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Polychlorinated Biphenyl and Hexachlorobenzene Induced Humoral Immunosuppression

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INTRODUCTION

The influence of pesticides and other environmental chemicals on immune responses has received scant attention. Immunosuppression by chemicals of environmental importance is a developing concern in chronic toxicity assessment. Also, chemicals that are persistent in the environment are of particular interest because of their possible accumulation in animal tissues and subsequent magnification in the food chain (4, 23, 28). Indeed, most feared and least understood are the possible effects of the chemicals on cell regulatory processes which Golberg (13) has termed "the three geneses" — carcinogenesis, teratogenesis and immunogenesis (15).

Several environmental contaminants have been reported to be synergistic with infectious agents. Polychlorinated biphenyls (PCB's) (8), arsenicals (12), cobalt sulphate (11) and sulphur dioxide (5) increased the mortality of animals infected with viral agents. Lead nitrate (14) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (24) enhanced the mortality of mice to *Salmonella* sp. challenge. Evidence indicating an impaired host resistance to protozoan or rickettsial infections is presently unavailable. The enhanced susceptibility to bacterial and viral challenge in animals exposed to environmental chemicals may be related to a suppression in antibody (Ab) formation previously reported in animals administered either lead, cadmium, mercury (17), PCB's (18), or DDT (30, 31), or to an impaired cell-mediated immunity (9, 10, 28, 29) in organo-chlorine treated animals.

Two environmental chemicals of importance which have not been extensively investigated with regard to their impact on immune responsiveness are hexachlorobenzene (HCB) and PCB's. Polychlorinated biphenyls are extremely stable compounds, are not hydrolyzed by water and have high thermal stability. They have a low acute toxicity and are poorly metabolized, particularly those with more than 4 chlorine atoms on the phenyl rings. Hexachlorobenzene (C₆Cl₆) is used agriculturally as a fungicide and industrially as a chemical intermediate in organic synthesis.

The increased mortality observed in ducks fed a diet containing 25, 50 or 100 ppm of Aroclor 1254 for 10 days and subsequently challenged with duck hepatitis virus (8), coupled with the demonstrated reduction in neutralizing Ab titers to pseudorabies virus in rabbits administered 300 mg of either PCB 1221, 1242, or 1254 once a week for 4 weeks (18), suggests an immunosuppressive action of PCB's. Therefore, the present studies were conducted to evaluate the influence of dietary administration of PCB's (Aroclor 1242) and HCB on Ab production and immunoglobulin concentrations. Concurrent analysis of tissue levels of the chemicals and histopathology of select lymphoid tissues and reticuloendothelial organs were utilized to relate tissue concentration and morphology to functionality.

MATERIALS AND METHODS

Animals. Male Balb/c mice (18 to 20 g) were used throughout. Food and water were provided *ad libitum*.

Test Chemicals. The polychlorinated biphenyl Aroclor 1242 (Monsanto) and HCB were mixed in the diet at a concentration of 167 ppm. Hexachlorobenzene (Eastman practical grade) was first passed through activated charcoal and then 2X crystallized from boiling benzene. Control animals received the normal Wayne mouse diet. Diet analyses were conducted to confirm the correct concentration of Aroclor 1242 and HCB in the food.

Immunologic Parameters. Six weeks after being placed on diets containing 167 ppm of either PCB 1242 or HCB, all mice, including control animals, received an i.v. injection of 0.1 ml of 10% sheep erythrocytes (SRBC). The primary Ab response was evaluated by the splenic direct plaque-forming cell (PFC) method as described by Jerne and Nordin (16). The plaques formed in this technique have been suggested to represent 19S or IgM Ab producing cells (3, 20). The primary PFC response was measured on days 4, 5, 6 and 7 following immunization. Liver, lung, spleen, mesenteric lymph nodes and thymus tissue samples were also evaluated at this time for histopathologic changes and for concentrations of PCB 1242 and HCB. Serum IgG, IgA and IgM concentrations were quantitated by radial immunodiffusion (RID) using commercially prepared (Mely Laboratories) RID plates for mouse immunoglobulins. A secondary i.v. challenge of 0.1 ml of 10% SRBC was administered 2 weeks following the primary immunization. Direct splenic PFC formation, serum immunoglobulin (IgG, IgM and IgA) concentrations, histopathology of liver, lung, spleen, thymus and mesenteric lymph nodes and tissue levels of PCB 1242 and HCB were evaluated on days 3, 4, 5 and 6 following the secondary immunization.

To determine the effect of the PCB 1242 and HCB on Ab memory cells, a separate group of animals was immunized with 0.1 ml of 10% SRBC 2 weeks prior to being placed on the diets containing either PCB or HCB and then challenged with SRBC 6 weeks later while still on their respective diets. The parameters measured were the same as previously described.

Analysis of HCB and Aroclor 1242. Measured samples of spleen, thymus, liver, lung and serum were placed into separate glass vials. Two ml of acetonitrile and one ml of 2% sodium sulfate were added to each sample. The samples were allowed to stand overnight, shaken and were extracted with one 5 ml and two 2 ml portions of hexane. The hexane extracts were evaporated over a stream of nitrogen to a volume

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of 1 to 2 ml and then passed through a microcolumn fitted with a glass wool plug and containing 1.6 g of anhydrous sodium sulfate above 1.6 g of PR grade Florisil (100/120 mesh, Sigma Chemical Co.). The column was pretreated with 10 ml of hexane and the extracts were eluted from the column with 10 ml of hexane. The eluate was brought to an appropriate volume with hexane for gas-liquid chromatographic (GLC) analysis.

