Immunological Abnormalities in Humans Chronically Exposed to Chlorpyrifos

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ABSTRACT. Twenty-nine individuals with chronic health complaints following exposure to chlorpyrifos were compared with 3 control groups (i.e., 1 positive and 2 negative) with respect to the following: (1) peripheral lymphocyte phenotypes; (2) autoantibodies (nucleic acids and nucleoproteins, parietal cell, brush border, mitochondria, smooth muscle, thyroid gland, and central nervous system/peripheral nervous system myelin); (3) mitogenesis to phytohemagglutinin and concanavillin. The data revealed an increase in CD26 expression, a decrease in percentage of CD5 phenotype, decreased mitogenesis in response to phytohemagglutinin and concanavillin, and an increased frequency of autoantibodies. The alterations in these peripheral blood markers were unaffected by medications, age, sex, or season. The authors concluded that chronic exposure to chlorpyrifos causes immunological changes.
<Key words: antimyelin, antithyroid, autoimmunity, CD26 cells, chlorpyrifos, mitogenesis>

CHLORPYRIFOS (CPS; 0,0-diethyl-0-[3,5,6-trichloropyridinol] phosphorothionate) is a chlorinated organophosphate insecticide that is a moderate inhibitor via CPS oxon of acetylcholinesterase. Inhibition of this enzyme leads to signs and symptoms of overstimulation of the cholinergic system.[1,2] The commercial product contains several chemicals, including, but not limited to, alkyl phosphates, alkyl phosphorothioates (sulfotepp), 3,5,6-trichloropyridinol (TCP), and solvents.[3] Other adverse effects of CPS in animals and humans are (1) developmental neurotoxicity[4–7]; (2) targeting of deoxyribonucleic acid (DNA), ribonucleic acid, protein, nuclear transcription, and adenosine 3′5′-cyclic monophosphate (cyclic AMP) signaling cascade in postnatal brain neurogenesis[8–12]; (3) mitotic abnormalities, apoptosis, and cytoxicity in rat embryos and midbrain micromass cultures[13,14]; (4) birth defects in humans[15]; (5) decreased T-cell blastogenesis to phytohemagglutinin (PHA) and concanavillin (ConA), with increased expression of CD4 and CD8 surface makers in rats[16]; and (6) generation of reactive oxygen species, DNA damage, and lactate acid dehydrogenase leakage in rat brain and liver.[17]

In a previous study, we reported immunological alterations in 12 individuals who were chronically exposed to CPS.[18] In this article, we have confirmed and have extended our earlier observations of changes in peripheral blood immunologic phenotype frequencies, and we have described the presence of autoantibodies following exposure to CPS. In addition, these data showed that the observed changes in peripheral blood phenotype frequencies were not affected by medications, age, season, or sex. Finally, perhaps immune alterations, increased frequency of autoantibodies, and increased rate of apoptosis are associated with chronic illness following exposure to CPS.

Method and Material

Subjects and controls. Twenty-nine individuals (10 males, 19 females) with chronic health complaints were

evaluated for immunologic abnormalities following exposure to CPS. All subjects were diagnosed by their treating physicians as having multiple chemical hypersensitivity. Common symptoms were an initial flu-like illness, followed by multiorgan symptoms that included headache, loss of memory, dizziness, gastrointestinal disturbance, arthralgia, menstrual irregularities, fatigue, and heightened olfactory sensitivity to low concentrations of chemicals. All were nonsmokers. To the best of our knowledge, none of the 29 individuals took either prescribed or over-the-counter medications. We compared the data to 2 negative (unexposed to CPS) and 1 positive control (exposed to CPS) groups. Group A controls were volunteer asymptomatic chiropractic students (aged 29 ± 9 yr; 15 males, 13 females) who were exposed to formaldehyde in an anatomy course 1 yr prior to immune testing. Group B controls were healthy volunteer home dwellers (aged 54 ± 19 yr; 13 males, 16 females) who gave informed consent for immunological testing. History of atopy (i.e., asthma and hay fever) was unknown for students, but it was reported previously for the home dwellers.[18]

CPS sample collection. In some cases, concentrations of CPS were measured. Most subjects were exposed in either their home or workplace following commercial misapplication of the insecticide. One subject experienced a CPS spill in his truck; 2 males were exposed to wet CPS-treated lumber while working at a lumber mill. Two individuals (male and female) were exposed by a sprayer that left puddles of insecticide and vegetation concentrations of CPS that were between 0.013 and 0.074 parts per million (ppm). The detection of CPS in the homes was done by either Occupational Safety and Health Administration method 62 or U.S. Environmental Protection Agency method 8080 at independent laboratories.

