

Ovicidal Efficacy of Abametapir Against Eggs of Human Head and Body Lice (Anoplura: Pediculidae)

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Abstract

Studies were undertaken to determine the ovicidal efficacy of 5,5'-dimethyl-2,2'-bipyridyl (abametapir) against eggs of both human head and body lice. Head lice eggs of different ages (0–2, 3–5, and 6–8-d-old eggs) were exposed to varying concentrations of abametapir in isopropanol and concentration-dependent response relationships established based on egg hatch. One hundred percent of all abametapir-treated eggs failed to hatch at the 0.74 and 0.55% concentrations, whereas 100% of 6–8-d-old head louse eggs failed to hatch only at the 0.74% concentration. The LC₅₀ value for abametapir varied, depending on the age of the head lice eggs, from ~0.10% recorded for 0–2-d-old eggs and increasing to ~0.15% for 6–8-d-old eggs. Abametapir was also evaluated once formulated into a lotion referred to as Xeglyze (0.74% abametapir) and serial dilutions made. Ovicidal efficacies were determined against head lice eggs 0–8-d-old. Results indicated 100% ovicidal activity at the 0.74, 0.55, 0.37, and 0.18% concentrations. Additional studies undertaken using body lice eggs also demonstrated that abametapir was 100% ovicidal against eggs of all ages when evaluated at a concentration of 0.37 and 0.55%. Given that ovicidal activity is a critical component of any effective treatment regime for louse control, the data presented in this study clearly demonstrate the ability of abametapir to inhibit hatching of both head and body louse eggs as assessed in vitro.

Key words: Head louse (*Pediculus capitis*), Body louse (*Pediculus humanus*), ovicide, abametapir

Infestation by head lice or pediculosis is caused by the ectoparasitic louse, *Pediculus humanus capitis* (De Geer), which infests the hair and scalp of humans. Infestations are most commonly found in children aged from 4 to 13, but can occur in any age group with no particular limitations on sex, race, or social standing (Burkhart et al. 1998). In an attempt to control head lice, a range of treatment options exist in the market place from topical pediculicide therapies to physical removal (Smith and Goldman 2012). In the United States alone, pediculicide sales were estimated at greater than US\$350 million/yr in 2003 (Jones and English 2003, Frankowski et al. 2010), with infestation rates ranging from 6–12 million cases annually (Gratz 1997).

In the case of topical treatment regimes, the over-the-counter (OTC) pediculicides generally require two treatments 7–10 d apart (Burkhart and Burkhart 2006, Feldmeier 2012). This treatment regime is based around the life cycle of the louse, in which the first

treatment is directed against the crawling stages, whereas the second treatment is designed to kill the newly emerged nymphs. The need for a second treatment is driven by a general lack of ovicidal efficacy of OTC head louse products (Mumcuoglu 2006) necessitating a second application to kill newly emerged nymphs that hatched from treated eggs. Furthermore, the requirement for a second application also can lead to issues of compliance, whereby the second treatment may be overlooked or even administered too early after the first treatment, resulting in eggs hatching and the life cycle of the louse perpetuated. Another well-documented issue contributing to treatment failure in the field is resistance to many of the conventional topical insecticides currently in use, including permethrin and malathion (Durand et al. 2012). Given the number of issues that exist with currently marketed pediculicides, further research has been directed toward developing additional novel products in terms of chemistry and mode of action for treating this persistent condition

(Stough et al. 2009, Meinking et al. 2010, Pariser et al. 2012). While several new prescription products have entered the market place recently, the issue associated with the lack of ovicidal efficacy still remains for some.

