Associations between Plasma DDE Levels and Immunologic Measures in African-American Farmers in North Carolina

Glinda S. Cooper,[1] Stephen A. Martin,[2,3] Matthew P. Longnecker,[1] Dale P. Sandler,[1] and Dori R. Germolec[4]

[1]Epidemiology Branch, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, Research Triangle Park, North Carolina, USA; [2]University of Michigan School of Public Health, Ann Arbor, Michigan, USA; [3]Cook County Department of Public Health, Chicago, Illinois, USA; [4]Laboratory of Molecular Toxicology, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, Research Triangle Park, North Carolina, USA

Experimental studies in rodents demonstrate evidence of immunosuppressive effects of dietary exposure to DDT [2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane], but human data pertaining to immunomodulating effects of DDT exposure are limited. In this study we examined the association between the persistent organochlorine breakdown product 1,1-dichloro-2,2,bis(*p*-chlorophenyl)ethylene (*p,p′*-DDE) and immunologic measures using blood samples in a relatively highly exposed population of farmers in the United States. Levels of serum immunoglobulin A (IgA) and IgG and the prevalence of antinuclear antibodies in relation to plasma *p,p′*-DDE levels were evaluated in samples from 137 African-American male farmers (30–88 years of age; median, 64 years). Participants were recruited through black churches in four rural counties in eastern North Carolina. Data collection included a telephone interview pertaining to farming practices and health history, and one blood sample was collected from each participant. Linear and logistic regression, adjusting for age, cholesterol, triglycerides, smoking status, and years of any kind of pesticide use, was used to assess the association between immunologic parameters and plasma levels of *p,p′*-DDE. The median plasma *p,p′*-DDE concentration was 7.7 μg/L (range, 0.6–77.4 μg/L). There was no association between *p,p′*-DDE and IgA in any of the models. IgG levels decreased with increasing *p,p′*-DDE levels, with a statistically significant decrease of approximately 50% in the highest two categories of exposure (≥ 6.0 μg/L) compared with values of < 3.0 μg/L. Sixteen (12%) were positive for antinuclear antibodies. The prevalence of antinuclear antibodies was somewhat elevated in the highest category of *p,p′*-DDE exposure (odds ratio, 1.9; 95% confidence interval, 0.32–11.3; for ≥ 12.0 μg/L compared with < 3.0 μg/L *p,p′*-DDE), but this difference was not statistically significant. These analyses provide evidence that *p,p′*-DDE modulates immune responses in humans. *Key words:* African American, autoantibodies, DDE, epidemiology, farmers, IgA, IgG, immunotoxicology. *Environ Health Perspect* 112:1080–1084 (2004). doi:10.1289/ehp.6892 available via *http://dx.doi.org/* [Online 3 May 2004]

DDT [2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane] is an organochlorine pesticide that was widely used in the 1950s and 1960s but is now restricted from use in the United States because of its persistence in the environment and effects on wildlife. 1,1-Dichloro-2,2-bis(*p*-chlorophenyl)ethylene (*p,p′*-DDE) is a particularly long-lasting, lipophilic breakdown product of DDT. Immunosuppressive effects, including reduction in immunoglobulin levels and decreased response to bacterial challenges, have been demonstrated in experimental studies of dietary DDT exposure in mice and rats (Banerjee 1987a, 1987b; Banerjee et al. 1997; Gabliks et al. 1975; Rehana and Rao 1992). Laboratory and clinical studies have demonstrated the dual potential for immune suppression and dysregulation (e.g., autoantibody production) in response to chemical exposures, including the organochlorine hexachlorobenzene (Loose et al. 1978; Michielsen et al. 1999; Schielen et al. 1993) and mercury (Bagenstose et al. 1999; Pollard et al. 2001; Via et al. 2003). There are relatively few data pertaining to immunologic effects of DDT or DDE exposure in humans, and no studies have examined antinuclear antibodies in relation to biologic measures of exposure.

