TOXICOLOGY AND APPLIED PHARMACOLOGY 21, 549–555 (1972)

Immunosuppressive Activity of a Polychlorinated Biphenyl Preparation on the Humoral Immune Response in Guinea Pigs

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Received July 13, 1971

Immunosuppressive Activity of a Polychlorinated Biphenyl Preparation on the Humoral Immune Response in Guinea Pigs. (1972). Vos, J. G., and de Roij, Th. *Toxicol. Appl. Pharmacol.* **21**, 549–555. Three groups of 12 female albino guinea pigs were fed 0, 10 and 50 ppm Aroclor 1260 (PCB) for 8 wk. In half of the animals a function test of the immunological system was carried out: tetanus toxoid injection was used to study the humoral response. At the end of the experiment cellulose acetate electrophoresis was used to determine the serum proteins, including the γ-globulin level. The number of γ-globulin-containing cells in popliteal lymph nodes was determined semiquantitatively with the direct fluorescent antibody technique. Both these techniques seemed to be sensitive parameters for the immunosuppressive action of PCB in the tetanus toxoid-stimulated animals.

In the unstimulated guinea pigs no suppressive activity could be found. Other parameters, such as leukocyte counts, weights of thymus, spleen and lymph nodes and number of follicles and pyroninophilic cells, were in general not sensitive enough to detect an effect of PCB feeding. Absence of effects on weight and histomorphology of thymus and adrenal gland indicated that not stress but PCB itself was responsible for the immunosuppressive activity.

Residues of polychlorinated biphenyls (PCB) have been widely found in tissues of fish and wildlife (Jensen, 1966; Holmes *et al.*, 1967; Holden and Marsden, 1967; Koeman *et al.*, 1967; Risebrough *et al.*, 1968; Koeman *et al.*, 1969; Jensen *et al.*, 1969; Duke *et al.*, 1970). Contamination of human adipose tissue and human milk is also reported (Biros *et al.*, 1970; Acker and Schulte, 1970).

PCB compounds are industrial chemicals that are used as lubricants, heat-transfer agents and insulators; they are also added to paints, synthetic resins, varnishes and waxes to improve their properties. They are highly stable compounds, so PCB can persist in the environment. One source of environmental contamination, dumping of sewage sludge from an industrial area into the sea, has recently been determined (Holden, 1970).

Feeding of PCB to chicks resulted in small spleens (Flick *et al.*, 1965) showing atrophy of the lymphoid tissue (Vos and Koeman, 1970). Lymphopenia, atrophy of the cortex of the thymus and a reduction in the number of the germinal centers in the spleens and

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lymph nodes were found after dermal application of PCB in rabbits (Vos and Beems, 1971). The purpose of the present study was to investigate whether or not these findings were due to an immunosuppressive action of PCB. The experimental design was in part according to Verschuuren *et al.*, (1970). Tetanus toxoid was used to study the humoral response of the lymphoid system.

METHODS

Aroclor 1260, a 60% chlorinated PCB mixture, was obtained from the Monsanto Chemical Co., St. Louis, Missouri (Lot. No. AK-3). This sample was, according to our analytical procedure, free from contamination with chlorinated dibenzofurans (Vos *et al.*, 1970). Biological testing on the skin of rabbits indicated a minor contamination with acnegenic impurity (Vos and Beems, 1971).

The experiment was started with 4-wk-old female albino guinea pigs. The mean body weight was 220 g (189–251 g). The animals were housed in groups of 6. Standard diet (Hope Farms, Woerden) and water were provided ad libitum. The 36 animals were distributed at random into 3 groups receiving diets of 0, 10, and 50 ppm PCB, respectively, for 8 wk. Half of the animals in each group received sc injections of aluminum phosphate-adsorbed tetanus toxoid (5LF) (National Institute of Public Health, Utrecht) in the right footpad at days 35 and 49 to stimulate the lymphoid system, in particular the draining popliteal lymph node. Weight gain was determined weekly. Leukocyte and differential leukocyte counts were made on day 56.

The animals were killed with carbon dioxide gas. Blood was taken from the heart for the determination of serum proteins. Electrophoretic separation was done on cellulose acetate strips (Cellogel Chemetrion, Milan, Italy) in a Veronal buffer (pH 8.6) at a constant voltage of 200 v. Before use, the strips were submerged in the buffer for 10 min. The separation was finished after 15 min, at which time the strips were colored with a solution of 0.5% Ponceau in trichloroacetic acid for 5 min. Then the strips were decolored with a 5% solution of acetic acid in water and were made transparent by treatment with methanol (30 sec), and with a mixture of 87 ml methanol and 13 ml acetic acid for 1 min. After drying at 100°C for 5 min, the fully transparent strips were scanned on a Rotorscan apparatus (Automazione Industriale, Milan).