Testing of 5 different houses following commercial pesticide application revealed the following concentrations of CPS: (1) wipe samples (n = 4), 50.7 ± 57.6 ng/in^2; (2) wipe samples (n = 15) ranged from nondetectable to 150 ppm (mean [$\bar{x}$] ± standard deviation [SD] = 18.9 ± 38.5 ppm); (3) wipe samples (n = 7) ranged from nondetectable to 128.7 ppm ($\bar{x}$ = 22.2 ± 44.7 ppm; (4) 2 carpet samples contained 16 mg/kg and 316 mg/kg, respectively; and (5) attic bulk samples (n = 6) ranged from 3.9 mg/kg to 190 mg/kg ($\bar{x}$ = 37.4 ± 90 mg/kg), attic wipe samples (< 1.6 µg/kg), and attic air samples (< 0.008 mg/m^3). There were 2 males and 4 females who resided in the aforementioned homes. The houses were tested up to 4 yr after application of CPS. In addition, all subjects were healthy prior to exposure, and they expressed only common health problems (e.g., colds, hay fever). Exposure to CPS was verified through records maintained by the subjects. No attempt was made to correlate symptoms with the data because this was beyond the scope of our study. However, it has been ascertained that at least 3 individuals (2 males and 1 female) were subsequently diagnosed with lupus erythematosus (LE) -like disease, and a 4th female died from complications of chronic seizures believed to be kindling of the central nervous system (CNS).

Blood collection. Venous blood samples were drawn from all individuals 1–4.5 yr following exposure. All samples, including controls, were collected under the supervision of an attending physician in silicon-treated, sodium heparinized glass evac-tubes®. The blood samples were transported to the laboratory and were used within 24 hr; there was a cell viability of ≥ 90%. All samples were assigned a computer-generated number for identification. Quality assurance was performed by positive and negative controls, which were run simultaneously with the unknown samples. The mononuclear cells were isolated using Ficoll-isopaque density gradient centrifugation.[19]

Lymphocyte markers. All subjects had the following immunologic tests performed: phenotyping for surface makers CD4, CD5, CD8, CD19, CD25, CD26, and CD4/CD8, as has been reported previously.[18] We selected these phenotypes for comparison with our previous observations.[18] The data were subjected to analysis of variance (ANOVA) so that we could determine the effects of age, sex, and seasonal variation on lymphocyte markers.

Autoantibody determinations. Autoantibodies against (1) smooth muscle (ASM), (2) nucleic acids and nucleoprotiens (ANA), (3) mitochondria (AMA), (4) parietal cells (APC), and (5) brush border (ABB) were performed with standard indirect immunofluorescence.[20] Antibodies to myelin sheath were detected with frozen spinal cord (CNS) and sciatic nerve (i.e., peripheral nervous system) as substrates with an indirect immunofluorescence technique for which antihuman immunoglobulin conjugated to fluorescein was used.[21]

Thyroid antibodies were measured by an immunoradiographic procedure using thyroglobulin and purified thyroid peroxidase in 11 subjects.[22]

Mitogenesis. Mitogenesis to PHA and ConA were performed on peripheral lymphocytes from 13 individuals (colorimetric MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (tetrazolium) assay].[23] Viable cells activate the MTT, which is measured colorimetrically at 570 nm. Mononuclear cells were isolated and suspended in 0.1 ml at 10^6 cells. They were cultured in RPMI 1640 medium, 10% fetal calf serum, and antibiotics (i.e., penicillin and streptomycin). Cells from each individual were tested at 3 different concentrations of the mitogens for optimum stimulation. The tests were performed in triplicate at the optimum concentration and reported as the average of the 3 concentrations.

Results

Effects of age, sex, and season on peripheral lymphocyte markers. The effects of age, sex, and season on peripheral blood phenotypes were analyzed. ANOVAs we used to compare the 3 age groups showed no significant differences for total counts or percentages of each cell type (Table 1). All data were then pooled and analyzed for effects of sex and season on the mean and percentage cell counts. The data were not affected by either sex or season (data not shown).

