in the Japanese population, it may exist given that the total blood level of dioxins (which includes 2,3,4,7,8-PeCDF in its composition) showed variation in relation to residential district of Japan [14]. The geographical difference of 2,3,4,7,8-PeCDF level in humans, however, may not be common, even if it does exist. Two studies examining the regional difference in 2,3,4,7,8-

PeCDF level in the general population in Spain and Northern Taiwan showed no evidence for it [15,16]. Even if an apparent variation in the concentration of the congener was observed among the regions of a country, the difference was attributed to the geographical differences in age of the inhabitants [17]. It is not clear whether the geographical difference of the two populations in this study caused the deviation of the age-adjusted baseline values of blood 2,3,4,7,8-PeCDF level.

Another possible problem in comparability of blood 2,3,4,7,8-PeCDF level was the difference in age distribution between the controls and the participants of the nationwide annual health examination for Yusho. The controls were younger than 60 years, whereas 43% of the participants of the health examination were 60 years or older. The age dependency of the blood level of this congener has been shown by previous studies [17—19]. In this study, a multiplicative regression model was used to estimate age-adjusted values and the prediction limit of 2,3,4,7,8-PeCDF. The regression curve for controls, therefore, should be extrapolated to ages older than 60 years. The extrapolation may result in an increase of error in both the adjustment for age and estimation of prediction limit. Despite the possible errors due to extrapolation, the false positives (the non-Yusho patients who were wrongly diagnosed as Yusho patients) classified by diagnostic criterion 1 are expected to be minimal because of the high cut-off level of the 99 percentile. With a trade-off between sensitivity and specificity, the presumably high specificity implies low sensitivity of the criterion and, consequently, a reduction in the Yusho patients who could be correctly identified as Yusho patients. In this study, the high cut-off level was provisionally set, because few data are available on 2,3,4,7,8-PeCDF level in the Japanese population and its relation to age. Considering the extreme difficulty in canceling registration once a person is officially registered as a Yusho patient, it may be justifiable that specificity was given priority over sensitivity.

It must be recognized that an individual cannot be disproved of being a Yusho patient on the grounds that the individual was not diagnosed as a patient by the proposed criteria. There may be large interpersonal variation in the half-life of blood 2,3,4,7,8-PeCDF level. Even if a person's blood 2,3,4,7,8-PeCDF level was high at the time of the Yusho incident, it can be reduced to a low level compared with other Yusho patients at present by a more rapid reduction rate specific to the individual. There may also be variation in the physiological sensitivity to 2,3,4,7,8-PeCDF among people. Some people with currently lower levels of the congener compared with other Yusho patients may have suffered from Yusho because of higher sensitivity to the congener than others.

To improve the precision of the diagnostic criteria, more data on blood dioxin levels in controls are required. Ideally, the controls should be randomly selected from the general population, and they should have a distribution of age, sex and place of residence similar to that of the Yusho patients. There is also potential for improvement in the statistical methodology of diagnosis. Inclusion of other factors that possibly influence blood dioxin levels to the diagnostic criteria may improve the precision of the criteria. A combination of levels of other congeners in addition to 2,3,4,7,8-PeCDF level may also improve precision. With such efforts, the sensitivity of the diagnosis may increase without sacrificing the specificity.

Acknowledgements

The authors are grateful to Professor Suminori Kono, Department of Preventive Medicine, Kyushu University, Japan, for providing the data on blood dioxin levels in the residents of Fukuoka prefecture, Japan. We would also like to thank Dr. Masanori Kuratsune for his invaluable comments on the draft of this paper. This work was supported in part by a Grant-in-Aid for scientific research from the Ministry of Health, Labour and Welfare, Japan.

