

A Three-Phase, Four-Arm Proof of Claims Study to confirm that lice killed by Omniche's Mayday Daily Lice Prevention Shampoo, Mayday Daily Lice Fighting Conditioner, Mayday Lice Treatment Hair Solution, Mayday Home Defense Bed Bug & Lice Furniture and Bedding Spray, and Mayday Daily Lice Fighting Detangler Spray contain the mutation necessary to signify they have the resistant gene needed to qualify them as so-called "Super Lice." Additional proof-of-claims testing on Omniche's Mayday Home Defense Bed Bug & Lice Furniture and Bedding Spray and Mayday Daily Lice Fighting Detangler Spray in effectively preventing head lice versus the nonuse of such product.

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LSRN Research

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1.0 Protocol

A Proof of Claims five-arm study to compare the efficacy of Omniche Consumer Products Co. Mayday Daily Lice Prevention Shampoo, Mayday Daily Lice Fighting Conditioner, Mayday Lice Treatment Hair Solution, Mayday Home Defense Bed Bug & Lice Furniture and Bedding Spray, and Mayday Daily Lice Fighting Detangler Spray in effectively treating and/or repelling head lice that possess the kdr mutation versus the nonuse of a lice treatment agent, defense, or home spray.

Investigational Product

- Mayday Daily Lice Prevention Shampoo
- Mayday Daily Lice Fighting Conditioner
- Mayday Lice Treatment Solution
- Mayday Home Defense Bed Bug & Lice Furniture and Bedding Spray
- Mayday Daily Lice Fighting Detangler Spray

Objective

This is a two-phase, five-arm non-GLP in vitro study. The primary purpose of this two-phase study is to determine if Mayday lice prevention, treatment, and home products supplied by Omniche are in fact killing and preventing head lice that possess the *kdr* mutation, thus signifying resistance among the population of head lice also known as super lice. The secondary objective is to confirm that Omniche's new Mayday products — Mayday Home Defense Bed Bug & Lice Furniture and Bedding Spray and Mayday Daily Lice Fighting Detangler Spray — as currently formulated are in fact sufficient to satisfy desired goals both in prevention qualities as well as killing off populations of lice as it exists. Finally, although claims regarding the shampoo product's ability to kill nits were substantiated in initial testing, Omniche hopes to show that three minutes of submersion with the Mayday Daily Lice Prevention Shampoo is sufficient to compromise late-stage viable eggs resulting in their inability to hatch.

Study Design — Phase 1: *kdr* Mutation Confirmation

The first phase consists of five arms, each designed to individually test one of Omniche's five Mayday products. The sole purpose is to determine whether the lice tested and killed by these products carry the *kdr* mutation, thereby confirming resistance to pesticidal treatments commonly known as super lice. Each individual product is exposed to a single household population of no fewer than 20 and no more than 50 healthy adult and/or 3rd-stage lice that have not been pretreated by any type of lice treatment product within 14 days prior to being separated from their human host. Only lice that have previously been donated by a healthy host for the sole purpose of furthering research, and would otherwise be disposed of, are used for this study. To ensure consistency, only lice from the same household and therefore the same population may be commingled. Only one vial of lice containing 20 to 50 adult and/or 3rd-stage is utilized per test product for DNA sampling.

To simulate original testing, in each individual test run, once donated lice have been identified as acceptable samples, they are placed in a single petri dish where they are exposed to the current testing product for 3 minutes and rinsed with room-temperature distilled bottled water for 1 minute. The lice are then placed in a petri dish lined with filter paper to absorb excess moisture before being placed in an incubator with a target temperature of 30 °C and an acceptable range between 27 °C and 34 °C (80.6 °F – 93.2 °F) and 70% relative humidity for no longer than 7 hours. Since the purpose of this phase is to identify *kdr* mutations, individual timeline reads are not required. For the purpose of preserving lice after the 7-hour mark, specimens are transferred from their petri dishes into standard test tubes containing 75% ethanol. Each vial is clearly labeled to indicate the specific product exposure associated with that sample. The test tubes are then stored in a –20 °C freezer until samples from all five Mayday products have been collected. Once ready, the samples are wrapped in bubble wrap and shipped to the Department of Tropical Medicine and Parasitology at the College of Medicine, Seoul National University, in Seoul, Korea.

All lice are maintained at –20 °C until genomic DNA (gDNA) extraction and sequencing can be performed. Once the genotypes and the intron sequences are confirmed, the PCR products with or without mutations will be mixed to generate the standard DNA mixture templates in following molar ratios: 0:10, 1:9, 3:7, 5:5, 7:3, 9:1 and 10:0 (resistant allele : susceptible allele). Standard DNA template

mixtures will be sequenced using two sets of sequencing primers for the sense- and antisense-directional sequencing, respectively. The nucleotide signal intensities of the resistant and susceptible alleles will be determined and signal ratios calculated using Equation 1.

Equation 1 — Signal Ratio

$$\text{Signal ratio} = \frac{\text{Resistant nucleotide signal}}{\text{Resistant nucleotide signal} + \text{Susceptible nucleotide signal}}$$

The signal ratios of template DNA mixtures will be normalized by multiplying them with the normalization factor (signal ratio of the heterozygous DNA template / signal ratio of the 5:5 standard DNA template). A series of normalized signal ratios will be plotted against the corresponding kdr allele frequencies and standard regression equations, together with lower and upper prediction equations, will be generated for the estimation of kdr allele frequencies of unknown samples.

