Effectiveness of One Percent Sodium Chloride Spray and Gel Against Resistant Head Lice

Authors: Katie Shepherd[a], J Marshall Clark[b], Jeffery C. Seller[a], Amy Botello[a], Evengy Adischey[c], John Mark Christensen[d], Jessica Smith[e]
a- Lice Solutions Resource Network, West Palm Beach Florida 33407
b- Department of Veterinary and Animal Science, University of Massachusetts, Amherst MA, 01003
c- Adisdshchev Consulting Services, Moscow, Russia
d- Department of Pharmaceutical Sciences, College of Pharmacy, Oregon State University, Corvallis Oregon 97330
e- Tec Laboratories, Albany, Oregon 97321

Abstract

An in-vitro study was performed to evaluate the effectiveness of one percent sodium chloride spray and gel pediculicidal products against pyrethroid-resistant head lice. A two-part, double-arm study utilizing a total of 40 repeatability runs were performed. Each test run included 12 healthy mature adult and/or 3rd stage lice (7 for each petri dish product exposure test and 5 for genetic testing) for a minimum of 240 head lice in each arm (overall inclusion of 480 head lice). The pyrethroid resistance was determined by QS genotyping of the kdr-type mutations associated with pyrethroid resistance. All head lice but one tested 100 percent positive for all three allele genotype mutations.

The results of the study found that 98.6 + 4.4 percent of the head lice exposed to the sodium chloride spray product and 99.3 + 3.2 percent of the lice exposed to the sodium chloride gel product were dead within six hours after treatment application.

Exposure of pyrethroid resistant head lice to one percent sodium chloride spray and gel products demonstrated a rapid and severe effect on the mobility of the lice, leading ultimately to death.

Exposure of pyrethroid resistant head lice to one percent sodium chloride spray and gel products demonstrated a rapid and severe effect on the mobility of the lice, leading ultimately to death.

Introduction

Pediculus Capitus
(head lice)

Pediculosis, the infestation of humans by lice, has been well documented throughout human history [1]. Humans are hosts to three types of lice: Pediculus Capitus, Pediculus humanus, and Pthirus pubis [1]. The worldwide prevalence of Pediculus Capitus (head lice) is documented in current scientific literature [2] and its incidence is widespread, crossing all socio-economic strata [3]. Cost of treatment, retreatments, loss of workdays, as well as consumer costs such as cleaning, doctor visits, and medication also contribute to the economic burden of battling head lice infestations, driving up overall treatment expenses [3].

In 2013, the CDC estimated 6 - 12 million lice infestations annually [4]. Infestations are found primarily in children ages 3 to 11 [3, 5, 6], but siblings and parents may become infested with head lice as well [6, 7]. As there is a lack of concrete current data on the overall number of cases in the US, this number may or may not overstate the actual infestation rate. The CDC estimate needs to be updated. The estimate does not take into consideration the number of cases treated by lice professionals, product sales through the internet, the large variety of homeopathic methods used, or that some individuals choose no treatment at all.

In treatment of infestations of head lice it is important to consider safety, efficacy, availability, expense, and ease of use of the remedy [3, 10]. Treatment options available include over-the counter drug products [5, 6, 7], prescription products [5, 6, 7] and mechanical devices [8, 9]. Drug products that are usual front line insecticide treatments include topical permethrin, malathion, and lindane [8]. In treatment failure with permethrin, malathion is the second treatment of choice [8]. Lindane should be avoided when possible [6]. Other treatments include topical ivermectin, benzyl alcohol, or spinosad [7, 8], however, treatment costs to the patient needs to be assessed before their use [3]. Treatment failure occurs frequently with insecticide products. As such, other remedies are sought due to the public belief that insecticide treatments are unsafe or the assumption that pesticide-resistant head lice are present [8]. This assumption leads many to seek out other alternative treatments or home remedies that may or may not work. Most often, these treatments lack definitive safety or efficacy data for the treatment of head lice infestation [8, 10].

The continuing concerns of pyrethrum-resistant head lice led Dr. Kyong Sup Yoon, at the University of Massachusetts to determine and publish the first of a series of studies on head lice with mutated genes. The specific three mutations that provide head lice with the ability to develop resistance to the commonly used OTC pyrethroid products [11] was followed by the QS method developed to rapidly detect these three mutations [12}. 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 [13]. Resistance to pyrethroid head lice products has now been shown to exist extensively throughout the world [14, 15, 16, 17 and 18]. Furthermore, worldwide resistance of head lice to malathion is also increasing. This, in combination with pyrethroid pesticide resistance, amplifies a real need to find more effective treatment options [19, 20 and 21].

