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Comparative Efficacy of Treatments for Pediculosis Capitis Infestations

Update 2000

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Objective: To evaluate the pediculicidal and ovicidal activity of 5 head lice products.

Design: In vitro pediculicidal and ovicidal product comparison.

Setting: Tropical field station in Panama.

Participants: Head lice and eggs were harvested from healthy children infested with *Pediculus capitis*.

Intervention: Within 2 hours of capture, lice were placed in continuous, direct contact with the pediculicide products and observed at regular intervals. Fresh, viable eggs were immersed in the pediculicides for 10 minutes, rinsed, air-dried, and incubated for 2 weeks.

Main Outcome Measures: Percentage of lice dead at regular observation intervals between 5 minutes and 3 hours of continuous exposure to the pediculicide and percentage of eggs not hatched after 2 weeks.

Results: All lice treated with Ovide lotion (0.5% malathion) were dead within 10 minutes and none of the eggs hatched. There was no significant change in the effectiveness of 0.5% malathion lotion or A-200 shampoo compared with the results of an earlier study (1986). There were significant declines in the pediculicidal activity of RID and the ovicidal activity of lindane. Nix (1% permethrin), which was not on the market at the time of the original study, killed lice in less than 30 minutes, and ovicidal activity ranged from 73% to 90% (diluted and undiluted, respectively).

Conclusions: Ovide lotion (0.5% malathion) was the fastest-killing pediculicide and the most effective ovicide. One percent lindane shampoo was the slowest-acting pediculicide and least effective ovicide. Nix was highly effective in both undiluted and diluted forms.

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PEDICULOSIS CAPITIS is the most prevalent parasitic infestation of humans in the United States and Europe.¹ The Field Epidemiology Survey Team (FEST), headed by Profs David Taplin and Terri Meinking, has been conducting studies on head lice for almost 20 years. From our many years of experience, it appears that infestation with pediculosis capitis was probably at an all time high in the United States in the year 2000, perhaps due to the development of head lice resistant to currently available products. Reliable estimates of the incidence and prevalence of pediculosis are lacking. Traditionally, market sales of pediculicides have been used to estimate the incidence of head lice infestations. However, with the advent of pediculicide resistance, market sales may be an unreliable indicator of incidence rates because a child with permethrin-resistant head lice may

be treated several times with different products and still not be cured. The usefulness of market sales as an indicator is further limited by the booming trend toward "natural" head lice remedies, including a variety of essential oils that may be purchased at health food stores or over the Internet. The number of units of natural products sold for pediculosis is unknown. Few, if any, efficacy or toxicity studies have been conducted for most of these products.²

Development of head lice (*Pediculus capitis*) resistant to current therapies, including lindane, permethrin, and malathion, has become a worldwide problem.^{1,3-13} It is clear that lindane and permethrin resistance is on the rise in the United States.^{1,2,13} The extent of this resistance is uncertain.

The issue of head lice resistance is also complicated by the many pediculicide product formulation changes that have oc-

MATERIALS AND METHODS

Five currently available pediculicides and a water control were evaluated for both pediculicidal and ovicidal activity. Pediculicides tested included 2 prescription products, 1% lindane shampoo (Alpharma, Baltimore, Md) and Ovide lotion (0.5% malathion; Medicis), and 3 OTC products, Nix (diluted and undiluted; Pfizer Inc), A-200 (Hogil Pharmaceutical Corp, Purchase, NY), and RID (Bayer Corporation, Morristown, NJ). Products to be tested were purchased in various pharmacies in Miami, Fla, and transported to Panama. Materials and supplies used in the experiments were protected at all times from extreme temperatures, pesticides, or other forms of contamination.

The protocol for this *in vitro* study was reviewed and approved by the Panamanian Ministry of Health, the island's Congreso (local governing body), and the local health committee. Informed consents were obtained from the participants in their own language (Tule). No identifying information was recorded. Kuna women with prior experience in FEST lice studies harvested live lice and viable nits from healthy Kuna children.