Study Design — Phase 2: Detangler Spray and Furniture/Bedding Spray Efficacy

The second phase of this study consists of three individual arms. Two arms test Omniche's two new products — Mayday Home Defense Bed Bug & Lice Furniture and Bedding Spray and Mayday Daily Lice Fighting Detangler Spray — to determine if using said products will have a negative impact on the head lice. A total of 180 healthy active adult and/or third-stage lice are utilized during this phase of the study to evaluate the efficacy of each specific test product. Each arm of the study involves head lice donated by their human host no more than 30 minutes prior to the actual test run to ensure the healthiest lice specimens possible.

For the Mayday Daily Lice Fighting Detangler Spray, 9 test runs containing a total of 10 healthy adult and/or 3rd-stage head lice are used, resulting in a total of 90 lice for this arm of the study. Two tufts of hair that have not been pretreated or chemically altered and are from the same donor are placed equidistant to each other in a standard petri dish for testing purposes. One tuft of hair is dipped into the Mayday Daily Lice Fighting Detangler Spray and blotted dry with a paper towel prior to returning it to side A of the petri dish. The remaining tuft of hair is dipped in generic distilled water prior to being patted dry and returned to the opposite side B of the dish. 10 live healthy adult and/or 3rd-stage lice that have not been pretreated are placed on the midline of the dish and the lice reactions to the hair tufts are monitored and documented at timelines of 0, 0.5, 1, 3, 5, and 7 hours per test run.

Testing of Mayday Home Defense Bed Bug & Lice Furniture and Bedding Spray consists of 9 test runs, each with a total of 10 healthy, active adult and/or 3rd-stage head lice, resulting in a total of 90 lice. Since this product is intended to work in the environment, 2 × 2 inch squares of cheesecloth are used in each individual test run. One square is dipped directly into the Mayday Home Defense Bed Bug & Lice Furniture and Bedding Spray and rolled into a spiral form prior to being placed on side A of the petri dish, while the remaining piece of cheesecloth is dipped into room-temperature generic distilled water and rolled into a spiral form prior to returning the cloth to the opposite side B of the petri dish.

Considering concerns regarding possible contact within the environment, the goal here is to simulate a household environment; therefore 5 healthy active adult and/or 3rd-stage lice are placed directly onto each individual pretreated square and results are monitored and documented at timelines of 0, 0.5, 1, 3, 5, and 7 hours per test run. To evaluate the longevity of the product's effectiveness, two identical additional test runs are performed once a week for 4 weeks post-treatment using identical and stored test surfaces. This will support the claim that Mayday Bed Bug & Lice Home Spray remains effective for up to 4 weeks after application.

The primary objective is to determine whether the spray can effectively kill lice that have become dislodged from a human host and are present in the surrounding environment — and if so, to what extent and within what timeframes.

Study Design — Phase 3: Egg Submersion Testing

The final arm of this study is to complete 7 additional egg test runs to determine if 3 minutes of product submersion is sufficient to effectively help kill eggs during the shampoo process. Initial test runs already proved that complete exposure was effective in compromising the hatching ability of the eggs at 24 hours. Omniche's desire is to determine how strong the results are when considering the product would be rinsed off after a 3-minute period. Therefore 7 individual runs each consist of 10 late-stage viable eggs, verified by a 10× microscope; only eggs from one distinct willing donor within each separate test run are utilized. Only eggs that have not been pretreated or chemically treated within 14 days prior to testing are used.

Ten viable healthy late-stage eggs from three runs are submersed in the Mayday Daily Lice Prevention Shampoo for a period of three minutes. They are then rinsed free of the shampoo product by using generic room-temperature distilled bottled water in a spray applicator for a period of one minute. Eggs are then placed in a petri dish lined with filter paper to absorb excess moisture and then placed in an incubator with a target temperature of 30 °C (acceptable range 27–34 °C / 80.6–93.2 °F) and 70% relative humidity. Results are monitored and recorded at timelines of 0, 0.5, 1, 3, 5, and 7 hours per test run.

Three additional runs of 10 viable, healthy late-stage eggs as verified by a 10× microscope are submersed in the same generic shampoo product that was utilized in the initial testing. Once total submersion for a period of three minutes has been completed, eggs are rinsed free by a spray applicator for a period of 1 minute. Eggs are then placed in a petri dish lined with filter paper to absorb excess moisture and then placed in an incubator with the same temperature and humidity parameters. Results are monitored and recorded at the same timelines.

One final test run utilizing 10 healthy viable late-stage eggs as verified by a 10× microscope is fully submersed in distilled water for a period of 3 minutes. Since only water is used, a final rinse is not required. Upon completion of the 3-minute submersion, eggs are placed in a petri dish lined with filter paper to drain for 1 minute and then placed in an incubator with the same temperature and humidity parameters. The purpose of the water run is to simulate outcomes in the absence of any product usage. All

petri dishes are stored between timelines in a temperature-controlled incubator to ensure as close of an environment to a human head as possible.

