

# Differential Permethrin Susceptibility of Head Lice Sampled in the United States and Borneo

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**Background:** Pediculiasis is treated aggressively in the United States, mainly with permethrin- and pyrethrin-containing pediculicides. Increasingly frequent anecdotal reports of treatment failure suggest the emergence of insecticidal resistance by these lice.

**Objective:** To confirm or refute the susceptibility of head lice sampled in the United States to permethrin.

**Design:** Survey. Head lice were removed from children residing where pediculicides are readily available and where such products are essentially unknown. Their survival was compared following exposure to residues of graded doses of permethrin in an in vitro bioassay.

**Setting:** School children from Massachusetts, Idaho, and Sabah (Malaysian Borneo).

**Subjects:** In the United States, 75 children aged 5 to 8 years. In Sabah, 59 boys aged 6 to 13 years. Virtually all sampled US children had previously been treated with pediculicides containing pyrethrins or permethrin; none

of the Sabahan children were so exposed.

**Main Outcome Measure:** Survival of head lice exposed to permethrin.

**Results:** Permethrin did not affect head lice sampled from chronically infested US children who had previously been treated for pediculiasis. The slope of the dose-response regression line for these lice did not differ significantly from zero ( $P = .66$ ). This pediculicide immobilized lice sampled in Sabah. Mortality correlated closely with permethrin concentration ( $P = .008$ ).

**Conclusions:** Head lice in the United States are less susceptible to permethrin than are those in Sabah. The pyrethroid susceptibility of the general population of head lice in the United States, however, remains poorly defined. Accordingly, these relatively safe over-the-counter preparations may remain the pediculicides of choice for newly recognized louse infestations.

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**Editor's Note:** It looks like we're very far from scratching lice off the list of minor(?) "plagues."

Catherine D. DeAngelis, MD

**V**ARIOUS PYRETHROID formulations began to replace lindane in the United States during the past decade as the treatment of choice against head lice (*Pediculus capitis*). Preferred treatments came to include such synthetic permethrin-containing products as Nix (Warner-Lambert Co, Morris Plains, NJ) and synergized pyrethrum extracts as RID (Pfizer Inc, New York, NY). Their relative absence of recognized mammalian toxicity contributed to the acceptability of these products for application to the heads of children. By the mid 1990s, however, so many anecdotal reports of treatment fail-

ure had accumulated that the efficacy of these pediculicides seemed questionable and the lay press began to suggest that head lice no longer were susceptible to these chemicals. These commonly applied pediculicides may have begun to fail.

Permethrin-resistant head lice elsewhere in the world had become evident at about this time. Thus, lice taken from residents of Israel<sup>1</sup> in 1994 survived longer exposure to this pediculicide than did lice sampled and tested in 1989. Those sampled in the United Kingdom<sup>2</sup> in 1994 to 1995 and in the Czech Republic<sup>3</sup> in 1992 similarly survived such exposure. Each of these studies, however, defined susceptibility in terms of mortality of lice confined in contact with a single standardized concentration of insecticide. This time-dependent protocol is confounding because pediculicide dose would increase with duration of survival of the test

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## SUBJECTS AND METHODS

### POPULATION SAMPLED

Lice were sampled from the heads of US children by nurses assigned to schools in Massachusetts (in Cambridge and Brookline, located near Boston) and in Idaho (in Boise). These lice mainly came from children aged 5 to 8 years who were referred to their school nurse because their teachers suspected that lice might be present in their hair. Additional head lice were sampled from children residing near Boston whose parents reported to us directly. The study was approved by the human subjects committee of the Harvard School of Public Health, Boston, and the appropriate institutional review board of each cooperating school district.

In Sabah, head lice were sampled from children pre-screened by nurses employed by the regional department of health who searched for lice in an elementary school and a residential community located in Kota Kinabalu (the capital of Sabah) and in 3 schools in Telupid. The human subjects committee of the Harvard School of Public Health and the appropriate authority in the Sabah Department of Health approved this study and authorized the nurses' services.

