Effect of Organochlorine Compounds on Serum Proteins

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Of the organochlorine compounds (OCC), the organochlorine insecticides (OCI),(DDT, Dieldrin, BHC etc.) are used as economic poisons, and the polychlorinated biphenyls (PCBs) as industrial materials.

PCBs and OCI persist in nature due to their chemical stability. Their liposolubility explains their storage in animal tissues especially in adipose tissue with increasing concentration in the food chain.

The use of OCI began with DDT spraying of crops and exanthematous typhus prophylaxis towards the end of World War II. DDT was first demonstrated in the adipose tissue of sheep in 1947 and in a professional sprayer in 1948 (9). Since then a large number of publications has proven the ubiquity of OCI in the ecosystem and in the animal body. The toxicity and biological effects of these insecticides have been widely documented.

Although the use of PCBs in industry began about forty years ago and increased in many industrial fields (due to many unique properties), their presence in the animal body was proven only in 1966 (11). At this time the occupational hazards of these compounds were already known (10). The hepatotoxicity of PCBs was experimentally demonstrated as early as 1937 (5) and 1944 (15).

It was only after 1966 that the ubiquitous pollution of the environment with these OCC was demonstrated (1, 3, 4, 8, 12, 13, 16, 17, 18, 20).

The production of PCBs totals hundreds of millions of pounds annually in the United States alone (6). PCB pollution of the global ecosystem has reached the same proportions as that of DDE (6). Contamination is greatest in water animals near industrial areas. The highest residues were found in fish-eating birds and marine mammals.

During the last decade it was demonstrated that relatively small quantities of OCC (which would not have been detectable by means available 15 - 20 years ago) may influence natural metabolic

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processes and thus some physiological phenomena in the animal body. Some of these biological effects are the consequences of the action of OCC as inducers of liver microsomal enzymes. It seems that PCBs are more powerful inducers of liver hydroxylases than OCI (20).

In previous studies on the immunological impact of OCI we demonstrated the action of OCI on serum proteins in lowering γ-globulin and raising albumin (23, 24, 25) and moderating the immunological response to antigens (soluble or particulate)(23, 24).

This paper reports serum protein level changes in animals receiving OCI or/and PCBs in a comparative study of the two groups of OCC, using a low chlorinated group of PCBs, namely Arochlor-1221.

Materials and Methods.

Sixty young local strain male rabbits weighing between 1400 - 2120 g were housed two in a cage and fed an ordinary diet ad libitum. The rabbits were divided into six groups of 10 animals each. The organochlorine compounds were given in the drinking water for three months as follows:

Group 1: controls
Group 2 received 200 ppm recrystallized pp'-DDT
Group 3 received 50 ppm Dieldrin
Group 4 received 200 ppm PCBs 1221
Group 5 received 100 ppm pp'-DDT and 100 ppm PCBs 1221
Group 6 received 25 ppm Dieldrin and 100 ppm PCBs 1221

The organochlorine compounds being insoluble in water, were first dissolved in 6 ml ethyl alcohol and diluted to 1 liter with tap water so that the drinking water of all the groups contained 6‰ ethyl alcohol.

During the experiment 19 rabbits died, namely 5, 3, 3, 2, 2, 4 in groups 1, 2, 3, 4, 5, 6 respectively. Since the greatest number of rabbits died in the control group, we cannot assign the cause to the treatment received by the animals nor can we argue for a positive influence, since figures are more or less close one to another.

The animals were weighed at the beginning of the experiment and one and three months after it.

Blood samples were taken one and three months after the beginning of the experiment. The serum was separated, inactivated at 56^0 C for 30 minutes and kept at -20^0 C. The plasma was separated after centrifugation and kept at the same temperature.

