

Comparative In Vitro Pediculicidal Efficacy of Treatments in a Resistant Head Lice Population in the United States

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Objective: To compare the pediculicidal activity of 5 head lice products available in the United States on head lice from south Florida.

Design: In vitro pediculicidal product comparison.

Setting: Lice Source Services, Inc, located in Plantation, Fla, a clinic for the treatment and grooming of individuals with pediculosis capitis.

Participants: Head lice were collected from healthy clients with *Pediculus capitis* that came to Lice Source Services, Inc, to seek their services.

Interventions: Within 2 to 6 hours of capture, lice were placed in continuous direct contact with the pediculicide products and observed at regular intervals. Results were compared with findings of a recent study of a treatment-sensitive population of head lice conducted in Panama.

Main Outcome Measure: Percentage of lice dead at regular observation intervals between 5 minutes and 3 hours of continuous exposure to the pediculicides.

Results: Two prescription products, Ovide lotion (0.5% malathion) and 1% lindane shampoo, were ranked in the same order as in 2 previous studies (first and last, respectively). The order of effectiveness from most to least effective was as follows: Ovide lotion, A-200 shampoo (a natural pyrethrin product synergized with piperonyl butoxide), undiluted Nix (1% permethrin), diluted Nix, RID (a natural pyrethrin product synergized with piperonyl butoxide), and 1% lindane shampoo.

Conclusions: There were statistically significant differences in the efficacy of all the products when compared with the results found in the recent study in Panama, except for Ovide lotion. Of those tested, Ovide was the only pediculicide in the United States that had not become less effective. The difference in efficacy of 1% lindane, Nix, and pyrethrins between the Panama and Florida studies supports the argument that some head lice in the United States have become resistant to these treatments.

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INFESTATION with head lice is on the rise in the United States and worldwide.¹⁻³ The most likely targets are school-aged children between 3 and 11 years²⁻⁴ and their immediate contacts (such as day care providers, family members, and playmates). In the United States, families spend more than \$160 million annually on head lice treatments purchased from pharmacies and supermarkets.⁵ The total cost of products purchased at health food stores or over the Internet is unknown, but these sales seem to be increasing. Each head lice product ranges in price from approximately \$10 to \$28 per person per treatment, and most products require 2 treatments. People who visit Lice Source Services, Inc (LSS), a lice and nit removal clinic located in Plantation, Fla, a suburb of Fort Lauderdale, have treated themselves an average of 5 times

before seeking its services. Unlike prescription pediculicides, insurance plans, Medicare, or Medicaid do not cover over-the-counter (OTC) products. The cost of purchasing head lice treatments could amount to much more when consumers find that most currently marketed products do not cure them even when used as directed.

In 1986, when Nix (1% permethrin) (Warner Lambert Co, Morris Plains, NJ [now manufactured by Pfizer Inc, New York, NY]) crème rinse was approved by the Food and Drug Administration (FDA) and came on the market as a prescription product, lindane and pyrethrin products had an average ovicidal activity of only 70%.^{6,7} Thus, a second treatment was recommended 1 week later to kill any nymphs that hatched from eggs, surviving treatment. Also, nit removal with a fine-

METHODS

The lice used in the present study were collected from over 25 children and adults seen at LSS. The study was conducted between July and November 2000. Individuals using the services of LSS are usually residents of Palm Beach, Broward, or Miami-Dade counties. These clients are often referred to LSS by school nurses, pediatricians, child care centers, public health departments, and foster care agencies.

From our experience in south Florida, we have found that resistant and sensitive lice can live on the same person's head at the same time.^{3,17} Because some individuals had self-treated their infestation and some had not, lice collected from LSS clients may include both treatment resistant and treatment sensitive. To ensure that this did not skew the results, we pooled all lice collected on study days and tested all products on that day's pool of lice. To provide sufficient numbers of lice, we conducted numerous runs on different days using lice from several infested individuals. The number of dead lice was recorded at each predetermined interval.

