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Effect of DDT on the Immune System in Swiss Albino Mice during Adult and Perinatal Exposure: Humoral Responses

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Although, the use of 1,1,1 trichloro 2-2 bis (chlorophenyl) ethane (DDT) as a pesticide is banned in western countries, it is still widely used in developing coutries like India. DDT's effects on immune responses has received only scarce attention so far. However immunostimulatory properties (Lukic et al 1973) and immunosuppressive properties (Wassermen et al 1969, Street & Sharma 1975, Kannan & Sharma 1979, Banerjee et al 1986, Banerjee 1987) were suggested in animal models. Nevertheless these studies provide only limited information on the dose and time-dependence both features repeatedly shown to be of prime importance. The present study was thus undertaken to investigate the effect of graded doses of DDT on the immune system over a period of six months. As high DDT residues are present in fat and milk (Laug et al 1951; Lactatia Kanja 1986), effects in weaned mice exposed pre and postnatally to DDT were also assessed.

MATERIALS AND METHODS

Female Swiss albino mice weighing around 18-20 gms. were purchased from the National Institute of Nutrition, Hyderabad, India. Initially the LD 50 value of DDT (Aldrich Chemical Co., 99% pure) in these animals was determined and was found to be 316 mg/kg body weight. The concentrations that we have chosen in the present investigation are in the range of 6 to 666 times the acceptable daily intake (ADI) value and cover the range in which it is present in the population of some countries (GEMS report 1983). The animals were divided into four groups. Control animals received normal feed and the other groups received 0.0316 (0.001 LD 50), 0.316 and 3.16 mg/kg mixed in feed. After six months some animals from 0.316 and 3.16 mg/kg groups were mated with normal male mice. After weaning, the litters within each group were divided into two groups. One group was given the control feed and the other group was give the same concentration of DDT as their mothers. These two groups are referred to as Prenatal exposure and Pre and postnatal exposure groups. Six mice from each group were immunized intraperitoneally with 0.2 ml of 10% sheep red blood cells (SRBC) v/v in saline. Five days later, the animals were sacrificed and the spleen cell suspension was made in RPMI-1640 medium (Gibco, U.S.A.) with 10% foetal calf serum (FCS) (Sera Labs. U.K.).

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The plaque forming cells (PFC) were determined by modified method of Jerne's haemolytic plaque forming assay (Jerne & Nordin 1963).

The primary IgM T independent antibody secreting cells were evaluated by injecting SRBC coated with LPS according to the method of Halliday and Weab (1965). The mice were immunized with 0.2 ml of 10% LPS-SRBC (v/v) in saline intraperitoneally. On day four, the animals were sacrificed and the T independent plaque forming cells were enumerated as above.

Spleen cells were prepared from control and DDT exposed mice and suspended in RPMI-1640 medium containing 10% FCS. The cells were cultured in 96 well microtitre plate at 37°C under 5% CO_2 in air with or without LPS. After two days, 3H thymidine (0.5 uC)[2](BARC, India) was added and the cells were cultured for another 24 hr. The cells were harvested on to glass fibre filters by a harvester (PHD, Carbridge) and the incorporated 3H thymdine was counted in Packard B scintillation counter. The results were expressed as stimulatory index (S.I).

RESULTS AND DISCUSSION

Our results indicate that the exposure of adult mice to DDT at high concentrations suppress the primary IgM PFC response to SRBC and lymphoproliferative response. Mice exposed to 0.0316 mg/kg for 4 to 16 weeks did not show any significant alteration (Tables 1, 3 & 5) but further exposure showed stimulation of responses. In contrast mice exposed to 0.316 and 3.16 mg/kg for 4 to 16 weeks showed stimulation

