Associations of Blood Levels of PCB, HCHs, and HCB with Numbers of Lymphocyte Subpopulations, *in Vitro* Lymphocyte Response, Plasma Cytokine Levels, and Immunoglobulin Autoantibodies

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Pentachlorophenol (PCP), hexachlorocyclohexane-α, -β, and -γ (HCH-α, -β, and -γ), polychlorinated biphenyls (PCBs), and hexachlorobenzene (HCB) are widely distributed industrial chemicals. They are suspected to induce immunologic impairments in exposed individuals. We examined dose–response relationships of blood levels of these chemicals with cellular (numbers of lymphocyte subpopulations, *in vitro* lymphocyte response) or humoral (plasma cytokine levels, immunoglobulin autoantibodies) immunologic dysfunctions. We studied 146 patients who had been occupationally exposed primarily to PCBs for more than 6 months. Lymphocyte subpopulations, *in vitro* responses to mitogens and allogeneic stimulator cells, plasma neopterin, cytokines, soluble cytokine receptors, soluble adhesion molecules, anti-Ig autoantibodies, and liver transaminases were determined. Blood levels of the different compounds were strongly correlated with one another. There were only weak dose–response relationships between blood levels of PCBs with cellular immune parameters, and of HCHs and HCB with humoral immune parameters. An exception was the statistically significant negative association of HCB with interferon-γ (IFN-γ), indicating that HCB has a significant impact on Th1 lymphocytes. Patients with HCB blood levels above the mean of 1,109 ng/L more often had undetectable IFN-γ blood levels than patients below the mean. Patients with increased PCB 138 (> 710 ng/L) had more frequently undetectable interleukin-4 blood levels than patients with PCB 138 below the mean, and patients with increased PCB 101 (> 31 ng/L) more often had low DR+ cell counts in the blood (< 190/μL) than patients with PCB 101 below the mean. To assess possible cumulative effects, we compared patients who had blood levels of all compounds below background with patients who had blood levels of all compounds above background. Patients with low or absent blood levels of the compounds studied had higher IFN-γ plasma levels, providing some evidence for a cumulative effect of several weakly active compounds. In conclusion, exposure to PCBs, HCB, or HCHs is associated with weak immunologic abnormalities. These results contrast with those obtained in earlier studies of blood levels of PCP, which showed a strong dose-dependent relationship with immunologic impairments. Our data suggest that long-term exposure of patients to HCB suppresses IFN-γ production. *Key words*: cytokines, hexachlorobenzene, hexachlorocyclohexane, immune function, lymphocytes, polychlorinated biphenyls. *Environ Health Perspect* 109:173–178 (2001). [Online 25 January 2001]
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Pentachlorophenol (PCP), hexachlorocyclohexane-α, -β, and -γ (HCH-α, -β, and -γ), polychlorinated biphenyls (PCBs), and hexachlorobenzene (HCB) are widely distributed industrial chemicals. PCBs are used as components of mobile oils, elastic sealing compounds, dielectric fluids in older transformers, heat exchangers, paints and printing inks, and pesticide extenders and protection colors for concrete (*1–3*). The fungicidal chemicals PCP and HCH were used in wood preservatives. PCP was banned in Germany in 1989, and the use of HCH-γ in pesticides was strongly reduced since 1991 (*4*). HCB is an organochlorine fungicide and is also used as a softening agent for plastics (*5*). PCBs are inert, lipid-soluble molecules and tend to accumulate in food chains in animals such as fish (*6*). PCBs accumulate in the human body, and their blood levels can be measured (*2,7–10*). In cell cultures as well as in animal experiments, all of these substances were toxic for lymphocytes in many studies (*11–38*), with some notable exceptions (*39–43*). Although there are reports of immunologic dysfunctions in patients with high exposure to these compounds (*44–48*), it is controversial whether the chemicals are toxic for humans. The compounds occur as mixtures, and studies on their possible interactive effects are therefore important. In previous studies we demonstrated a suppressive effect of PCP blood levels of > 10 μg/L on certain cellular and humoral immune parameters (*38,49*). To avoid an interference of high PCP levels, we excluded patients with PCP blood levels > 10 μg/L from the present analysis.