### DISK ASSAY AGAINST PERMETHRIN RESIDUES

Our "disk assay" test for evaluating the susceptibility of head lice to permethrin residues used a graded array of impregnated filter paper disks. To prepare these papers, 10  $\mu$ L of serial semilogarithmic dilutions of permethrin ([3-phenoxyphenyl] methyl  $\pm$  *cis-trans*-3-[2,2-dichloroethenyl]-2,2 dimethyl-cyclopropanecarboxylate; *cis-trans* isomers ratio: min 35%  $\pm$  *cis* and max 65%  $\pm$  *trans*; Coulston International Corp, Easton, Pa) dissolved in acetone were evaporated onto 1.5-cm disks of filter paper placed in the bottoms of wells of 24-well flat-bottom cell culture plates (Linbro; Flow Laboratories Inc, McLean, Va). The plates were then covered, the wells coded, and the plates stored in the dark at ambient temperature (24°C-26°C) for

no longer than 1 month. Lice were combed gently from the heads of children, and those that appeared undamaged were transferred by means of fine forceps to the treated papers within minutes of sampling. Lice were randomly distributed onto filter disks, ensuring that some were always deposited onto nontreated disks. The preparations were then maintained in the dark at ambient temperature in a water-saturated atmosphere within sealed polyethylene bags. The activity of these lice was monitored after specified periods of continuous contact with these residue-containing papers. Lice that were mobile were considered to be alive, and those not moving in response to a probe to be dead.

In Massachusetts, school nurses were supplied with our disk assay kits and instructed in their use. They were asked to place lice in the assay plates and convey the kits to us by courier within 1 hour. In Idaho, lice were sampled directly by one of us (C.H.) and evaluated after 18 hours. In Sabah, assays were conducted by several of us (A.S., S.R.T., H.A.R., N.W.) and insecticidal effect recorded after 6 hours.

### QUESTIONNAIRE

After US children were examined for pediculiasis, a questionnaire was sent home with each louse-infested student to be completed by his or her caregiver. The questionnaire solicited information concerning each student's prior experience with louse infestations, that of other members of the household, and any pediculicidal treatments that may have been applied.

### STATISTICAL METHODS

We sought to test at least 5 lice to each concentration of insecticide in each assay, and each test was replicated 3 times. To describe patterns of mortality of lice exposed to graded doses of pediculicide, linear regression coefficients were compared by means of probit analysis.<sup>4</sup> Abbott's formula was used to adjust data relevant to insecticide-associated mortality with mortality due to all other factors.<sup>4</sup>

insect. The frequency of permethrin susceptibility could not, therefore, directly be described. Therapeutic recommendations based on either study would further be burdened by their passive mode of sampling, a procedure that might bias them toward lice taken from children who were repeatedly exposed to pediculicide. Although head lice infesting certain residents of Europe and the Middle East have become insusceptible to permethrin during the past decade, the therapeutic efficacy of this pediculicide in the United States remains uncertain.

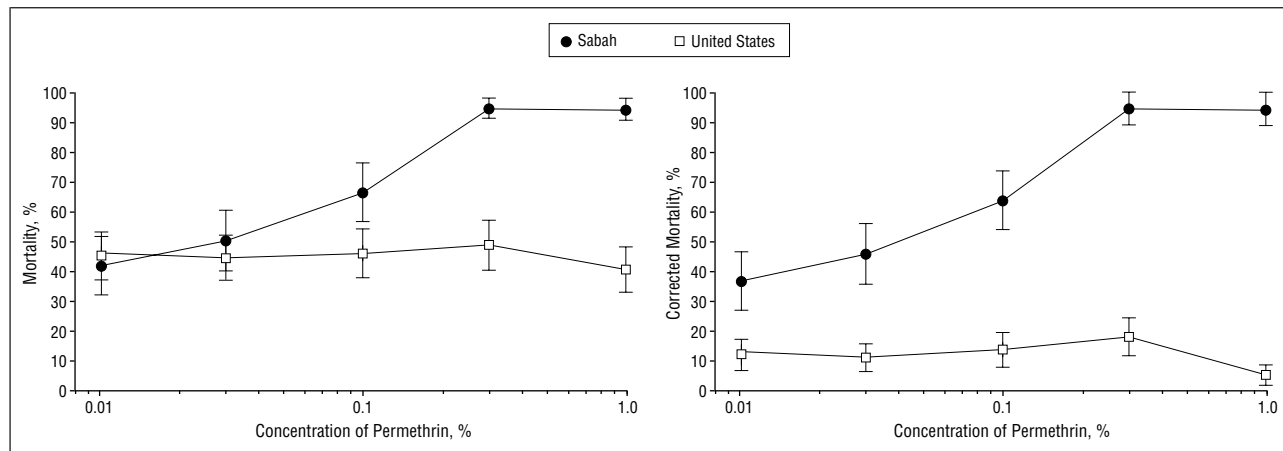
It may be that lice infesting the heads of children residing in the United States are more frequently insusceptible to permethrin than are those sampled where similar pediculicides may be less readily applied. Thus, we determined whether lice sampled passively from the heads of US residents of Massachusetts and Idaho were as susceptible to permethrin as lice infesting residents of Sabah (Malaysian Borneo). We sampled head lice from children residing in each of these sites and recorded survival of these lice following exposure to graded doses of permethrin residues. We anticipated that the resulting mortality pattern of permethrin-susceptible lice would

