A recent World Resources Institute (WRI) report concluded that pesticides are a likely cause of immune suppression for millions of people throughout the world. The gravity of this conclusion motivated us to review the scientific evidence cited in the report. The predominant human evidence came from cross-sectional studies conducted in the former Soviet Union. These studies were difficult to evaluate due to incomplete reporting and had obvious limitations in terms of subject selection, exposure assessment, lack of quality control, statistical analysis, adequacy of the comparison group, and confounding. The toxicologic evidence was comprised mainly of acute high-dose studies in which the exposure conditions resulted in systemic toxicity. The relevance of these studies to effects at typical human exposure levels is questionable. We did not find consistent, credible evidence to support the conclusion of widespread pesticide-related immune suppression. Nonetheless, the WRI report is an important document because it focuses attention on a potentially important issue for future research and brings a substantial literature of foreign language studies to the attention of Western scientists. Key words: immune effects, pesticides. Environ Health Perspect 106:51-54 (1998). [Online 15 January 1998] http://ehpnet1.niehs.nih.gov/docs/1998/106p51-54acquavellabstract.html

A recent World Resources Institute (WRI) report, “Pesticides and the Immune System: The Public Health Risks” (1), represents a broad attempt to synthesize the available evidence on the potential effects of pesticides on the human immune system. The authors make a commendable effort to compile a wide range of epidemiologic evidence, including a large number of studies from the former Soviet Union that were previously unknown to most Western scientists. In addition, they review a significant portion of the available immunotoxicology data on pesticides.

A major conclusion of the report was that pesticides are a likely cause of immune suppression for millions of people throughout the world, especially in developing countries, and, thereby, cause (or exacerbate) various infectious and chronic diseases (e.g., cancer) in these populations. Quoting from the report (1):

The scientific evidence (referring to animal studies) suggesting that many pesticides damage the immune system is impressive... There is convincing direct and indirect evidence that these findings carry over to human populations exposed to pesticides.

In light of the gravity of this conclusion, the American Crop Protection Association convened a task group of industrial scientists with expertise in immunotoxicology and epidemiology to review the WRI report. The purpose of the current paper is to summarize the task group’s assessment of the scientific evidence cited in the WRI report. In addition, we hope to stimulate a critical evaluation by others of the science in this important area.

The Human Evidence

There are few studies of immunologic parameters or infectious disease rates for pesticide-exposed workers in the developed world. There have, however, been a sizable number of studies in the former Soviet Union. These studies comprise the primary human evidence in the WRI report.

Epidemiologic research in the former Soviet Union takes place in a very different milieu to that in developed countries. According to the WRI authors (1):

malnutrition and unsanitary living conditions are so widespread [in developing countries and countries of the former Soviet Union] that distinguishing any greater susceptibility due to immune deficiencies is difficult.

Others have noted deficiencies in epidemiologic research methodology in the former Soviet Union. In a recent commentary on Eastern Bloc epidemiologic research, Little et al. (2) commented:

Population-based studies are rare at best... The idea of a denominator has not been part of the standard Eastern training. Nor has the necessity for incorporating measures of quality control... and avoiding systematic bias.

We arranged for English translations of many of the Soviet epidemiologic studies cited in the WRI report in order to review their research methods, results, and conclusions. These studies were difficult to evaluate, however, due to incomplete reporting of research methods to an extent that would preclude publication in most western journals. Characteristically, there were no descriptions of the study design, subject selection methodology, or statistical analysis. Procedures for collection of specimens and laboratory analytical methods were not detailed or were described inadequately, and there was no mention of quality control measures. There was no attention to confounding and other potential sources of bias, either in the study design or in statistical analysis. Several of the Soviet references were commentaries and not actual studies.

The predominant study design was the cross-sectional approach. The limitations of this study design, versus longitudinal designs, for etiologic research are well described (3). Many of the Soviet studies did not have an unexposed control group, relying instead on unspecified external normal values. This raises two important potential sources of bias: 1) demographic or other risk factor differences between exposed and referent populations; and 2) differences in specimen collection procedures and analytic methodology between exposed and referent populations. Also, given the lack of a control group, special precautions should be taken to ensure unbiased evaluation of specimens; no such procedures were specified.