Materials and Methods

Patients. From 1992 to 1998 blood levels of PCP, PCBs, HCHs, and HCB were determined in 146 patients. Immune parameters were measured in 141 individuals. Two patients were investigated in 1992, 2 in 1993, 6 in 1994, 10 in 1995, 30 in 1996, 44 in 1997, and 52 in 1998. We studied 12 patients at least twice during a mean time interval of 936 days. In these patients, only the results of the last determination were used for statistical analysis. The patients had various clinical symptoms: 82% of the patients complained about a lack of mental concentration, 80% about rapid exhaustion, 50% had frequent common cold diseases, 39% had bronchitis, 14% experienced sleeplessness, 8% had irritations of mucous membranes of the throat and nose, 7% complained about general fatigue, and 2% about nausea. These clinical symptoms are in accordance with other reports of PCP- (*50,51*) and PCB-exposed patients (*52,53*). As determined by a questionnaire, 144 of 146 patients in the study were nonsmokers at the time of the investigation because they tried to avoid any contact with pollutants. The mean (± 1 SD) age of the patients was 46.8 ± 12.5 years (range, 8.6–74.2 years; ≤ 18 years, n = 3; ≤ 30 years, n = 9; ≤ 40 years, n = 36; ≤ 50 years, n = 87; ≤ 60 years, n = 124); 71 were male and 75 were female. Patients with chronic diseases such as hepatitis B virus infection, renal failure, diabetes mellitus, or rheumatism were excluded from the study. The patients were occupationally exposed to PCBs for more than 6 months. Most of them were teachers, construction workers, or telecommunication technicians who were exposed to PCBs in schools, building sites, or factories. Ninety percent of the patients were exposed to PCBs for more than 20 years. Half of the patients were still exposed at the time of investigation. We did not determine blood levels of chemicals in controls.

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Determination of plasma cytokines, soluble cytokine receptors, soluble cytokine receptor antagonists, and soluble adhesion molecules. Values obtained in 40 healthy controls are given in Table 1. Interleukin (IL)-1α, IL-1β, soluble interleukin receptor antagonist (sIL-[1]RA), IL-2, IL-3, IL-4, IL-6, sIL-6R, IL-8, transforming growth factor (TGF)-β_2, and tumor necrosis factor (TNF)-α were measured by ELISA using Quantikine kits (R&D Systems, Firma Biermann, Bad Nauheim, Germany), soluble intercellular adhesion molecule (sICAM)-1 with Cellfree kits (T Cell Diagnostics, Biermann), sIL-2R with Immunotech kits (Dianova, Hamburg, Germany), interferon (IFN)-γ with HBT kits (Holland Biotechnology BV, Biermann), and IL-10 with Cytoscreen kits (Laboserv, Giessen, Germany). Plasma was snap frozen within 2 hr after blood was drawn and stored at –30°C until testing.

Determination of lymphocyte subpopulations. Lymphocyte subpopulations were determined as described previously (*38*). The mean ± 1 SD of the absolute lymphocyte count in 40 healthy controls was 1,804 ± 576/μL. Other values are given in Table 1. The following panel of antibodies was used: CD3 (OKT3, pan T lymphocytes); CD4 (OKT4, helper/inducer T lymphocyte subset); CD8 (suppressor/cytotoxic T lymphocyte subset); OK-DR (MHC class II positive T and B lymphocytes, monocytes); CD25 (OKT26a, IL-2 receptor positive T lymphocytes); CD16 [OK-NK, natural killer (NK) cells]; CD19 (OKB19, B lymphocytes); CD11b (OKM1, monocytes, granulocytes, NK cells); and CD56 (NK cell subset), all from Ortho (Raritan, NJ, USA). Ten microliters of mononuclear antibody were added to 100 μL of whole blood and incubated for 30 min at 4°C. Erythrocytes were lysed with NH_4Cl. The cells were washed and incubated with 50 μL fluorescein isothyocyanate-conjugated goat–anti-mouse Ig (Medac, Hamburg, Germany) for another 30 min at 4°C, washed again, and analyzed with an Ortho Cytoron flow cytometer using the same lymphocyte window for all patients. CD8+/CD56+ NK cell subsets were determined using double fluorescence flow cytometry with a FACScan flow cytometer (Becton Dickinson, Sunnyvale, CA, USA). The mean ± 1 SD range of the CD4/CD8 ratio was 2.0 ± 0.6.