correlate more closely with pediculicide dose than would that of insusceptible lice. We also estimated the intensity of exposure of head lice in each of these sites to pyrethrin-containing pediculicides.

## RESULTS

### INTENSITY OF INFESTATION

Head louse infestations detected passively by school nurses in our US study sites were surprisingly infrequent. Although school nurses in the participating Brookline and Cambridge school districts sampled lice from infested children, few reports were received throughout this 2-year study, and many of these proved to be spurious. In all, we discovered only 11 louse infestations in Massachusetts, comprising a total of 48 lice, and only 64 in Idaho, comprising 215 lice. An average of 3.5 discernible trophic-stage lice were present in each confirmed infestation. Parents of these infested children who responded to a questionnaire (56% of sample) all reported that they were aware of and had treated these



**Figure 1.** Effect of increasing permethrin concentration on mortality of head lice taken from the heads of children residing in Sabah, Malaysian Borneo, and the United States. Left, Data not corrected for nonspecific mortality. Right, Data corrected for nonspecific mortality by means of Abbott's formula.<sup>4</sup>

infestations within the previous month, generally with a pediculicide containing pyrethrins or permethrin. Passive sampling detected remarkably few cases, and these infestations were apparent to caregivers.

Louse-infested children were readily identified in Sabah. The Sabah Department of Health routinely monitored pediculiasis in schoolchildren, and we chose to sample children from particular schools in Kota Kinabalu and Telupid because health records indicated that children in these schools were particularly subject to this condition. We detected nymphal or adult lice in the hair of 59 (61%) of 97 children who had been selected because louse eggs were apparent in their hair. An average of 5.1 such trophic-stage lice were found on each of these 59 children. Head louse infestations seem relatively commonplace in Sabah and are apparent to school health workers.

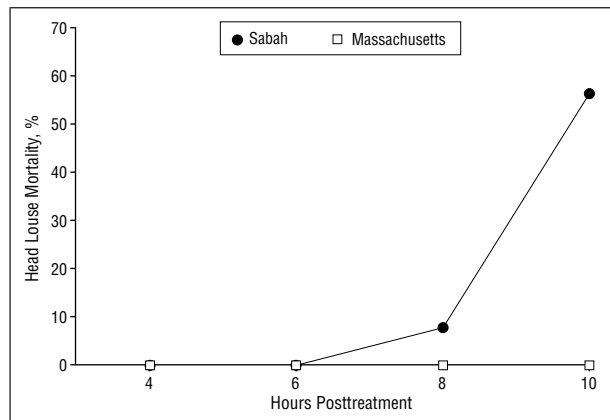
#### HISTORY OF EXPOSURE TO PEDICULICIDES

To describe the perceived history of pediculiasis in louse-infested US children, we administered a questionnaire to those who were infested. Of 75 questionnaires that were distributed, 42 (56%) were completed and returned to us. The respondents informed us that these 42 children had experienced a mean of 2 prior infestations during their lifetimes and had missed an average of 3.8 days (SD, 6.4 days) of school owing to louse infestations during the preceding year. Of these 42 infested children, the caregivers of 39 stated that at least 1 other family member was infested at the time of the survey, and all but 2 indicated that pediculicide had been applied during the course of the current infestation. Nix was the most frequently used pediculicide, comprising more than a third of all treatments, with the pyrethrin-based alternatives RID, A-200 (Hogil Pharmaceutical Corp, Purchase, NY), and Pronto (Del Laboratories, Farmingdale, NY) comprising 17%, 4%, and 4% of treatments, respectively. Home remedies included vinegar, rubbing alcohol, margarine, and laundry detergent. Pediculiasis is perceived as a heavy social burden by parents of louse-infested US children and is treated aggressively, mainly with the use of pyrethroid-containing pediculicides.

