Chapter 1
Introduction and Summary

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INTRODUCTION

The potential for substances used in industry, transportation, and households to be simultaneously beneficial and toxic to human life creates a legislative and regulatory dilemma. The challenge of balancing a strong economy, one that delivers products that people need and desire, with the health and safety of the populace sometimes seems to be a tremendous burden.

Technological advances add to the weight of that burden. Thousands of new, potentially toxic substances enter the market annually. Advanced instruments help scientists measure the presence of new and existing substances in minute quantities. Substances formerly unknown or undetected suddenly become worrisome as technology provides the means to predict human risks from these substances.

Governmental concern that a substance might create an adverse health effect historically concentrated on carcinogenicity. Most Federal legislative and regulatory efforts to prevent or minimize human exposure to toxic substances have focused on identifying and controlling carcinogens. Physicians and researchers now recognize the noncancer, toxic effects of many substances. Some of these effects, for instance teratogenicity, have become the subject of specific legislative concern. Federal regulatory attention to other types of toxic injury, e.g., to the nervous system, the immune system, the respiratory system, depends on the more general mandate to protect human health. Some observers fear that historical emphasis on carcinogenicity, combined with limited agency resources, has led to neglect of problems such as neurotoxicity, immunotoxicity, or pulmonary toxicity– problems that may be as widespread and severe as carcinogenicity.

The Senate Committee on Environment and Public Works, and its Subcommittee on Toxic Substances, Environmental Oversight, Research and Development, asked for assistance from the Office of Technology Assessment (OTA) in evaluating technologies to identify and control noncancer health risks in the environment. The committee's interests include advances in toxicology, research and testing programs in the Federal agencies, and the consequences of exposure to toxic substances.

This background paper, which describes the state-of-the-art of identifying substances that can harm the immune system, represents one response to the committee's request. Chapter 2 provides basic information about the principal components of the immune system and the general consequences that stem from perturbations to it. Chapter 3 describes methods for evaluating chemical immunotoxicity and reports on some known or suspected immunotoxicants. Chapter 4 summarizes Federal research and regulatory activities related to immunotoxicity. Appendix A provides a very brief synopsis of income replacement programs available to persons disabled by toxic exposures.

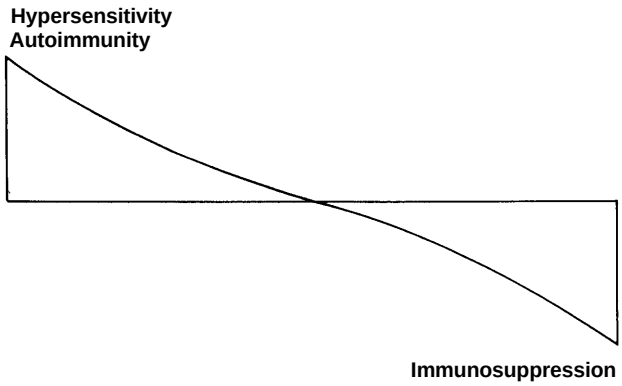
TERMINOLOGY

This background paper examines a field of study rife with jargon. Not all specialists in the field of immunotoxicology--which combines the expertise of immunologists and toxicologists — use terminology in precisely the same manner. The following definitions explain how OTA applies the terms-of-art essential to this study.

immunotoxicity, or an immunotoxic effect, is defined as an adverse or inappropriate change in the structure or function of the immune system after exposure to a foreign substance. Foreign substances capable of adversely affecting the immune system may be of synthetic or natural origin. This study focuses on chemicals, including industrial, transportation, agricultural, and household chemicals, drugs, and food additives.

immunotoxicity manifests at both ends of a spectrum— as an enhanced but inappropriate immune response, and as a failure to mount an appropriate immune response. (See figure l-l.) This background paper examines three types of adverse effects: immunosuppression, hypersensitivity, and autoimmunity. *Immunosuppression is* a generalized decrease in immune responsiveness that may result in increased incidence of

Figure 1-1- Spectrum of *Adverse* Immune System Effects



SOURCE: Office of Technology Assessment, 1991.

infection or tumors. Immunologic hypersensitivity is allergy-an overreaction of the immune system to a substance. Hypersensitivity is a normal immune response to a foreign substance, but one with deleterious consequences. *Autoimmunity* results from a breakdown in the ability of the immune system to distinguish "self' from "nonself." An autoimmune reaction involves the body mounting an immune response against some of its own components.