Plasma OCC levels were assessed in order to determine at what

concentrations of OCC the biological effects detected took place. They also facilitated the evaluation of metabolic interrelationships between different OCC. 1 -2 ml plasma were used for PCB and OCI assessments. PCBs were separated from the OCI by column chromatography on silicic acid - celite. PCBs were eluted from the column with petroleum ether and afterwards the OCI with a mixture of acetonitrile 1%, hexane 19%, and methylene chloride 80% (2). The OCC levels were determined by gas chromatography with electron capture detection and a 6 inch/4 mm spiral glass column containing a mixture of equal parts of 15% QF-1 and 10% DC-200 on 80-100 mesh chromosorb W HP. For quantitation the picks obtained for samples were compared as against picks from standards.

Quantitation of serum immunoglobulins and albumin was carried out by single radial immuno-diffusion method (7).

Four µl of each serum, undiluted and diluted 1/5, using phosphate buffer saline, ph 7.2, 0.15 M (PBS) were put into wells punched out in 1.5% agar plates containing goat anti-rabbit IgG* or IgM *, (diluted 1/10 with PBS). Purified rabbit 7S and 19 S (fraction I from Sephadex G-200 of rabbit serum prepared by us) were used for standard curves. The agar plates were incubated at room temperature. Results were read after 24 hours for the 7 S fraction and after 72 hours for the 19 S fraction of gamma globulins.

Lyophilized IgG fraction of Goat anti Rabbit Albumin serum** was dissolved in 5 ml PBS (5mg Ab/ml) and diluted 1/10 in the same buffer. The tested rabbit sera were diluted 1/10 and 1/20. Crystallized rabbit serum albumin* was used for the standard curve. The results were read after 12 hours.

Results and Comments.

Table 1 summarizes the gain in weight of the rabbits during the three months of exposure to OCC. The gain in weight of rabbits receiving Dieldrin for 1 month (group 3) was 20% smaller when compared to the control group. The weight of the animals in group 3 was improved in the following two months. The gain in weight in the fourth group (PCBs receiving rabbits) was less than in controls after one and three months of exposure respectively.

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* Miles Lab.

** Cappel Lab.

Tables 2 and 3 show the OCC plasma levels reached after one and three months of administration of OCC.

Administration of p,p'-DDT during one month led to a mean plasma level of 584 ppb total DDT. After two more months it reached about 3 ppm. At this latter stage we observed considerable rise in the plasma level of Dieldrin, BHC and PCBs which are current constituents of the food and water the animals received.

The plasma level of Dieldrin after three months of Dieldrin administration was 258 ppb. The total DDT plasma level was higher in these animals than in the controls.

Exposure to PCBs leads to a rise in plasma level of PCBs to 227 and 1098 ppb after one and three months respectively. The amount of total DDT rose significantly in these animals, but less than after Dieldrin administration.

When DDT and PCB were administered concomitantly (group 5), but in half the dosages received by the groups 2 and 4, lower plasma values for DDT and PCBs (although progressively increasing) were reached after three months. The Dieldrin and BHC plasma levels were increased when compared to the control group, as happened in the rabbits which received only DDT (group 2).

Dieldrin and PCBs administered concomitantly in half doses led to a certain rise of Dieldrin and PCBs plasma level in these rabbits (group 6). The accompanying increase in plasma levels of total DDT occurred as in the rabbits receiving only Dieldrin (group 3) although to a lesser extent. BHC plasma level was comparable to the controls, as in the rabbits receiving Dieldrin (group 3) or PCBs (group 4).

Metabolic interactions between different OCC compounds are exemplified by our findings. The enhanced toxicity of DDT and Dieldrin by PCBs, beyond an additive effect, found in flies (14) may be explained as a result of such metabolic interactions which lead to higher plasma levels of some OCI than those expected from the exposure to a given single compound.

The mean serum protein levels in the different groups are shown in tables 4 and 5. There was a slight reduction in the IgG fraction of γ-globulins after one month of OCC administration, which was accentuated after two months, and especially in the group receiving PCBs (p=2). The serum levels of IgM fraction of gamma globulins are lowest in the groups which received two OCC concomitantly (groups 5 and 6) ($p < 0.01$).

