Int Arch Occup Environ Health (2014) 87:125–135
DOI 10.1007/s00420-012-0841-x

ORIGINAL ARTICLE

Accidental exposure to polychlorinated biphenyls (PCB) in waste cargo after heavy seas. Global waste transport as a source of PCB exposure

Lygia Therese Budnik · Ralf Wegner · Ulrich Rogall · Xaver Baur

Received: 14 May 2012 / Accepted: 13 December 2012 / Published online: 6 January 2013

Abstract
Objectives After cargo with PCB-containing transformer oil waste was damaged in heavy seas, the vessel crew exposed to PCB developed itching and acne-form eruption of the skin. The objective of our study was to analyse this work-related incident and its effects on health.
Methods Air and wipe test samples were taken in the ship for analysis of PCB (28/52/101/138/153/180); clinical investigations of all seafarers ($n = 6$) included lung function, chest X-ray, clinical chemistry and biomonitoring (plasma PCBs, chlorophenols in urine) measured after a latency of 7 weeks. The biomonitoring data were adjusted according to age-related reference values and validated against controls ($n = 96$).
Results \Biomonitoring showed elevated PCB-28-/52/-102/-138 congeners (mean 1.16/0.91/136, $\sum$PCB: 5.82 µg/l), which correlates with the dust samples from the cargo hold ($\sum$PCB. 9,440 mg/m^2) and with 6.1 and 5.0 µg/m^3 in stern and bow cargo air samples. IgE elevation in two seafarers and substantial blood sedimentation rate increase with anaemia or pulmonary emphysema were unlikely to be caused by PCB exposure. Although two members showed slightly elevated airway resistance values, other lung function parameters were normal and reactive airways dysfunction syndrome due to PCBs could be excluded. Elevated chlorophenols in urine could contribute to the manifestation of chloracne.
Conclusions PCB-52/-101/-138 found in plasma and in air samples confirm exposure to PCB. Acne-form skin eruptions were from occupational exposure to polychlorinated biphenyls in the spilt transformer oil. There were no other abnormal findings in medical and clinical examinations that could be attributed to PCBs. This does not exclude possible long-term effects.

Keywords Polychlorinated biphenyls (PCB) · Seafarers · Chlorophenols · Accidental exposure · Clinical and laboratory diagnosis · RADS (reactive airways dysfunction syndrome)

Abbreviations

PCB	Polychlorinated biphenyls
CP	Chlorophenols
PCP	Pentachlorophenol
Chest X-ray	Chest radiograph
CDT	Carbohydrate-deficient transferring
FEV_1/FVC %	Forced expiratory volume in 1 s/inspiratory vital capacity

Introduction

Chlorinated aromatic hydrocarbon compounds such as polychlorinated biphenyls (PCBs) used to be frequently

L. T. Budnik · R. Wegner · X. Baur
Institute for Occupational and Maritime Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

L. T. Budnik (✉)
Institute for Occupational and Maritime Medicine (ZfAM), Division of Occupational Toxicology and Immunology, Department of Occupational Health, University of Hamburg, School of Medicine, Marckmannstrasse 129 b, Bld. 3, 20539 Hamburg, Germany
e-mail: LBudnik@uke.uni-hamburg.de

U. Rogall
Consultant in Occupational Medicine, Hamburg, Germany

X. Baur
Charité Institute for Occupational Medicine, School of Medicine, Charité - Universitätsmedizin, Berlin, Germany

applied in hydraulic fluids, paints, inks, adhesives, paper products, building sealants (Robson et al. 2010), electrical and hydraulic liquids, and used as cooling and insulating agents in electrical transformers (NIOSH 2006; Prince et al. 2006; Ruder et al. 2006). Aroclors (Arochlors) are industrial multi-component mixtures, containing approximately 20–70 % of PCB mixture with various levels of chlorine substituent, that is Aroclor 1254 contains 12 carbon atoms and 54 % chlorine by mass, with 17–392 μg PCB–TEQ/g Aroclor (TEQ, dioxin-like toxic equivalents) (Rushneck et al. 2004; UBA 2007). Poisoning of humans was reported for the first time in 1968 after the consumption of rice oil prepared from PCB-containing heat exchanger oil (Onozuka et al. 2009), which produced extensive and widespread acne-form skin eruptions, with cysts or comedones in face and auricles, retro-auricular, axillary spaces and the trunk. The PCB concentrations in the blood of these "Yusho" patients were a factor of 3.6 above those of a control group (Onozuka et al. 2009). These poisoning of 14,000 people resulted from PCB-contaminated rice bran oil used as chicken feed. Guo et al. (1999) reported additional occurrence of joint and spine complaints, skin alterations, anaemia and pathological thyroid enlargement of the exposed people 15 years after mass intoxication in Taiwan (Yucheng cohort). Serious outbreaks of poisoning in humans and domestic animals from the ingestion of food contaminated with PCB resulted in legislation for biomonitoring of contamination in humans and the subsequent banning of PCB production and restricting its use in Europe and North America in the 1980s (Diamond et al. 2010; NIOSH 2006). PCBs belong to the group of 'Persistence Organic Pollutants (POPs)', which were classified as particularly dangerous industrial chemicals by the Environmental Protection Authority of the United Nations.

In modern times, occupational exposure to PCBs is largely restricted to construction site workers removing building seals (Robson et al. 2010; Herrick et al. 2007; Selden et al. 2008) or opening hydraulic or heat transformer systems, with some residual contamination in mining, recycling and the burning of waste (Breivik et al. 2011). In 2010, workers from a German transformer recycling company and their families (as well as all individuals working close by) were reported to display accidentally elevated PCB levels (Schettgen et al. 2012a, b). There is also evidence that unknown amounts of industrial organic contaminants exported outside former regions of use as well as their re-import provide new opportunities for environmental spillage and occupational exposure, especially with the increases in transport of PCB-containing industrial waste (Fig. 1).

PCBs are well absorbed through lungs, gastrointestinal tract and skin. Earlier studies with workers from the electrical industry reported the high prevalence of comedones and acne as well as cases of pathological liver changes with abnormal values for liver-sensitive enzymes (Maroni et al. 1981; Zhang et al. 2012). Smith et al. (1982) also reported elevated GOT and GPT values in 321 workers exposed to PCBs in the electrical industry.