The pesticide sodium chloride is listed in the international Pesticide Assistance Network (PAN) [22], the US EPA list of Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) under the Minimum Risk Exemption regulation as an active insecticide ingredient [23], and has gained use as a head lice treatment [24]. Serrano et al, [24] reported successful treatment of 85 percent of subjects with head lice treated with a one percent sodium chloride spray compared to 45 percent of permethrin treated subjects fifteen days after initial treatment.

Retreatment is common due either to reinfestation or failure of initial treatment of head lice [3, 6, 24]. Seventy percent of permethrin subjects in the Serrano et al study needed retreatment with permethrin at day 8 compared to twenty five percent of sodium chloride treated subjects. [24]. A second treatment of permethrin increased the overall effectiveness, but only forty five percent of subjects were free of head lice after 15 days. The inefficacy of the permethrin treatment warranted additional treatments in those subjects. Retreatment with currently available pesticides, poses a safety concern among children without the proper waiting period [3]. The Serrano study was performed in South Florida where pyrethroid-resistant lice are prevalent, however this study did not encompass resistance testing.

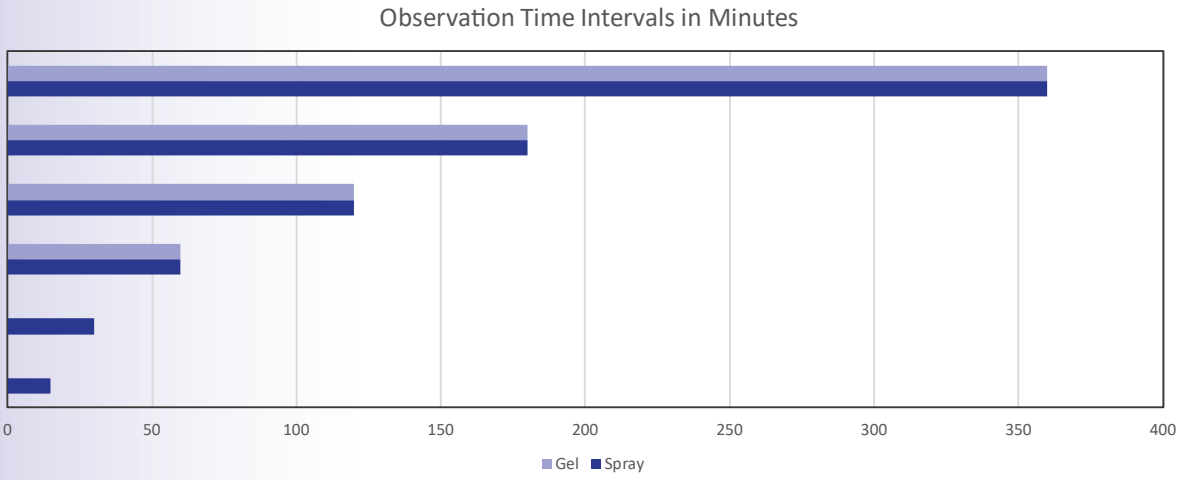
Articles have been written that claim OTC sodium chloride products are not effective treatments against head lice [23, 24]. However, with the global advance of pyrethroid-resistant head lice, a reexamination of sodium chloride as a pediculicide is being initiated. Therefore, the purpose of this in-vitro study was to determine if head lice collected from human hosts were in fact pyrethroid-resistant head lice and if one percent sodium chloride spray and gel products have positive pediculicidal activity against pyrethroid-resistant head lice.

Materials and Methods

This was a two-part, double-arm study utilizing a total of 40 repeatability runs. Each test run included 12 healthy mature adult and/or 3rd stage lice for a minimum of 240 head lice in each arm (overall inclusion of 480 head lice). In each unique collection 7 mature, active healthy head lice were utilized for product testing in an in-vitro setting; while the remaining lice from the same unique collection (each unique collection contained head lice from only one individual) were sent to Massachusetts Pesticide Analysis Laboratory (MPAL) to determine if the head lice were pyrethroid-resistant head lice.

The ingredients of the sodium chloride spray include one percent sodium chloride, poloxamer 188, benzyl alcohol, fragrance, and water. The ingredients in the sodium chloride gel include one percent sodium chloride, anise oil, aminomethyl propanol, carbomer, cocamidopropyl betaine, PEG-6 caprylic/caproic glycerides, and water.

