AMERICAN JOURNAL OF INDUSTRIAL MEDICINE 49:1046–1055 (2006)

Immunotoxic Effects of Chemicals: A Matrix for Occupational and Environmental Epidemiological Studies

Angela Veraldi,[1] Adele Seniori Costantini,[1]* Vanessa Bolejack,[1] Lucia Miligi,[1] Paolo Vineis,[2,3] and Henk van Loveren[4,5]

Background *Many biological and chemical agents have the capacity to alter the way the immune system functions in human and animals. This study evaluates the immunotoxicity of 20 substances used widely in work environments.*

Methods *A systematic literature search on the immunotoxicity of 20 chemicals was performed. The first step was to review literature on immunotoxicity testing and testing schemes adopted for establishing immunotoxicity in humans. The second step consisted of providing a documentation on immunotoxicity of substances that are widely used in work environment, by building tables for each chemical of interest (benzene, trichloroethylene, PAHs, crystalline silica, diesel exhausts, welding fumes, asbestos, styrene, formaldehyde, toluene, vinyl chloride monomer, tetrachloroethylene, chlorophenols, 1,3-butadiene, mineral oils, P-dichlorobenzene, dichloromethane, xylene, 1,1,1-trichloroethane, ethylene oxide). The third step was the classification of substances; an index (strong, intermediate, weak, nil) was assigned on the basis of the evidence of toxicity and type of immunotoxic effects (immunosuppression, autoimmunity, hypersensitivity) on the basis of the immune responses. Finally substances were assigned a score of immunotoxic power.*

Results *Tables have been produced that include information for the 20 substances of interest, based on 227 animal studies and 94 human studies. Each substance was assigned an index of immunotoxic evidence, a score of immunotoxic power and type of immunotoxic effect.*

Conclusions *This matrix can represent a tool to identify chemicals with similar properties concerning the toxicity for the immune system, and to interpret epidemiological studies on immune-related diseases.* Am. J. Ind. Med. 49:1046–1055, 2006. *© 2006 Wiley-Liss, Inc.*

KEY WORDS: immunotoxicity; immune effects; immunostimulation; immunosuppression; autoimmunity; hypersensitivity; chemicals; epidemiological studies

The supplemental table appendices described in this article can be found at http://www.interscience.wiley.com/jpages/0271-3586/suppmat.

[1]Centre for Study and Cancer Prevention, Unit of Environmental and Occupational Epidemiology, Florence, Italy

[2]Department of Biomedical Sciences and Human Oncology, AOS Giovanni Battista, University of Turin, Turin, Italy

[3]Imperial College London, South Kensington Campus, United Kingdom

[4]Dutch National Institute of Public Health and the Environment (RIVM), Bilthoven, The Netherlands

[5]Department of Health Risk Analysis and Toxicology, Maastricht University, Maastricht, The Netherlands

Contract grant sponsor: The "Fondazione S. Paolo" in Turin.

*Correspondence to: Adele Seniori Costantini, Centro per lo Studio e la Prevenzione Oncologica, UO Epidemiologia ambientale e occupazionale, via di S. Salvi, 12, 50135 Florence, Italy. E-mail: a.seniori@cspo.it

Accepted 23 May 2006

DOI 10.1002/ajim.20364. Published online in Wiley InterScience (www.interscience.wiley.com)

INTRODUCTION

Consequences of Immunotoxicity

A number of biological or chemical agents have the capacity to alter the functionality of the immune system in humans and animals, potentially compromising the organism's ability to recognize or neutralize infectious agents or neoplastic cells.

A number of chemical and environmental agents as well as pharmaceuticals may lead to autoimmune diseases in experimental animals or humans [Bigazzi, 1988; Kammuller et al., 1989]. The consequences regarding the effects of immunosuppression/depression in humans have been extensively studied. On the basis of epidemiological studies, a reduction in resistance to infections produced by biological agents (virus, bacteria, fungi) has been extensively described [Bowler et al., 1997]. Neoplasia can also be the result of a compromised surveillance mechanism on the part of the immune system responsible for the elimination of neoplastic cells [Tryphonas and Feeley, 2001]. Severe immunosuppression represents a well-described risk factor for the development of non-Hodgkin's lymphomas (NHL). An increase in incidence for NHL was found in AIDS patients [Beral et al., 1991; Obrams and Grufferman, 1991], and in patients that have had immunosuppressive therapies following organ transplants [Hoover and Fraumeni, 1973; Anonymous, 1984]. Conclusive evidence is lacking, however, regarding risks associated with factors that have a modest but prolonged capacity to induce immunosuppression. Regarding chemical agents, there is some epidemiological evidence that suggests that exposure to various agents with a potential of immunotoxicity, for example, pesticides and solvents, may be associated with an increase in risk of NHL [Chiu and Weisenburger, 2003]. However, the data is insufficient to support a causal relationship.

Immunotoxicity Testing

Due to the complexity of the immune system, the identification of substances that induce adverse effects on the human immune system requires considering the markers according to each specific immunologic effect.

Various organizations (Organization for Economic Cooperation and Development, OECD, U.S. National Toxicology Program, NTP, Dutch National Institute of Public and the Environment, RIVM, US Food and Drug Administration, FDA, U.S. Environmental Protection Agency, EPA) that conduct testing for immunotoxicity have proposed different approaches to immunotoxicity testing that include validated immune testing protocols for both animals, as reviewed by Luster et al. [1988] and Van Loveren and Vos [1989], and humans, as reviewed by Tryphonas, 2001. OECD have proposed guidelines for testing the toxicity of chemicals (guideline number 407) that were adopted in many countries.