All lice and nits were protected from heat and light and were delivered to the field laboratory within 1 hour of capture. Lice were tested within 2 hours of collection. Viable eggs were dipped within 8 hours.

Because Nix is to be applied to shampooed, towel-dried hair, this product was tested in dilution (9 parts Nix to 1 part water) and in its undiluted form. We believe that *in vitro* tests conducted with the diluted solution more accurately simulate *in vivo* use of Nix than tests conducted with the undiluted product.

PEDICULICIDAL ACTIVITY

Before the *in vitro* testing, new 100% cotton "flour sack" kitchen towels were washed in an anionic shampoo (Prell) to remove any sizing or other residues, cut to 5-cm disks, and stored in clear Nalgene screw-cap jars to prevent contamination. For each experiment, adult male and female head lice and nymphs were collected from the heads of 6 or more infested children. The lice were pooled in a Petri dish containing a dampened cloth disk and were not identified as coming from any particular individual.

At the time of testing, investigators using forceps placed a single dry cloth disk (5-cm diameter) in sterile Petri dishes (15 × 60 mm). The pediculicide products to be tested were shaken several times, and a 1-mL syringe (without needle) was used to apply 0.5 mL of product (or water control) to each cloth disk. The products were applied in a circular motion to ensure even distribution. The product code number, application time, and exposure time were recorded on a data form and on the individual Petri dishes.

The amount of product (0.5 mL) applied to each cloth disk was half the amount used in the 1984 study. The amount of pediculicide used was decreased because the cotton towels used for the disks in the present study were thinner than those used previously. We have been using these "flour sack towel" disks for more than 2 years. Thus, this method has been validated and used extensively before the present study. In both studies, the amount of product applied was sufficient to saturate the cloth disk and to produce a wet surface on which the lice could walk.

Fifteen lice, including nymphs and adults of both sexes, were transferred from the collection disk to the lid of the Petri

currant in recent decades. Since the mid-1980s, a number of the most commonly used head lice treatments have undergone various formulation changes, including changes in vehicle components and sources of active ingredients. The effects of these reformulations on product efficacy are not well documented or understood. Indeed, most health care professionals and consumers are unaware that such changes have occurred. Therefore, although there has been a decline in product efficacy over the years, how much of this decline is attributable to resistance and how much is merely a reflection of changes in formulation is still unknown.

The primary goal of this study was to assess the extent to which product efficacy has changed since the early 1980s due to changes in formulation. To assess the effects of formulation changes, while controlling for the effects of resistance, FEST conducted *in vitro* comparative efficacy tests using "sensitive" (nonresistant) head lice collected from infested children in Panama. Results of these tests, performed in April 2000, were then compared with baseline efficacy data collected by us in the same region between April and August 1984.¹⁴ By comparing product efficacy data obtained in 2 similar studies conducted 16 years apart, we aimed to assess not only changes in efficacy, but also the validity of manufacturers' claims, which have changed little in the last 2 decades.

A secondary objective of this study was to establish baseline efficacy data for Nix (Warner Lambert Co [Morris Plains, NJ], now Pfizer Inc [New York, NY]), which had never been tested against other pediculicide products using this *in vitro* method. Baseline data on the effectiveness of pediculicides in a sensitive population of lice are essential to determine the relative roles of formulation vs resistance in treatment failures.

PEDICULICIDAL EFFICACY IN 1984

In the early 1980s, anyone (ie, school nurse, parent, physician) looking through the many pages of pediculicide advertisements appearing in the medical journals or parenting magazines would have been confused and doubtful about which head lice treatment was most safe and effective.¹⁴ At that time, Nix had not been approved by the Food and Drug Administration (FDA) and, therefore, was not an option for consumers. The prescription products, Kwell shampoo (1% lindane) (Reed & Carnrick, Jersey City, NJ) and Prioderm lotion (Purdue Frederick Company, Norwalk, Conn), now Ovide (0.5% malathion) (Medicis Pharmaceutical Corp, Phoenix, Ariz), were FDA approved. The over-the-counter (OTC) natural pyrethrin products synergized with piperonyl butoxide (PBO), such as RID (Pfizer Inc), A-200 (Norcliff Thayer, Inc [company extinct]), R&C shampoo (Reed

