Topical Permethrin Exposure Causes Thymic Atrophy and Persistent Inhibition of the Contact Hypersensitivity Response in C57Bl/6 Mice

Korawuth Punareewattana,[1] Bonnie J. Smith,[1] Benny L. Blaylock,[2] John L. Robertson,[1] Robert M. Gogal, Jr.,[1] M. Renee Prater,[1] Janice Longstreth,[3] Hubert L. Snodgrass,[4] and Steven D. Holladay[1]

[1]*Department of Biomedical Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA*
[2]*College of Pharmacy and Health Sciences, University of Louisiana at Monroe, Monroe, Louisiana, USA*
[3]*The Institute for Global Risk Research, LLC, Bethesda, Maryland, USA*
[4]*U.S. Army Center for Health Promotion and Preventive Medicine, Health Effects Research Program, Aberdeen Proving Ground, Maryland, USA*

Permethrin was applied to the shaved dorsal interscapular region of female C57Bl/6 mice at doses of 0.5 or 1.5 θl/day in corn oil and neat 5.0 θl/day. These doses corresponded to approximately 22, 66, and 220 mg/kg/day topical permethrin. Mice were exposed in this manner either daily for 10 or 30 consecutive days, or every other day for 7 or 14 exposures. Body weight was not affected by any of the treatment regimens. However, thymic weight was decreased and splenic weight was increased by 1.5 or 5.0 θl permethrin/day, 2 days after termination of 10 consecutive days of topical chemical exposure. Cell surface antigen expression did not change in any treatment group on thymocytes (CD4, CD8), splenocytes (CD45R, Thy 1.2), or bone marrow cells (CD45, CD45R). A persistent, dose-related inhibition of the contact hypersensitivity (CH) response occurred in mice at all exposure levels of permethrin tested.

Keywords Contact Hypersensitivity, Immune Suppression, Immunotoxicity, Permethrin

Permethrin, a photostable class I pyrethroid insecticide, is effective against a wide range of insects and has been widely used in agriculture, animal husbandry, and human medicine (Taplin and Meinking 1990). Humans are exposed to relatively high levels of topically applied permethrin for treatment of head lice and scabies (Llewellyn et al. 1996; Asakawa et al. 1996; Fuortes 1999). Permethrin has also been extensively used to treat military clothing for prevention of insect-borne disease (Schreck, Snoddy, and Spielman 1986; Schreck and Kline 1989; Sholdt et al. 1989). Snodgrass (1992) found that wearing permethrin-treated clothing (0.125 mg/cm^2) resulted in topical exposure to permethrin at about 34 μg/kg/day. The latter author estimated the systemic dose to humans wearing permethrin- treated military clothing at 6×10^{-4} mg/kg/d.

During the recent Persian Gulf War, service personnel were concurrently exposed to chemical agents (e.g., permethrin, pyridostigmine, chlorpyrifos) as well as stress (Zhang et al. 1999). Veterans of this war have reported persistent symptoms including headache, loss of memory, fatigue, muscle and joint pain, ataxia, skin rash, respiratory difficulties, and gastrointestinal disturbances (collectively referred to as Persian Gulf War Syndrome) (Murphy et al. 1999). An alteration in immune function has been suggested as a possible contributing cause to some of these illnesses (Doucet 1994). In support of this hypothesis, certain immune parameters were found to be significantly different between veterans with syndrome and controls (Zhang et al. 1999). These alterations included increased total T cells and major histocompatibility complex (MHC) class II–restricted T cells, decreased natural killer (NK) cells, and increased levels of interleukin-2 (IL-2), IL-10, interferon gamma (IFN-γ), and tumor necrosis factor alpha (TNF-α).

Permethrin has been extensively studied in laboratory rodents to determine its neurotoxic effects (reviewed by Vijverberg and van den Bercken 1990). Studies examining possible immunotoxicity of permethrin have been limited to a few reports,

Received 17 May 2000; accepted 14 August 2000.
This work was supported by U.S. Army DAADO5-96-P-3975 and NIH RO1 ES09642-02.
Address correspondence to S.D. Holladay, PhD, Department of Biomedical Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Phase II, Southgate Drive, Blacksburg, VA 24061-0442, USA. E-mail: holladay@vt.edu

1091-5818/00 $12.00 + .00

which suggest effects on the immune system. In vitro, permethrin inhibited the mitogenic response of murine lymphocytes to concanavalin A, lipopolysaccharide (Stelzer and Gordon 1984), or phytohemagglutinin (Diel et al. 1998). Several immune responses were inhibited in mice exposed to 0.004 to 0.4 mg/kg permethrin by oral gavage, including mixed lymphocyte response, cytotoxic T lymphocyte response, and natural killer cell activity (Blaylock et al. 1995). Deltamethrin (structurally similar to permethrin) has been shown to affect both cellular and humoral immune responses in mice (6–16 mg/kg; Lukowicz-Ratajczak and Krechniak 1992). To date, the dermal route of exposure to pyrethroid insecticides has not been evaluated for capacity to alter immune responses.

Mice in the present study were topically exposed to permethrin to mimic the most common route of human exposure (Snodgrass 1992). Immune function was evaluated using selected tests from the immunotoxicity testing battery of the National Toxicology Program (NTP) (Luster et al. 1992).

