Bull. Environ. Contam. Toxicol. (1993) 51:795–802

Environmental Contamination and Toxicology

Effect of Sub-Chronic Lindane Exposure on Humoral and Cell-Mediated Immune Responses in Albino Rats

Sutapa Saha[1] and B. D. Banerjee[2]

[1]Department of Biochemistry and Nutrition, All India Institute of Hygiene and Public Health, 110, Chittaranjan Avenue, Calcutta-700 073 and [2]Department of Biochemistry, University College of Medical Sciences and G.T.B. Hospital, University of Delhi, Shahdara, Delhi-110 095, India

Lindane, the gamma isomer of 1,2,3,4,5,6 hexachloro cyclohexane (HCH), is one of the most widely used organochlorine insecticides for agriculture and public health programs. The large scale application of lindane over a number of years, coupled with its extreme stability and slow metabolism, has lead to environmental contamination and potential health hazards. These residues find their ways into various food commodities forming an important source of dietary lindane and ultimate carry-over from food chain into the mammalian system.

The analysis of human body fat, blood, milk and food stuffs both in India and abroad, has revealed the presence of significant amounts of lindane (Ramachandran et al. 1984; WHO 1991). A serious outbreak of poisoning due to prolonged consumption of lindane contaminated food stuffs occured in India (Ramachandran et al. 1982). Human health affects of these pesticide residues are yet to be answered satisfactorily. Inadvertent alteration of the immune responses by organochlorine pesticide residues has been of increasing concern (Banerjee and Hussain 1986; Banerjee et al. 1986, Banerjee 1987a, 1987b) At present very little is known about the effects of lindane on the immune res- ponse. The findings of Desi et al. (1978) demonstrated a decrease in serum antibody of Salmonella typhi in rabbits after sub-acute treatment with lindane. Further, Dewan et al. (1980) also observed similar inhibition of the specific production of antibodies against Salmonella typhi and Salmonella paratyphi in rats acutely intoxicated by lindane. These studies have indicated that important changes in humoral immune response may occur after acute or chronic exposure to lindane. However, the effect of lindane on cell-mediated immune response remain largely unanswered. Furthermore, the consequence to prolonged dietary exposure to low doses (sub-toxic) of lindane on

Send reprint requests to B D Banerjee at above address.

the immune system has not been studied so far. Hence, systematic studies on dose-time relationship in different experimental animals appear to be essential before evaluating the potentiality of lindane on immune system of mammalian host. Keeping this in view the present study was designed to examine the effect of sub-chronic doses of lindane on humoral and cell-mediated immune responses in albino rats.

MATERIALS AND METHODS

Lindane (97% purity) was obtained from Aldrich Chemical Company Ltd., Gillingham, Dorset, U.K. The sources of other chemicals were the same as presented in an earlier paper (Banerjee et al. 1992). Wister male albino rats weighing 85-90 grams were divided randomly into four groups. The animals were maintained under similar condition as reported earlier (Banerjee and Hussain 1986) and provided with standard laboratory diet containing 0 (control), 5, 20 or 30 ppm of lindane and water *ad libitum* for 22 weeks. The preparation of the diets and exposure of animals to the toxicant has been described previously (Banerjee et al. 1986; Banerjee and Hussain 1986). Food consumption, general condition and any other clinical symptoms were looked for daily. Body weights were recorded weekly.

Animals were immunized subcutaneously with tetanus toxoid (0.2 ml) mixed with an equal volume of Freund's complete adjuvant, 20 days before terminating the exposure. These immunized rats were injected intraperitoneally with 5 ml sterile liquid paraffin 48 hr before use. An equal number of animals in each dose group were maintained unstimulated. Unstimulated rats were treated similarly except they were not immunized with tetanus toxoid. Rats (10-12 animals/group) were randomly selected from each group and sacrificed at 8, 12, 18 and 22 weeks of exposure. Blood samples, peritoneal macrophages, liver, spleen and thymus were collected as described earlier (Banerjee and Hussain 1986). The serum antibody titre to tetanus toxoid was estimated by indirect haemagglutination technique, quantitation of serum immunoglobulin (IgM and IgG) was carried out by single radial immunodiffusion, serum albumin and globulin fractions were studied by zone electrophoresis, and leucocyte and macrophage migration inhibition were assayed *in vitro* by capillary method according to the procedures as described in detail earlier (Banerjee and Hussain 1986).