In order to investigate the potential for improving ovicidal efficacy, an alternate approach to investigate the biochemistry associated with egg hatching in lice was adopted. These studies focused on the hatching process and involved collecting water washings from recently hatched body louse eggs. Once collected, these egg shell washings were analyzed by gelatin zymography for the presence of proteases (Bowles et al. 2008). A number of proteases were identified with further characterization identifying them to be of the metalloprotease class. In addition, their presence in the washings from newly hatched louse eggs indicated that they may play a role in egg hatching in body lice. Data were subsequently obtained showing that when particular metalloproteinase inhibitors were incubated with body louse eggs, the treated eggs failed to hatch. This key finding provided an opportunity to investigate the role of metalloproteinases as novel ovicidal targets in lice. In an extension of this research, we used the model insect, *Drosophila melanogaster*, as a means to better understand the ovicidal effects of the compound 5,5'-dimethyl-2,2'-bipyridyl (formerly termed Ha44, now referred to as abametapir; Van Hiel et al. 2012). Abametapir is a heterocyclic organic molecule that is capable of chelating heavy metal ions, including iron, copper, and zinc, and is therefore able to interact with a range of targets within the insect that require metal co-factors for function, including metalloproteinases. These experiments demonstrated that abametapir was able to arrest *D. melanogaster* embryo development at the blastoderm stage, during the germ band stage, and at a very late stage near the point of hatching, providing further evidence for the ovicidal efficacy of this compound in insects.

This manuscript describes the ovicidal activity of abametapir against eggs of both the head and body louse. The results indicate that abametapir is able to inhibit all stages of embryo development and therefore has the potential to be a highly effective ovicidal pediculicide.

Materials and Methods

Louse eggs from the DDT- and permethrin-resistant SF-HL strain of human head louse (*Pediculus humanus capitis*, De Geer, Anoplura: Pediculidae) were oviposited on tufts of human hair (Lee et al. 2000, Yoon et al. 2003, Yoon et al. 2006). The eggs were topically treated with either abametapir alone in isopropanol or in the formulation referred to as Xeglyze containing 0.74% abametapir. Serial dilutions for 0.74% abametapir were prepared in isopropanol in the following concentrations: 0.55, 0.37, 0.18, 0.09, 0.009, and 0.0009%. Serial dilutions were also prepared from Xeglyze, resulting in the following concentrations: 0.55, 0.37, 0.18, and 0.09%. The Xeglyze formulation without abametapir (termed vehicle: an oil-in-water emulsion containing the following inactive ingredients: water, light mineral oil, polysorbate 20, carbomer 980, trolamine, butylated hydroxytoluene, and benzyl alcohol) was also evaluated, as was the commercially available pediculicide treatment Nix (1% permethrin; INSIGHT Pharmaceuticals, LLC., Trevose, PA). For assessing the efficacy of abametapir alone, a solvent-only control (isopropanol) was included in addition to a distilled, deionized water control (ddH₂O).

The procedure for setting up the ovicidal assay involved using eggs that were laid on hair tufts (~300 strands, ~4 cm in length)

over a 48-h period and collected from rearing units of the in vitro rearing system containing male and female adult lice (~30 of each sex; Yoon et al. 2006, Strycharz et al. 2011, Strycharz et al. 2012). The day that adults were placed into feeding cups of the rearing system was designated Day 0 and development stages were determined from this day onward. After 48 h, adults were removed and the hair tufts with ~150–180 eggs/rearing unit divided in three equal groups (~50–60 eggs per group) and were designated as Group 1 (0–2-d-old eggs), Group 2 (3–5-d-old eggs), or Group 3 (6–8-d-old eggs). Group 1 eggs were treated on Day 2, Group 2 on Day 5, and Group 3 on Day 8, post-infestation of the tufts by adults. In each group, 349–530 eggs/biological replicates were used for ovicidal bioassay with abametapir. Also, 273–363 eggs/biological replicates were used for ovicidal bioassays with Xeglyze formulation in each age group. Tufts with attached eggs were immersed into 0.5 ml of the various treatments for 30 s with swirling to ensure saturation of the tuft with treatment and complete egg coverage and then placed onto a glass petri dish for 10 min at 31°C and 70–80% relative humidity (RH). To ensure saturation and complete egg coverage, the tufts were visually inspected under a stereomicroscope. At the end of the exposure period, treated tufts with attached eggs were sequentially washed in three separate ddH₂O baths (100 mL ddH₂O each bath) with gentle swirling for 30 s per wash, placed on filter paper for blotting, and air-dried for 5 min at room temperature. Dried tufts with treated eggs were then placed into covered sterile glass petri dishes and moved to an incubator at 31°C and 70–80% RH and incubated for 14 d. Egg viability was recorded daily by examining individual eggs for proper size, shape, and color to determine survivorship of eggs throughout their development before and after treatment. The number of lice that hatched from the eggs was recorded and used to determine the percent egg hatch. Undeveloped eggs and stillborn lice were recorded as dead.