Polychlorinated biphenyls have an irritating effect on mucous membranes with the potential for lesions in the airways (Reggiani and Bruppacher 1985). Although not of primary importance for disease, the lung can also be one of the target organs (Rallis et al. 2012; Recio-Vega et al. 2012). Obstructive ventilation patterns were described by Lawton et al. (1986), and neurological changes after substantial or long-term chronic exposure to PCBs have been reported (Altenkirch et al. 1996). Both animal experiments and human studies indicate endocrinological effects of chlorinated biphenyls (Leijs et al. 2009; Garner et al. 1999; Brouwer et al. 1999; Hany et al. 1999; Weisglas-Kuperus et al. 2004). The outcome of environmental or occupational exposure to polychlorinated biphenyls continues to be controversial, and some questions remain unanswered. Medical information from accidental poisoning, which can provide much-needed information, is rarely published. The description of the outcome (Table 1) of accidental PCB exposure, on a ship transporting PCB-containing waste, may help to resolve some of these questions.

Methods

Ambient monitoring

Seven weeks after the incident, air and dust samples were taken from the tween deck of the stern and bow cargo holds of the vessel upon arrival in the harbour of Hamburg. The ambient monitoring measurements were conducted after cleaning and sand blasting (Table 1). The measurements of polychlorinated biphenyls in ambient air and the wipe tests were performed by certified commercial laboratories (Fresenius laboratories, Frankfurt, Germany and Dr. Wiertz Eggert, and Dr. Jörissen, Fresenius laboratories, Hamburg) using extraction techniques and gas–liquid chromatography analysis confirmed by mass fragmentography. Air measurements from the tween deck of the stern and bow cargo holds were performed for 2 h each and were conducted on the same day, 7 weeks after the incident. The stern cargo hold was empty, and the hatch remained closed. The total PCB in the air was calculated as the sum of six indicator compounds ($\sum$congeners 28, 52, 101, 138, 153, 180) multiplied by a factor of 5.

Fig. 1 Potential new occupational health hazard: global transport of industrial waste: containers in European harbours upon inspection. The picture shows similar cargo type and damage described herewith to illustrate a typical workplace situation

Table 1 Case description

Accidental occurrence
A small coaster of 400 twenty–foot equivalent, manned by 6 seafarers, took 19 containers of transformers on board in the Thai port of Bangkok. Two transformers in one of these containers still contained transformer oil (Aroclor 1254) to be disposed in Europe; the ship was in a heavy storm, which badly damaged the cargo. One of the two transformers detached from its restraints and leaked. About 400 l of transformer oil containing Aroclor 1254 flowed into the cargo hold, leading to continuous inhalative exposure by those on board. During a recovery operation and after arriving in the next harbour, the crew received significant skin exposure to the transformer oil. After the vessel arrived at an Arab port, more cargo was taken on board and the defective transformer was newly fixed within the container. After the coaster reached its destination port in Europe, 7 weeks after leaving Bangkok, a thorough cleaning of the hatches followed, and the walls of the vessel were also sandblasted
Exposure-associated job description
Exposed subjects
With the exception of the cook, the crew performed a recovery operation in the cargo hold for 30 min on the day of the incident (A, B, D, E, F). They *did not wear protective clothes*. During these activities, the crew members *inhaled and were percutaneously exposed to Aroclor 1254 to a great extent.*
For degassing, the hatches of the *cargo hold were permanently opened at outside temperatures of* up to 40 °C for nearly 2 weeks, 1 week after the leakage. Due to the high temperatures, *the crew members slept on deck during this period*. Thus, they were exposed to Aroclor 1254 by inhalation for a longer time. During this time, all crew members developed itchy papule vesicles all over the body. On arriving in the next harbour, treatment with fucidine (sodium fusidate topical) was started and the symptoms receded. When the vessel arrived at an Arab port, the crew performed further recovery operations in the cargo hold, this time *using breathing masks and protective suits*
Exposure-associated symptoms: all subjects developed slightly itchy papule vesicles all over the body (A, B, C, D, E, F). Treatment with the chemotherapeutic agent fucidine reduced the symptoms

Subjects

The examination of the whole crew was performed 8 weeks after the accident upon arrival in the harbour of Hamburg. All seafarers from the ship—six men (aged 25–50 years), two from northern Europe (A + B) and four from southern Asia (C–F)—took part in the investigation (all gave written permission). They underwent medical examination and donated blood and urine.

Control reference subjects ($n = 96$) were men (aged 47 ± 10 years) from northern Germany, working in administrative jobs, not exposed to PCB.

Lung function testing and chest X-rays (CXR)

Spirometry and body plethysmography were performed with a Jaeger Masterlab (Jaeger, Würzburg, Germany). The variables assessed were as follows: specific airway resistance (sRt), airway resistance (Rt), inspiratory vital capacity (IVC), residual volume as % of total lung capacity (RV/TLC%) and forced expiratory volume in 1 s as % of inspiratory vital capacity (FEV_1/IVC%). Diffusing capacity for CO ($T_{L,CO,SB}$) was measured by the single breath method (Compact transfer, Jaeger, Würzburg, Germany). According to the European Respiratory Society recommendations, the best values were taken from at least three acceptable and reproducible manoeuvres (IVC, FEV_1) and compared with reference values (Quanjer et al. 2012). For airway resistance and TGV (thoracic gas volume), the median of at least three measurements was chosen. Routine posterior–anterior chest radiographs were obtained and evaluated by an expert radiologist.

Clinical laboratory parameters

Considering the variety of health risks, the seafarers exposed to PCB underwent an examination programme with extensive, differentiated diagnostic aspects. Tests for hepatitis infections or alcohol abuse (CDT) were performed. The intention was to differentiate between non-occupational infections or nutritional liver diseases and potential liver disorders due to PCB exposure.