The results are expressed as mean and standard deviation (S.D). The student's t-test and one-way analysis of variance [ANOVA] were employed to assess the statistical significance of the treatment effects.

RESULTS AND DISCUSSION

Exposure of rats to lindane in the diet at levels of 5-30 ppm for 8-22 weeks produced no overt toxicity signs and symptoms. No significant differences were noted in mortality rate, body growth rates and food intake between controls and treated rats. These animals did not show any significant alteration in spleen and thymus weights throughout the course of study (Table-1). The absence of any effect of lindane on body and organ (spleen and thymus) weights at these exposure level indicates that this compound itself did not produce any stress responsible for the observed immuno-suppressive effect in the present study. Further the results suggest that change in immune competence occured prior to any changes in spleen and thymus weights.

The serum albumin versus globulin ratio, antibody titre and immunoglobulin levels were studied for the assessment of humoral immune response. The effect of lindane

Table 1. Relative organ weights and serum albumin versus globulin ratio in tetanus toxoid stimulated rats exposed to various concentrations and durations of lindane*

Duration of exposure (wk)	Level of exposure (ppm)	Spleen wt/BW ratio x 10^{-3}	Thymus wt/BW ratio x 10^{-3}	Albumin/Globulin ratio Unstimulated**	Stimulated
8	0	3.77 ± 0.23	2.45 ± 0.15	1.25 ± 0.20	1.10 ± 0.20
	5	3.70 ± 0.66	2.40 ± 0.42	1.25 ± 0.25	1.00 ± 0.15
	20	3.80 ± 0.60	2.11 ± 0.82	1.30 ± 0.10	1.05 ± 0.10
	30	3.61 ± 0.12	2.15 ± 0.21	1.28 ± 0.14	1.00 ± 0.12
12	0	3.60 ± 0.24	2.20 ± 0.41	1.15 ± 0.20	0.98 ± 0.22
	5	3.61 ± 0.25	2.12 ± 0.34	1.20 ± 0.18	1.00 ± 0.13
	20	3.44 ± 0.23	2.15 ± 0.44	1.28 ± 0.32	1.10 ± 0.20
	30	3.70 ± 0.55	2.00 ± 0.50	1.30 ± 0.12	1.05 ± 0.10
18	0	3.35 ± 0.12	1.90 ± 0.20	1.18 ± 0.10	0.96 ± 0.26
	5	3.38 ± 0.18	1.76 ± 0.12	1.20 ± 0.12	0.95 ± 0.31
	20	3.41 ± 0.55	1.71 ± 0.50	1.32 ± 0.23	0.95 ± 0.25
	30	3.48 ± 0.45	1.77 ± 0.38	1.30 ± 0.15	1.02 ± 0.07[a]
22	0	3.12 ± 0.20	1.80 ± 0.25	1.12 ± 0.13	0.80 ± 0.14
	5	3.00 ± 0.19	1.78 ± 0.12	1.20 ± 0.11	1.08 ± 0.17
	20	3.17 ± 0.41	1.70 ± 0.17	1.29 ± 0.10	0.98 ± 0.10[b]
	30	2.88 ± 0.30	1.78 ± 0.14	1.34 ± 0.10	1.05 ± 0.14[b]

* Data presented as the mean value ± S.D. of 10-12 rats in each group. a. Significantly different from the respective control, $p<0.02$ (t-test), $p<0.05$ (ANOVA); b. $p<0.01$ (t-test), $p<0.05$ (ANOVA)