Human body louse eggs were from the S.C. Barker isolate that was originally adapted from the Orlando strain of body louse, *P. b. humanus*, at the University of Queensland. The Barker isolate was founded with lice from the isolate of Dr. K. Mumcuoglu from the Hebrew University, Jerusalem. The Orlando strain was originally founded from body lice from a small, but unspecified, number of people in Washington DC and Orlando, Florida, USA, around 1942. The ovicidal assay was conducted using body louse eggs based on the ASTM “Standard test method for effectiveness of liquid gel, cream or shampoo against human louse ova,” designation E-1517-99 (reapproved 2006). Louse eggs were obtained from gravid female *P. b. humanus* by incubating adult lice on cotton cloth at 32°C and 50% RH for 12–16 h. After this period, the lice were removed and eggs still attached to the cloth were collected, counted, and placed in a 12-well tissue culture plate (Falcon; ~20 eggs per well). In each age group, 68–137 eggs/experimental replications were used in ovicidal bioassays with the experimental abametapir formulations. Eggs of different ages were then treated with either an experimental formulation containing 0.55% abametapir or serial dilutions (0.37, 0.18, 0.09, and 0.02%). In addition, the experimental formulation without abametapir (vehicle) and a water control were also evaluated. Treatment involved immersing the eggs into the various formulations for 10 min, followed by removing the treatment and rinsing the cloth containing the eggs for 1 min in 100 mL of distilled water. The treated and rinsed eggs were then placed on a paper towel and blotted dry before each piece of cloth was placed into a clean well of a 12-well tissue culture plate and incubated at 32°C and 50% RH for ~12 d to enable all eggs time to hatch. Undeveloped eggs and stillborn lice were recorded as dead.

The number of lice that hatched from the eggs was recorded and used to determine the percent egg hatch.

Statistical Analysis

The mean percent egg hatch (\pm SD) was determined from three replicate experiments and statistically analyzed using one-way analysis of variance (ANOVA) to determine differences between treatment groups. Tukey's test was performed with ANOVA to determine differences between means if the overall *F* value is significant. Statistical significance was established at the *P* < 0.05 level for all tests. Log % abamectin concentration versus Logit % egg hatch regression lines were generated for the three aged egg groups to determine lethal concentration 50% values (LC₅₀) with their 95% confidence limits (Polo PC, LeOra Software, 1987). Maximum log-likelihood ratio tests were performed on the regression lines to test the equality (slope and y-intercept) between aged egg groups. The null hypothesis that the lines were equal was rejected at a *P* value < 0.05.

Results

Ovicidal Efficacy of Abamectin Against Eggs of the Head Louse

Treatment of head louse eggs with abamectin (0.74% in isopropanol) resulted in complete inhibition of hatching (100% ovicidal) in all stages of egg development from Day 0 to Day 8 (Table 1). At 0.55% abamectin, approximately 5.4% of the 6–8-d-old eggs hatched, in contrast to none of the 0–2- and 3–5-d-old eggs. As the concentration of abamectin declined, egg hatch increased in a concentration-dependent manner. A statistically significant concentration-dependent response was observed for all egg stages, with younger eggs (0–2 d) proving to be the most susceptible (LC₅₀ = 0.108 (0.079–0.13) % abamectin concentration), followed by the 3–5-d-old eggs (LC₅₀ = 0.121 (0.093–0.146) % abamectin concentration), whereas the oldest aged eggs (6–8 d) were most refractory to the treatment (LC₅₀ = 0.160 (0.147–0.174) % abamectin concentration; Fig 1). All three aged egg regression lines were significantly different from each other, as determined by the maximum log-likelihood ratio test (egg

group 1 vs group 2; $\chi^2 = 17.5$, *df* = 2, *P* < 0.001; group 1 vs group 3; $\chi^2 = 107$, *df* = 2, *P* < 0.001; group 2 vs group 3; $\chi^2 = 31.3$, *df* = 2, *P* < 0.001).

Results from this study indicated that the vehicle isopropanol had no significant effect on the egg hatch of the 0–2, 3–5, and 6–8-d-old eggs when compared with their respective ddH₂O controls or between these two age groups (Table 1).