Currently in the United States, *p,p′*-DDE exposure occurs primarily through contamination via the food chain. Because of the persistence of *p,p′*-DDE in the body, past occupational exposure to DDT can also be relevant. Biologic measurement of DDE levels in the general population has demonstrated higher levels among African Americans compared with whites [Centers for Disease Control and Prevention (CDC) 2003] and higher levels among people born in the southern and western regions of the United States compared with other regions (James et al. 2002). Reasons for these differences have not been established but could include differing exposure experience with respect to farming and diet.

We used data from a population of African-American farmers from North Carolina to examine the association between *p,p′*-DDE levels and immunologic parameters. Because of the historical use of DDT on cotton, tobacco, and other crops grown in this region, we anticipated that exposure levels in this study population would be higher than in general population samples. The immunologic measures we used were serum levels of immunoglobulin A (IgA) and IgG, as measures of mucosal and humoral immune responses. A previous study of residents living around a dump site had reported a positive association between DDE and IgA but no association between DDE and IgG (Vine et al. 2001). We measured antinuclear antibodies to assess the potential effect on autoimmunity. Antinuclear antibodies may occur after infections and with some chronic diseases, particularly among the elderly (Juby and Davis 1998), but high-titer levels of antinuclear antibodies are a distinctive feature of the autoimmune disease systemic lupus erythematosus. The potential androgen-agonist properties of *p,p′*-DDE (Kelce et al. 1995) can be hypothesized to increase the risk of this disease (Cooper et al. 1998).

Materials and Methods

Study design. The Agricultural Health Study is a prospective study of licensed pesticide applicators from Iowa and North Carolina (Alavanja et al. 1996). Recruitment took place at the time of licensure or renewal, with enrollment of approximately 52,000 applicators (farmers and commercial applicators) and 32,000 spouses between 1994 and 1997. Because only a small percentage (2.5%) of applicators were African American, a separate recruitment effort was undertaken to identify additional African-American farmers who did not currently hold a pesticide applicator's license but who may have previously applied pesticides. This add-on study was designed to look at DDE and androgens in men (Martin et al. 2002). Because men were more likely than women to have been engaged in farming activities including pesticide application, it was anticipated that their levels of DDE would be higher, making it easier to detect any associations with immunologic parameters.

Address correspondence to D.R. Germolec, Laboratory of Molecular Toxicology, NIEHS, MD C1-04, PO Box 12233, Research Triangle Park, NC 27709 USA. Telephone: (919) 541-3230. Fax: (919) 541-0870. E--mail: germolec@niehs.nih.gov

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Farmers and retired farmers were recruited through 118 predominantly black churches in five rural North Carolina counties (Warren, Halifax, Northampton, Bertie, and Sampson) in 1995 and 1996. This analysis is based on the sample of African-American male farmers or farmworkers ≥ 18 years of age who lived in one of these counties, had at least 2 years of farmwork experience as an adult, and completed a follow-up telephone interview that included more detailed questions on previous farming, pesticide use, and DDT. Details of the recruitment and data collection procedures for this sample have been previously described (Martin et al. 2002). Of the 334 men who were eligible for the telephone follow-up study, 275 (82%) completed this portion of the study. Blood sample collection was limited to participants from four adjacent counties (Warren, Halifax, Northampton, and Bertie counties) because of distance and budgetary constraints. Of the 228 men who were eligible for the blood draw, 30 were excluded because of medical conditions such as use of anticoagulant medications or high blood pressure that precluded blood collection. Blood samples were collected from 138 (70%) of 198 eligible persons. The final sample (n = 137) excluded 1 participant who was found to have a plasma *p,p′*-DDE concentration of 232 μg/L, three times higher than the maximum value among all other samples. The study protocol was approved by the institutional review boards at the National Institute for Environmental Health Sciences and the University of Michigan.

Total years of pesticide use were obtained in the follow-up telephone interview. Additional data collected in this follow-up interview included age at time of blood draw, education level, total years smoked, alcohol consumption in the last 12 months, physical activity on current or last job, and physical activity for recreation. Height and weight were measured using a standard protocol before blood was drawn and were used to calculate body mass index (kilograms per square meter). History of immune-mediated diseases and other medical conditions was obtained in the enrollment and telephone interviews. None of the participants in this study reported a history of leukemia, Hodgkin disease, non-Hodgkin lymphoma, or lupus.