Mitogen stimulation and mixed lymphocyte culture. Mononuclear cells obtained from heparinized blood were adjusted to 1×10^6 cells/mL in culture medium TC-199 (Gibco, Grand Island, NY, USA) containing 20% heat-inactivated human AB-serum, 20 mM/L HEPES buffer, 100 U/mL penicillin, and 100 μg/mL streptomycin. We tested *in vitro* stimulation of lymphocytes using pokeweed mitogen (PWM; Gibco, Paisley, Scotland), concanavalin A (Con A; Pharmacia, Uppsala, Sweden), phytohemagglutinin (PHA; Wellcome, Dartford, UK), or OKT3 mAb (anti-CD3; Ortho) in three different concentrations. We assessed the mixed lymphocyte culture (MLC) response using allogeneic MHC-incompatible stimulator cells pooled from three healthy donors. We added 100 μL of irradiated pooled allogeneic stimulator cells (10^6 cells/mL) to wells of a microtray. All cultures were performed in triplicate by standard methods. Mitogen stimulation assays were incubated at 37°C for 3 days and MLCs for 6 days. The cultures were pulsed with 20 μL ^{3}H-thymidine (1 mCi/mL), harvested after an additional 16 hr, and the incorporation of ^{3}H-thymidine was measured. We calculated relative reponses (RR) as counts per minute of patient lymphocytes cultured with mitogen minus counts per minute of patient lymphocytes in medium, divided by counts per minute of control lymphocytes cultured with mitogen minus counts per minute of control lymphocytes in medium. The maximum RR of each mitogen was used for statistical analysis. Based on control measurements in 52 healthy individuals, an RR < 0.5 was considered abnormally low.

Determination of plasma neopterin. Plasma neopterin was measured initially with the neopterin-RIAcid assay and since 1995 with the Neopterin ELISA (Brahms, Berlin, Germany). Based on control measurements in 70 healthy individuals, we considered > 15 nmol/L abnormally high for both tests (*54*).

Enzyme immunoassay for determination of anti-Ig activities. Anti-Ig autoantibodies were determined as previously described (*54*). We coated 96-well microtiter plates (Nunc, Roskilde, Denmark) at 37°C for 16 hr with either 0.5 μg/well of human IgG-Fab (ICN Biochemicals, Costa Mesa, CA, USA) or IgG-F(ab´)$_2$ fragments (Dianova). Uncoated sites were blocked for 3 hr at 37°C with 1% phosphate-buffered saline–bovine serum albumin.

To determine IgG–anti-Fab and IgG–anti-F(ab´)$_2$ activities, 50 μL of 1:64 diluted test serum was added; to determine IgA–anti-Fab and IgA–anti-F(ab´)$_2$ activities, 50 mL of 1:32 diluted test serum was added; and to determine IgM–anti-Fab and IgM–anti-F(ab´)$_2$ activities, 50 mL of 1:16 diluted test serum was added to the Fab- or F(ab´)$_2$-coated wells in the first step. In the second step, the reaction was developed with 50 μL of alkaline phosphatase-conjugated goat antibodies specific for the Fc fragments of IgG, IgA, or IgM molecules (Dianova; working dilutions 1:5000). In all assays phosphate-buffered saline-Tween 0.05% was used as washing buffer and *p*-nitrophenyl phosphate disodium solution (Sigma Chemical Co., St. Louis, MO, USA) as substrate. All incubation steps with test sera and antibodies were performed at 22°C for 1 hr. After each step the plates were washed four times with washing buffer. The optical density (OD) was measured (± SEM) at 405 nm using a 340 ATTC Microplate Reader (SLT, Crailsheim, Germany).