Serum samples of 0.03 to 0.10 ml were pipetted into glass extraction tubes and 2 ml of 2% sodium sulfate were added. The samples were extracted with three 2 ml aliquots of hexane and evaporated to an appropriate volume by drying over a stream of nitrogen.

Known amounts of HCB or Aroclor 1242 were added to control tissue or serum samples and analyzed simultaneously with experimental samples and direct standards.

A Hewlett-Packard gas chromatograph (Model 5713A), equipped with a ^{63}Ni electron capture detector held at 300 C and containing a 6' x $\frac{1}{8}$ " (0.25 mm I.D.) glass column filled with 3% OV-1 on Chromosorb Q, 100/120 mesh, was maintained at an oven temperature of 210 C for analysis of Aroclor 1242. A carrier gas of 5% methane in argon was used at a flow rate of 45 ml/min. The GLC conditions for HCB analysis required the use of the above glass column filled with 1.5% OV-17/1.95% QF-1 on Chromosorb W HP, 80/100 mesh, maintained at an oven temperature of 190 C. Two μ liters of each sample were used for the analysis of either Aroclor 1242 or HCB.

A regression line was calculated from peak area of Aroclor 1242 x attenuation or peak height of HCB x attenuation vs. concentration of Aroclor 1242 or of HCB for extraction standards, and the resulting slopes and intercepts were used to determine the concentrations of the compounds in the tissue and serum samples. Peak area for Aroclor 1242 was determined using a Sargent-Welch dual pen recorder and integrator. The percent extraction, determined by comparing the slopes of the regression lines for extracted and direct standards, was found to range between 90 and 100%.

Histopathology — General. Mice were anesthetized with ether and killed by exsanguination. The peritoneal and thoracic cavities were opened and their contents examined *in situ*. The spleen, mesenteric lymph nodes, liver, thymus and lungs were removed in the stated order. The spleens were cut longitudinally and one half was used for immunologic studies and the other half was fixed for histology. Two representative samples of liver were fixed in 10% buffered formaldehyde. The lungs were injected with a small amount of formaldehyde to expand the pulmonary parenchyma to its normal size; all other organs were fixed *en bloc*. The tissue samples were embedded in paraffin and cut at 6 μm . Hematoxylin-eosin was used as a general stain for all organs. A parallel set of lung sections was stained according to the method of Cason (2) for the demonstration of type II pneumocytes.

Spleen. Each hematoxylin-stained section was projected on a sheet of millimeter paper at 40X magnification. The outline of the entire section and the areas of white pulp were drawn in, resulting in a topographical map of every spleen section. Planimeter values expressed in mm^2 were obtained for the entire area of the section and for all follicles (which were individually numbered at the time). The total area of follicles was then expressed in percent of total area of the spleen section. The composition of the follicles which contained both small and large lymphocytes, or large lymphocytes alone, was determined by a point sampling method (1) at a

magnification of 400X. Each follicle was represented by 100 test points and 10 follicles were chosen from each section, resulting in a total of 1,000 test points for each spleen. The results were expressed as percent of total individual follicular volume. These 10 values were then averaged to give a percent volume figure for the entire section. Increases in number of pyknotic and amounts of nuclear debris in splenic follicles were graded as: normal, minimal, slight, moderate and marked.

Thymus and Mesenteric Lymph Nodes. Hematoxylin-eosin stained sections were examined microscopically and the presence or absence of some morphologic details was noted without further quantification.

Liver. Hematoxylin-eosin stained sections were used to quantify microscopic changes, using the following scores: normal, minimal, slight and moderate.

Lungs. Hematoxylin-eosin stained slides and parallel sections stained with Casson's method (2) were used to enumerate microscopic changes, using the scores: normal, minute, slight (few) and moderate (some), the terms in parentheses being used to describe numbers, the terms outside the parentheses indicating degrees of change.

Statistical Evaluation. Data are presented as the mean $\pm$ standard error with significance, as determined by the Student t test, denoted by the lettered superscript.

RESULTS

The dietary administration of 167 ppm of Aroclor 1242 or HCB to male Balb/c mice for 6 weeks resulted in a $> 2x$ reduction in peak primary (1°) splenic Ab PFC response to SRBC antigen (Ag) (Table 1). The peak PFC response occurred on day 5 following Ag injection in PCB, HCB and control mice, suggesting that there was no delay in response time. Also, the total yield of mononuclear spleen cells in PCB or HCB treated mice was unaltered from control values (Table 1). A concomitant reduction in serum IgG₁, IgA and IgM was demonstrated in PCB and HCB treated mice throughout the measured 1° response period. Although all the immunoglobulin concentrations measured were decreased, the most consistently and significantly reduced was IgA. Polychlorinated biphenyl and HCB treated mice had serum IgA levels which were consistently 40 to 80 mg/dl lower than control values throughout the 1° response. Serum IgA levels were more profoundly reduced in HCB treated mice than in those animals which received PCB 1242 (Table 2).

Lung, thymus and spleen weights in the PCB and HCB treated mice were comparable to control values. A significant increase (111 to 160% of control values) in relative liver weight in mice receiving PCB or HCB was attributed to centrilobular and pericentral hepatocyte hypertrophy (Table 3). Histopathologic examination of the lung, spleen, thymus, or mesenteric lymph nodes did not reveal any histologic alterations. Morphometric analysis of the spleens from PCB or HCB treated mice did not indicate any changes from control values in the number, size or cellular composition of the germinal follicles.