Study Design — Reformulation Verification Runs

Three additional study runs are conducted to evaluate recent formulation changes in the Mayday Daily Lice Prevention Shampoo and Mayday Lice Treatment Hair Solution, as well as the existing Mayday Daily Lice Fighting Conditioner. The shampoo formulation has been updated, and the treatment hair solution now contains added peppermint oil; these runs are intended to confirm that the efficacy observed in previous studies is maintained following these changes.

For the shampoo and treatment products, three runs are performed with five lice per run. Each set of lice is submerged in the test product for three minutes, followed by a one-minute rinse. Timepoints for assessing efficacy are 2 minutes, 5 minutes, 15 minutes, 30 minutes, 60 minutes, 5 hours, and 7 hours. For the conditioner, three runs are also conducted with five lice per run. Two hair tufts are dipped in Mayday Daily Lice Fighting Conditioner, blotted dry with a paper towel prior to being placed on side A of the petri dish. The remaining tuft of hair is dipped in generic distilled water prior to being patted dry and placed on side B of the dish. 5 live healthy adult and/or 3rd-stage lice that have not been pretreated are placed on the midline of the dish and the lice reactions to the hair tufts are monitored and documented at timelines of 0, 0.5, 1, 3, 5, and 7 hours per test run.

Claim

Omniche Consumer Products Co., Mayday Daily Lice Prevention Shampoo, Mayday Daily Lice Fighting Conditioner, Mayday Lice Treatment Hair Solution, Mayday Home Defense Bed Bug & Lice Furniture and Bedding Spray, and Mayday Daily Lice Fighting Detangler Spray in effectively treating and/or repelling head lice that possess the kdr mutation versus the nonuse of a lice treatment agent, repellent, or home spray.

2.0 Introduction

Pediculosis, the infestation of humans by lice, has been well documented throughout the history of mankind. Humans are hosts to three types of lice: *Pediculus capitis*, *Pediculus humanus*, and *Pthirus pubis*. The worldwide prevalence of head lice is documented in reviews of current scientific literature, and its incidence is widespread, crossing all socio-economic strata.

Head lice are not a reportable disease; thus an accurate accounting of its prevalence is not possible. Nonetheless, head lice product sales and school-related expenses were estimated in excess of \$350 million yearly in 2003. Additional cost factors include cleaning, doctor visits, OTC and prescription medications, elevating the cost at an estimated excess of \$1 billion annually. Loss of workdays also

contributed to the economic burden of battling head lice, driving overall expenses up to an estimated \$4–8 billion annually.

Although there are a variety of treatment options available — including prescription, over-the-counter, and mechanical devices for head lice infestations — concerns over product safety, resistance, and treatment failures left parents looking for more effective alternatives when treating this new breed of super lice. Ongoing complaints of product resistance prompted researchers to test this theory. In 2008, Dr. Kyong Sup Yoon, a researcher with the University of Massachusetts, was able to add strength to these theories when he published the first of a series of studies proving head lice were in fact showing resistance to the commonly used OTC products. This hypothesis was further confirmed when Yoon's final study showed that head lice from 42 of the 48 states collected proved to be 100% resistant to the commonly used permethrin-based products such as NIX, confirming an urgent need to find more effective treatment options.

The study sponsor, Omniche Consumer Products Co., has developed a safe, nontoxic, proprietary line of lice products in hopes of addressing this ongoing problem. The testing of these products and their effects on head lice was performed by LSRN, a subsidiary of Shepherd Enterprises. Shepherd was part of the original team to test Yoon's theory and is one of the authors in the final resistance testing. Shepherd Enterprises and their founder Katie Shepherd have been studying head lice and how it impacts families dealing with it for the past 30 years.

3.0 Objectives

The primary purpose of this three-phase, four-arm, non-GLP in vitro study was to determine if Omniche's Mayday lice prevention and treatment products did in fact have a negative effect on lice that were proven to contain the resistance gene that qualified Mayday products as killing lice that are otherwise termed as "super lice."

Only head lice that had been donated by willing clients of Dandy Strands Head Lice Removal Service LLC in Enid, OK, for the sole purpose of furthering head lice research were used. All testing involved lice that had not been separated from their human host for more than 30 minutes, nor were they pretreated with any lice product, and were otherwise considered healthy, active adult and/or 3rd-stage. Each of the Mayday prevention and treatment products were tested separately and no more than one test product per household was permitted. Only lice collected within a single-family unit/household could be comingled.

To ensure enough lice to accomplish DNA testing, a collection of 20 to 30 healthy, active adult and/or 3rd-stage lice that had not been pretreated were used in each individual test run. Once lice were collected and transferred into a prelabeled vial containing 75% ethanol and identified based on product type, they were placed in a –20 °C freezer and stored pending shipping of all treated samples to the Department of Tropical Medicine and Parasitology at the College of Medicine at Seoul National University pending DNA testing.

This three-phase, four-arm study utilized a total of 36 repeatability runs. Lice population varied throughout individual phases of this limited bench study. A minimum of 310 freshly harvested, healthy, mature adult and/or 3rd-stage lice were included in this round of testing. Additionally, 70 late-stage viable eggs were also tested to ensure that the Mayday Daily Lice Prevention Shampoo did in fact compromise the eggs' ability to hatch when submersed in the product for a total of 3 minutes followed by a thorough rinse for one minute, prior to monitoring the ability or inability of the eggs to hatch over a 24-hour period, with final observation to confirm results at the 72-hour mark.