Few studies discussed the specific pesticides under investigation or the potential extent of exposure. None of the studies detailed how the participants were selected or how the investigators protected against selection bias.

A few examples are illustrative. Ivashina and Komarova (4) investigated characteristics of cellular and humoral immunity among 20 controls and 40 workers involved in the manufacture of a fungicide. Selection of subjects, specimen collection procedures, and statistical methodology were not described. No mention was made of confounding factors or other potential sources of bias.

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The potential for selection bias is conspicuous in the study by Katsenovich et al. (5), which compared the absolute numbers and functional characteristics of T and B cells from workers with pesticide exposure and an unspecified control group. In this study, exposed subjects were selected because they were ill with disorders of the kidney and liver; controls were selected because they were healthy and unexposed. In this case, the selection of subjects probably contributed to the outcome of the study.

Lack of control for potential confounding factors was apparent in most of the Soviet studies. For example, Palvanova (6) compared rates of gastritis for 116 longer term (male and female) workers and 20 shorter term (male) workers without controlling for age or sex. Palvanova noted several other potential uncontrolled confounding factors including abnormal work, smoking, and incomplete protein nutrition (6). It is likely that there were many nonimmunologic local causes of gastritis that needed to be considered in this study before a putative effect of pesticides could be evaluated. Another example is the report by Kozlyuk et al. (7); the authors measured immunoglobulin concentrations and lymphocyte numbers in children from three population centers with 5-fold, 6-fold, and 12-fold higher pesticide use compared with the Soviet average. Results for these children were compared to unspecified external normal values. No additional description of the population centers was given. One must question whether the use of pesticides is an indicator or a correlate of factors that might affect childhood immune status (e.g., an appropriate immune response to a locally common parasite).

Finally, Anismarova et al. (8) studied tractor operators and warehouse workers with exposure to pesticides. The authors compared blood lymphocyte and leukocyte numbers in individuals with less than 10 years work experience and those with 10 or more years work experience. There was no control for age and no discussion of potential confounding by smoking (9) or other external risk factors. In addition, there were apparent differences in laboratory analyses for the two groups: only 60% of lymphocytes were described as T cell or B cell for workers employed less than 10 years, compared with 70% for workers employed more than 10 years. The percent of lymphocytes described as T or B cell was unknown for the population that constituted the normal values (8).

Perhaps the strongest Soviet study was that of Laknova et al. (10). This study had an unexposed control group, and both the control group, and the exposed group were employed similarly as machine operators. Outcomes were determined from medical records for disability claims. The exposed group had higher rates of illnesses such as gastritis, acute and chronic respiratory diseases, and allergic diseases of the skin, while controls had higher rates of diseases of the nerves and peripheral ganglia, hypertonic diseases, and diseases of the kidneys and urinary tract (10). These results speak to widespread health differences between the exposed and control groups and complicate the evaluation of putative effects related to pesticide exposures.

Non-Soviet Studies

The major non-Soviet evidence cited in the WRI report (1) was a study of Inuit Indians in remote areas of northern Canada (11). Chronic otitis media is described as epidemic among Inuit children and related to organochlorine exposure, including dioxins, polychlorinated biphenyls, and pesticides, in the mainstays of their diet—fish, whale, seal, walrus, and bear meat (11). The issue of confounding exposures aside, two aspects of this reference deserve comment. First, this study did not address dietary exposures at all. Rather, the authors set out to collect information on the natural course of otitis media among the Inuits and the Cree Indians and to assess how to offer more adequate preventive and curative services. Second, the authors’ finding that otitis media was 6.5-fold less prevalent among Cree Indians, who shared the same settlement, lead them to conclude that “… it seems reasonable to rule out environmental factors …” as a cause of the high prevalence of otitis media among the Inuits (11). Additional research on this population is ongoing, but, at present, there are no research findings that implicate pesticides as a cause of immune deficiency among the Inuits.