Investigators from LSS and FEST conducted the assessments under the supervision of the principal investigator (T.L.M.). Four members of our team who conducted *Update 2000*,¹ also did some of the experiments in the present study and worked closely with the LSS researchers to ensure that the methods were conducted exactly as in *Update 2000*.

Pediculicidal activity was tested by exposing lice continually to cotton disks impregnated with the pediculicide products. The procedure for preparing and impregnating the cotton disks and the rationale for using this methodology has been described extensively.^{1,6,18} The same bottles tested in *Update 2000*¹ in Panama were returned to south Florida and used to impregnate the cloth disks tested in the present study, and new bottles recently purchased locally were also tested. As in the Panama studies,^{1,6} lot number, expiration date, place of purchase, and date opened were recorded for each bottle used in each experiment to ensure that differences in results could not be attributed to poor stability, oxidation, storage problems, or "bad" batches.

Because Nix is to be applied to clean "towel-dried" hair, there is a dilution factor to account for, although the dilution will vary by user. We took a conservative dilution, consisting of 9 parts Nix to 1 part water (90% Nix solution), as in *Update 2000*,¹ and for the US studies, we also added a more "realistic" dilution of 3 parts Nix to 1 part water (75% Nix solution).

Depending on the amount of lice collected from the clients of the clinic on each day of the study, usually between 10 and 15 lice were placed on the impregnated disk and observed at regular intervals for 3 hours. The lab at LSS was maintained at the same temperature and humidity range as our field station in Panama, even though the screening and treatment rooms were air conditioned.

toothed comb or "fingernail" technique was necessary to prevent reinfestation. The introduction of Nix revolutionized the management of pediculosis capitis. Although Nix was only 70% ovicidal,⁷ the residual perme-

thrin bound to the hair and scalp was sufficient to kill lice that hatched from the eggs, providing almost 100% efficacy with a single 10-minute application.⁷ Many schools allowed children back in class without nit removal if they brought in the used bottle of Nix. However, because of the currently poor pediculicidal and ovicidal activity of Nix and other treatments, even more time and money is now spent on special nit combs, grooming services, environmental sprays, laundry, dry cleaning, vacuuming, bagging stuffed animals, and replacing earphones, helmets, and other head gear suspected of being sources of reinfestation. When adding the time spent and the salary lost from work, many parents have estimated the cost of their "never-ending" head lice experience to be in the thousands of dollars.

In the last 5 years, when a treatment failure is reported the blame seems to fall on the consumer, suggesting that parents did not adhere to the instructions, remove all nits, or adequately clean the environment. However, in the original Nix studies submitted for FDA approval,^{8,9} there was no nit removal, combs, or environmental treatment. Our study⁷ was conducted in Panama in populations with no electricity or running water, making it impossible to wash bedding or vacuum homes. In the double-blind, placebo-controlled clinical trial, children slept 4 to a hammock, yet participants who received active crème rinse remained free of lice 2 weeks after a single treatment.⁷

There are several reasons for the increasing frequency of treatment failures reported worldwide.² In the United States, our 2 primary reasons of concern are subtle changes in product formulation over the years¹ and the increasing development of pesticide resistance.^{4,10-16} To address these questions, investigators from the Field Epidemiology Survey Team (FEST) at the University of Miami, Miami, Fla, conducted an in vitro baseline study in Panama (which we refer to as *Update 2000*¹ to prevent confusion with the 1986 study⁶), where head lice are still sensitive to prescription and OTC products. The primary goal of this study was to assess the extent to which possible formulation, ingredient sources, or manufacturing changes over the last 16 years may have affected product efficacy.¹

In *Update 2000*,¹ we compared the killing time of 5 widely sold products: 2 prescription products, Ovide lotion (0.5% malathion) (Medicis Pharmaceutical Corp, Phoenix, Ariz), which in 1986 was formerly available as Prioderm lotion (Purdue Frederick Company, Norwalk, Conn), and 1% lindane shampoo, which until 1995 was formerly available under the brand name Kwell shampoo (Reed & Carnrick, Jersey City, NJ); 2 OTC synergized natural pyrethrins, RID (Pfizer, Inc, New York, NY) and A-200 shampoo (Hogil Pharmaceutical Corp, Purchase, NY); and a synthetic pyrethroid, Nix. Both pediculicidal and ovicidal activity were evaluated¹ and compared with the data of the 1986 study.⁶