Fasting blood samples were drawn before 1100 hr. Plasma was separated from the whole-blood sample on the day of the blood draw, and aliquots were frozen at –20°C. Serum samples were obtained from a separate tube of blood immediately after clotting. Aliquots (1 mL) of serum were refrigerated at 4°C until delivery within 48 hr of collection to the Duke University CARL Clinical Laboratory (Durham, NC) for immediate analysis of lipids.

Laboratory analyses. DDE was extracted from 2 mL of plasma using solid-phase extraction (C_{18}) by the Centre de Toxicologie du Québec (Sainte-Foy, Québec, Canada). After a washing step, *p,p′*-DDE was eluted with isooctane. The extract was then analyzed by gas chromatography with electron capture detection. Identification and quantification of DDE were confirmed by mass spectrometry. ^{13}C-labeled *p,p′*-DDE was used as an internal standard. The limits of quantification and detection for total *p,p′*-DDE were 0.5 μg/L and 0.2 μg/L, respectively. All study samples were above the detection limit for *p,p′*-DDE. For purposes of quality control, a 5 μg/L *p,p′*-DDE standard and a 25 μg/L *p,p′*-DDE standard were analyzed with each batch. The between-batch coefficient of variation for the standards was 5.2% and 8.3%, respectively, and recovery averaged 97%.

Total cholesterol was quantified using a cholesterol oxidase/cholesterol esterase fully enzymatic procedure using the Hitachi 911 Automatic Chemistry Analyzer (Roche Diagnostic Corporation, Indianapolis, IN). Triglycerides were determined enzymatically with a glycerol phosphate oxidase/peroxidase system after blanking for endogenous free glycerol concentration (before hydrolysis of the triglycerides by lipase). All study samples were within the reportable ranges of 3–800 mg/dL for total cholesterol and 4–1,000 mg/dL for triglycerides. The mean within-batch coefficient of variation was 0.61% for total cholesterol and 1.02% for triglycerides.

Serum was analyzed for total IgG and IgA using a sandwich ELISA assay. Polyclonal affinity-purified goat anti-human IgG or IgA (ICN Biomedicals, Inc., Aurora, OH) was bound to a solid phase using Immulon 1 plates (Dynex Technologies, Chantilly, VA) at a concentration of 150 μg in 150 μL phosphate-buffered saline (PBS). Excess antibody was removed by washing, and nonspecific binding was blocked by incubation with PBS/0.05% Tween 20 containing 0.5% bovine serum albumin. Test serum (200 μL) was then added to each well. After a 2-hr incubation, the plates were washed and the bound immunoglobulins were labeled with 150 μg peroxidase-conjugated goat anti-human IgG/IgA/IgM antibody (ICN Biomedicals, Inc.). Unbound antibody was removed by repeated washing, and immunoglobulins were detected using the horseradish peroxidase substrate kit (Bio-Rad, Hercules, CA) according to the manufacturer's instructions.

Serum antinuclear antibodies specific to Sm, double-stranded DNA, SSA/Ro, SSB/La, histone, RNP, Scl-70, Jo-1, and centromeric antigens were analyzed using Hep-2 nucleus antigens with the BINDAZYME ANA (antinuclear antibody) EIA Kit (The Binding Site, Birmingham, UK). Antinuclear antibodies were detected with peroxidase-labeled anti-human IgG conjugate and 3,3′,5,5′ tetramethylbenzidine. Positive, cutoff, and negative controls were included on each plate. The ANA result for each sample was calculated as the ratio of the test sample to the cutoff control (optical density of test sample/optical density of cutoff control). An antinuclear antibody score of ≥ 1.0 was interpreted as positive, as suggested by the manufacturer. The scores for positive individuals ranged from 1.13 to 4.70. Four known positive sera (samples from patients with systemic lupus erythematosus) and four known negative sera were included in the assays in a blind fashion. All known positive samples tested positive for antinuclear antibodies, and all known negative samples tested negative.