The WRI report (1) proposed a link between pesticides and the production of autoimmune antibodies, citing McConnachie and Zahalsky (12) who found autoantibodies in 8 of 32 subjects exposed to pentachlorophenol—a frequency slightly higher than expected in an age-matched control population. However, conflicting results from Colosio et al. (13) were not cited. Those authors found no evidence of autoantibodies in 32 subjects with exposure to pentachlorophenol. In animal studies, Kerkvliet et al. (14) and Holsapple et al. (15) showed that the apparent effects of pentachlorophenol on the immune system were due to contaminants and not to the parent compound.

The WRI report also cited indirect evidence of a link between pesticide exposure and immune effects based on studies of cancer rates among pesticide-exposed populations. A recent farming and cancer meta-analysis (16) was cited for showing an approximate 10% elevation of rates for farmers of some lymphopoeitic malignancies seen in immunosuppressed patients [especially non-Hodgkin’s lymphoma (NHL)]. This finding was used to advance the viewpoint that pesticides increase susceptibility to certain cancers by weakening immune surveillance against cancer cells or reducing host resistance to cancer-causing viruses. However, of the four references credited with proposing (or supporting) this viewpoint (17–19), three do not address pesticides at all (17–19).

Two specific studies of farmers were cited as indicative of even stronger relationships between pesticide use and NHL when the frequency of pesticide use was considered (21,22). However, our review of these references found no analyses by frequency of pesticide use and generally null findings across numerous analyses.

Whether farmers’ cancer rates are affected by pesticide exposures remains an unresolved area of active research. At present, therefore, this literature is too uncertain to support a theory of human immune suppression from pesticide exposures.

Toxicologic Evidence

The WRI report (1) reviewed a sizable number of pesticide immunotoxicologic studies. The focus was on acute high-dose studies in which the exposure conditions resulted in systemic toxicity and, in some instances, lethality. In our opinion, studies that reflect the human experience in terms of doses, routes of administration, and exposure duration are more relevant. As pointed out previously, pesticides can modulate the immune system of laboratory animals at doses that are orders of magnitude higher than reported human exposures (23). Such studies, however, provide a questionable basis for extrapolation and generalization to human exposure scenarios. In current practice, immunotoxicity is usually evaluated at doses lower than those producing overt toxicity (24).

A number of examples from the WRI report (1) are illustrative. In vivo data from Casale et al. (25) and Krajnc et al. (26) were cited as evidence supporting pesticide (parathion) or biocide (bis-tri-N-butyltin oxide; TBTO) induced decreases in splenic weight. Casale et al. (25) observed effects only in the high-dose treatment group. The high dose approached the median lethal dose (LD50), and 36–42% of the test animals died within 12–48 hr. Studies were performed on the remaining animals at 96 hr. Immunotoxic effects were associated with severe cholinergic stimulation. Thus, immunosuppression may have been secondary to toxic chemical stress due to cholinergic poisoning (25). Similarly,
Krajcic et al. (26) administered TBTO at concentrations of 80 and 320 mg/kg. Dietary administration of TBTO at the 320 mg/kg level has been shown to have lethal effects in test animals. At the high concentration, mice were emaciated and weak, had demonstrable nasal and ocular discharges, and showed reduced activity. Reduced activity was also seen at 80 mg/kg (26).

**In vitro** evidence was cited to support the view that allethin and cypermethrine (the cyano-derivative of permethrin) inhibit the proliferation of T and B lymphocytes. Data from Stelzer and Gordon (27) show that allethin, the most potent inhibitor, blocked proliferation over a limited dose range from 1.5 × 10⁻⁵ M to 1 × 10⁻⁶ M. Similarly, cypermethrin inhibited the response over a concentration range from 1 × 10⁻⁵ to 5 × 10⁻⁶ M. In general, biologically relevant effects occur over a wider concentration range. Moreover, high concentrations (10⁻³-10⁻⁵ M) of test material are usually toxic in **in vitro** assays. In fact, the authors (27) note that 1 × 10⁻⁴ M permethrin will kill 60% of the test lymphocytes at 48 hr postplating.