Mice maintained on the diets containing 167 ppm of PCB 1242 or HCB and injected with a secondary challenge of SRBC 2 weeks following the primary immunization manifested a significant reduction in the secondary splenic Ab PFC response. Control mice elicited a maximal response of 133 ± 15 PFC/ 10^6 spleen cells while PCB treated mice had 52 ± 4 PFC/ 10^6 spleen cells and HCB treated mice had 79 ± 15 PFC/ 10^6 spleen cells. Since there was no difference in the yield of total

TABLE I
Effect of PCB 1242 or HCB on Splenic Anti-SRBC Primary (P*) Response in Mice^{a,b}

	Day 4			Day 5			Day 6			Day 7		
	Control	PCB	HCB	Control	PCB	HCB	Control	PCB	HCB	Control	PCB	HCB
Cells/Spleen X10 ⁶	2.91 ± 0.38	2.78 ± 0.31	2.16 ± 0.26	3.14 ± 0.75	2.60 ± 0.21	2.77 ± 0.41	1.75 ± 0.43	2.05 ± 0.29	1.64 ± 0.30	2.36 ± 0.23	1.98 ± 0.22	1.75 ± 0.19
PFC/Spleen	7,435 ± 1,159	5,787 ± 520	6,538 ± 706	65,980 ± 9,812	26,659 ^c ± 4,612	28,450 ^c ± 3,021	8,338 ± 2,214	4,384 ± 958	4,476 ± 984	11,081 ± 1,278	2,747 ^d ± 426	5,145 ^d ± 817
PFC/10 ⁶ Spleen cells	25 ± 2	22 ± 3	30 ± 2	233 ± 33	99 ^e ± 11	110 ^e ± 16	55 ± 13	21 ^b ± 3	31 ± 8	48 ± 5	15 ^f ± 2	29 ^f ± 4

^aThe primary direct splenic PFC to the SRBC Ag was evaluated in mice fed 167 ppm of PCB 1242 or HCB for 6 weeks, at which time all mice received a primary i.v. administration of 0.1 cc of 10% SRBC. The mice were maintained on their respective diets during the response period.

^bData are expressed as mean ± standard error with significance levels indicated as b) p < 0.05; c) p < 0.02; d) p < 0.01; e) p < 0.002; f) p < 0.001; n = 7 to 8 in all groups.

TABLE 2
Serum Immunoglobulin Concentrations in Mice Fed PCB or HCB and
Immunized (I^a) with SRBC^a

	Day 4			Day 5			Day 6			Day 7		
	Control	PCB	HCB	Control	PCB	HCB	Control	PCB	HCB	Control	PCB	HCB
IgG, mg/dl	300 ± 13	280 ± 40	260 ± 24	490 ± 35	290 ^c ± 31	360 ^b ± 38	510 ± 63	190 ^d ± 16	340 ± 12	540 ± 50	400 ± 69	320 ± 77
IgA, mg/dl	170 ± 12	130 ± 20	100 ^c ± 3	190 ± 13	140 ^c ± 5	110 ^c ± 2	340 ± 41	140 ^c ± 3	280 ^d ± 18	190 ± 11	150 ^d ± 9	110 ^d ± 4
IgM, mg/dl	430 ± 35	410 ± 72	390 ± 15	410 ± 27	360 ± 21	330 ± 12	380 ± 63	120 ^e ± 7	290 ^e ± 14	370 ± 18	310 ^e ± 17	290 ^e ± 21

^aHalf of male mice were fed Aroclor (PCB) 1242 or HCB in their diet at a concentration of 167 ppm for 6 weeks at which time they received a primary immunization (i.v.) of 0.1 cc of 10% SRBC. Serum immunoglobulins were determined on days 4, 5, 6 and 7 by RID.

^bData are expressed as mean ± standard error; b) p < 0.05; c) p < 0.02; d) p < 0.01; e) p < 0.002; f) p < 0.001; n = 6 to 8 in all groups.

TABLE 3
Effect of PCB and HCB on Body and Organ Weights in Mice Fed PCB and HCB and
Immunized (I^a) with SRBC^a

TABLE 3
Effect of PCB and HCB on Body and Organ Weights in Mice Fed PCB and HCB and
Immunized (I^a) with SRBC^{a,b}

	Day 4				Day 5			Day 6			Day 7	
	Control	PCB	HCB	Control	PCB	HCB	Control	PCB	HCB	Control	PCB	HCB
Body Wt. (g)	32.1 ± 0.35	31.9 ± 0.51	28.9 ^a ± 0.71	30.5 ± 0.69	31.8 ± 0.99	30.9 ± 0.46	29.3 ± 0.72	32.3 ^c ± 0.37	27.8 ± 0.85	29.1 ± 0.68	31.5 ^b ± 0.61	30.0 ± 0.64
Relative Liver Wt. %	5.89 ± 0.22	6.01 ± 0.09	6.76 ^c ± 0.12	5.50 ± 0.18	6.10 ^b ± 0.18	7.12 ^c ± 0.22	5.45 ± 0.21	6.32 ^b ± 0.21	8.74 ^d ± 0.83	5.37 ± 0.45	6.17 ± 0.15	7.76 ^c ± 0.22
Relative Spleen Wt. %	0.758 ± 0.03	0.708 ± 0.01	0.807 ± 0.05	0.716 ± 0.04	0.609 ± 0.06	0.717 ± 0.02	0.671 ± 0.07	0.524 ± 0.02	0.699 ± 0.03	0.679 ± 0.05	0.524 ± 0.01	0.577 ± 0.01
Relative Thymus Wt. %	0.107 ± 0.01	0.142 ^b ± 0.01	0.126 ± 0.01	0.128 ± 0.01	0.124 ± 0.01	0.153 ± 0.01	0.143 ± 0.01	0.110 ± 0.01	0.112 ± 0.01	0.155 ± 0.01	0.119 ± 0.01	0.118 ± 0.01
Relative Lung Wt. %	0.522 ± 0.02	0.532 ± 0.03	0.496 ± 0.01	0.562 ± 0.03	0.506 ± 0.01	0.514 ± 0.01	0.710 ± 0.02	0.616 ± 0.01	0.686 ± 0.04	0.635 ± 0.01	0.629 ± 0.02	0.626 ± 0.01

^aArcher 1242 and HCB were fed in the diet at a concentration of 167 ppm for 6 weeks, at which time all mice received a primary immunization (i.v.) with 0.1 cc of 10% SRBC. Organ weights, histopathology, chemical analysis and measurements of immune parameters were conducted on days 4, 5, 6 and 7 following immunization.

