The Immune System and Pesticides

by Leon John Olson

The following article is not easy for a non-scientist to read because of its multitude of technical terms and density of information. With this article in hand, however, a reader will be able to decipher most discussions of pesticide effects on immune systems.

Since very little consideration is currently given to whether pesticide exposure will weaken people's (or other animals') immune system, Olson's discussions of the limitations of standard toxicology tests and the need for including immunotoxicity assays in pesticide testing offer pesticide reform activists a challenge. Olson's overview of research on pesticide immunosuppression demonstrates that the potential for this form of pesticide damage is real. —*Ed.*

Abstract

Immunotoxicology is a relatively new field of toxicology that studies the effect of chemicals and environmental contaminants on immune systems of animals and humans. Although the field is young, a significant body of information has been developed on a wide variety of compounds that affect immune systems and functions of both animals and humans.

Immune system effects are often subtle but can have very serious consequences to an animal's health, such as increased infections, cancers, allergies and autoimmune diseases. An overview of immunology and immunological tests is presented to help understand the issues. The damaging effects of numerous pesticides on various immune components in both animals and humans are discussed.

For several reasons, immunotoxicology testing is rarely required prior to a chemical's use in the United States. However, immunotoxicology is now felt by the author to be at, or rapidly approaching, the point where routine immune system testing can make a valuable contribution to determining a chemical's potential safety for humans and other animals.

Routine toxicity testing currently does not deal with factors such as multiple compound interactions, assaying with either old or very young test animals, or using stressed animals, all factors which greatly increase toxicant effects. These factors can be incorporated into routine testing and would provide far more realistic predictions of a chemical's effects.

Introduction

The effect of environmental contaminants on the immune system of animals or humans is an area of increasing scientific interest. A properly functioning, intact immune system is essential for protection against diseases, cancer, autoimmunity and certain allergic reactions. In the past, toxicologists have been concerned with more classic and easily measurable toxicities such as death, gross organ damage, overt illness or increased cancer rates. The new field of immunotoxicology studies the subtle effects that compounds can exert on the immune system. These effects are more complex, diffuse, and difficult to detect than the classical toxicities.

There is a prevalent philosophy among many toxicologists that if a chemical is going to be restricted or banned because of its overt toxicities, why examine its effects on immune systems? Although at appropriate times there is little argument with this belief, immune system tests can be very useful in determining overall safety when a chemical is not readily seen as toxic. Another generally accepted philosophy in toxicology contends that immunotoxicity would be observed in standard long-term or lifetime animal tests due to increased deaths and severe illnesses by bacteria, viruses and cancers over the study's duration. However, these tests for general toxicity may not pick up immune system damage.

To put this belief into perspective, one should be aware of certain facets of standard toxicity tests. Test animals, such as rats, mice, and rabbits, are chosen with great care for excellent health and normality within the breed. Any animals that appear sick, abnormal, or outside the parameters set up for a healthy animal, are removed before the study starts. The animals are acclimated carefully to any new facilities prior to testing. They are usually started on the test compounds as extremely healthy young adults, fed carefully selected and nutritious diets, and kept in very clean, stable facilities with the best of care. These facilities are maintained to prevent any undue stress on the animal, especially by keeping out any pathogens that could cause diseases. A great deal of money and effort is spent on keeping the animals as healthy as possible throughout the study.

These efforts result in an unexpected condition, i.e., these test animals are the least likely of an animal's population to develop problems from a chemical. If they do succumb, they would be the last in a normal wild population to be affected. In a normal, wild population, whether animal or human, the individuals most likely to be affected are those who are aged, very young, pregnant, sick, or otherwise stressed.

Cyclophosphamide, a very potent immunosuppressant, has been widely used in immunotoxicology as a known control chemical and is used in humans to control cancer (because it slows cell growth) and to prevent transplant rejection, which results from the host immune system trying to destroy the transplant. When cyclophosphamide is used in animal immunotoxicity studies at levels that cause immunosuppression, generally no unusual infectious diseases are encountered. When a pathogen (bacteria, virus, etc., that

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in test protocols [plans] and the extent to which such studies would identify potential immunotoxicities."[6] This lack of agreement is a partial reflection of the diversity of immune functions and available immune tests. The U.S. National Toxicology Program for Immunotoxicology has been working on this area for several years and has recently developed a battery of immune function tests that may be recommended for general use in assessing immune function.[7] The only currently required immune tests known to the author involve U.S. Environmental Protection Agency pesticide assessment guidelines for biorational pesticides (e.g., pheromones, bacteria, protozoa, viruses).[10]