Determination of PCP, PCBs, HCB, and HCHs in blood. Blood levels of PCP, PCBs, HCB, and HCHs were determined in the laboratory of K. Bauer (Saarbrücken, Germany) using gas chromatography (Hewlett Packard-GC 5890; Hewlett Packard, Palo Alto, CA, USA). Reference blood levels were determined by calculating the 95% quantile in 2941 randomly selected individuals who did not have a history of exposure to the chemicals.

Statistical analysis. Spearman rank correlation, Wilcoxon signed-rank test, and Fisher's exact test were applied using SPSS (Chicago, IL, USA). Adjustment for multiple testing was done according to the method of Bonferroni.

Results

Most patients were exposed to several of the investigated chemicals as indicated by increased blood levels of these chemicals above background (Table 2). Background blood levels were determined by calculating the 95% quantile in 2,941 randomly selected individuals who did not have a history of exposure to the chemicals. Means and ranges

Table 1. Mean values for studied parameters in control subjects.

Parameter	Mean ± SD
IL-1α	0 ± 0 pg/mL
IL-1β	0 ± 1 pg/mL
sIL-1RA	670 ± 1,164 pg/mL
IL-2	24 ± 38 pg/mL
sIL-2R	2,085 ± 2,126 pg/mL
IL-3	27 ± 50 pg/mL
IL-4	6 ± 17 pg/mL
IL-6	2 ± 12 pg/mL
sIL-6R	28,212 ± 7,836 pg/mL
IL-8	51 ± 128 pg/mL
IL-10	0 ± 1 pg/mL
TGF-β_2	4 ± 7 pg/mL
IFN-γ	383 ± 859 ng/mL
sICAM-1	263 ± 173 pg/mL
TNF-α	12 ± 55 pg/mL
CD3	1,380 ± 432 μL
CD4	837 ± 297/μL
CD8	451 ± 176/μL
OK-DR	317 ± 127/μL
CD25	119 ± 57/μL
CD16	183 ± 110/μL
CD19	228 ± 118/μL
CD11b	216 ± 176/μL
CD8+/CD56+ NK subset	134 ± 113/μL

Abbreviations: IFN, interferon; IL, interleukin; sICAM-1, soluble intercellular adhesion molecule 1; sIL, soluble IL-1 receptor antagonist; TGF-β_2, transforming growth factor β_2; TNF-α, tumor necrosis factor α.

of PCB 101, PCB 138, PCB 153, PCB 180, HCH-β, HCH-γ, and HCB blood levels in patients with PCP blood levels ≤ 10 µg/L are shown in Table 2. The blood levels of many compounds were correlated with one another, indicating multiple exposure (Table 3).

We found only weak dose–response relationships between blood levels of PCBs, HCHs, or HCB with cellular and humoral immune parameters (Table 4). PCBs were weakly associated with *in vitro* lymphocyte stimulation and the numbers of lymphocyte subpopulations in the blood, as well as titers of different autoantibody types against immunoglobulin components, whereas HCH-β (Spearman rank correlation, vs. IL-2: $r = 0.182$, $p = 0.040$; vs. IL-10: $r = 0.190$; $p = 0.032$), HCH-γ (Spearman rank correlation, vs. IL-1β: $r = -0.336$, $p = 0.024$), and HCB (Spearman rank correlation, vs. IL-6: $r = -0.189$, $p = 0.036$; vs. sIL-1RA: $r = 0.197$, $p = 0.029$) were weakly associated with plasma levels of cytokines and cytokine receptor antagonists. The most prominent finding was a strong negative association between HCB and IFN-γ blood levels (Spearman rank correlation: $r = -0.357$, $p < 0.0001$), indicating that HCB has a significant impact on Th1 lymphocytes (Table 4).