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Journal of Dermatological Science Supplement (2005) 1, S105—S112

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Effects of dioxins on stress-responsive systems and their relevance to toxicity

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KEYWORDS
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin;
Chaperone;
Heat-shock protein;
Reactive oxygen species

Summary

Background: Dioxins and related compounds, exemplified by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, are recognized as widespread, persistent and highly toxic environmental pollutants. Although numerous studies have been performed to clarify the mechanisms governing dioxin toxicity, these are not yet fully understood because of their complexity. In 1968, subacute poisoning by polychlorinated biphenyls, called 'Yusho', occurred in the southwest part of Japan. Although many of the Yusho patients appear to be free from any of the symptoms produced by the pollutant at present, they remain at high risk of dioxin toxicity because of the high concentrations present in the body. To date, no effective method for combating this toxicity has been developed.
Objective: In this review, we summarize dioxin toxicity by focusing on the quenching systems of reactive oxygen species and chaperone proteins. In addition, the possibility of the development of protective and therapeutic treatments for dioxin toxicity is discussed.

1. Introduction

Over 35 years ago, subacute poisoning (Yusho) by ingestion of food oil contaminated with some forms of dioxins, i.e. polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs), occurred in Japan. In this incident, almost 2000 people were affected. Although the patients exhibit almost no clinical signs at present (see other sections of this supplement), they still have higher levels of dioxins than normal people [1]. Because of the higher risk of damage to health due to the higher content of dioxins, Yusho patients require continuous attention.

Since the publication of 'Silent Spring' by Carson in 1962 [2], social interest in environmental pollutants has continued to increase. In that atmosphere, Yusho was one of the triggers for research addressing the effects of dioxins on health, and the number of

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1574-0757/$30.00 © 2004 Published by Elsevier Ireland Ltd on behalf of Japanese Society for Investigative Dermatology.
doi:10.1016/j.descs.2005.03.011

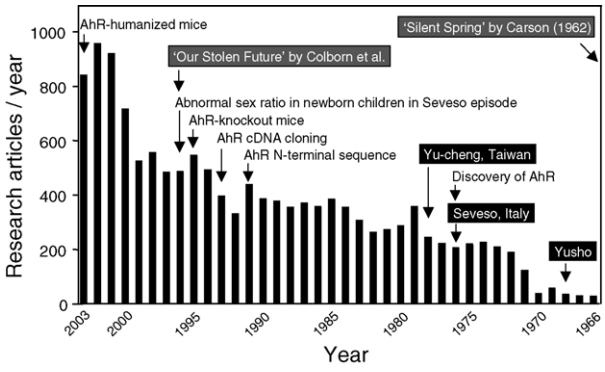

Fig. 1 Change in the number of articles devoted to dioxin. The number of articles on dioxins in a database (MEDLINE) was counted using the following keywords dioxin(s), tetrachlorodibenzo-*p*-dioxin, polychlorinated biphenyl, TCDD, PCB and PCDF. Epoch-making findings about the arylhydrocarbon receptor (AhR) and the effect of dioxin on reproduction are indicated in the Figure. The episodes of dioxin toxicity involving humans and the publication of books that had an impact on society are also shown in black and shaded boxes, respectively.

research articles about dioxins published a few years after the occurrence of this accident in 1968 increased significantly (Fig. 1). Following this, dioxin research increased steadily over the 1970s to the 1990s, and again has markedly increased in the 21st century. This jump in research studies is the result of the increased social interest in the reproductive toxicity of dioxins and other environmental contaminants. The reports about the abnormal sex ratio in newborn children from people affected by the incident in Seveso, Italy [3], and the publication of 'Our Stolen Future' [4] are among the driving forces that have stimulated dioxin research in the 21st century (Fig. 1).

Dioxins are believed to exert a wide range of toxic effects by initially binding to a specific receptor, arylhydrocarbon receptor (AhR) [5—7]. Fig. 1 also shows the epoch-making findings about AhR. The importance of this receptor is clearly evident from the observations that dioxin-induced symptoms, including organ atrophy and teratogenicity, are not present in AhR-knockout (KO) mice [8,9]. Thus, this receptor plays a crucial role in dioxin toxicity. However, immunosuppression produced by dioxins may occur by mechanism(s) not involving AhR [10]. In connection with this, dioxin-induced changes in protein kinases [11], phospholipase c [12] and low-density lipoprotein receptors [13,14] have been suggested to occur via an AhR-independent mechanism. Thus, some forms of dioxin toxicity do not seem to require AhR.