Substances that provoke an immune response are called *antigens.* Antigens trigger beneficial responses, e.g., destruction of an infectious agent, and adverse responses, e.g., hypersensitivity. Other substances can damage the immune system by destroying or inactivating specific elements within it, thereby suppressing immune response. Substances that elicit adverse immune responses or damage the immune system are called *immunotoxic substances,* or *immunotoxicants.*

THE IMMUNE SYSTEM

The immune system is a complex, cooperative effort among several types of cells, cell products, tissues, and organs in the body. The simplified description that follows should help the reader comprehend state-of-the-art immunotoxicology, but by no means represents an exhaustive examination of this complicated organ system.

The immune system involves several cell types. Most of them belong to the large group of cells commonly lumped together as white cells. The white cells of principle interest to immunotoxicologists are macrophages and lymphocytes. Macrophages ingest cellular debris and infecting organisms, and they process and present antigens to the lymphocytes. Lymphocytes include B cells, T cells, and natural killer (NK) cells. B cells secrete antibodies (immunoglobulins), substances that inactivate antigens in the body. Each B cell makes antibody specific to a given antigen; the body can make antibodies to thousands of antigens.

T cells regulate the immune system. T cells must interact with B cells for antibody production. Some T cells can directly kill cells presenting antigens. T cells also secrete cytokines, protein molecules that transmit signals to modulate (augment or suppress) immune response between cells of the immune system. (Macrophages also produce cytokines.) NK cells attack and destroy certain other cells. Most types of cells are resistant to NK cell activity, but tumor cells and virus-infected cells appear to be susceptible.

The organs of the immune system– the lymphoid organs– include the bone marrow, the thymus, the lymph nodes, and the spleen, as well as the tonsils, the appendix, and clumps of tissue in the small intestine and the respiratory tract. The lymphoid organs are compartmentalized collections of lymphocytes, macrophages, and other immune system cells. They produce, store, and distribute the immune system cells. The lymphatic vessel network also circulates lymphocytes.

Foreign substances provoke two basic types of immune response: nonspecific immunity and acquired immunity. Nonspecific immunity, which involves functions of the macrophages and NK cells, represents the body's first line of defense. The nonspecific immune response occurs without prior exposure to antigens. Acquired immunity develops after the initial exposure to a foreign

substance and can be subdivided into humoral immunity and cell-mediated immunity. humoral immunity involves antibody production, which generally requires macrophages (to process and present the antigen), T cells (to stimulate B cell production), and B cells (to produce antibodies). The other basic type of acquired immunity, cell-mediated immunity, also begins with the microphage but then relies on the T cell functions of cytotoxicity and cytokine secretion. Although scientists distinguish between humoral and cell-mediated immunity, most antigens can provoke both types of immune response.

IDENTIFYING IMMUNOTOXIC SUBSTANCES

The AIDS epidemic has heightened public interest in the immune system and its potential for injury. A virus, not a chemical, causes AIDS, but the disease illustrates the devastating effects of immunosuppression and suggests to people the importance of protecting the immune system. In response to increased public awareness, researchers have devoted greater effort in recent years to developing predictive tests to measure the potential immunotoxicity of chemicals before they enter commerce.

As a result of these efforts, a number of tests now exist to assess the effects of foreign substances on the components and processes of the immune system. Researchers testing a chemical's immunosuppressive potential often start with pathology, an examination of tissues and organs of the immune system for evidence of disease. The organs of experimental animals can be weighed or cells from organs or in peripheral blood can be counted to get preliminary indications of how a substance may affect the immune system. Other assays of immunosuppression assess whether exposure to a chemical affects the ability to mount a particular type of immune response. Measures of humoral, cell-mediated, and nonspecific immunity exist and can be applied in the laboratory. These tests usually involve experimental animals but occasionally direct assessment of humans.