Ovicidal Efficacy of the Xeglyze Formulation of Abamectin Against Eggs of the Head Louse

Abamectin was formulated into a lotion referred to as Xeglyze (0.74% abamectin). This formulation and its dilutions were assessed against head louse eggs of all ages, as described earlier. The results from these studies indicated that Xeglyze acted as a highly effective ovicide against all stages of eggs at the 0.74, 0.55, 0.37, and 0.18% abamectin concentrations, in which 100% of treated eggs failed to hatch. At the lowest concentration of Xeglyze tested (0.09% abamectin), only 1.4% of the 3–5-d-old eggs hatched

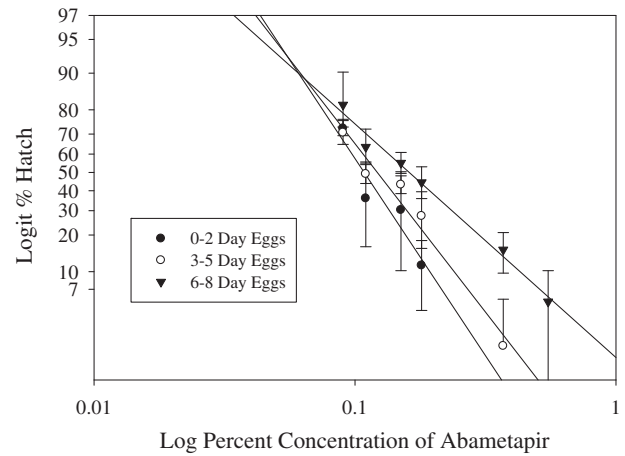


Fig. 1. Log percent abamectin concentration versus logit % hatch regression analysis of three groups of aged head louse eggs.

Table 1. Comparisons of the percent hatch of DDT- and permethrin-resistant head louse (SF-HL) eggs treated with various concentrations of abamectin in isopropanol

Treatment ^d	Group 1: 0–2-d-old eggs	Group 2: 3–5-d-old eggs Percent hatch, mean \pm SD ^{b,c} , (N ^d)	Group 3: 6–8-d-old eggs
ddH ₂ O	92.1 \pm 0.5 ^{a,A} , (163)	96.0 \pm 2.9 ^{a,A} , (123)	94.3 \pm 7.6 ^{a,A} , (112)
Isopropanol ^e	84.6 \pm 2.3 ^{a,A} , (129)	95.8 \pm 3.7 ^{a,B} , (81)	96.3 \pm 3.2 ^{a,B} , (103)
Abamectin concentration, %			
0.0009	82.0 \pm 1.2 ^{a,A} , (128)	94.2 \pm 2.5 ^{a,B} , (89)	93.8 \pm 2.1 ^{a,B} , (93)
0.009	75.3 \pm 2.3 ^{a,A} , (143)	82.5 \pm 11.2 ^{a,A} , (112)	89.6 \pm 1.7 ^{a,A} , (144)
0.09	72.6 \pm 3.3 ^{a,A} , (156)	70.7 \pm 5.7 ^{b,A} , (99)	81.7 \pm 8.5 ^{a,A} , (85)
0.11	36.0 \pm 19.8 ^{b,A} , (117)	49.1 \pm 5.2 ^{c,A} , (125)	63.7 \pm 8.6 ^{b,A} , (122)
0.15	30.3 \pm 20.1 ^{b,A} , (165)	43.3 \pm 4.8 ^{c,d,A} , (95)	55.2 \pm 5.7 ^{b,c,A} , (95)
0.18	11.3 \pm 6.8 ^{b,A} , (121)	27.6 \pm 11.9 ^{d,A,B} , (142)	44.5 \pm 8.6 ^{c,B} , (91)
0.37	0, (147)	2.1 \pm 3.6 ^c , (110)	15.3 \pm 5.6 ^d , (112)
0.55	0, (167)	0, (99)	5.4 \pm 4.8 ^d , (107)
0.74	0, (156)	0, (182)	0, (83)

^a In each treatment, data from three biologically replicated experiments were analyzed to obtain mean \pm SD.

^b Means \pm SD in the same column followed by the same lowercase letter are not statistically different by ANOVA (*P* > 0.05).

^c Means \pm SD in the same row followed by the same uppercase letter are not statistically different by ANOVA (*P* > 0.05).

^d N, total number of eggs.