Data from Casale et al. (25) and Vos et al. (28) were presented as evidence of immunotoxicity induced by zineb (a thio carbamate) and organophosphates such as azinophos and parathion. It is clear from the data that immunotoxicity occurred at concentrations that caused generalized toxicity or severe cholinergic stimulation, indicating secondary, not primary, effects on the immune system. Immunotoxicity per se is therefore not indicated by these results.

Pesticides are not routinely considered to be immunotoxins. Using structure-activity analogies to known immunotoxins, the National Toxicology Program (NTP) selected 53 compounds for use in a test validation battery (24); only 5 compounds were pesticides. This suggests that pesticides are not overly represented in the immunotoxicant family.

The NTP program tested the 53 chemicals at doses that avoided overt toxicity (i.e., defined as a reduction in body weight greater than 10%). Among pesticides, only pentachlorophenol was classified as positive. As mentioned previously, Kerkvliet et al. (14) and Holsapple et al. (15) showed that the apparent effects of pentachlorophenol were due to contaminants. Thus, the findings in the NTP database, while not a comprehensive evaluation of pesticides, are contrary to the conclusion from the WRI (1) report that there is a large body of experimental evidence indicating that pesticides are immunotoxic. Research on additional pesticides, at doses insufficient to produce overt toxicity, would be necessary to evaluate the general proposition that pesticides are likely to be immunotoxins at typical human exposure levels.

Another issue with the immunotoxicity review in the WRI report (1) is the lack of distinction between statistically significant and nonsignificant findings. The data of Lee et al. (29) are cited to support the viewpoint that organochlorines (DDT), organophosphates (crufomate and methyl parathion), and thiocarbamates (propam) decrease neutrophil chemotaxis. In fact, except for methyl parathion, these findings were within the range where chance precedes rejecting the null hypothesis.

Finally, our limited review of English language references found some papers that were cited incorrectly. For example, the authors suggested that studies by Kerkvliet and others (30,31) show that sodium arsenate impaired host resistance to tumor growth. In the summary, Kerkvliet stated: "Our results indicate that chronic exposure to sodium arsenate at arsenic levels as high as 100 ppm is not detrimental to mice in terms of tumor growth and immunosuppression" (31). Second, it was suggested that malathion and methyl parathion inhibited mitogenic responses of lymphocytes in **in vitro** assays. In fact, Lee et al. (29) used only methyl parathion and found no significant difference between the treated and control groups.

**Conclusions**

We consider "Pesticides and the Immune System: The Public Health Risks" (1) to be an important report. We hope it will begin a critical dialogue on the scientific evidence linking pesticides and human immune effects.

A thorough reading of the WRI report (1) finds frequent acknowledgments of the limitations of the available epidemiologic and immunotoxicologic research on pesticides. On balance, however, the authors concluded that immunosuppression probably occurs at pesticide exposure levels common to millions of people, especially in the developing world. We do not find consistent, credible evidence for this viewpoint, but we think that human pesticide immunotoxicity is an important area for critical scientific evaluation and future research. The conduct of epidemiologic studies of immune effects and pesticides is difficult, however, because exposure scenarios are complex and there are important issues of confounding and other biases to consider, especially in the developing world. Until some high quality research is available, it seems inappropriate to implicate pesticides as a widespread cause of immune suppression, infectious diseases, and cancer. Additional reviews of the literature would be beneficial from the perspective of consensus, research planning, and exposure prevention efforts.

We recommend that future reviews of the epidemiologic literature make distinctions, when possible, by type of pesticide or, at least, by pesticide class. The general trend in industry is decidedly toward marketing less toxic pesticides, and perhaps this trend could be accelerated by a more specific review.

Evaluation of the potential health effects of pesticides is only one aspect of improving the health and immunologic status of populations in the developing world. Risk/benefit evaluations should also consider the beneficial effect of pesticides in increasing food production and combating the spread of infectious diseases.

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