The most comprehensive tests of immunosuppression available to researchers today measure a subject's ability to fight infections or tumors after exposure to a suspected immunotoxicant. Scientists expose experimental animals to a toxic substance and subsequently expose (challenge) the animals to an infectious agent or a tumor. If animals exposed to the suspected toxicant show a significant increase in the incidence of disease or death following challenge compared to unexposed animals, the substance is suspected to be immunotoxic.

Researchers and manufacturers also use hypersensitivity assays. Whether a substance can induce hypersensitivity can be assessed using skin and respiratory tests. Antibodies can also be measured in the blood of animals or humans who have had an allergic reaction to an antigen. (Table l-l lists common immunotoxicological tests.)

immunotoxicological testing presents an investigator with significant challenges in interpretation. Test results can differ depending on the subject's age, species, sex, or recent illnesses. Environmental factors, such as diet or other chemical exposures, can also affect immune system performance. Choosing the appropriate test dose and the means and duration of exposure can prove difficult when the point of the exercise is to extrapolate from the test to the predicted consequences of actual human exposure. (See box l-A.) These considerations present themselves in all types of toxicology, but the complexity of the immune system compounds these difficulties for immunotoxicologists. immunotoxicology's fledgling status as a field of scientific inquiry and the fact that few toxicologists have training in immunology further complicate study of immunotoxicity.

The immune system is thought to have a reserve capacity, although the size of that reserve is as yet undetermined. Thus tests that measure impairment of one immune system component may not, in fact, indicate overall immunotoxicity, since other immune components or processes may compensate for the impairment. Frequently a decrease in a particular immune function is discerned but no clinical symptoms – certain infections or cancers, for instance– appear during the test period. There is still much to be learned about the long-term consequences of weakening an individual component of the immune system.

Relatively few data exist on immunotoxicity in humans. Studies of radiation victims and patients receiving immunosuppressive drugs provide evidence of the consequences of long-term immunosuppression. A few epidemiologic studies of toxic exposures that appeared to result in immunosuppression have been attempted,

Table I-l—Common immunotoxicological Tests

Category	Test
Pathology	Hematology
	Organ weights
	Histology
	Cellularity
humoral immunity	Antibody plaque forming cell (PFC) response
	B cell litogen response
	Immunoglobulin levels in serum
	Quantitation of splenic and/or peripheral blood B cells
Cell-mediated immunity	T cell mitogen response
	Cytotoxic T lymphocyte (CTL) cytolysis
	Delayed hypersensitivity response
	Mixed leukocyte response (MLR)
	Quantitation of splenic and/or peripheral blood T cells
Nonspecific immunity	Natural killer cell activity
	Microphage counts
	Neutrophil counts
Host-resistance . . .	Syngeneic tumor cells:
	. PYB6 sarcoma (tumor incidence)
	. B16F1O melanoma (lung burden)
	Bacterial models:
	• *Listeria monocytogenes* (mortality)
	• *Streptococcus* species (mortality)
	Viral models:
	. Influenza (mortality)
	• Mouse cytomegalovirus (mortality)
	Parasite models:
	. *Plasmodium yoelii* (parasitemia)
Hypersensitivity . . .	Draize test
	Open epicutaneous test
	Buehler test
	Freund's complete adjuvant test
	Optimization test
	Split adjuvant test
	Guinea pig maximization test
	Mouse ear swelling test
	Respiratory rate measurement
	Serum lgE measurement
	Local lymphnode proliferation assav

NOTE: immunotoxicologists seldom perform each test or type of test for a particular substance. They often divide the tests into tiers, using tests that indicate immune system damage at a fairly gross level to screen for potential immunotoxicants, and applying more sensitive and specific tests only to those substances that indicate possible toxicity in the primary screen. These tests are described inch. 3.