Lymphocyte and white cell counts. Table 2 summarizes the results on lymphocyte markers for the 29

Table 1.—Mean Cell Counts (cells/mm³) of Lymphocyte Phenotypes, by 3 Age Groups

Variable	Group 1	Group 2	Group 3	*F**
Age (yr)	27.4 ± 7.5	45.7 ± 3.8	65.8 ± 3.1	
WBCs ($\bar{x}$)	7,000 ± 1574	7,670 ± 954	5,820 ± 643	2.636 *ns*
Lymphocytes ($\bar{x}$)	2,968 ± 553	2,996 ± 589	2,195 ± 420	2.843 *ns*
CD5				
$\bar{x}$	2,126 ± 553	2,043 ± 505	1,989 ± 538	1.897 *ns*
%	68 ± 11	69 ± 12	72 ± 10	0.005 *ns*
CD4				
$\bar{x}$	1,423 ± 373	1,479 ± 288	1,197 ± 282	0.191 *ns*
%	48 ± 6.2	50 ± 9.9	43.3 ± 3.8	0.021 *ns*
CD8				
$\bar{x}$	794 ± 304	765 ± 291	515 ± 173	1.653 *ns*
%	26.8 ± 6.8	25.1 ± 6.8	24 ± 9.2	0.021 *ns*
CD4/CD8				
$\bar{x}$	1.89 ± 0.42	2.15 ± 0.65	2.58 ± 0.94	2.170 *ns*
CD25				
$\bar{x}$	67 ± 47	105 ± 61	69 ± 39	1.441 *ns*
%	2.1 ± 1.4	3.8 ± 1.6	3.2 ± 2	1.339 *ns*
CD26				
$\bar{x}$	490 ± 233	513 ± 292	369 ± 173	0.532 *ns*
%	17.4 ± 6.2	16.9 ± 9.3	17.3 ± 8	0.008 *ns*
CD19				
$\bar{x}$	159 ± 139	287 ± 173	141 ± 93	2.377 *ns*
%	4.9 ± 3.2	9.8 ± 4.7	9.2 ± 4.5	0.939 *ns*

Notes: ANOVA tests (*F* values) revealed that age had no effect on the mean cell counts. $\bar{x}$ = mean, WBCs = white blood cells, and *ns* = not significant.
*$p = .05$.

Table 2.—Mean Cell Counts (cells/mm³) of Lymphocyte Phenotypes, by Exposed Group, Positive Control Group, and 2 Negative Control Groups (A and B)

	Group				
Variable	Exposed (n = 29)	Positive control (n = 12)	Control A (n = 28)	Control B (n = 27)	*p*
WBCs ($\bar{x}$)	6,887 ± 754	7,011 ± 1584	6,824 ± 1742	6,886 ± 1527	*ns*
Lymphocytes ($\bar{x}$)	2,703 ± 754	2,845 ± 650	2,392 ± 707	2,517 ± 550	*ns*
CD5					
$\bar{x}$	1,876 ± 469	1,901 ± 533	1,772 ± 576	1,906 ± 462	*ns*
%	69 ± 11	66.6 ± 9.6	74.1 ± 7.7	75.6 ± 5.6	< .05
CD4					
$\bar{x}$	1,342 ± 367	1,336 ± 381	1,234 ± 421	1,336 ± 324	*ns*
%	50 ± 7.6	47.9 ± 9.6	51.3 ± 5.9	53.1 ± 4.7	*ns*
CD8					
$\bar{x}$	703 ± 293	640 ± 258	597 ± 219	597 ± 175	*ns*
%	26 ± 7.1	22.4 ± 6.4	25.2 ± 6.4	23.7 ± 4.5	*ns*
CD4/CD8					
$\bar{x}$	2.1 ± 0.7	2.3 ± 0.76	2.2 ± 0.72	2.3 ± 0.59	*ns*
CD25*					
$\bar{x}$	78 ± 55	47.3 ± 64.4	71.2 ± 45.6	61.8 ± 42.4	*ns*
%	2.8 ± 1.8	1.5 ± 1.8	3.3 ± 2.7	2.4 ± 1.6	*ns*
CD19*					
$\bar{x}$	205 ± 157	196 ± 167	143 ± 103	202 ± 65	*ns*
%	7.4 ± 4.8	7.0 ± 5.0	6.1 ± 4.5	8.0 ± 2.0	*ns*
CD26					
$\bar{x}$	438 ± 265	372 ± 392	122 ± 93	52 ± 43	< .001
%	16.1 ± 9	13 ± 13.4	5.1 ± 3.6	2.1 ± 1.8	< .001