For the sodium chloride spray in-vitro portion of the study, the lice were observed at 15 min., 30 min., 1 hr., 2 hr., 3 hr. and 6 hr. after product exposure. For the sodium chloride gel in-vitro portion of the study, the lice were observed at 1 hr., 2 hr., 3 hr. and 6 hr. after product exposure. The observation time points were selected so as to comply with the instructions for use for each sodium chloride product. Sodium chloride spray directions are to spray the infested area until saturated and let air dry naturally. The sodium chloride gel is left in the hair for one hour and then washed out. To maintain an environment similar to their human host, petri dishes were kept in an incubator (Clinical Laboratory Incubator/L-CU300 Unico, N.J.) between each end point of the study process. The incubator temperature was recorded on source documents and monitored throughout the 6-hour study period. All observations were made with a standard dissecting microscope (StereoZoom 4, Bausch & Lomb, Irvine, CA). Since many factors play a role in the ultimate position of head lice during a given testing period, the movements of the lice over a 6-hour time period were recorded; with the 6-hour position being used for the statistical analysis in determining the end results.



Study Procedures

LSRN Research (LSRN), a head lice awareness and control center located in West Palm Beach, FL, provided mature, freshly harvested adult and 3rd-stage instar head lice specimens. All phases of the testing process were done at the LSRN West Palm Beach location to ensure the head lice specimens remained healthy during the study. Procedures for obtaining head lice specimens were as follows: Head lice samples were collected from otherwise discarded head lice as a result of LSRN head lice removal services. Only head lice removed from hair that had not been pretreated within the seven days preceding head lice removal from the subjects were used. To prevent potential damage to the louse, all head lice were obtained as a result of combing dry hair with a rattail comb (Burmex Co. Holtsville, NY). 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 subjects who had previously agreed to allow their discarded head lice to be utilized for education and research purposes by signing an informed consent form detailing the purposes the head lice would be used for. The consent form was approved by LSRN's IRB. Subjects 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 (Fisherbrand, Pittsburg, PA) 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 being separated from their human host. At no time did LSRN Research staff interact with clients.

A Two-Part, Double-Arm "in vitro" study utilizing a minimum of 480 active and healthy adult and/or 3rd-stage head lice were used to complete a total of 40 individual test runs. Each of the 40 petri dishes contained one tuft of untreated and chemically unaltered human hair. In petri dishes numbered 1-20 the hair tuft was positioned on the midline of the petri dish while awaiting qualified head lice samples In Petri dishes numbered 21-40 a cheesecloth was secured to the petri dish by a rubber band and the hair tuft was set atop the cheese cloth in the middle of the dish.

One percent sodium chloride spray (Licefreee Spray!, Tec Laboratories Inc, Albany, Oregon) was tested in petri dishes 1 through 20 with a minimum of 240 lice selected for this phase of the project. Each unique, individual, collection of head lice contained 12 active healthy adult and/or 3rd-stage head lice. The 7 most active head lice from each collection were placed on the hair tuft in the petri dish for product testing. The hair tuft was then sprayed with five pumps of sodium chloride spray, from a distance of 12cm. The reaction of the head lice to the sodium chloride spray treatment was then recorded based on predetermined timelines listed above. The remaining five head lice were placed in a screw top vial (Cynken/Seoul, South Korea) containing 95% ethanol (Healthlink/Jacksonville, Fl). Corresponding numbers matching samples to their original tray runs were noted on each individual vial. At the request of the team at Massachusetts Pesticide Analysis Laboratory (MPAL) samples were shipped in blocks of 20 vials each.

One percent sodium chloride gel (Licefreee! Gel, Tec Laboratories Inc. Albany, Oregon) was tested in petri dishes 21 through 40 with a minimum of 240 head lice selected for this phase of the project. Each unique, individual, collection of head lice contained 12 active healthy adult and/or 3rd-stage head lice.

One drop of sodium chloride gel was then gently massaged onto hair tuft and placed back on cheesecloth, thus allowing for fluid run-off during the rinsing phase of the study. The 7 most active head lice from each collection were then placed on hair tuft for product testing. Per product instructions, the gel remained on the hair for 1 hour prior to rinsing off, exposing the lice to the product during that time period. After one hour, the hair was rinsed by spraying 10 pumps of distilled water from a 6 oz. spray bottle and from a distance of 12 cm. The cheesecloth allowed water to pass through to the petri dish below as the hair tuft was rinsed off. This rinsing process prevented the head lice from being submersed in the rinse water. The reaction of the head lice to the sodium chloride gel treatment was recorded beginning with the 1-hour reading and throughout the remaining predetermined intervals. The remaining five head lice were placed in a screw top vial containing 95% ethanol. Corresponding numbers matching samples to their original tray runs were noted on each individual vial. At the request of the team at MPAL samples were shipped in blocks of 20 vials each.