METHODS

Mice

Female C57Bl/6 mice (21.5 ± 1.0 g; Harlan Sprague-Dawley, Indianapolis, IN) were used in these studies. All studies were reviewed and approved by the Virginia Tech Animal Care and Use committee prior to purchasing mice. Mice were quarantined for 1 week prior to initiation of experiments. Following the quarantine period, mice were randomly assigned to treatment groups (one mouse per cage, six mice per treatment group). Mice were anesthetized by brief inhalation exposure to methoxyflurane, and the interscapular area was shaved of hair using electric clippers. Mice were maintained under controlled conditions of temperature (22 ± 1°C), humidity (40%–60%), and lighting (12/12-hour light/dark cycle) and provided with food and water ad libitum throughout the course of the experiments. The mice were examined daily for clinical signs.

All procedures involving animals were approved prior to initiation of experiments by the Virginia Tech Animal Care and Use Committee. Animals in all experiments were humanely cared for and maintained, in accordance with University and funding agency guidelines.

Permethrin Preparation and Treatment Protocols

Permethrin (91.6%; Coulston Products, Easton, PA) was provided by the U.S. Army Center for Health Promotion and Preventive Medicine (CHPPM; Aberdeen Proving Ground, MD). The isomeric composition of the permethrin was 42.3% cis isomer, 57.7% trans isomer. Mice were treated with permethrin using three dose solutions defined as low dose (0.5 ml permethrin in 4.5 ml corn oil), intermediate dose (1.5 ml permethrin in 3.5 ml corn oil), and high dose (5.0 ml neat permethrin). Corn oil was used as the control solution. All dosing solutions were maintained in the dark at room temperature. Mice were dosed by interscapular topical exposure with 5 μl of respective dosing solutions using an Eppendorf micropipettor. Following application of the permethrin solution to the shaved skin, the smooth side of the pipette tip was used to spread the solution over the skin. Daily treatment continued in this manner for 10 or 30 consecutive days, or every other day for 7 or 14 exposures. For convenience, the four dose groups were named as follows:

Exposure 1 = every day for 10 consecutive days
Exposure 2 = every other day for 7 exposures
Exposure 3 = every other day for 14 exposures
Exposure 4 = every day for 30 consecutive days

Mice were euthanized by CO_2 inhalation on the 2nd, 10th, or 30th day after termination of treatment, weighed, and evaluated for immune effects of the chemical exposure.

Thymus/Body Weight and Spleen/Body Weight Ratio

The thymus and spleen were removed by dissection. The spleen was cleaned of excess adipose and other connective tissues. Organs were weighed individually on an Ohaus TS-120 balance (Fisher Scientific, Pittsburgh, PA) and percent of total body weight calculated.

Cell Preparation and Cellularity

Thymuses, spleens, and both femurs and tibias from six mice per treatment group were collected and placed separately in 2 ml of culture medium (RPMI 1640; Mediatech, Cellgro, Herndon, VA) in a 60 × 15-mm culture dish (Fisher Scientific, Norcross, GA). Thymocytes and splenic cells were gently dissociated in the culture medium using a metallic sieve screen (Sigma Chemical Co., St. Louis, MO) and curved forceps. For bone marrow studies, femurs and tibias were stripped of associated muscles, and the proximal and distal ends of each bone removed. Marrow hematopoietic cells were collected and pooled by gently flushing the marrow cavity of each of the four bones collected per mouse with 2 ml culture medium, using a 25-gauge needle. Erythrocytes were removed from thymic, splenic, and bone marrow samples by suspending cells in lysing solution (0.015 M NH_4Cl, 1.0 mM $NaHCO_3$, 0.1 mM EDTA) for 5 minutes at room temperature. Cells were then washed twice in culture medium, resuspended in 2 ml standard buffer (Hank's balanced salt solution), and enumerated using a CASY-1 electronic cell counter (Scharfe System GmbH, Germany).

Immune Cell-Surface Marker Analysis Assays

Thymocytes, splenocytes, and bone marrow hematopoietic cells from control and permethrin-exposed mice were adjusted to 5×10^6 cells/ml in standard buffer. Expression of CD4 and CD8 surface antigens on thymocytes was determined by incubating 100 μl cell suspension (containing 5×10^5 cells) in the dark at 4°C for 30 minutes with 1.0 μg fluorescein isothiocyanate (FITC)-conjugated anti-mouse CD8 (clone 53-6.7) and

0.2 μg phycoerythrin (PE)-conjugated anti-mouse CD4 (clone H129.19) monoclonal antibodies (Boehringer Mannheim, Indianapolis, IN). Splenocytes (5×10^5) were incubated in the dark at 4°C for 30 minutes with either 1.0 μg PE-conjugated anti-mouse Thy 1.2 (clone 30-H12: Boehringer Mannheim), 1.0 μg PE-conjugated anti-mouse CD45R (Ly-5/B220; clone RA3-6B2; Pharmingen, Sandiago, CA), or 1.0 μg FITC-conjugated anti-mouse Mac1 (clone M1/70: Boehringer Mannheim). Marrow hematopoietic cells (5×10^5) were incubated in the dark at 4°C for 30 minutes with either 1.0 μg FITC-conjugated anti-mouse CD45 (clone IM7; Pharmingen), or 1.0 μg PE-conjugated anti-mouse CD45R. Following incubations cells were washed twice, resuspended in 0.5 ml standard buffer, and immediately analyzed using an Epics XL flow cytometer. Cell viability was verified by forward angle light scatter and ethidium bromide exclusion and was greater than 95% in all samples. The limited number of dead cells present were excluded from analysis with electronic gates. For each sample 10,000 events were collected and analyzed.