Effects of Pesticides on the Immune System

DDT has probably been the pesticide most studied for immunotoxic effects and for the longest period of time. DDT is still used in some parts of the world and its residues are commonly found in the United States (NCAP NEWS 4(2): 7-9). DDT was found to significantly lower the antibody titer (concentration) to the bacterium *Salmonella typhi* and decrease the gamma globulin (IgG) serum fraction to ovalbumin in rabbits after 35 days exposure to 200 parts per million (ppm) of DDT in the drinking water.[11,12] On the other hand, no consistent effect was found on the anti-*Salmonella pullorum* antibody titer of DDT-fed chicks.[13] DDT increased the mortality of young mice infected with encephalomyocarditis virus compared to mice exposed to the virus, but *not* to DDT.[14] Chickens were shown to have increased susceptibility to a protozoan parasite, *Histomonas meleagridis*, after DDT administration.[15] Increased susceptibility to duck hepatitis virus was found in ducks given DDT.[16] Tissue structure of the thymus and spleen of rabbits exposed to DDT were found to be adversely affected.[17] DDT decreased the phagocytic activity and cell viability of rat peritoneal (abdominal lining) macrophages.[18] However, rabbits, chickens, and rats were not found to change antibody titers to various antigens in several different tests following exposure to DDT.[17,19,20] After a review of the available DDT immunotoxicity data, Koller[4] felt DDT did not appear to seriously impede immune system activity in animals.

In the carbamate class of insecticides, a number of compounds have been shown to affect the immune system in a variety of ways. Methyl carbamate and pyrindol carbamate were found to have little or no effect on selected immune responses in mice or rabbits.[21,22] Carbofuran has been shown to variously affect immunoglobulin subclass levels in mice[22] and increase granulocyte levels, while decreasing lymphocyte and bone marrow cell populations of mice.[24] Carbofuran fed to mice was associated with decreased mitogen activity, reduced immunoglobulins, and increased mortality following challenge by *Salmonella typhimurium*, a bacteria.[25] Primicarb has induced immune hemolytic anemia (damage to the bone marrow affecting output of white and/or red blood cells) in dogs.[26] Ethyl carbamate caused severe myelotoxicity (bone marrow damage) in $B_6C_3F_1$ mice, depressed killer T-cell activity, reduced humoral immunity (B-cells) and increased susceptibility to tumor cell challenge.[21] Carbaryl was found to increase quail susceptibility to a protozoan parasite,[27] reduce splenic plaque-forming cell (spleen antibody-producing cell) numbers in mice[28] and enhance the *in vitro* infectivity of viruses to human lung cells and green monkey kidney cells.[29] Aldicarb was shown to significantly reduce the splenic plaque-forming cell response in outbred white mice fed 1, 10, 100, and 1000 parts per billion (ppb) in the drinking water. The effect was *greatest* at the 1 ppb aldicarb level.[30]

A wide variety of other pesticides have been shown to variously affect immune system components in animals. The fungicide triphenyltin acetate (TPTA) was associated with atrophy of the thymus, reduced plasma cell (B-cell) populations and decreased immunological response to tetanus toxoid in guinea pigs fed 12 ppm TPTA for several weeks.[31] Methyl parathion has been shown to increase the death rates of mice exposed to *Salmonella typhimurium*, decrease the mouse antibody response to *S. typhimurium*, reduce the mouse mitogenic response,[23] and suppress the tuberculin delayed hypersensitivity reaction in rabbits.[17] Dieldrin enhanced the lethal effects of duck hepatitis virus in ducklings,[32] and also reduced the phagocytic activity and viability of rat macrophages. Hexachlorobenzene administered by diet resulted in immune impairment in mice[33,34] and rats.[35] Technical grade pentachlorophenol (PCP) was shown to affect a variety of parameters in mice but analytical grade PCP (i.e., without contaminants) did not.[36] The difference was explained as the result of impurities. Dioxin is a known contaminant of PCP and is a very potent immunosuppressant.[35-39] Dioxin very likely caused the immunotoxicity noted with technical grade PCP. Another study of technical grade PCP in dairy cattle found no differences in the immune parameters tested.[40]