Statistical analysis. We used linear regression models of IgA and IgG levels to examine the association with plasma levels of *p,p′*-DDE adjusting for age, cholesterol, and triglycerides as continuous variables, and smoking status (current smoker, non-current smoker). We also adjusted for total years of pesticide use of any kind in these models. The $\log_e$ transformation of IgA and IgG variables was used to decrease the variance heterogeneity. The exponential of the β-coefficients from a linear regression using these transformed variables can be taken as an estimate of the percent change in IgA or IgG associated with the corresponding change in DDE. In addition, dichotomous variables were created to represent values of IgA and IgG above the 75th percentile cut points of the distributions (cut points are shown in Tables 1 and 2); we used logistic regression to examine the relation between *p,p′*-DDE and these dependent variables. Logistic regression was also used with prevalence of antinuclear antibodies as the outcome. The relation of outcome to *p,p′*-DDE was evaluated using the four categories of exposure (< 3, 3. 0–5.9, 6.0–11.9, and ≥ 12.0 μg/L) with indicator variables for the highest three groups, and by using three types of trend test: one with *p,p′*-DDE category as an ordinal variable, one with subjects in a given *p,p′*-DDE category assigned to the median exposure level for that category, and one with *p,p′*-DDE level as a continuous variable.

Results

The median age of the study participants was 64 years, and age ranged from 30 to 88 years (Table 1). Nine percent were current smokers. The median number of years smoked among the 81 ever-smokers was 12 years. Most participants (87%) were no longer working on a farm, but the mean number (± SD) of years farmed was 29.6 ± 16.7. The mean IgA level was 222.5 mg/dL, and the mean IgG was 1,665 mg/dL. Sixteen (12%) were classified as

positive for antinuclear antibodies. The median plasma *p,p′*-DDE concentration was 7.7 µg/L (range, 0.6–77.4 µg/L).

We found no association between IgA and *p,p′*-DDE in any of the models (Table 2). IgG levels, however, generally decreased with increasing *p,p′*-DDE levels. The association appears to be nonlinear such that the statistically significant differences (~ 50% decrease in IgG) were seen in the higher two categories of exposure and with the ordinal trend test but not with the analysis using median values per group in the trend test or using DDE as a continuous variable. The prevalence of antinuclear antibodies was elevated in the highest category of *p,p′*-DDE exposure (odds ratio, 1.9; 95% confidence interval, 0.32–11.3), but this association was not statistically significant within this group or in the trend tests across the four levels of exposure. We found no association between years of any kind of pesticide use and IgG or prevalence of antinuclear antibodies (data not shown), but there was some evidence that IgA was positively associated with years of pesticide use (ordinal trend test *p*-value = 0.03 in the analysis using log-transformed IgA as the dependent variable). Although adjusting for overall years of pesticide use could theoretically have attenuated any association between DDE and IgA levels if DDE levels were associated with years of pesticide use, we did not see an association with IgA even when overall years of use was excluded from the models.

Table 1. Characteristics of 137 African-American farmers in North Carolina, 1999.

	No. (%)	Mean ± SD	Median
Age		61.7 ± 13.1	63.7
Education			
Less than high school	67 (49)		
Completed high school	40 (29)		
More than high school	30 (22)		
Body mass index (kg/m^2)		28.7 ± 4.7	28.1
Smoking status			
Never	56 (41)		
Former	68 (50)		
Current	13 (9)		
Years of pesticide use		12.3 ± 13.4	8.0
Years of DDT use		2.2 ± 5.5	0.0
DDE (µg/L)		11.4 ± 12.2	7.7
Cholesterol (mg/dL)		208.29 ± 35.1	206.0
Triglycerides (mg/dL)		127.7 ± 84.1	103.0
IgA (mg/dL)[a]		222.5 ± 313.4	96.7
IgG (mg/dL)		1,665 ± 1,761	1,170
Positive antinuclear antibodies (≥ 1.0)[b]	16 (12)		

[a]One missing value. [b]Ratio of optical density of test sample to optical density of cutoff control sample.