Histopathology of Spleen and Thymus

For all histologic studies, three mice per treatment group were used. The spleen and thymus were removed from these mice immediately after sacrifice and placed into 10% neutral buffered formalin. Following a 24-hour fixation at 4°C, tissues were processed using routine histological techniques (Lund 1968). Sections (6 μm) were made and stained with hematoxylin and eosin. All tissue samples were evaluated using light microscopy.

Contact Hypersensitivity Assay

The contact hypersensitivity response was assayed by quantifying the ear swelling response to chemical exposure using the method originally published by Asherson and Ptak (1968). Briefly, mice in all treatment groups were sensitized by topical application in the shaved interscapular region of 25 μl of 2% (*w/v*) oxazolone dissolved in a 4:1 reagent-grade acetone/olive oil mixture. Five days after sensitization, the mice were challenged with 10 μl of 0.5% oxazolone by topical application to both the dorsal and ventral surfaces of the right ear. The acetone:olive oil vehicle was applied to the left ear as a control for vehicle-induced irritancy. Immunological responses to permethrin were assessed at 24 and 48 hours after challenge by measuring the thickness of the challenged ear with an Oditest D 1000 micrometer (The Dyer Co., Lancaster, PA). The response was quantified as the difference in the thickness of the challenged ear before and after challenge. The thickness of the left ear (vehicle-treated) was also determined before and after treatment with the acetone/olive oil vehicle to control for vehicle-induced irritation and swelling.

Statistical Analysis

Data were expressed as arithmetical mean ± *SEM*. Analysis of variance (ANOVA) was used with Dunnett's *t* test to establish significant differences among groups. Results described as different in this paper indicate significantly different at $p < .05$.

TABLE 1
Body weight in C57Bl/6 mice topically exposed to permethrin

Permethrin exposure	Mouse body weight (grams, means ± *SEM*)		
	Days after dosing termination		
	2 days	10 days	30 days
Exposure 1			
Control	20.81 ± 0.65	23.20 ± 0.60	22.40 ± 0.30
0.5 μl/day	21.70 ± 0.45	23.20 ± 0.20	23.29 ± 0.47
1.5 μl/day	20.61 ± 0.48	21.10 ± 0.40*	22.29 ± 0.48
5.0 μl/day	20.34 ± 0.43	22.90 ± 0.40	22.70 ± 0.35
Exposure 2			
Control	19.26 ± 0.43	21.59 ± 0.57	22.40 ± 0.67
0.5 μl/day	19.02 ± 0.56	21.60 ± 0.42	22.60 ± 0.50
1.5 μl/day	19.23 ± 0.64	21.15 ± 0.54	22.80 ± 0.40
5.0 μl/day	20.79 ± 0.60	21.33 ± 0.57	21.90 ± 0.78
Exposure 3			
Control	20.98 ± 0.60	21.46 ± 0.38	23.14 ± 0.34
0.5 μl/day	21.09 ± 0.22	20.67 ± 0.57	23.31 ± 0.15
1.5 μl/day	21.13 ± 0.52	21.96 ± 0.59	21.86 ± 0.60
5.0 μl/day	22.36 ± 0.50	21.94 ± 0.52	21.48 ± 0.31
Exposure 4			
Control	20.78 ± 0.51	21.58 ± 0.38	21.07 ± 0.44
0.5 μl/day	20.84 ± 0.49	20.60 ± 0.28	21.14 ± 0.81
1.5 μl/day	21.23 ± 0.51	21.27 ± 0.21	22.10 ± 0.45
5.0 μl/day	20.95 ± 0.59	21.09 ± 0.55	21.79 ± 0.52

Exposure 1 = every day for 10 consecutive days.
Exposure 2 = every other day for 7 exposures.
Exposure 3 = every other day for 14 exposures.
Exposure 4 = every day for 30 consecutive days.
N = 6 mice/treatment group in all cases; *statistically significant ($p < .05$).

RESULTS

Mouse Body and Organ Weights

Topical permethrin treatment did not appear to affect body weight of experimental mice (Table 1). In one experiment (1.5 μl/day, with evaluation at 10 days post exposure) body weight of experimental mice was less than control mice. No decrease or trend toward a decrease was present in the higher-dose group in this particular experiment, or in the other 11 experiments, thus this single observation was felt to be due to chance rather than a result of chemical exposure. Thymic/body weight ratio was decreased by Exposure 1, at 2 and 10 days post exposure (Table 2). Total numbers of leukocytic cells in the thymus (cellularity) also decreased in Exposure 1 mice 2 days after treatment (Table 3). Trends toward reduced thymic cellularity were present in all groups of Exposure 3 and Exposure 4 mice. Spleen/body weight ratio increased in mice exposed to the

