Prevention of head lice with the incorporation of menthol propyleneglycol carbonate in hair shampoo

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Well-documented risks to human health, increased awareness on the toxicity of pesticides, pest resurgence and resistance, environmental pollution and infrastructure degradation have prompted the search for environmentally-friendly, safe and non-toxic applications in pest management. To understand the processes influencing insect ecology and behavior, Poseidon embarked on a project that began in 1990 to identify a wide variety of natural biochemicals with the objective of selecting compounds that may be useful in practical situations. Analogues of natural compounds that showed promising results were evaluated for improved efficacy under laboratory conditions. The selection of these compounds took into account their potential for further development, their impact on the environment, and their safety for human use.

As a "generally recognized as safe" (GRAS) compound, menthol would have been an ideal eco-friendly material for a repellent. But studies have documented its low repellent potency. Depending upon formulations and concentrations, the repellent effect of menthol and its isomers are generally less than one hour and therefore are unsuitable for effective use. However, a novel compound derived from menthol has now been identified. This compound, menthol propyleneglycol carbonate (MR-08, MPC), demonstrated a high efficacy as repellent against a variety of insect species. It is referred in Poseidon's inventory and in succeeding references in this presentation as MR-08. The chemical structure is shown in Fig. 1.

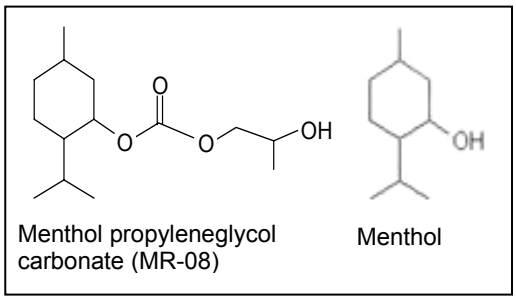

Fig. 1. Structure of Menthol propyleneglycol carbonate (MR-08) compared to menthol.

While ineffective against the Anopheles mosquito that transmits malaria, recent studies conducted by medical entomologists show that the chemical DEET *(N,N-diethyl-m-toluamide)* which is found in varying concentrations in most mosquito repellents on the market today is still the most effective in driving away mosquitoes, including *Aedes aegypti* , the species that carries yellow fever. Concerns over DEET's safety have diminished. However, repellents containing DEET tend to be sticky, have an unpleasant smell, burn when it gets into eyes and on lips, and tend to melt plastic objects and synthetic fabrics that it comes into contact with. Repellents based on plant extracts such as Citronella perform poorly in keeping away mosquitoes and bugs. Mosquito- and fly-borne diseases are still plaguing the world today.

MR-08 is also a GRAS (generally recognized as safe by the US FDA) food ingredient and is referenced as number 3806 in the U.S. FEMA GRAS list and as number 444 on the JECFA (Joint FAO/WHO Expert Committee on Food Additives) list. The latest JECFA assessment conducted in 1999 of menthol and its derivatives, including MR-08, reaffirmed this compound's safety for use as an ingredient in food and cosmetics. The known physical properties of this compound are described in Table 1.

Table 1. Physical, chemical and toxicological properties of menthol propyleneglycol carbonate.

Molecular Weight	Carbonic acid, 5-methyl-2-(1-methylethyl) cyclohexyl ester [1R-(1a, 2ß, 5a)]	
Common Name	menthol propyleneglycol carbonate	
CAS Number	0030304-82-6	
Trade Name	MR-08	
Physical Properties	Form /Color Boiling Point Range Melting Point Flashpoint Ignition Temperature Vapor Pressure Density Solubility in Water Partition Coefficient	liquid / colorless 158.0-173.^{0}C (9.3 mbar) < -21.0^{0}C 153^{0}C c.c. 276.0 ^{0}C < 0.1 mbar (25.0^{0}C) 0.014 g/cm^3 (20.0^{0}C) < 0.100 g/l (20.0^{0}C) n-octanol/water 0.70 (log POW)
Toxicity Profile	Acute Oral Toxicity Acute Dermal Toxicity Ames Test Sensitization Eye Irritation Skin Irritation	LD_{50} >2,000 mg/kg rat LD_{50} >2,000 mg/kg rat no mutagenicity non-sensitizing,guinea pig irritant, rabbit eye non-irritant, rabbit
Ecological Profile	Biodegradability Fish Toxicity Daphnia Toxicity Bacterial Toxicity Algal Toxicity	78% (Strum-Test, LC_{50} 24 mg/ l / 96h Daphnia magna EC_{50} 8.8 mg / l / 48h EC_{50} >62.5 mg / l / 3h EC_{50} 62 mg / l / 72h

Table 2 summarizes the effect of MR-08 in a wide variety of insect pests. In all species studied thus far, we have seen dramatic repellent effects that make this biochemical a unique product for many applications, especially those where food and human contact are involved.