Blood sedimentation rate was performed according to the Westergren method, and total IgE serum levels were measured (ImmunoCAP, Phadia, Freiburg, Germany), and urine stick analyses (Combur^9-Test, Roche Diagnostics, Mannheim, Germany) and flow cytometry were performed. Flow cytometry was performed on a FACSCalibur flow cytometer (Becton–Dickinson, Heidelberg, Germany): Two-colour direct immunofluorescence surface marker analysis was performed using fluorescein isothiocyanate (FITC) and phycoerythrin (PE) conjunctivated monoclonal anti-human antibodies, respectively. The lymphocyte subpopulations were measured by the use of the following pairs of antibodies: CD3/CD19; CD3/CD4; CD3/CD8; CD16/CD56 (Becton–Dickinson). The samples were stained for two-colour immunofluorescence as described in the manufacturers' protocol. Briefly, 100 µl of blood sample was incubated with each set of monoclonal antibodies for 20 min in the dark. All antibodies were used at optimal dilutions, and, after incubation, 1.9 ml of PBS (phosphate-buffered saline) was added before evaluating the results.

The following analyses were performed by a commercial laboratory: leucocytes, haemoglobin, erythrocytes, haematocrit, MCH, MCHC, thrombocytes, differential hemogram, GOT (AST), GPT (ALT), γ-GT, total bilirubin, alkaline phosphatase, cholinesterase, α-amylase, glucose, cholesterol, HDL cholesterol, LDL cholesterol, triglyceride, uric acid, urea, total protein, serum electrophoresis (albumin, $alpha_1$-globulin, α_2-globulin, β-globulin, γ-globulin), immunoglobulins (IgA, IgG, IgM), thyroid hormones (T3, T4), TSH; carbohydrate-deficient transferrin (CDT), hepatitis serology: hepatitis A (IgG and IgM antibodies), hepatitis B (antigen and antibodies, IgM antibodies), hepatitis C (antigen and antibodies).

Biomonitoring of polychlorinated biphenyls (PCB) and chlorophenols (CP)

PCB in plasma was measured as follows: One millilitre of plasma was treated with 1 ml HNO_3 (30 %) for 30 s and extracted with n-hexane/toluene (1:1) for 20 min. The organic phase was washed with 1 ml of K_2CO_3 (5 %), and 1 ml of organic phase was evaporated by gaseous nitrogen to the volume of 100 µl with 100 µl n-decane as keeper. Two microlitres of residues (splitless; auto sampler) was separated gas chromatographically on a HP 5 MS column (by Hewlett Packard; internal diameter 0.25 mm; film thickness 0.25 µm; temperature programme 2 min 100 °C, rate 7.5 °C/min to 295 °C, 3 min 295 °C isotherm). PCB 28/52/101/138/153/180 were quantified at retention times of 14.9, 15.8, 18.0, 20.8, 20.1, 22.3 min using the fragment ion of 256, 292, 326, 360, 360, 394 (m/z) respectively after electron impact ionisation at 70 keV (mass selective detector). Calibration was performed with PCB standards that were treated identically to the plasma samples. Linearity of the calibration line was observed in the range of 0.25–30 µg/l. Detection limit was estimated to 0.10–0.15 µg/l plasma by a signal to noise ratio of 3:1. Accuracy was established by certification for PCB in plasma within the runs of the German External Quality Assessment Scheme (G-EQUAS). Creatinine in urine was measured with HPLC method. As reference, newly established German biological workplace reference values (BAR) (Hartwig 2012) were applied (Table 2); if not

Table 2 Ambient and biological monitoring for polychlorinated biphenyls and chlorophenols

	28	52	101	138	153	180	∑	Total	Occupational limit values (μg/m³)
(a) Ambient monitoring PCB congeners									
Stern cargo air (μg/m³)	0.057	0.700	0.350	0.065	0.035	0.005	1.212	6.1	3.0 (MAK)
Bow cargo air (μg/m³)	0.047	0.600	0.270	0.050	0.025	0.03	0.995	5.0	3.0 (MAK)
Wipe test (mg/m²)	40	700	2,200	2,500	2,300	1,700	9,440		

	A	B	C	D	E	F	Mean values	Control reference group ($n = 96$)	Reference values (μg/l)
(b) PCB in plasma (μg/l)									
Study subjects (A–F)									
PCB 28	**0.16**	**0.18**	**0.16**	**0.16**	**0.21**	**0.17**	**0.17**	<0.1	**0.02** (BAR)
PCB 52	**0.88**	**1.38**	**1.00**	**1.04**	**1.74**	**0.89**	**1.16**	<0.1	**<0.01** (BAR)
PCB 101	**0.47**	**1.51**	**0.45**	**0.46**	**1.92**	**0.64**	**0.91**	<0.1	**<0.01** (BAR)
PCB 138	1.41	**2.46**	0.60	0.69	2.43	0.58	1.36	1.01–2.23	**0.6–1.7*** (UBA reference)
PCB 153	1.57	**1.98**	0.74	0.59	2.12	0.52	1.25	1.26–3.27	**0.9–2.1*** (UBA reference)
PCB 180	1.98	**1.03**	0.53	0.41	1.53	0.31	0.97	0.88–2.16	**0.6–2.1*** (UBA reference)
Σ PCB	6.47	**8.54**	3.48	3.35	**9.95**	3.11	5.82	**3.30–7.81**	

	A	B	C	D	E	F	Mean values	Control reference group ($n = 96$)	Occupational limit values (μg/l)	Reference values (μg/l)
(c) Chlorophenols in urine (μg/l)										
study subjects (A–F)										
2,6-DiCP	<LOD.	<LOD	<LOD	<LOD	<LOD	<LOD	<0.2	**<0.2**		
3,5-DiCP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<0.2	**<0.2**		
2,5-DiCP	1.2	5.1	1.4	2.4	2.6	4.9	2.93	6.2		**6.0** (UBA reference)
2,4/3,4-DiCP	0.3	0.6	0.7	<LOD	<LOD	1.1	0.48	**1.5**		
2,3-DiCP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<0.2	**<0.2**		
2,4,6-TriCP	0.4	1.0	1.2	0.6	0.4	1.5	0.85	**1.8**		
2,3,6-TriCP	1.3	1.5	0.6	0.7	0.7	1.1	0.98	**<0.3**		
2,4,5-TriCP	20.9	**30.9**	13.7	9.7	9.7	**25.4**	18.4	**Σ 24.5**		1.0 (UBA reference)
2,3,5-TriCP	1.8	10.0	4.0	3.6	1.7	7.3	4.73	**0.6**		
3,4,5-TriCP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<0.3	**<0.3**		
2,3,4-TriCP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<0.3	0.3		**<0.3** (UBA reference)
2,3,4,6/-TetraCP	**1.7**	**1.9**	**1.2**	**0.9**	**0.7**	**1.4**	**1.3**	1.3		**<0.3** (UBA reference)
2,3,4,5-TetraCP	**2.2**	**6.4**	**1.5**	**1.0**	**1.1**	**2.0**	**2.37**	**<0.5**		

