Pesticide-induced Immunotoxicity: Are Great Lakes Residents at Risk?

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Several organophosphate and organochlorine compounds, including pesticides commonly found in the Great Lakes basin, have the potential to induce immunotoxicity. Because of biomagnification and accumulation in the food chain, Great Lakes residents may inadvertently be exposed to these compounds and thus face increased risk of immune dysfunction. In spite of the laboratory animal data and evidence from occupational exposures that suggest immunotoxicity, there is no definitive evidence as yet that environmental exposure to these xenobiotics poses a significant threat to the human immune system. Although the laboratory studies addressed are limited, this review will attempt to bridge the gap between the laboratory data (1–5) and real-life exposure by highlighting the potential for human immunological changes caused by ingestion of fish and wildlife harvested from the region. This paper was prepared for the Great Lakes Health Effects Program which is part of a Canadian Department of Health Initiative established in 1989.

Introduction

Pesticides are among the many chemicals present in the Great Lakes basin. Four of these pesticides, including hexachlorobenzene (HCB), mirex, dieldrin, and dichlorodiphenyldichloroethane (DDT) and its metabolites, have the potential to produce immunotoxicity and are thus among the 11 critical environmental pollutants identified by the International Joint Commission (1). Although not considered as critical pollutants, other organochlorine and organophosphate pesticides such as chlordane and malathion have the potential for human exposure through the food chain (2). In addition to the presence of measurable residues in fish and wildlife as well as people living in the Great Lakes basin, the pesticides listed above have documented immunotoxic effects in laboratory animal studies. The effects range from an increased incidence of experimental infection to specific effects on immune system structure (histopathology) and function (3–5).

Due to the persistent nature of these compounds and biomagnification and accumulation in the food chain, Great Lakes residents who consume large amounts of contaminated fish and wildlife may ingest greater amounts of these compounds than the general population. At the present time there is no clear evidence that environmental exposure to these pesticides through consumption of contaminated fish or wildlife in the Great Lakes Basin poses a threat to the human immune system. Currently, the best source of information concerning the potential immunotoxic effects of these pesticides in humans comes from laboratory animal studies. These studies have shown that under defined conditions these compounds can modulate the immune system. Generally, the effects are seen only in carefully controlled laboratory studies using inbred or hybrid strains of experimental animals. Furthermore, the doses of pesticide required to modulate the immune response are often orders of magnitude higher than those reported for human exposure.

A review of pertinent laboratory animal studies, as well as epidemiology reports of occupational exposure to pesticides, and potential immunotoxicity is presented. There are data that suggest that occupational exposure to pesticides, especially in situations where high concentrations of material are present, can induce contact and immediate (respiratory) sensitization. For recent reviews on this subject, see Edmiston and Maddy (3), Thomas et al. (5), and Murray and Thomas (6). Emphasis in this review is placed on data concerning the potential immunosuppressive effects of pesticides and related compounds that tend to persist in the environment and may be particularly important under chronic exposure conditions such as those encountered in and around the Great Lakes Basin.

Animal Studies

Several organophosphate and organochlorine compounds, including pesticides commonly found in the Great Lakes basin, have been shown to alter immune function in laboratory animals. Typically, laboratory animal studies are conducted under carefully controlled conditions with acute or relatively short-term exposures at concentrations considerably higher than those encountered in the environment. Considerable variation exists in animal species used and in route, duration, and level of exposure, as well as end point examined. In almost no case were efforts made to verify actual delivered dose. In studies involving dose–response experiments, little effort is ever made to determine a lowest observable effect level (LOEL). In some cases, increased susceptibility to an infectious agent and alterations in immune-function parameters were reported; in others, only changes in immune function were examined. In spite of these experimental uncertainties, it is clear that many organochlorine and organophosphate compounds, including pesticides, are capable of modulating the immune system. Since some of these materials have the potential to persist in the environment, they are particularly important from a health hazard perspective under chronic exposure conditions such as those encountered by individuals consuming fish from the Great Lakes. For a comprehensive review of the literature on pesticide immunotoxicity, see Thomas et al. (5), Luster et al. (7), Newcombe and
Although with colleagues thought complemented machinery humoral dence dimethyl mg/kg), malathion fact the may noncholinergic treated presented below.