^bData are expressed as mean ± standard error with significance levels indicated as b) $p < 0.05$; c) $p < 0.02$; d) $p < 0.01$; e) $p < 0.002$; f) $p < 0.001$; n = 5 to 8 in all groups.

TABLE 4
Effect of Aroclor 1242 or HCB on Splenic Anti-SRBC^c
Secondary (II^o) Response in Mice^{a,b}

	Day 3			Day 4			Day 5			Day 6		
	Control	PCB	HCB	Control	PCB	HCB	Control	PCB	HCB	Control	PCB	HCB
Cells/Spleen X10 ⁶	3.15 ± 0.31	3.09 ± 0.16	2.94 ± 0.27	3.01 ± 0.39	2.52 ± 0.20	3.40 ± 0.81	1.72 ± 0.14	2.00 ± 0.32	2.12 ± 0.28	1.69 ± 0.26	1.82 ± 0.34	1.60 ± 0.09
Pl-C/Spleen	40,737 ± 4,120	15,724 ^c ± 855	22,034 ^c ± 3,022	26,590 ± 4,299	14,926 ± 4,946	19,152 ± 9,210	11,859 ± 1,566	3,175 ^e ± 493	3,494 ^e ± 659	6,710 ± 1,147	7,478 ± 1,613	5,047 ± 1,647
Pl-C/10 ⁶ Spleen Cells	133 ± 15	52 ^c ± 4	79 ^b ± 15	91 ± 10	52 ^b ± 13	47 ^c ± 10	70 ± 9	17 ^c ± 2	17 ^c ± 3	45 ± 10	44 ± 8	30 ± 9

^aThe secondary direct splenic Pl-C to the SRBC Ag was evaluated 2 weeks after primary immunization. At this time all mice received a second challenge of 0.1 cc of 10% SRBC. The mice were maintained on their diets of 167 ppm of PCB or HCB during the response period.

^bData are expressed as mean ± standard error with significance levels indicated as b) p < 0.05; c) p < 0.02; d) p < 0.01; e) p < 0.002; f) p < 0.001; n = 7 to 8 in all groups.

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TABLE 5
Serum Immunoglobulin Concentrations in Mice Fed PCB and HCB
and Immunized (IP) with SRBC**

	Day 3			Day 4			Day 5			Day 6		
	Control	PCB	HCB	Control	PCB	HCB	Control	PCB	HCB	Control	PCB	HCB
IgG (mg/dl)	320 ± 36	300 ± 24	490 ± 81	380 ± 61	460 ± 54	330 ± 42	440 ± 47	460 ± 82	540 ± 68	340 ± 30	260 ^a ± 19	510 ± 98
IgA (mg/dl)	140 ± 7	140 ± 5	110 ± 3	160 ± 11	190 ± 19	150 ± 13	190 ± 20	170 ± 13	140 ^b ± 3	180 ± 14	170 ± 11	130 ^c ± 6
IgM (mg/dl)	350 ± 13	340 ± 25	300 ± 23	380 ± 17	370 ± 18	450 ± 43	400 ± 35	370 ± 26	300 ^d ± 20	330 ± 14	310 ± 28	300 ± 22

*Serum immunoglobulin concentrations were determined by RID in mice challenged with SRBC 2 weeks following their IP immunization. All mice were maintained on their diets containing 167 ppm of PCB or HCB during the secondary response period.

**Data are expressed as mean ± standard error with levels of significance indicated as: b) $p < 0.05$; c) $p < 0.02$; d) $p < 0.01$; e) $p < 0.002$; f) $p < 0.001$; n = 7 to 8 in all groups.

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TABLE 6

Effect of PCB and HCB on Body and Organ Weights in Mice during the Secondary Response to SRBC^{a,b}

	Day 3			Day 4			Day 5			Day 6		
	Control	PCB	HCB	Control	PCB	HCB	Control	PCB	HCB	Control	PCB	HCB
Body Wt. (g)	29.9 ± 0.52	30.8 ± 0.48	28.5 ± 0.84	29.5 ± 0.55	31.2 ± 0.71	28.6 ± 0.86	30.1 ± 0.56	32.0 ^b ± 0.61	30.9 ± 0.12	29.8 ± 0.33	30.4 ± 0.76	26.7 ^a ± 0.67
Relative Liver Wt. %	5.41 ± 0.28	6.50 ^c ± 0.22	8.61 ^a ± 0.22	5.55 ± 0.64	6.80 ^c ± 0.17	10.42 ^a ± 0.23	5.33 ± 0.30	7.04 ^c ± 0.15	9.53 ^a ± 0.22	5.35 ± 0.81	6.65 ^c ± 0.12	8.58 ^a ± 0.47
Relative Spleen Wt. %	0.724 ± 0.06	0.638 ± 0.02	0.709 ± 0.03	0.536 ± 0.06	0.692 ± 0.05	0.754 ^b ± 0.05	0.569 ± 0.03	0.583 ± 0.04	0.691 ^b ± 0.03	0.644 ± 0.03	0.577 ± 0.05	0.645 ± 0.03
Relative Thymus Wt. %	0.110 ± 0.01	0.112 ± 0.01	0.105 ± 0.01	0.086 ± 0.01	0.086 ± 0.01	0.088 ± 0.01	0.085 ± 0.01	0.090 ± 0.01	0.076 ± 0.01	0.082 ± 0.01	0.090 ± 0.01	0.092 ± 0.01
Relative Lung Wt. %	0.696 ± 0.04	0.782 ± 0.05	0.768 ± 0.03	0.593 ± 0.01	0.576 ± 0.01	0.679 ± 0.04	0.662 ± 0.06	0.604 ± 0.02	0.650 ± 0.08	0.636 ± 0.03	0.653 ± 0.04	0.674 ± 0.03