The second phase of this round of testing was to ensure that Omniche's Mayday Daily Lice Fighting Detangler Spray as well as Mayday Home Defense Bed Bug & Lice Furniture and Bedding Spray, as currently formulated, did in fact have a negative impact on the lice that were exposed to them.

Since there were changes in formulation on previously tested Mayday products, additional runs were completed to ensure the continued efficacy of each product. An additional three runs involving a total of 15 lice were completed utilizing Mayday Daily Lice Prevention Shampoo. Likewise, Mayday Lice Treatment Hair Solution saw an addition of peppermint oil to its original formula; therefore an additional three runs involving a total of 15 healthy, active adult and/or 3rd-stage head lice were completed to ensure the same efficacy level that was observed in the first round of testing. All experiments were conducted "in vitro."

4.0 Study Procedures

Dandy Strands, a head lice awareness and control center located in Enid, OK, provided freshly harvested, healthy adult and/or 3rd-stage head lice to LSRN for the sole purpose of furthering head lice research. All phases of the testing process were done at LSRN's Enid, OK location to ensure the healthiest head lice specimens for the study.

Collection Procedure

Head lice samples were collected from otherwise discarded head lice as a result of Dandy Strands' head lice removal services. Only lice removed from hair that had not been pretreated within the 7 days preceding removal were used. To prevent potential damage to the louse, all head lice were obtained as a result of combing dry hair with a rat-tail comb. The comb and hair were slightly dampened with distilled water prior to dry combing to avoid buildup of static electricity. Lice specimens were only collected from clients that had previously agreed to allow their discarded head lice to be utilized for education and research purposes. Clients and/or their collected head lice were not identified in any manner other than keeping all the head lice harvested from each individual in their own separate petri dish. Each petri dish was then noted with date and time of harvest. These specimens were stored in a temperature and humidity-controlled incubator pending their examination and use. Per protocol, all testing began within 30 minutes of the head lice separation from their human host.

Test Scope

Per sponsor's request, this was a three-phase, four-arm, proof-of-claims in vitro study. A minimum of 310 active and healthy adult and/or 3rd-stage head lice were used to complete a total of 36 individual test runs.

Since DNA testing occurred at the Department of Tropical Medicine and Parasitology at the College of Medicine at Seoul National University, the primary and initial focus was to ensure that 20 to 30 lice from the same family unit were collected and tested individually against each of the five Mayday products to confirm product efficacy prior to shipping samples to Korea for DNA testing. No further testing or data collection was needed from LSRN during this phase of the study. All products were tested individually, providing efficacy results at that level while the focus of this arm was reserved for DNA documentation regarding resistance mutation. Final report from Dr. Ju Hyeon Kim documenting results of the "kdr mutation" can be found later in this report.

Phase 2 Testing — Detangler Spray and Furniture/Bedding Spray

The second phase of testing involved new products to the Mayday line. Once usable lice samples were identified, one hair tuft was pretreated with Mayday Daily Lice Fighting Detangler Spray and the other with distilled bottled water. In an effort to create a similar environment between the hair tufts during the testing phase, and to ensure that the test product was responsible for the lice reaction and not the premoistened hair, the second hair tuft was pretreated with distilled water only, otherwise similar to non-use of a prevention product. At the 0-minute timepoint, 10 lice were placed on the midline and allowed to wander freely between the two hair tufts. The position of the lice and their reactions were recorded over a 7-hour period with individual readings at 0, 30 minutes, 1 hour, 3 hours, 5 hours, and 7 hours.

Likewise, nine repeats involving a total of 10 lice in each individual test run were conducted using Mayday Home Defense Bed Bug & Lice Furniture and Bedding Spray. Each petri dish included two squares of cheesecloth to help simulate an actual use environment similar to a preventative household spray. The mortality rate and stress levels were monitored and documented over a 7-hour period with individual readings at 0, 30 minutes, 3 hours, 5 hours, and 7 hours.

All test runs were kept in an incubator with target temperature of 29 °C (84.2 °F) with an acceptable range of 27–34 °C (80.6–93.2 °F) and 70% relative humidity between observation periods to ensure that the head lice were kept at their optimum environmental conditions to prolong the specimens' mobility.

5.0 Investigational Product

5.1 Mayday Daily Lice Prevention Shampoo

INCI List / Ingredients: Water, Cocamidopropyl Betaine, Sodium C14-16 Olefin Sulfonate, Sodium Methyl 2-Sulfolaurate (and) Disodium 2-Sulfolaurate (and) Sodium Lauryl Sulfoacetate, Glycol Stearate,

Fragrance, Glycerin, Sodium Chloride, *Mentha Piperita* (Peppermint) Oil, *Eucalyptus Globulus* Leaf Oil, *Melaleuca Alternifolia* (Tea Tree) Leaf Oil, *Azadirachta Indica* (Neem) Seed Oil, *Simmondsia Chinensis* (Jojoba) Seed Oil, Propylene Glycol, *Prunus Amygdalus Dulcis* (Sweet Almond) Oil, Phenoxyethanol, Polyquaternium-7, Maltodextrin, Polyquaternium-10, Ethylhexylglycerin, PEG-150 Pentaerythrityl Tetrastearate, Citric Acid, Tetrasodium Glutamate Diacetate.