After one month of exposure the albumin serum level shows a rise in rabbits receiving DDT (group2) and Dieldrin (group 3)($p < 0.01$)

TABLE 1

Gain in Weight (G). Mean ± SD.

Group	Gain in weight after one month of exposure to OCC	Gain in weight after three months of exposure to OCC
1. Control	1018 ± 143	1642 ± 200
2. DDT	955 ± 255	1553 ± 317
3. Dieldrin	809 ± 320	1531 ± 257
4. PCBs	868 ± 191	1464 ± 251
5. DDT+PCBs	926 ± 146	1635 ± 262
6. Dieldrin+PCBs	1055 ± 146	1655 ± 278

TABLE 2

OCC (ppb) plasma levels after 1 month of OCC administration. Mean ± SD.

Group	Total DDT	Dieldrin	γ-BHC	PCBs
1. Control	14.4 ± 4.0	2.20 ± 0.85	3.90 ± 1.20	8.20 ± 3.69
2. DDT	584.4 ± 943.2	1.50 ± 1.00	2.04 ± 1.83	11.40 ± 5.83
3. Dieldrin	80.3 ± 133.4	0.67 ± 1.19	4.27 ± 1.85	9.85 ± 3.99
4. PCBs	12.0 ± 10.0	1.35 ± 0.83	1.21 ± 0.37	127.10 ± 62.40
5. DDT+PCBs	75.8 ± 61.6	1.80 ± 1.18	2.10 ± 2.65	49.80 ± 37.40
6. Dieldrin+PCBs	75.6 ± 118.3	1.86 ± 0.45	4.05 ± 2.17	35.20 ± 10.00

TABLE 3

OCC (ppb) plasma levels after 3 months of OCC administration. Mean ± SD.

Group	Total DDT	Dieldrin	γ-BHC	PCBs
1. Control	6.2	0.1	3.3	5.7
2. DDT	2949.4 ±1041.2	12.4 ± 14.4	30.4 ± 26.4	64.2 ± 24.6
3. Dieldrin	62.2 ± 38.7	257.7 ± 80.6	5.6 ± 4.9	7.3 ± 0.7
4. PCBs	24.2 ± 24.4	1.1 ± 1.2	3.5 ± 0.6	1098.9 ± 990.3
5. DDT+PCBs	322.6 ± 183.5	6.6 ± 5.7	33.3 ± 28.2	243.4 ± 143.5
6. Dieldrin+PCBs	27.4 ± 26.4	24.6 ± 33.1	3.4 ± 4.4	207.9 ± 182.7

TABLE 4

Serum protein fractions (mg/ml), after 1 month of OCC administration. Mean ± SD.

Group	No. of cases	Gamma globulins				Albumin	%
		7 S	%	19 S	%		
1. Control	9	12.38 ± 2.50	100.00	1.68 ± 0.20	100.00	38.33 ± 2.01*	100.00
2. DDT	10	10.90 ± 1.41	88.05	1.70 ± 0.10	101.19	43.50 ± 2.87	113.49
3. Dieldrin	8	11.20 ± 0.57	90.46	1.69 ± 0.10	100.59	43.37 ± 2.16	113.15
4. PCBs	9	10.95 ± 1.22	88.44	1.62 ± 0.12	96.43	40.60 ± 2.87	105.92
5. DDT+PCBs	10	10.92 ± 1.47	88.21	1.64 ± 0.12	97.62	40.20 ± 2.56	104.88
6. Dieldrin+PCBs	6	10.80 ± 0.97	87.24	1.60 ± 0.01	95.24	41.66 ± 3.28	108.69

* Gr.1 vs gr.2 and 3: $p < 0.01$, gr.1 vs gr.4: $p < 0.10$.

TABLE 5

Serum protein fractions (mg/ml), after 3 months of OCC administration. Mean ± SD.