Table 2. Summary of the insect repellent characteristics of menthol propyleneglycol carbonate.

Organisms	Delivery System	Protection Time
Mosquitoes *Culex quinquefasciatus*	Cream/lotion Laboratory test on human volunteers	5 hours, 5% to 40% depending on formulation
	Cream Field test on human volunteers	4 hours, 10% in cream
Houseflies *Musca domestica*	Spray on bait, up to 50% concentration in ethanol	4 hours
Stable flies	50% concentration in ethanol	8 hours
Ants *Monomonium pharaonis*	Impregnation of paper at 30 µg/cm^2	12 hours
Fire Ants *S. invicta*	50% in alcohol, spray	5 hours
Termites *Coptotermes vastator*	24 hour soaking of wood at 20% in ethanol	> 6 months
	Sand barrier test	Complete prevention of termite migration

The Head Lice Problem

The head louse (*Pediculus capitis*) has been part of human existence for millennia (see structure of head lice, Fig. 2). It has been able to adapt to human hair and hold on to the hair for most of its life. When taken away from the human head, the lice typically live for less than 24 hours. Although head lice cause no serious medical problems, head lice infestation, particularly in children, remains a public health issue because of the itching and social stigma attached to their presence in the scalp. Resistance to the typical pyrethrins and pyrethroids normally used to kill head lice are increasing so that management problems now exist. Some lice populations have been documented to show resistance to as many as five different chemicals. Although there has been many reports on the use of 'natural" products, these are typically anecdotal and proven to have no practical value in head lice management. The use of DEET in hair shampoo as a method of deterrence was not effective as well. Thus, the concept of using repellents as part of the management of head lice is not considered a viable one.

Fig. 2. A close up look at the head lice with the egg sac using scanning electron microscope as it grips human hair (from New Scientist magazine, 2004).

Although the use of repellents has not been promising, the potential of MR-08 as an adjunct therapy for head lice was investigated because this chemical has been demonstrated to have multi-functional characteristics. It has been found to be effective against other insects, such as sand flies, that are normally resistant to pesticides and repellents. Also, this chemical is known to have anti-angiogenic and anti-inflammatory activity. It is also known that menthol and related compounds produce a mild analgesic effect. Altogether, this multi-functionality may work in concert to produce a useful compound to treat pesticide resistant head lice populations by incorporating into a shampoo. As a food ingredient, the safety issues on use of MR-08 is considerably reduced compared to other toxic chemicals still in use today.

Experimental Results

Chemicals

Menthol propyleneglycol carbonate (MR-08) was supplied through Poseidon Sciences at >99% purity and added directly (v/v) to Johnson's Baby Shampoo (Johnson & Johnson) with constant mixing. The solution was prepared each day prior to use. The control group comprised those treated with Baby Shampoo alone.

The results in Fig. 3, demonstrated that head lice avoided crawling over surfaces that were impregnated with MR-08.

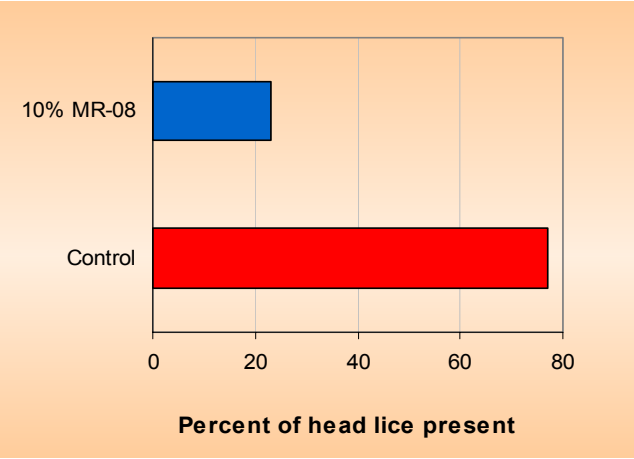

Fig. 3. Repellency of MR-08 in the in vitro filter paper test.

In vitro hair repellency test. Study A. Black human hair in a bundle (10 cm in length) was treated by dipping half of the hair in shampoo containing 10% MR-08 for five seconds and the excess shampoo was removed by wiping with paper towel. The treated hair was air dried for 1 hour at room temperature. The control hair consisted of those treated with shampoo without MR-08. The hair was fixed flat on a plastic petri dish by taping both ends on the surface. After collection, 10 lice were placed at a location between the treated and control hairs and the number of lice that migrated were counted. All the studies were conducted in a darkened room at ambient temperature. The test was conducted in triplicate.

Table 3. The preference of head lice in MR-08 treated and control hair. N=5 samples per group.