SOURCE: Office of Technology Assessment, 1991.

but with inconclusive results. On the other hand, workers and consumers have provided conclusive evidence that several chemicals can induce hypersensitivity. At least one epidemiologic study has examined humans for signs of autoimmunity resulting from chemical exposure. However, the overall lack of data makes it problematic to extrapolate from the results of tests of substances in experimental animals to the likely effects of human exposure.

EXAMPLES OF immunotoxic SUBSTANCES

Scientists have identified several substances that suppress the immune system, induce hypersensitivity, or cause autoimmunity in laboratory animals. This is true despite the fact that testing for immunotoxicity has been done on very few of the chemical substances, regulated or unregulated, in commerce.

Substances That Suppress Immune Response

Substances that can suppress immune responses fall into several general categories, including: therapeutics, solvents, pesticides, halogenated aromatic hydrocarbons, polycyclic aromatic hydrocarbons, airborne pollutants, and metals. Testing has not been conducted on all individual substances in any particular category, and not all substances tested within each of these categories have been shown to be immunotoxic. Not all categories of chemicals have been scrutinized.

Therapeutic drugs designed to prevent transplant rejection or treat cancer or autoimmune disorders are the most thoroughly studied immunosuppressive chemicals. The most frequently used immunosuppressive drugs fall into four basic categories-–alkylating agents (e.g., cyclophosphamide), glucocorticosteroids (e.g., prednisolone), antimetabolites (e.g., azathioprine), and natural products (e.g., cyclosporin A).

Benzene, a solvent commonly used in many industrial processes, has been linked to immune dysfunction. Workers chronically exposed to high levels of benzene (> 100 ppm in the air) experienced increased rates of agranulocytosis and myelogenous leukemia, accompanied by an increased risk of infection. The immunotoxicity of benzene at levels closer to the current regulatory level (1 to 5 ppm in the air) has not been demonstrated.

Animal studies of, pesticides show evidence of immunosuppressive potential, but little analysis of the effects of human exposure has been done. Among the commonly found halogenated aromatic hydrocarbons (HAHs), polybrominated biphenyls (PBB), polychlorinated

Box 1-A—General Principles of Toxicology

To evaluate the toxic nature of a substance, including its immunotoxicity, scientists have developed several general criteria for consideration, including:

Nature of the Toxic Substance—Toxicologists try to determine the characteristics that render a chemical toxic. Individual molecules may be nontoxic in their native states but become toxic after being metabolized.

Dose and Length of Exposure—These parameters, together with rates of metabolism and excretion, determine what quantity of a substance is actually affecting the body. A given substance may be toxic in high doses but nontoxic under conditions of chronic low-dose exposure.

Route of Exposure—The pathway by which a toxicant enters the body (e.g., skin, eye, lungs, or gastrointestinal tract) affects its toxicity. The amount of absorption, ability of the toxicant to combine with native molecules at the entry point (e.g., heavy metals with skin collagen), vulnerability of sensitive areas (e.g., lining of the lung), and condition of the organ at time of contact (e.g., pH and content of the stomach) all play a role in subsequent toxicity.

Species Affected—Toxicants exhibit different levels and effects of toxicity depending on the species on which it is tested.

Age—Susceptibility to a toxicant varies with age—the young and the old generally being the most susceptible.

State of Health—The health status of an individual, including the presence of disease, can greatly affect toxicity response. For example, substances that are detoxified by individuals with normal liver functions may cause adverse effects in people with liver diseases.

Individual Susceptibility—Host factors such as genetic predisposition affect the response of an individual to a toxicant.

Presence of Other Agents—Toxicology often involves evaluating one substance in isolation, yet the body is seldom exposed to agents in this manner. Knowledge about toxic effects of multiple substances is not well-developed because of the practical limitations of testing the infinite number of combinations.

Adaptation/Tolerance—Biological adaptation to a toxicant often occurs when chronically low doses are presented. Adaptation/tolerance must be factored into evaluating the range of individual responsiveness to a toxicant.

SOURCE: Office of Technology Assessment, 1991, based on M.A. Ottoboni, *The Dose Makes the Poison* (Berkeley, CA: Vincente Books, 1984).

biphenyls (PCBs), and dioxin have shown evidence of immune suppression in animal studies. However, the few human studies on these substances have either been inconclusive or have contradicted the animal evidence.