Popliteal lymph nodes were weighed and frozen in isopentane suspended in liquid nitrogen. Acetone-fixed cryostat sections (7μ) were incubated with fluorescein-isothiocyanate labeled rabbit antiguinea pig γ-globulin serum (RaGp/FITC; Nordic Diagnostics, Tilburg) for the detection of all γ-globulin-containing cells. These sections were studied in a fluorescence microscope. The number of γ-globulin-containing cells was arbitrarily graded from + to ++++. Photographs were taken from a representative area of all sections. These photographs were ranged in order of increasing number of γ-globulin-containing cells to make statistical evaluation possible.

Thymus, spleen, and cervical and mesenteric lymph nodes were fixed in Carnoy fluid for 24 hr at 4°C and embedded in Paraplast. Serial sections (5 μ) were stained with hematoxylin-eosin, for reticulin, and with methyl green-pyronin according to Elias (1969) using pyronin Y (Gurr Ltd., London). The sections stained for reticulin were used for an estimation of the number of the follicles as a measure of stimulation of the

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humoral system (C. J. Meijer, personal communication). Methyl green-pyronin stain was used to grade from + to ++++ for number (and location) of pyroninophilic cells.

The liver, heart, kidneys and adrenals were weighed, and, along with lung, pancreas, esophagus, stomach, small intestine, cecum, large intestine, skin of back and ear skin, fixed in 10% buffered formalin. Paraplast sections were stained with hematoxylin-eosin.

The significance of any difference between treatment and control groups was measured using the Wilcoxon test for 2 unrelated samples on a 1-tail significance level (van der Waerden, 1957).

RESULTS

Weight gain of guinea pigs receiving PCB was less than that of controls. Terminal body weights of unstimulated animals of the 50 ppm group were significantly lower ($P \leq 0.025$) than the weights of the unstimulated controls. Liver weights of the stimulated 50 ppm group were significantly increased ($P \leq 0.025$). The relative liver weights of both the stimulated 10 and 50 ppm groups were also significantly higher ($P \leq 0.005$). Absolute and relative weights of the mesenteric lymph nodes in the stimulated 10 and 50 ppm groups were significantly increased ($P \leq 0.05$). Absolute and relative weights of the cervical lymph nodes in the unstimulated 10 ppm group were significantly decreased ($P \leq 0.005$ and $P \leq 0.05$, respectively). The significantly reduced weights of kidneys ($P \leq 0.05$) in the stimulated 10 ppm and the unstimulated 50 ppm group, of adrenals ($P \leq 0.025$) in the unstimulated 50 ppm group, and of spleens ($P \leq 0.05$) in the unstimulated 10 ppm group were probably due to the lower terminal weights of PCB-fed animals; no significant differences were seen in the organ: body weight ratios. The total number of leukocytes were not altered significantly. Differential leukocyte counts did not differ between the controls and PCB-fed groups.

The results of the electrophoresis are given in Table 1. The effect of tetanus toxoid injections on the humoral immune response can be seen readily from the increased

TABLE 1

SERUM PROTEINS CONTENTS OF TETANUS TOXOID-STIMULATED AND UNSTIMULATED GUINEA PIGS FED 0.10 AND 50 PPM PCB FOR 8 WK[a]

Dietary (mg/kg)	No. of animals	Total protein (g/100 ml)	Albumin[b] (%)	α-Globulin[b] (%)	β-Globulin[b] (%)	γ-Globulin[b] (%)
0	6	5.15 ± 0.27	63.3 ± 2.2	16.3 ± 3.0	9.3 ± 0.8	11.1 ± 2.7
10	6	5.13 ± 0.38	67.2 ± 3.6[c]	12.8 ± 1.1	8.2 ± 1.4	11.8 ± 2.6
50	6	5.28 ± 0.37	63.6 ± 2.4	14.8 ± 3.2	8.9 ± 1.3	12.6 ± 1.4
0 + TT[f]	6	5.33 ± 0.20	57.7 ± 3.1	16.9 ± 2.7	8.6 ± 1.7	17.0 ± 2.9
10 + TT	6	5.48 ± 0.10	62.9 ± 3.1[c]	19.7 ± 1.1[c]	7.1 ± 1.3	10.4 ± 2.2[e]
50 + TT	6	5.42 ± 0.38	55.7 ± 3.1	21.0 ± 2.1[d]	7.2 ± 1.8	16.3 ± 3.2

[a] Mean values ± SD.
[b] Percentage of the total protein content.
[c] Significantly different from controls at the level $P \leq 0.05$.
[d] Significantly different from controls at the level $P \leq 0.01$.
[e] Significantly different from controls at the level $P \leq 0.005$.
[f] TT = tetanus toxoid.