Maleic hydrazide and cycloheximide, two plant growth regulators, were found to decrease the splenic plaque cell count to srbc's in white mice while maleic hydrazide increased the titer (concentration) of hemolysin, the antibody formed in response to srbc's.[41] Chlorocholine chloride and glyphosine were fed to wild deer mice and significantly suppressed the white blood cell count, splenic plaque-forming cell response, and the srbc hemolysin antibody titer. At low levels, glyphosine stimulated the antibody titer, while depressing it at higher concentrations.[42] Dinoseb (a dinitrophenol herbicide), triiodobenzoic acid (TIBA), and gibberellic acid (a synthetic plant hormone) were examined for immunotoxicity in wild deer mice.[43] Dinoseb and TIBA were found to suppress selected immune functions while gibberellic acid generally enhanced the immune assays. Chlorocholine chloride, when tested in combination with a polychlorinated biphenyl (PCB) and other stress conditions such as reduced lactation (milk production), reduced feed, and reduced water, caused significantly increased susceptibility to challenge by a virus.[44] This review, because of limited space, cannot include all possible references to pesticides and immune system dysfunctions. Other references provide additional information.[4,45-48]

To this point, only animal studies have been discussed. Unfortunately, there are only a limited number of human studies available and very few on pesticide effects on immune systems. In the non-pesticide area, smoking,[49] asbestos,[50,51] benzene,[52] lead,[53] marijuana,[54] PCBs,[55-57] PBBs,[58] ozone,[59] and phencyclidine,[60] among other agents, have been shown to affect human immune responses, either directly or *in vitro*. Most pesticides and other chemicals can also elicit allergic reactions if the dose and exposure time are appropriate. This may be the most widespread of all toxic reactions, although underreported and understudied.

Malathion was found to cause contact sensitivity in almost 1/2 of the human subjects tested, although no one was sick enough to leave their job.[61] Another study found antibodies formed to DDT and malation in volunteer subjects. It was speculated that this could potentially result in conditions of hypersensitivity.[62]

In 1959, a patient died of aplastic anemia (a generalized anemia involving reduced white and red blood cells) suspected of being acquired from working with several different insecticides.[63] A case of aplastic anemia with immune abnormalities associated with occupational exposure to pentachlorophenol and tetrachlorophenol was described.[64] Dieldrin apparently induced a case of immunohemolytic anemia (similar or equivalent to aplastic anemia) in a factory worker.[65] A number of cases of aplastic anemia associated with the insecticides DDT, chlordane, benzene hexachloride, and arsenical sprays have been described.[66] Household ogranophosphates were speculated to be the cause of aplastic anemia and acute leukemia in British children,[67] although this was disputed by some.[68,69] A 1967 study in southern California failed to uncover any hematologic abnormalities associated with pesticides.[70] Several pesticides including DDT, parathion, trithion, and butoxide were found to inhibit various human lymphocyte functions *in vitro*, while other pesticides had only marginal effects in the assays.[71] In a clinical review of 56 very divergent cases of complaints about environmentally induced illness, no significant correlation could be drawn between immune status and seriousness of the complaints.[72] As a last note, a recent study found that long-term exposure to 2,3,7,8-TCDD (dioxin) in Missouri was associated with depressed cell-mediated immunity, although the results have not yet caused excess clinical illnesses in the exposed people (see p. 36).[73] Dioxins are widespread contaminants in PCBs, 2,4,5-T, silvex, hexachlorobenzene and pentachlorophenol. Dioxins and related compounds may well be responsible for the immune perturbations observed with PCBs and the other contaminated chemicals.

Conclusion

In conclusion, although the field of immunotoxicology is perhaps the newest in toxicology; its information base is becoming extensive and rapidly expanding, primarily due to the efforts of individual scientists rather than industrial or governmental testing. Numerous compounds have now been shown to affect the immune system in a variety of ways. Although there is some question about certain compounds, there is little doubt that in general, the effects are real. Several recent findings discussed, such as aldicarb causing immune disruption at 1 ppb,[30] TCDD being associated with depressed human cell-mediated immunity, and herbicides acting in conjunction with other compounds and stressors to unexpectedly reduce survival to a viral challenge,[44] all combine to suggest that as more information is gained, documentation of these phenomena will increase. In particular, the simultaneous effects of multiple compounds and various stressors on animals mimic the real world situation for environmental contaminants far more closely than the routine, one-step-at-a-time, ideal conditions of toxicity testing currently in standard use.