dish using curved entomologic forceps to avoid physical damage to the lice. This group of 15 lice was then immediately inverted onto the impregnated disk, the time was recorded, and a timer was started. Immediately after contact with the impregnated disk, the lice grasp the fibers of the impregnated cloth as if they were human hairs. This open system simulates an in vivo treatment but allows the lice to remain in intimate contact with the product until death or up to 3 hours, which is not possible in an in vivo situation due to possible toxic effects to the human subject.¹⁴

Lice on the impregnated disks were observed at regular intervals for up to 3 hours using a $\times 10$ magnifying hand lens. Death was determined when all movement and peristalsis of the gut had ceased. If lice were still living at the end of 3 hours, the number of live lice was recorded as a percentage of the total number of lice tested. If all lice were dead in less than 3 hours, observation ceased when the death of the final louse was observed. A confirmatory reading, however, was done at 3 and 6 hours after initial exposure to ensure that the lice had not revived. The "resurrection effect" is common with many pediculicides, especially natural pyrethrins and some synthetic pyrethroids (eg, *d*-phenothrin), and may lead to erroneous conclusions. Sometimes lice that are exposed to these products appear dead after several minutes and then revive after 1 or 2 hours. Thus, it is necessary to observe lice for 3 to 6 hours after exposure to pediculicides.

For a period of 4 days, investigators repeated this protocol 6 or more times for each product, testing each pediculicide on a total of at least 90 (6×15) lice. All tests were conducted by 1 of 4 team members with previous experience in this protocol. Every product was tested by each person. Also, each team member tested 15 lice as a water

control on each day they were involved in the study. All tests were conducted under artificial, solar-powered lighting at ambient temperatures (30°C - 34°C ; 86°F - 93°F) and relative humidity, which ranged from 70% to 90% at the study site.

OVICIDAL ACTIVITY

To evaluate ovicidal activity, single hairs, each with a viable-appearing egg attached, were snipped from infested children and placed in a clean container. Groups of 10 to 15 hairs with viable eggs were attached to small adhesive labels, allowing a 2-cm-long strand of hair to protrude, with eggs aligned at the distal end. When clamped with hemostat forceps, the label formed a convenient holder for transferring a group of eggs simultaneously to the test solutions and rinses. Each set of 10 to 15 eggs was immersed in the product for exactly 10 minutes. The eggs were then agitated in several changes of filtered water and air-dried at ambient temperatures.

When dry, all sets of hairs with eggs were transferred to small, sterile screw-cap glass vials (15×45 mm), which were tightly capped and incubated in the dark for 2 weeks at the ambient temperature and humidity of the tropical research station. The 4 evaluators replicated this protocol 8 or more times for each pediculicide product. On each day that experiments were conducted, 3 or more sets of 10 to 15 eggs were dipped into filtered water for 10 minutes to serve as "wet controls." In addition, 4 sets of 10 to 15 eggs were transferred to vials and incubated without irrigation or agitation to serve as "dry controls." Our control hatch rate in in vitro and clinical trials generally runs from 90% to 95%.

& Carnrick), and others, had been registered through the Environmental Protection Agency and "grandfathered in" by the FDA monograph.¹⁵ Most of the data on file referenced in most advertisements for these products were taken from studies conducted on a laboratory colony of body lice fed on rabbits. The lice from the laboratory colony, which was developed in the early 1940s,¹⁶ are no longer like their "wild" body lice counterparts and are quite different from head lice.¹⁴ The laboratory body lice are useful in preliminary screening of potential new products, because a product that is not highly effective in the body lice colony is unlikely to be effective against head lice.