5.2 Mayday Daily Lice Fighting Conditioner

INCI List / Ingredients: Water, Propylene Glycol, Cetearyl Alcohol (and) Behentrimonium Methosulfate, Cetyl Alcohol, Glyceryl Stearate, Dimethicone, *Helianthus Annuus* (Sunflower) Seed Oil, Behentrimonium Chloride, Fragrance, *Mentha Piperita* (Peppermint) Oil, *Azadirachta Indica* (Neem) Seed Oil, *Melaleuca Alternifolia* (Tea Tree) Leaf Oil, *Eucalyptus Globulus* Leaf Oil, Cetrimonium Chloride, Phenoxyethanol, Ethylhexylglycerin, Citric Acid.

5.3 Mayday Lice Treatment Hair Solution

INCI List / Ingredients: Dimethicone, Cocamidopropyl Betaine, *Mentha Piperita* (Peppermint) Oil.

5.4 Mayday Home Defense Bed Bug & Lice Furniture & Bedding Spray

Active Ingredients: Sodium Lauryl Sulfate (7%), Sodium Chloride (1%), Citric Acid (0.2%).

Inert Ingredients: Water, Sodium Benzoate, Potassium Sorbate (q.s. to 100%).

5.5 Mayday Daily Lice Fighting Detangler Spray

INCI List / Ingredients: Water, C12-15 Alkane, Propanediol, Cetrimonium Chloride, Polyglyceryl-6 Laurate, Cetearyl Alcohol, Behentrimonium Methosulfate, Glycerin, Ethylhexyl Oliviate, Phenoxyethanol, Dimethicone, Lauryl Glucoside, Behentrimonium Chloride, *Mentha Piperita* (Peppermint) Oil, Fragrance, Panthenol, *Eucalyptus Globulus* Leaf Oil, *Melaleuca Alternifolia* (Tea Tree) Leaf Oil, *Rosmarinus Officinalis* (Rosemary) Leaf Oil, Polyquaternium-10, Citric Acid.

6.0 Data Collection and Documentation

Source documents were maintained for each numbered tray run. Raw data was tabulated and summarized with graphs.

7.0 Results

Results of the Mayday product test runs were as follows.

7.1 Mayday Daily Lice Fighting Detangler Spray

Nine repeats were completed with a total of 10 lice in each individual test run. Each petri dish included two tufts of hair — one that was pretreated with the Mayday Detangler Spray and the other with distilled bottled water. In an effort to create a similar environment between hair tufts during the testing phase, and to ensure that the test product was responsible for the lice reaction and not the premoistened hair, the second hair tuft was pretreated with distilled water only, otherwise similar to non-use of a prevention product. At the 0-minute timepoint, 10 lice were placed on the midline and allowed to wander freely between the two hair tufts. The position of the lice and their reactions were recorded over a 7-hour period with individual readings at 0, 30 minutes, 1 hour, 3 hours, 5 hours, and 7 hours.

It was noted that lice moved quickly at the start of each individual run, looking for hair to attach to, as would be a normal reaction in the movement of head lice. Although initially some lice did wander towards hair tufts that had been treated with the Mayday Daily Lice Fighting Detangler Spray, they quickly vacated the treated hair for safer ground. By the seventh hour reading of the 90 lice utilized in this arm of the study, only one louse remained on the treated hair. Additionally, this louse remained immobile throughout the 7-hour observation, signifying that the product had an almost immediate negative effect on the lice that were exposed to it. Based on this information, it left no doubt that the Mayday Daily Lice Fighting Detangler Spray did indeed both stun and repel the lice that had been exposed to it.

Statistician Report

The percentage distribution of lice across Test Product, Control, and Off Hair/Dead is shown in Figure 1. A consistent pattern emerges in which the Test Product produces a much more rapid reduction in lice remaining on the hair compared with the Control. In the early exposure periods (30 minutes to 3 hours), a substantially higher proportion of lice have already transitioned to the Off Hair/Dead category in dishes treated with the Test Product, while lice in the Control group generally remained attached, indicating lower efficacy in dislodgement and mortality. By the later time points (5–7 hours), most dishes treated with the Test Product achieve 70–100% lice removal or death, whereas the Control group shows only 20–60% lice removal or death, demonstrating slower and less complete action. Overall, these findings indicate that the Test Product demonstrates a markedly superior performance in promoting both rapid dislodgement and mortality of lice compared with no treatment.

In practical terms, based on the differences in remaining attached lice over time, individuals using the Test Product can expect approximately a 97% greater level of safety and protection from lice persistence and spread than those using only water (control) or no treatment at all. This enhanced efficacy reduces both the duration of infestation risk and the likelihood of continued transmission.

Percentage Safety Improvement — Detangler Spray

To quantify how much safer an individual could expect to be using the test product versus the control or nothing at all, the relative reduction in live lice at the 7-hour endpoint is calculated.

- Average % Live Lice (on hair) at 7 hours — Control Group: ~**37%**
- Average % Live Lice (on hair) at 7 hours — Test Product Group: ~**1%**

Relative Risk Reduction (Detangler Spray)

$$\text{Safety Improvement} = \frac{\text{Control Live \%} - \text{Test Product Live \%}}{\text{Control Live \%}} \times 100\% = \frac{37\% - 1\%}{37\%} \times 100\% = \mathbf{97\%}$$

Figure 1 — Percentage distribution of lice across Test Product, Control, and Off Hair / Dead, across nine dishes over 7 hours of exposure. Original chart available in source PDF; data summarized above and in Section 8 Conclusion.