Discussion

In this study of African-American farmers from the southeastern United States, we observed an inverse association between levels of *p,p′*-DDE and IgG. We found no significant association between the concentration of *p,p′*-DDE and IgA, the immunoglobulin class that is primarily responsible for protecting mucosal surfaces (e.g., the respiratory and gastrointestinal tract). Few other studies have focused on immunologic parameters and DDE or DDT exposure in humans. In contrast to our findings, Vine et al. (2001) reported an increase in total lymphocytes and higher IgA levels, but not IgG levels, in relation to increasing *p,p′*-DDE levels in a study of 302 adults residing around a pesticide dump site in North Carolina. In that study, exposure levels were lower (DDE median, 2 µg/L) compared with our study population (median, 7.7 µg/L). In the general population, the main source of *p,p′*-DDE is diet (Gunderson 1995), and diet undoubtedly contributed to exposure among the subjects in the present study of African-American farmers. In addition, subjects in our study who had used DDT on crops in the past had slightly higher serum *p,p′*-DDE levels (data not shown). Although DDT was banned in 1972, the half-life is relatively long (> 5 years), and previous use likely accounts for the elevated levels in our subjects (Wolff 1999). DDE is stored more tenaciously in humans than is

Table 2. Regression analyses of *p,p′*-DDE in relation to immunologic measures among African-American farmers in North Carolina, 1999.[a]

Exposure measure		Linear regression			Logistic regression		
DDE (µg/L)	No. per group	Dependent variable	β (SE)	*p*-Value	Dependent variable	No. (%) "positive"[b]	Odds ratio (95% CI)
< 3.0	28	Log-transformed IgA	Referent		Highest quartile IgA	6 (21)	1.0 (referent)
3.0–5.9	28		–0.113 (0.324)	0.73	(≥ 251.5 mg/dL)	4 (14)	0.53 (0.12–2.3)
6.0–11.9	39		0.308 (0.305)	0.32		13 (33)	1.8 (0.54–5.9)
≥ 12.0	41		0.043 (0.318)	0.89		11 (27)	1.2 (0.34–4.3)
Ordinal trend *p*-value[c]				0.59			0.38
Median-value trend *p*-value[d]				0.82			0.51
Continuous: per unit increase			–0.003 (0.009)	0.72		34 (25)	1.00 (0.97–1.04)
< 3.0	29	Log-transformed IgG	Referent		Highest quartile IgG	11 (38)	1.0 (referent)
3.0–5.9	28		–0.463 (0.294)	0.12	(≥ 2,110 mg/dL)	10 (36)	0.89 (0.27–2.9)
6.0–11.9	39		–0.717 (0.276)	0.01		8 (21)	0.34 (0.10–1.1)
≥ 12.0	41		–0.609 (0.289)	0.04		7 (17)	0.36 (0.10–1.3)
Ordinal trend *p*-value[c]				0.03			0.05
Median-value trend *p*-value[d]				0.13			0.10
Continuous: per unit increase			–0.005 (0.008)	0.57		36 (26)	0.99 (0.95–1.05)
< 3.0	29				Positive antinuclear	2 (7)	1.0 (referent)
3.0–5.9	28				antibodies (≥ 1.0)	2 (7)	0.75 (0.09–6.1)
6.0–11.9	39					4 (10)	1.1 (0.17–7.2)
≥ 12.0	41					8 (20)	1.9 (0.32–11.3)
Ordinal trend *p*-value[c]							0.33
Median-value trend *p*-value[d]							0.25
Continuous: per unit increase[e]						16 (12)	1.01 (0.97–1.05)

CI, confidence interval.
[a]Adjusted for age, smoking status, total years of pesticide exposure (quartiles), cholesterol, and triglycerides; one value was missing for IgA, for a total number of 136 for IgA analyses and 137 for IgG and antinuclear antibody analyses. [b]"Positive" denotes those within each DDE group that are in the highest quartile of IgA, highest quartile of IgG, or "positive" for antinuclear antibodies, respectively. [c]Trend test using values of 1, 2, 3, and 4, respectively. [d]Trend test using median value per group. [e]Ratio of optical density of test sample to optical density of cutoff control sample.

DDT, and *p,p′*-DDE levels increase in plasma after DDT intake has decreased (Smith 1991). Besides diet, additional exposure in both our study and the study by Vine et al. (2001) was through contaminated air; thus, the expected routes of inhalation and/or dermal absorption were similar. We do not know how differences in dose or other study characteristics contributed to the associations with different types of immunoglobulins observed in these two studies.