TABLE 2
Thymus/body weight ratio in C57Bl/6 mice topically exposed to permethrin

Permethrin exposure	Thymus/body weight ratio (%, means ± *SEM*)		
	Days after dosing termination		
	2 days	10 days	30 days
Exposure 1			
Control	0.266 ± 0.041	0.261 ± 0.017	0.239 ± 0.012
0.5 μl/day	0.253 ± 0.026	0.263 ± 0.018	0.298 ± 0.056
1.5 μl/day	0.142 ± 0.019*	0.217 ± 0.028*	0.250 ± 0.008
5.0 μl/day	0.151 ± 0.012*	0.249 ± 0.015	0.304 ± 0.021
Exposure 2			
Control	0.306 ± 0.020	0.371 ± 0.023	0.261 ± 0.016
0.5 μl/day	0.353 ± 0.036	0.318 ± 0.018	0.272 ± 0.021
1.5 μl/day	0.242 ± 0.038	0.333 ± 0.019	0.290 ± 0.009
5.0 μl/day	0.256 ± 0.020	0.361 ± 0.016	0.238 ± 0.021
Exposure 3			
Control	0.282 ± 0.023	0.223 ± 0.012	0.156 ± 0.010
0.5 μl/day	0.301 ± 0.016	0.245 ± 0.016	0.153 ± 0.011
1.5 μl/day	0.281 ± 0.025	0.249 ± 0.015	0.152 ± 0.014
5.0 μl/day	0.252 ± 0.016	0.243 ± 0.014	0.165 ± 0.010
Exposure 4			
Control	0.243 ± 0.005	0.185 ± 0.015	0.145 ± 0.010
0.5 μl/day	0.209 ± 0.014	0.215 ± 0.020	0.155 ± 0.011
1.5 μl/day	0.211 ± 0.013	0.217 ± 0.019	0.179 ± 0.016
5.0 μl/day	0.225 ± 0.028	0.221 ± 0.007	0.146 ± 0.012

Exposure 1 = every day for 10 consecutive days.
Exposure 2 = every other day for 7 exposures.
Exposure 3 = every other day for 14 exposures.
Exposure 4 = every day for 30 consecutive days.
N = 6 mice/treatment group in all cases; *statistically significant ($p < .05$).

TABLE 3
Thymic cellularity in C57Bl/6 mice topically exposed to permethrin

Permethrin exposure	Thymic cellularity (decimal portion of control)		
	Days after dosing termination		
	2 days	10 days	30 days
Exposure 1			
Control	1.00 ± 0.15	1.00 ± 0.11	1.00 ± 0.22
0.5 μl/day	0.74 ± 0.15	0.80 ± 0.11	1.06 ± 0.08
1.5 μl/day	0.38 ± 0.18*	0.84 ± 0.12	0.97 ± 0.10
5.0 μl/day	0.49 ± 0.13*	1.04 ± 0.11	0.80 ± 0.20
Exposure 2			
Control	1.00 ± 0.17	1.00 ± 0.39	Not recorded**
0.5 μl/day	0.97 ± 0.30	1.00 ± 0.24	
1.5 μl/day	1.02 ± 0.20	1.04 ± 0.15	
5.0 μl/day	1.17 ± 0.27	1.24 ± 0.14	
Exposure 3			
Control	1.00 ± 0.10	1.00 ± 0.14	1.00 ± 0.13
0.5 μl/day	1.00 ± 0.09	0.98 ± 0.14	0.66 ± 0.11
1.5 μl/day	0.83 ± 0.25	0.80 ± 0.17	1.02 ± 0.14
5.0 μl/day	0.66 ± 0.21	0.60 ± 0.08	0.76 ± 0.07
Exposure 4			
Control	1.00 ± 0.13	1.00 ± 0.13	1.00 ± 0.19
0.5 μl/day	0.86 ± 0.07	1.01 ± 0.06	0.69 ± 0.12
1.5 μl/day	0.83 ± 0.10	0.86 ± 0.08	0.68 ± 0.12
5.0 μl/day	0.83 ± 0.15	0.64 ± 0.13	0.62 ± 0.11

Exposure 1 = every day for 10 consecutive days.
Exposure 2 = every other day for 7 exposures.
Exposure 3 = every other day for 14 exposures.
Exposure 4 = every day for 30 consecutive days.
N = 6 mice/treatment group in all cases; *statistically significant ($p < .05$); **a major snowstorm prevented this evaluation.

middle- and high-dose levels of permethrin for 10 consecutive days, 2 days after termination of treatment (Table 4). Cellularity of the spleen and bone marrow was not affected by permethrin treatment (data not shown).

Histopathology

In blinded studies, light microscopy of thymus and spleen revealed no morphologic effects of the topical permethrin. The ratio of the cortex to medulla in the thymus of experimental animals was similar in all groups, as was degree of lymphocyte degeneration (apoptosis) (data not shown). In the spleens, no difference in the ratio of red pulp to white pulp, or in cellular degeneration, was observed (data not shown).

Cell-Surface Antigen Expression

Exposure to permethrin did not alter expression of thymocyte CD4, CD8, or CD45R markers. The expression of each CD4, CD8 marker ($CD4^+8^-$, $CD4^+8^+$, $CD4^-8^-$, and $CD4^-8^+$) was the same among control and treated groups in all exposures (data not shown). In the spleen, CD45R (identifies B lymphocytes), Thy 1.2 (identifies T lymphocytes), and Mac-1 (identifies macrophages) marker expression was not altered by exposure to permethrin (data not shown). In the bone marrow, CD45R (identifies B lineage cells) and CD45 (identifies white blood cells) marker expression was also not altered by permethrin exposure (data not shown).

Contact Hypersensitivity Assay

Consistent inhibition of the ear swelling response was observed in permethrin-exposed mice (Table 5). This effect showed a dose-response relationship in all 12 experiments, and persisted to day 30 after termination of exposure. Mice were not examined at end points more than 30 days post exposure, thus it is not known if this effect would have persisted past 30 days.