The incubator was prepared with a target temperature of 29°C (84.2°F) with an acceptable range of 27°C to 34°C (80.6°F – 93.2°F). Temperatures were recorded on source documents at start of the testing period and monitored throughout the duration of each individual test run. The incubator was used to hold harvested head lice until the test petri dishes were prepared and throughout the 6-hour testing period. Test petri dishes were kept in the incubator between observations to ensure that the head lice were kept at their optimum environmental conditions to prolong the mobility of the specimens.

Forty individual head lice sample collection kits were prepared by the research team at LSRN. Sample collection and the mailing of collected samples to MPAL was performed by LSRN. Received samples were stored at -20°C until genomic DNA extraction. The determination of kdr allele frequencies from 40 vials of harvested human head louse populations was performed using the quantitative sequencing method.

Genomic DNA extraction and amplification of 908bp PCR fragment

Genomic DNA (Gdna) was extracted from pooled lice from each single unique collection following homogenization using a 0.2 mL glass-glass homogenizer (Kontes Glass Co., Vineland, NJ). The extraction was performed with the Qiagen DNeasy blood and tissue kit (Qiagen Inc., Valencia, CA) following the manufacturer's instructions. A 908 bp length fragment of the head louse VSSC α-subunit gene, encompassing the M815I, T917I and L920F mutations sites, was amplified using PCR [12, 14]. The concentration of the fragment was quantified using Picogreen dsDNA quantification kit (Thermo Fisher Sci., Waltham, MA). The DNA fragment was diluted in nuclease-free water to a final concentration of 27 ×g/L and QS reactions performed for the detection of the three kdr mutations principally responsible for permethrin resistance in head lice [12].

QS Genotyping of the kdr-type mutations associated with permethrin resistance

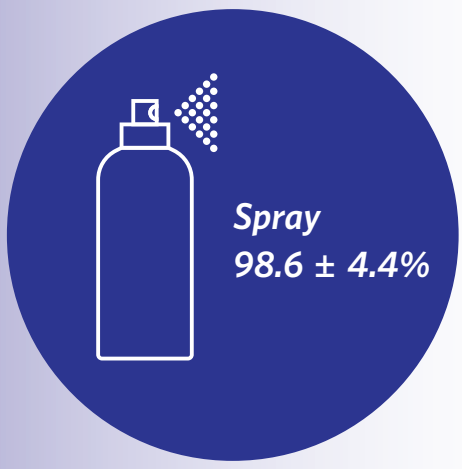
The QS protocols were as initially reported by Kwon et al [11]. Briefly, a 908 bp genomic DNA fragment of the VSSC α-subunit gene, encompassing the M815I, T917I and L920F mutation sites, was PCR-amplified from the previously known susceptible and resistant reference populations of head lice. Once the genotypes and the intron sequences were confirmed, the PCR products with or without mutations were 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 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, and signal ratios calculated using Equation 1.

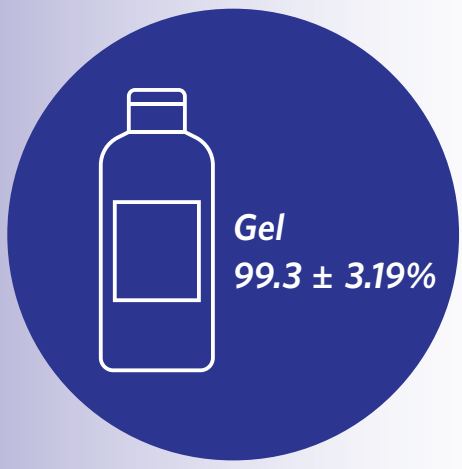
Eq. 1

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

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 normalized signal ratios were plotted against the corresponding kdr allele frequencies to generate standard regression equations, together with lower and upper prediction equations, for the estimation of kdr allele frequencies of unknown samples [12].

Percent of the lice exposed that were dead by the 6-hour time point:





Results

Determination of kdr allele frequencies from collected head louse samples: Samples of head lice from each unique collections were tested for the presence and frequency of pyrethroid-resistant alleles.

Ninety-six lice were collected in 20 separate samples collected from hair of subjects that were treated with sodium chloride spray, including 3 larva, 73 females and 20 males (4.8 lice per sample 4 0.52, mean 4 S.D.) (Table 1). Seventy-three lice were collected in 20 separate samples in samples collected from hair of subjects treated with sodium chloride gel, including 12 larva, 44 females and 17 males (3.65 lice per sample 4 0.75, mean 4 S.D.) (Table 2). A total of 169 lice were collected in 40 samples.