Notes: $\bar{x}$ = mean, WBCs = white blood cells, and *ns* = not significant.
*There were 26 subjects in the exposed group for CD25 and CD19.

Table 3.—Values Following Mitotic Stimulation with Phytohemagglutin (PHA) and Concanavillin (ConA) for 13 Individuals

Value	PHA		ConA	
	Normal (%)	Below normal (%)	Normal (%)	Below normal (%)
Expected	96–195		94–354	
Observed	114	81	94	62
	128	74	138	65
	106	27	130	75
	107	74	169	25
	104	85	103	76
	117	95	103	77
	103			77
	111 ± 9	73 ± 24	123 ± 38	65 ± 9

Notes: The 6 individuals with normal values had a stimulation index of 111 ± 9% (PHA) and 123 ± 38% (ConA). Five of the remaining 7 individuals had abnormally low values for both PHA and ConA, whereas each had a value below expected for PHA and ConA, respectively. Comparison of the 6 normal vs. 7 abnormal individuals revealed no difference in CD26 and CD5 cells, or in autoantibodies.

subjects, the 12 positive controls (exposed to CPS), and 2 control groups previously reported.[18] No significant differences were observed in the absolute and percentage counts of each cell types except as follows: (1) The percentage of CD5 was significantly lower than in the 2 control groups ($p < .05$), and it was no different from the positive control group; (2) both the percentage and absolute numbers of CD26 cells were significantly greater than in the 2 control groups ($p < .001$), and they were no different from the positive control group.

Mitogenesis. The results of stimulation of peripheral lymphocytes by PHA and ConA in 13 subjects are presented in Table 3. Five individuals had stimulation indices below expected values for both PHA and ConA, 1 had a lower value for PHA, and 1 had a lower value for ConA. The average lowered stimulation indices were 73 ± 24% ($\bar{x} \pm SD$) for PHA and 65 ± 19% for ConA, compared with the expected values of 96 ± 195% and 94 ± 354%, respectively. The absolute numbers, percentage of CD26 cells, and autoantibodies were not significantly different among individuals with abnormal mitogenesis ($n = 7$) vs. those with normal stimulation ($n = 6$).

Autoantibodies detected in the peripheral blood. Autoantibodies detected in the peripheral blood are

Table 4.—Number and Percentage of Autoantibodies Found in Exposed, Positive Control, and 2 Negative Control (A and B) Groups, and Odds Ratios (ORs) at 95% Confidence Intervals (CIs) for Exposed vs. Control A and B Groups

Antibody	Exposed ($n = 27$)		Positive control ($n = 12$)		Control A ($n = 28$)		Control B ($n = 27$)		Control A		Control B	
	n	%	*n*	%	*n*	%	*n*	%	OR	95% CI	OR	95% CI
ANA	5	19	3	25	1	3.6	0	0	5.63	1.76, 23.58	—*	
ASM	14	52	5	42	4	14.3	4	14.8	6.14	2.96, 13.28	6.14	2.96, 13.18
AMA	1	3.8	0	0	0	0	0	0	—*		—*	
APC	7	27	4	33	1	3.6	1	3.7	8.88	2.88, 13.18	8.88	2.88, 13.18
ABB	9	35	7	58	4	14.3	7	24.0	3.31	1.57, 7.19	1.71	0.88, 3.33

Notes: ANA = antibodies against nucleic acids and nucleoproteins, ASM = antibodies against smooth muscle, AMA = antimitochondrial, APC = antibodies against parietal cells, and ABB = antibrush border.
*Odds ratios could not be calculated because zero values existed in Controls A and B.