^a Aroclor 1242 and HCB were fed in the diet at a concentration of 167 ppm for 6 weeks, at which time all mice received a primary immunization of 0.1 cc of 10% SRBC. Two weeks later all mice received a secondary challenge of 0.1 cc of 10% SRBC. Body and organ weights, histopathology, chemical analyses and measurements of immune parameters were determined on days 3, 4, 5 and 6 following 11th challenge.

^b Data are expressed as mean ± standard error with significance levels indicated as: b) p < 0.05; c) p < 0.02; d) p < 0.01; e) p < 0.002; f) p < 0.001; n = 5 to 8 in all groups.

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TABLE 7

Effect of PCB or HCB on the Secondary (II*) Splenic Anti-SRBC Response
in Mice Primed with SRBC Prior to Dietary Administration of the Chemicals^{a,b}

	Day 4			Day 6			Day 8			Day 11		
	Control	PCB	HCB	Control	PCB	HCB	Control	PCB	HCB	Control	PCB	HCB
Cells/Spleen X10 ⁶	2.38 ± 0.18	2.73 ± 0.36	2.86 ± 0.35	1.75 ± 0.15	1.62 ± 0.13	2.21 ± 0.28	1.54 ± 0.15	1.46 ± 0.16	1.58 ± 0.14	1.68 ± 0.13	2.14 ± 0.68	2.00 ± 0.24
PLC/Spleen	4,065 ± 575	2,910 ± 512	1,728 ^c ± 335	5,886 ± 682	1,718 ^c ± 196	2,308 ^c ± 459	6,418 ± 1,179	2,461 ^b ± 710	2,113 ^c ± 480	972 ± 315	2,092 ± 1,480	1,232 ± 192
PLC/10 ⁶ Spleen Cells	17 ± 4	12 ± 3	6 ^b ± 1	36 ± 5	10 ^c ± 1	12 ^c ± 3	41 ± 6	18 ^b ± 5	13 ^b ± 2	5 ± 1	6 ± 2	6 ± 2

^aMice were immunized with 0.1 cc of 10% SRBC 2 weeks prior to being placed on diets containing 167 ppm of PCB or HCB. After 6 weeks on the dietary regimen, the mice received a secondary challenge of SRBC. Responses were evaluated on days 4, 6, 8 and 11.

^bData are expressed as mean ± standard error with levels of significance indicated as: b) $p < 0.05$; c) $p < 0.02$; d) $p < 0.01$; e) $p < 0.002$; f) $p < 0.001$; n = 5 to 8 in all groups.

TABLE 8
Serum Immunoglobulin Concentrations During Secondary Response to SRBC in Mice
Primed with SRBC Prior to Being Placed on the Dietary Regimen Containing PCB or HCB^{a,b}

	Day 4			Day 6			Day 8			Day 11		
	Control	PCB	HCB	Control	PCB	HCB	Control	PCB	HCB	Control	PCB	HCB
IgG (mg/dl)	380 ± 29	360 ± 15	450 ± 30	460 ± 14	460 ± 45	490 ± 90	480 ± 31	420 ± 31	470 ± 61	480 ± 35	410 ± 43	600 ± 75
IgA (mg/dl)	140 ± 7	110 ^c ± 2	110 ^c ± 3	150 ± 5	130 ^c ± 8	110 ^c ± 4	130 ± 6	110 ^c ± 4	110 ^c ± 2	130 ± 7	130 ± 4	120 ± 4
IgM (mg/dl)	400 ± 19	340 ^c ± 7	350 ^c ± 7	300 ± 13	290 ± 10	290 ± 13	390 ± 37	300 ^c ± 13	290 ^c ± 25	320 ± 19	280 ± 24	320 ± 14

^aMice were immunized with 0.1 cc of 10% SRBC 2 weeks prior to being placed on diets containing 167 ppm of PCB or HCB. After 6 weeks on the dietary regimen, the mice received a secondary challenge of SRBC. Responses were evaluated on days 4, 6, 8 and 11.

^bData are expressed as mean ± standard error with levels of significance indicated as: b) $p < 0.05$; c) $p < 0.02$; d) $p < 0.01$; e) $p < 0.002$; f) $p < 0.001$; n = 5 to 8 in all groups.