Organophosphate pesticides were first reported as immunotoxicants in the early 1970s by Ercogovich (10) and Street and Sharma (11). The latter study reported thymic atrophy and reductions in splenic germinal centers in rabbits following exposure to 1.5 mg/kg/day of malathion. Reductions in protective responses in mice to the Gram-negative bacterium Salmonella typhimurium were reported following malathion exposure (12). Studies by Casale et al. (13) reported suppression of primary humoral immune responses to a T-cell-dependent antigen in rodents treated orally with cholinergic doses of parathion (16 mg/kg), malathion (720 mg/kg), or dimethyl dichlorovinyl phosphate (DDVP; 120 mg/kg). Suppression was absent at noncholinergic doses, suggesting that stress may have played a role. Casale and colleagues (14) compared several organophosphate compounds for their ability to inhibit human serum complement-mediated lysis of sheep red blood cells (SRBC). Although dose-dependent inhibition of complement activity was noted, potencies of these compounds to inhibit acetylcholinesterase activity did not correlate with classical complement inactivation. The authors concluded that the risk of complement inactivation leading to increased incidence of infection is small in workers occupationally exposed to anticholinesterase compounds. An immunosuppressive mechanism involving cholinergic stimulation is thought by some to be unlikely, given the fact that cholinergic agonists fail to suppress the immune system in vivo (15−17).

Extensive studies by Rodgers and colleagues (18−23) have focused on defining the impact of the trialklyphosphorothioate contaminant O,O,S-trimethyl phosphorothioate (OOS-TMP) on the rodent immune system. This compound is formed as an impurity during the manufacture and storage of certain organophosphate pesticides such as malathion and fenitrothion. Briefly, dose-dependent suppression of both humoral immunity and inability to generate cytotoxic T-lymphocytes was noted. Further, decreased antigen presentation and IgA antigen expression was noted in splenic macrophages from OOS-TMP treated mice (18−23). The dose levels with which effects were seen ranged from 0.5 to 10 mg/kg. Rats treated orally with a single dose of 20 mg/kg OOS-TMP exhibited altered macrophage cytotoxicity and esterase activity, which may contribute to the pathogenesis of lung injury seen in rats treated with these compounds (24).

Hinton et al. (25) illustrated that toxicity observed in health effect studies of these compounds is often due to technical-grade contaminants. No statistically significant suppression of immune status (antibody titers, serum immunoglobulins, immunohistopathology) was noted in Sprague-Dawley rats receiving pure triphenyl phosphate (TPP) at doses up to 10,000 ppm in the diet for 120 days. Although the data suggest that TPP has no effect on innate or adaptive immunity, the extremely small numbers of animals/dose/end point (i.e., two to three rats per point for antibody titers) limit the statistical power of this study.

Hexachlorobenzene

Hexachlorobenzene (HCB) is one of the most persistent and ubiquitous chemicals in the environment. It is no longer manufactured in Canada or the United States for use as a fungicide, although it is generated in significant quantities as a by-product (26). The acute toxicity of HCB is relatively low in laboratory animals, and the oral LD$_{50}$ (lethal dose, 50%) for rats is greater than 3000 mg/kg (27).