In view of the need for accurate and reliable efficacy data, FEST conducted a study in 1984 to evaluate the comparative efficacy of the most widely used pediculicides on the US market at that time.¹⁴ These studies were conducted at our tropical research station in Panama on head lice from a population with no prior exposure to pediculicides or pesticides. FEST has found the method developed for this study to be an accurate means of simulating clinical use of pediculicides. A detailed description of the methods can be found in the original comparative efficacies study published in 1986.¹⁴

The 1984 study revealed that the synergized pyrethrin products tested (RID, R&C shampoo, A-200 Pyri-nate shampoo and liquid) had an average killing time that

ranged from 10 to 23 minutes. Prioderm alcoholic lotion (0.5% malathion) killed lice within 5 minutes. Kwell shampoo (1% lindane) required approximately 3 hours to kill all lice. The comparative ranking of the tested products in 1984 and 2000 appear in **Table 1**.

PEDICULICIDAL EFFICACY IN 2000

Sixteen years have passed since the first comparative efficacy study was conducted. In that time, Nix has been approved by the FDA, become available by prescription, gone OTC, changed manufacturers (from Burroughs Wellcome, now Glaxo Wellcome Inc [Research Triangle Park, NC] to Warner Lambert Co and now Pfizer Inc), and decreased in effectiveness due to resistance in head lice in the United States^{1,13} and other countries (ie, Israel,³ France,⁵ Czech Republic,⁶ and Britain^{4,7,8}). Since the mid-1980s, there have been formulation changes in the various synergized natural pyrethrin products. Malathion had been removed from the market years ago because of poor sales, but at the time there were no resistant lice in the United States. Ovide was reintroduced to the prescription market last year. Also, during this time, the 1% lindane shampoo brand Kwell, which had been approved by the FDA and available since 1948, was taken off the market for "commercial reasons"¹⁷ and replaced with generic brands.

In light of these changes, FEST was prompted to return to Panama in April 2000 to conduct a second comparative efficacy study in a sensitive population of head lice. This study was conducted on Niadup, an isolated island off Panama's Caribbean coast inhabited by approximately 800 Kuna Indians. Unlike the population at the site of the earlier comparative efficacy study, which was treatment naive, Kuna Indians on Niadup had been previously exposed to permethrin and pyrethrins, but only under the strict supervision of FEST and the Panamanian Ministry of Health.¹⁸ All treatments have been meticulously timed and conducted and no permethrin has been "given out" or "left behind." Lindane and malathion have not been used on this island. Recent studies by Lee et al¹ have shown that the head lice in this population are still sensitive to permethrin.

RESULTS

PEDICULICIDAL ACTIVITY

Although in the 1986 comparative efficacy article¹⁴ pediculicidal activity was reported as mean killing time, in the present study product efficacy was assessed at predetermined intervals throughout 3 hours (**Table 2**). Pediculicidal activity was expressed as the percentage of lice that appeared dead at each interval. This allowed for comparison of the killing time of products during a 3-hour

Table 1. Comparative Ranking of Pediculicidal Activity (Most Effective to Least Effective)

Pediculicide	1984 Rank	2000 Rank
0.5% Malathion	1	1
RID	2	5
A-200	3	4
Lindane	4	6
Nix (undiluted)	NA*	2
Diluted Nix	NA	3

*NA indicates not applicable because the pediculicide was not on the market at the time of study.

Table 2. In Vitro Formulation Pediculicidal Test Results*

Pediculicide	Total No. of Lice Tested	% of Lice That Appeared Dead						
		5 min	10 min	20 min	30 min	1 h	2 h	3 h
Lindane	90	7	9	17	14	15*†	39	61
Undiluted Nix	93	30	53	90	99	100	100	100
Diluted Nix	80	11	44	73	83	94	100	100
Ovide	152	98	100	100	100	100	100	100
RID	119	16	49	73	75‡	50*§	41	53*
A-200	109	43	86	97*¶	99	99	100	100
Wet control	120	0	0	0	0	0	0	0

*To ensure that all lice were exposed to the pediculicides while they were still healthy (<120 minutes after harvesting), it was necessary to run concurrent tests, which sometimes created a need to evaluate different batches of lice simultaneously. Where simultaneous evaluation was not possible, the percentage of lice that appeared dead is based on the total number of lice for which an evaluation was made, rather than the total number of lice tested.