It should not be forgotten that the potential exists for serious damage to nonhuman populations through immunosuppression as well as to humans. Animals, by their sheer numbers, appetite, and environment; are at a considerably greater risk from immunotoxicants and pathogens than humans. The combined fields of environmental toxicology and immunotoxicology will undoubtedly demonstrate serious environmental health impacts of contaminants in the future.

The contention that standard toxicity tests will detect any immunotoxicologic effect (or the converse argument, that any immunotoxicity not picked up is thus inconsequential) is no longer valid. Research has demonstrated that subtle immune system defects can have serious, even fatal consequences to both animals and humans. The information base on immune dysfunction, environmental contaminants, and immune function testing is significant and warrants routine testing for immunotoxicity effects prior to using a chemical in either the workplace or the environment.

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effects have been noted as a result of ingestion of carbamate contaminated cucumbers in Nebraska (Centers for Disease Control, 1979). Reversible acute anticholinergic poisoning was also noted in a human study (Haines, 1971). Little is known about the potential chronic immune system effects associated with long-term ingestion of aldicarb in humans or animals.

Aldicarb was first detected as a groundwater contaminant in the late 1970s when more than 1100 wells tested positive for aldicarb residues above 7 parts per billion (ppb) in New York's Suffolk County, a potato farming region on rural Long Island (Zake *et al.*, 1982). Since then, aldicarb has been found in the groundwater of many other states including Maine, Florida, California, Arizona, North Carolina, Virginia, and Wisconsin (McWilliams, 1984).

The Central Sands area of Wisconsin, an agricultural region in the middle of the state with sandy soils and high water tables, is a center for extensive central-pivot irrigation potato farming. The pesticide aldicarb has been widely used in this region since 1980. In 1981, testing of residential drinking-water wells first detected aldicarb in the groundwater. Subsequently, a Wisconsin law was enacted which regulates and restricts the use of the pesticide throughout the state. An enforcement standard of 10 ppb was established for aldicarb in groundwater. Households with aldicarb levels above 10 ppb were advised to refrain from drinking the contaminated groundwater. Since the pesticide was first detected in Wisconsin groundwater, more than 300 wells have tested positive for aldicarb residues at levels ranging from 1 to 100 ppb. Most of these wells have been located in Portage County, Wisconsin, one of the leading potato farming regions in the Central Sands area.

Recently, Olson and others at the University of Wisconsin, Madison, described an immune system suppressant effect in laboratory mice fed water containing aldicarb at levels of 1, 10, 100, and 1000 ppb (Olson *et al.*, 1986). The aldicarb levels which caused immunomodulation included doses below the currently used Wisconsin groundwater enforcement standard of 10 ppb. To assess possible immunodysfunction in humans exposed to aldicarb-contaminated groundwater, we performed a cross-sectional study of exposed and unexposed residents of Portage County, Wisconsin.

METHODS

Subjects. A list of Portage County households for potential study participants was obtained through a review of the Wisconsin Department of Natural Resources (DNR) groundwater survey data. This list included household wells that had tested positive for aldicarb residues in the groundwater and household wells that had tested negative (nondetectable) for aldicarb residues (limit of laboratory detection—1 ppb). To be eligible for study inclusion, household wells must have been tested for aldicarb residues on at least two occasions within the last 4 years, including once within the previous 12 months.

Subject selection was limited to women, ages 18 to 70 years old. Potential households from the DNR groundwater survey list were telephoned and queried as to the presence of an age-eligible woman. If an age-eligible woman resided in perature to the laboratory for immune function tests performed 18 to 24 hr after phlebotomy. Tests included (a) complete blood counts (CBC: Coulter Counter. Model S-PLUS4[3] with manual differential counts). (b) quantitative immunoglobulin assays (Beckman Auto-ECS Rate nephelometer.[3] Beckman Instruments, Inc., Immuno Systems Operations, Brea, Calif.). (c) T-cell subsets and β-cell enumeration including T4, T8, T11, and B1 (modified Coulter Clone Procedure,[3] Coulter Corporation, Hialeah, Fla.). (d) mitogenic (phytohemagglutinin, concanavalin A, and pokeweed mitogens) and antigenic (*Candida* and tetanus antigens, MLC alloantigens) (Oppenheim and Schecter, 1980) lymphocyte stimulation assays, and (e) tetanus toxoid antibody levels prior to and 14 days after immunization with tetanus toxoid booster by an ELISA assay (Moen, 1986) (0.5 ml aluminum phosphate adsorbed ultrafined tetanus toxoid administered intramuscularly, Lot No. 4848101, Wyeth Laboratories Inc.,[3] Marietta, Pa.). Laboratory quality control procedures for immune function tests included daily standardization of study results to reference normal controls. Laboratory technicians completed all analyses while blinded to exposure status of study participants.