Animal studies have demonstrated species-specific effects of HCB on the immune system. Based upon studies by Loose et al. (28), increased susceptibility to infection and increased sensitivity to endotoxic challenge were noted in mice fed approximately 30 mg HCB/kg/day. Unfortunately, a no observable adverse effect level (NOAEL) could not be estimated from these studies since single concentrations of HCB were administered. Effects on the generation of humoral immunity are less clear cut (29). Barnett et al. (30) reported that in the absence of changes in splenic B- and T-cell mitogenesis, antigen-specific delayed-type hypersensitivity and mixed lymphocyte culture responses were suppressed in mice exposed perinatally to up to 5 mg/kg HCB. On the other hand, combined prenatal and postnatal exposure of rats to as little as 4 mg/kg HCB significantly increased immunoglobulins M and G (IgM, IgG) antibody titers to tetanus toxoid as well as delayed hypersensitivity to ovalbumin in a dose-dependent manner relative to controls (31). In these studies at dose levels that appear to stimulate the immune system, no changes in host defense against experimental infection with *Trichinella spiralis* was seen. Recent studies have confirmed and extended those earlier observations by demonstrating increases in IgM serum and autoantibody levels in rats fed approximately 18 mg HCB/kg per day for 13 weeks. Furthermore, biotransformation studies suggest that HCB-induced porphyria is not involved as a mechanism of immune modulation by this compound (32).

Short-term exposure to 35 mg/m$^3$ HCB aerosols for 1 or 4 days significantly reduced the capacity of alveolar macrophages to phagocytize radiolabeled red cells as well as viable Klebsiella pneumoniae bacteria, suggesting impaired pulmonary host defense (33). Lymphoproliferative responses to T- and B-cell mitogens in these studies in the lung-associated and mesenteric lymph nodes exhibited differential effects depending upon location and duration of exposure. In other studies, evidence of reduced pulmonary natural killer cell activity was observed in rats following oral exposure to 150 mg/kg HCB in the diet for 6 weeks (34); unfortunately, a NOAEL could not be determined from this study. Proliferation of lymph node, high endothelial venules following HCB exposure noted by Kitchin et al. (35), as well as Vos et al. (36), in rats at prenatal and postnatal HCB doses of 50 mg/kg/day may alter T- and B-lymphocyte trafficking and explain these differential effects. Hyperplasia of gut-associated lymphoid tissues was also noted in dogs administered as little as 1 mg/kg/day (37), providing further histological evidence for an effect of HCB on the immune system.

Mirex

Mirex is a cyclodiene contact insecticide, although it has had several other applications. Its use in Canada has been banned since 1978. It is one of the most stable chemicals known, but it does slowly break down to photomirex (8-monohydromirex), a toxic chemical that contains one less chlorine atom than mirex.

Single doses of mirex are moderately acutely toxic in laboratory animals. The acute oral LD$_{50}$ of mirex in rats is 600 to 700 mg/kg. Mirex causes several effects in laboratory animals, including morphological changes in the liver (0.05 mg/kg/day), fetotoxicity (1−2 mg/kg/day), and carcinogenicity (5−10 mg/kg/day) (38). A no observable effect level (NOEL) for the immune system can be estimated at 40 mg/kg in chickens following chronic (5-week) dietary exposure to the pesticide (39).
Dieldrin and Aldrin
Dieldrin and aldrin are persistent chlorinated hydrocarbon insecticides that were used in Canada and the United States for control of soil insects and mosquitoes. Both were manufactured on a commercial scale between 1950 and 1990. Most uses of aldrin and dieldrin were banned in the United States in 1975. In Canada, all food uses for dieldrin were prohibited in 1978 and only limited applications are now permitted (1).

Aldrin is rapidly metabolized to dieldrin in plants, animals, and humans. The modes of action of both compounds, including acute and chronic toxicity, are nearly identical. Single doses of dieldrin have a high acute toxicity in laboratory animals, and the acute oral LD₅₀ in rats is approximately 50 mg/kg (40). The overall NOEL for chronic toxicity in rodents is approximately 1 mg/kg/day (40). Relative to the NOEL, the immune system appears to be fairly resistant to the effects of this pesticide. Inhibition of the ability of murine macrophages to restrict intracellular viral replication of mouse hepatitis virus 3 (MHV3) was seen in mice receiving a single ip injection of 36 mg/kg dieldrin, a very high acute dose (41). In related studies, impairment of macrophage phagocytosis, as well as suppression of antibody responses to both T-dependent and T-independent antigens, was also observed, albeit at similar high acute ip doses (42,43). The investigators suggested that the former changes contribute to increased susceptibility of C57Bl/6 mice to challenge with MHV3 in vivo seen in earlier studies (44).