Collection information of human head lice, grouping of lice into samples for QS analysis, determination of percent resistance allele frequency (RAF) at each allele, and determination of mean percent RAF are given in Table 1. Table 2 reports the results for samples collected from hair of subjects treated with sodium chloride gel.

In-Vitro Lethality Tests on Head Lice: Twenty test runs each of one percent sodium chloride spray and gel products were completed for a total of 40 test runs. Table 3, (Figure 1) and Table 4, (Figure 2) give the average results of lethality of the test runs, which show that the one percent sodium chloride gel and spray products produced an overwhelmingly negative impact on the head lice on or before the 6-hour time point. The impact of sodium chloride treatment on pyrethroid-resistant head lice was seen at the one hour time point. More than fifty percent of head lice in both spray and gel treatment groups were severely impacted. At three hours after exposure, more than ninety percent of the head lice were showing marked deleterious effects in appearance an behavior, or were dead. Additional testing of the head lice collected with each of these same individual, unique, head lice collections found that they were pyrethroid-resistant head lice as demonstrated in the results from the resistant testing phase of the study.

For the purpose of final results, the 6-hour time frame was utilized to show the lethal effect of sodium chloride, as head lice can survive for 1-2 days off of a host. [3, 4, 27]. For the one percent sodium chloride spray product, results revealed that in 18 out of 20 test runs, all 7 lice, or 100 percent of the head lice in the petri dish were found dead at or before the 6 hour time point. In the case of the one percent sodium chloride gel product, results showed that in 19 out of 20 test runs, all 7 lice, or 100 percent of the head lice in the petri dish were found dead at or before the 6-hour time point. Further examination of lice either actively moving or dead at 6 hours was performed to determine whether any slight movement was a result of spasm or similar involuntary movement, or that of a louse that was merely stunned in the number count of dead lice. This revealed that 98.6 ± 4.4 percent of the lice exposed to the sodium chloride spray were dead by the 6-hour time point (two head lice were considered still alive). Similarly, 99.3 ± 3.19 percent of the lice exposed to the sodium chloride gel were dead at the time of the 6-hour time point one head louse was considered alive). The head lice collections were found to be pyrethroid-resistant head lice, as shown in Table 1 and Table 2 test results.

The head lice tested in vitro against one percent sodium chloride were taken from the same unique sample collections tested to be pyrethroid-resistant. These lice were shown to be susceptible to treatment with one percent sodium chloride spray and gel products, providing evidence that sodium chloride head lice products are effective against pyrethroid-resistant head lice.

NaCl

Sodium chloride treatments:
- **are effective against pyrethroid-resistance lice**
- **are affordable, available OTC's**
- **do not require a waiting period between treatments**
- **warrant further research**

Conclusions

All 20 samples collected from hair of subjects that were treated with sodium chloride spray had the resistant genotype at all three alleles. The mean percent RAF was 100% for all samples. Nineteen samples collected from hair of subjects treated with sodium chloride gel (1-18 and 20) had the resistant genotype at all three alleles. The mean percent RAF was 100% for these 19 samples. Sample 19 had the resistant genotype at the M815I and L920F alleles but the T917I was heterozygous with a sequence signal ratio of 0.67 and a RAF of 73%. This result is consistent with reports from studies showing the kdr-type mutations in head lice are widely and uniformly present in the US [14] and around the world [15, 16, 17, 18, 19, 20, 21] and pyrethroid susceptible alleles are rare.

This "in vitro" study reports results that 98.6% of the pyrethroid-resistant head lice exposed to sodium chloride spray died at or before the 6-hour time point. Additional results found that 99.3% of the lice exposed to sodium chloride gel were dead at or before the 6-hour time point. Furthermore, of the 40 individual test runs completed, head lice collected from those same samples for resistance testing found that 39 out of 40 samples collected were 100% pyrethroid-resistant and would be resistant to pyrethroid products currently available in US market.

Optimal treatment for head lice infestation should be readily available, safe, and effective in quickly eliminating live lice [6]. Removal of live lice should be easy, and the products used to perform this must be affordable [6]. Head lice treatments that meet this criteria are preferred. Erasing the stigma of head lice infestation and keeping children in school should be the goal of health professions and parents [5]. As head lice infestation is not a public health concern, keeping children in school while treating is preferred to other alternatives for the child [5].