** Unstimulated rats were treated similarly except immunization.

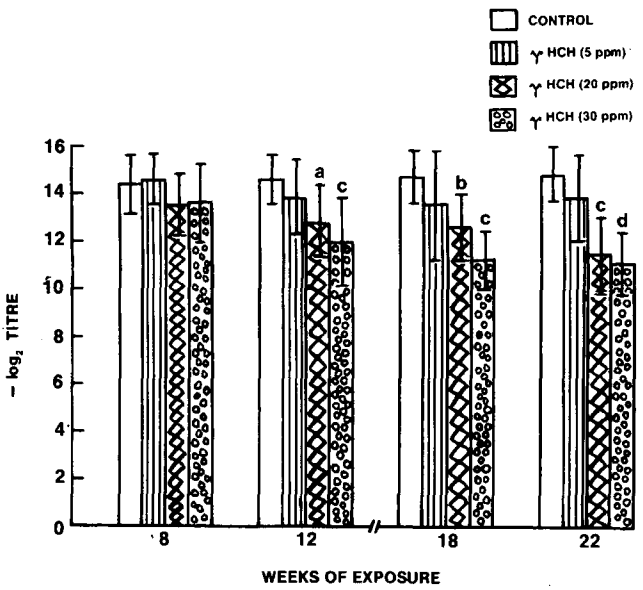

Figure 1. Antibody response to tetanus toxoid in rats exposed to vrious concentrations and duration of lindane (τ-HCH). Results are expressed as - $\log_2$ antibody titre. Height of the bar represants mean ± SD of 10-12 rats in each group, a-significantly lower than respective control by statistical comparison, $p<0.05$ (t-test or ANOVA); b-$p<0.02$ (t-test), $p<0.05$ (ANOVA); c-$p<0.01$ (t-test), $p<0.05$ (ANOVA); d-$p<0.01$ (t-test or ANOVA).

on serum albumin and globulin in tetanus toxoid-stimulated and unstimulated rats are given in Table 1. Albumin and globulin concentrations in serum were consistent and unaffected by lindane treatments imposed. However, the effect of tetanus toxoid injection in humoral immune response can be seen readily from increased globulin level (decreased A/G ratio) in stimulated animals. Globulin level was significantly decreased (increased A/G ratio) in the stimulated 20 ppm lindane group exposed for 22 weeks and 30 ppm lindane group exposed for 18-22 weeks when compared with the stimulated control groups. A similar decrease in serum globulin fraction has been reported in rats exposed to organochlorine pesticides (Banerjee 1987b).

Rats exposed to 20 or 30 ppm lindane for 12-22 weeks showed significant decrease in serum antibody titre to tetanus toxoid (Figure 1). Results indicated consistent and significant effects on antibody response, particularly when considered in relation to dose levels and duration of exposure. A similar decrease in serum antibody titre to Salmonella typhi or Salmonella paratyphi was observed in rats and rabbits after chronic treatment with lindane (Desi et al. 1978; Dewan et al. 1980).

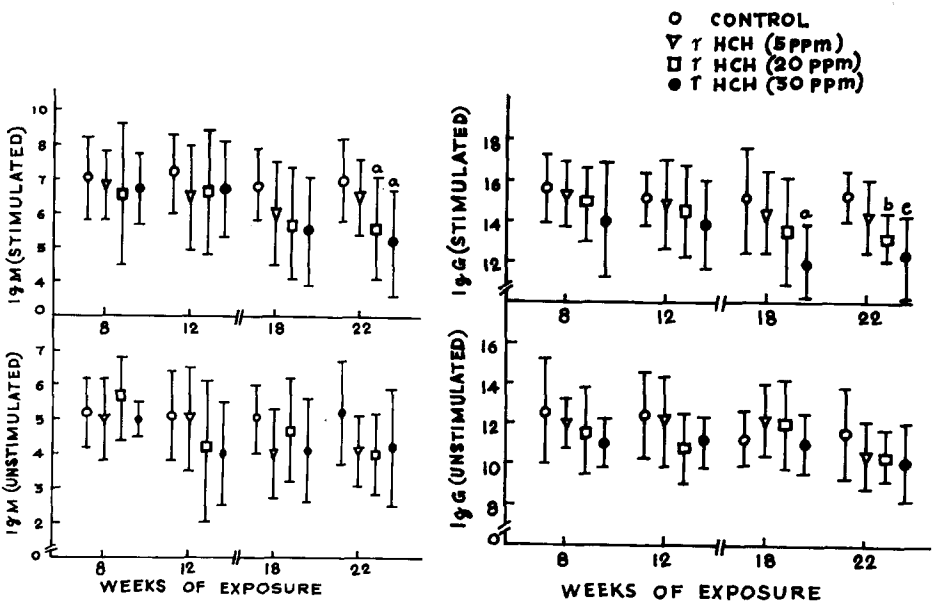

Figure 2. Serum immunoglobulin (IgM and IgG) concentrations in tetanus toxoid unstimulated and stimulated rats exposed to various levels of lindane (γ-HCH). Immunoglobulin concentrations are presented as mean ± SD in mg/ml of 10-12 rats per group. a-$p<0.05$ (t-test or ANOVA), b-$p<0.02$ (t-test), $p<0.05$ (ANOVA); c-$p<0.01$ (t-test or ANOVA).