The study we report here is a replication of *Update 2000*¹ in Panama, conducted in a population of head lice in south Florida, where permethrin- and lindane-resistant head lice have been reported.^{3,15} Ovicidal activity was not evaluated in Florida because previous treatments and combing could damage nits and result in unreliable information. Our main goal was to compare

Table 1. Pediculicidal Activity, South Florida

Pediculicide Tested	Lice Tested, No.	Lice That Appeared Dead, %						
		5 min	10 min	20 min	30 min	1 h	2 h	3 h
1% Lindane shampoo	118	0	0	2	3	8	16	17
Undiluted Nix	137	1	5	10	17	49	66	74
Diluted Nix (90% Nix)	114	2	4	8	11	18	32	46
Diluted Nix (75% Nix)	90	2	3	9	14	22	36	42
Ovide lotion	163	30	88	100	100	100	100	100
RID	203	1	7	8	11	21	25	34
A-200 shampoo	136	3	23	60	67	82	96	100
Water control	185	0	0	0	0	0	0	0

Table 2. Florida and Panama¹ Comparative Efficacy Results*

Pediculicide Tested	Dead at 20 min		Dead at 1 h		Dead at 3 h	
	Panama	South Florida	Panama	South Florida	Panama	South Florida
Ovide lotion	100†	100†	100†	100†	100†	100†
A-200 shampoo	97	60‡	99	82‡	100	100†
Undiluted Nix	90	10‡	100	49‡	100	74‡
Diluted Nix (90%)	73	8‡	94	18‡	100	46‡
RID	73	8‡	50§	21‡	53	34‡
1% Lindane shampoo	17	2‡	15	8	61	17‡

*Data are percentage of lice.

†All lice were dead at this time interval in both sites; χ^2 statistic cannot be calculated.

‡ $P < .001$.

§Resurrection effect (knocked down and appeared dead, but revived).

the results of these 2 identical studies to assess the degree of resistance in south Florida. It is evident from *Update 2000*¹ that some currently available pediculicides differ from the formulations we tested 16 years ago.⁶ We attribute the differences in results from the lice tested in Florida and those of the recent study in Panama to resistance because we have already controlled for formulation changes.

RESULTS

Ovide (0.5% malathion) was the fastest and most effective pediculicide tested, killing 88% of lice at 10 minutes and 100% at 20 minutes. The second best product, the synergized natural pyrethrin A-200, killed 60% of lice at 20 minutes, 82% at 1 hour, and 100% at 3 hours. Although RID has the same active ingredients as A-200 (pyrethrum extract equivalent to 0.33% plus 4% piperonyl butoxide), it had a slow killing time with only 8% of lice dead in 20 minutes and only 34% dead after 3 hours of continuous exposure (**Table 1**). We attribute this large difference of efficacy between the synergized natural pyrethrin products A-200 and RID to formulation and vehicle differences.

Nix was tested at 3 concentrations: undiluted and 2 dilutions (90% Nix [9 parts Nix to 1 part water] and 75% Nix [3 parts Nix to 1 part water]). As expected, undiluted Nix was the most effective of the 3, killing 84% of the lice at 3 hours. The difference between 90% and 75% Nix dilutions was not significant; however, there was a highly significant difference between undiluted and diluted Nix at the 1-hour and 3-hour intervals ($P < .001$ for

both dilutions at 1 and 3 hours). After 10 minutes, which is the indicated application time, only 3% to 5% of lice exposed to Nix were dead, regardless of whether or not it was diluted, and only 8% to 10% at 20 minutes. Even after 3 hours of continuous exposure to undiluted Nix, over 25% of lice were still alive. With diluted Nix used to simulate towel-dried hair, more than half of the lice were still alive at 3 hours (Table 1).