The results of this study suggest that sodium chloride treatment of head lice infestation is effective against pyrethroid-resistance lice. Additionally, sodium chloride treatment products are affordable, available over-the-counter, and do not require a waiting period between treatments. While the results are significant, caution should be taken in that this is a limited study, in a lab setting, and intended to determine if further research, particularly in an actual clinical environment is justified. Based on results derived from this two-part, double-arm study, further research is warranted.

BIBLIOGRAPHY

1-Raoult, Didier., Reed, David L; Ditmer, Katharina; Kirchman, Jeremy J; Roalin, Jean-Marc; Guillen, Sonia; and Light, Jessica E. "Molecular Identification of Lice from Pre-Columbian Mummies. "The Journal of Infectious Diseases 197 (2008): 535-43.

2-Falagas, Matthew E; Matthaiou, Dimitrios K; Rafailidis, Petros I; Panos, George; and Pappas, Georgios. "Worldwide prevalence of head lice [letter]." Emerging Infectious Disease. Sep (2008). 14: 1493-1494.

3-Cynthia D. Devore, Gordon E. Schutz and the Council on School Health and Committee on Infectious Diseases "Head Lice" The Journal of the American Academy of Pediatrics Vol 135-5 (205): 1355-1365.

4-Centers for Disease Control and Prevention (CDC) September 24, 2013, Epidemiology and Risk Factors, www.cdc.gov/parasites/lice/head/epi.html

5-Mumcuoglu, Kosta Y., Meinking,Terry A.; Burkhart, Craig N, and Burkhart, Craig C. (2006). Head louse infestations: The "no nit" policy and its consequences. International Journal of Dermatology. 45:891-896.

6-Jones, Kimberly N, and Joseph C English III. "Review of common therapeutic options in the United States for the treatment of pediculous capitis." Clinical Infectious Diseases 36 (Nov 2003): 1355-361.

7-Feldmeier, Hermann. Treatment of Pediculosis Capitis: A critical review Appraisal of the current Literature, American Journal of Clinical Dermatology 214. 15:401-412. doi:10.1007/s40257-014-0094-4.

8-Bohl, Brittany; Evetts, Jessica; McClain, Kymberli; Rosenauer, Amanda; and Stellitano, Emily. Clinical Practice Update: Pediculosis Capitis. Pediatric Nursing 2015. 41 (5) 227-234.

9-Rush, Sarah E; Rock, Alex n; Jones, Sherri L; Malenke, Jael R; Clayton, Dale E. Efficacy of the LouseBuster, a New Medical Device for treating head Lice (Anoplura: Pediculidae) Journal of Medical Entomology 2011. 48(1):67-72. DOI.org/10.1063/ME10122

10-Jahangiri, Farhad, Case report: a new method for treatment of permethrin resistant head lice, Clinical Case Reports (2017) 5(5): 601-604. doi: 10.1002/ccr3.899

11-Yoon, Kyong Sup, Symington, S.B., Lee, Sy Hyock, Soderlund, D. M., and Clark, J. Marshall. Three Mutations identified in the Voltage-sensitive Sodium Channel α-subunit Gene of Permethrin-resistant Human Head Lice Abolish Permethrin Sensitivity of the House Fly Vssc1 Expressed in Xenopus Oocytes. Insect Biochemistry and molecular Biology (2008) 38:296-306.

12-Kwon, Deok Ho, Yoon Kyong Sup, Strycharz, Joseph P, Clark J Marshall; Lee Si Hyeock, Determination of permethrin resistance allele frequency of human head louse population by quantitative sequencing, Journal of Medical Entomology (2008) 45:912-920.

13-Gellatly, Kyle J; Kim, Sarah; Palenchar, Daniel J; Shepard Katie; Yoon, Kyong Sup; Rhodes, Christopher J; Lee, Si Hyeock; Clark, J Marshall. Expansion of the Knockdown Resistance Frequency map for Human Head Lice (Phthiraptera Pediculidae) in the United States Using Quantitative Sequencing. Journal Medical entomology 2016. 53(3):653-659. doi:10.1093/jme/tiw023.

14-Hodgdon, Hilliary E; Yoon Kyong Sup; Previte, Domenic J; Kim, Hyo Jeong; Aboelghar Gamel E; Lee Si Hyeock; Clark, J Marshall: Determination of knockdown resistance allele frequencies in global human head louse populations using the serial invasive signal amplification reaction. Pest Management Science 2010, 66(9): 1031 – 1040.

15-Durand, Remy: Bouvresse, Sophie; Berdjane, Zohra; Izri, Arezeki; Clark J M. Insecticide Resistance in Head Lice: Clinical, Parasitological and Genetic Aspects, Clinical Microbial and Infection (2012) 18:338-344. doi: 10.111/j.1469.2012.03806.x.