Figure 1. Lice distribution across the Detangler Spray test, Control, and Off Hair / Dead categories during the 7-hour exposure period.

7.2 Mayday Home Defense Bed Bug & Lice Furniture and Bedding Spray

Nine repeats were completed with a total of 10 lice in each individual test run. Each petri dish included two squares of cheesecloth to help simulate an actual use environment similar to a prevention household spray. Mortality and distress were higher across all nine runs on the treated (product-sprayed) side of testing. Surviving lice generally avoided the treated cheesecloth, clustering instead on the control side or midline of the petri dish. Visible symptoms such as twitching, reduced mobility, and movement away from the treated area indicated both toxic and repellent effects. By the seventh hour, treated surfaces consistently showed a stronger negative impact, while the control sections maintained higher survival rates. Based on the seventh-hour read of the 90 lice exposed to the Mayday Lice & Bed Bug Spray, only one louse remained alive, although severe twitching, similar to an electrical shock, was evident — confirming that exposure to the Mayday Home Defense Bed Bug & Lice Furniture and Bedding Spray did in fact have a negative effect on the lice. Test results confirmed that the Mayday Home Defense Bed Bug & Lice Furniture and Bedding Spray is an effective tool when it comes to killing and/or preventing the spread of lice within a home environment.

Statistician Report

Figure 2 illustrates the percentage of lice remaining on the cheesecloth as Test Product, Control, or Dead over a 7-hour exposure period across nine dishes. Across nearly all dishes and time points, the Test Product consistently demonstrates a more rapid and sustained reduction in lice on the cheesecloth compared with the control. During the early intervals (30 minutes to 3 hours), a greater proportion of lice exposed to the Test Product are already removed from the cloth or dead, whereas lice in the control

condition largely remain attached, indicating limited efficacy. By 5 to 7 hours, the Test Product results in 0–30% of lice still alive and attached in most dishes, while control dishes frequently show 40–80% of lice still alive and attached. This trend highlights the superior ability of the Test Product to both eliminate and dislodge lice more efficiently than the control solution.

Based on the reduction in lice remaining on the cloth across the full time course, an individual using the Test Product could expect to be approximately 92% safer from ongoing lice attachment and transmission compared with using the control or no treatment at all. This increased protection reflects both faster action and greater overall effectiveness in reducing viable lice capable of reinfestation.

Percentage Safety Improvement — Bed Bug & Lice Spray

- Average % Live Lice (on cloth) at 7 hours — Control Group: **~48%**
- Average % Live Lice (on cloth) at 7 hours — Test Product Group: **~4%**

Relative Risk Reduction (Bed Bug & Lice Spray)

$$\text{Safety Improvement} = \frac{\text{Control Live \%} - \text{Test Product Live \%}}{\text{Control Live \%}} \times 100\% = \frac{48\% - 4\%}{48\%} \times 100\% = \mathbf{92\%}$$

Figure 2 — Percentage of lice remaining on the cheesecloth, divided into Test Product, Control, and Off cloth / Dead categories, across nine dishes over 7 hours. Original chart available in source PDF; data summarized above.

Figure 2. Lice distribution on cheesecloth — Bed Bug & Lice Spray vs Control — across nine dishes during the 7-hour exposure period.

7.3 Late-Stage Egg Submersion — Mayday Daily Lice Prevention Shampoo

The final arm of this project involved seven additional test runs of late-stage viable eggs to ensure that three minutes of submersion in the Mayday Daily Lice Prevention Shampoo was sufficient to effectively help terminate late-stage eggs during the shampoo process. Although initial test runs already proved that complete and total exposure to the shampoo product was effective in compromising the hatching ability of the lice eggs, Omniche desired to determine how strong the results are when considering that the product would be rinsed off after a three-minute period. Therefore, seven additional individual runs, each containing 10 late-stage viable eggs, were performed. Three runs were exposed to the Mayday Daily Lice Prevention Shampoo product. Three additional runs were exposed to the same generic shampoo that was utilized in the first round of testing, and one final run was exposed solely to distilled bottled water.

The following test runs were completed:

Egg-hatch outcomes after 3-minute submersion + 1-minute rinse

Product	Eggs Tested	Hatched	Failed to Hatch	% Failed to Hatch
Mayday Daily Lice Prevention Shampoo (reformulated)	30	8	22	73.3%
Generic Shampoo	30	17	13	43.3%
Distilled Water (control)	10	9	1	10%

The control run demonstrates that non-use of a lice prevention shampoo is only 10% effective as a means of prevention. Since the end goal was to help prevent hatching, the back-up testing where the eggs were rinsed after three minutes of exposure to the Mayday Daily Lice Prevention Shampoo did in fact have a negative effect on the majority of the late-stage viable eggs, rendering them unable to hatch.

Figure 3 — Percentage of hatched and unhatched eggs out of 30 per product and 10 per control. Original chart available in source PDF; data summarized in the table above.

Figure 3. Egg-hatch percentages across Mayday Daily Lice Prevention Shampoo (reformulated), Generic Shampoo, and Distilled Water control.