The slowest and least effective of all products tested was once again 1% lindane shampoo, killing only 2% of lice at 20 minutes and 8% at 1 hour; after 3 hours of continuous exposure only 17% of the lice tested were dead (Table 1). These results, which confirm the findings in the previous 2 studies,^{1,6} are of concern, considering that 1% lindane shampoo has an indicated application time of less than 10 minutes. Increasing the treatment time, which we have seen many parents do in an effort to increase efficacy, could result in increased percutaneous absorption and toxic effects on the central nervous system.^{3,19}

Ovide lotion was equally effective in the present study as in *Update 2000*,¹ killing 100% of lice within 20 minutes at both sites. The only other product tested that killed all lice in both sites at 3 hours was A-200 shampoo. When comparing the efficacy of all other products in *Update 2000*¹ with the present study, we found that all but 1% lindane shampoo (exposed for 1 hour) yielded statistically significant differences (**Table 2**).

COMMENT

It is important for treatments to kill, or at least immobilize, lice within the application time because parents, chil-

dren, and infested individuals experience anxiety and psychological stress when they see lice crawling and walking after treatment. All of the treatments tested in the present study, with the exception of Ovide, are labeled to be rinsed off within 10 minutes. The present study demonstrates that many of the pediculicides sold in the United States do not meet their advertising claims that they “kill lice and eggs on contact.” Slow-killing pesticides, poor formulations, and residual pediculicide concentrations on the hair that expose lice to sublethal doses are more likely to encourage the development of resistance.^{2,3,20}

In the present study conducted in south Florida, we found that Ovide was the fastest killing pediculicide and the only one of the 5 products tested that had not become less effective compared with the findings from the Panama studies.^{1,6} There has been confusion among consumers and health care professionals regarding the safety of malathion, the active ingredient in Ovide. Negative publicity and statements made concerning “toxicity” rarely distinguish between pharmaceutical and agricultural grade malathion. Products for nonhuman use may contain impurities that have been associated with reported “toxicity” or adverse experiences. The pharmaceutical grade malathion used in Ovide has a high level of purity. Its safety and efficacy have been well documented.^{2,21} Although Ovide (formerly Prioderm) was approved by the FDA 2 decades ago, it has spent a relatively short time on the US market, while in many countries it has remained one of the treatments of choice for decades. Malathion lotion has been on the market twice in the United States and withdrawn in 1995 by the manufacturer (prior to the development of resistant lice) because of commercial failure.^{2,3} The long application time, odor, and flammability of this prescription alcoholic lotion was not appealing to consumers who could successfully cure an infestation with a 10-minute application of an OTC product. Unlike permethrin that originally provided “residual activity” on the hair and scalp within 10 minutes, malathion takes several hours to achieve this action. The overnight application time was originally chosen by the Purdue Frederick Company for clinical trials and FDA submission because 20 years ago residual activity on the hair and scalp was considered to be a “good thing.” However, it is now recognized that residual activity and prophylactic use only add to the development of resistant lice.^{3,20} The FDA approval confirms that Ovide lotion is the only product we tested with the toxicity profile and data to support an 8- to 12-hour application, even though our *in vitro* results show that a much shorter exposure time is highly effective. Resistance to malathion has developed in France and the United Kingdom^{2,16,22}; however, the 0.5% malathion products available in these countries are not the same as Ovide lotion. The Ovide vehicle contains components such as dipentene, terpineol, and 78% isopropanol, which increase the pediculicidal and ovicidal activity of the malathion. Further studies to better define the enhancing activity of the vehicle are in progress.

The other prescription product, 1% lindane shampoo, was the least effective pediculicide tested, performing poorly in Panama in a population with treatment-sensitive lice infestation and even worse in south Florida. Lindane resistance has been a worldwide problem for decades.^{3,17} We encountered lindane treatment failures in

Florida 16 years ago and find it to be increasingly ineffective at curing infestations. Because of the increasing treatment failures of OTC products over the past 6 years and the trend toward prescription products under insurance and other health care plans, lindane is still widely used in the United States. Lindane, like DDT (chlorophenothane), is an organochlorine insecticide, and recently California has banned the sale of lindane for head lice and scabies because of environmental problems.²³ In view of extremely poor pediculicidal and ovicidal activity, potential toxic effects on the central nervous system, resistance, and environmental contamination, we see no reason for continued use of lindane in the United States, and as stated in *Update 2000*,¹ we strongly recommend its removal from the market.