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TABLE 9
Effect of PCB 1242 or HCB on Body and Organ Weights During the Secondary Response
in Mice Primed with SRBC Prior to Dietary Administration of PCB or HCB^{a,b}

	Day 4			Day 6			Day 8			Day 11		
	Control	PCB	HCB	Control	PCB	HCB	Control	PCB	HCB	Control	PCB	HCB
Body Wt. (g)	31.9 ± 0.33	30.0 ^a ± 0.73	30.4 ± 0.64	29.8 ± 0.71	30.1 ± 1.00	29.1 ± 1.44	30.0 ± 0.73	30.3 ± 0.57	29.0 ± 0.87	29.4 ± 0.68	31.4 ± 0.83	30.1 ± 0.95
Relative Liver Wt. %	6.08 ± 0.37	6.27 ± 0.16	7.46 ^c ± 0.21	5.54 ± 0.12	6.31 ^b ± 0.27	7.43 ^c ± 0.24	5.19 ± 0.17	6.14 ^a ± 0.12	7.58 ^b ± 0.77	4.93 ± 0.05	6.11 ^a ± 0.24	7.24 ^c ± 0.42
Relative Spleen Wt. %	0.559 ± 0.03	0.750 ^a ± 0.04	0.595 ± 0.03	0.578 ± 0.04	0.453 ± 0.02	0.680 ± 0.04	0.560 ± 0.04	0.500 ± 0.04	0.559 ± 0.02	0.464 ± 0.01	0.461 ± 0.04	0.520 ± 0.02
Relative Thymus Wt. %	0.187 ± 0.05	0.154 ± 0.03	0.129 ± 0.01	0.110 ± 0.01	0.136 ± 0.01	0.149 ^b ± 0.01	0.125 ± 0.01	0.137 ± 0.01	0.137 ± 0.01	0.141 ± 0.01	0.123 ± 0.01	0.120 ± 0.01
Relative Lung Wt. %	0.571 ± 0.03	0.526 ± 0.05	0.505 ± 0.01	0.646 ± 0.03	0.695 ± 0.07	0.702 ± 0.06	0.656 ± 0.06	0.649 ± 0.05	0.609 ± 0.03	0.768 ± 0.02	0.648 ^c ± 0.02	0.816 ± 0.06

^aMice were immunized with 0.1 cc of 10% SRBC 2 weeks prior to being placed on diets containing 167 ppm of PCB or HCB. After 6 weeks on the dietary regimen, the mice received a secondary challenge of SRBC. Responses were evaluated on days 4, 6, 8 and 11.

^bData are expressed as mean ± standard error with levels of significance indicated as b) p < 0.05; c) p < 0.02; d) p < 0.01; e) p < 0.002; f) p < 0.001, n = 5 to 8 in all groups.

TABLE 10

Electron-Capture Gas Chromatographic Analysis of Aroclor 1242 and HCB in Tissues of Mice Fed Diets Containing 167 ppm of PCB or HCB^a

		Tissue Concentration (μg/g)									
		Serum		Liver		Lung		Spleen		Thymus	
	Day	PCB	HCB	PCB	HCB	PCB	HCB	PCB	HCB	PCB	HCB
Group 2-IP ^b	4	0.84 ± 0.03	12.0 ± 0	2.8 ± 0.35	56 ± 9.2	7.0 ± 1.9	269 ± 36	2.2 ± 0.33	47 ± 2.8	7.1 ± 1.1	152 ± 36
	5	1.0 ± 0.12	9.1 ± 3.0	4.4 ± 0.53	80 ± 7.5	5.8 ± 0.90	243 ± 25	3.0 ± 0.38	52 ± 6.2	4.5 ± 0.90	180 ± 29
	6	1.0 ± 0.17	12.0 ± 1.4	3.4 ± 0.21	87 ± 7.4	6.4 ± 0.68	214 ^c ± 14	3.6 ± 0.11	51 ± 3.6	7.3 ± 0.47	121 ± 7.9
	7	0.95 ± 0.16	9.6 ± 0.8	4.3 ± 0.43	75 ± 5.2	7.6 ± 1.6	157 ± 15	4.4 ± 0.58	52 ± 4.3	5.9 ± 1.0	153 ± 8.7
	3	0.63 ± 0.13	11 ± 0.35	6.9 ± 0.59	99 ± 12	5.4 ± 0.53	265 ± 21	2.8 ± 0.74	54 ± 7.5	6.2 ± 1.4	145 ± 33
	4	0.51 ± 0.04	15 ± 1.6	5.2 ± 0.75	111 ± 9.1	12 ± 2.3	320 ± 72	1.7 ± 0.30	60 ± 6.0	9.0 ± 1.4	224 ± 54
	5	0.57 ± 0.01	11 ± 0.64	6.0 ± 0.48	94 ± 9.0	6.5 ± 0.58	217 ± 54	1.5 ± 0.22	69 ± 4.6	10 ± 1.1	72 ± 15
Group 2-IP ^b	6	0.46 ± 0.02	14 ± 1.4	7.8 ± 0.82	99 ± 4.7	7.1 ± 1.2	208 ± 37	2.6 ± 0.40	77 ± 8.5	9.3 ± 1.2	126 ± 30
	7	0.76 ± 0.11	—	4.0 ± 0.65	—	5.7 ± 2.8	—	3.0 ± 0.53	—	7.9 ± 0.30	—
Group 1-IP ^b	4	0.86 ± 0.13	8.8 ± 0.73	2.6 ± 0.23	60 ± 3.2	9.1 ± 3.2	247 ± 44	3.0 ± 0.50	41 ± 8.0	5.8 ± 0.84	195 ± 30
	6	1.3 ± 0.14	9.7 ± 0.20	3.2 ± 0.38	67 ± 5.4	9.6 ± 1.4	183 ± 16	3.6 ± 3.9	50 ± 4.8	3.7 ± 0.35	137 ± 31
	8	1.5 ± 0.22	13 ± 0.84	4.0 ± 0.45	63 ± 8.7	7.8 ± 2.3	198 ± 30	3.3 ± 0.24	67 ± 5.5	7.5 ± 1.0	244 ± 20
	11	0.86 ± 0.30	11 ± 0.41	2.9 ± 0.32	82 ± 7.7	8.7 ± 1.2	250 ± 42	3.0 ± 0.79	66 ± 6.9	8.4 ± 1.2	190 ± 32