Currently, there are a number of test procedures that measure diverse immunological end points, mainly in laboratory animals, but also in humans. Tiered testing schemes, organized in stepwise protocols, have been successfully used to identify and characterize immunotoxic substances. These tests are designed to detect a change in the number of cells or the weight of an immune system organ, or to evaluate altered functionality of its components. Various agencies have adopted protocols that include specific tests classified by levels of increasing complexity. Level (or Tier) 1 is comprised of a series of preliminary tests that should indicate the absence of toxicity to the immune system, or suggest a direction for further study. The tests included in level (or Tier) 2 are meant to enhance the understanding of the nature of effect when level one tests are positive. Testing schemes for establishing immunotoxicity in humans have also been proposed [Colosio et al., 1999; Van Loveren et al., 1999] according to a 3-tier approach. There is not always general agreement, however, as to which tests fall into which levels. In any case, it is agreed that the most effective approach to immunotoxicity testing is to perform a battery of tests, and interpret them in their entirety, not on the analysis of a single parameter [Dean, 1979; Vos, 1980; Luster et al., 1988].

Objectives

The purpose of this study is to provide a documentation on immunotoxicity of substances that are widely used in work environment, by building tables for each chemical of interest and the preparation of a matrix that includes information on the immune category affected, the type of effect, and the strength of evidence for each chemical, and subsequent scoring the substances for their immunotoxicity evidence, power and type of effect. It is intended to use the matrix in interpreting the role of adverse immune effects of exposures to a variety of industrial chemical compounds on the induction of different diseases including neoplasms. The matrix will be used to estimate relative risks by considering the immunotoxicity evidence, the power and type of immunotoxic effect of each agent to which an individual has been exposed in the context of epidemiological studies, as the ''Italian multicenter case-control study on hematolymphopoietic malignancies in Italy and exposures to solvents and pesticides'' [Seniori Costantini et al., 2001] where detailed information on chemical exposures (intensity and probability) has been collected. Substances has been selected on the basis of the number of workers exposed. Currently only chemical compounds used in industrial settings have been considered. Subsequently it is intended to take pesticides into consideration as well.

MATERIALS AND METHODS

The first step was to prepare summary tables on immunotoxicity testing given the complexity of the immune

system. A large part of information came from the Environmental Health Criteria documents published by the World Health Organization, ''Principles and Methods for Assessing Direct Immunotoxicity Associated with Exposure to Chemicals'' [International Programme on Chemical Safety [WHO, 1996], and ''Principles and Methods for Assessing Hypersensitization Associated with Exposure to Chemicals'' [International Programme on Chemical Safety [WHO, 1999]. A section of these texts provides an overview of the testing strategies for detecting immunotoxicity adopted by various organizations. These testing methods are discussed, often with an evaluation of their efficacy. In constructing the tables, these two texts were consulted to identify and describe test characteristics and the immune response category that is represented by each test. The report of validation study of assessment of direct immunotoxicity in the rat by the International Collaborative Immunotoxicity Study (ICICIS) group investigators was also consulted in order to collect evaluation of performance of various experimental techniques used in the rats to indicate toxic effects on the immune system [ICICIS Group Investigators, 1998]. This information has been updated with more current information obtained from consulting the web sites of the various organizations cited, when available, and with other information obtained by Medline searches for immunotoxicity testing procedures.

In these summary tables, various tests that are used in immunotoxicology research in animals and in humans are summarized. They include, in addition to the principal characteristics regarding each test, basic information on the validation that each test has undergone regarding its ability to determine adverse effects on the immune system. The following information was included: (i) the immune category evaluated by each test; (ii) specific test; (iii) organs, cells or other parameters affected; (iv) principal effects on the immune system; (v) organizations that recommend or mention the use of these tests, and when considered, the level or tier attributed to this test.

The second step consisted in the preparation of substance-specific tables for 20 substances of interest. Substances were chosen on the basis of the most frequent chemicals reported in the working histories of cases and controls enrolled and interviewed in the context of the ''Multicenter case-control study on hematolymphopoietic malignancies in Italy.'' For each substance the literature available was reviewed and results were summarized taking into account the type of immunotoxicity and strength of evidence and power. The ''substance tables'' have been realized essentially with the information collected from documents of the Agency for Toxic Substances and Disease Registry (ATSDR). In particular the paragraphs dealing with immunological and lymphoreticular effects in the toxicological profiles on the each substances have been considered. Other information has been found from the environmental health criteria (EHC) monographs and from ''abstracts'' and/or from ''full texts'' of relevant papers through consultation of ''Pubmed'' and ''Toxnet.''

The third step was the classification of the 20 substances of interest on the basis of the evidence of immunotoxicity, expressed by end-points, considering the capacity of the tests to predict the immunotoxicity (for instance: the effects on host resistance weigh more heavily than effects on pathology). The end-points have been identified on the basis of the existing literature and indications supplied from an expert immunotoxicologist. Criteria to define evidence of immunotoxicity are shown in Table I. An index was assigned in order to classify different substances: strong, intermediate, weak, nil.