Group	No. of cases	Gamma globulins				Albumin	%
		7 S	%	19 S	%		
1. Control	5	13.20 ± 2.28*	100.00	1.74 ± 0.01**	100.00	44.00 ± 2.53***	100.00
2. DDT	7	12.31 ± 1.32	93.26	1.71 ± 0.17	98.27	45.43 ± 3.44	103.25
3. Dieldrin	8	10.15 ± 1.66	76.89	1.66 ± 0.03	95.40	45.87 ± 2.88	104.25
4. PCBs	7	9.26 ± 2.21	70.15	1.70 ± 0.14	97.70	38.00 ± 2.53	86.36
5. DDT+PCBs	8	9.87 ± 1.95	74.77	1.57 ± 0.15	90.23	35.87 ± 2.59	81.52
6. Dieldrin+PCBs	6	9.20 ± 2.21	69.70	1.50 ± 0.06	85.21	34.50 ± 2.06	78.41

*Gr.1 vs gr.3,5,6: $p < 0.05$, gr.1 vs gr.4: $p < 0.02$. **Gr.1 vs gr.5,6: $p < 0.01$

***Gr.1 vs gr.4,5,6: $p < 0.01$

which is slowed during the two following months. The administration of PCBs alone (group 4) or together with DDT (group 5) or Dieldrin (group 6) led, after three months of exposure, to a decrease in serum albumin level ($p < 0.01$).

PCBs act in the same way as OCI in lowering serum gamma globulin levels and in different ways in serum albumin levels. This is an example of dissimilar effects of OCI and PCBs.

These effects of OCI on serum proteins are in keeping with our previous findings (23, 24, 25). The findings regarding the effect of PCBs with low chlorine content (Arochlor 1221) on serum protein fractions, demonstrate their influence on one of the most important defense systems of the animal body: the immunological system.

Recently a lowering of the gamma globulin fraction of serum proteins was described in guinea pigs fed 10 ppm PCBs-1254 (22).

Enlargement of the liver and kidneys and atrophy of the spleen are common findings in PCB feeding studies in birds (6). Thymus atrophy and lymphopenia in PCBs receiving rabbits (21) and increased mortalityfrom hepatitis virus in ducklings receiving PCBs (13) has also been described.

These findings emphasize the noxious hazard of OCC pollution of the environment and raise the problem of efficient control.

Summary

Many OCC are at present current constituents of the global ecosystem. Their biological effects are of great interest to ecologists and environmental toxicologists.

This paper deals with the interrelationship between OCC (p,p'-DDT, Dieldrin and Arochlor 1221) and the serum level of protein fractions in rabbits receiving the above-mentioned compounds.

Sixty rabbits were divided into 6 groups given respectively no OCC, 200 ppm p,p'-DDT, 50 ppm Dieldrin, 200 ppm Arochlor-1221, 100 ppm p,p'-DDT and 100 ppm Arochlor-1221, 25 ppm Dieldrin and 100 ppm Arochlor in their drinking water which contained 6‰ ethyl alcohol in all groups.

After p,p'-DDT administration the increased plasma levels of DDT were accompanied by increased levels of Dieldrin,γ-BHC and PCBs, although the animals did not receive extra dosage of these OCC, except the amounts currently present in food and water. Dieldrin

administration led to a concomitant increase in total DDT plasma level. A moderate increase in total DDT plasma level occurred also after the administration of PCBs. When administered concomitantly, in half doses, the same results were obtained at a lower level.

Variations in plasma level of different OCC as a result of feeding a given compound may explain differences in the degree of toxicity based on the resultant of metabolic interrelationships of OCC in the animal body.

The serum level of gamma globulin fractions (IgG and IgM) shows a tendency to decrease in rabbits given OCC. The serum albumin level rose in rabbits receiving OCI and fell in those receiving PCBs. In regard to the biological effects of OCC these facts point to a moderation of the activity of the immunological system.

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