Treatment	Number of attached lice Mean ± SEM	P*
MR-08	1.0 ± 0.6	P <0.006
Control	6.7± 0.9	

** Student's t-test*

In vitro hair test. Study B. Hair preparations were as described in Study A. Here, the collected head lice were placed directly on either MR-08 treated or control hair. After 30 minutes, the total numbers of -head lice that have fallen off were counted when the hair was lifted off the surface.

Table 4. The efficiency of attachment of head lice when placed directly on hair treated with or without MR-08. N=5 per group

GROUP	Number of head lice	Number attached	Number detached
Control	50	50	0
3% MR-08	50	49	1
10% MR-08	50	24	26

The data in Table 4 showed that all head lice will completely and firmly attach to the control hair. When 3% MR-08 was added to the shampoo, there was no significant effect on the prior attachment. However, when the concentration was increased to 10%, approximately 52% of the head lice actively detached from the hair.

In vitro hair test. Study C. The study was again repeated with 50 head lice comparing control and 10% MR-08 treated hair using similar procedures as in Study B. The results of this study are presented in Table 5.

Table 5. The ability of head lice to remain attached on the surface in MR-08 treated and control hair. The study was conducted with 10 head lice per test and the data represent the results of 5 replicates with total of 50 head lice per group.

Treatment	Number of detached lice Mean ± SEM	P*
MR-08	13.6 ± 1.4	P <0.006
Control	2.6± 1.1	

** Student's t-test*

Clinical Studies

Twenty-five female children (ages 4 to 12) with confirmed head lice infestation were recruited from the town of Miagao (Iloilo, Philippines) from July to September, 2006. Randomly assigned groups of five children each were placed in both the control group that received only the shampoo and the test groups that received MR-08 shampoo at various concentrations. The scalp of each child was shampooed for total of 3 minutes as shown in Figure 4, rinsed with tap water for 2 minutes and towel dried. Immediately after towel drying, the hair is combed with either a regular comb for one group or a fine-toothed comb in another. The number of head lice that falls out of the hair after combing were counted for each child. The head lice were collected, examined visually and also under a dissecting microscope. The treatments were continued daily, once each day. Each child was interviewed for any comments associated with the treatment process. Special attention was placed on any side effects, possible irritation of the scalp or any relief from the itch associated with head lice infestation.

Fig. 4. A child being shampooed.

MR-08 treatment and wet combing with standard comb.

The effect of low concentrations of MR-08 (1% and 3%) was tested by incorporation in shampoo. In this study, the hair was combed using a standard comb which typically is ineffective in removing head lice. The purpose is to determine if the treatment will enable use of a more convenient hair comb used everyday for normal grooming. The data shown on (Fig.5) demonstrate that 1% MR-08 had minimal effect in removing head lice.

However, increasing the concentration three fold dramatically increased the lice removal process. At 3% MR-08, approximately 72% of the head lice were removed by just normal grooming (Fig. 6).

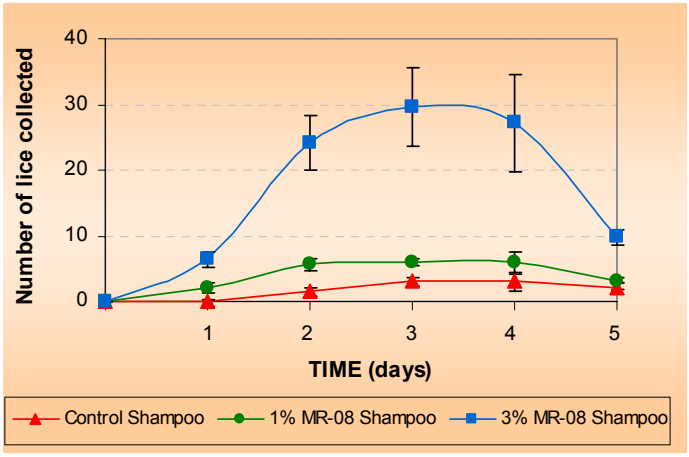

Fig. 5. Removal of head lice in children by treatment with MR-08 and wet combing with a standard comb. N= 5 volunteers per group.

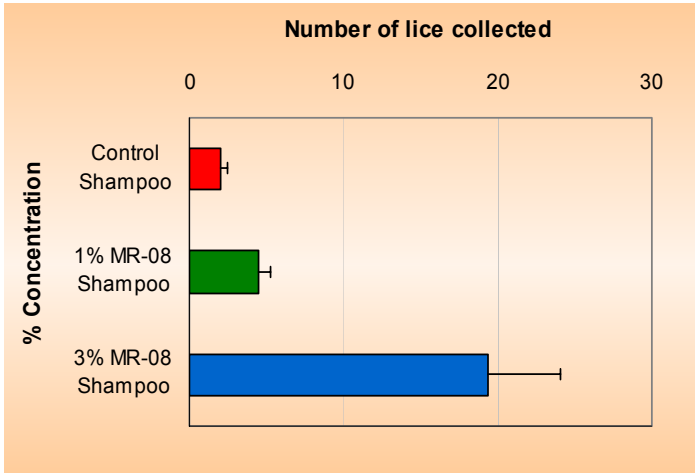

Fig. 6. The total amount of head lice collected using combination of MR-08 shampoo and wet combing with a standard comb.