When we calculated the frequency of individuals with impaired (below the mean –1 SD level) immune parameters in relation to blood levels above the mean, there were few associations that remained significant after adjustment for multiple testing ($n = 38$ analyzed parameters). Patients with HCB blood levels above the mean of 1,109 ng/L more often had undetectable IFN-γ blood levels than patients below the mean (23/52 vs. 11/72; Fisher's exact test: $p = 0.0005$). Patients with increased PCB 138 (> 710 ng/L) more frequently had undetectable IL-4 blood levels than patients with PCB 138 below the mean (29/47 vs. 23/78; Fisher's exact test: $p = 0.0007$), and patients with increased PCB 101 (> 31 ng/L) more often had low DR+ cell counts in the blood (< 190/µL) than patients with PCB 101 below the mean (12/50 vs. 2/81; Fisher's exact test: $p = 0.0002$).

To assess possible cumulative effects, we compared patients who had blood levels of all compounds below background with patients who had blood levels of all compounds above background. Patients with low or absent blood levels of the chemicals studied had higher T lymphocyte counts (CD3/µL: 1,772 ± 606 vs. 1,297 ± 496; $p = 0.051$), higher IFN-γ plasma levels (IFN-γ: 638 ± 737 pg/mL vs. 178 ± 313 pg/mL; $p = 0.016$), and lower GGT plasma levels (γ-glutamyl transpeptidase; 8 ± 2 U/mL vs. 15 ± 9 U/mL;

Table 2. Number and percentage of patients with PCB, HCB, or HCH blood levels above background.

Background level of chemical (ng/L)[a]	No. of patients with blood level above background (%) ($n = 141$)	Mean blood level	5% Quantile of blood level	95% Quantile of blood level
PCB 28 < 10	1 (1)			
PCB 52 < 10	1 (1)			
PCB 101 < 100	0	31.4	13.0	66.0
PCB 138 < 500	98 (70)	710.1	269.0	1444.0
PCB 153 < 600	111 (79)	1025.1	389.0	2221.0
PCB 180 < 300	107 (76)	599.1	141.0	1066.0
HCB < 1,000	60 (43)	1109.4	228.7	2973.7
HCH-α <10	0	< 10		
HCH-β <350	13 (9)	256.2	101.0	513.5
HCH-γ <70	6 (4)	26.9	11.0	54.9

All patients analyzed had a PCP blood level < 10 µg/L.
[a]The 95% quantile was defined in 2,941 randomly selected individuals who did not have a history of exposure to the chemical.

Table 3. Cross-associations (Spearman rank correlations; *r*) of PCB 101, PCB 138, PCB 153, PCB 180, HCB, PCP, HCH-β, and HCH-γ blood levels in 141 patients.

	PCB 101	PCB 138	PCB 153	PCB 180	HCB	PCP	HCH-γ
PCB 138	0.247**						
PCB 153	0.183*	0.959##					
PCB 180	0.201*	0.827##	0.828##				
HCB	0.103	0.506##	0.473##	0.334##			
PCP	0.166	0.082	0.079	0.051	0.315##		
HCH-β	0.009	0.471##	0.454##	0.347##	0.543##	0.003	
HCH-γ	–0.039	–0.008	0.007	0.004	0.064	0.154	0.152

All patients analyzed had a PCP blood level < 10 µg/L.
*$p < 0.05$; **$p < 0.01$; ##$p < 0.0001$.

Table 4. Association (Spearman rank correlations) of PCB 101, PCB 138, PCB 153, PCB 180, HCB, HCH-β, and HCH-γ plasma levels with numbers of lymphocyte subpopulations, *in vitro* lymphocyte response, plasma cytokine levels, and immunoglobulin autoantibodies in 141 patients.