^e Isopropanol is a solvent vehicle for application.

whereas, no eggs hatched in either the 0–2- or 6–8-d-old eggs at this concentration (Table 2).

For the vehicle formulation, which lacked abametapir, a significant ($P < 0.05$) reduction in the percent egg hatch was observed compared with the ddH₂O control, where approximately 70% of the 0–2-d-old eggs hatched compared with ~90% in the ddH₂O control. Percent hatch declined to 55% in the 3–5-d-old eggs and to 45% for the 6–8-d-old eggs. A very similar response in egg hatching was also observed for louse eggs treated with Nix, in which there was a trend for the older eggs to be more susceptible to the treatment.

Ovicidal Efficacy of Abametapir in a Prototype Formulation Against Eggs of Body Louse

Body louse eggs of different ages were assessed for their susceptibility to a prototype formulation containing from 0.02% through to 0.55% abametapir (Table 3). Both the 0.55 and 0.37% concentrations of the formulation containing abametapir resulted in 100% ovicidal activity against 0–7-d-old body louse eggs. Egg hatching was observed at 0.18% abametapir and continued to increase as the concentration of abametapir in the formulation was reduced. As observed previously with the head louse eggs, the trend for the younger eggs to be more susceptible to treatment than the older eggs

was also observed for the body louse eggs. Approximately 86% of the eggs hatched in the vehicle treated groups, with a very similar level of hatch being observed across all ages of eggs. The percent hatch in the presence of vehicle was also similar to that observed for the ddH₂O control for the 3–5-d-old eggs, but was significantly lower ($P < 0.05$) for the 0–2- and 6–7-d-old eggs.

It was interesting to note that at concentrations of abametapir that were 100% ovicidal, the development within the eggs appeared arrested at the stage of treatment. This effect was observed for both head and body louse eggs (data not shown).

Discussion

The present study established concentration-dependent ovicidal responses in all three different developmental stages of DDT- and permethrin-resistant head louse (SF-HL) eggs using abametapir dissolved in isopropanol. This finding suggests that head lice may have orthologues of the pharmacological targets, metalloproteinases, mentioned above in *D. melanogaster*. While 100% mortality responses at 0.74% abametapir were determined regardless of their developmental stages (Table 1), we found statistical significances in different ages of eggs, younger the eggs, more susceptible to abametapir treatments (Fig. 1). However, differences of LC₅₀ values

Table 2. Comparisons of the percent hatch of DDT- and permethrin-resistant head louse (SF-HL) eggs treated with various concentrations of Xeglyze formulation, vehicle (a vehicle formulation without the active ingredient, abametapir), Nix (a formulation containing 1% permethrin) and ddH₂O

Treatment ^a	Group 1: 0–2-d-old eggs	Group 2: 3–5-d-old eggs Percent hatch, mean \pm SD ^{b,c} , (N ^d)	Group 3: 6–8-d-old eggs
ddH ₂ O	90.1 \pm 1.9 ^{a,A} , (112)	80.0 \pm 10.1 ^{a,A} , (135)	89.7 \pm 3.9 ^{a,A} , (89)
Vehicle	69.6 \pm 5.5 ^{b,A} , (94)	55.3 \pm 2.5 ^{b,B} , (214)	44.6 \pm 3.5 ^{b,C} , (94)
Nix	72.8 \pm 3.4 ^{b,A} , (145)	63.3 \pm 7.1 ^{a,b,B,C} , (155)	41.5 \pm 14.8 ^{b,C} , (71)
Xeglyze, %			
0.09	0, (111)	1.4 \pm 2.4 ^c , (126)	0, (138)
0.18	0, (91)	0, (130)	0, (107)
0.37	0, (88)	0, (93)	0, (120)
0.55	0, (122)	0, (111)	0, (98)
0.74	0, (131)	0, (125)	0, (103)

^a In each treatment, data from three biologically replicated experiments were analyzed to obtain mean \pm SD.

^b Means \pm SD in the same column followed by the same lowercase letter are not statistically different by ANOVA ($P > 0.05$).

^c Means \pm SD in the same row followed by the same uppercase letter are not statistically different by ANOVA ($P > 0.05$).

^d N, total number of eggs.