7.4 Reformulation Verification Runs

Mayday Daily Lice Prevention Shampoo (reformulated)

In an effort to provide accurate information to the end user and as a result of changes in formulation on previously tested Mayday products, additional runs were completed to ensure the continued efficacy of each product. An additional three runs involving a total of 15 lice were completed utilizing Mayday Daily Lice Prevention Shampoo. At the 7-hour reading, results showed **100% mortality**, confirming existing claims that the Mayday Daily Lice Prevention Shampoo does in fact continue to kill the lice that have also been confirmed and identified as frequently termed "super lice."

Mayday Lice Treatment Hair Solution (reformulated with peppermint oil)

Mayday Lice Treatment Hair Solution saw an addition of peppermint oil to its original formula; therefore, an additional three runs involving a total of 15 healthy, active adult and/or 3rd-stage head lice were completed to ensure the same efficacy level seen in the first round of testing. At the 7-hour reading, results showed all lice were dead with absolutely no movement from the first reading at 30 minutes on. Such results leave no doubt that the use of the Mayday Lice Treatment Hair Solution product is an effective and useful tool when it comes to eliminating head lice. Based on these results, product claims have been substantiated as to Mayday Lice Treatment Hair Solution's ability to effectively kill lice that have also been proven to qualify as "super lice."

Mayday Daily Lice Fighting Conditioner

Mayday Daily Lice Fighting Conditioner was previously tested, and since no changes in formula were made, no further testing was required. Based on original testing and confirmed through DNA testing to be lice containing the mutation signifying product resistance, it is safe to refer to the product's ability to stun and repel lice, otherwise known as "super lice."

7.5 DNA Testing Report — Seoul National University

Determination of kdr allele frequencies from 5 human head louse samples / Work-for-Hire Project

Sponsor	Omniche Consumer Products Co.
Performing Site	Laboratory of Medical Entomology Department of Tropical Medicine and Parasitology Seoul National University College of Medicine Seoul, 03080 South Korea
Principal Investigator	Ju Hyeon Kim, Ph.D. Assistant Professor, Seoul National University College of Medicine
Objective	Determine kdr mutation allele frequencies from 5 human head louse populations using the quantitative sequencing (QS) method.

Experimental Design, Methods and Materials

Head Louse Collections

After treatment with the test products, head lice samples were collected in 5 mL screw tubes containing 75% ethanol. The tubes were wrapped in protective bubble wrap and shipped to Korea with a sufficient amount of packing peanuts to prevent breakage during transport. Sample collections and mailing collected samples to Seoul National University were performed by Katie Shepherd, LSRN Maitland, FL. Received samples were stored at -20°C until genomic DNA (gDNA) extraction.

Genomic DNA Extraction and Amplification of 908 bp PCR Fragment

The pooled lice samples from a single collection were rehydrated in $1\times$ TE buffer at 4°C overnight and then homogenized using 0.9–2.0 mm stainless steel beads and Bullet Blender Tissue Homogenizer (Next Advance, Troy, NY, USA). gDNA was extracted with the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions, including a 3-hour proteinase K treatment.

A 908 bp fragment of the head louse voltage-sensitive sodium channel (VSSC) α -subunit gene, encompassing the M815I, T917I, and L920F mutation sites, was amplified by PCR [1, 2]. The PCR product was confirmed by gel electrophoresis and purified using the Monarch® Spin PCR & DNA Cleanup Kit (New England Biolabs, Ipswich, MA, USA). The concentration of the purified PCR fragment was quantified using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

The DNA fragment was diluted in nuclease-free water to a final concentration of $27 \mu\text{g L}^{-1}$ and QS reactions were performed for the detection of the three *kdr* mutations principally responsible for permethrin resistance in head lice [2].

QS Genotyping of the *kdr*-Type Mutations Associated with Permethrin Resistance

The QS protocols were as initially reported by Kwon et al. [2]. Briefly, 908 bp genomic DNA fragments of the VSSC α -subunit gene, encompassing the M815I, T917I, and L920F mutation sites, were PCR-amplified from the previously known permethrin-susceptible (EC-HL) and permethrin-resistant (SF-HL) head lice. Once the genotypes and the intron sequences were confirmed, the PCR products with or without mutations were mixed to generate standard DNA mixture templates in the following molar ratios: 0:10, 1:9, 3:7, 5:5, 7:3, 9:1, and 10:0 (resistant allele : susceptible allele). Standard DNA template mixtures were sequenced using two sets of sequencing primers for the sense- and antisense-directional sequencing, respectively. The nucleotide signal intensities of the resistant and susceptible alleles were determined by the sequencing chromatograms and signal ratios were calculated using Equation 1 (above).

The signal ratios of template DNA mixtures were normalized by multiplying them with the normalization factor (signal ratio of the heterozygous DNA template / signal ratio of the 5:5 standard DNA template). A series of the normalized signal ratios were plotted against the corresponding *kdr* allele frequencies (resistance allele frequency, RAF) and standard regression equations, together with lower and upper prediction equations, were generated for the estimation of *kdr* allele frequencies (RAF) of unknown samples [2, 3].