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TABLE 2

γ-GLOBULIN-CONTAINING CELLS IN POPLITEAL LYMPH NODES OF TETANUS TOXOID-STIMULATED AND UNSTIMULATED GUINEA PIGS FED 0.10 AND 50 PPM PCB FOR 8 WK.

Dietary conc. of PCB (ppm)	No. of animals	γ-Globulin-containing cells in popliteal lymph nodes							
		Stimulated (right lymph node)				Unstimulated (left lymph node)			
		±[a]	++	+++	++++	±	−	++	+++
0 + TT[b]	6	1	1	2	2	0	4	2	0
10 + TT	6	3	3	0	0[c]	5	0	0	1[d]
50 + TT	6	1	4	1	0[c]	4	1	1	0[d]
0	6					2	2	2	0
10	6					4	2	0	0
50	6					2	1	3	0

[a] + to ++++ = a comparative estimate of the number of cells.
[b] TT = tetanus toxoid.
[c] Significantly different from controls at the level $P < 0.005$.
[d] Significantly different from controls at the level $P < 0.05$.
[e] Significantly different from controls at the level $P < 0.10$.

γ-globulin levels (Table 1) and the increased number of γ-globulin-containing cells (Table 2) in the stimulated animals when compared with the unstimulated animals. γ-Globulin level was very significantly decreased in the stimulated 10 ppm group when compared with the stimulated control group. Significantly increased α-globulin values were found in the 10 and 50 ppm stimulated groups, as well as significantly increased albumin values in the stimulated and unstimulated 10 ppm group. A comparative estimate of the number of γ-globulin-containing cells in popliteal lymph nodes, visualized by the direct fluorescent antibody technique, is given in Table 2. Statistical evaluation, made possible by arranging the photographs of representative areas of the lymph nodes in order of increasing number of γ-globulin-containing cells, revealed a significantly reduced number of these cells in the stimulated right lymph nodes of the 10 ppm tetanus toxoid-stimulated group (Figs. 1 and 2) and in the unstimulated left lymph nodes of the stimulated 10 and 50 ppm groups.

Estimation of the number of pyroninophilic cells in the cervical lymph nodes indicated a decreased number of these cells in the stimulated 50 ppm group and in the nonstimulated 10 ppm group when compared with their respective control groups. No shift in the number of pyroninophilic cells could be found in the spleen and mesenteric lymph nodes between controls and PCB-fed animals, with or without antigenic stimulation. Nor was a general trend found in the number of follicles in the spleen and the right popliteal, cervical and mesenteric lymph nodes. No differences could be found in the number and morphology of Hassal corpuscles and the cortex–marrow relation in the thymuses of control and PCB-fed groups.

No evidence of any PCB-induced change was observed at histological examination of the hematoxylin-eosin stained sections of the other organs, including liver, kidney, adrenal and skin.

DISCUSSION

The significantly reduced number of γ-globulin-containing cells in popliteal lymph nodes of PCB-fed guinea pigs stimulated with tetanus toxoid (Table 2; Figs. 1 and 2),