Substances with the same index of evidence (i.e., Benzene, Trichloroethylene, Crystalline silica, and PHAs that have all been classified ''strong'') were compared pair-wise in order to produce a score for each chemical. Scoring was

TABLE I. Index of Chemicals by Immunotoxic Evidence

Immunotoxic index	Immunotoxic identification criteria (high predictability test)
Strong	Host resistance[a] (viral, bacterial, parasite and tumoral model) and delayed type hypersensitivity[a] (DTH)
	Host resistance[a] (viral, bacterial, parasite and tumoral model) and plaque forming cells[a] (PFC) +cytotoxicT lymphocyte[a] (CTL)
	Host resistance[a] (viral, bacterial, parasite and tumoral model) and CTL[a] + surface marker analysis[b]
	Host resitstance[a] (viral, bacterial, parasite and tumoral model) and lymphoproliferative response (LPS)[a] + (CTL)[a]
Intermediate	Pathology1: Organ weight, histopathology cellularity, hematology (thymus, spleen, bone marrow, lymph nodes) and delayed type hypersensitivity[a] (DTH) (or MLR)[a], and Natural Killer cell assay[a] (NK), and Plaque forming cells[a] (PFC) (or Antigen specific antibody responses)[a]
	Delayed type hypersensitivity (DTH)[a] (or mixed leukocyte response)[a], and natural killer cell assay (NK)[a], and plaque forming cells (PFC)[a] (or Antigen specific antibody responses)
Weak	Pathology: Organ weight, histopathology cellularity, haematology (thymus, spleen, bone marrow, lymph nodes) Surface marker analysis[b]
	Surface marker analysis[b] (or cytochines, interleukins expression patterns)[b] mitogen response[a]
Nil	Toxicity but no immunotoxicity: Organ weight, histopathology, cellularity, hematology of non immune tissue (i.e., liver, kidney)

[a]Functional tests.
[b]Non-functional test.

done considering doses (minimal doses at which the effects are seen), but the route of administration was also considered (main route of exposure). The number of positive studies and of species (mice, rats, pigs, humans) was also taken into account in scoring substances. Formally chemicals can be adequately compared for their effects (comparable magnitude of effects and of doses that produce same effects) if similar endpoints are compared and if tests are performed under similar conditions and similar routes of exposure (i.e., inhalation compared with inhalation). This is not always possible in a straightforward way, and experts have provided further contributions.

Substances were classified also for the type of immunotoxic effects. The type of immunotoxic effect has been assigned independently from the immunotoxic evidence. This work has taken in consideration also those studies that provide indications on the various types of immunotoxicity, (as an example the test that finds the level and the type of autoantibody provides information on alterations of autoimmunity type). Several positive studies with highly predictive tests for autoimmunity evaluation have been taken into consideration, even if these tests are not always recommended by the agencies for immunotoxicity evaluation. As an example, crystalline silica has been classified as ''strong,'' because two tests (Host resistance and DHR) that satisfy criteria of high predictability are positive. Its effects on the immune system are mainly of autoimmunity. A document of the U.S. FDA (FDA, 1999; Guidance for Industry and FDA reviewers-Immunotoxicity testing) has used potential immunotoxic effects associated with immune responses to evaluation of type of immunotoxic effect.

Criteria for classification of immune response associated with potential immunotoxic effects are shown in Table II.

RESULTS

A total 321 studies were reviewed of which 227 were animal studies and 94 human studies related to the 20 substances of interest. Human studies were related mainly to crystalline silica and asbestos. Human studies included tests on humoral and cellular mediated immunity (antibody levels in serum or in fluid, surface marker analysis, cytokine synthesis patterns, cytokine expression patterns, T- and B-cell mitogen assay), and non-specific cellular mediated immunity (NK cells activity: cytotoxicity, degranulation of granulocytes: basophils or eosinophils), and test on autoimmunity evaluation (autoantibodies titers in serum). Twenty tables have been produced that include information collected for the 20 substances of interest. These tables include information on the immune category evaluated by each test, organs, cells, or other targets, principal effects on the immune system, doses, routes of exposure for each specific assay. References for each study and specific to the organizations that recommend or mention the use of these tests, and, when considered, the

TABLE II. Classification of Immune Responses Associated With Potential Immunotoxic Effects*

	Immune responses							
			Cellular Responses					
Immunotoxic effects	Histopathology	Humoral response	T-cells	Natural killer cells	Macrophages	Granulocytes[a]	Host resistance	Observe for signs of illness
Hypersensitivity	NC	C (IgE in Type I reactions only)	C (Type IV reactions only)	NA	NA	C	NA	
Inflammation	C	NC	C	NA	C	C	NA	
Immunosuppression	NC	C	C	C	C	C	C	
Immunostimulation	NC	C	C	NA	NC	NA	NC	
Autoimmunity[b]	C	C	C	NA	NA	NC	NA	

C, critical.
NC, non-critical.
NA, not applicable or not needed.
*FDA 1999 Guidance for Industry and FDA reviewers-Immunotoxicity testing.
[a]Basophils, eosinophils, and/or neutrophils.
[b]Routine testing for autoimmunity is not recommended.