The effect of lindane on serum immunoglobulin (IgM and IgG) levels are shown in Figure 2. The serum immunoglobulin levels did not show any significant change after lindane exposure. The IgG and IgM fractions of serum immunoglobulin tend to increase after tetanus toxoid administration. This increase in IgG level after tetanus toxoid immunization was significantly lower in rats exposed to 20 ppm lindane for 22 weeks or 30 ppm lindane for 18-22 weeks as compared to antigen stimulated control and found to be well correlated with decreased globulin levels in these animals (Table 1). However increase in IgM level after tetanus toxoid immunization was only significantly lower after 22 weeks in rats exposed to 20 or 30 ppm lindane. Such an increase in serum IgG level after Salmonella typhi administration was also inhibited in rabbits exposed to 50 ppm lindane for five weeks (Wassermann et al. 1972).

The effect of lindane on cell-mediated immune response was evaluated with the help of macrophage migration inhibition (MMI) and leucocyte migration inhibition (LMI) tests. Figure 3 summarizes the effect of lindane exposure on cell-mediated immune response. Rats exposed

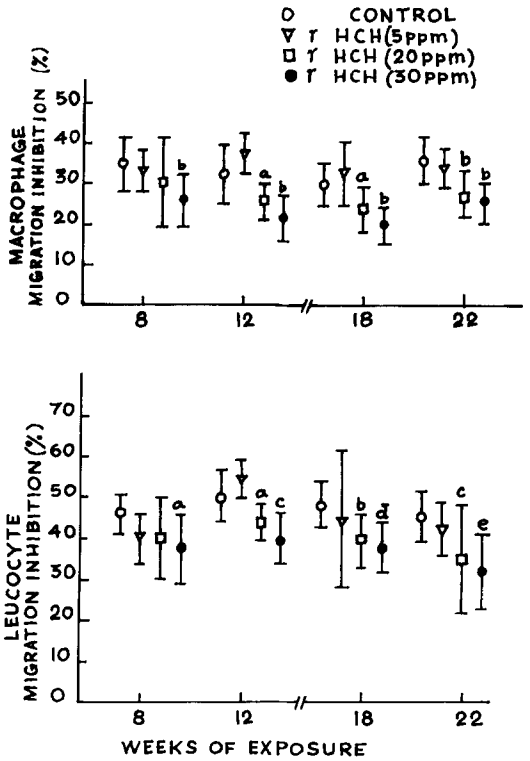

Figure 3. Leucocyte and macrophage migration inhibition tests in rats exposed to various concentrations and durations of lindane (τ-HCH). Each value respresent the mean percentage migration inhibition ± SD of 10-12 rats per group. a-$p<0.05$ (t-test or ANOVA); b-$p<0.02$ (t-test), $p<0.05$ (ANOVA); c-$p<0.02$ (t-test), $p<0.05$ (ANOVA); d-$p<0.01$ (t-test), $p<0.05$ (ANOVA); e-$p<0.01$ (t-test or ANOVA).

to 20 ppm lindane for 12-22 weeks or 30 ppm lindane for 8-22 weeks showed significant decrease in LMI and MMI responses. Lindane appeared to suppress lymphokine production as suggested earlier for other organochlorine compounds (Banerjee and Hussain 1986; Banerjee 1987b). Dose-time dependent inhibition were found when both the ability to produce MMI and LMI were evaluated.

Appreciable quantities of lindane residues have been detected in air, water, soil sediment, aquatic and terrestrial organism from India (Dikshith et al. 1990; Jani et al. 1991; Kausik et al. 1991). The persistence and extreme stability of lindane in the environment is the ultimate source of contamination in the diet. Significant amount of lindane residues have been reported in food grains, animal products, oil and vegetable from India (Krishnamurti 1984; WHO 1991). Further, relatively high level of lindane has been reported in

body fat, blood and milk of Indian population (Ramachandra et al. 1984, Krishnamurti 1984). In a separate study Ramachandran et al. (1982) investigated an out-break of poisoning, in an Indian village population, due to the prolonged consumption of HCH-contaminated food stuffs over a period of nearly two years. It is intriguing to note from this study that the six food stuffs predominantly consumed were rice, wheat, wheat flour, black gram and peas and containing respectively 9.05, 62.26, 8.32, 4.63 and 3.01 mg/kg of HCH. Furthermore, Indians have been reported to carry the highest body burden of HCH in addition to the DDT content (Ramachandran et al. 1982; 1984; Krishnamurti 1984). Keeping in view the residue levels in food commodities, it was considered appropriate to incorporate lindane 5 to 30 ppm in the diet of experimental animals for the purpose of sub-chronic toxic study.