Higher levels of prenatal *p,p′*-DDE were associated with an increased incidence of otitis media in a study of 171 Inuit infants (Dewailly et al. 2000). Studies of individuals occupationally exposed to DDT also suggest that long-term exposure may lead to altered resistance to infectious diseases. Hermanowicz et al. (1982) found a higher prevalence of infectious diseases in workers who had directly worked with DDT and lindane for 12–30 years compared with a control population of 1,000 individuals. Upper respiratory tract infections such as tonsillitis, bronchitis, and pharyngitis were the most frequently observed. These investigators also found deficits in neutrophil function, including decreased chemotaxis, phagocytic activity, and respiratory burst (Hermanowicz et al. 1982). Similar associations between increased infectious disease and pesticide exposure were later reported for a larger cohort (Hermanowicz and Kossman 1984); however, there were no significant differences in neutrophil function in the larger study.

The function of immunoglobulin is to inactivate or eliminate pathogenic organisms, and individuals with severely reduced levels of serum IgG due to primary immunodeficiencies suffer from recurring infections (Schur et al. 1970). However, the clinical significance of modestly reduced serum IgG or IgG subclass levels remains controversial and is evidenced by the identification of asymptomatic individuals with abnormally low serum IgG subclass concentrations who do not have increased rates of infectious disease (Maguire et al. 2002). At 4–6 months of age, neonates lose the protection of maternally derived IgGs, and at 7–12 months of age, IgG and IgM levels are approximately 50% of adult levels (Stiehm and Fundenberg 1966). Infants of this age have been shown to be particularly susceptible to infections with encapsulated bacterial pathogens associated with upper respiratory tract infections. In the studies described above, the observed decrease in serum IgG levels with increasing *p,p′*-DDE suggests the potential for increased susceptibility to pathogens whose clearance is IgG mediated, such as *Haemophilus influenzae* and *Streptococcus pneumoniae* (Maguire et al. 2002). Our study was designed to examine immunologic parameters rather than clinical end points, but some medical history data were collected as part of the screening and enrollment process. Seventeen (12%) of the study participants reported a history of pneumonia. This prevalence did not vary by DDE level (14, 21, 10, and 15% in the lowest to highest DDE group, respectively). A larger study focusing on specific infectious diseases (and including validated medical history data) would be needed to examine the association between DDE and clinical outcomes.

Several studies have examined the relation between use of specific pesticides and presence of autoantibodies with inconsistent results (Colosio et al. 1993; McConnachie and Zahalsky 1991, 1992; Rosenberg et al. 1999; Thrasher et al. 1993). In the largest study of this type, self-reported use of some organochlorine pesticides (aldrin, chlordane, dieldrin, endrin, heptachlor, and lindane) was associated with increased prevalence of low-titer (1:40) antinuclear antibodies in a farming community (Rosenberg et al. 1999). The authors noted that the association with diphenyl chlorines (DDT and methoxychlors) was not statistically significant, but data pertaining to the prevalence of this exposure and the magnitude and precision of the observed association were not given. Although we observed the highest prevalence of antinuclear antibodies in the highest category of *p,p′*-DDE exposure in our study, this association was not statistically significant. Larger and expanded studies that are able to identify and quantify specific autoantibody levels are needed to more clearly examine this relationship.

Study participants were ambulatory members of the community, recruited through churches rather than through a hospital or clinic setting. When we excluded the six individuals who reported a history of kidney failure or dialysis or cancer, the results pertaining to the association between DDE and IgG levels were essentially unchanged (data not shown). Based on these self-reported data pertaining to medical conditions, we believe that major illnesses (cancer, malnutrition) were unlikely to be influencing the IgG levels seen in this study population.

A strength of this study is that the levels of *p,p′*-DDE observed in study participants were higher than those reported by Vine et al. (2001) in community residents and higher than in the general population (CDC 2003). This difference in exposure level may reflect the influence of occupational exposure through farmwork, in addition to background exposure from the food supply and other environmental sources. This variability in exposure improves our ability to detect an effect of *p,p′*-DDE that occurs with higher exposures, such as those that may occur in occupationally exposed populations. Although our study sample size was modest, our failure to confirm the association between *p,p′*-DDE and IgA observed previously by Vine et al. (2001) is unlikely to be due to limited statistical power because we saw no evidence of a trend that would have been strengthened with greater precision.