	PCB 101 *p* (*r*)	PCB 138 *p* (*r*)	PCB 153 *p* (*r*)	PCB 180 *p* (*r*)	HCB *p* (*r*)	HCH-β *p* (*r*)	HCH-γ *p* (*r*)
CD4/CD8	NS	NS	NS	NS	NS	NS	0.035 (–0.183)
CD4/µL	0.017 (0.208)	NS	NS	NS	NS	NS	NS
CD16/µL	NS	NS	NS	0.049 (0.172)	NS	NS	NS
CD25/µL	0.029 (0.191)	0.043 (–0.177)	0.036 (–0.183)	NS	NS	NS	NS
CD8/56/µL	0.023 (–0.495)	NS	NS	NS	NS	NS	NS
CD11b/µL	NS	NS	NS	NS	NS	NS	0.015 (0.366)
PHA (RR)	NS	NS	0.029 (–0.192)	0.018 (–0.207)	NS	NS	NS
CD3 mab (RR)	NS	NS	0.047 (–0.176)	NS	NS	NS	NS
IL-1β	NS	NS	NS	NS	NS	NS	0.024 (–0.336)
IL-2	NS	NS	NS	NS	NS	0.040 (0.182)	NS
IL-6	NS	NS	NS	NS	0.036 (–0.189)	NS	NS
IL-10	NS	NS	NS	NS	NS	0.032 (0.190)	NS
INF-γ	NS	NS	NS	NS	0.0001[a] (–0.357)	NS	NS
TGF-β2	NS	0.039 (0.194)	NS	0.035 (0.199)	NS	NS	NS
sIL-1RA	NS	NS	NS	NS	0.029 (0.197)	NS	NS
IgA–anti-Fab	NS	NS	NS	0.021 (0.219)	NS	NS	NS
IgM–anti-Fab	NS	0.004 (–0.271)	0.001 (–0.307)	0.004 (–0.272)	NS	NS	NS
IgM–anti-F(ab')$_2$	NS	0.021 (–0.218)	0.006 (–0.256)	0.041 (–0.193)	NS	NS	NS
GGT (U/mL)	NS	0.009 (0.238)	0.017 (0.217)	0.050 (0.179)	NS	0.016 (0.218)	NS

Abbreviations: GGT, γ-glutamyl transpeptidase; NS, not significant. Only *p*-values < 0.05 are listed. *p*-Values were calculated using the Spearman rank correlation test. Adjustment for multiple testing was done according to the method of Bonferroni. All patients analyzed had a PCP blood level of < 10 µg/L.
[a]Significant ($p < 0.05$) after adjustment for multiple testing (analyzed parameters; $n = 38$).

$p = 0.055$), which provides some evidence for a cumulative effect of several weakly active compounds (Table 5).

We studied 12 patients twice during a mean time interval of 936 days. HCH-γ blood levels decreased from a mean of 36.3 ng/L to a mean of 19.2 ng/L ($p = 0.033$), whereas the mean PCB 153 blood level increased from 857.0 ng/L to 1024.4 ng/L ($p = 0.050$). The blood levels of the other chemicals were not significantly different between the two measurements, suggesting rather constant exposure (Table 6).

Discussion

PCP, PCBs, HCB, and HCHs are incorporated into human tissues transdermally or via inhalation, or they can become part of the food chain and accumulate in the body, especially in lipophilic body tissues. During periods of starvation or after cessation of exposure, the chemicals are redistributed to the blood from lipophilic tissues. Long-term, low-dose exposure is suspected to cause clinical symptoms, such as chronic infections of the upper respiratory tract, general fatigue, and neurotoxicity.