Statistical analysis. All data were keyed into a SAS data base. Statistical analyses were executed under SAS 82.4 using an IBM 370/3083 mainframe computer.[3] The distributions of single continuous variables were compared between the exposed and unexposed groups with a two-tailed unpaired Student's t test and the Spearman Rank Order correlation coefficient test. Immune function test results for continuous variables were transformed to natural log values as another test of statistical significance. Differences in categorical variables were assessed by a χ^2 analysis and Fisher exact test. A P value less than 0.05 was considered statistically significant.

RESULTS

Subject Selection

Among 85 household wells in Portage County that had been tested twice over the previous 4 years for aldicarb residues, 37 households with an age-eligible woman fulfilling the study protocol requirements were enrolled. Enrollment was completed in a blinded manner with respect to exposure status. Of the remaining 48 households, 8 contained a woman who fulfilled eligibility criteria but who refused to participate in the study; 8 could not be contacted (moved, had unlisted telephone numbers, or did not answer the telephone on at least six different attempts); and 32 were excluded because they did not contain a woman who fulfilled the protocol requirements (27 did not have an age-eligible female who drank the household tap water, 2 had a pregnant female, 2 had a female ill within the last 4 weeks, and 1 had a female on phenytoin).

The second group of unexposed females from the municipal water supply listing included 20 women. Thirteen fulfilled the eligibility requirements of the study and were enrolled. Seven of the women were excluded (three due to preg-

[3] Tradename is given for identification only and does not imply endorsement by the U.S. Department of Health and Human Services or the U.S. Public Health Service.

TABLE 1
SUBJECT VARIABLES AND LABORATORY MEASURES AS A FUNCTION OF EXPOSURE STATUS, PORTAGE COUNTY, WISCONSIN, 1985

Variable	Exposed (n = 23)	Unexposed (n = 27)	Significance
Mean age	37.6 ± 12.6	42.7 ± 11.3	NS†
Mean number of M.D. visits over past year	1.3 ± 1.6	2.0 ± 2.2	NS†
Mean number of hospital admissions over the past 5 years	0.2 ± 0.4	0.4 ± 0.6	NS†
% Currently under medical care	21.7	33.3	NS‡
% Present smokers	39.1	33.3	NS‡
Mean white blood cell count (× 10³)	7.3 ± 2.7	6.9 ± 1.9	NS†
Mean red blood cell count (× 10⁶)	4.5 ± 0.4	4.5 ± 0.4	NS†
Mean daily tapwater ingested (oz/day)	37.3 ± 15.1	35.8 ± 26.4	NS†
Mean hematocrit %	40.7 ± 3.2	40.6 ± 2.7	NS†
Mean corpuscular volume (fl)	91.1 ± 4.8	90.0 ± 5.3	NS†
Platelet count (× 10³)	325.4 ± 79.4	348.1 ± 106.7	NS†
% Segmented neutrophils	49.0 ± 9.5	48.6 ± 10.5	NS†
% Lymphocytes	40.7 ± 10.2	40.1 ± 10.4	NS†
% Monocytes	6.1 ± 2.2	6.6 ± 3.2	NS†
Quantitative IgG level (mg/dl)	1039.2 ± 227.1	1037.1 ± 291.1	NS†
Quantitative IgM level (mg/dl)	125.8 ± 69.1	135.1 ± 62.3	NS†
Quantitative IgA level (mg/dl)	201.7 ± 48.5	198.8 ± 84.7	NS†

Note. Values expressed as means ± one standard deviation.
† t test, two-tailed, NS = not significant at the P = 0.05 level.
‡ Fisher exact test, two-tailed, NS = not significant at the P = 0.05 level.

measures, quantitative immunoglobulin measures (Table 1), or B-cell subset measures (Table 2).