In spite of these effects, the biological significance of these observations in light of the comparatively low toxicity of dieldrin can be questioned.

In continued studies, impairment of T-cell-mediated graft-versus-host reactions was seen in recipient mice receiving allo geneic lymphoid cell suspensions from dieldrin-treated mice (45). Hugo et al. (45) ruled out changes in lymphocyte subsets, cytotoxicity, and inhibition of T-cell proliferation potential as mechanisms.

Since they did not fully characterize the donor cell population, the contribution of macrophages to this effect cannot be discounted. Treatment of C57Bl/6 mice with mixtures of dieldrin, malathion, and carbophuran did not result in synergy or additivity of effects attributed to single compound exposures (46). As to the LD₅₀ value above, the doses used in the above studies are relatively high. Furthermore, the LOEL could not be estimated from these studies.

Dichlorodiphenyltrichloroethane
Dichlorodiphenyltrichloroethane (DDT) was developed in the late 1930s and it was used extensively as a broad-spectrum contact organochlorine insecticide. Its use peaked in the early 1960s, with U.S. production at 176 million pounds in 1963. In 1973, DDT was banned in the United States except for essential public health purposes. In the early 1970s, most uses of DDT were banned in Canada, but the last remaining product registration was not discontinued until 1989.

Although DDT is relatively stable, it undergoes a relatively complex series of biological degradative changes. The most important of these is dehydrochlorination to dichlorodiphenyldichloroethylene (DDE). DDE is more lipid soluble and, as a result, is much less toxic to mammals than DDT. Like the parent compound, DDE is retained in tissues for long periods of time.

The acute oral LD₅₀ for DDT in rats is approximately 100 mg/kg. In contrast to the other priority pesticides found in the Great Lakes Basin, numerous studies have been conducted on the effect of DDT on the immune system of laboratory animals (39,47–50).

In general, the immune system is not a sensitive target organ for toxicity. Differences in species used, dose, duration, routes of exposure, and parameters evaluated make it difficult to estimate a LOEL for the immune system. However, the lowest in vivo immunosuppressive effects of DDT were based upon histopathological changes in lymphoid organs in rabbits fed the equivalent of 0.18 mg/kg DDT/day for up to 8 weeks (41).

Acute DDT poisoning in humans is rare, considering its widespread use. A single oral dose of 10 mg/kg produces toxicity in humans. There is no evidence of any adverse long-term effects resulting from small daily doses of DDT and no conclusive evidence that DDT is immunosuppressive in humans inadvertently exposed through the food chain. Recent reports (51) have cited an association between DDT exposure and appearance of breast cancer, but the relationship of this observation to changes in tumor surveillance mechanisms has not been established.

Chlordane
Chlordane has had a wide variety of applications since its development as an insecticide in the mid-1940s. It was heavily used without restriction until the sole U.S. manufacturer voluntarily stopped production in 1988. Chlordane is absorbed and retained in lipid-containing tissues following any route of exposure; therefore, the potential exists for immunotoxicity. Laboratory animal studies have suggested that this compound adversely affects the immune system, especially in the fetus. In 1982, Spyker-Kranmer et al. (52) reported that the offspring of mice treated perinatally with chlordane exhibited suppressed cell-mediated immunity (CMI) compared to controls. In the absence of effects on adult animals (53), chlordane appears to exert immunotoxic effects in vivo. Menna et al. (54) demonstrated that, compared to controls, chlordane-exposed offspring are capable of withstanding a higher challenge dose of influenza virus in the absence of effects on viral replication in vivo (55).

Diminution of viral specific delayed-type hypersensitivity responses in the absence of changes in T-cell blastogenesis at maternal chlordane dose levels of 4 mg/kg/day may have minimized infectious pathogenesis and contributed to increased survival (56).

In further studies, persistent suppression of adult bone marrow colony formation was noted in perinatally treated animals (57) with females exhibiting greater sensitivity (58). Furthermore, recent studies suggest that 8 mg/kg maternal doses of chlordane differentially introduce defects in macrophage biochemical mechanisms associated with tumor cell killing in offspring (59).