Table 5.—Number and Percentage of Individuals with 1, 2, and 3 Autoantibodies in Exposed, Positive Control, and 2 Negative Control (A and B) Groups, and Odds Ratios (ORs) at 95% Confidence Intervals (CIs) for Exposed vs. Control A and B Groups

Autoantibody	Exposed ($n = 27$)		Positive control ($n = 12$)		Control A ($n = 28$)		Control B ($n = 27$)		Control A		Control B	
	n	%	*n*	%	*n*	%	*n*	%	OR	95% CI	OR	95% CI
1 positive	19	70.00	9	83.30	5	14.80	4	14.54	14.33	6.72, 31.31	14.33	6.73, 31.31
2 positive	16	58.00	6	50.00	2	7.10	1	3.70	18.35	7.42, 50.89	33.14	11.01,131.30
3 positive	3	12.00	3	25.00	0	0.00	1	3.70	—*		13.50	1.91,583.78

*Odds ratio could not be calculated because zero values existed in Control A.

Table 6.—Percentages of Individuals (n = 26) with Antithyroid Autoantibodies to Central and Peripheral Myelin in Exposed vs. Control Subjects

Individual	Antithyroglobulin	Antimicrosomal
1	0.33	0
2	0	110*
3	2.6	16*
4	0.8	1.6*
5	0.32	0
6	0	11*
7	1.9	12*
8	0.35	0
9	1:10	1:80*
10	22	1,671*

Notes: Individuals 1–8 were tested by Antibody Assay Laboratories, Santa, California, as described previously.[18] Expected values in MRC are 0.0–0.33 (antithyroglobulin) and 0.0–0.35 (antimicrosomal). Individual 9 was tested by Immunsciences Lab., Inc., Beverly Hills, California. Rabbit red blood cell agglutination was used. Expected titers are < 1:20 (antithyroglobulin) and < 1:80 (antimicrosomal). Individual 10 was tested by Immunodiagnostic Laboratories, San Leandro, California. Fluorescence-activated microsphere assay was used for the test. The expected values are < 1 for both antithyroglobulin and antimicrosomal.
*Six of the individuals had sufficient antibodies indicative of autoimmune thyroiditis, either clinical or subclinical. None were tested for thyroid disease.

presented in Tables 4 through 7. Odds ratios for ANA, ASM, and APC were significantly different from the unexposed control subjects, but they were no different from the exposed group. The odds ratio could not be determined for AMA because of zero values for the controls (Table 4). In addition, the odds ratios for individuals with multiple autoantibodies (i.e., at least 1, 2, or 3 different autoantibodies) were significantly different from the nonexposed controls (Table 5).

Antithyroid autoantibodies detected in 10 of 26 individuals are presented in Table 6. Ten (38.5%) had antibodies directed toward the thyroid gland. Four had both antithryoglobulin and antimicrosomal antibodies, 3 had only antimicrosomal antibodies, and 3 had only antithyroglobulin antibodies.

The titers in 6 individuals were sufficiently indicative of autoimmune thyroiditis. No subjects were tested for thyroid hormone abnormalities.

In Table 7 are summarized the percentages of individuals with antimyelin autoantibodies to central and peripheral myelin in the exposed vs. control subjects. Antimyelin antibodies were considered positive at titers of 1:8 or greater. The critical Z values were all significant at $p < .05$ (i.e., 1.645).

Table 7.—Percentage and Z Values of Individuals with Autoantibodies for Each Isotype (IgG, IgM, IgA) Against Myelin of the Central and Peripheral Nervous Systems (CNS and PNS, respectively) in the Exposed Group vs. Control Group

	Exposed		Control		Z*	
Isotype	CNS %	PNS %	CNS %	PNS %	CNS	PNS
IgG	76	35	28	15	3.532	1.698
IgM	35	44	15	20	1.698	1.891
IgA	53	50	20	21	2.513	2.313

Note: Ig = immunoglobulin.
*All Z values were significant at $p \geq .05$. Critical Z values (2-sided significance level) at $p = .1,0, .05, .02$, and .01 were 1.645, 1.960, 2.326, and 2.576, respectively.