AhR is a transcription factor, and microarray experiments suggest that this receptor regulates more than 100 genes following activation by ligand binding [15]. Although the role of this receptor in dioxin toxicity has been established as mentioned above, very little is known about the gene products governed by AhR contributing to the toxicity. This laboratory identified many proteins the expression of which is changed by dioxins. They include stress-quenching systems as well as the proteins necessary for the utilization of nutrients. The details and significance of these changes have been reviewed elsewhere [16]. In this review, we focus on chaperone proteins and quenching systems of reactive oxygen species (ROS), and discuss in more detail the relevance of their alteration by dioxins in relation to toxicity.

2. Effects of dioxins on the quenching systems of ROS

Since the first mention made by Stohs et al. [17], a number of studies have been undertaken to clarify the relationship between oxidative stress and dioxin toxicity. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and related chlorinated aromatic hydrocarbons, such as 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) and 3,3′,4,4′,5-pentachlorobiphenyl (PeCB), produce oxidative stresses in animals [18—20]. Cellular signs of such oxidative stress involve production of ROS including lipid peroxide, reduction in sulfhydryl groups, increase in membrane fluidity and DNA strand breakage. Butylated hydroxyanisole, an antioxidant, reduced TCDD-induced death of mice [21]. Although the above investigators failed to detect a protective effect of α-tocopherol on the in vivo toxicity of TCDD, this vitamin has been reported to protect human cells (conjunctival epithelial cells) from TCDD-induced cell death [22]. Also, it is well known that iron facilitates the production of hydroxy radicals, a candidate for the ultimate toxic species of ROS. In agreement with this, TCDD-induced lipid peroxidation in liver is abolished in rats given an iron-deficient diet [23]. These observations suggest that the acute toxicity of TCDD is at least partially due to the increase in ROS. Because ascorbic acid has no protective effect on TCDD-induced acute toxicity [24], the lipid soluble nature of the antioxidant is important for combating dioxin toxicity.

Vitamin A also exhibits a protective effect on TCDD-induced death [21]. Because Vitamin A has a wide range of physiological roles as well as its effect as an antioxidant, its protective effect against dioxin toxicity cannot be attributable only to a reduction of ROS. Keeping this in mind, the magnitude of the TCDD-induced reduction in hepa-

tic Vitamin A correlates with species differences in the acute toxicity of this compound [25,26]. However, it should be noted that, in contrast to the above data, no correlation between the serum level of retinoids in rats and differences in toxic manifestation has been reported [27]. Thus, it is unlikely that a change in Vitamin A homeostasis is the sole mechanism for acute dioxin toxicity. TCDD causes a reduction in the storage of tissue Vitamin A and increases its excretion [28]. The reduction in Vitamin A in the liver of animals following administration of TCDD is suggested to be due to the increase in glucuronidation of this vitamin [29].

One of the mechanisms for the oxidative stress produced by dioxins is assumed to be alteration in ROS quenching systems. This is partially supported by the observation that glutathione peroxidase, one of the important quenchers of hydrogen peroxide, is reduced by dioxins [18,19,21]. In addition, the Cu/Zn-containing isoform of superoxide dismutase is reduced by treatment of mice with 3,3′,4,4′,5-PeCB (PCB 126) (Fig. 2). In support of the general concept that the AhR plays an important role in dioxin toxicity, the change in the function of glutathione peroxidase and superoxide dismutase is partially dependent on AhR; thus, the TCDD-mediated reduction is specific in AhR-responsive mice but not in less responsive mice (Fig. 2, and data reported previously [18,19]). The involvement of AhR in the change in superoxide dismutase has also been demonstrated by other investigators [30,31]. Thus, it is likely that at least some forms of dioxin toxicity are caused by a change in ROS quenching systems. However, it is unlikely that we can explain all forms of dioxin toxicity by a change in ROS quenchers. For example, although the guinea pig is well-known to be the most sensitive animal to acute dioxin toxicity, glutathione peroxidase in this species is not reduced by coplanar PCB administration [19]. With regard to catalase, another important ROS quencher, dioxins have some effect on the expression of this enzyme in mouse liver (Fig. 3). However, the change is not great, and it seems to be independent of AhR (Fig. 3). Catalase activity has been shown to be unaffected or elevated by coplanar PCBs in guinea pigs [32]. In AhR-deficient mice, mitochondrial production of hydrogen peroxide is one-fifth of that seen in wild-type mice, and this ROS is not elevated in AhR-KO mice even after TCDD administration [33]. This observation clearly shows that ROS production in mitochondria, a putative major source of ROS, depends on AhR function. This paper describes that the lack of a TCDD-induced increase in hydrogen peroxide in AhR-deficient mice is not due to a change in superoxide dismutase and glutathione peroxidase.