In vitro studies suggest that chlordane impairs early activational events in antibody synthesis, albeit at relatively high concentrations of up to 10 µM (60).

Unlike other organochlorine compounds, the above studies suggest that perinatal exposure to chlordane induces long lasting and subtle effects on immune function. The in vivo perinatal exposure model used in many of the above studies, however, achieves a total maternal chlordane dose that approaches 3 mg, an amount that may not be environmentally realistic with regard to human exposure potential.

Human Studies
In the case of occupational pesticide exposure, few examples have been reported for which a direct correlation of immunotoxic effects in man and experimental animals can be made. Studies based on data derived from company and municipal death records suggest an association between occupational exposure to pesticides in grain mills, for example, and higher incidence of neoplasms in the hemopoietic and lymphatic tissue than...
observed in other tissues. Similarly, increases in the incidence of leukemia and myeloid leukemia occurred among pesticide workers in Florida (61). Pearce et al. (62) suggested that pesticides act additively or synergistically with other chemical agents and concluded that agricultural workers have an increased risk of multiple myeloma, not because of any obvious patterns of pesticide use but perhaps because of other chemicals used in their industry. Recently a cohort mortality study among more than 5000 chemical-manufacturing workers showed increased mortality due to pancreatic cancer (63). Among subjects with a mean exposure to DDT of 47 months, the authors claim that the cancer risk was 7.4 times greater than among subjects with no exposure.

A recent review and metaanalysis of epidemiologic studies of human pesticide exposure performed from 1975 to 1991 revealed no significant decrease in life expectancy or increase in cancer incidence as a result of exposure (64). However, the authors noted an increased risk of myeloproliferative disorders associated with exposure among manufacturers, applicators and farmers. Marconi and Fait (64) caution that other etiologic agents (e.g., viruses) that may be responsible cannot be ruled out.

Although there is evidence that pesticides affect certain functions of the immune and hematopoietic systems that may predispose to neoplasia, it remains difficult to relate these changes to clear-cut adverse health risks. For example, even though morphological changes in blood were observed in special occupational conditions such as pesticide application within greenhouses where high concentrations of pesticide were present, there was no indication of altered immune status (65). However, in a study of pesticide workers exposed to combinations of four widely used organophosphate and organochlorine pesticides (malathion, parathion, DDT, and hexachlorocyclohexane), 73% showed signs of toxicity, including altered levels of serum immunoglobulin levels (66). Increases in serum IgG but decreases in serum IgM and complement C3 were reported in a study of 51 men exposed to chlorinated pesticides as compared to a 28-man control group. Immune complexes were suggested as a possible significant factor in occupational diseases (67). In each of these studies, a direct association with a change in immune status was absent. Some in vitro studies complement these findings by showing that either paraxon or malathion reduces both erythrocyte and granulocyte stem-cell colony formation and thus support the principle that pesticides affect stem cell growth (68).

Although it is well documented that exposure to organophosphorus compounds alters human monocyte carboxylesterase activity (69–71), the biological significance of this effect is still unclear. The concentrations of organophosphorus compounds causing 50% inhibition of monocyte esterase activity have been determined to be in the 1 to 10 μM range (72). Although no measurements were taken, Newcombe and Esa (4) concluded that exposed workers presenting decreased monocyte esterase activity may have circulating concentrations of organophosphorus compounds in the same concentration range. A possible mechanism for inhibition of monocyte-non-specific esterases was suggested by Paxman et al. (73). Adherent human monocytes (not lymphocytes or neutrophils) released phenol upon exposure to radiolabeled (TPP). The cellular degradation of TPP was inhibited by diisopropylfluorophosphate (DFP), an esterase inhibitor. Catechol, hydroquinone, and biphenyls were also produced. It was concluded that TPP inactivates nonspecific esterase by phosphorylation after release of phenol.