Table 3. Comparisons of the percent hatch of body louse eggs treated with various concentrations of an experimental abametapir formulation, vehicle (a vehicle formulation without the active ingredient, abametapir) and ddH₂O

Treatment ^a	Group 1: 0–2-d-old eggs	Group 2: 3–5-d-old eggs Percent hatch, mean \pm SD ^{b,c} , (N ^d)	Group 3: 6–7-d-old eggs
ddH ₂ O	96.8 \pm 3.9 ^{a,A} , (151)	89.4 \pm 7.4 ^{a,A} , (173)	95.3 \pm 0.58 ^{a,A} , (64)
Vehicle	85.4 \pm 6.2 ^{b,A} , (159)	86.4 \pm 8.6 ^{a,A} , (189)	87.9 \pm 3.9 ^{a,A} , (113)
Abametapir concentration, %			
0.02	84.4 \pm 6.1 ^b , (73)	84.3 \pm 2.2 ^a , (101)	N/A
0.09	39.5 \pm 24.9 ^c , (89)	66.6 \pm 26.4 ^a , (100)	N/A
0.18	5.3 \pm 4.7 ^d , (86)	11.2 \pm 6.1 ^b , (103)	N/A
0.37	0, (147)	0, (183)	0, (114)
0.55	0, (120)	0, (171)	0, (117)

^a In each treatment, data from six to nine experimental replicates were analyzed to obtain mean \pm SD.

^b Means \pm SD in the same column followed by the same lowercase letter are not statistically different by ANOVA ($P > 0.05$).

^c Means \pm SD in the same row followed by the same uppercase letter are not statistically different by ANOVA ($P > 0.05$).

^d N, total number of eggs.

between the three age groups are only 1.1- to 1.3-fold, indicating that these differences may be negligible for the purpose of controlling head lice that are sensitive to abametapir.

During *in vitro* studies comparing the formulated product Xeglyze to the unformulated abametapir in isopropanol, complete ovicidal activity was observed across all stages of egg development at concentrations as low as 0.18% in the formulated product and 0.74% abametapir in isopropanol. This result indicates that a component(s) in the Xeglyze formulation enhances the ovicidal effect of abametapir.

The body louse has long been recognized as a surrogate for evaluating active ingredients for the control of head lice, and hence, it was interesting to compare the activity of abametapir against these two subspecies. The ovicidal results obtained indicate that body louse eggs of all ages were also susceptible to abametapir. Given the known similarities between these two subspecies/ecotypes (Olds et al. 2012), it is perhaps not surprising that both head and body louse eggs are susceptible to this compound.

The inability to develop effective ovicides has been the “Achilles heel” of products for treating louse infestations where it is generally recognized that no topical OTC pediculicide (usually containing natural pyrethrins or synthetic pyrethroids such as permethrin) is 100% ovicidal (Barker and Altman 2011). As a result, the recommended treatment regimens typically advise that pediculicides should be applied twice with a gap of approximately 7–10 d following the first treatment to kill newly emerged nymphs (Burkhart and Burkhart 2006, Feldmeier 2012).

Compounds that have been used for treating head louse infestations have traditionally targeted the nervous system of the insect and include lindane, pyrethrin, pyrethroid, organophosphorus, and carbamate insecticides. While the precise timing of the development of the nervous system in lice has not been described, this phenomenon is known to be highly conserved among insects (Thomas et al. 1984). An examination of the neuronal system development of the locust *Locusta migratoria*, which hatches after 13 d at 30°C, reported that neuron differentiation in the ganglia of the thorax and anterior abdomen to produce axons occurs 96 h after egg laying (Bate and Grunewald 1981). From these studies, it is not unreasonable to assume that the louse neuronal system would be developing in the first 2–3 d post laying (early stage; Cueto et al. 2006). Therefore, these neurotoxic compounds would not be expected to affect freshly laid eggs. A reliance on persistence, however, is one means by which this issue has been partially addressed (Meinking et al. 1986). If we examine the pyrethroid insecticide, permethrin, for example, this compound acts on the voltage-sensitive sodium channels (VSSC) of the nervous system of insects, where it prolongs inward sodium currents, leading to nerve cell depolarization and hyperexcitation, followed by muscle paralysis and death (Yoon et al. 2014). It is therefore not surprising that permethrin is not highly ovicidal, given its site of action (VSSC) and presumably the low abundance of these targets during early embryo development. More recently, data were published on the efficacy of ivermectin as a topical pediculicide (Pariser et al. 2012), with this compound now also approved for use in treating head louse infestations in the United States. Ivermectin increases chloride permeability in insect nerve and muscle membranes (Kane et al. 2000) through binding to glutamate-gated chloride channels. It also affects the nervous system via *gamma*-aminobutyric acid (GABA)-gated chloride channels in vertebrates and invertebrates (Bloomquist 2003) and histamine-gated chloride channels in insects (McCavera et al. 2007). In the case of the head louse, ivermectin is not directly ovicidal (Strycharz et al. 2011). Ivermectin does, however, adversely affect the newly

