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Humoral Immunity in Mice Following Oral Administration of Selected Pesticides

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Introduction

Various pesticides or their degradation products have been implicated as potential causes of several detrimental biological effects. For example, many pesticides are extremely toxic to mammals or other non-target organisms (GAINES 1968; TREON and CLEVELAND 1955). In addition, some pesticides have been demonstrated to be either carcinogenic (TOMATIS et al. 1972; TOMATIS et al. 1974; TURUSOV et al. 1973) or mutagenic (KELLY-GARVERT and LEGATOR 1973; LEGATOR et al. 1969). Pesticides have also been implicated as potential inducers of hypersensitivity in humans (MILBY and EPSTEIN 1964). Several recent studies have appeared which indicate that certain pesticides may also have the potential to influence a host's ability to mount a immune response (BOLKHOVITIANOVA and ALEXEVICH 1968; WASSERMANN et al. 1969a; WASSERMAN et al. 1969b). The impetus for this study was a recent review article by ERCEGOVICH (1973) which revealed the relative paucity of information regarding the immunological aspects of pesticide exposure. The present study was designed to investigate the effects of representatives of several widely used pesticide classes on the primary humoral immune response of an experimental animal. The time of pesticide exposure, relative to immunization and the subsequent immune response, as well as the effect of pesticide dose were investigated.

Materials and Methods

Female Balb/c mice, weighing between 12 and 25 grams and obtained from Cumberland View Farms (Clinton, Tenn.) or Laboratory Supply, Inc. (Indianapolis, Ind.), were used in this study. Within individual experiments all animals which served as either immunized controls or experimental (pesticide treated and immunized) animals, were of the same weight, ±0.5 g. Test compounds were selected for this study on the basis of their importance in the field of pesticide chemistry. All pesticides used in this study were obtained from the Pesticide Research Laboratory, The Pennsylvania State University, University Park, PA. The following pesticides were used in this investigation: DDT, [1,1,-trichloro-

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2,2-bis(p-chlorophenyl) ethane]; Parathion [0,0-diethyl-0-p-nitrophenylphosphorothioate]; Ametryne [2-ethylamino-4-isopropylamino-6-methylmercapto-s-triazine]; Carbaryl [1-naphthyl-N-methylcarbamate]; and Chlordimeform [N'-(4-chloro-o-toyl)-N,N-dimethylformamidine]. All pesticides used in this study were administered to test animals via the oral route. Administrations were made by syringe and 18 gauge stomach tube in a total volume of 0.5 ml. Chlordimeform is extremely water soluble when formulated as the HCl salt and was administered in distilled water. The other four test compounds were all highly water insoluble. These were either dissolved, suspended, or emulsified in corn oil and administered in this manner. Appropriate controls were performed for each compound.

The effect of pesticide administration on humoral immune competence was measured by the immunoplaque in gel technique originally described by JERNE (1963). The number of antibody plaque forming cells (PFC) per plate was counted and the PFC/spleen calculated for each animal. The results are expressed as the ratio of the PFC/spleen when each experimental group is compared to its appropriate control.

For the studies of the effects of pesticide administration on humoral immune competence, three types of experiments were performed. The first experiments involved the single administration of a pesticide, at various times relative to immunization. In these studies the pesticide was administered either five days before (-5 day), on the day of (0 day), or two days after (+2 day) immunization. In the second type of study animals received daily doses of the pesticide for eight consecutive days and were immunized on day nine. In the third type of experiment animals received daily doses of pesticide for 28 days and were immunized on day 29. The time of assay for all studies of humoral immunity was four days post-immunization (DPI) which has been demonstrated to be the peak of the primary humoral response (CEGLOWSKI and FRIEDMAN, 1968).

All statistical analyses of the data were performed on a programmed Monroe model 1930 calculator. Values of p below 0.05 were regarded as statistically significant.

Results

The data obtained when mice received a single dose of pesticide are presented in Table 1. The immune responses observed following repeated daily doses of the pesticides are presented in Table 2.

In the case of carbaryl the only treatment which resulted in a statistically significant effect on the primary humoral immune response occurred when 1 LD_{50} was administered 2 DPI. Carbaryl in this case resulted in a statistically significant suppression of the humoral immune response as quantitated by PFC/spleen.

As in the case of carbaryl, the only instance in which a statistically significant suppressive effect of DDT administration occurred when 1 LD_{50} DDT was administered 2 DPI. This statistically significant suppression was accompained by a marked decline in the total number of splenic lymphocytes present. Administration of a single dose of DDT under conditions other than 1 LD_{50} at 2 DPI failed to result in any statistically significant effects on humoral immune competence. Repeated administration of 0.1 LD_{50} each day also had no statistically significant effect on the humoral immune response.