†n = 75 instead of 90.
‡n = 89 instead of 119.
§n = 103 instead of 119.
||n = 75 instead of 119.
¶n = 94 instead of 109.

period. After many years of conducting this type of research, we have found that it is more efficient and equally informative to record observations at specific intervals than to watch the lice continuously.

As in the 1984 study, 0.5% malathion lotion (Prioderm in 1984, Ovide in 2000) was the fastest-acting pediculicide tested. The 1% lindane shampoo (Kwell in 1986, generic in 2000) was the slowest-acting product tested, killing only 39% of lice within 2 hours and 61% within 3 hours. A-200 likewise performed much the same as it did in the earlier comparative efficacy study, killing 97% of lice within 20 minutes.

RID was the only product tested to show a sharp decline in pediculicidal efficacy. RID killed only 49% of lice in 10 minutes, and 47% of lice exposed to RID survived more than 3 hours. Lice exposed to RID demonstrated a "resurrection effect." Although after 30 minutes of testing, 75% of lice appeared to be dead (all movement and peristalsis of the gut had ceased), this percentage decreased to 50% after 1 hour of exposure and to only 41% after 2 hours. At 3 hours, the percentage of lice "killed" (53%) once again appeared to be on the rise.

Diluted Nix (10:1) was consistently slower killing than Nix in its undiluted form. At 30 minutes, 83% of lice exposed to the diluted solution were dead compared with 99% of lice exposed to the undiluted product.

OVICIDAL ACTIVITY

Ovicidal test results are displayed in **Table 3**. Stillbirths represent embryos that continued to develop inside the egg after exposure to the pediculicide but did not successfully hatch. In these cases, the newborn nymph was able to lift the operculum but did not fully emerge from the egg. Ovicidal activity was expressed as the total number of nits that were either stillborn or unhatched (nonviable) divided by the total number of nits tested, multiplied by 100.

Ovicidal activity rates for Ovide (0.5% malathion), RID, and A-200 were similar to the ovicidal activity rates reported for those products in the 1984 study

Table 3. In Vitro Formulation Ovicidal Test Results

Pediculicide	No. of Nits Hatched/No. Tested	Nits Hatched, %	Nonviable Nits, %		Ovicidal Activity, % of Nits Stillborn or Unhatched
			Stillborn	Unhatched	
Lindane	91/120	76	12	12	24
Undiluted Nix	12/110	11	16	73	89
Diluted Nix	22/114	19	9	72	81
Ovide	0/110	0	0	100	100
RID	36/115	31	9	60	69
A-200	20/119	17	4	79	83
Wet control	119/131	91*	0	9	NA†
Dry control	53/57	93*	2	5	NA

*A total of 90% to 95% of control head lice eggs will hatch under normal circumstances.

†NA indicates not applicable.

(**Table 4**). Only 1% lindane shampoo showed a highly significant change in efficacy, with its ovicidal activity rate decreasing from 70% in the 1986 study to only 25% in 2000.

COMMENT

Ovide continues to be the fastest-acting pediculicide, killing 98% of lice tested in less than 5 minutes and 100% of lice tested in less than 10 minutes. Ovide is also the most effective ovicide, showing 100% ovicidal activity after a 10-minute exposure. Ironically, Ovide, the fastest-killing pediculicide and ovicide tested, also has the longest recommended application time. Because the original malathion studies for FDA approval involved an 8- to 12-hour treatment, this is the application time approved by the FDA. At the time of the original approval, a long application time was desired to produce residual activity, which was then thought to be beneficial. We believe, however, that residual activity may lead to development of resistance. Our in vitro findings suggest that a much shorter application time would be effective to kill lice and eggs.

