Effects of Dieldrin and Gamma BHC on Serum Proteins and PBI

by M. Wassermann, Dora Wassermann, E. Kedar, M. Djavaherian, and Simi Cucos

Department of Occupational Health
Hebrew University-Hadassah Medical School
Jerusalem, Israel

Previous studies regarding the effect of DDT on defence systems of the animal body revealed: A. A decrease of gamma globulins serum level in p,p'-DDT receiving rats. B. A moderation of the antibody titer to ovalbumin (1) and an activation of DDT detoxication in p,p'-DDT - ovalbumin receiving rats (3). C. In rabbits exposed to p,p'-DDT, an impaired immunological response occurred. The serum gamma globulins (especially the 7 S fraction) as well as the antibody titer against Salmonella typhi were significantly decreased (2). D. The plasma total DDT level was smaller in p,p'-DDT - Salmonella receiving rabbits than in those receiving only p,p'-DDT (2). Our similar findings in rats and rabbits suggested the occurrence of a bidirectional metabolic relationship between the fate of a toxic compound in the animal body and the answer to the presence of an antigen (soluble or particulate): The presence of a toxic compound (p,p'-DDT) in the body leads to a moderation in the response to an antigen, while the presence of an antigen leads to an increased detoxication of the toxic compound. E. DDT moderated the adrenal reaction to surgical stress (1).

F. In people occupationally exposed to DDT, cholesterol (4), and serum PBI (5) homeostasis were concerned.

This paper reports on some biological effects of Dieldrin and gamma BHC, as far as rabbit serum proteins and protein bound iodine (PBI) are concerned.

Materials and Method

Sixty young local strain male rabbits (body weight 1.350 - 2.400 kg.) were used. The animals were housed two in a cage and fed an ordinary diet ad libitum.

The animals were distributed into six groups.

Group 1. (10 rabbits) - controls.
Group 2. (10 rabbits) - received 3 weekly injections of Salmonella typhi starting two weeks after the beginning of the experiment.
Group 3. (8 rabbits) - received 50 ppm Dieldrin in their

Bulletin of Environmental Contamination & Toxicology,
Vol. 8, No. 3, 1972, © by Springer-Verlag New York Inc.

drinking water during the five weeks of the experiment.

Group 4. (12 rabbits) - received Dieldrin as group 3 and Salmonella typhi injections as group 2.

Group 5. (8 rabbits) - received 50 ppm gamma-BHC in their drinking water during the five weeks of the experiment.

Group 6. (12 rabbits) - received gamma-BHC as group 5 and Salmonella typhi injections as group 2.

The Dieldrin and the gamma BHC used in these experiments were 95 % technical grade. 50 mg Dieldrin or gamma-BHC were dissolved in 3 cc ethyl alcohol and brought to 1 liter with tap water. A 3 %o solution of ethyl alcohol was given also to the control groups (groups 1 and 2).

Salmonella typhi (standard antigen) was washed with saline and brought to a concentration of $1x10^8$ and $5x10^8$ bacteria per ml. $1x10^8$ bacteria were given intravenously in the first injection, $5x10^8$ intravenously, in the second, and $5x10^8$ intraperitoneally in the third injection.

During the experiment four rabbits died; one in group 2 (Salmonella typhi receiving rabbits) and 3 in group 4 (Dieldrin plus Salmonella typhi group).

The animals were weighed at the beginning and at the end of the experiment.

They were bled one week after the last injection. A serum aliquote for each rabbit was separated, inactivated at 56^0 C for 30 minutes and kept at -20^0 C for serum proteins estimations. Another serum aliquote was kept at -20^0 C for PBI assessment. Plasma was separated and kept at the same temperature for OCI assessments.

Quantitation of serum immunoglobulins by single radial immunodiffusion method. Quantitation of rabbits 7 S and 19 S fractions of gamma globulins was done by the method of Fahey (6). Four µl of each serum, undiluted and diluted 1/5 using phosphate buffer saline, Ph 7.2 , 0.15 M (PBS) were inserted into wells punched out in 1.5 % agar plates containing goat anti-rabbit IgG* or IgM*. (diluted 1/10 with PBS). Purified rabbits 7 S* 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.