Chronic exposure of mice to low levels of TCDD is known to cause significant production of ROS (0.45 ng TCDD/kg/day) or to reduce the level of glutathione (<0.15 ng/kg/day) in tissues [34,35]. In addition, antioxidants abolish the tumor-promoting effect caused by a low concentration (1.5 pM) of TCDD, which was estimated using malignant transformation of mouse fibroblasts as the index [36]. It is therefore likely that low levels of dioxins produce oxidative stress in the animal body, and antioxidants are effective in reducing this. Shimizu et al. [37] reported that the content of nitric oxide (NO) in serum that was obtained in 1999 from Yusho patients tended to be higher compared with controls. This seems to be inconsistent with the observation that TCDD downregulates NO synthase in the brain of rats

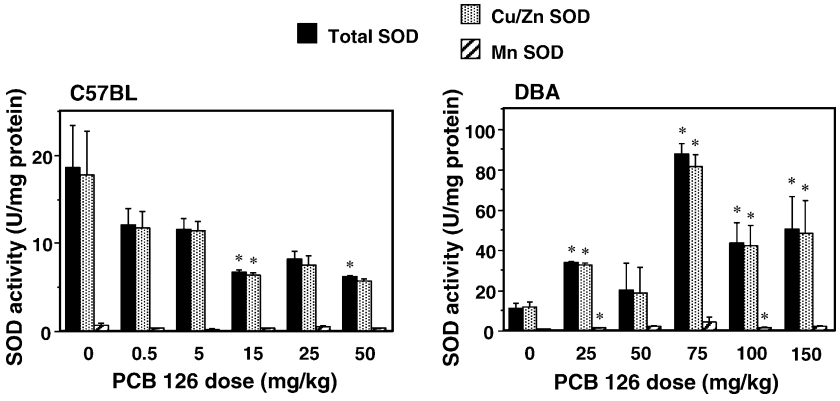

Fig. 2 Change in hepatic superoxide dismutase (SOD) activity in mice following administration of 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126). Five-week-old C57BL/6j and DBA/2N mice were treated once with PCB 126 at the doses indicated. A corn oil solution of PCB 126 (5 ml/kg body weight) was given to mice. The liver was removed 5 days after treatment, and homogenized in 50 mM Tris–HCl (pH 7.4) containing 1.15% KCl. The homogenate was stored at −80 °C before assay of SOD activity. Each bar represents the mean ± S.E. of four or five mice. $^{*}P < 0.05$ compared with the control.

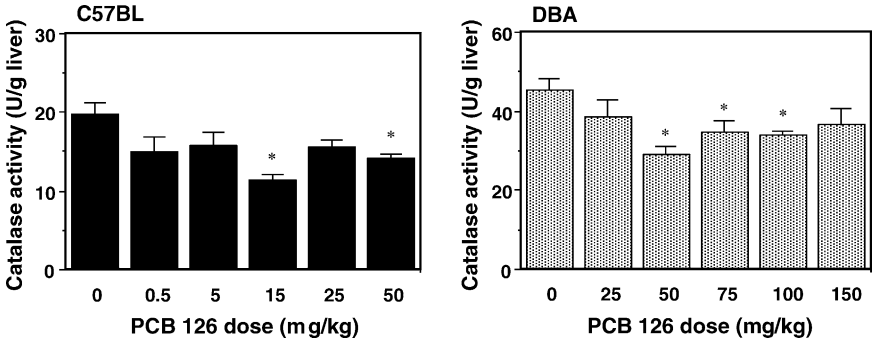

Fig. 3 Change in hepatic catalase activity following treatment of mice with 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126). See the legend to Fig. 2 for details of the animal treatment. Each bar represents the mean ± S.E. of four or five mice. $^{*}P < 0.05$ compared with the control.