Table 2 continued

	A	B	C	D	E	F	Mean values	Control reference group ($n = 96$)	Occupational limit values (µg/l)	Reference values (µg/l)
PCP	**20.3**	**10.8**	1.5	**6.2**	1.0	1.9	**6.95**	5.3	6 µg/l is equiv. to 1 µg/m^3 air (EKA)	**2** (reference) 25 (HBM I) 40 (HBM II)

[a] Ambient monitoring in air and wipe tests for PCB content in ship cargo areas (µg/m^3)

[b] Biological monitoring for PCB congeners: 28, 52, 102, 138, 153, 180 in plasma (µg/l)

[c] Biological monitoring for chlorophenols in urine (µg/l)

LOD < Limit of detection, *MAK* maximal workplace concentrations, *BAR* biological workplace reference values, *EKA* exposure equivalent for carcinogenic substances (Hartwig 2012) *Age-dependent values (the lower value refers to the youngest crew member with 25 year, the higher to the oldest with 50 year). Bold typing shows values above the respective reference values (age adjusted) and the reference used

available, German reference levels for the general populations (UBA 2012) or alternative (if no reference values were available) mean values of the control reference group were used.

Urinary excretion of chlorophenols was analysed with a sensitive gas chromatography–mass spectrometry method according to the validated methods from the German Research Council, DFG methods (DFG 2012). Briefly after hydrolysis, clean-up by steam distillation, extraction steps, enrichment and derivatization (limits of detection 0.2–0.5 µg/l). The steam eluate was concentrated with reverse-phase column (RP-18). The chlorophenols plus internal standards (see below) underwent derivatisation with diazomethane, and the samples were separated with gas chromatographically on DB-5 ms 30 m column (with 0.5 film thickness as internal standards 13C-2,4-dichlorophenol and 13C-2,4,6 trichlorophenols were used). For the analysis of pentachlorophenols, the samples that underwent acid hydrolysis (DFG 2012) were extracted with *n*-hexane and separated with gas chromatographically DG-5 ms 30 m column, and for the detection quadruples, MSD was applied.

The results were compared with the data of 96 men (aged 47 ± 10 years) from northern Germany, working in administrative jobs, not exposed to PCB (control reference group). The samples from the control reference group were measured in parallel with the exposed subjects.

Results

Clinical and occupational case histories

Details of the exposure and working conditions are presented in the Table 1. The crew members were heavily exposed to PCB-containing vapour by inhalation and percutaneous from the spilled transformer oil at outside temperatures of up to 40 °C. All developed acne-form non-infectious skin eruptions with multiple itching papulovesicles. Topical treatment with the agent fucidine (sodium fusidate topical) reduced the symptoms.

Exposure assessment: ambient and biomonitoring

Air samples and wipe tests could only be taken in the empty cargo area after extensive cleaning activities. The air and wipe samples taken 7 weeks after the accident demonstrated PCB $\sum$ concentrations of 0.995 and 1.212 µg/m^3 in stern and bow areas, respectively (with total PCB concentration of 6.1 and 5.0 µg/m^3 in stern and bow cargo air). The wipe surface values were from 13.8 to 9,440 mg/m^2 ($n = 8$, mean 2,000 ± 3,113 ± SD, median 1,053 mg/m^2). The highest concentration was found at the hatch cover under the transformer. More results are given in Table 2a.

Biomonitoring of PCB and chlorophenols: Samples for all crew members exceeded the ranges of normal for PCB congeners 28 and especially 52 and 101 (Table 2b). Based on our previous experience with patients exposed to similar waste cargo in the harbour areas and industrial waste facilities, we had decided to perform additional biological monitoring for possible incorporation of chlorophenols (Table 2c).

Analysis for chlorophenols: Chlorophenol screening was performed in urine samples from both patients and the reference group. We found that, as expected, crew members had enhanced secretions of trichlorophenols, tetrachlorophenols and pentachlorophenols in urine, when compared with a control reference group (Table 2c).

Table 3 Personal data and the results of lung function testing

Subject	A	B	C	D	E	F
Age (year)	50	25	43	35	33	41
BMI	17	24	**34**	23	23	23
Smoking	yes	yes	ex	ex	no	ex
Rt (kPa*s/l)	0.15	0.23	**0.38**[b]	**0.34**[b]	0.24	**0.34**[b]
sRt (kPa*s)	0.92	0.63	1.17	1.04	0.66	0.91
FVC (% mean predicted[a])	**64**[b]	104	85	81	**76**[b]	81
RV/TLC (%)	**52**[b]	18	**29**[b]	**29**[b]	**30**[b]	22
FEV_1/FVC (%)	77	83	75	87	83	78
$D_{L,CO}$ SB (mmol/min/kPa)	5.9	10.4	8.9	11.5	7.9	9.0

[a] Reference values: (Quanjer et al. 2012), [b] Abnormal value

Rt airway resistance airways, *sRt* specific airway resistance, *IVC* inspiratory vital capacity, *RV* residual volume, *TLC* total lung capacity, FEV_1 forced expiratory volume in 1 s, $T_{L,CO}$ *SB* lung diffusing capacity for CO, single breath method

Clinical examinations eight weeks after the incident

Acne-form alterations persisted in two patients (B, F) with one of them showing single comedones (F). One of these seafarers had a mild eczema on the palms of the hand (B) and the other had slightly squamous palms (D). None of the examined seafarers demonstrated severe pathological skin alterations, generalised swollen lymph nodes and thyroid gland increase or organic changes in thorax,