TABLE III. Immunotoxicity of Benzene

Benzene						
Evidence/Type (level)	**Test (positive response)**	**Effects**	**Dose/Duration**	**Route/Species**	**Study**	**Notes (negative study at all doses)**
Strong/suppression (1)	**(a) Organ weight** (spleen)	(a) Decreased	(a) 25 ppm 5 days.	(a-b) Inhalation/mice	(a) Wells et al. [1991]	No effects
	(b) Body weight	(b) Decreased weight gain	(b) 100 or 300 ppm (6 h/day), 5 day/weeks over their lifetime		(b) Snyder et al. [1978, 1980][a]	Body weight: at 12.52 ppm, 2h/day, 6 day per week for 30 days (inhalation) in mice [Li, 1992][a]
	Cellularity					Body weight: up to 100 mg/kg/day, 12 months or 2 years, (oral) in rats [NTP, 1986]
	(a) B and T lymphocyte number	(a) Reduced at all conc. (B-cell more sensitive than T-cell)	(a) 30–300 ppm, 5–12 days.	(a-b) (1–2) Inhalation/mice	(a) Rosenthal and Snyder [1985]	Cellularity (spleen) T-cell: at 31 ppm, 6 days, and at 300 ppm, 2–5 weeks, (inhalation) in mice [Rozen et al., 1984]
	(b) Granulocytes (spleen)	(b) (1) Reduced at all levels except 9.9 ppm;	(b) (1) 1.1–4.862 ppm (6h/day), 5 days.	(c) (1–3) Inhalation/mice	(b) (1) Green et al. [1981][a]	Cellularity (bone marrow): at 25 ppm, 2 weeks
	(c) (1–3) Bone marrow	(2) Decreased	(2) 300 ppm, 2 weeks	(d) Oral/mice	(2) Chertkov et al. [1992]	Cellularity B, T and T cell subset (spleen): at 100 ppm, 20 days (inhalation) in mice [Rosenthal and Snyder, 1987]
	(d) Cell numbers (spleen)	(c) (1–3) Decreased	(c) (1) 100 ppm, 8 days,	(e) Inhalation/mice	(c) (1) Gill et al. [1980][a]	Cellularity B lymphocytes (bone marrow): at 10 ppm, 6 days, (inhalation) in mice [Rozen et al., 1984]
	(e) Cell numbers (bone marrow and spleen)	(d) Decreased	(2) 300–400 ppm, 16 weeks	(f) Intraperitoneal /mice	(2) Cronkite [1986]	Cellularity (bone marrow) at 25 ppm, 2 weeks (inhalation) in mice [Cronkite et al., 1985]
	(f) Bone marrow (nucleated cells, granulocytes, lymphocytes)	(e) Depressed	(3) 300 ppm		(3) Neun et al. [1992]	Cellularity (bone marrow) B-cell, (spleen) B and T-cell, (thymus)
		(f) Decreased (nucleated cells, at 2 days, lymphocytes, at 7 days) increased (granulocytes, at 7 days)	(d) 27–154 mg/kg/day, 28 days		(d) Fan [1992]	T-cell: 10 ppm, up to 8 weeks (inhalation) in mice [Farris et al., 1997]
			(e) 400 ppm, up to 9 weeks		(e) Cronkite et al. [1982]	Mitogenic response (T-cell) lymphocyte to PHA (peripheral blood): (occupational) humans [Yardley-Jones et al., 1988]
			(f) 600 mg/kg 2 days (nucleated cells); 600 mg/kg 7 days (granulocytes, lymphocytes)		(f) Niculescu and Kalf [1995]	PFC to SRBC: at 400 ppm, 2 or 4 weeks (inhalation) in rats [Robinson et al., 1997]
	Hematology					Host resistance s. zooepidemicus: at 10 ppm, 1 or 5 days (inhalation in mice) [Aranyi et al., 1989]
	(a) (1–2) Leukocyte count (peripheral blood)	(a) (1–2) Decreased	(a) (1) 25 ppm, 5 days	(a) (1) Inhalation/mice	(a) (1) Wells and Nerland [1991]	
		(b) Decreased (B-cell depression dose-dependent, more intense)	(2) 800 mg/kg, 5 days	(2) Intraperitoneal /mice	(2) Klan et al. [1990]	
	(b) B and T cell count (peripheral blood)	(c) Leucopoenia	(b) 48 ppm, 7–14 days	(b) Inhalation/mice	(b) Aoyama [1986]	
	(c-d) Circulating leucocytes	(d) Decreases in the levels of circulating leukocytes	(c) 15 to 210 ppm	(c) Occupational/human	(c) Aksoy et al. [1987][a]	
	(e) (1–10) Lymphocytes	(e) (1–9) Decreased lymphocytes and other blood elements	(d) 30 ppm	(d) Occupational/human	(d) Aksoy et al. [1971, 1972, 1974][a]	
	(f) Leucocytes	(10) Lower in exposed	(e) (1–6) From 1 to 1,060 ppm.	(e) (1–10) Occupational/human	(e) (1) Cody et al. [1993][a]	
	(g) Blood cell count (red and white)	(f) Increase	(7) 6 ppm mean (0,69–140 ppm for an average period of 5–6 years);	(f) Occupational/human	(2) Goldwater [1941][a]	
		(g) Decreased	(8) 31 ppm;	(g) Occupational/human	(3) Greenburg et al. [1939][a]	
			(9) 300 ppm, 2 weeks;		(4) Kipen et al. [1989][a]	
			(10) up to 15 ppm/17 years)		(5) Lange [1973a][a]	
			(f) **0,9 ppm**		(6) Ruiz et al. [1994][a]	
			(g) Ranged from 1 to 1,060 ppm; from 1940 to 1975		(7) Xia et al. [1995][a]	
					(8) Yin et al. [1982][a]	
					(9) Chertkov et al. [1992]	
					(10) Bogadi-Sare et al. [2000]	
					(f) Froom et al. [1994]	
					(g) Cody et al. [1993]	
	(a) Macrophage activity (bone marrow)	(a) Marked increase	(a) 660 mg/kg	(a) Injection/mice	(a) MacEachern et al. [1992]	
	(b) Macrophage activity	(b) Decreased (50%)	(b) 800 mg/kg, 5 days	(b) Intraperitoneal /mice	(b) Klan et al. [1990]	
	Serum immunoglobulin level	(c) Increased IgM and decreased IgG and IgA	(c) Painters: exp. at 3–7 ppm/for 1–21 years (benzene in a mixture with xylene and toluene)	(c) Occupational/human	(c) Lange et al. [1973a][a]	