The reduced IgG levels seen with increasing *p,p′*-DDE provides evidence of potential immunosuppression associated with this exposure. These findings are consistent with data from experimental studies in rats and mice (Banerjee 1987a, 1987b; Banerjee et al. 1997; Gabliks et al. 1975; Rehana and Rao 1992) and with an *in vitro* study reporting decreased macrophage activation with DDT/DDE exposure (Nunez et al. 2002). Thus, in addition to potential effects on reproductive outcomes, including preterm birth (Longnecker et al. 2001) and impaired lactation (Gladen and Rogan 1995; Rogan et al. 1987), immune-mediated health effects such as infectious diseases and autoimmune diseases should be considered when evaluating the long-term consequences of DDT use.

References

Alavanja MC, Sandler DP, McMaster SB, Zahm SH, McDonnell CJ, Lynch CF, et al. 1996. The Agricultural Health Study. Environ Health Perspect 104:362–369.

Bagenstose LM, Salgame P, Monestier M. 1999. Murine mercury-induced autoimmunity: a model of chemically related autoimmunity in humans. Immunol Res 20:67–78.

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

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

Banerjee B, Koner B, Pashsa S. 1997. Influence of DDT exposure on susceptibility to human leprosy bacilli in mice. Int J Lepr 65:97–99.

CDC. 2003. Dichlorodiphenyltrichloroethane. In: Second National Report on Human Exposure to Environmental Chemicals. NCEH 02-0716. Atlanta, GA:National Center for Environmental Health, Centers for Disease Control and Prevention, 190–193. Available: http://www.cdc.gov/exposurereport/pesticides/organochlorine/pdf/Dichlorodiphenyltrichloroethane.pdf [accessed 1 December 2003].

Colosio C, Maroni M, Barcellini W, Meroni P, Alcini D. 1993. Toxicological and immune findings in workers exposed to pentachlorophenol (PCP). Arch Environ Health 48:81–88.

Cooper GS, Dooley MA, Treadwell EL, St Clair EW, Parks CG, Gilkeson GS. 1998. Hormonal, environmental, and infectious disease risk factors for the development of systemic lupus erythematosus. Arthritis Rheum 41:1714–1724.

Dewailly É, Ayotte P, Bruneau S, Gingras S, Belles-Isles M, Roy R. 2000. Susceptibility to infections and immune status in Inuit infants exposed to organochlorines. Environ Health Perspect 108:205–211.

Gabliks J, Al-zubaidy T, Askari E. 1975. DDT and immunological responses. 3. Reduced anaphylaxis and mast cell population in rats fed DDT. Arch Environ Health 30:81–84.

Gladen BC, Rogan WJ. 1995. DDE and shortened duration of lactation in a northern Mexican town. Am J Public Health 85:504–508.

Gunderson EL. 1995. FDA Total Diet Study, July 1986-April 1991, dietary intakes of pesticides, selected elements, and other chemicals. J AOAC Int 78:1353–1363.

Hermanowicz A, Kossman S. 1984. Neutrophil function and infectious disease in workers occupationally exposed to phosphoorganic pesticides: role of mononuclear-derived chemotactic factor for neutrophils. Clin Immunol Immunopathol 33:13–22.

Hermanowicz A, Nawarska Z, Borys D, Maslankiewicz A. 1982. The neutrophil function and infectious diseases in workers occupationally exposed to organochloride insecticides. Int Arch Occup Environ Health 50:329–340.

James RA, Hertz-Picciotto I, Willman E, Keller JA, Charles MJ. 2002. Determinants of serum polychlorinated biphenyls

and organochlorine pesticides measured in women from the child health and development study cohort, 1963–1967. Environ Health Perspect 110:617–624.

Juby AG, Davis P. 1998. Prevalence and disease associations of certain autoantibodies in elderly patients. Clin Invest Med 21:4–11.

Kelce WR, Stone CR, Laws SC, Gray LE, Kemppainen JA, Wilson EM. 1995. Persistent DDT metabolite *p,p´*-DDE is a potent androgen receptor antagonist. Nature 375:581–585.