Exposed and unexposed subjects differed in their measures of T-cell subsets (Table 2). Exposed subjects tended to have an increased absolute number of T8

TABLE 2
B- AND T-CELL SUBSET ANALYSIS, PORTAGE COUNTY, WISCONSIN, 1985

B- or T-cell subset	Laboratory normal values*	Exposed* (n = 23)	Unexposed* (n = 27)	Significance† (exposed vs unexposed)
Mean B1 (cell/mm³)	168.0 ± 10.0	160.4 ± 22.1	150.7 ± 16.4	NS
Mean B1 percentage	7.0 ± 0.4	5.4 ± 0.5	5.4 ± 0.3	NS
Mean T11 (cells/mm³)	1980.0 ± 83.3	2404.0 ± 210.1	2306.1 ± 162.0	NS
Mean T11 percentage	82.0 ± 0.9	83.2 ± 1.1	83.7 ± 1.0	NS
Mean T4 (cells/mm³)	1049.0 ± 53.8	1293.6 ± 123.3	1372.8 ± 107.4	NS
Mean T4 percentage	43.0 ± 1.2	44.7 ± 1.5	49.5 ± 1.3	P < 0.02
Mean T8 (cells/mm³)	588.0 ± 45.9	815.3 ± 99.2	586.1 ± 47.2	P < 0.05
Mean T8 percentage	23.0 ± 0.8	27.8 ± 2.1	21.5 ± 1.1	P < 0.02

* Values expressed as means ± one standard error of the mean.
† t test, two-tailed; NS = not significant at the P = 0.05 level.

subjects with ratios <1.0 (Fisher exact test, $P < 0.09$). To assess the possibility of transient effects on T-cell subsets, we retested five of the six subjects with low T4:T8 ratios for a third time approximately 2 months after the initial testing (one refused a third venipuncture). Results of the third testing were essentially identical to previous results (Table 3).

The lymphocyte proliferation assays showed no significant differences between exposed and unexposed participants with the phytohemagglutinin, concanavalin A, pokeweed, tetanus, or MLC assays (Table 4). In the *Candida* antigen stimulation assay, the mean counts-per-minute (cpm) measure for exposed subjects was significantly higher than the cpm for unexposed subjects. The stimulation index for the *Candida* antigen was also significantly higher in the exposed group when compared to the unexposed group (Table 4).

For the tetanus toxoid antibody measures prior to and 14 days after immunization with 0.5 ml of tetanus toxoid booster, titers for IgM and IgG were measured and compared to a reference standard (Moen *et al.*, 1986). Mean antibody levels for specific IgG and IgM antibodies both before and after immunization with tetanus toxoid did not significantly differ (Table 5).

Dose–Response Analysis

The dose–response relationships between immune function tests and both household well aldicarb level and average daily aldicarb ingestion were examined. Using the Spearman rank order correlation analysis, a statistically significant negative correlation was noted between household well aldicarb levels (ppb) and T4:T8 ratio values ($r = -0.34$, $P < 0.02$). Additionally, significant positive correlations were noted between household well aldicarb levels (ppb) and both the *Candida* proliferation assays ($r = +0.41$, $P < 0.01$) and the *Candida* stimulation indices ($r = -0.36$, $P < 0.02$). No significant correlations were noted for the other antigen or mitogen assays.

A statistically significant negative correlation was observed between average

TABLE 3
T-CELL SUBSETS IN THE SIX SUBJECTS WITH REPEATEDLY DECREASED T4:T8 RATIOS, PORTAGE COUNTY, WISCONSIN, 1985

Age	Aldicarb level (ppb)[b]	Estimated average daily aldicarb ingestion (μg)	T4 value[a]	T8 value[a]	T4:T8 ratio 1st[c]	2nd[c]	3rd[c]
36	7	12.1	766	1402	0.5	0.6	0.6
66	61	32.2	833	986	0.9	0.8	0.9
33	1	0.9	1127	1627	0.7	0.7	0.7
33	3	0.3	951	794	1.2	1.2	1.2
31	24	32.6	1733	1671	1.0	1.1	refused
29	none	none	1009	853	1.2	1.2	1.1

[a] Mean number of cells/mm³ from the three different testing dates.
[b] Aldicarb groundwell testing result from September/October, 1985.
[c] First testing, Day 1; second testing, Day 14; third testing, approximately Day 60.