We inquired into the use of pediculicides in Sabah. Each of the 3 pharmacies that we visited displayed shampoos containing either lindane or malathion, and none marketed louse treatments containing pyrethrins or permethrin. Two traditional herbalists that we visited said that they marketed no products designed for lice and suggested that their customers would perceive no need for such a product. Indeed, we noted no burden associated with head louse infestations in our subjects. Although no signs of inflammation were apparent, nor were cases of louse-borne rickettsiosis or relapsing fever reported to public health officials, these authorities placed sufficient priority on this condition that they monitored prevalence of head lice in each school in Sabah. Pyrethroids seem to be rarely applied as remedies against head lice in Sabah.

#### SUSCEPTIBILITY OF HEAD LICE TO PERMETHRIN RESIDUES

The susceptibility of head lice removed from children in the United States and Sabah was evaluated by disk assay. The 215 lice sampled from Idaho were exposed to permethrin in groups of 28 to 55 per treatment and the 48 from Massachusetts in groups of 5 to 11 per treatment. An exposure interval of 18 hours seemed to be satisfactory for recording mortality because virtually all nontreated lice survived in our disk assay devices this long. By then, about half of the permethrin-exposed lice had become immobile, regardless of insecticide concentration (**Figure 1**). The slope of the dose-response regression line for these lice did not differ significantly from 0 ( $P = .66$ ); no differences were apparent between lice sampled from Massachusetts and Idaho. Although virtually all nontreated head lice removed from Sabahan children survived for 8 hours thereafter, about half became immobile after 10 hours (**Figure 2**). We chose, therefore, to record insecticide-induced mortality in Sabah after 8 hours of exposure. A total of 239 lice in cohorts of 12 to 41 were tested. About half of the lice become immobile after exposure to the 0.03% concentration of permethrin, and virtually all become immobile after exposure to the 0.3% residue. Mortality of Sabah-derived head



**Figure 2.** Survival of head lice harvested in Sabah, Malaysian Borneo, and in Massachusetts confined in disk assay devices containing no pediculicide.

lice correlated closely with permethrin concentration ( $P = .008$ ). Whereas permethrin seemed not to affect US head lice in our disk assay, mortality of those from Sabah correlated closely with the dose of this pediculicide.

#### COMMENT

Our system of exposing head lice to graded concentrations of permethrin deposited on paper disks provided a useful measure of insecticide susceptibility. Mortality among fully susceptible lice from Sabah following exposure to these papers correlates closely with permethrin content. A classic linear relationship links the logarithm of the dose with the probit of percent mortality. Interpretation of this assay system is somewhat limited in that tested lice remain in continuous contact with the insecticidal residues throughout the holding period. Although this practice effectively delivers the greatest insecticidal dose to those lice that survive longest, the effect does not seem to have modified the linearity of the response of Sabah-derived lice. This practice was dictated by our requirement for simplicity, reflecting our need for a system of testing that can be executed by generalist personnel working in remote locations. Assays performed in Israel,<sup>1</sup> in comparison, relied on just 1 insecticide-treated surface and recorded mortality in terms of duration of survival. Our disk assay system seems to be valid.

Our practice of holding lice sampled in the United States for a longer exposure period than for lice sampled in Sabah requires comment. The duration of this period, in both cases, was dictated by the condition observed in lice not exposed to pediculicide. Lice sampled in the United States survive host deprivation longer (18 hours) than do those in Sabah (8 hours), and those in the United Kingdom<sup>2</sup> survive even longer (24 hours). Although differing ambient temperatures may account for these differences, the effect may also reflect some inherent biological difference between these populations of lice. In either event, our use of longer postexposure holding periods in the United States than in Sabah seems justified.