Longnecker MP, Klebanoff MA, Zhou H, Brock JW. 2001. Association between maternal serum concentration of the DDT metabolite DDE and preterm and small-for-gestational-age babies at birth. Lancet 358:110–114.

Loose LD, Silkworth JB, Pittman KA, Benitz KF, Mueller W. 1978. Impaired host resistance to endotoxin and malaria in polychlorinated biphenyl- and hexachlorobenzene-treated mice. Infect Immun 20:30–35.

Maguire GA, Kumararatne DS, Joyce HJ. 2002. Are there any clinical indications for measuring IgG subclasses? Ann Clin Biochem 39:374–377.

Martin SA Jr, Harlow SD, Sowers MF, Longnecker MP, Garabrant D, Shore DL, et al. 2002. DDT metabolite and androgens in African-American farmers. Epidemiology 13:454–458.

McConnachie PR, Zahalsky AC. 1991. Immunological consequences of exposure to pentachlorophenol. Arch Environ Health 46:249–253.

McConnachie PR, Zahalsky AC. 1992. Immune alterations in humans exposed to the termiticide technical chlordane. Arch Environ Health 47:295–301.

Michielsen C, van Loveren H, Vos J. 1999. The role of the immune system in hexachlorobenzene-induced toxicity. Environ Health Perspect 107:783–792.

Nunez GMA, Estrada I, Calderon-Aranda ES. 2002. DDT inhibits the functional activation of murine macrophages and decreases resistance to infection by *Mycobacterium microti*. Toxicology 5:201–210.

Pollard KM, Pearson DL, Hultman P, Deane TN, Lindh U, Kono DH. 2001. Xenobiotic acceleration of idiopathic systemic autoimmunity in lupus-prone BXSB mice. Environ Health Perspect 109:27–33.

Rehana T, Rao PR. 1992. Effect of DDT on the immune system in Swiss albino mice during adult and perinatal exposure: humoral responses. Bull Environ Contam Toxicol 48:535–540.

Rogan WJ, Gladen BC, McKinney JD, Carreras N, Hardy P, Thullen J, et al. 1987. Polychlorinated biphenyls (PCBs) and dichlorodiphenyl dichloroethene (DDE) in human milk: effects on growth, morbidity, and duration of lactation. Am J Public Health 77:1294–1297.

Rosenberg AM, Semchuk KM, McDuffie HH, Ledingham DL, Cordeiro DM, Cessna AJ, et al. 1999. Prevalence of antinuclear antibodies in a rural population. J Toxicol Environ Health 56:225–236.

Schielen P, Schoo W, Tekestra J, Oostermeijer HH, Seinen W, Bloksma N. 1993. Autoimmune effects of hexachlorobenzene in the rat. Toxicol Appl Pharmacol 122:233–243.

Schur PH, Borel H, Gelfand EW, Alper CA, Rosen FS. 1970. Selective gamma-G globulin deficiencies in patients with recurrent pyogenic infections. N Engl J Med 283:631–634.

Smith AG. 1991. Chlorinated hydrocarbon insecticides. In: Handbook of Pesticide Toxicology. Vol. 2: Classes of Pesticides (Hayes WJ, Laws ER, eds). San Diego, CA:Academic Press, 731–915.

Stiehm ER, Fundenberg HH. 1966. Serum levels of immune globulins in health and disease. Pediatrics 37:715–727.

Thrasher JD, Madison R, Broughton A. 1993. Immunological abnormalities in humans exposed to chlorpyrifos—preliminary observations. Arch Environ Health 48:89–93.

Via CS, Nguyen P, Niculescu F, Papadimitriou J, Hoover D, Silbergeld EK. 2003. Low-dose exposure to inorganic mercury accelerates disease and mortality in acquired murine lupus. Environ Health Perspect 111:1273–1277.

Vine MF, Stein L, Weigle K, Schroeder J, Degnan D, Tse CK, et al. 2001. Plasma 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE) levels and immune response. Am J Epidemiol 153:53–63.

Wolff MS. 1999. Half-lives of organochlorines (OCs) in humans [Letter]. Arch Environ Contam Toxicol 36:504.