Results — Determination of *kdr* Allele Frequencies (RAF) from 5 Human Head Louse Samples

All insect specimens provided were individually examined to determine their developmental stage prior to gDNA extraction (Table 1). Chromatograms corresponding to each sample obtained from the QS are shown in the source figure. Signal ratios were calculated from the relative peak intensities of resistant and susceptible nucleotides in the sequencing chromatograms, and resistance allele frequencies for each sample were estimated using the best-fit equations derived from the standard curves of the three *kdr* mutations.

Table 1. Collection information of human head lice

No.	Sample Name	Larva	Female	Male	Total
1	Tx product 23	—	10	16	28
2	Enviro spray 28	—	16	12	28
3	Shampoo 20	2	11	9	22
4	Repel spray 26	1	9	16	26
5	Conditioner 24	1	11	12	24

All head lice in the five samples exhibited **100% resistance allele frequency (RAF)** at each of the three kdr loci, yielding mean RAF values of 100% across all samples (Table 2). No susceptible alleles (M815, T917, or L920) were detected in any of the samples analyzed.

Table 2. The determination of % resistance allele frequency by QS

No.	Sample Name	No. of lice used for QS			% Resistance Allele Frequency (RAF)			Mean % RAF
		Larva	Female	Male	M815F	T917I	L920F	
1	Tx product 23	—	10	16	100	100	100	100
2	Enviro spray 28	—	16	12	100	100	100	100
3	Shampoo 20	2	11	9	100	100	100	100
4	Repel spray 26	1	9	16	100	100	100	100
5	Conditioner 24	1	11	12	100	100	100	100

Conclusions — Seoul National University DNA Report

DNA-based resistance analysis was performed on head lice tested with five products (Tx product, Enviro spray, Shampoo, Repel spray, Conditioner) using the QS method. The analysis detected three mutations (M815I, T917I, L920F) in the voltage-sensitive sodium channel, the target site of pyrethroid insecticides, indicating that all head lice used in this study exhibit a high level of pyrethroid resistance.

References (DNA sub-report)

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Signed: Dr. Ju Hyeon Kim, Ph.D., Principal Investigator, Seoul National University College of Medicine, Seoul, South Korea 03080 — Date: October 17, 2025.

8.0 Conclusion

Omniche Consumer Products' goal was to develop a line of lice treatment and prevention products that their customers could rely on. As parents who have personally experienced the challenges of fighting head lice, they knew firsthand how important safe, yet effective products are for families that need them. It is with this thought in mind that they have continually tested the efficacy and safety of their products, even altering formulations when they believed they could do better.

Their efforts have paid off. Continued testing has found that their products did in fact kill super lice. A fact that is important to parents when product resistance is a growing issue. And while bench studies are always considered a stepping stone towards more conclusive results, testing has found enough evidence to ensure parents that their Mayday line is both safe and effective.

Headline Efficacy Findings

Summary of Mayday product efficacy from this study (in vitro, LSRN 2025)

Product	Efficacy	Mechanism
Mayday Daily Lice Prevention Shampoo	99.05% effective in controlling head lice; 100% mortality at 7-hour reading in reformulation verification runs	Kills head lice; in limited bench studies, proven to kill both super lice and their eggs
Mayday Daily Lice Fighting Conditioner	95.24% effective in stunning and repelling head lice	Stuns and repels head lice
Mayday Daily Lice Fighting Detangler Spray	97% effective in stunning and repelling head lice	Stuns and repels head lice (used outside the bath)
Mayday Lice Treatment Hair Solution	100% mortality in limited lab studies when used properly	Dimethicone-based suffocation; kills head lice quickly while providing a slick formula for comb-outs
Mayday Home Defense Bed Bug & Lice Furniture and Bedding Spray	92% effective in protecting the family's home from head lice	Kills and repels head lice on cheesecloth-simulated household surfaces; primary product goal focuses on bed bug treatment

Lice experts suggest using the combined shampoo and conditioner products 2–3 times a week as the best way to improve your odds in the fight against lice.

Lice are however a fact of life and contact is inevitable. For this reason, Omniche felt the need to provide families with Mayday Daily Lice Prevention (Detangler) Spray. Lab testing found it to both stun and repel lice, a fact that is increasingly important in today's active lifestyles. Study results found the detangler product to be 97% effective in the fight against head lice.

Even with great products, the risk of lice crossing over still exists. Mayday Lice Treatment Hair Solution was found to be a great resource when it came to eliminating head lice. Limited lab studies found when used properly it could provide parents with 100% results in bringing a quick and effective end to their head lice problem. Mayday Lice Treatment Hair Solution killed lice quickly while the slick formula provided parents with a slippery slope when it came to effective comb-outs. And although many lice treatment products can leave hair dry and dull, researchers were pleased to see that Mayday Lice Treatment Hair Solution left the hair both shiny and smooth.

It's a well-known fact that lice need blood to survive and they rarely leave their food source by choice. Still there are those families that need the comfort of checking all boxes when it comes to head lice. Therefore Omniche felt that their Mayday Home Defense Bed Bug & Lice Furniture and Bedding Spray was the ultimate tool when it came to offering peace of mind for parents looking at every alternative. While the primary goal of this new product may focus more on the treatment of bed bugs, it was found to be **92% effective** in protecting the family's home from head lice as well.

Based on study results, Mayday's combined treatment options, with their promising results, provide parents with effective ways in which to treat lice while developing a prevention regime as they move forward.

9.0 Bibliography

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