16-Kasai, Shinji; Ishii, Norihisa; Natsuaki, Nasuru; Fukutomi, Hiroyuki; Komagata, Osamu; Kobayashi, Mutsdo and Tomita, Takashi. Prevalence of kdr-like Mutation Associated with Pyrethroid Resistance in Human Head Louse Populations in Japan. Journal of Medical Entomology 2009. 46(1):77-82. doi: org/10.1603/033.046.0110

17-Toloza, Ariel Ceferino;Ascunce, Marina S; Reed, David, and Picollo, Maria Ines. Geographical distribution of Pyrethroid Resistance Allele Frequency in Head Lice (Phthiraptera: Pediculidae) from Argentina. Journal of Medical Entomology 2014. 51(1): 139-144. doi.org/10.1603/me13138

18- Durand, Remy; Bouvresse, Sophie; Andriantsoanirina, Valerie; Berdjane, Zohra; Chosidow, Olivier; and Izri, Arezki. High Frequency of Mutations Associated with Head Lice Pyrethroid Resistance in Schoolchildren from Bobigny, France. Journal of Medical Entomology 2011. 48(1): 73-75. doi:org/10.1603/ME10115

19- Ross, Kirstin, Head Lice, Resistance, and Malathion, Pediatrics (2008) 121:222. Doi:10.1542/peds.2007-3116

20- Bouvresse, Sophie; Berjanc, Zohra; Durand, Remy; Bouscaillou, Julie; Izri, Arezki; and Chosidow, Olivier, Permethrin and Malathion resistance in head Lice: Results of ex-Vivo and molecular assays, Journal of the Academy of Dermatology (2012) 67:1143-50. doi:10.1016/j.jaad.2012.04.011

21-McNair, CM. Ectoparasites of medical and veterinary importance: drug resistance and the need for alternative control methods. Journal of Pharmacy and Pharmacology 2015. 67(30: 351-363. doi: 10.1111/jphp.12368

22- PAN (Pesticide Assistance Network) Chemical Summary for the pesticide Sodium Chloride http://www.pesticideinfo.org/Summary_Chemical.jsp?Rec_Id=PRI5761 or http://www.pesticideinfo.org/Detail_Chemical.jsp?Rec_Id=PRI5761

23- United States Environmental Protection Agency (EPA) Active Ingredients Eligible for Minimum Risk Pesticide Products (Updated December 2015) From Federal Insecticide, Fungicide and Rodenticide ACT (FIFRA) under the Minimum Risk Exemption regulation in 40 CFR 152.25 (f). https://www.epa.gov/sites/production/files/2018-01/documents/minrisk-active-ingredients-tolerances-jan-2018.pdf

24-Serrano, Lidia: Deceasar, Lorraine, and Pham, Loam: Evaluation of the Efficacy and Safety of 1% Sodium Chloride (LiceFreee Spray) against 1% Permethrin Crème Rinse on Head Lice Infested Individuals. Pharmacology & Pharmacy 2013. 04(02):266-273. doi:10.4236/pp.2013.42038

25-Burgess, Ian F, head Lice: Resistance and Treatment Options, The Pharmaceutical Journal7-September 2016, 5:16.

26-Burgess, Ian F, Lice, Damned Li..., and Regulatory Confusion. Outlooks on Pest Management, (2014) 25 (6): 352-355.doi.org/10.1564/v25_dec_02

27- Centers for Disease Control and Prevention (CDC), Biology, September 2, 2015 http://dpd.cdc.gov/paracites/head/lice/biology.html

Table 1. Collection information of human head lice samples collected from hair of subjects that were treated with sodium chloride spray, grouping of lice into samples for QS analysis, and the determination of percent resistance allele frequency by QS for tests of sodium chloride spray on head lice.

Head Lice Sample	Number of Lice used for QS			Percent Resistance Allele Frequency (RAF)			
Number	larva	Female	Male	M815I	T917I	L920F	Mean percent RAF
1	-	5	-	100	100	100	100
2	-	5	-	100	100	100	100
3	-	5	-	100	100	100	100
4	-	3	2	100	100	100	100
5	-	5	-	100	100	100	100
6	-	5	-	100	100	100	100
7	-	3	2	100	100	100	100
8	-	5	-	100	100	100	100
9	-	5	-	100	100	100	100
10	-	5	-	100	100	100	100
11	-	3	2	100	100	100	100
12	-	-	5	100	100	100	100
13	-	5	-	100	100	100	100
14	-	2	2	100	100	100	100
15	-	5	-	100	100	100	100
16	3	2	-	100	100	100	100
17	-	3	2	100	100	100	100
18	-	4	1	100	100	100	100
19	-	3	2	100	100	100	100
20	-	2	2	100	100	100	100

Table 2. Collection information of human head lice samples collected from hair of subjects treated with sodium chloride gel, grouping of lice into samples for QS analysis, and the determination of percent resistance allele frequency by QS for tests of sodium chloride gel on head lice.