[38]. The reason for this inconsistency is unknown, because of a lack of available information about the effect of dioxins on tissue NO. Further studies are needed to help our understanding of the role of NO in dioxin toxicity.

It is of great interest whether antioxidants reduce the reproductive toxicity exhibited by dioxins. TCDD reduces ROS quenching with a concomitant increase in ROS in the gonadal tissues of the rat [39,40]. Maternal exposure to TCDD produces an increase in oxidative stress in rat fetuses [41]. Furthermore, when male adult rats are chronically exposed to TCDD, an increase in hydrogen peroxide production and a reduction in catalase and glutathione peroxidase in their sperm are observed [39]. From these data, it is likely that oxidative stress by dioxins is one of the possible mechanisms explaining their reproductive toxicity. Although tocopherol has been reported to have little effect on the TCDD-induced atrophy of reproductive organs and on the reduced production of sperm in male adult rats [39,40], this vitamin reduces fetal toxicity induced by in utero exposure to TCDD. Thus, tocopherol combats the fetal growth retardation and death by TCDD, although it fails to prevent the formation of cleft palate and hydronephrosis [41]. Protective effects of tocopherol and Vitamin A have been observed in chicken embryos administered with TCDD [42]. Similarly to tocopherol, Vitamin A cannot attenuate the teratogenicity of dioxin in mice and actually increases its frequency [43]. Thus, it is expected that combating oxidative stresses is a useful method for preventing the occurrence of reproductive injuries as well as acute damage by dioxins.

3. A brief survey of heat-shock proteins

In general, it is well known that various exogenous factors, such as high or low temperature, radiation, infection, inflammation, heavy metals and alcohol, adversely affect all forms of animal life including humans. These stress factors are believed to cause toxicity by unfolding functional cellular proteins. To combat this, cells produce stress-responsive proteins. The heat-shock proteins (HSPs) have been studied as one of the typical stress-responsive proteins which play a role in protecting cells from a variety of stresses. This protein was discovered as a high-temperature-inducible protein in *Drosophila melanogaster* in the 1970s. Tissiéres et al. [44] showed that exposure of the insect to heat shock of 37.5 °C for 20 min leads to the rapid appearance of six rather abundant and a few less abundant proteins that seem to cause the activation of chromosome puffs. In the year after this finding, it was suggested that the heat-shock-inducible mRNA, accumulated in the puffs located at subdivision 87B on the right arm of the third chromosome, coded the 70 kDa protein [45]. Subsequently, it was reported that the 70 kDa HSP family (called the HSP70 family) is required for folding, assembly and translocation of newly synthesized proteins [46]. The HSPs are now classified as one of the chaperone proteins due to their biological functions in the quality control of de novo synthesized cellular proteins [47]. The chaperone proteins including HSPs have been shown to be protective proteins the production of which is increased in response to a variety of stresses [48]. In mammals, chaperone proteins contain several members, such as HSP60, HSP70, HSP90 and TriC, according to their molecular weights and biological functions [49]. In general, although the expression of HSPs is very low under normal physiological conditions [50], the production of these proteins is induced by various stresses including xenochemicals [51]. The biological function, classification and character of chaperone proteins including HSPs have been reviewed elsewhere in more detail (e.g. [49,52]).

Among the various HSP isoforms, the HSP70 family has been assigned a critical role in cellular response to acute stress, which is abundant and highly conserved in eukaryotic cells. This family is encoded in at least seven different mammalian genes. In the mouse, heat shock cognate protein (Hsc) 70 [53], participating in the regulation of Hsps and two Hsp70 members, glucose-regulated proteins (Grp) 75 [54] and Grp78 [55], are thought to be ubiquitously expressed in the different cell types. In contrast, spermatocyte-specific Hsp70.2 [56,57] and testis-specific Hsc70t [58,59] are expressed during the meiotic and postmeiotic phases, respectively. Furthermore, most cells express two intronless genes, HSP70.1 and HSP70.3, in response to acute stress stimuli [60]. They are considered to play an important part in quenching acute damage to cells. It is suggested that the binding of HSP70 to stress-damaged proteins reduces the availability of HSP70, thereby inducing the expression of new HSP70 [61]. The binding of HSP70 to damaged proteins is believed to assist in preventing their aggregation and promoting correct refolding ('molecular chaperoning') as well as facilitating their degradation [62].