TABLE 6
T4:T8 RATIO VALUES AS A FUNCTION OF AVERAGE ALDICARB INGESTION LEVEL (μg/DAY), PORTAGE COUNTY, WISCONSIN, 1985 (n = 50)

	Average aldicarb ingestion level (μg/day)		
	0	0.1–10.0	10.1–48.3
Number and percentage of participants with normal[a] T4:T8 ratio values	26 (96.3%)	10 (83.3%)	8 (72.7%)
Number and percentage of participants with decreased[b] T4:T8 ratio values	1 (3.7%)	2 (16.7%)	3 (27.3%)
Totals	27	12	11

[a] Normal T4:T8 = 1.4 or greater.
[b] Decreased T4:T8 = 1.3 or less.

or self-evaluation of past and present health status. In the laboratory assessment of immune function, most of the tests completed showed no statistically significant differences between exposed and unexposed females. However, in measures of T-cell subsets and lymphocyte proliferation assays to *Candida* antigenic stimulation, the exposed and unexposed women did differ significantly and dose–response effects were noted.

A limited number of reports have been published on immune system effects of pesticides such as aldicarb or environmental contaminants such as PCBs and PBBs. Vos and De Roij (1972) noted humoral immunosuppression in guinea pigs fed PCBs and challenged with a tetanus toxoid injection. Wiltrout *et al.* (1978) observed humoral immunosuppression in mice given near-lethal oral doses of five separate pesticides (carbaryl, DDT, parathion, chlordimeform, and ametryne). Bekesi *et al.* (1979) first described human immune dysfunction in a group of Michigan farmers who ingested farm products contaminated with PPBs. Exposed farmers showed abnormal lymphocyte blastogenesis in response to challenge by selected mitogens when compared with unexposed farmers. Yau-Chin Lu and Ying-Chin Wu (1985) have followed a large number of Taiwan residents for acute and chronic health effects after the ingestion of rice bran oil contaminated with PCBs. An evaluation of these subjects 3 years later showed continued detectable levels of PCB in the blood; although the percentage of total T-cells was normal, there was, as with our study, an increased percentage of T8 cells. Lu and Wu also noted an enhancement of lymphocyte proliferation in response to various mitogens. Finally, Olson *et al.* (1986) have investigated the effects of aldicarb on selected immune parameters of mice. Aldicarb was administered via drinking water at concentrations of 1–1000 ppb for 14 to 34 days. These authors found that aldicarb significantly suppressed the splenic plaque-forming cell response to sheep red blood cells at all concentrations of aldicarb tested.

In our study, we noted a significant difference between the exposed and unexposed groups as well as a positive dose–response effect in the mean lymphocyte stimulation assay for one of the antigens (*Candida*). However, the values for both groups for this antigen were within normal limits as routinely observed at the lective tropism and the decreased T4:T8 ratio can be ascribed to a decrease in the numerator (Quinnan *et al.*, 1985). With aldicarb exposure, there apparently is an increase in T8 cells resulting in a low ratio due to an increase in the denominator. It is important to distinguish the cause of the T4:T8 ratio decrease as the implications are quite different depending upon the etiology (Fahey *et al.*, 1983). In our study, the T4:T8 ratio decrease was due to an absolute increase in numbers of T8 cells. No clinically apparent immunodeficiency was noted.

In assessing a system as complex as the lymphoid network, minimal measures may not reveal subtle differences. Lane *et al.* (1985) have recently shown that, in following patients with AIDS, antigen-response testing revealed defects that would have been missed by looking only at mitogen-induced proliferation. In the study of the effects of toxic exposures, we believe that as complete an assay as possible should be performed on the immune system to bring out more subtle differences.

The alteration in T-cell subsets noted in our study appears to be an observation that was not accompanied by any obvious present clinical implications. What is less clear, however, is whether this observation represents an early subclinical manifestation of immunotoxicity resulting from low-level and relatively short-term (<5 years) exposure to the aldicarb-contaminated groundwater. Further epidemiologic studies with larger numbers of participants are needed to resolve this question.

Because of the high solubility of aldicarb in water and its presence in drinking water in several states (McWilliams, 1984), the public health implications of continuing to expose large populations to a potential immunomodulating environmental contaminant warrants careful review. We may need to reevaluate present state and national policies which allow trace quantities of toxic chemicals such as aldicarb in the groundwater. Finally, we believe that there is a need to consider immunologic effects in any future assessment of the public health risks associated with potential environmental contaminants such as aldicarb, and that a protocol such as as the one used in this study can provide a model for informative assessments in the future.

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