TABLE III. (*Continued*)

Benzene						
Evidence/Type (level)	**Test (positive response)**	**Effects**	**Dose/Duration**	**Route/Species**	**Study**	**Notes (negative study at all doses)**
	PFC to SRBC	Response was suppressed	50–200 ppm, 7–14 days	Inhalation/mice	Aoyama [1986]	
	MLR assay	Enhanced at low dose, depressed in higher doses	8-40-180 mg/kg/day, 4 weeks	Oral/mice	Hsieh [1988][a]	
	Mitogeneses					
	(a) to LPS	(a) Depression in femoral B-colony forming ability	(a) 10 ppm, 6 days	(a) Inhalation/mice	(a) Rozen et al. [1984]	
	(b) Bone marrow spleen	(b) Depressed	(b) 300 ppm, up to 23 weeks	(b) Inhalation/mice	(b) Rozen and Snyder [1985]	
	(c) B and T cell	(c) Suppressed	(c) 166 mg/l/4 weeks	(c) Oral/mice	(c) Hsieh et al. [1990]	
	Surface markers:					
	(**a**) CD4/CD8 ratio (peripheral blood)	(**a**) Increase in CD4, increase in ratio	(**a**) 300 or 900 ppm, 5 days.	(**a**) Inhalation/mice	(**a**) Plappert et al. [1994]	
	(**b**) B-CD4+/CD5+, CD5+ T lymphocyte numbers (spleen)	(**b**) Reduced	(**b**) 400 ppm, 4 weeks	(**b**) Inhalation/rats	(**b**) Robinson et al. [1997]	
	CTL assay	Reduced tumor lytic abilities	100 ppm, 4 weeks	Inhalation/mice	Rosenthal and Snyder [1987]	
	(a) Cytokine expression: IL-2 production	(a) Inhibited	(a) 27–154 mg/kg/day, 28 days	(a) Oral/mice	(a) Fan [1992]	
	(b) Cytokine synthesis patterns	(b) Suppressed	(b) 166,790 mg/l, 28 days	(b) Oral/mice	(b) Hsieh et al. [1991]	
	Macrophage bactericidal activity: K pneumoniae	Decreased % bacteria killed	10 ppm, 5 days	Inhalation/mice	Aranyi et al. [1986]	
	DTH (contact sensitivity): picril chloride	Enhanced at higher dose	200 ppm, 14 days	Inhalation/mice	Aoyama [1986]	
	Host resistances					
	(a) L. monocytogenes)	(a) Increased susceptibility	(a) 30–300 ppm, 5–12 days (continuous exposure)	(a) Inhalation/mice	(a-b) Rosenthal and Snyder [1985]	
	(b) L. monocytogenes)	(b) Increased susceptibility	(b) 300 ppm, 5–12 days (pre-exposure)	(b) Inhalation/mice	(c) Rosenthal and Snyder [1987]	
	(c) (PYB6 tumor cells)	(c) Reduced resistance to tumors	(c) 100 ppm, 20 weeks			

[a]This information is taken directly the publication: US Department of Health and Human Services, Public Health Services, Agency for Toxic Substances and Disease Registry (ATSDR) "Toxicological Profile for Benzene". September 1997.

TABLE IV. Immunotoxicity Scoring for the 20 Substances in the Study

Substance	Formula	Synonym	Index (immunotoxicity evidence)	Score (Immunotoxicity power)	Main effects on immune system	Species	Notes
Benzene	C_6H_6		Strong	1	Suppression	Human, animal	
Trichloroethylene	$CHCl_3$		Strong	2	Hypersensitivity, Suppression	Human, animal	
PAHs			Strong	3	Suppression	Animal	(DMBA or its metabolites)
Crystalline silica	SiO_2	Quartz	Strong	4	Autoimmunity	Human, animal	
Diesel exhausts			Intermediate	1	Dysregulation	Human, animal	Higher levels of exposure to diesel exhaust particles are at increased risk for hypersensitivity to specific allergens (adjuvant activity)
Welding fumes			Intermediate	2	Suppression	Human, animal	
Asbestos			Intermediate	3	Suppression, Enhancement	Human, animal	
Styrene	C_8H_8		Intermediate	4	Suppression	Human, animal	
Formaldehyde	CH_2O		Weak	1	Hypersensitivity	Human, animal	
Toluene	C_7H_8	Dimethyl benzene	Weak	2	Suppression	Human, animal	Oral exposure: only animal study
Vinyl chloride monomer	C_2H_3Cl	Chloroethylene	Weak	3	Enhancement	Human, animal	
Tetrachloroethylene	C_2Cl_4	Perchloroethylene	Weak	4	Suppression		
Chlorophenols			Weak	5	Immunotoxycity		2-CP; 4-CP; 2,4-DCP; 2,4,5-TCP; 2,4,6-TCP;2,3,4,6-TeCP
1,3-butadiene	C_4H_6		Weak	6	Suppression		
Mineral oils			Weak	7	Immunotoxycity		Used mineral based crankcase oil
P-dichlorobenzene	$C_6H_4Cl_2$	1,4 dichlorobenzene					Inadequate data
Dichloromethane	CH_2Cl_2	Methylene chloride					Inadequate data
Xylene	C_8H_{10}						Inadequate data
1,1,1-trichloroethane	$C_2H_3Cl_3$	Methyl chloroform					Inadequate data
Ethylene oxide	C_2H_4O						Inadequate data

level or tier attributed to this test, were also included. In Table III a synthesis of the summary table for benzene is reported. Benzene was classified as ''strong'' because the end points affected (DTH and Host resistance). In Table IV the summary evaluations for the 20 substances are shown. An index was assigned on the basis of evidence of immunotoxicity and a score on the basis of potency within the index of evidence (defined by the dose, route of exposure and species). Substances were classified also on the basis of the specific effect on the immune system, main type of immunosuppressive effects with the ''strong'' and ''intermediate'' index, are highlighted in the heavy type. Substances evaluated with immunosuppressive effects and with ''strong'' and ''intermediate'' index are: benzene, trichloroetylene, PHAs, welding fumes, asbestos, and styrene.