Table 1. Number of Ig M plaques/10^6 lymphocytes to SRBC antigen in adult mice

DDT mg/kg/bw	Duration of Exposure in weeks					
	4	8	12	16	20	24
Control	550 ±40.8	519 ±14.4	445 ±8.1	433 ±12.4	513 ±36.3	488 ±74.8
0.0316	552^{ns} ±19.2	641* ±26.7	670** ±36.8	669** ±23.1	786** ±34.7	901* ±28.0
0.316	668* ±20.4	825** ±38.6	1432** ±39.6	670** ±11.0	426$^{£}$ ±37.7	228* ±43.9
3.16	737* ±9.5	1094** ±69.5	2103** ±167	953* ±158	325$^{£}$ ±55.1	98** ±11.5

Values expressed are no. of PFC/10^6 lymphocytes.
**P < 0.001, *P < 0.01, £P < 0.05, ns = not significant

Table 2. Number of Ig M plaques/10^6 lymphocytes to SRBC antigen following pre and postnatal exposure

Time after birth	0.316 mg/kg/bw		3.16 mg/kg/bw	
	DDT-	DDT+	DDT-	DDT+
8 Wks	260 ±19.2	279 ±11.1	179 ±15.7	162 ±44.3
12 Wks	266 ±33.0	153 ±28.6	147 ±13.8	61 ±13.6

Values expressed are no. of PFC/10^6 lymphocytes and significant at $P < 0.001$. DDT-=prenatal exposure. DDT+=pre and postnatal exposure

Table 3. Number of Ig M plaques/10^6 lymphocytes to T independent antigen in adult mice

DDT mg/kg/bw	Duration of Exposure in weeks					
	4	8	12	16	20	24
Control	2126 ±125	2113 ±83	2042 ±29	2084 ±63.4	1937 ±50.2	2048 ±106
0.0316	2294^{ns} ±19.1	2556** ±45	2766* ±52.7	2618* ±67	2867* ±482	3206** ±155
0.316	$2423^{\$}$ ±15	2909** ±89	3529** ±115	3720* ±86	1356* ±86	574** ±203
3.16	3171* ±138	3753** ±73	3298** ±61	2531* ±107	1133** ±77.7	538* ±27.7

Values expressed are no. of PFC/10^6 lymphocytes
**$P < 0.001$, *$P < 0.01$, \$$P < 0.02$, ns = not significant

and then sharp reduction in responses up to 24 weeks. Such reduction in PFC numbers was also reported (Wassermen et al 1969) in mice exposed to 20, 50 and 100 ppm of DDT for 12 weeks only. This early suppression might be due to the high concentration of DDT used which corresponds to 31.6, 79 and 158 mg/kg in our case. In another study (Luckic et al 1973) in which the rats were fed with 40 mg/kg of technical DDT, a potentiating influence on both humoral and cellular immune response was observed. Further, the enhancement was attributed to the removal of normal inhibitory homeostatic activity of the adrenal gluco-corticoids. Our findings, however suggest that DDT residues in the lower concentration or in the higher concentration groups,

Table 4. Number of Ig M plaques/10^6 lymphocytes to T independent antigen following pre and postnatal exposure

Time after birth	0.316 mg/kg/bw		3.16 mg/kg/bw	
	DDT-	DDT+	DDT-	DDT+
8 Wks	771 ±135	545 ±43.0	664 ±67.9	463 ±23.4
12 Wks	591 ±39.2	351 ±17.6	641 ±48.8	198 ±61.9

Values expressed are no. of PFC/10^6 lymphocytes and significant at $P < 0.001$, DDT-=prenatal exposure. DDT+=pre and postnatal exposure

Table 5. Effect of DDT on lymphoproliferative responses to LPS in adult mice

DDT mg/kg/bw	Duration of Exposure in weeks					
	4	8	12	16	20	24
Control	18.3 ±0.40	17.0 ±0.80	19.8 ±1.28	16.9 ±1.28	17.9 ±0.92	14.0 ±1.0
0.0316	18.4^{ns} ±0.43	18.4^{ns} ±0.43	20.4^{ns} ±1.65	16.4^{ns} ±0.71	$19.7^{£}$ ±0.57	17.3** ±1.2
0.316	23.3* ±0.86	$24.7^{\$}$ ±2.9	$24.8^{£}$ ±2.1	20.9* ±0.98	9.43** ±0.12	5.76** ±0.28
3.16	$28.9^{£}$ ±4.75	30.2** ±2.20	33.4* ±2.25	18.9^{ns} ±1.45	10.0** ±0.53	6.6** ±1.63

Values expressed are stimulatory indices
**$P < 0.001$, *$P < 0.01$, \$$P < 0.002$, £$P < 0.05$ ns = not significant

must reach certain threshold levels to cause either stimulation or suppression of the immune system.