emerged nymph’s ability to feed, leading to speculation that ivermectin acts as a posteclosion nymphicide by targeting the glutamate-gated chloride channels of the piercing–sucking mouthparts of the nymph (Strycharz et al. 2011). Exposure of the nymph was considered to be either through the ivermectin penetrating the egg itself or through residual ivermectin located on the outside of the egg that the newly emerged nymph comes into contact upon hatching.

Benzyl alcohol (5%) has also been approved as a prescription head louse treatment in the United States and while not acting as a neurotoxin, is considered to kill lice through asphyxiation. This product is also not ovicidal (Frankowski et al. 2010). Another approved prescription head louse product contains spinosad (Stough et al. 2009). Spinosad has been reported to exhibit some ovicidal activity *in vitro* against both head and body louse eggs (Cueto et al. 2006) and is known to alter the function of nicotinic acetylcholine receptors and GABA-gated chloride channels (Sparks et al. 2001). Both targets are present in nymphs and adult louse and would be expected to be present in older eggs as their nervous system develops, but not in very young eggs. The finding that all stages of louse eggs treated with spinosad were apparently of similar susceptibility was considered to be associated with poor metabolism of the insecticide in the immature embryos enabling an accumulation of the insecticide within the developing egg. As the embryo matured and the relevant targets became present in the more mature eggs, spinosad was able to exert its effects. This hypothesis was consistent with the finding that nymphs within treated eggs almost completed their development before they died *in situ*.

In contrast, abametapir-treated eggs failed to develop beyond the stage at which they were treated, suggesting that targets, perhaps multiple targets, are present within the developing louse embryo over time that are affected by abametapir. Previous studies in *D. melanogaster* indicated that abametapir was capable of arresting a number of stages during embryogenesis. These inhibitory effects, however, were reversible following the addition of certain metal ions (Fe, Cu, and Zn; Van Hiel et al. 2012). This finding is consistent with the proposed mechanism of action of abametapir, which, as a metal chelating agent, is capable of inhibiting metal-dependent processes, including metalloproteinases, in the louse egg. Indeed, abametapir has previously been shown to inhibit a purified metalloproteinase Meprin A (Van Hiel et al. 2012), which has a similar protease domain structure to Zn metalloproteinase, Astacins, which are known to be involved in developmental morphogenesis in a number of species (Sterchi et al. 2008). Interestingly, the effects of the additions of Fe, Cu, and Zn to first-instar larvae differed from those additions made to eggs. In larvae, the addition of Fe greatly reduced the mortality response to abametapir as it did in eggs. However, the effects of Cu and Zn additions were much less dramatic than in eggs, indicating that the targets in eggs and larvae may differ (Van Hiel et al. 2012).

Given that the majority of insecticides commonly act against a very limited number of targets (Casida 2009), the potential for the selection of mutations giving rise to target site insensitivity in a particular target following widespread use is predicted to be higher than that seen with compounds that target multiple targets, increasing the probability of resistance developing against a single target compound (Perry et al. 2007, Wang et al. 2009). In the case of head lice, resistance has already been reported to a number of the currently used active ingredients that target one or a limited number of targets (Durand et al. 2012). Therefore, compounds that work against multiple targets are critical for the development of more effective and sustainable pediculicides for treating this ectoparasitic infestation.

The results of the current *in vitro* studies comprehensively demonstrate the ovicidal activity of abametapir. The novel chemistry and unique predicted mechanism of action of abametapir represents a new approach that addresses ovicidal efficacy as a key component of an effective treatment that targets the entire life cycle of the head louse. Additional studies will assess the safety and efficacy of this new approach to treating head louse infestations in the field.

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