Head Lice Sample	Number of Lice used for QS			Percent Resistance Allele Frequency (RAF)			
Number	larva	Female	Male	M815I	T917I	L920F	Mean percent RAF
21	-	3	-	100	100	100	100
22	-	3	1	100	100	100	100
23	3	-	3	100	100	100	100
24	-	4	-	100	100	100	100
25	-	2	2	100	100	100	100
26	-	1	3	100	100	100	100
27	-	4	-	100	100	100	100
28	-	4	-	100	100	100	100
29	-	3	-	100	100	100	100
30	-	2	1	100	100	100	100
31	-	3	-	100	100	100	100
32	-	1	2	100	100	100	100
33	3	-	-	100	100	100	100
34	-	3	-	100	100	100	100
35	2	1	-	100	100	100	100
36	-	4	-	100	100	100	100
37	1	3	-	100	100	100	100
38	-	1	2	100	100	100	100
39	-	1	3	100	73*	100	91
40	3	1	-	100	100	100	100

Table 3. Percentage of Head Lice highly active, slightly active or dead over time after one hour exposure to One Percent Sodium Chloride Gel.

Time	Highly Active	Slightly Active	Dead
1 hr	31.43 + 20.52	31.43 + 19.44	37.14 + 18.77
2 hr	10.71 + 17.26	40.71 + 25.08	48.58 + 19.33
3 hr	3.57 + 7.86	32.86 + 15.44	63.57 + 17.01
6 hr	0.00 + 0.00	0.71 + 3.19	99.29 + 3.19

Table 4. Percentage of Head Lice highly active, slightly active or dead over time after exposure to One Percent Sodium Chloride Liquid Spray.

Time	Highly Active	Slightly Active	Dead
15 min	95.71+ 19.17	0.0 + 0.0	4.29 + 19.17
30 min	70.72 + 18.23	17.14 + 14.36	12.14 + 20.87
1 hr	48.57 + 18.77	19.29+ 14.86	32.14 + 19.17
2 hr	27.86 + 23.39	25.71 + 19.99	46.43 + 17.26
3 hr	7.14 + 12.69	25.71 + 14.36	67.15 + 16.78
6 hr	0.00 + 0.00	1.43 + 4.4	98.57 + 4.40

Figure 1. Percentage of head lice highly active, slightly active or dead over time after exposure to One Percent Sodium Chloride Liquid Spray.

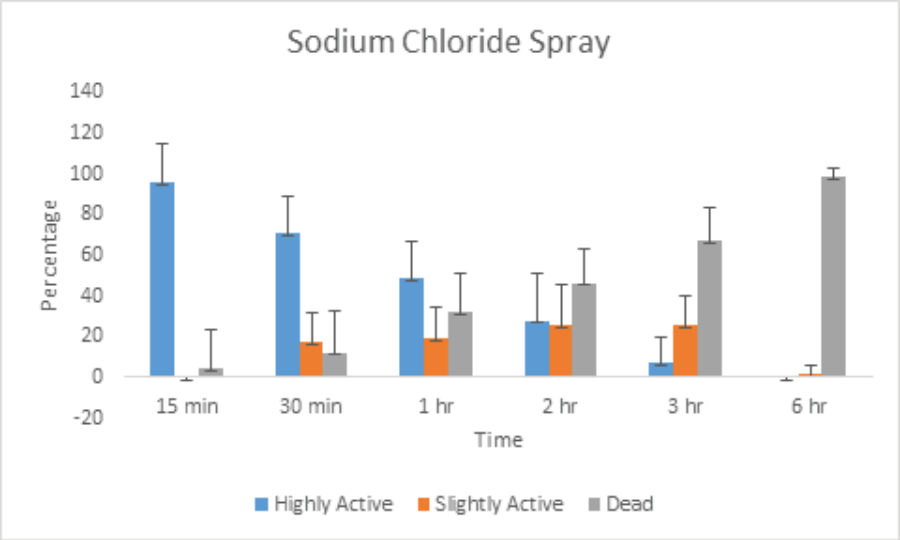


Figure 2. Percentage of head lice highly active, slightly active or dead over time after exposure to One Percent Sodium Chloride Liquid Gel.