The apparently greater prevalence of head louse infestations in Sabah than in the United States may mirror a sharply differing set of attitudes toward this condition in these societies and probably reflects the effect of pe-

diculicidal interventions. The louse-tolerant attitude of certain traditional societies is exemplified by the Bedouin proverb<sup>5</sup> equating a “deserted head [with] an ungenerous mind.” A societal abhorrence of ectoparasites, on the other hand, drives the intensive and extensive use of pediculicides in the United States as well as the various “no nits” policies that prevail in many local schools. Sabahan school children seem similar to some Bedouin in their acceptance of these ectoparasites, and no pyrethroid-containing pediculicides seem to be marketed in Sabah. These considerations persuade us that pyrethroid selection pressure on head lice infesting children residing in Massachusetts and in Idaho greatly exceeds that in Sabah, and they are consistent with our finding that head lice in the United States are less susceptible to permethrin than those infesting children in Sabah. Indeed, head lice from relatively poor Argentinian children, who were not likely to have been treated, were more susceptible to pediculicides than those sampled from families with greater resources.<sup>6</sup>

Insect populations exposed to a pyrethroid insecticide tend to lose susceptibility to that general class of chemicals. This insusceptibility is generally attributed to an amino acid substitution in a protein that induces “knockdown resistance,” thereby desensitizing cholinergic nerves to the activity of these toxins as well as to that of DDT.<sup>7</sup> Such toxins bind to paratype sodium channel membrane proteins, thereby blocking nervous conduction or inducing tetanus by delaying signal inactivation.<sup>8</sup> Somewhat narrower spectra of cross-resistance accompany more specific enzymatic detoxification such as that due to DDT dehydrochlorinase or malathion carboxylesterase. Broader spectra characterize less specific detoxifying or sequestering enzymes such as hydrolases or cytochrome-dependent P450 oxidase.<sup>9</sup> Lindane, the main organochlorine pediculicidal alternative to the pyrethroids, is detoxified in resistant houseflies by conjugation to a glutathione transferase.<sup>10</sup> Interestingly, the same enzyme specifically degrades methyl parathion. Lindane resistance has been documented in head lice.<sup>11</sup> Synergists generally, but not always, fail to restore pyrethroid effectiveness.<sup>12</sup> Although the specificity of permethrin insusceptibility in head lice remains unexplored, permethrin insusceptibility probably extends to related pyrethroid compounds, even when synergized, and perhaps even further.

Our demonstration of permethrin insusceptibility in US head lice must be interpreted cautiously, because our system of passive sampling may have biased our observations toward insusceptible lice. Many of the infested residents sampled were infested repeatedly or chronically, and virtually all had previously been treated for pediculiasis. Few susceptible lice would remain in the “problem infestations” that comprise such a sample. This sampling limitation similarly burdens other recent attempts to measure susceptibility of head lice, including those performed in Israel<sup>1</sup> and the United Kingdom.<sup>2</sup> Although the degree of pyrethroid susceptibility of the US head louse population was not recorded before these pediculicides became available there, the frequency of pyrethroid resistance seems to have increased. It is evident that lice infesting pediculicide-exposed US children are



far less susceptible to permethrin than are lice infesting nontreated Sabahan children.

The pyrethroid susceptibility of the general population of head lice in the United States, however, remains poorly defined. These relatively safe over-the-counter preparations may remain the pediculicides of choice for newly diagnosed louse infestations on the heads of US residents. Repeated applications or the use of more concentrated pyrethroid formulations, however, are ill advised because those US head lice that are insusceptible to permethrin seem solidly resistant, regardless of dose. The current susceptibility of these insects to the organochlorine insecticide lindane and the organophosphate insecticide malathion has not yet been analyzed in the United States. Prescription preparations containing these insecticides should be considered as alternative pediculicides if live lice persist after treatments with pyrethroid-based pediculicides. Such potentially toxic materials must be applied conservatively, particularly when the diagnosis rests solely on the discovery of nits by nonspecialized personnel. We note that desperate parents or caregivers whose children have been excluded from school and generally ostracized owing to perceived pediculiasis may apply inappropriate and potentially damaging remedies. Court orders may compel action, and the problem may become pernicious if diagnosis rests on the discovery of spurious objects. Indeed, of 56 diagnostic samples from children with presumed pediculiasis submitted to us for verification from residents scattered across the United States between April and August of 1998, 37 (66%) proved to be spurious. Even when correctly diagnosed, hatched or dead eggs may remain in the hair long after living lice have been eliminated; such findings do not justify pediculicidal treatment nor exclusion of a child from school. In addition to pediculicidal treatment, the hair of chronically infested children should be groomed manually, perhaps with the aid of a fine-toothed comb.

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