4. The role of chaperone proteins in dioxin toxicity

To date, numerous studies have been performed that aimed to clarify the relationship between the expression of HSPs and toxicity caused by exogenous factors, such as heavy metals, xenobiotic organic or inorganic compounds. However, little is known about the role of HSPs in the adverse effects produced by dioxins. To address this issue, we examined whether the expression of cellular HSPs is changed by dioxins. Our results showed that HSP70 as well as HSP90 are induced in the hepatic cytosol of rats treated with PCB 126 [63]. Hepatic Hsp70 was also found to be one of the TCDD-inducible genes in mice [64]. In contrast, our studies indicated that the expression of rat microsomal GRP78 and GRP94 is markedly reduced by PCB 126 administration [65,66]. The reduction in GRP78 was suggested not to be due to the reduced transcription of this gene because its mRNA remained unchanged following administration of PCB 126 [66]. GRP78 and GRP94 have a role in maintaining the quality of proteins in the endoplasmic reticulum [67,68]. In agreement with this, the hepatic microsomal content of albumin, which is processed by chaperones in the endoplasmic reticulum before secretion, was also reduced by administration of PCB [66]. It is therefore conceivable that the reduction of GRPs seems to be one of the mechanisms of dioxin toxicity. In contrast, we observed that the Hsp70 inducers curcumin [69] and geranylgeranylacetone [70] reduce some forms of TCDD toxicity such as the reduction in body weight gain and lethality. Although the mechanism underlying the protective effects of the above agents remains unclear, these studies lead to the hypothesis that the induction of HSP70 by dioxin is a defensive response to protect cells from dioxin damage. If this were the case, it would be expected that inducer-assisted reinforcement of HSP70 would reduce dioxin toxicity. As mentioned previously, adverse effects produced by TCDD are caused by mechanisms involving the AhR-dependent pathway. In the absence of activation by ligands, AhR exists as a complex(es) with HSP90 [71], cochaperone p23 [72] and an immunophilin-like protein called the hepatic B virus protein X associated protein 2 (XAP2) [73,74] or AhR activated 9 (ARA9) [75]. It has been suggested that HSP90 regulates AhR function, such as ligand binding and location to the nucleus [76,77]. Subcellular localization, signal transduction and functional activation of AhR are also mediated through processes involving p23 cochaperone [72] and XAP2 [74,75]. XAP2 has a role in the protection of AhR from degradation by proteasome, which requires ubiquitination of the target proteins [74,78]. Because the ubiquitination of AhR requires ubiquitin ligase bound to HSP70, it is likely that this chaperone is one of the members acting as an AhR downregulator [78]. This seems to be consistent with the observation that HSP70-inducers reduce dioxin toxicity [69,70]. However, it should be noted that HSP70-inducers exhibit their protective effect without affecting gene regulation in the liver by AhR [69,70]. Thus, the effect of HSP70-inducers cannot be simply attributable to the reduction in hepatic AhR content. In contrast to HSP70, HSP90 is suggested to have a role as a stabilizer and cytosolic-retention factor for AhR [74,75,78]. From recent studies using KO mice, it is suggested that the expression of chaperone proteins is regulated by other chaperones. For example, Park et al. [79] reported a reduction in the expressions of Hsp60 and Hsp90 in Hsp70 KO mice compared with wild-type mice. This observation suggests that there is cross-talk of chaperones in their expression as well as function. The toxic mechanisms produced by dioxins are still unclear because of their complexity. However, the hypothesis that HSPs, especially HSP70, protect cells from dioxin toxicity warrants further study to provide new insights into the development of therapeutic and preventive measures to combat dioxin toxicity.

Acknowledgement

This work was supported in part by a Grant-in-Aid for scientific research from the Ministry of Health, Labour and Welfare, Japan.

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