Discussion

Human head lice infestation is a common condition worldwide, but more prevalent in children. The louse clings to the hair near the scalp and the multiple obligatory feeding each day of the host's blood results in pruritus reaction from the lice saliva. This ectoparasite is totally dependent on being attached to the scalp hair and die within a few hours if not able to re-infect its host. Secondary infection results from the bites being scratched. Scalp itch and erythema are the common external hallmark of lice infestation, with the associated social stigma to the sufferer. Pyoderma of the scalp in Western countries is thought to be primarily caused by lice infestation.

Pesticides, such as pyrethroids and pyrethrins, are the most commonly used chemical method of eradication. In the last decade, the industry has switched to malathion because of the increased tolerance to conventional pesticides. In vitro levels of resistance to permethrin and malathion can reach as high as 87% and 64%, respectively. However, toxicity of the increasingly stronger pesticides is also becoming a concern because the majority of users are children. There are no adequate clinical studies available to document the treatment claims made by companies that promote herbal treatment methods. Even DEET, which is commonly used as an insect repellent, has also been ineffective as a curative treatment of lice infestation.

Menthol is used for centuries for a wide variety of ailments, including as repellent against various types of insects. It has gained a much wider use as a cooling agent for foods and cosmetics. As the industry attempts to develop even better cooling agents, new synthetic molecules have been developed in the past that increased the cooling effect while reducing the associated heavy menthol smell. Since menthol has weak insect control properties, it became apparent that the new synthetic molecules that possess higher cooling efficiency may also have higher efficiency as a repellent than the parent compound. As the recent research on menthol derivatives show, these compounds also have heightened efficacy not only as an insect repellent, but also as anti-inflammatory chemical as well.

However, not all synthetic cooling agents show efficacy in other applications outside of the cooling effect. Structure activity studies show that only certain conformations of menthol derivatives are suitable for non-cooling applications.

While the repellency activity may likely be insufficient as a stand alone a curative for head lice, the combination of cooling, analgesia, anti-inflammatory and repellency may provide a multi-target approach within a single molecule. This concept was explored in this study with the use of menthol propyleneglycol carbonate (MR-08), a GRAS chemical in current use as a cooling agent.

The data presented in this report described the inhibitory effect of MR-08 on attachment of head lice when added to a common shampoo both in laboratory experiments and in clinical studies. In laboratory studies, head lice will preferentially avoid surfaces containing this chemical when presented in a two-choice test with the control hair. When head lice were collected and placed on the surface of hair treated with MR-08 shampoo, the head lice were not able to cling to the hair efficiently and easily fall off the hair. The louse show erratic movements when it tries to attach to MR-08 treated hair. It is important to note that the effect is a transitory one since the louse recovers from this effect and will cling to the hair after a few minutes. Also important to note that the lice remained active and do not show any signs of toxic effects from the MR-08.

The transitory nature of the effect and the nontoxic action of this compound therefore necessitates removal of the lice from the hair as soon as possible. Combing after shampooing and rinsing with water is a common activity. The use of fine toothed comb is typically a cumbersome activity for parents and children alike. The clinical study with MR-08 shampoo and use of a standard comb show that standard combing practice is adequate to remove head lice. Once out of the hair, it is less likely for re-infestation and the lice typically dies in a few hours outside the human scalp. All the children involved in the study did not experience any discomfort or side effects with the MR-08 shampoo treatment.

Besides the more pleasant smell, the MR-08 treated group showed reduced scalp itch at night. The use of MR-08 in shampoo in shampoo therefore introduces a non-toxic, easy to use therapeutic method especially for children and would not introduce an added burden to normal grooming activities. Moreover, the itch that normally comes with head lice infestation is considerably reduced because of the anti-inflammatory action of this compound.

With the use of fine-toothed comb, a more efficient collection of head lice is also possible enabling both options to be available to parents of affected children.

In summary, MR-08 containing shampoo presents a simple, effective, nontoxic alternative to the use of pesticidal compounds in the treatment of head lice infestation.

Selected References

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Intellectual Property Rights

The international patent for MR-08 was published in 24 March 2005 (International Publication Number WO 2005/025313 A1). This PCT patent went into its national phase filings in various countries in March 2006.

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