The test summary tables and the substance summary tables are presented in Appendix A and B. These are available at http://www.interscience.wiley.com/jpages/0271-3586/suppmat.

DISCUSSION

A matrix on immunotoxicity that can be used in epidemiological studies in which information on chemical exposures has been constructed. This matrix can represent a tool to identify chemicals with similar immunotoxic properties and thus to improve risk estimations for immune-related diseases. Information was systematically reviewed and summarized. Weight of evidence (end points), doses to which adverse effects were observed and type of immunotoxic effects were considered in order to classify chemicals by their immunotoxic potential. The presence of positive studies that examined immunotoxicity in highly predictive tests provided greater weight, as well as positive studies in human beings.

Limitations of this study include some discrepancies in the results of studies on which the matrix is based. Some effects of a given agent on immune function may result from differences in the experimental system used by different investigators and laboratories that generated the published information examined (there can be significant variability in some of these tests, from laboratory to laboratory), rather than reflect real differences in the biological effects of these toxic agents. There is a random aspect in the weight of evidence: some substances may have been studied less broadly or have gained less attention just by chance, selection of types of studies depends on research group.

A second problem consists in the fact that different tests have been performed for different chemicals in most cases, making it difficult to compare substances. Moreover, most chemicals tested can show more different types of immunotoxic effects (i.e., immunosuppression and stimulation), depending on dose. Another problem is that conflicting results for the same substances emerged for different endpoints depending on different doses, route of exposure, species (human, animal).

Finally, the issue of potential differences in how toxic agents may affect murine versus human immune systems is raised. The proposed classification provides more information than that usually offered by the current literature on toxicity of chemicals as for immunotoxicants estimates risk in epidemiological studies.

CONCLUSION

This matrix will be used in the ''Multicenter case-control study on hematolymphopoietic malignancies in Italy'' to estimate relative risks by considering the immunotoxicity evidence and type of immunotoxic effects of each agent to which individuals have been exposed. Cumulative exposure to each chemical classified for its effect on immune system will be estimated, in addition to peaks of exposure to strong immunotoxic agents. Once validated the matrix will be made available for other epidemiological studies in which immunotoxicity of chemicals is of concern.

ABBREVIATIONS

NHL	non-Hodgkin's lymphomas
AIDS	acquired immunodeficiency syndrome
ICICIS	International Collaborative Immunotoxicity Study
OECD	Organization for Economic Co-operation and Development
NTP	U.S. National Toxicology Program
RIVM	Dutch National Institute of Public Health and the Environment
FDA	U.S. Food and Drug Administration
EPA	U.S. Environmental Protection Agency
NK	natural killer
DTH	delayed type hypersensitivity
PFC	plaque forming cell
CTL	cytotoxic T-lymphocyte
MLR	mixed leucocyde response
LPS	lipopolysaccharide
DMBA	dimethylbenzanthracene
2-CP	2-chlorophenol
4-CP	4-chlorophenol
2,4-DCP	2,4-dichlorophenol
2,4,5-TCP	2,4,5-trichlorophenol
2,4,6-TCP	2,4,6-trichlorophenol
2,3,4,6-TeCP	2,3,4,6-tetrachlorophenol

ACKNOWLEDGMENTS

We thank Luigi Rigacci and Otonie Martinez-Maza for their helpful suggestions. This research has been supported by the ''Fondazione S. Paolo'' in Turin.

REFERENCES

Aksoy M, Ozeris S, Sabuncu H, et al. 1987. Exposure to benzene in Turkey between 1983 and 1985: A hematological study on 231 workers. Br J Ind Med 44:785–787.

Aksoy M, Erdem S, Dincol G. 1972. Details of blood changes in 32 patients with pancytopenia associated with long-term exposure to benzene. Br J Ind Med 29:56–61.

Aksoy M, Erdem S, Dincol G. 1974. Leukemia in shoe workers exposed chronically to benzene. Blood 44:837–841.

Anonymous. 1984. Lymphoma in organ transplant recipients. Lancet 17:601–603.

Aoyama K. 1986. Effects of benzene inhalation on lymphocyte subpopulations and immune response in mice. Toxicol Appl Pharmacol 85(1):92–101.

Aranyi C, O'Shea WJ, Graham JA, Miller FJ. 1986. The effects of inhalation of organic chemical air contaminants on murine lung host defenses. Fundam Appl Toxicol 6(4):713–720.

Beral V, Peterman T, Berkelman R, Jaffe H. 1991. AIDS-associated non-Hodgkins lymphoma. Lancet 337:805–809.

Bigazzi PE. 1988. Autoimmunity induced by chemicals. J Toxicol Clin Toxicol 26:125–156.

Bogadi-Sare A, Zavalic M, Trosic I, Turk R, Kontosic I, Jelcic I. 2000. Study of some immunological parameters in workers occupationally exposed to benzene. Int Arch Occup Environ Health 73:397–400.

Bowler RM, Ngo L, Hartney C, Lloyd K, Tager I, Midtling J, Huel G. 1997. Epidemiological health study of a town exposed to chemicals. Environ Res 72:93–108.