Discussion

Previously, we noted that lymphocyte activation markers and autoantibodies can occur from chronic illness (e.g., autoimmune disease), as well as from toxic exposure.[18,26–28] However, we considered each subject in this study to be healthy, and each led a normal life prior to CPS exposure. Concomitant with the exposure, they developed multiorgan symptoms that have persisted for several years. Most common among these symptoms were an initial flu-like illness. A flu-like illness is a common symptom following exposure to organophosphates, as well as to toxins in general (for a review, see Gordon and Rowsey[24]). Although some of the individuals in this study were eventually diagnosed with an LE-like illness, and others may have an autoimmune thyroiditis,[25] their initial illness and symptoms began when CPS exposure began. Thus, we conclude that the immunologic observations in this study, as well as in previous studies, are a result of the exposure leading to subsequent health problems.

In addition, the data on lymphocyte phenotypes in the peripheral blood of these individuals corroborate and extend previous observations on humans with chronic illness following exposure to CPS.[18] The data also show that the lymphocyte markers are not affected by age, sex, season, over-the-counter medicines, or prescription drugs. Thus, the increase in CD26 cells, the decrease in percentage of CD5 cells, as well as the presence of multiorgan autoantibodies, are associated with a chronic exposure to CPS—an organophosphate.

Multiorgan autoantibodies and an increased expression of CD26 and other activation markers on peripheral lymphocytes have been reported for other xenobiotics: formaldehyde,[26,27] chlordane/heptachlor,[28,29] pentachlorophenol,[30] industrial solvents,[31] silicone breast implants,[32] and methyl tertiary-butyl ether/benzene.[33] From these observations, it appears that immune activation and the development of multiorgan autoantibodies are common manifestations of exposure to xenobiotics. Such immune alterations (e.g., appearance of immune activation markers, abnormal mitogenesis, and multiple autoantibodies) may suggest the presence of an atypical autoimmune disease process and/or immune dysregulation.[31–36] Several recent observations on altered immune parameters in chronically ill individuals may shed some light on possible causative mechanisms, which require further examination and research; these include Gulf War veterans, apoptosis, and respiratory burst.

Gulf War veterans with chronic fatigue have higher levels of T cells, major histocompatibility complex (MHC) II+T cells, interleukin-2 (IL-2), IL-10, interferon-gamma, and tumor necrosis factor (TNF) -alpha, and decreased Natural Killer cells, compared with healthy controls.[37] Ill veterans with neurological symptoms also have a lower than expected activity of paraoxonase (PON1) (Type Q Type A) arylesterase.[38] PON1 hydrolyzes several organophosphates, including CPS, sarin, diazinon, and soman.[38,39] Moreover, Au et al.[40] have shown that farmers with "unfavorable" versions of polymorphic metabolizing enzymes (cytochrome P4502E1, glutathione-S-transferases mu and theta, and paroxonase genes) expressed greater cytogenetic effects following exposure to mixed pesticides, compared with individuals with "favorable" genes. Thus, it appears that the association between genetic susceptibility and alterations in various immune parameters in relation to toxic exposure requires additional research.

Seven of the 13 individuals tested had abnormally low mitogenesis in response to stimulation of peripheral blood lymphocytes by PHA and ConA. Abnormal mitogenesis and an increased rate of apoptosis in some individuals may also be an underlying mechanism in altered immune parameters in humans following chemical exposure.[33–35] Individuals with chronic fatigue induced by chemical exposure have an increase in serum TNF-alpha and an increased rate of apoptosis of peripheral lymphocytes.[35] Humans exposed to gasoline-contaminated water also have increased rates of apoptosis.[33] CPS in rat embryo cultures is a mitotic spindle poison that causes apoptosis and affects neurogenesis in vivo and in vitro.[8,10,13,14] Thus, immune dysregulation caused by xenobiotics, including CPS, may occur via an increased rate of apoptosis. Finally, an increase in lymphocyte surface-marker expression and a decreased lymphocyte blastogenic response following CPS exposure may result in a compensatory shift of the immune response toward certain stimuli.[16] Therefore, increased expression of CD26, decreased percentage of CD5, and multiple autoantibodies in exposed individuals are compatible with these observations.