The effect observed in response to acute parathion administration is very similar to results observed for the previous two compounds, carbaryl and DDT. One LD_{50} parathion, administered 2 DPI, resulted in statistically significant suppression of the humoral immune response expressed as PFC/spleen. Accompanying this suppression was a marked decrease in the total number of splenic lymphocytes. The administration of a single dose of parathion under all other conditions, had no statistically significant effect on humoral immune competence.

The administration of 0.1 LD_{50} parathion for 8 days resulted in a statistically significant suppression of the humoral immune response, in terms of PFC/spleen. These animals also exhibited a 20% loss of body weight during the course of the experiment. Weight loss was not evident in either the immunized control group used in this experiment or in any other group of animals used in our studies. The planned 28-day experiment had to be prematurely terminated (10 days) because of the death of some experimental animals, presumably due to accumulated toxic effects of the parathion. This experiment did not reveal any statistically signficant suppression of the humoral immune response. The reasons for this are not clear, since the animals did appear "sick" and indeed several had died.

TABLE 1

Humoral Immune Response of Mice Receiving a Single Dose of Pesticide.

Pesticide	Treatment: Dose LD_{50}	Treatment: Time	Ratio of Antibody Plaque Forming Cells: Experimental/Control
Ametryne	1.0	-5	0.49**
	0.1	-5	1.02
	1.0	0	0.48*
	0.1	0	1.07
	1.0	+2	0.47**
	0.1	+2	0.72
Chlordimeform	1.0	-5	1.08
	0.1	-5	1.05
	1.0	0	0.75*
	0.1	0	0.79
	1.0	+2	0.44**
	0.1	+2	1.11
Parathion	1.0	-5	0.75
	0.1	-5	0.85
	1.0	0	1.16
	0.1	0	0.86
	1.0	+2	0.29**
	0.1	+2	1.02
DDT	1.0	-5	0.77
	0.1	-5	0.97
	1.0	0	0.74
	0.1	0	0.69
	1.0	+2	0.54*
	0.1	+2	0.83
Carbaryl	1.0	-5	0.91
	1.0	0	1.04
	1.0	+2	0.34**

* $p < 0.05$

** $p < 0.01$

LD_{50} - Ametryne ($870_{mg/kg}$), Chkordimeform ($148_{mg/kg}$), Parathion ($22.3_{mg/kg}$), DDT ($300_{mg/kg}$), Carbaryl ($153_{mg/kg}$).

TABLE 2

Humoral Immune Response of Mice Receiving Repeated Daily Doses (0.1 LD_{50}) of Pesticide.

Pesticide	Days Exposed Prior to Immunization	Ratio of Antibody Plaque Forming Cells: Experimental/Control
Ametryne	8	0.76
	28	0.97
Chlordimeform	8	0.89
	28	1.03
Parathion	8	0.66*
	10	0.90
DDT	8	0.88
	28	1.26
Carbaryl	8	0.94
	28	0.97

* $p < 0.05$

The oral administration of 1 LD_{50} chlordimeform, 2 DPI, resulted in statistically significant suppression of the humoral immune response (Table 1 & 2). In addition administration of 1 LD_{50} chlorodimeform on the day of immunization also caused a statistically signficant suppression of humoral immunity. Neither 8 days nor 28 days of administration of chlordimeform at a daily rate of 0.1 LD_{50} had a statistically significant effect on humoral immune competence.

The administration of a single dose of 1 LD_{50} ametryne caused a statistically significant suppression of the humoral immune response at all times tested relative to immunization. Ametryne was the only compound tested for which this type of effect was observed.

Eight-day and 28-day administration of 0.1 LD_{50} ametryne each day caused no statistically significant effect on humoral immune competence. These results are comparable to those noted for carbaryl, DDT, and chlordimeform.

Discussion

Experiments, involving administration of a single pesticide dose, were performed at various times relative to immunization. No precedent for this type of study, in relation to effects of pesticides on immunocompetence, was detected in the literature. As far as can be determined, this is the first time single high dose administrations of pesticides have been monitored for their effects on immunocompetence.