There were statistically significant differences in the efficacy of pyrethrin and permethrin products when compared with the results found in *Update 2000*.¹ One interesting observation between the 2 studies was that the 2 synergized natural pyrethrin products, RID and A-200 shampoo, which contain the same active ingredients and concentrations, performed so differently. The RID used in the 1986 study⁶ was in a brown glass bottle and contained benzyl alcohol as an inactive ingredient, probably due to its properties as a preservative. However, 20 years ago we found this particular alcohol alone to have quick knockdown, but not kill. Nix was introduced on the market in 1986 in a plastic bottle similar to the packaging of other *crème* rinses, not in a glass bottle like a pediculicide. The OTC products soon picked up this safety component for use in the bath or shower with children, and they soon came out in plastic bottles. However, when the container for RID was changed to a plastic bottle sometime after 1986, it no longer contained benzyl alcohol and was inferior to the prior product. Even at small concentrations, benzyl alcohol, which A-200 shampoo and many generic products still contain in their vehicle, seems to have some synergistic activity, perhaps slowing down the development of resistance as piperonyl butoxide (PBO) is now known to do.^{3,15} Over a half a century ago, PBO was added as a synergist to the relatively expensive pyrethrum extract, so less extract was needed to produce the same insecticidal activity.²⁴ Although originally PBO was added purely for financial reasons, it is now known to slow down the development of resistance at the mixed function oxidase (MFO) pathway.³

The difference in efficacy between undiluted and diluted Nix is important. Because Nix is indicated for use on “damp” hair, there is a dilution effect when used *in vivo*. This effect will vary from user to user because damp or towel dried can mean different things to different people and certain hair types retain more water. The dilution factor could be in part responsible for the development of permethrin resistance because some lice may have been exposed to sublethal doses of this insecticide. The first Nix clinical trial published found that a single 10-minute treatment of Nix was 100% effective at 1 week and 97% at 2 weeks due to the residual activity of permethrin on the hair shaft.⁷ In this first Nix study,⁷ the product was applied to slightly damp hair that had recently been shampooed with Prell concentrate (Proctor & Gamble, Cincinnati, Ohio), an anionic stripping sham-

poo, to prepare the hair shaft for the bonding of the permethrin. In 1985, shampoos with built-in conditioners were rare. The addition of silicon or conditioning agents to shampoos may have prevented the permethrin from firmly bonding to the hair shaft and not providing sufficient residual activity to kill hatching nymphs.

The difference in the efficacy of Nix between the Panama and Florida studies adds to the growing body of evidence that permethrin resistance is reaching chronic levels in the United States. Through a collaborative effort with the Entomology Department at the University of Massachusetts, Amherst, a considerable amount of resistance data has been generated using knockdown and mortality assays on lice from LSS.¹⁵ Furthermore, through molecular cloning and sequencing from LSS lice, 2 point mutations have been identified, therefore confirming true permethrin resistance in head lice from south Florida.¹⁵

From our experience and that of other investigators, combing alone is not sufficient treatment for the treatment of pediculosis capitis and is also time-consuming, frustrating, and painful.^{2,3,20} Since combing alone cannot guarantee cure, treatment must also be used. The successful off-label use of ivermectin in the United States and worldwide for ectoparasites suggests that this be clinically evaluated for head lice.^{2,17,25} And in the United Kingdom, Roberts et al²² found that a malathion lotion similar to that tested here was about 3 times more effective than extensive wet combing or "bug busting" even in a malathion-resistant population.

Unfortunately, with the increase of resistance and the decrease of efficacy of certain commercially available pediculicides, we are finding an increasing number of severe infestations with multiple pyodermas and excoriations of the scalp. Thus, there is an urgent need for lice treatments that are safe, effective, rapid killing, ovicidal, and easy to use; that do not require nit removal for success; and that also incorporate a resistance prevention strategy.

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