Polychlorinated pesticides, including organophosphates and organochlorines, cause generation of reactive oxygen species both in vitro and in vivo.[17,41] These species are apparently capable of causing (1) damage to single-stranded DNA, (2) increased leakage of lactate dehydrogenase from mitochondria, and (3) an apparent increased rate of apoptosis in rat brain and liver.[17] CPS is a chlorinated compound, and 3,5,6-trichlorpyridinol is the major metabolite.[42] Thus, the roles that reactive oxygen species, mitochondrial damage, leakage of mitochondrial proteins (e.g., lactate acid dehydrogenase, cytochrome c), and TNF-alpha have in apoptosis and in the release of self-antigens should be examined closely, particularly because mitochondria are damaged by cytotoxic agents that release several proteins, including cytochrome c, that lead to cell death.[43]

In conclusion, it is increasingly apparent that certain individuals are more susceptible to the adverse effects of xenobiotics, and they experience chronic adverse health effects following exposure. Altered frequency and function of biomarkers, such as certain genes, enzymes, lymphocyte phenotypes, lymphokines, multiple autoantibodies, as well as increased apoptosis, appear associated with exposure to xenobiotics and subsequent chronic health problems.

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References

1. Fukoto TR. Mechanism of action of organophosphorus and carbamate insecticides. Environ Health Perspect 1990; 87:245–54.
2. Kaloyanova FP, El Batawi M. Human Toxicology of Pesticides. Boca Raton, FL: CRC Press, 1991; pp 3–41.
3. Sherman JD. Chlorpyrifos (Dursban) -associated birth defects: a proposed syndrome, report of four cases, and discussion of the toxicology. Int J Occup Med Toxicol 1995; 4:417–31.
4. Whitney KD, Seidler FJ, Slotkin TA. Developmental neurotoxicity of chlorpyrifos: cellular mechanisms. Toxicol Appl Pharmacol 1995; 134:53–62.
5. Moser VC. Dose-response and time-course of neurobehavioral changes following oral chlorpyrifos in rats of different ages. Neurotoxicol Teratol 2000; 22:713–23.
6. Chanda SM, Pope CN. Neurochemical and neurobehavioral effects of repeated gestational expoure to chlorpyrifos in maternal and developing rats. Pharmacol Biochem Behavior 1996; 53: 771–76.
7. Chakraborti TK, Farrar JD, Pope CN. Comparative neurochemical and neurobehavorial effects of repeated chlorpyrifos exposures in young and adult rats. Pharmacol Biochem Behav 1993; 46:219–34.
8. Dam K, Seidler FJ, Slotkin TA. Developmental neurotoxicity of chlorpyrifos: delayed targeting of DNA synthesis after repeated administration. Develop Brain Res 1998; 108:39–45.
9. Campbell CG, Seidler FJ, Slotkin TA. Chlorpyrifos interferes with cell development in rat brain regions. Brain Res Bull 1997; 43:179–89.
10. Song X, Seidler FGJ, Saleh J, et al. Cellular mechanisms for developmental toxicity of chlorpyrifos: targeting the adenyl cyclase signaling cascade. Toxicol Appl Pharmacol 1997; 145:158–74.
11. Johnson DE, Seidler FJ, Slotkin TA. Early biochemical detection of delayed neurotoxicity resulting from developmental exposure to chlorpyrifos. Brain Res Bull 1998; 45:143–47.
12. Crumpton TL, Seidler FJ, Slotkin TA. Developmental neurotoxicity of chlorpyrifos in vivo and in vitro: effects on nuclear transpcription factors involved in cell replication and differentiation. Brain Res 2000; 857:87–98.
13. Roy TS, Andrews JE, Seidler FJ, et al. Chlorpyrifos elicits mitotic abnormalties and apoptosis in neuroepithelium of cultured rat embryos. Teratol 1998; 58:62–68.
14. Cozena ME, Bidanset J. Effects of chlopyrifos on neuronal development in rat embryo midbrain micromass cultures. Vet Human Toxicol 1995; 37:118–21.
15. Sherman JD. Chlorpyrifos (Dursban) exposure and birth defects: report of 15 incidents, evaluation of 8 cases, theory of action, and medical and social aspects. Eur J Oncol 1999; 4:53–59.
16. Blakely BR, Yole MJ, Brousseau P, et al. Effect of chlorpyrifos on immune function in rats. Vet Human Toxicol 1995; 41:140–44.
17. Bagchi D, Bagchi M, Hassound EA, et al. In vitro and vivo generation of reactive oxygen species, DNA damage and lactate dehyrogenase leakage by selected pesticides. Toxicol 1995; 104:129–40 .