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FIG. 1

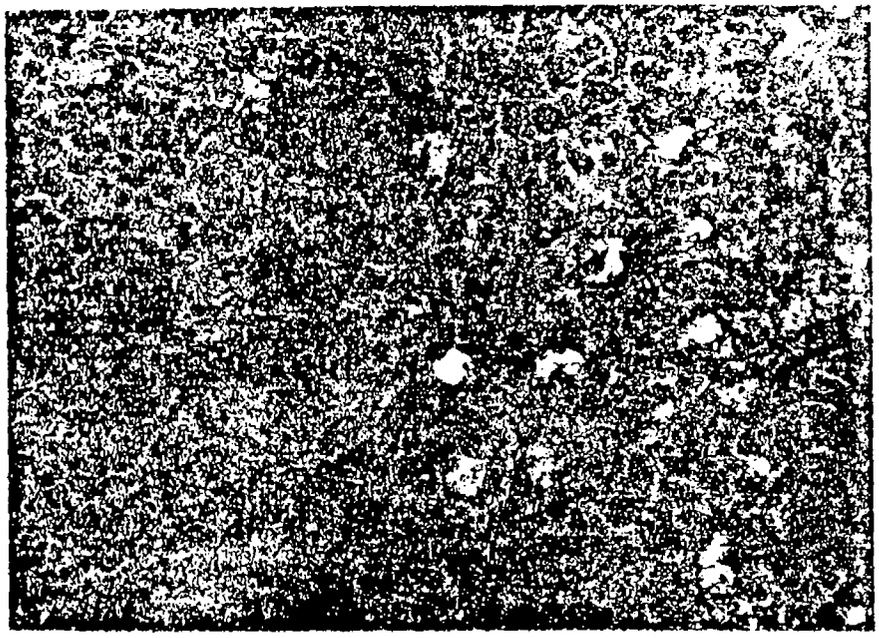


FIG. 2.

FIGS. 1 and 2. γ-Globulin-containing cells in tetanus toxoid-stimulated right popliteal lym node of an animal from the control (Fig. 1) and from the 10 ppm group (Fig. 2). The figures sh representative numbers of immunologically active cells as visualized with the direct fluorescent antibo technique. Cryostat sections. ×320.

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together with the significantly decreased γ-globulin level in the serum of stimulated animals fed 10 ppm PCB (Table 1), demonstrate that some immunosuppression is produced by PCB-feeding. The decrease of the γ-globulin level was not found to be dose-related. An explanation could be the presence of antigenic stimulation, perhaps due to viruses. To elucidate this point, a more specific technique such as the determination of tetanus antitoxin titer could be necessary. Further study will be conducted. In the popliteal lymph node no decrease in number of follicles could be observed, although the number of follicles is a parameter for humoral immunity. Probably, this parameter is not sensitive enough to detect an immunosuppressive action by this particular compound.

At histological examination of spleen, thymus, cervical and mesenteric lymph nodes, no differences in number of follicles and pyroninophilic cells could be detected, except for a decrease of these cells in the cervical lymph nodes of the stimulated 50 ppm group. The present results show that immunosuppression by PCB at a low feeding level could only be demonstrated using sensitive techniques (fluorescent antibody technique and serum electrophoresis) in tetanus toxoid-stimulated animals.

During the course of the present study, more evidence from the literature for a suppressive action of PCB was obtained (Friend and Trainer, 1970). In their study, feeding of Aroclor 1254 in concentrations of 25, 50 and 100 ppm for 1 wk to ducklings (20 animals per group) did not result in clinical intoxication. Body weights of PCB-fed animals were even higher. Inoculation of the birds with duck hepatitis virus resulted in a significantly higher mortality in the PCB-fed birds compared with the controls. Also in this study, a clear dose-effect relation was absent. Hansen *et al.* (1971) suggest that chronic exposure to Aroclor 1254 increases the susceptibility of fish to disease.

Absence of effects on weight and histomorphology of thymus and adrenal gland indicate that PCB itself, not stress, is responsible for the effect found in the present study. Stress could have been a factor, perhaps, in addition to the immunosuppressive action of PCB in the lymphopenia, thymus atrophy and decreased spleen weights found in previous studies (Vos and Koeman, 1970; Vos and Beems, 1971). The rabbits, used in the latter study also showed severe skin, liver and kidney lesions.

This study clearly demonstrates the danger of immunosuppression by this widespread pollutant even at a low feeding level. In a prior study it was found that feeding of PCB to Japanese quail resulted in increased levels of the enzyme δ-aminolevulinic acid synthetase (Vos *et al.*, 1971). This induction, considered to be responsible for the porphyrogenic action of PCB, was similarly found at low level feeding.

It should be borne in mind that the present study concerns only the immunosuppressive activity of PCB on the humoral immune response. A possible influence on the cell-mediated immunity will be the subject of further study.

ACKNOWLEDGMENTS

The authors are grateful to Dr. E. J. Ruitenberg (National Institute of Public Health, Utrecht) for his help and discussion and to Mr. Th. Wensing (Laboratory of the Clinic of Veterinary Internal Disease, Utrecht; Head: Dr. A. J. H. Schotman) for the cellulose acetate electrophoresis. Mr. A. Janmaat, B.V.Sc., has made valuable linguistic contributions.

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