DISCUSSION

A limited number of investigations have examined parameters of immune function in permethrin-exposed animals, and have all reported immunotoxicity. To date, there have been no investigations examining potential immunotoxicity following

TABLE 4
Spleen/body weight ratio in C57Bl/6 mice topically exposed to permethrin

Permethrin exposure	Spleen/body weight ratio (%, means ± *SEM*) Days after dosing termination		
	2 days	10 days	30 days
Exposure 1			
Control	0.226 ± 0.025	0.317 ± 0.051	0.242 ± 0.035
0.5 μl/day	0.268 ± 0.011	0.320 ± 0.070	0.284 ± 0.051
1.5 μl/day	0.468 ± 0.067*	0.328 ± 0.053	0.253 ± 0.026
5.0 μl/day	0.392 ± 0.050*	0.330 ± 0.065	0.252 ± 0.020
Exposure 2			
Control	0.208 ± 0.044	0.235 ± 0.020	0.272 ± 0.014
0.5 μl/day	0.244 ± 0.018	0.240 ± 0.029	0.263 ± 0.024
1.5 μl/day	0.316 ± 0.036*	0.224 ± 0.035	0.275 ± 0.054
5.0 μl/day	0.278 ± 0.024	0.200 ± 0.019	0.448 ± 0.112
Exposure 3			
Control	0.192 ± 0.047	0.212 ± 0.046	0.207 ± 0.025
0.5 μl/day	0.223 ± 0.031	0.227 ± 0.040	0.266 ± 0.073
1.5 μl/day	0.178 ± 0.020	0.188 ± 0.031	0.242 ± 0.040
5.0 μl/day	0.222 ± 0.013	0.249 ± 0.040	0.242 ± 0.031
Exposure 4			
Control	0.153 ± 0.012	0.176 ± 0.016	0.257 ± 0.044
0.5 μl/day	0.228 ± 0.024	0.159 ± 0.012	0.183 ± 0.024
1.5 μl/day	0.199 ± 0.029	0.218 ± 0.044	0.196 ± 0.017
5.0 μl/day	0.165 ± 0.026	0.192 ± 0.037	0.230 ± 0.032

Exposure 1 = every day for 10 consecutive days.
Exposure 2 = every other day for 7 exposures.
Exposure 3 = every other day for 14 exposures.
Exposure 4 = every day for 30 consecutive days.
N = 6 mice/treatment group in all cases; *statistically significant ($p < .05$).

TABLE 5
Contact hypersensitivity response (CH) in C57Bl/6 mice topically exposed to permethrin

Permethrin exposure	Ear thickness (μm) Days after dosing termination		
	2 days	10 days	30 days
Exposure 1			
Control	0.21	0.27	0.24
0.5 μl/day	0.14*	0.19*	0.20*
1.5 μl/day	0.09*	0.12*	0.18*
5.0 μl/day	0.06*	0.10*	0.18*
Exposure 2			
Control	0.18	0.28	0.26
0.5 μl/day	0.10*	0.26	0.24
1.5 μl/day	0.06*	0.19*	0.21*
5.0 μl/day	0.06*	0.17*	0.17*
Exposure 3			
Control	0.16	0.20	0.18
0.5 μl/day	0.13*	0.17	0.14
1.5 μl/day	0.10*	0.10*	0.12*
5.0 μl/day	0.06*	0.07*	0.06*
Exposure 4			
Control	0.26	0.19	0.19
0.5 μl/day	0.20*	0.17	0.14
1.5 μl/day	0.18*	0.16*	0.11*
5.0 μl/day	0.12*	0.08*	0.06*

Exposure 1 = every day for 10 consecutive days.
Exposure 2 = every other day for 7 exposures.
Exposure 3 = every other day for 14 exposures.
Exposure 4 = every day for 30 consecutive days.
*Statistically significant ($p < .05$).

dermal exposure to permethrin. Topical exposure to this insecticide is the most common route of exposure in humans. Further, permethrin has been shown to cross the skin in mice (Grissom, Brownie, and Guthrie 1987; Baynes, Halling, and Riviere 1997), rats and monkeys (Sidon, Moody, and Franklin 1988), rabbits (Snodgrass 1992), guinea pigs and humans (Franz et al. 1996), suggesting possible systemic effects. The two most noteworthy observations from the present studies were thymic atrophy and inhibited contact hypersensitivity (CH) response in mice following topical exposure to permethrin.

Thymic atrophy in the present mice occurred only after 10 consecutive days of permethrin treatment. Specific reasons for why this effect was not seen after every-other-day exposure or after 30 days of exposure using the same dose levels have not been identified. Permethrin is rapidly metabolized by ester hydrolysis and oxidation (Casida et al. 1983), resulting in short half-life (12.37 hours in rats; Anedon et al. 1991). Thus, the present every-other-day topical application of low levels of permethrin may have been insufficient to produce some effects seen with 10 consecutive days of exposure (i.e., thymic atrophy). Permethrin also has hepatic enzyme-inducing ability (Anedon et al. 1988), which may have led to diminution of effect with 30 day exposure. It was noted that nonsignificant trends toward reduced thymic cellularity were present in all groups of Exposure 3 or Exposure 4 mice. Further, in subsequent acute-exposure studies using higher doses of topical permethrin (15 or 25 μl), we observed both significant thymic atrophy and reduced $CD4^+8^+$ thymocytes (unpublished data).