Table 4 Results of clinical laboratory parameters

Study subjects	A	B	C	D	E	F	Normal range
Haematological findings							
Leucocytes (/nl)	8.8	7.9	**11.8**	7.5	6.3	7.5	4–10
Erythrocytes (Mio/µl)	4.8	4.7	5.0	5.9	4.9	4.4	4.7–6.1
Haemoglobin (g/dl)	14.5	14.6	15.0	17.2	15.0	13.9	14–18
Lymphocytes (/nl)	1.9	1.9	3.0	1.8	1.9	2.0	1.0–4.8
T-lymphocytes CD3+ (%)	75	75	59	70	64	71	59–85
B-Lymphocytes CD19+ (%)	17	8	**27**	10	13	17	6–23
Helper T cells CD3+/CD4+ (%)	**64**	42	42	36	31	47	29–61
Suppressor T cells CD3+/CD8+ (%)	10	30	19	30	33	23	11–38
Natural killer cells CD16+/CD56+ (%)	7	15	14	19	24	10	6–31
Clinical chemistry (serum)							
GOT (U/l)	8	14	13	12	12	10	<19
GPT (U/l)	6	21	18	**26**	**24**	12	<23
γ-GT (U/l)	9	**31**	25	**30**	16	15	<29
Phosphatase alcaline (U/l)	91	88	126	126	89	109	<190
Glucose (mg/dl)	100	79	92	61	99	102	60–100
Cholesterol (mg/dl)	168	**213**	171	**211**	**211**	168	<200
HDL cholesterol (mg/dl)	54	61	44	47	67	35	>35
LDL cholesterol (mg/dl)	93	137	102	141	89	113	<150
Triglyceride (mg/dl)	103	75	124	116	**275**	192	<200
Creatinine (mg/dl)	1.1	1.2	1.2	1.1	0.9	1.1	0.5–1.2
Immunoglobulins							
IgA (mg/dl)	194	202	**403**	294	264	**822**	70–400
IgG (mg/dl)	1,081	1,149	1,498	1,793	1,030	1,506	700–1,600
IgM (mg/dl)	103	86	85	126	92	158	40–230
IgE (mg/dl)	30	14	50	**299**	**195**	8	<100

abdomen or nervous systems. Blood pressure was normal with the exception of crew member C (190/110 mmHg).

Lung function (Table 3) and chest X-rays (CXR): The lung function tests of seafarer C and D showed slightly elevated airway resistance values, and seafarer E revealed marginally reduced vital capacity. The chest X-ray of seafarer A indicated presumed pulmonary emphysema with elevated residual volume and reduction in vital capacity and diffusion capacity, presumably due to long-term cigarettes smoking (45 pack years). The chest X-ray findings of the other subjects were undistinguished.

Considering the variety of health risks, the seafarers exposed to PCB underwent an examination programme of extensive, differentiated diagnosis. For instance, tests on hepatitis infections and the alcohol marker CDT were performed. The intention was to differentiate between non-occupational infections or nutritional liver diseases and potential follow-up liver disorders on account of the PCB exposure.

Clinical parameters: Four (C − F) of the six seafarers had indications of a previous hepatitis B infection, and two of them (C + D) still in a contagious state. The seafarers were informed about these findings and the risk of infection. One of the seafarers (D) with active hepatitis B had increased liver-sensitive enzymes. Another one (B) without detectable hepatitis B had a mild liver function disorder, presumably from elevated alcohol consumption (CDT positive). Five of the six seafarers (B–F) showed antibodies against hepatitis A. Three of the seafarer demonstrated mild cholesterolemia (D–F), and one of them also had a distinctly elevated triglyceride level (E). One seafarer exhibited anaemia (F) with a high blood sedimentation rate (36/58 mm n. W.), an elevated uric acid level (8.5 mg/dl) and a decrease in thyroid-stimulating hormone. The latter was also informed about the findings and instructed to undergo further medical examination at home. Two subjects (D + E) demonstrated high total IgE levels as evidence of atopy or previous worm parasite infections. One seafarer (C) showed an enhanced number of leucocytes of unknown reason. He was distinctly overweight (BMI = 34) and had arterial hypertonia. All other laboratory findings (Table 4) were within normal ranges.

After a latency time of 7 weeks from the incident and the local anti-inflammatory treatment, none of the examined seafarers exhibited severe skin disorders. The significant chloracne characteristic of PCB exposure had receded and only some mild acne-form skin eruptions and single comedones persisted in two of the seafarers.

Discussion

The temporal relationship of the exposure to the leaking transformer oil, with the occurrence of acne-form skin eruptions and the detection of enhanced values of both polychlorinated biphenyls and chlorinated phenols in blood and urine samples, indicated that the seafarers incorporated these substances after the incident. The aim of the study was to evaluate whether the consequences of the PCB exposure contribute to their health status or not.

The PCB concentrations in respiratory air measured at the end of the cruise, 7 weeks after the accident (and after safety measures), were 5 or 6 $\mu g/m^3$, which are above the German threshold limit value (MAK, maximum workplace concentration of 3 $\mu g/m^3$) (Hartwig 2012). They were similar to the concentration range that Bent et al. (2000) and Gabrio et al. (2000) measured in the ambient air of schools contaminated with PCB (mean value 6–7 $\mu g/m^3$; maximum values between 1.59–10.66 $\mu g/m^3$). The PCB measurements show that total air concentration in the hatches of the vessel consisted of more than 80 % of the PCB congeners 52 and 101. The corresponding PCB congeners 52, 101 and 28 found in plasma of six patients confirm the occupational exposure to the polychlorinated biphenyls from ship cargo by the seafarers. The values exceeded not only the normal range of subjects occupationally non-exposed to PCB (control reference group) but also the German occupational reference values, BAR (Hartwig 2012). The source of additional contamination with chlorophenols could also be the transformer oil as PCB mixtures are often contaminated with other polychlorinated substances, such as polychlorinated naphthalenes, or the exposure could come from other waste cargo present on board. Small quantities of polychlorinated dibenzofurans and dioxins cannot be excluded either. A comparison of the PCB values found in the plasma with data from the literature suggests that these values are not in a range that might constitute a health risk. However, the extensive exposure to PCB took place several weeks before; therefore, the measured values do not represent immediate post-exposure values. Smoking (Deutch and Hansen 1999) was shown to be a confounder, and the frequent consumption of PCB-contaminated food (i.e., fish) (Badia-Vila et al. 2000; Consonni et al. 2012) can increase the internal PCB load (Crinnion 2011; Lazaro et al. 1999; Fitzgerald et al. 1999; Sala et al. 1999). There was no evidence for such exposure by the crew members. Accordingly, the temporary acne-form skin eruptions and liver function disorders belong to frequently observed effects of polychlorinated biphenyls. Chloracne is one of the most common occupational dermatoses caused by systemic poisoning with dioxin-like compounds or pentachlorophenol (Cheng et al. 1993; Adams et al. 2011). It is characterised histologically by an acne-like eruption of comedones. The exact mechanism by which the skin lesions characteristic of chloracne develops is still obscure; it is thought to be mediated by the binding of these compounds to the aryl hydrocarbon receptor, leading to up-