In weaned mice in which the mothers were exposed to 0.316 and 3.16 mg/kg of DDT for six months, the PFC numbers to SRBC as well as LPS-SRBC and lymphoproliferative responses to LPS were decreased by around 37% irrespective of the concentration of DDT exposed (Table 2, 4 & 6). At the end of 8 and 12 weeks these responses were further decreased (around 60-80%) in both prenatal and combined pre and post-natal groups irrespective of the concentration of DDT. As there is no significant difference between prenatal and combined

Table 6. Effect of pre and postnatal exposure of DDT on lymphoproliferative response to LPS

Time after birth	0.316 mg/kg/bw		3.16 mg/kg/bw	
	DDT-	DDT+	DDT-	DDT+
8 Wks	4.7 ±0.4	4.0 ±0.41	3.9 ±0.54	4.0 ±0.41
12 Wks	3.0 ±0.56	2.7 ±0.24	2.5 ±0.30	2.2 ±0.33

Values expressed are stimulatory indices and significant at $P < 0.001$ DDT-=prenatal exposure. DDT+=pre and postnatal exposure

pre and postnatal groups, these results indicate that the DDT residues that got accumulated during embryogenesis through placenta and during nursing (Suckling) period through milk, are high enough to cause suppression of the immune system. As the development of the immune system in mouse is mostly a postpartum event, we assume that the DDT residues have interfered at some stage in the maturation of hematopoietic stem cells. Therefore additional exposure to DDT in the weaned mice has no significant effect on the immune system.

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REFERENCES

Banerjee BD, Ramachandran M, Hussaian QZ (1986) Subchronic effect of DDT on humoral immune responses in mice. Bull Environ Contam Toxical 37: 433-440

Banerjee BD, (1987) Subchronic effect of DDT on humoral immune responses to a thumus - independent antigen (Bacterial lipopolysacaccharide) in mice. Bull Environ Contam Toxical 39: 822

Global Environmental Monitoring System (Gems) Report (1983) Assessment of human exposure to selected organochlorine compounds through biological monitoring. Prepared for United Nations Environment Programme and the World Health Organization by the Swedish National Food Administration

Halliday WJ, Weab M (1965) A plaque technique for counting cells which produce antibacterial antibody. Eur J Immunol 6: 58

Jerne NK, Nordin AA (1963) plaque formation in agar by antibody producing cells. Science 140: 405

Kannan K, Sharma JD (1979) Defective lymphocyte transformation by DDT: in vitro responsiveness of rabbit peripheral blood lymphocytes to PHA Ind J Exp Biol 17: 805-806

Lactitia Kanja (1986) Organochlorine pesticides in human milk from different areas of Kenya 1983-84 J Toxical Environ Health 19: 449-464

Laug EP, Kunz FM, Prickett CS (1951) Occurence of DDT in human fat and milk Arch Ind Hygiene Occup Med 3: 245
Lukic ML, Popeskovic LJ, Jamkovic BD (1973) Potentiation of immune responsiveness in rats treated with DDT. Fed Proc Fen Ann Soc Exp Biol 32: 1037
Street JC, Sharma RP (1975) Alterations of induced cellular and humoral immune response by pesticites and chemicals of environmental concern: quantitative studies of immuno suppression by DDT, aroclor 1254, carbaryl, carbofuran and methylparathion. Toxicol Appl Pharmacol 32: 587-602
Wasserman M, Wasserman D, Geralon Z, Zellermover L (1969) Effects of organochlorine insecticides on body defense system. Annn NY Acad Sci 160: 393-401

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