Albumin was determined using the Fahey method (6). 5 mg leophylised µ-globulin fraction of anti-rabbit serum albumin**

TABLE 1. Body weight (g)

Group	Statistical Evaluation	Body Weight		Gain in weight
		Initial	Final	
1. Control	Range	1400 - 2300	2400 - 2950	500 - 1400
	Mean ± SD	1610 ± 254	2685 ± 167	1075 ± 285
2. Salmonella (S)	Range	1350 - 1900	2200 - 2880	700 - 1380
	Mean ± SD	1622 ± 161	2585 ± 220	952 ± 214
	Group 1 vs. group 2			$p > 0.10$
3. Dieldrin	Range	1350 - 2400	2100 - 2750	350 - 1050
	Mean ± SD	1694 ± 300	2419 ± 171	725 ± 215
	Group 1 vs. group 3			$p < 0.02$
4. Dieldrin + S	Range	1450 - 2050	2150 - 2650	520 - 1050
	Mean + SD	1756 ± 224	2436 ± 173	680 ± 152
	Group 1 vs. group 4			$p < 0.01$
5. Gamma BHC	Range	1400 - 2200	2440 - 3000	800 - 1450
	Mean + SD	1756 ± 264	2781 ± 188	1025 ± 192
	Group 1 vs. group 5			$p > 0.10$
6. Gamma BHC + S	Range	1350 - 2050	2350 - 2800	700 - 1220
	Mean + SD	1725 ± 225	2568 ± 156	843 ± 187
	Group 1 vs. group 6			$p < 0.05$

were dissolved in 5 ml. PBS and diluted 1/10 in the same buffer. Crystalized rabbit albumin* was used for the standard curve. The results were read after 12 hours.

Protein bound iodine (PBI) was assessed using the Barber Dry-Ash method (7) partly modified.

Plasma OCI levels. One to two ml of plasma were extracted by shaking followed by centrifugation three times with a total of 20 ml n-hexane. The 20 ml extract was evaporated to 2 ml and cleaned on a florisil column. The volume of the extract was then concentrated to 0.5 ml. 5 µl were injected into a Packard dual electron capture gas chromatograph model A-7420-99, having a QF 5 % and a SE 3% chromosorb column.

Results and Comments.

Table 1 shows the gain in weight for the six groups of rabbits. The Dieldrin receiving groups (group 3 and 4) have a smaller gain in weight which differs statistically from the controls (group 1 and 2 respectively).

Table 2 summarizes the ranges and mean values of plasma organochlorine insecticides (OCI). The groups receiving Dieldrin (Group 3 and 4) have Dieldrin plasma levels of 1.7 and 1.3 ppb which are significantly different from the other groups (groups 1, 2, 5 and 6) which did not receive special Dieldrin dosage. Nevertheless the other OCI naturally occurring in food and water were present in an amount more or less comparable to the other groups. In the gamma BHC receiving groups (group 5 and 6), the plasma level of BHC (of respectively 22.9 and 18.8 ppb) were the highest and significantly different from the other groups which did not receive special gamma BHC dosage. The plasma level of the other OCI, naturally occurring in the animal body, was more or less similar to the other groups.

Table 3 shows the mean values of serum albumin and serum globulins (7 S and 19 S fractions).

The 7 S fraction of serum gamma globulins has the tendency to increase after Salmonella typhi administration. This increase is inhibited by the presence of the given level of Dieldrin or gamma BHC. These compounds do not affect the increase of the 19 S fraction in rabbits receiving Salmonella. The administration of Dieldrin and gamma-BHC brought about an increase of serum albumin (group 3 and 5). ($p<0.01$, $p<0.05$). The serum level of albumin decreased in a certain degree in rabbits receiving Salmonella (group 2). This depressing effect of Salmonella is better evidenced in the groups 4 and 6, which received respectively Dieldrin and gamma BHC plus Sal-

*Miles Lab. **Cappel Lab.

TABLE 2.

Plasma OCI levels. Mean ± SD (ppb)