^aGroup 2-IP received a primary immunization (IP) of SRHC following 6 weeks dietary administration of 167 ppm of PCB or HCB. Group 2-IP^b received a booster, booster of SRHC 2 weeks following the primary immunization. Group 1-IP^b received the primary immunization of SRHC 2 weeks prior to being

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spleen cells obtained from control versus PCB or HCB treated animals and since both the control group and experimental groups had the greatest PFC response on day 3 following 11th challenge, either an alteration in the splenic Ab producing cell population and/or an impaired cell function may be suggested (Table 4).

No consistent significant alterations, as compared to control values, in serum IgG₁ or IgM concentrations in the PCB or HCB treated mice, were demonstrated during the 11th response to SRBC. However, a significant decrease (72 to 79% of control values) in IgA was measured in HCB treated mice on days 3, 5 and 6 during the secondary response period; day 4 IgA concentration was decreased but was not statistically significant. IgA levels in PCB treated mice were comparable to controls (Table 5).

Hepatomegaly, attributed to a centrilobular and pericentral hepatocyte hypertrophy, was a consistent finding in the PCB and HCB treated mice during the 11th response period. Control liver weights were unaltered. Significant splenomegaly (21 to 41% of control values) was observed in HCB treated mice on days 4 and 5 following the 11th SRBC administration. However, this change was not reflected in the splenic germinal follicle number, size or cellular composition. Thymus and lung weights and histology were unaltered from control tissue (Table 6).

To ascertain the influence of the environmental chemicals on memory cell function, one group of mice was immunized (1st) with SRBC 2 weeks prior to being placed on the diets containing PCB 1242 or HCB. Six weeks following dietary administration of the chemicals, a 11th challenge of SRBC was administered. Control animals manifested a direct splenic Ab response of 41 ± 6 PFC/10⁶ spleen cells, whereas PCB treated mice had only 18 ± 5 PFC/10⁶ spleen cells and HCB animals had 13 ± 2 PFC/10⁶ cells. Maximal response in all groups was achieved on day 8 following the 11th immunization. The yield of spleen cells in the environmental chemical-treated mice was unaltered from control values (Table 7). A consistent and significant decrease of 20 to 40 mg/dl from control IgA concentration was demonstrated on days 4, 6 and 8 in mice receiving PCB or HCB. IgA values returned to normal by day 10. IgG₁ and IgM levels in the PCB and HCB treated animals were unaltered from control levels (Table 8).

A significant increase in liver weight in PCB and HCB treated mice (117 to 149% of control values, respectively) was determined to be a result of hypertrophy of centrilobular and pericentral hepatocytes. Thymus, liver, lung and spleen weights and histopathology were unaltered from control tissues (Table 9).

Electron-capture gas chromatographic analysis of PCB 1242 and HCB in serum and tissues revealed: 1) an approximate steady state condition occurred by the 6 week period, i.e., no significant increase in tissue chemical levels occurred between 6 and 8.5 weeks in lung, thymus, spleen or serum; however, liver content of PCB and HCB did increase; 2) liver, spleen and thymus manifested an approximate 20:1 concentration of HCB/PCB while the lung HCB/PCB ratio was ~38:1; and 3) maximal tissue uptake of PCB occurred equally in the thymus and lung while the principle tissue site of HCB localization was the lung, with the second largest deposition being present in the thymus (Table 10).

DISCUSSION

Clinically subtoxic levels of PCB 1242 or HCB were profoundly immunosuppressive when fed to mice for 6 weeks prior to and during the Ag challenge (Tables 1 and

Group 2: received a primary immunization (1st) of SRBC following a 6 weeks dietary administration of 1% ppm of PCB or HCB. Group 2: received a secondary immunization (11th) of SRBC 2 weeks prior to being placed on the diets containing PCB 1242 or HCB. Six weeks following dietary administration of the chemicals, a 11th challenge of SRBC was administered. Control animals manifested a direct splenic Ab response of 41 ± 6 PFC/10⁶ spleen cells, whereas PCB treated mice had only 18 ± 5 PFC/10⁶ spleen cells and HCB animals had 13 ± 2 PFC/10⁶ cells. Maximal response in all groups was achieved on day 8 following the 11th immunization. The yield of spleen cells in the environmental chemical-treated mice was unaltered from control values (Table 7). A consistent and significant decrease of 20 to 40 mg/dl from control IgA concentration was demonstrated on days 4, 6 and 8 in mice receiving PCB or HCB. IgA values returned to normal by day 10. IgG₁ and IgM levels in the PCB and HCB treated animals were unaltered from control levels (Table 8).