Enan et al. (1996) reported thymic atrophy in mice exposed to deltamethrin by intraperitoneal injection. These authors demonstrated apoptosis in the thymus of treated animals, apparently an effect of deltamethrin on the Ca^{2+}/calmodulin-dependent protein kinase-phosphatase cascade. Similar to deltamethrin, permethrin is also a potent inhibitor of calmodulin (Rashatwar and Matsumura 1985). However, histologic evaluations of the thymus in the present study did not detect increased numbers of apoptotic or necrotic cells. In what may be a related observation, increased thymocyte apoptosis could be detected cytometrically but not histologically in mice exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (Kamath et al. 1997). The latter

authors hypothesized that rapid clearance of apoptotic cells by phagocytic cells occurred in vivo, precluding morphologic demonstration of these apoptotic cells.

The CH response is a T cell–mediated immune response that requires antigen recognition by memory T cells, proliferation of these cells and migration to site of antigen, and cytokine secretion resulting in influx of other inflammatory cells. This response has been included as a Tier II assay in the National Toxicology Program testing battery to assess chemical-induced immunotoxicity, and may be indicated when Tier I testing suggests effects on T cells (e.g., reduced thymic weight) (Luster et al. 1988, 1992). A persistent inhibition of the CH response occurred in the present mice, in all exposure groups, and even at the lowest dose of topical permethrin studied (0.5 μl/day).

It is difficult to compare human permethrin exposure to the present mouse exposure; however, some estimates can be made. Military personnel in the Gulf War had access to permethrin (0.5%) in spray aerosol cans. Following label directions, the spray application resulted in a permethrin concentration of about 0.025 mg A.I./cm^2 of fabric (Snodgrass 1992). The California Environmental Protection Agency (CEPA) estimated a similar fabric concentration of 0.021 mg A.I./cm^2 using 0.5% permethrin aerosol to treat civilian clothing (CEPA 1992). Based on earlier risk calculations developed for military personnel wearing permethrin-treated uniforms, the predicted absorbed dose by the wearer of such clothing would be 0.14 μg/kg/day (Snodgrass 1992). This value represents a worst-case scenario because the data were derived from tests using fabric nearly saturated with the permethrin solution, rather than cloth treated only on the outside with an aerosol spray.

In the present study, permethrin in corn oil was applied on the interscapular skin of mice at doses ranging from 22 to 220 mg/kg/day. Shah, Monroe, and Guthrie (1981) reported skin penetration of permethrin in acetone in mice, measured as disappearance of label from application site, at 88% within 8 hours of treatment. In a subsequent publication, however, the same laboratory (Grissom, Brownie, and Guthrie 1987), using a nearly identical protocol, reported skin penetration of about 2.5% in 8 hours and 26% in 48 hours. Sidon, Moody, and Franklin (1988) studied percutaneous absorption of permethrin in monkeys and rats after 24 hours and reported absorptions of 22%, 9%, and 44%, for the pesticide applied (in propylene glycol) to monkey forehead, monkey forearm, and rat back, respectively. In in vitro studies, Baynes, Halling, and Riviere (1997) reported that 1.2% to 1.7% of permethrin, administered in DMSO or acetone, crossed the skin of mice. (This is close to the 1.25% reported in humans; however, it is unclear whether the duration of exposure in mice was the same as that reported in humans.) Grissom, Brownie, and Guthrie (1987) reported similar data (2.5% absorption) if applying permethrin in acetone to full-thickness skin with exposure durations ranging between 8 and 48 hours. However, parallel studies in vivo, and using split thickness skin, although demonstrating similar degrees of absorption at 8 hours, at 48 hours found much higher degrees of absorption: 24% and 15% absorption, respectively.

No data are available for percutaneous absorption of permethrin from corn oil (the present vehicle). Assuming the 88% absorption rate reported by Shah, Monroe, and Guthrie (1981), the absorbed dose of permethrin in low-dose mice in the present study would be estimated at 19 mg/kg/day. This is about 135,000 times higher than that predicted for humans wearing permethrin-treated clothing. Assuming the 24% absorption rate reported by Grissom, Brownie, and Guthrie (1987), the absorbed dose of permethrin in the same mice would be estimated at 4.84 mg/kg/day, still about 35,000 times higher than that predicted for humans wearing permethrin-treated clothing.

Higher levels of permethrin exposure occur acutely in humans (especially children) with the use of permethrin-containing products as pediculicides and scabicides (Vander Stichele, Dezeure, and Bogaert 1995). These products, containing 1% to 5% permethrin, are prescribed for application to the head or body. The scabicide Elimite (5% permethrin) is described as safe and effective in children two months of age and older (*Physicians Desk Reference* [PDR] 1995). The PDR indicates that for adults, a 30-g application of Elimite should be thoroughly massaged into the skin from the head to the soles of the feet, and washed off after 8 to 14 hours. In adult volunteers treated with Elimite, an average of 1.25% of permethrin (i.e., 10 mg of 800 mg applied) was absorbed (COT 1994).

Assuming application size varies with surface area, a 2-year-old child weighing 11.2 kg and having a surface area 35% that of an average adult (EPA 1985) would require approximately a 10 g application of Elimite, containing 500 mg permethrin. Applying the 1.25% absorption figure (COT 1994) to an 11.2-kg child treated with 500 mg permethrin suggests a 6.25-mg absorbed dose and, thus, systemic exposure of about 0.56 mg/kg. Assuming the 88% absorption rate, the present low-dose mice received 33.9 times this dose every other day for 14 or 28 days or daily for 10 or 30 days. Assuming the 24% absorption rate, the present low-dose mice received 9 times this dose/day.