regulation of multiple genes involved in xenobiotic metabolism (Adams et al. 2011). Coenraads et al. (1999) have analysed blood levels of PCDDs and PCDFs in the chemical factory workers and concluded that the exposure levels required to cause chloracne should exceed 650 pg/g blood lipids. In our case, the exposure was lower. It should, however, be noted that the seamen were also exposed to pentachlorophenol, which might presumably have cumulative effect.

The other marked outcomes, especially IgE elevations in two seafarers and substantial blood sedimentation rate increase with anaemia or pulmonary emphysema, are unlikely to be from PCB exposure. Three seamen had slightly higher cholesterol levels (211–213 mg/d), other lipids were in a normal range, and their BMI range was normal (with exception of one seaman), indicating that body fat unlikely influenced the PCB serum levels. Some crew members showed slight increases in airway resistance, but specific airway resistance and FEV_1/IVC% were within the normal ranges. From the absence of respiratory symptoms and the lung function data, we can exclude RADS.

The occasionally observed abnormal liver changes after occupational intoxication with chlorinated compounds are in general mild and reversible functional disorders and only rarely constitute severe changes. In our study, three of the six seafarers exhibited a mild elevation of liver-sensitive enzymes, obviously at least partially due to alcohol abuse in one of them (elevated CDT levels). The other two seafarers showed evidence of previous hepatitis B infection, which would account for the enzyme elevations. It is more difficult to clarify lipidosis in three patients. The enhanced triglyceride blood level of one of them might be from their diet. The cholesterolemia also affecting these two seafarers was probably of dietary origin as they were of normal weight. Cholesterol levels were mildly enhanced (211–213).

Enhanced lipid levels in blood, especially triglyceride elevation (Hirota et al. 1993; Masuda 2001; Hsieh et al. 1996; Tokunaga et al. 1999), were described for Yusho and Yu-Cheng patients who were orally exposed to the PCB-contaminated rice. Some studies report, in addition, immunodepressive effects, mostly reductions in the number of T-lymphocytes and helper T cells (Lemesh 1992; Nakanishi et al. 1995; Hoffmann 1996). Although former Yusho patients showed enhanced immunoglobulin levels, any correlation with PCB content in blood was not discernible (Tsuji et al. 1999). Other authors have discussed possible effects on thyroid function or metabolism (Smits et al. 2002).

The World Health Organisation (WHO) has identified twelve specific congeners as dioxin-like with toxicity ranging from 0.00003 to 0.1 times the standard 2,3,7.8-tetrachlorodibenzo-p-dioxin(2,3,7,8-TCDD) toxicity. Burgin et al. (2001) (Kodavanti et al. 2001) have shown that two lots of Aroclor 1254 may have differential effects on enzyme induction, thyroid hormones, oxidative stress and neurochemical effects. Waste mixtures, such as the case described herewith, may contain various Aroclor mixtures with various TEQs, thus having presumably different pathophysiological effects. Unfortunately, we could not determine the TCDD amounts in our study. On the other hand, we could confirm our clinical observations by showing co-exposure with chlorophenols, which might contribute to the observed manifestation of chloracne. With respect to malignant tumours (PCBs are probable carcinogens, IRAC classification 2A), only long-term follow-up can provide information about residual risks. In the United States, the numbers of deaths from melanoma skin cancer and brain cancer were higher in PCB-exposed workers than expected (NIOSH 2006). The latency time for exposure to carcinogenic substances is typically 10 or more years.

In summary, the temporal connection between occupational PCB exposure and acne-form skin eruptions in all of the exposed and examined patients concurs with a causal relationship. From the latency period of 7 weeks and the appropriate immediate treatment, the major effects were ameliorated and only residual effects remained at the time of the examinations. The detection of chlorinated phenols as well as of polychlorinated biphenyls in biological material provides the evidence for the incorporation of these hazardous substances from the leaked transformer oil.

Acknowledgments We acknowledge the laboratories Fresenius, Frankfurt/Main, and Dr. Wiertz Eggert and Dr. Jörissen, Hamburg, who did the PCB monitoring in ambient air and in the wipe test samples; ZfAM colleagues for help in supporting the collection of the clinical data; Dr. Wurm for his X-ray investigations; Mrs. Johannsen for lung function testing; Mrs. Koops for flow cytometry; Mrs. Tieu for analysis of the PCBs and chlorophenols; and Dr. Kevan Willey for critical appraisal of the manuscript. The study was funded by the State Ministry for Health and Consumer Protection (XB, RW) and is a part of the WHO GPA (global plan of action) project: New chemical health risk hazards in transportation and warehousing of marine cargo because of globalisation (LTB).

Conflict of interest The authors declare that they have no conflicts of interest.