In this study we analyzed individuals with a documented history of PCB, HCB, and HCH exposure who had PCP blood levels in the background range of ≤ 10 µg/L. Because PCP was shown in previous studies to be strongly associated with humoral and cellular dysfunction, we excluded individuals with PCP blood levels > 10 µg/L from the analysis (*38,49*). The blood levels of PCBs, HCB, and HCHs were often associated with one another, suggesting simultaneous exposure, making it difficult to dissociate the impact of individual compounds. The observed positive and negative associations of cellular and humoral immune parameters with blood levels of PCBs, HCB, and HCHs were relatively weak, with the exception of a strongly negative association of IFN-γ with HCB. This finding indicates that HCB has a significant impact on Th1 lymphocytes *in vivo*. IFN-γ, which is produced by Th1 lymphocytes, is involved in the induction of cellular immune responses against antigens such as viruses by activating NK cells, monocytes/macrophages, and granulocytes, and in humoral immune responses by increasing the immunoglobulin secretion of plasma cells. Impaired IFN-γ production might favor infections. These data are in agreement with reports that oral exposure to HCB at concentrations of 150 and 450 mg/kg food for 6 weeks suppressed NK activity in rat lungs in a dose-related manner (*56*). Immunotoxic effects of HCB on the pathogenesis of systemic, pneumonic, and hepatic viral infections were described in the mouse (*57*). Moreover, HCB has been reported to suppress humoral and cell-mediated immunity to protozoan (malaria and leishmania) infections and to tumor cell challenges in the mouse (*58*). Gram-negative endotoxin (*Salmonella typhosa*) sensitivity in PCB- and HCB-treated mice was increased 5.2- and 32-fold, respectively (*59*). Mice fed PCB or HCB for 3–6 weeks and inoculated with *Plasmodium berghei* had decreased survival times (*59*). Workers exposed to HCB showed an impaired lytic activity of neutrophils in the presence of *Candida albicans* and *Candida pseudotropicalis* (*60*). It is noteworthy that NK cells seem to have a role in the pathogenesis of chronic fatigue syndrome (CFS) (*61*) and that patients with CFS showed elevated HCB serum levels (*62*). The incidence of HCB contamination (> 2.0 ppb) was 45% in a CFS group compared with 21% in a non-CFS control group ($p < 0.05$) (*62*).

Table 5. Immune and enzymatic parameters (means ± 1 SD) of patients with blood levels of all compounds below background versus immune and enzymatic parameters of patients with blood levels of all compounds above background.

	Patients with blood levels of all compounds		
Parameter	Below background ($n = 9$)	Above background ($n = 40$)	p
Lymphocytes/µL	2,357 ± 897	1,868 ± 824	NS
CD3/µL	1,772 ± 648	1,297 ± 502	0.051
CD4/µL	1,120 ± 539	788 ± 323	NS
CD8/µL	537 ± 207	466 ± 250	NS
CD16/µL	259 ± 279	243 ± 143	NS
CD19/µL	305 ± 157	250 ± 234	NS
CD25/µL	128 ± 62	114 ± 113	NS
DR/µL	384 ± 127	360 ± 351	NS
Con A (RR)	1.2 ± 0.9	1.1 ± 0.6	NS
PHA (RR)	2.0 ± 2.9	0.9 ± 0.5	NS
PWM (RR)	1.7 ± 1.8	1.4 ± 1.0	NS
CD3 mab (RR)	7.5 ± 13.7	3.1 ± 6.6	NS
MLC (RR)	2.1 ± 1.6	1.4 ± 2.0	NS
Neopterin (nmol/L)	21 ± 37	8 ± 4	NS
IL-1α (pg/mL)	1 ± 1	1 ± 1	n.s
IL-1β (pg/mL)	1 ± 1	0	NS
sIL-1RA (pg/mL)	431 ± 145	646 ± 704	NS
IL-2 (pg/mL)	10 ± 10	12 ± 11	NS
sIL-2R (pg/mL)	1,919 ± 1,422	2,192 ± 2,021	NS
IL-3 (pg/mL)	68 ± 146	16 ± 26	NS
IL-4 (pg/mL)	87 ± 247	5 ± 11	NS
IL-6 (pg/mL)	216 ± 442	62 ± 112	NS
sIL-6R (pg/mL)	35,781 ± 17,854	30,498 ± 14,369	NS
IL-8 (pg/mL)	49 ± 12	37 ± 43	NS
IL-10 (pg/mL)	3 ± 5	14 ± 48	NS
TNF-α (pg/mL)	41 ± 72	78 ± 245	NS
TGF-β_2 (pg/mL)	2 ± 2	12 ± 20	NS
IFN-γ (pg/mL)	638 ± 737	178 ± 318	0.016
sICAM-1 (µg/mL)	185 ± 42	348 ± 146	NS
SGOT (U/mL)	10 ± 7	10 ± 3	NS
GPT (U/mL)	14 ± 10	14 ± 7	NS
GGT (U/mL)	8 ± 3	15 ± 10	0.055
IgA–anti-Fab (OD)	0.1 ± 0.1	0.2 ± 0.1	NS
IgA–anti-F(ab')$_2$ (OD)	0.1 ± 0.1	0.2 ± 0.2	NS
IgG–anti-Fab (OD)	0.4 ± 0.4	0.3 ± 0.3	NS
IgG–anti-F(ab')$_2$ (OD)	0.7 ± 0.4	0.6 ± 0.4	NS
IgM–anti-Fab (OD)	0.2 ± 0.1	0.1 ± 0.1	NS
IgM–anti-F(ab')$_2$ (OD)	0.1 ± 0.1	0.1 ± 0.1	NS