Undiluted Nix (1% permethrin) creme rinse, which was not available when the earlier comparative efficacy study was conducted, killed 99% of the lice tested in less than 30 minutes. When this product was diluted (9 parts Nix to 1 part water, 10:1), 83% of lice exposed to Nix were dead after 30 minutes. We believe that the results obtained with diluted Nix more accurately reflect real-world use of this product. The manufacturer recommends that Nix creme rinse be applied after a shampoo to towel-dried hair. In our experience, towel dried can mean different things to different people, and a 10:1 dilution may be a very conservative product-to-water ratio. The dilution factor may partly explain why we have received so many reports of treatment failures with Nix and may also have helped lead to the development of resistance by delivering a sublethal dose of permethrin. We believe that if the directions for Nix had originally called for application on dry hair, the "dilution factor" would not have arisen. Also, because Nix is a creme rinse to be applied after shampooing hair, parents may use it like regular conditioner on wet hair.

The results for A-200 were similar to those found in the earlier study. These findings suggest that,

Table 4. Comparison of Ovicidal Activity in 1984 and 2000

Pediculicide	Ovicidal Activity, % of Nits Nonviable		Significance	
	1984	2000	χ^2	P
Lindane, Kwell brand (1984) vs generic (2000)	70	24	55.00	<.001
0.5% Malathion, Prioderm lotion (1984) vs Ovide (2000)	95	100	NA*	NA
RID	74	69	0.66	.43
A-200	77	83	1.50	.22

*NA indicates not applicable because a χ^2 analysis could not be done for this product because a cell contained a value of less than 5.

although its formulation has changed since the 1986 study, A-200 continues to be a very effective pediculicide and a moderately effective ovicide in a sensitive population.

RID, which like A-200 contains synergized natural pyrethrins, demonstrated decreased efficacy in comparison to the original study. At 30 minutes, RID appeared to have killed 75% of the lice tested, but this percentage decreased over time as lice experienced a "resurrection effect," recovering after all peristalsis of the gut had ceased. After 3 hours, only 53% of the lice exposed to RID were dead. The ovicidal activity was 69%. The product was reformulated when it changed from a brown glass bottle to a plastic bottle many years ago. We believe that one of the vehicle components that was removed at that time contributed to this product's efficacy.

The 1% lindane shampoo displayed poor pediculicidal and ovicidal activity. After 3 hours of observation, lindane had killed only 61% of the lice tested, and its ovicidal activity (24%) was the lowest of all the products tested. Lindane also performed poorly in comparison with results from the previous study, showing a sharp decline in ovicidal activity. In 1984, 1% lindane shampoo was available as a brand name product (Kwell). The decline in ovicidal activity may be due to changes in the sourcing of ingredients that have occurred over the years or possibly to the development of resistance.

In light of the consistently poor pediculicidal and ovicidal activity of lindane, even in a sensitive popula-

tion, in our opinion, there seems to be no reason for keeping this product on the market in the United States, where lindane resistance has been documented.^{1,19,20} Beginning in 2002, the sale of lindane for head lice and scabies will be prohibited in California under legislation signed into law in September 2000 by Gov Gray Davis. Banning of lindane in California was due to environmental toxic effects and the fact that it was less effective and has more potential toxicity than other available pediculicides and scabicides.²¹ The risk of central nervous system toxicity in children associated with this product far outweighs any potential benefits of its use, especially when more effective products are available.^{22,23}

We performed this study to assess how changes in the formulation of the most widely sold pediculicides in the United States may have affected their efficacy. To do this, we conducted an in vitro comparative efficacy study in a sensitive population.

The in vitro method used in this study (disk method) simulates in vivo pediculicide use. Based on our experiences in more than a decade of conducting in vitro studies, we believe that this method most closely simulates actual pediculicide use in humans.

Recently, we began a similar study in a population in South Florida, where cases of lindane- and permethrin-resistant lice have been documented.^{1,2} Comparing efficacy results obtained in South Florida with those obtained among a sensitive population of lice in Panama should increase our understanding of the extent of the pesticide resistance problem in head lice in the United States.

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