Carcinogenic polycyclic aromatic hydrocarbons (PAHs), the products of fossil fuel combustion, create clear signs of immunosuppression in animal studies. Humans are exposed to PAHs in the workplace and as airborne pollutants, but few data on their effects on humans exist. Other airborne pollutants with immunosuppressive potential include ozone, nitrogen dioxide, cigarette smoke, and trace metals. Metals shown to suppress immune function in experimental animals include lead, cadmium, mercury, and organotins.

Substances That Induce Hypersensitivity

Contact sensitivity and other immune mediated skin disorders often arise from exposure to environmental

agents. A variety of substances, including drugs, cosmetics, and certain metals can give rise to allergic contact dermatitis, which manifests itself with a red rash, swelling itching, and sometimes blisters. Numerous inhalants cause immune-mediated respiratory disorders, including some types of asthma, hypersensitivity pneumonitis, allergic rhinitis, bronchopulmonary aspergillosis, silicosis, asbestosis, coalworkers' pneumoconiosis, and possibly byssinosis.

Among the known human allergens are certain prescription drugs, including penicillin and methyldopa. Over-the-counter drugs, cosmetics, and the metals found in costume jewelry can also cause allergic reactions. Plastics and resins, particularly the isocyanates, cause both asthma and contact dermatitis. Pesticides, too, have provoked hypersensitivity under certain conditions.

Substances That Induce Autoimmunity

Several drugs and heavy metals have been implicated in autoimmune processes. Genetic susceptibility also plays an important role in autoimmunity. Because of this strong genetic component and a generally poorer understanding of autoimmunity compared to other immune responses, deciphering the exact role of toxic chemicals in the induction of autoimmunity is difficult.

FEDERAL ATTENTION TO immunotoxic SUBSTANCES

The Federal Government is actively involved in advancing the state-of-the-art of immunotoxicology. The Environmental Protection Agency (EPA), Food and Drug Administration (FDA), Centers for Disease Control, and National Institutes of Health have immunotoxicological research programs. Each of these agencies also contributes to the work of the National Toxicology Program (NTP), whose immunotoxicological research program was the first coordinated Federal effort to evaluate toxic substances for adverse effects on the immune system.

Current Federal research in immunotoxicology is directed toward developing and validating tests for evaluating substances for immunotoxic potential. NTP has published a panel of tests for immunosuppressive potential that has been validated in the mouse, and it continues to work on validating immunotoxicity tests in other species and on improving its current panel of tests. NTP is also applying the battery of tests for immunosuppressive potential and standard hypersensitivity assays to various substances. EPA is working independently and with NTP on developing and validating immunotoxicity tests using the rat and the mouse, and has undertaken human inhalation studies on substances regulated under the Clean Air Act. EPA has also published immunotoxicity testing guidelines for certain pesticides. FDA considers the immune system an important target of toxicological assessment and is involved in developing and applying tests to measure the potential immunotoxicity of foods and drugs. (See table 1-2.)

Few substances have been regulated by the Federal Government on the basis of immunotoxicity. The Occupational Safety and Health Administration (OSHA) has issued regulations for certain substances because they can provoke hypersensitivity. FDA has restricted the use of sulfites in foods because they can induce asthmatic attacks insensitive individuals. These agencies and EPA regulate additional substances that have shown evidence of immunotoxicity, but other health effects serve as the basis for those regulations.