Compound \ Group	1. Control	2. Salmonella (S)	3. Dieldrin	4. Dieldrin + S	5. Gamma BHC	6. Gamma BHC + S
Total p,p'-DDT	5.3 ± 2.8	5.5 ± 3.0	6.0 ± 4.2	4.4 ± 2.2	4.7 ± 3.3	3.4 ± 1.4
Total o,p'-DDT	1.4 ± 0.2	2.9 ± 1.2	1.6 ± 1.2	1.2 ± 1.2	1.6 ± 1.5	1.5 ± 1.1
Total DDT	6.7 ± 3.2	8.4 ± 4.1	7.5 ± 4.8	5.6 ± 3.2	6.3 ± 4.8	4.9 ± 5.0
α-BHC	4.0 ± 2.2	3.4 ± 2.0	2.7 ± 1.7	2.7 ± 2.4	2.4 ± 2.0	3.0 ± 2.0
β-BHC	3.1 ± 4.0	1.8 ± 2.6	1.4 ± 2.6	2.3 ± 5.5	1.0 ± 1.3	1.0 ± 1.4
γ-BHC	4.4 ± 2.0	11.3 ± 10.0	4.5 ± 2.8	8.3 ± 7.2	22.9 ± 10.0	18.8 ± 10.0
Total BHC	11.5 ± 2.2	16.5 ± 12.2	8.7 ± 4.3	13.2 ± 13.7	26.3 ± 2.4	22.7 ± 2.4
Dieldrin	0.2 ± 0.2	0.5 ± 0.4	1.7 ± 1.1	1.3 ± 1.1	0.1 ± 0.1	0.3 ± 0.5

TABLE 3.
Serum Protein Fractions

Group	Statistical Evaluation	Gamma Globulins 7 S	%	Gamma Globulins 19 S	%	Albumin	%	%
1. Control	Range	9.0 - 17.0		1.7 - 2.2		33 - 41		
	Mean + SD	12.60 ± 2.22	100	1.93 ± 0.15	100	37.2± 5.3	100	100
2. Salmonella (S)	Range	9.0 - 22.5		2.0 - 3.3		33 - 38		
	Mean + SD	15.10 ± 4.92	120	2.40 ± 0.61	124	35.0± 5.9	94.1	
	Gr. 1 vs. Gr. 2			$p < 0.05$		$p > 0.10$		
	Gr. 1 vs. Gr. 3					$p < 0.01$		
	Gr. 1 vs. Gr. 5					$p < 0.05$		
3. Dieldrin	Range	8.0 - 16.0		1.8 - 2.4		37 - 48		
	Mean ± SD	12.10 ± 2.51	100	2.10 ± 0.22	100	44.0± 3.74	100	117.7
4. Dieldrin + S	Range	8.5 + 16.0		1.9 + 2.8		35 + 44		
	Mean ± SD	12.50 ± 2.66	103.3	2.38 ± 0.36	113.3	38.0± 3.8	86.8	
	Gr. 3 vs. Gr. 4			$p < 0.05$		$p < 0.01$		
5. Gamma BHC	Range	10.0 - 16.5		1.7 - 2.3		36 - 48		
	Mean ± SD	13.60 ± 2.36	100	1.86 ± 0.22	100	43.1± 4.8	100	115.9
6. Gamma BHC + S	Range	10.0 - 19.0		2.0 - 2.6		34 - 38		
	Mean ± SD	13.30 ± 2.68	97.8	2.30 ± 0.17	123.7	35.0± 5.7	81.3	
	Gr. 5 vs. Gr. 6			$p < 0.02$		$p < 0.01$		

monella. In other words, the tendency of increase in serum levels of gamma globulins after administration of a particular antigen (Salmonella typhi) was prevented by the presence of an increased level of Dieldrin or gamma-BHC in the animal body, while the increase in serum albumin induced by Dieldrin and gamma-BHC was cancelled by the administration of Salmonella typhi. These findings are in accordance with our previous work. (1, 2).

A tendency to the decrease of Dieldrin or gamma-BHC plasma levels was observed in the Dieldrin-Salmonella and the gamma-BHC-Salmonella receiving groups, when compared to the groups receiving only Dieldrin or gamma-BHC. These results confirm the bidirectional metabolic relationship between the detoxication of a foreign compound (OCI) in the presence of an antigen and the response of the animal body to an antigen in the presence of a foreign compound, as we described in our previous work.

Table 4 shows that Dieldrin and gamma-BHC lead to a significant decrease of the serum PBI when compared to the control group ($p<0.01$). The administration of Salmonella also produced a decrease in the serum PBI level when given alone (group 2) or together with Dieldrin (group 4) or gamma-BHC (group 6) ($p<0.01$).

The decrease of PBI in the case of a high Dieldrin or gamma-BHC level, may be the result of an increased metabolisation of thyroxine, since OCI produce liver SER proliferation and as a consequence increased microsomal enzyme activity. A hyperfunctional morphologic appearance of thyroids from Dieldrin treated rats (8) suggests a compensated hypothyroidism in Dieldrin receiving rats.