Abbreviations: GPT, glutamic pyruvic transaminase; NS, not significant; SGOT, serum glutamic–oxaloacetic transaminase; sICAM-1, soluble intercellular adhesion molecule 1. All patients analyzed had a PCP blood level < 10 µg/L. Background levels: PCB 138 < 500 ng/L, PCB 153 < 600 ng/L, PCB 180 < 300 ng/L, HCB < 1,000 ng/L.

Table 6. Blood levels (mean ± 1 SD) of PCB 101, PCB 138, PCB 153, PCB 180, HCB, HCH-β, and HCH-γ in 12 patients who were tested twice (mean time interval, 936 days).

	First determination	Second determination	p
PCB 101 (ng/L)	46.5 ± 26.7	28.9 ± 16.4	0.221
PCB 138 (ng/L)	598.0 ± 160.1	700.5 ± 315.9	0.084
PCB 153 (ng/L)	857.0 ± 261.2	1024.4 ± 518.1	0.050
PCB 180 (ng/L)	437.0 ± 191.5	474.0 ± 296.9	0.610
HCB (ng/L)	1240.8 ± 1150.4	879.4 ± 461.9	0.155
HCH-β (ng/L)	188.0 ± 66.4	186.0 ± 58.6	0.625
HCH-γ (ng/L)	36.3 ± 25.6	19.2 ± 9.2	0.033

p-Values were calculated using the Wilcoxon signed-ranks test. All patients analyzed had a PCP blood level < 10 µg/L.

The impact of HCB on humoral immune responses is controversial, especially concerning autoimmune responses. Our finding of a positive association of IgA–anti-Fab serum autoantibodies and the lack of an association of IgG–anti-Fab and IgG–anti-F(ab´)$_2$ with HCB blood levels are in partial agreement with observations describing dose-dependent increases of serum levels of IgM, IgA, and autoantigen-specific IgM as well as unchanged levels of IgG and IgG autoantibody levels in rats given HCB in their diet (*13*,*63*). The negative associations of IgM–anti-Fab and IgM–anti-F(ab´)$_2$ with PCBs in our patients vary with findings in animals on IgM responses against single-stranded DNA, native DNA, rat IgG (representing rheumatoid factor), and bromelain-treated mouse erythrocytes (*13*). However, others reported that antibody synthesis to sheep red blood cells was significantly depressed in PCB- and HCB-treated mice, and serum IgA concentrations were consistently 40–80 mg/dL lower than controls (*59*). The ability of HCB to elevate serum antibody levels against autoantigens but not against foreign antigens suggests that HCB does not act as a polyclonal B cell activator (*13*). HCB activates rat splenic B-1 cells committed to produce autoantibodies associated with systemic autoimmune diseases (*13*,*64*). It is of interest that increased IgG and IgM serum levels were reported in HCB-exposed workers (*65*). However, the workers were not examined for the presence of autoimmune phenomena.

The weak associations of PCBs, HCB, and HCHs with immunologic abnormalities described herein are in stark contrast to the striking abnormalities observed in individuals with high blood levels of PCP (*38*,*49*). The clinical relevance of HCB-associated suppression of IFN-γ awaits further clarification.

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