Approximately 1 oz (29.5 g) of the crème rinse NIX, containing 280 mg permethrin, is used in humans in one 10-minute application as a pediculicide. The reported dermal absorption of permethrin in adults following treatment with NIX is less than 1% (CEPA 1992). If it is assumed that 280 mg permethrin are applied according to label to the head and neck (approximately 1000 cm^2) of a 6-year-old child weighing 22.5 kg (EPA 1985; ICRP 1975), and that about 1% is absorbed, a 2.8-mg absorbed dose would be estimated. This corresponds to systemic exposure of 0.124 mg/kg. Again assuming an 88% percutaneous absorption rate, the present low-dose mice received about 158 times this dose/day. Assuming the 24% absorption rate, the present low-dose mice received 40 times this dose/day.

In summary, topical exposure to permethrin caused limited but significant thymic atrophy in mice, as well as persistent inhibition of the CH response. Available data suggest that systemic exposure in humans wearing permethrin-treated clothing is considerably less than that experienced by the present mice. An accurate comparison of systemic permethrin exposure in humans treated for lice or mites to that occurring in the present mice is

important but difficult, due to the range of percutaneous transfer rates reported in the literature. The upper end of estimated transfer rates across mouse skin (88%) in acetone is 70.4 times the estimated transfer rate across human skin (1.25%) from a crème vehicle, which could overestimate the rate of permethrin absorption from corn oil. The lower end of estimated transfer rates across mouse skin (1.2%–1.7%) is similar to that reported for human skin, and results in estimated systemic exposure in the present mice in the range of that reported in humans. Accurately determining percutaneous transfer of permethrin across mouse skin from a corn oil vehicle is therefore an important next step for estimating risk to human immune health from use of permethrin-based products.

REFERENCES

Anedon, A., M. J. Diez, M. Sierra, J. A. Sanchez, and M. T. Teran. 1988. Microsomal enzyme induction by permethrin in rats. *Vet. Hum. Toxicol.* 30:4309–4312.

Anedon, A., M. R. Martinez-Larrañaga, M. J. Diaz, and P. Bringas. 1991. Toxicokinetics of permethrin in the rat. *Toxicol. Appl. Pharmacol.* 110:1–8.

Asakawa, F., F. Jitsunari, K. Miki, J. O. Choi, N. Takeda, T. Kitamado, S. Suna, and Y. Manabe. 1996. Agricultural worker exposure to and absorption of permethrin applied to cabbage. *Bull. Environ. Contam. Toxicol.* 56:42–49.

Asherson, G., and W. Ptak. 1968. Contact and delayed hypersensitivity in the mouse. I. Active sensitization and passive transfer. *Immunology* 15:405–416.

Baynes, R. E., K. B. Halling, and J. E. Riviere. 1997. The influence of diethyl-*m*-oluamide (DEET) on the percutaneous absorption of permethrin and carbaryl. *Toxicol. Appl. Pharmacol.* 144:332–339.

Blaylock, B. L., M. Abdel-Nasser, S. M. McCarty, J. A. Knesel, K. M. Tolson, P. W. Ferguson, and H. M. Mehendale. 1995. Suppression of cellular immune responses in BALB/c mice following oral exposure to permethrin. *Bull. Environ. Contam. Toxicol.* 54:768–774.

Casida, J. E., D. W. Gammon, A. H. Glickman, and L. J. Lawrence. 1983. Mechanisms of selective action of pyrethroid insecticides. *Annu. Rev. Pharmacol. Toxicol.* 23:413–438.

CEPA (California Environmental Protection Agency). 1992. *Permethrin (Permanoner® Tick Repellant) Risk Characterization Document.* Sacramento, CA: Department of Pesticide Regulation, California Environmental Protection Agency. Medical Toxicology and Worker Health and Safety Branches.

COT (Committee on Toxicology). 1994. *Health Effects of Permethrin-Impregnated Army Battle-Dress Uniforms.* Subcommittee to Review Permethrin Toxicity from Military Uniforms, Committee on Toxicology, Board on Environmental Studies and Toxicology, Commission on Life Sciences, National Research Council. Washington, DC: National Academy Press.

Diel, F., M. Detscher, H. Borck, D. Schrimpf, E. Diel, and H. W. Hoppe. 1998. Effects of permethrin on human basophils and lymphocytes in vitro. *Inflamm Res.* 47 (Suppl 1):S11–S12.

Doucet, I. 1994. Desert Storm syndrome: Sick soldiers and dead children? *Med. War* 10:183–194.

Enan, E., K. E. Pinkerton, J. Peake, and F. Matsumura. 1996. Deltamethrin-induced thymus atrophy in male Balb/c mice. *Biochem. Pharmacol.* 2351: 447–454.

EPA (U.S. Environmental Protection Agency). 1985. Development of statistical distributions of ranges of standard factors used in exposure assessments. Washington, DC: Office of Health and Environmental Assessment, Exposure Assessment Group. EPA/600/8-85/010.

Franz, T. J., P. A. Lehman, S. F. Franz, and J. D. Guin. 1996. Comparative percutaneous absorption of lindane and permethrin. *Arch. Dermatol.* 132: 901–905.