References

Adams ET, Auerbach S, Blackshear PE, Bradley A, Gruebbel MM, Little PB, Malarkey D, Maronpot R, McKay JS, Miller RA, Moore RR, Morrison JP, Nyska A, Ramot Y, Rao D, Suttie A, Wells MY, Willson GA, Elmore SA (2011) Proceedings of the 2010 national toxicology program satellite symposium. Toxicol Pathol 39(1):240–266

Altenkirch H, Stoltenburg G, Haller D, Hopmann D, Walter G (1996) Clinical data on three cases of occupationally induced PCB-intoxication. Neurotoxicology 17(3–4):639–643

Badia-Vila M, Ociepa M, Mateo R, Guitart R (2000) Comparison of residue levels of persistent organochlorine compounds in butter from Spain and from other European countries. J Environ Sci Health B 35(2):201–210

Bent S, Rachor-Ebbinghaus R, Schmidt C (2000) Decontamination of highly polychlorinated biphenyl contaminated indoor areas by complete removal of primary and secondary sources. Gesundheitswesen 62(2):86–92

Breivik K, Gioia R, Chakraborty P, Zhang G, Jones KC (2011) Are reductions in industrial organic contaminants emissions in rich countries achieved partly by export of toxic wastes? Environ Sci Technol 45(21):9154–9160

Brouwer A, Longnecker MP, Birnbaum LS, Cogliano J, Kostyniak P, Moore J, Schantz S, Winneke G (1999) Characterization of potential endocrine-related health effects at low-dose levels of exposure to PCBs. Environ Health Perspect 107(Suppl 4): 639–649

Burgin DE, Diliberto JJ, Derr-Yellin EC, Kannan N, Kodavanti PR, Birnbaum LS (2001) Differential effects of two lots of Aroclor 1254 on enzyme induction, thyroid hormones, and oxidative stress. Environ Health Perspect 109(11):1163–1168

Cheng WN, Coenraads PJ, Hao ZH, Liu GF (1993) A health survey of workers in the pentachlorophenol section of a chemical manufacturing plant. Am J Ind Med 24(1):81–92

Coenraads PJ, Olie K, Tang NJ (1999) Blood lipid concentrations of dioxins and dibenzofurans causing chloracne. Br J Dermatol 141(4):694–697

Consonni D, Sindaco R, Bertazzi PA (2012) Blood levels of dioxins, furans, dioxin-like PCBs, and TEQs in general populations: a review, 1989–2010. Environ Int. doi:10.1016/j.envint.2012.01.004

Crinnion WJ (2011) Polychlorinated biphenyls: persistent pollutants with immunological, neurological, and endocrinological consequences. Altern Med Rev 16(1):5–13

Deutch B, Hansen JC (1999) High blood levels of persistent organic pollutants are statistically correlated with smoking. Int J Circumpolar Health 58(3):214–219

DFG (2012) Analytical methods from the working group on analytical chemistry, AibM, DFG German Research Council (MAK commission) publications. http://onlinelibrarywileycom/book/101002/3527600418/topics

Diamond ML, Melymuk L, Csiszar SA, Robson M (2010) Estimation of PCB stocks, emissions, and urban fate: will our policies reduce concentrations and exposure? Environ Sci Technol 44(8):2777–2783. doi:10.1021/es9012036

Fitzgerald EF, Deres DA, Hwang SA, Bush B, Yang BZ, Tarbell A, Jacobs A (1999) Local fish consumption and serum PCB concentrations among Mohawk men at Akwesasne. Environ Res 80(2 Pt 2):S97–S103

Gabrio T, Piechotowski I, Wallenhorst T, Klett M, Cott L, Friebel P, Link B, Schwenk M (2000) PCB-blood levels in teachers, working in PCB-contaminated schools. Chemosphere 40(9–11): 1055–1062

Garner CE, Jefferson WN, Burka LT, Matthews HB, Newbold RR (1999) *In vitro* estrogenicity of the catechol metabolites of selected polychlorinated biphenyls. Toxicol Appl Pharmacol 154(2):188–197

Guo YL, Yu ML, Hsu CC, Rogan WJ (1999) Chloracne, goiter, arthritis, and anemia after polychlorinated biphenyl poisoning: 14-year follow-Up of the Taiwan Yucheng cohort. Environ Health Perspect 107(9):715–719

Hany J, Lilienthal H, Sarasin A, Roth-Harer A, Fastabend A, Dunemann L, Lichtensteiger W, Winneke G (1999) Developmental exposure of rats to a reconstituted PCB mixture or aroclor 1254: effects on organ weights, aromatase activity, sex hormone levels, and sweet preference behaviour. Toxicol Appl Pharmacol 158(3):231–243

Hartwig A (2012) The MAK-collection for occupational health and safety. Villey-VCH http://onlinelibrarywileycom/book/101002/3527600418/topics

Herrick RF, Meeker JD, Hauser R, Altshul L, Weymouth GA (2007) Serum PCB levels and congener profiles among US construction workers. Environ Health 6:25

Hirota Y, Hirohata T, Kataoka K, Shinohara S, Tokiwa H (1993) Laboratory findings in the medical examination of chronic "Yusho" (PCB poisoning) patients with special reference to blood PCB and serum triglyceride. Fukuoka Igaku Zasshi 84: 287–293

Hoffmann W (1996) Organochlorine compounds: risk of Non-Hodgkin's lymphoma and breast cancer? Arch Environ Health 51:189–192

Hsieh SF, Yen YY, Lan SJ, Hsieh CC, Lee CH, Ko YC (1996) A cohort study on mortality and exposure to polychlorinated biphenyls. Arch Environ Health 51(6):417–424

Kodavanti PR, Kannan N, Yamashita N, Derr-Yellin EC, Ward TR, Burgin DE, Tilson HA, Birnbaum LS (2001) Differential effects of two lots of aroclor 1254: congener-specific analysis and neurochemical end points. Environ Health Perspect 109(11): 1153–1161

Lawton RW, Ross MR, Feingold J (1986) Spirometric findings in capacitor workers occupationally exposed to polychlorinated biphenyls (PCBs). J Occup Med 28(6):453–456

Lazaro R, Herrera A, Conchello MP, Arino AA, Bayarri S, Yague C, Peiro JM (1999) Levels of selected polychlorinated biphenyl congeners in total diet samples from Aragon, Spain. J Food Prot 62(9):1054–1058

Leijs MM, Koppe JG, Olie K, van Aalderen WM, de Voogt P, ten Tusscher GW (2009) Effects of dioxins, PCBs, and PBDEs on immunology and hematology in adolescents. Environ Sci Technol 43(20):7946–7951. doi:10.1021/es901480f

Lemesh RA (1992) Polychlorinated biphenyls: an overview of metabolic toxicologic and health consequences. Vet Hum Toxicol 34(3):256–260

Maroni M, Colombi A, Arbosti G, Cantoni S, Foa V (1981) Occupational exposure to polychlorinated biphenyls in electrical workers. II. Health effects. Br J Ind Med 38(1):55–60

Masuda Y (2001) Fate of PCDF/PCB congeners and change of clinical symptoms in patients with Yusho PCB poisoning for 30 years. Chemosphere 43(4–7):925–930