Several Federal activities are designed to enhance public awareness of the hazards of toxic substances, including immunotoxicants. OSHA'S hazard communication standard requires that workers be provided with information about known health hazards in their jobs. However, since so little information is available regarding immunotoxic effects, and since the standard cannot be used to compel testing, the standard does little at present to protect workers from immunotoxic hazards. Community right-to-know legislation requires EPA to collect information about substances that pose potential toxic hazards to local communities and make that information available to the public. As with the OSHA stan-

Table 1-2—Federal Agencies Engaged in immunotoxicological Research or Regulation

Consumer Product Safety Commission
Department of Agriculture
Department of Defense
Department of Health and Human Services
Agency for Toxic Substances and Disease Registry
Alcohol, Drug Abuse, and Mental Health Administration
Centers for Disease Control
Food and Drug Administration
National Institutes of Health
Department of Labor
Mine Safety and Health Administration
Occupational Safety and Health Administration
Environmental Protection Agency

SOURCE: Office of Technology Assessment, 1991.

dard, however, this program does not permit EPA to require that health effects information be developed The Federal Government also supports information dissemination activities related to toxic substances undertaken by the National Library of Medicine and the Agency for Toxic Substances and Disease Registry.

RELATED ISSUES

As a science develops, experts continually disagree on whether currently available evidence supports a particular conclusion. In the ease of immunotoxicology, a growing number of medical practitioners are drawing conclusions regarding the immunotoxicity of chemicals in the environment that other practitioners and researchers believe to be unjustiled on the basis of current knowledge. This disagreement has had at least two interesting effects on governance in the past few years.

One effect is the increasing public demand for governmental attention to a problem frequently identified as multiple chemical sensitivity (MCS) but also called environmental illness and several other names. MCS remains a poorly defined problem — experts disagree on what causes the problem and whether it is physical or mental—but sufferers generally exhibit symptoms that they attribute to immune system dysfunction when exposed to very low levels of common industrial, environmental, and household chemicals. Two States, Maryland and New Jersey, commissioned studies of MCS as a public health problem but have not proceeded with screening or regulatory programs. California has considered (but not passed) legislation that would encourage research and education regarding environmental illness and MCS. Within the Federal Government, EPA is funding a study by the National Academy of Sciences that will assess the type of research needed to determine whether MCS exists and its possible causes. Constituents at the State and Federal level are appearing before their elected representatives to demand attention to this problem, which some medical practitioners attribute to immunotoxicity. This background paper does not attempt to weigh the merits of arguments supporting or denying the existence of MCS or of claims that MCS is an immune system dysfunction. The analysis examines state-of-the-art methods for identifying substances that may damage the immune system but does not evaluate diseases of or involving the immune system.

A second effect is the introduction of immunotoxicological test results to support claims of disability. Individuals with diseases they believe to be linked to immune system impairment are making claims against Social Security, workers' compensation programs, and in tort suits for injuries caused by chemicals frequently used in commerce. Here, too, experts disagree on whether available evidence supports these claims. However, most of the experts contacted by OTA believe that data currently available from immunotoxicological testing are limited in the support they provide to plaintiffs' claims of immune system injury from toxic substances. Appendix A provides a brief description of the compensation programs and a synopsis of a few claims based on immune system injury from toxic chemicals.

SUMMARY

immunotoxicology has advanced rapidly as a distinct field of study over the last several years. Scientists now recognize the immune system as an important target organ for toxicity. Tests to determine whether a substance can suppress the immune system or induce hypersensitivity or autoimmunity have been developed and applied by manufacturers and by regulatory agencies. Yet the growing database of immunotoxicological information remains insufficient for most regulatory purposes.

immunotoxicology is complicated by several factors: the complex nature of the immune system, limited (historically) attention to immunotoxicity research, and lack of human exposure data stand out among them. Regulators at the FDA have the opportunity to observe the effects of drugs on humans in clinical trials before they are marketed. However, when FDA, EPA, OSHA, or the other regulatory agencies try to decide whether a food additive or pesticide, for instance, poses an undue risk to human health, they face the problem of trying to extrapolate from animal test results to the likely consequences of human exposure to immunotoxic substances. Very few useful human data are available to support these attempts.

The science of immunotoxicology is sufficiently advanced for regulators to begin to consider use of its results in the decisionmaking process, but much research remains to be done. Current law permits Federal agencies to require immunotoxicological testing-either by the government or by industry-on substances suspected of posing a potential threat to the human immune system. The agencies are devoting resources to developing and applying immunotoxicological tests in areas where the potential for human exposure is thought to be significant,