4). Both the 1st and 11th splenic PFC responses to SRBC were impaired in the environmental chemical-treated mice. Animals immunized 2 weeks prior to being placed on the diets containing either the PCB or HCB and challenged with a 1st SRBC injection 6 weeks after the dietary administration of the chemicals manifested a significantly depressed secondary response, suggesting either that Ab memory cell function was impaired or that there was an alteration in cellular cooperation (Table 7). Peak Ab formation occurred on the same day in experimental animals as in control mice, indicating that there was no delay in Ab formation. Also, since the total number of mononuclear spleen cells obtained from the PCB or HCB treated mice was essentially the same as the number obtained from controls, the decreased PFC response in the treated mice may be due to an impaired cell function and/or an alteration in the composition of the spleen cell population, i.e., a change in the macrophage, T and B lymphocyte ratios. A PCB induced immunosuppression was also noted by Koller and Thigpen (18), who demonstrated a significant 50 to 59% reduction in neutralizing Ab titers to pseudorabies virus in rabbits administered 300 mg of either PCB 1221, 1242, or 1254 once a week for 14 weeks; PCB 1242 treated rabbits had the lowest Ab titers. Vos and van Driel-Grootenhuys (27) previously observed a decrease in cellular immunity and humoral Ab formation in guinea pigs exposed to a 60% solution of PCB 1260. Street and Sharma (23) did not detect any change in hemolysin or hemagglutinin titers in rabbits fed 3.7 to 170 ppm of PCB 1254 for 4 weeks. However, since Ab titers were measured only at 10 and 17 days post-1st injection, it is possible a change in peak titers could have been missed.

An interaction between organochlorines and infectious diseases was determined by Friend and Trainer (8), who demonstrated a significant ($p < 0.01$) increase, as compared to control values, in mortality of ducklings fed 25, 50 or 100 ppm of Aroclor 1254 for 10 days prior to i.p. inoculation of duck hepatitis virus. A 2 to 4X increase in mortality rates over control (duck hepatitis virus alone) mortality was observed in the PCB plus virus group. There was no significant difference in mortality among ducklings receiving the different concentrations of PCB 1254 plus virus. A 3 to 9X increase in mortality rates, over control (duck hepatitis virus alone) values, was manifested in ducklings receiving p,p'-DDT or dieldrin and inoculated with duck hepatitis virus. The suppressed host resistance to the duck hepatitis virus may be associated with the suppressed immune response demonstrated in the present study and also observed by Vos and van Driel-Grootenhuys (27) and by Koller and Thigpen (18).

The reduction in primary direct splenic PFC formation, a measurement primarily of IgM Ab producing cells (3, 20), was associated with a significant decrease in serum IgM on days 6 and 7 following immunization in PCB and HCB treated mice. IgM concentration was reduced from control values by approximately 23% in HCB treated mice, whereas the IgM levels in PCB treated mice were reduced 69% and 16% on days 6 and 7, respectively. Serum IgG₁ was significantly reduced, 41% and 63%, from control values on days 5 and 6, respectively, in PCB treated mice; a slight, but significant decrease of 27% was observed on day 5 in the HCB treated animals. The most consistent alteration in immunoglobulins was a decrease in serum IgA in the HCB treated animals during both the 1st and 11th immunization periods. A mean decrease of 43% below control concentrations was measured on days 4 to 7 in the HCB-treated animals during the 1st response and a mean decrease of approximately 23% during both 11th responses, i.e., in animals which received the

¹p prior to being placed on the diets and in animals which received the ¹p while on their respective diets. Polychlorinated biphenyl-treated mice also manifested a significant decrease in serum IgA of approximately 35% during the ¹p response and a slight, but significant, decrease of 16% below control values, in animals primed prior to dietary administration of the PCB (Tables 2, 5 and 8). It is significant to note that Shigematsu *et al.* (22) reported that IgG in the sera of 203 victims of the rice oil poisoning incident (Yusho disease) was increased by 23.8%, whereas IgM was decreased by 18.6% and IgA by 28.5%.

Extensive histopathologic examination of the lung, thymus, mesenteric lymph nodes and spleen did not reveal any tissue alterations different from controls. However, a significant and consistent hepatomegaly, attributed to a centrilobular and pericentral hepatocyte hypertrophy, was observed. This type of hepatic response is consistent with that seen following exposure to organochlorines. Detailed morphometric analysis of the spleens from PCB or HCB treated mice throughout the experimental period did not indicate any alteration from control values in the germinal follicle numbers, size or cellular composition. In guinea pigs fed 10 or 50 ppm of PCB 1260 for 8 weeks Vos and De Rooij (26) also noted an absence of any histomorphologic changes in the spleen, thymus, cervical and mesenteric lymph nodes. Similarly, Koller and Thigpen (18) did not detect any pathologic changes in the spleen, thymus or lymph nodes in rabbits fed 300 mg of PCB 1221, PCB 1242 or PCB 1254 by gavage once a week for 14 weeks. However, in rabbits fed PCB 1254 in their diet at doses ranging from 3.7 to 170 ppm for 4 weeks, Street and Sharma (23) suggested that alterations in lymphoid organ histology were the most sensitive indicators of immunosuppression, i.e., treatment with PCB 1254 resulted in a decreased count of plasma cells in the popliteal lymph nodes, reduction of splenic germinal centers and cortical atrophy of the thymus.

Tissue localization of the PCB 1242 and HCB in the liver, lung, spleen and thymus is of 2-fold importance. First, the liver, lung and spleen contain the principle populations of fixed tissue macrophages which have been suggested to act as initiators of the immune response (6, 7, 25) and to be essential in thymocytic mitogenesis (19, 21); second, the thymus is a primary lymphoid organ in addition to SRBC being a thymic or T dependent Ag. It is interesting to speculate that the approximate 20:1 ratio in tissue concentration of HCB and PCB is correlative with the increased immunosuppressive effects of HCB as compared to PCB. The question remains that, in the absence of histopathologic changes, excepting the hepatocyte hypertrophy, and with the demonstrated immunosuppression, is the tissue concentration of PCB and HCB in lymphoid and reticuloendothelial organs inducing a functional but not morphologic alteration and is the functional change a more sensitive indicator of toxicity than a pathologic alteration?

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