The same findings in Salmonella receiving rabbits raise the problem of the implications for thyroid hormones homeostasis in the presence of an antigen.

Summary.

Dieldrin and gamma BHC biological effects in rabbits were investigated as far as serum proteins and protein bound iodine (PBI) are concerned.

Sixty rabbits were distributed in six groups. Groups 1, 3, 5 received respectively 3%o alcohol, 50 ppm Dieldrin in 3%o alcohol and 50 ppm gamma BHC in 3%o alcohol, as drinking water for the five weeks of the experiment. Groups 2, 4, 6 similar respectively to groups 1, 3, 5 received in addition Salmonella typhi in three weekly injections beginning with the third week of the experiment. The Dieldrin receiving groups (group 3 and 4) had a smaller gain in weight.

The tendency of the 7 S fraction of serum gamma globu-

TABLE 4.
PBI Serum Levels (µg/100 ml)

Group	Statistical Evaluation	
1. Control	Range	7.2 - 7.8
	Mean ± SD	7.38 ± 0.17
2. Salmonella (S)	Range	4.1 - 6.0
	Mean ± SD	5.15 ± 0.71
	Group 1 vs. group 2	$p \leq 0.01$
3. Dieldrin	Range	4.0 - 7.0
	Mean ± SD	5.60 ± 1.00
	Group 1 vs. group 3	$p \leq 0.01$
4. Dieldrin + S	Range	4.4 - 7.0
	Mean ± SD	5.31 ± 0.81
	Group 1 vs. group 4	$p \leq 0.01$
5. Gamma BHC	Range	5.6 - 7.0
	Mean ± SD	6.31 ± 0.55
	Group 1 vs. group 5	$p \leq 0.01$
6. Gamma BHC + S	Range	4.2 - 6.6
	Mean ± SD	5.40 ± 0.95
	Group 1 vs. group 6	$p \leq 0.01$

lins to increase after Salmonella typhi administration (group 2) was inhibited in the presence of Dieldrin and gamma-BHC, received respectively by groups 4 and 6.

Dieldrin and gamma-BHC receiving groups had a statistically significant increase of serum albumin which was inhibited in the groups receiving in addition Salmonella typhi injections (group 4 and 6).

The amount of plasma Dieldrin or plasma gamma BHC was lowered in the presence of Salmonella typhi.

These findings confirm the bidirectional metabolic relationship between the detoxication processes of a foreign compound (Dieldrin and gamma-BHC) in the presence of an antigen, and the response of the animal body to an antigen in the presence of a foreign compound as we described in our previous work.

Dieldrin and gamma-BHC caused a significant decrease of the serum PBI, when compared to the control group ($p<0.01$).

Salmonella administered alone or in association with Dieldrin or gamma-BHC also produced a decrease in the serum PBI level ($p<0.01$).

The decrease of serum PBI level in OCI receiving rabbits confirms the findings we described in people occupationally exposed to OCI.

References.

1. WASSERMANN, M., WASSERMANN, DORA, GERSHON, ZIPORA and ZELLERMAYER, L., Ann. N.Y. Acad. Sci. 160, 393 (1969)
2. WASSERMANN, M., WASSERMANN, DORA, KEDAR, E. and DJAVAHERIAN, M., Bull. Environ. Cont and Toxicol. 6, 426 (1971)
3. WASSERMANN, M., WASSERMANN, DORA and LAZAROVICI, S., Proc. XVIth Intl. Cong. Hlth., Tokyo (Sept. 1969)
4. WASSERMANN, M., WASSERMANN, DORA, ARONOVSKI, ITA, IVRIANI, J. and ROSENFELD, DEBORA, Bull. Environ. Cont. and Toxicol. 5, 368 (1970)
5. WASSERMANN, DORA, WASSERMANN, M. and DJAVAHERIAN, M., Bull. Environ. Cont. and Toxicol.6, 85 (1971)
6. FAHEY, J.L. and MCKELVEY, E. M., Immun. 94, 98 (1965)
7. BARKER, S. B., HUMPHREY, M.J. and SALEY, M.H., J. clin. Invest 30, 55 (1951)
8. WASSERMANN, DORA and WASSERMANN, M., Unpublished data.

Supported by Grant 06-004-3 from the USA Department of Health, Education and Welfare, Public Health Service, Bureau of Occupational Safety and Health, Environmental Control Administration.