Chertkov JL, Lutton JD, Jiang S, da Silva JL, Abraham NG. 1992. Hematopoietic effects of benzene inhalation assessed by murine long-term bone marrow culture. J Lab Clin Med 119(4):412–419.

Chiu BC, Weisenburger DD. 2003. An update of the epidemiology of non-Hodgkin's lymphoma. Clin Lymphoma 4:161–168.

Cody RP, Strawderman WW, Kipen HM. 1993. Hematologic effects of benzene. Job-specific trends during the first year of employment among a cohort of benzene-exposed rubber workers. J Occup Med 35(8): 776–782.

Colosio C, Corsini E, Barcellini W, Maroni M. 1999. Immune parameters in biological monitoring of pesticide exposure: Current knowledge and perspectives. Toxicol Lett 108:285–295.

Cronkite EP. 1986. Benzene hematotoxicity and leukemogenesis. Blood Cells 12(1):129–137.

Cronkite EP, Inoue T, Carsten AL, Miller ME, Bullis JE, Drew RT. 1982. Effects of benzene inhalation on murine pluripotent stem cells. J Toxicol Environ Health 9(3):411–421.

Cronkite EP, Drew RT, Inoue T, Bullis JE. 1985. Benzene hematotoxicity and leukemogenesis. Am J Ind Med 7(5–6):447–456.

Dean JH. 1979. Assessment of immunobiological effects induced by chemicals, drugs or food additives. I. Tier testing and screening approach. Drug Chem Toxicol 2:5–17.

Fan XH. 1992. Effect of exposure to benzene on natural killer (NK) cell activity and interleukin-2 (IL-2) production of C57BL/6 mice. Nippon Ika Daigaku Zasshi 59(5):393–399.

Farris GM, Robinson SN, Gaido KW, Wong BA, Wong VA, Hahn WP, Shah RS. 1997. Benzene-induced hematotoxicity and bone marrow compensation in B6C3F1 mice. Fundam Appl Toxicol 36(2): 119–129.

Froom P, Dyerassi L, Cassel A, Aghai E. 1994. Erythropoietin-independent colonies of red blood cells and leukocytosis in a worker exposed to low levels of benzene. Scand J Work Environ Health 20(4):306–308.

Gill DP, Jenkins VK, Kempen RR, et al. 1980. The importance of pluripotential stem cells in benzene toxicity. Toxicology 16:163–171.

Goldwater LJ. 1941. Disturbances in the blood following exposure to benzol. J Lab Clin Med 26:957–973.

Green JD, Snyder CA, LoBuc J, et al. 1981. Acute and chronic dose-response effects of inhaled benzene on multipotential hematopoietic stem (CTU-S) and granulocyte/macrophage progenitor (CMCFUC) cells in CD-1 mice. Toxicol Appl Pharmacol 58:492–503.

Greenburg L, Mayers MR, Goldwater L, et al. 1939. Benzene (benzol) poisoning in the rotogravure printing industry in New York City. J Ind Hyg Toxicol 21:395–420.

Hoover R, Fraumeni JF Jr. 1973. Risk of cancer in renal-transplant recipients. Lancet 2:55–57.

Hsieh GC, Sharma RP, Parker RDR. 1988. Subclinical effects of groundwater contaminants. I. Alteration of humoral and cellular immunity by benzene in CD-1 mice. Arch Environ Contam Toxicol 17:131–138.

Hsieh GC, Parker RD, Sharma RP, Hughes BJ. 1990. Subclinical effects of groundwater contaminants. III. Effects of repeated oral exposure to combinations of benzene and toluene on immunologic responses in mice. Arch Toxicol 64(4):320–328.

Hsieh GC, Sharma RP, Parker RD. 1991. Hypothalamic-pituitary-adrenocorticoal axis activity and immune function after oral exposure to benzene and toluene. Immunopharmacology 21(1):23–31.

ICICIS Group Investigators. 1998. Report of validation study of assessment of direct immunotoxicity in the rat. Toxicology 125:183–201.

International Programme on Chemical Safety (WHO). 1996. Environmental Health Criteria 180. Principles and methods for assessing direct immunotoxicity associated with exposure to chemicals. World Health Organization, Geneva.

International Programme on Chemical Safety (WHO). 1999. Environmental Health Criteria 212. Principles and Methods for assessing direct hypersensitization associated with exposure to chemicals. Geneva: WHO.

Kammuller ME, Bloksma N, Seinen W. editors. 1989. Autoimmunity and toxicology. Immune disregulation induced by drugs and chemicals. Amsterdam: Elsevier.

Kipen HM, Cody RP, Goldstein BD. 1989. Use of longitudinal analysis of peripheral blood counts to validate historical reconstructions of benzene exposure. Environ Health Perspect 82: 199–206.

Klan MJ, Adams DO, Lewis JG. 1990. Effects of exposure to benzene in vivo on the murine mononuclear phagocyte system. Toxicol Appl Pharmacol 103(2):198–205.

Lange A, Smolik R, Zatonski W, et al. 1973a. Leukocyte agglutinins in workers exposed to benzene, toluene, and xylene. Int Arch Arbeitsmed 31:45–50.

Lange A, Smolik R, Zatonski W, et al. 1973b. Serum immunoglobulin levels in workers exposed to benzene, toluene, and xylene. Int Arch Arbeitsmed 31:37–44.

Li L, Sun W, Gong Z, et al. 1992. Effect of low benzene exposure on neurobehavioral function. AchE in blood and brain and bone marrow picture in mice. Biomed Environ Sci 5:349–354.