Fuortes, L. 1999. Urticaria due to airborne permethrin exposure. *Vet. Hum. Toxicol.* 41:92–93.

Grissom, R. E., C. Brownie, and F. E. Guthrie. 1987. *In vivo* and *in vitro* dermal penetration of lipophilic and hydrophilic pesticides in mice. *Bull. Environ. Contam. Toxicol.* 38:917–924.

ICRP (International Commission on Radiological Protection). 1975. *Report of the Task Group on Reference Man.* New York: Pergamon Press.

Kamath, A. B., X. Huimin, P. S. Nagarkatti, and M. Nagarkatti. 1997. Evidence for the induction of apoptosis in thymocytes by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin *in vivo*. *Toxicol. Appl. Pharamcol.* 142:367–377.

Llewellyn, D. M., A. Brazier, R. Brown, J. Cocker, M. L. Evans, J. Hampton, B. P. Nutley, and J. White. 1996. Occupational exposure to permethrin during its use as a public hygiene insecticide. *Ann. Occup. Hyg.* 40:499–509.

Lukowicz-Ratajczak, J., and J. J. Krechniak. 1992. Effects of deltamethrin on the immune system in mice. *Environ. Res.* 59:467–475.

Lund, L. G. 1968. *Manual of histologic staining methods of the Armed Forces Institute of Pathology*, 3rd ed. New York: McGraw-Hill.

Luster, M. I., A. E. Munson, P. T. Thomas, M. P. Holsapple, J. D. Fenters, K. L. White, Jr., L. D. Lauer, D. R. Germolec, G. J. Rosenthal, and J. H. Dean. 1988. Methods evaluation. Development of a testing battery to assess chemical-induced immunotoxicity: National Toxicology Program's guidelines for immunotoxicity evaluation in mice. *Fundam. Appl. Toxicol.* 10:2–19.

Luster, M. I., C. Portier, D. G. Pait, K. L. White, Jr., C. Gennings, A. E. Munson, and G. J. Rosenthal. 1992. Risk assessment in immunotoxicology. I. Sensitivity and predictability of immune tests. *Fundam. Appl. Toxicol.* 18:200–210.

Murphy, F. M., H. Kang, N. A. Dalager, K. Y. Lee, R. E. Allen, S. H. Mather, and K. W. Kizer. 1999. The health status of Gulf War veterans: Lessons learned from the Department of Veterans Affairs Health Registry. *Mil. Med.* 164:327–331.

PDR (*Physicians' Desk Reference*, 49th ed., 488.). 1995. Eliminite® Cream. Montvale: Medical Economics.

Rashatwar, S. S., and F. Matsumura. 1985. Interaction of DDT and pyrethroids with calmodulin and its significance in the expression of enzyme activities of phosphodiesterase. *Biochem. Pharmacol.* 34:1689–1694.

Schreck, C. E., and D. L. Kline. 1989. Personal protection afforded by controlled-release topical repellents and permethrin-treated clothing against natural populations of *Aedes taeniorhynchus*. *J. Am. Mosq. Control Assoc.* 5:77–80.

Schreck, C. E., E. L. Snoddy, and A. Spielman. 1986. Pressurized sprays of permethrin or DEET on military clothing for personal protection against *Ixodes dammini* (Acari: Ixodidae). *J. Med. Entomol.* 2823:396–399.

Shah, P. V., R. J. Monroe, and F. E. Guthrie. 1981. Comparative rates of dermal penetration of insecticides in mice. *Toxicol. Appl. Pharmacol.* 59:414–423.

Sholdt, L. L., E. J. Rogers, Jr., E. J. Gerberg, and C. E. Schreck. 1989. Effectiveness of permethrin-treated military uniform fabric against human body lice. *Mil. Med.* 154:90–93.

Sidon, E. W., R. P. Moody, and C. A. Franklin. 1988. Percutaneous absorption of cis- and trans-permethrin in rhesus monkeys and rats: Anatomic site and interspecies variation. *J. Toxicol. Environ. Health* 23:207–216.

Snodgrass, H. L. 1992. Permethrin transfer from treated cloth to the skin surface: Potential for exposure in humans. *J. Toxicol. Environ. Health.* 35:91–105.

Stelzer, K. J., and M. A. Gordon. 1984. Effects of pyrethroids on lymphocyte mitogenic responsiveness. *Res. Commun. Chem. Pathol. Pharmacol.* 46:137–150.

Taplin, D., and T. L. Meinking. 1990. Pyrethrins and pyrethroids in dermatology. *Arch. Dermatol.* 126:213–221.

Vander Stichele, R. H., E. M. Dezeure, and M. G. Bogaert. 1995. Systematic review of clinical efficacy of topical treatments for head lice. *BMJ* 311:604–608.

Vijverberg, H. P., and J. van den Bercken. 1990. Neurotoxicological effects and the mode of action of pyrethroid insecticides. *Crit. Rev. Toxicol.* 21:105–126.

Zhang, Q., X. D. Zhou, T. Denny, J. E. Ottenweller, G. Lange, J. J. LaManca, M. H. Lavietes, C. Pollet, W. C. Gause, and B. H. Natelson. 1999. Changes in immune parameters seen in Gulf War veterans but not in civilians with chronic fatigue syndrome. *Clin. Diagn. Lab. Immunol.* 6:6–13.