It is clear from our study that immune system may be a sensitive target for lindane. Immunotoxicity of lindane in rats was observed at concentrations which have been reported to be no-observed adverse effect level (WHO 1991). It is apparent that a more complete understanding of the toxicity of lindane is necessary to study human health hazards and establish guidelines for acceptable residues in the environment. The effect of lindane on immune responses are more time dependent than dose, and suggest a threshold susceptibility to exposure. It is emphasized that the threshold levels of the lindane below which no effect would be seen depends on the method of testing for immune responses, animal species, endocrine and nutritional status of the host and type of antigen against which the responses are studied (Banerjee et al. 1986; Banerjee and Hussain 1986; Banerjee 1987a, 1987b, Banerjee et al. 1992). Although it appears that the depression of cell-mediated immunity extends to the primary humoral response, more light can be thrown in this direction by studying the response to a thymus independent antigen (Banerjee 1987a). It is now important to elucidate the phenomenon in order to understand its mechanism of immunosuppression and the possible health hazards due to continued use of lindane.

Acknowledgment. The authors are grateful to Professor S. Chaudhuri, School of Tropical Medicine, Calcutta, Professor B.N. Ghosh, Director, and Professor Indira Chakravarty, Head, Department of Biochemistry and Nutrition, AIIHPH, Calcutta for providing facilities.

REFERENCES

Banerjee BD, Hussain QZ (1986) Effect of sub-chronic endosulfan exposure on humoral and cell-mediated immune responses in rats. Arch Toxicol 59:279-284

Banerjee BD, Ramachandran M, Hussain QZ (1986) Sub-chronic effect of DDT on humoral immune response in mice. Bull Environ Contam Toxicol 37:433-441

Banerjee BD (1987a) Sub-chronic effect of DDT on humoral immune response to a thymus-independent antigen (bacterial lipopoly-saccharide) in mice. Bull Environ Contam Toxicol 39:822-826

Banerjee BD (1987b) Effects of sub-chronic DDT exposure on humoral and cell-mediated immune responses in albino rats. Bull Environ Contam Toxicol 39:827-834

Banerjee BD, Saha S, Ghosh KK, Nandy P (1992) Effects of Tri-cresyl phosphate on humoral and cell mediated immune responses in albino rats. Bull Environ Contam Toxicol 49:312-317

Desi I, Varga L, Farkas I (1978) Studies on the immunosuppressive effect of organochlorine and organophosphoric pesticides in subacute experiments. J Hyg Epidemiol Microbiol Immunol 22:115-123

Dewan A, Gupta SK, Jain JP, Kashyap SK (1980) Effect of Lindane on antibody response to typhoid vaccine in weanling rats. J Environ Sci Health, 15:395-402

Dikshith TSS, Raizada RB, Kumar SN, Srivastava MK, Kulshrestha SK, Adholia U N (1990) Residues of DDT and HCH in major sources of drinking water in Bhopal, India. Bull Environ Contam Toxicol 45:389-393

Jani JP, Raiyani CV, Mistry JS, Patel JS, Desai NM, Kashyap SK (1991) Residues of organochlorine pesticides and polycyclic aromatic hydrocarbons in drinking water of Ahmedabad city, India. Bull Environ Contam Toxicol 47:381-385

Kausik CP, Agarwal HC, Pillai MKK (1991) Dry aerial fallout of organochlorine insecticide residues in Delhi, India. Environ Pollut 71:83-86

Krishnamurti CR (1984) Pesticide residues in food and biological tissue. Indian National Science Academy, New Delhi

Ramachandran M, Banerjee BD, Gulati M, Grover A, Zaidi SSA, Hussain QZ (1984) DDT and HCH residues in body fat and blood samples from Delhi hospitals. Indian J Med Res 80:590-593

Ramachandran M, Chand B, Pyarelal A, Hussain QZ (1982) An out break of poisoning due to consumption of HCH admixed food stuffs in an Indian population. Nutr Reports Int 26:377-380

Wassermann M, Wassermann D, Kedar E, Djdvaherian M, Cucoss S (1972) Effects of dieldrin and gamma BHC on serum proteins and PBI. Bull Environ Contam Toxicol 8:177-185

WHO (1991) Lindane. Environmental Health Criteria 124. World Health Organization, Geneva

Received January 28, 1993; accepted May 1, 1993.