Nakanishi Y, Nomoto Y, Matsuki A, Kunitake R, Hara N (1995) Effect of polychlorinated biphenyls and polychlorinated dibenzofurans on leukocyte in peripheral blood and bronchoalveolar lavage fluid. Fukuoka Igaku Zasshi 86(5):261–266

NIOSH C (2006) Exposure to Polychlorinated Biphenyls and the Risk of Disease. http://wwwcdcgov/niosh/pgms/worknotify/PCB2html

Onozuka D, Yoshimura T, Kaneko S, Furue M (2009) Mortality after exposure to polychlorinated biphenyls and polychlorinated dibenzofurans: a 40-year follow-up study of Yusho patients. Am J Epidemiol 169(1):86–95

Prince MM, Ruder AM, Hein MJ, Waters MA, Whelan EA, Nilsen N, Ward EM, Schnorr TM, Laber PA, Davis-King KE (2006) Mortality and exposure response among 14,458 electrical capacitor manufacturing workers exposed to polychlorinated biphenyls (PCBs). Environ Health Perspect 114(10):1508–1514

Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver B, Enright PL, Hankinson JL, Ip MS, Zheng J, Stocks J (2012) Multi-ethnic reference values for spirometry for the 3–95 year age range: the global lung function 2012 equations. Eur Respir J. doi:10.1183/09031936.00080312

Rallis GN, Sakkas VA, Boumba VA, Vougiouklakis T, Albanis TA (2012) Determination of organochlorine pesticides and polychlorinated biphenyls in post-mortem human lung by matrix solid-phase dispersion with the aid of response surface methodology and desirability function. J Chromatogr A 1227:1–9

Recio-Vega R, Mendez-Henandez A, Gabriel AP, Jacobo-Avila A, Portales-Castanedo A, Hernandez-Gonzalez S, Gallegos-Arreola MP, Ocampo-Gomez G (2012) Potentially estrogenic polychlorinated biphenyls congeners serum levels and its relation with lung cancer. J Appl Toxicol. doi:10.1002/jat.2763]

Reggiani G, Bruppacher R (1985) Symptoms, signs and findings in humans exposed to PCBs and their derivatives. Environ Health Perspect 60:225–232

Robson M, Melymuk L, Csiszar SA, Giang A, Diamond ML, Helm PA (2010) Continuing sources of PCBs: the significance of building sealants. Environ Int 36(6):506–513

Ruder AM, Hein MJ, Nilsen N, Waters MA, Laber P, Davis-King K, Prince MM, Whelan E (2006) Mortality among workers exposed to polychlorinated biphenyls (PCBs) in an electrical capacitor manufacturing plant in Indiana: an update. Environ Health Perspect 114(1):18–23

Rushneck DR, Beliveau A, Fowler B, Hamilton C, Hoover D, Kaye K, Berg M, Smith T, Telliard WA, Roman H, Ruder E, Ryan L (2004) Concentrations of dioxin-like PCB congeners in unweathered Aroclors by HRGC/HRMS using EPA Method 1668A. Chemosphere 54(1):79–87

Sala M, Sunyer J, Otero R, Santiago-Silva M, Camps C, Grimalt J (1999) Organochlorine in the serum of inhabitants living near an electrochemical factory. Occup Environ Med 56(3):152–158

Schettgen T, Alt A, Preim D, Keller D, Kraus T (2012a) Biological monitoring of indoor-exposure to dioxin-like and non-dioxin-like polychlorinated biphenyls (PCB) in a public building. Toxicol Lett 213(1):116–121

Schettgen T, Gube M, Esser A, Alt A, Kraus T (2012b) Plasma polychlorinated biphenyls (PCB) levels of workers in a transformer recycling company, their family members, and employees of surrounding companies. J Toxicol Environ Health A 75(8–10):414–422

Selden AI, Lundholm C, Johansson N, Wingfors H (2008) Polychlorinated biphenyls (PCB), thyroid hormones and cytokines in construction workers removing old elastic sealants. Int Arch Occup Environ Health 82(1):99–106

Smith AB, Schloemer J, Lowry LK, Smallwood AW, Ligo RN, Tanaka S, Stringer W, Jones M, Hervin R, Glueck CJ (1982) Metabolic and health consequences of occupational exposure to polychlorinated biphenyls. Br J Ind Med 39(4):361–369

Smits JE, Fernie KJ, Bortolotti GR, Marchant TA (2002) Thyroid hormone suppression and cell-mediated immunomodulation in American kestrels (Falco sparverius) exposed to PCBs. Arch Environ Contam Toxicol 43(3):338–344

Tokunaga S, Hirota Y, Kataoka K (1999) Association between the results of blood test and blood PCB level of chronic Yusho patients twenty five years after the outbreak. Fukuoka Igaku Zasshi 90(5):157–161

Tsuji H, Hirahashi T, Ogata H, Fujishima M (1999) Serum immunoglobulin concentrations and autoantibodies in patients with Yusho. Fukuoka Igaku Zasshi 90(5):147–149

UBA (2007) Health evaluation of dioxin-like polychlorinated bisphenols in indoor air. German Joint Working Group on Indoor Guidelines of the Federal Environment Agency, Department of Health; http://wwwumweltbundesamtde/gesundheit/innenraumhygiene/ dioxinaehnliche_polychlorierte_biphenylepdf

UBA (2012) Referenzvalues for organochlride substances, polychlorinated bisphenyls, chlorophenols (UBA, German Environmental Protection Ageny) http://wwwubade/gesundheit/publikationen/indexhtml/khb

Weisglas-Kuperus N, Vreugdenhil HJ, Mulder PG (2004) Immunological effects of environmental exposure to polychlorinated biphenyls and dioxins in Dutch school children. Toxicol Lett 149(1–3):281–285

G-Equas. External quality assessment scheme and certification for occupational-medical and environmental-medical toxicological analyses in biological materials. www.g-equas.de/

Zhang W, Sargis RM, Volden PA, Carmean CM, Sun XJ, Brady MJ (2012) PCB 126 and other dioxin-like PCBs specifically suppress hepatic PEPCK expression via the aryl hydrocarbon receptor. PLoS ONE 5(7):e37103