An early report by Lee et al. (74) demonstrated that for malathion, the in vitro IC50 (inhibitory concentration, 50%) for T-lymphocyte responses to phytohemagglutinin was 35 μM. Furthermore, a 10-μM concentration of methylparathion in culture significantly reduced neutrophil chemotaxis. Esa et al. (72) reported studies on the modulation of CMI responses and monocyte accessory cell function following in vitro exposure of human cells to a number of organophosphate compounds. Of the four compounds tested, dose-related suppression of antigen-induced human lymphocyte proliferation was seen only following pretreatment with tetra-α-cre-sylpiperazinyl diphasophoamide (TCPD) for 16 hr. Although dose related, this effect was not striking. If the incubation time is reduced from 16 to 6 hr, the effect is not apparent at the same concentrations (10 μM) as above. Partial restoration of cell cultures containing normal lymphocytes and TCPD-treated monocytes that allows a response to antigen suggests that the defect resides in the monocyte. The fact that restoration was only 70% of control implies that other factors may be involved. Antigen presentation studies with monocytes indicate that there may be a delay in the kinetics for antigen processing and presentation at chemical concentrations as low as 1 μM. These data may correlate with those of Paxman et al. (75), suggesting that organophosphorus compounds induce down regulation of monocyte surface HLA-DR antigen expression, a function critical to normal immune responsiveness.

Although considerable information is known about the molecular mechanisms of esterase inhibition and functional immune impairment by organophosphorus compounds, there is no convincing evidence that humans occupationally exposed to these materials have higher incidences of infection or disease (69,70). Hermanowicz and Kossman (76) examined neutrophil function and prevalence of respiratory infections in workers occupationally exposed to organophosphate pesticides compared to controls. Inhibition of serum and red blood cell cholinesterase correlated with degree of exposure. In contrast to the studies described above, increased overall incidence of respiratory infection was observed in exposed workers compared to controls. In addition, the incidence of recurrent infection was positively correlated with length of exposure. Impairment of neutrophil chemotaxis was also observed; however, in workers exhibiting increased morbidity, neutrophil function was similar to those that were healthy. It was concluded that inhibition of host defense was not due to impairment of neutrophil function but may have resulted from other nonspecific effects on the lung.

Summary and Conclusions

In summary, it is clear that under carefully controlled laboratory conditions pesticides and related compounds associated with the Great Lakes basin are capable of modulating the immune system of experimental animals. However, in assessing potential risks to the immune system of Great Lakes residents from environmental exposure to these compounds, problems include determining the exposure variables related to a particular compound, identifying the appropriate immunologic end points for evaluation, and interpreting the data gathered as a result of these evaluations. Subtle perturbations in immune function following exposure to environmental chemicals may not, in every instance, result in a relevant health effect. Alternatively, subtle changes in certain immune functions could conceivably increase the likelihood of adverse immune-related health effects only during the brief period when these effects are present, or these changes may result in minor health
A pesticide with xenobiotic activity should be objectively assessed using standardized methods of exposure studies. Xenobiotics are generally selective, causing subtle immunotoxicity that can manifest as changes in the immune system without overt symptoms. Laboratory studies can help identify xenobiotics that may cause immune dysfunction or modify immune responses. However, confounding factors must be considered when interpreting study results. Studies in humans and animals indicate that xenobiotics can cause profound, yet subtle, changes in the immune system.

Variability in population exposure and biological differences complicate the assessment of pesticide immunotoxicity. Although many pesticides are toxic to laboratory animals, their effects on humans may be subtle and difficult to quantify. Diseases associated with immune dysfunction may become detectable only after prolonged latency. The risk of human exposure to pesticide residues is not well documented, as most studies have been conducted in controlled settings.

The immune system is complex and variable, making risk assessment difficult. Although no substantial evidence exists to date, exposure to pesticides, either in the workplace or through contact in the environment, induces significant immune dysfunction in humans. Requirements for risk assessment should include identifying pesticide-related exposures and assessing the potential for immune dysfunction in susceptible populations. Future research should focus on determining the variability of pesticide immunotoxicity and improving risk assessment methods.
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