Luster MI, Munson AE, Thomas PT, Holsapple MP, Fenters JD, White KL, Lauer LD, Gennotec DR, Rosenthal GJ, Denn JH. 1988. Development of a testing battery to assess chemical immunotoxicity. National toxicology program's guidelines for immunotoxicity evaluation in mice. Fundam Appl Toxicol 10:2–19.

MacEachern L, Snyder R, Laskin DL. 1992. Alterations in the morphology and functional activity of bone marrow phagocytes following benzene treatment of mice. Toxicol Appl Pharmacol 117(2):147–154.

National Toxicology Program. 1986. Toxicology and carcinogenesis studies of benzene (CAS No. 71-43-2) in F344/N rats and B6C3F1 mice (Gavage Studies). USDHHS, PHS, NIH. NIH Pub No. 86-2545.

Neun DJ, Penn A, Snyder CA. 1992. Evidence for strain-specific differences in benzene toxicity as a function of host target cell susceptibility. Arch Toxicol 66(1):11–17.

Niculescu R, Kalf GF. 1995. A morphological analysis of the short-term effects of benzene on the development of the haematological cells in the bone marrow of mice and the effects of interleukin-1 alpha on the process. Arch Toxicol 69(3):141–148.

Obrams GI, Grufferman S. 1991. Epidemiology and biostatistics program, National Cancer Institute. Cancer Surv 10:91–102.

Plappert U, Barthel E, Raddatz K, Seidel HJ. 1994. Early effects of benzene exposure in mice. Hematological versus genotoxic effects. Arch Toxicol 68(5):284–290.

Robinson SN, Shah R, Wong BA, Wong VA, Farris GM. 1997. Immunotoxicological effects of benzene inhalation in male Sprague-Dawley rats. Toxicology 119(3):227–237.

Rosenthal GJ, Snyder CA. 1985. Modulation of the immune response to Listeria monocytogenes by benzene inhalation. Toxicol Appl Pharmacol 80(3):502–510.

Rosenthal GJ, Snyder CA. 1987. Inhaled benzene reduces aspects of cell-mediated tumor surveillance in mice. Toxicol Appl Pharmacol 88(1):35–43.

Rozen MG, Snyder CA. 1985. Protracted exposure of C57BL/6 mice to 300 ppm benzene depresses B- and T-lymphocyte numbers and mitogen responses. Evidence for thymic and bone marrow proliferation in response to the exposures. Toxicology 37(1–2):13–26.

Rozen MG, Snyder CA, Albert RE. 1984. Depressions in B- and T-lymphocyte mitogen-induced blastogenesis in mice exposed to low concentrations of benzene. Toxicol Lett 20(3):343–349.

Ruiz MA, Augusto LG, Vassallo J, Vigorito AC, Lorand-Metze I, Souza CA. 1994. Bone marrow morphology in patients with neutropenia due to chronic exposure to organic solvents (benzene): Early lesions. Pathol Res Pract 190(2):151–154.

Seniori Costantini A, Miligi L, Kriebel D, Ramazzotti V, Rodella S, Scarpi E, et al. 2001. A multicenter case-control study in Italy on hematolymphopoietic neoplasm and occupation. Epidemiology 12:78–87.

Snyder CA, Goldstein BD, Sellakumar A. 1978. Hematoxicity of inhaled benzene to Sprague-Dawley rats and AKR mice at 300 ppm. J Toxicol Environ Health 4:605–618.

Snyder CA, Goldstein BD, Sellakumar A, et al. 1980. The inhalation toxicology of benzene: Incidence of hematopoietic neoplasms and hematoxicity in AKR/J and C57BL/6J mice. Toxicol Appl Pharmacol 54:323–331.

Tryphonas H. 2001. Approaches to detecting immunotoxic effects of environmental contaminants in humans. Environ Health Perspect 109(Suppl 6):877–884.

Tryphonas H, Feeley M. 2001. Polychlorinated biphenyl-induced immunomodulation and human health effects. In: Robertson L, Hansen LG, editors. PCBs recent advances in the environmental toxicology and health effects. Lexington, KY: University Press of Kentucky. p 193–209.

Van Loveren H, Vos JG. 1989. Immunotoxicological considerations: A practical approach to immunotoxicity testing in the rat. In: Dayan AD, Paine AJ, editors. London, UK: Taylor and Francis. pp 143–163.

Van Loveren H, Germolec D, Koren HS, Luster MI, Nolan C, Repetto R, et al. 1999. Report of the Bilthoven Symposium: Advancement of epidemiological studies in assessing the human health effects of immunotoxic agents in the environment and the workplace. Biomarkers 4:135–157.

Vos JG. 1980. Immunotoxicity assessment: Screening and function studies. Review. Arch Toxicol Suppl 4:95–108.

Wells MS, Nerland DE. 1991. Hematotoxicity and concentration-dependent conjugation of phenol in mice following inhalation exposure to benzene. Toxicol Lett 56(1–2):159–166.

Xia ZL, Jin XP, Lu PL, Gu XQ, LaPorte RE, Tajima N. 1995. Ascertainment corrected prevalence rate (ACPR) of leukopenia in workers exposed to benzene in small-scale industries with capture-recapture methods. Biomed Environ Sci 8(1):30–34.

Yardley-Jones A, Anderson D, Jenkinson P. 1988. Effect of occupational exposure to benzene on phygohaemagglutinin (PHA) stimulated lymphocytes in man. Br J Ind Med 45(8):516–522.

Yin S, Li Q, Liang Y. 1982. Significance of leukocyte alkaline phosphatase in the diagnosis of chronic benzene poisoning. Reg Toxicol Pharmacol 2:209–212.