Our current studies indicate that single high dose administrations of all the test pesticides have the potential to suppress an ongoing primary humoral immune response. Chlordimeform and ametryne also caused a statistically significant suppression of the humoral immune response when 1 LD_{50} was administered on the day of immunization. Ametryne, when administered 5 days prior to immunization at a dose of 1 LD_{50} significantly suppressed the humoral immune response. This data indicates that in general, immunologic recovery from this type of pesticide administration is rather rapid. In all cases where the administration of 1 LD_{50} of a pesticide resulted in a statistically significant depression of the PFC/spleen level, there was also a concomitant decline in the total splenic lymphocyte population. This suggests that lymphocyte depletion of the spleen, possibly due to pesticide cytotoxicity, may be the mechanism responsible for the reduced PFC/spleen levels in the pesticide treated animals. Support for this hypothesis comes from work performed by GABLIKS and FRIEDMAN (1969). These investigators found that several pesticides, among them DDT, had *in vitro* cytopathic effects on both HeLa and Chang liver cells. *In vitro* results of this type may not always be comparable to results or effects observed *in vivo*.

Studies involving a single administration of pesticide were also performed using 0.1 LD_{50} as the dose. No statistically significant effect was observed in any of these studies, regardless of pesticide used or the time of administration relative to immunization.

Few investigations have been performed in relation to the potential effects of pesticide exposure on host immune competence. Of the work which has been initiated in this area, the largest portion has been directed toward examining the effects of repeated pesticide exposure. Consequently, our studies were extended to examine the effects of daily sublethal administration of the five test compounds on humoral immune competence. Only one compound, parathion, had a statistically significant effect on the humoral immune response under these conditions. When administered daily for 8 days, 0.1 LD_{50} parathion resulted in a statistically significant decrease in PFC/spleen. One possible explanation for this effect is expressed by O'BRIEN (1967), who states that during degradation, parathion undergoes activative metabolism which can result in the formation of degradation products which would be more toxic than the parent compound. This increased toxicity

might be the cause of a possible cytotoxic effect resulting in a diminution of lymphoid tissue, including lymphocytes. Indeed there was an observed decline in the total splenic lymphocyte population of animals chronically treated with parathion. GABLIKS and FRIEDMAN (1969) have demonstrated that certain pesticides, among them malathion, an organophosphorus compound, become more toxic to chronically exposed HeLa cells cultured *in vitro*. Parathion was the only compound to cause a statistically significant effect on the primary immune response when administered over the long-term. In evaluating the results of these parathion studies it should be noted that the dose of parathion used (0.1 LD_{50}) is a relatively high one, and due to rather rapid environmental degradation of this compound, is not an exposure which would be consistently contacted by many organisms in normal life situations.

Exposure to 0.1 LD_{50} of carbaryl, DDT, chlordimeform, or ametryne for either 8 or 28 days had no significant effect on the humoral immune response in our experimental system. BOLKHOVITIANOVA and ALEXEVICH (1968) have observed a suppression of the humoral immune response to tetanus toxin in mice exposed for 15 days to daily oral administrations of 0.1 LD_{50} carbaryl. Our studies with carbaryl do not present any evidence for suppressive potential under our experimental conditions. WASSERMANN *et al.* (1969a) observed suppression of antibody levels in response to ovalbumin in rats exposed to 200 ppm DDT in their drinking water for 35 days. Again our experiments utilizing DDT for shorter periods of time did not demonstrate any statistically significant effect on the humoral immune response. It should be noted that comparisons between our experimental results and the results presented in the studies just cited are difficult to evaluate due to substantial differences in experimental designs. Biological effects of long-term pesticide exposure, other than effects on immunocompetence should not be discounted. However, our experiments were not designed to investigate nonimmunologic effects such as potential carcinogenesis or mutagenesis.

Our studies indicate that the post-immunization administration of high pesticide concentrations is capable of causing a marked depression in the primary humoral immune response. A determination of the effects of lower dose, long-term, pre-immunization exposure to pesticides on immune competence is indicated. Studies concerning the effects of pesticide exposure on immunologic memory, in terms of the secondary immune response, might be of particular importance. Other immunologic aspects of pesticide exposure such as effects on the reticuloendothelial system or on antigen uptake and processing should also be resolved. Since aerosol exposure to pesticides is widespread, the effects of aerosol exposure on respiratory defense systems, particularly on the IgA system, should be investigated. In summary, a complete and systematic evaluation of the immunologic consequences of pesticide exposure appears warranted.

Summary

Five pesticides (carbaryl, DDT, parathion, chlordimeform, and ametryne) were tested for their effects on the immunocompetence of Balb/c mice. All five of the test pesticides were observed to induce statistically significant suppression of the humoral immune response if administered orally, at near lethal doses, during an ongoing immune response. Ametryne and chlordimeform were also observed to have a suppressive effect on humoral immune competence if orally administered in a sufficiently large quantity, 1 LD_{50}, at the time of, or prior to, immunization. Administration of 0.1 LD_{50} parathion per day, over an extended period of time, resulted in a statistically significant suppression of the humoral immune response.

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