

Recommendation to Standardize Pediculicidal and Ovicidal Testing for Head Lice (Anoplura: Pediculidae)

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ABSTRACT *Pediculus capitis* is a prevalent and highly communicable condition infesting millions of elementary school students annually. Topical insecticides are the present standard treatment for this condition. Because resistance of head lice to insecticides is a growing concern, assessment of efficacy of pediculicidal and ovicidal activity of the various agents is needed for public health interests. Given the number of anecdotal and market-driven reported studies, assessment of topical lice therapies requires standardized testing. Evaluations based on adaptations of World Health Organization guidelines are not ideal, whereas a protocol reflecting clinical exposure to insecticides is preferable.

KEY WORDS *Pediculus humanus capitis*, pediculicidal testing, ovicidal testing, *pediculosis capitis*, head lice

Pediculus capitis, or head lice, is caused by the highly host-specific insect, *Pediculus humanus capitis* De Geer. These wingless insects are obligate parasites of humans and have been a common companion of the human species since antiquity (Filer 1996). There are several treatment options for head lice, which include topical and oral, prescription and over-the-counter treatments for head lice (Burkhart and Burkhart 1998). With growing concerns of resistance of head lice to specific insecticides, a standardized in vitro technique to determine efficacy of various insecticides should be adopted by the World Health Organization (WHO) and professed experts in the field. Given the number of anecdotal and market-driven reported studies, and in the interests of public health, rational assessment of topical lice therapies requires standardized testing to determine pediculicidal and ovicidal function for the benefit of the public.

In the case of body lice, *Pediculus humanus humanus* L., the WHO developed a standardized in vitro resistance test in 1953 that used stocking cloth with dried insecticide powders (Rao 1958). WHO later revised the test on two occasions by using insecticide-impregnated papers but at concentrations of insecticide that were not necessarily at clinical or therapeutic doses (WHO 1976, 1981). Exposure times to various insecticides varied under WHO guidelines from 5 h to 1 d, and mortality was determined 24 h after exposure. Difficulties with the WHO protocol have surfaced, such as excessive control mortality within the testing time frame, and the mechanics of placing lice on rectangular test paper and covering them with a petri dish

weighted down to prevent escape (Zeichner 1999). Zeichner (1999) has recently modified the WHO test to allow for a shorter holding period and more practical handling of lice.

Of note, resistance testing is designed to determine shifts in population's susceptibility to a given insecticide. A particular treatment may be efficacious despite development of resistance mechanisms. However, the presence of low-level resistance may result in a rapid selection of higher-level resistance reducing the efficacy of treatment after continued selection pressure. Colonized body lice, which are available for body lice (Zeichner 1999), are relevant in comparing field lice populations, thereby gaining insight into whether or not any resistance has developed. In the interests of public health, however, efficacy testing is key. Efficacy testing concerns itself with whether a given insecticide dose controls head lice in a given population at the current time. Efficacy can be present even in the presence of some resistance mechanisms developing. Because continued usage of insecticides likely would enhance resistance mechanisms to levels in which the dose is no longer effective, efficacy testing would need to be monitored in specific populations. Moreover, efficacy testing could be used to monitor resistance. In this scenario, the lowest effective dose of insecticide would be monitored within a designated population over time to determine when efficacy was no longer achieved. Notably, a standardized method for determining efficacy of various head lice therapies for public assessment has not been adopted by the WHO or research community.

With head lice, no laboratory colonies exist because all attempts to colonize have failed. However, reasonable numbers of insects for testing can be obtained by finger grasping individual head lice while foraging through patients' scalps, or by raking a comb, such as

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Lice Meister (Stock #50197, National Pediculosis Association, Newton, MA), through sections of the scalp and removing adult lice captured between the metal teeth of the comb. Lice eggs (unhatched nits) from patients' scalps are easily removed for testing as well, by sniping individual hairs with nits attached from infested individuals.

For the most part, testing protocols for body lice relating to resistance testing have been the prototype for all lice species (Rao 1958; WHO 1976, 1981). Zeichner's (1999) modifications to the WHO 1981 protocol does offer a standardizable test, but it is not optimum for head lice analysis. Two recent clinical *in vitro* studies have attempted to adapt WHO guidelines for application to resistance testing with head lice (Downs et al. 1999, Pollack et al. 1999). However, a protocol for efficacy usage with head lice should more closely parallel the anticipated exposure to insecticide in clinical usage. Because head lice are treated usually with medicated shampoos, placing insects on insecticide-impregnated paper for several hours (Downs et al. 1999, Pollack et al. 1999), is not as appropriate as immersing insects for the clinical duration of insecticide exposure. After such a dip method of insecticidal exposure, the lice are then rinsed, dried, and observed for several hours. Moreover, by only exposing lice to treated paper, one neglects possible penetration of insecticides via the alimentary tract, as well as distorts normal diffusion kinetics through the cuticle. Additionally, in assays with impregnated discs, lice dwell in a fully enclosed environment with a petri dish upside down, seemingly giving an advantage to more volatile agents that could be absorbed through the spiracles. A perfect protocol would not offset the normal pharmacokinetics of absorption, distribution, metabolism, and excretion observed in the clinical arena. In short, *in vitro* testing, which emulates clinical usage with the therapeutic agent for head lice, produces results that mirror full-scale clinical trials (Meinking et al. 1986) and provides usable information of efficacy to the general public.

Returning to the modifications offered to WHO body lice testing (Zeichner 1999), after 6 h "the petri dish is turned on its side and lightly tapped on the table. Those lice that are unable to cling to the chromatography paper are counted as knocked down." Given that head lice claws are designed to grab hair, this method may be inappropriate because they may not be able to sufficiently grip the paper. More importantly, the term 'knocked down' needs clarification for proper assessment of insect viability. In adult insects, the 'knockdown' action of insecticides such as with pyrethroids, is defined as the rapid loss of coordinated locomotion and posture (Bloomquist and Miller 1986a). Knockdown resistance is a mechanism associated with the *kdr* and super-*kdr* genes, by which insects develop resistance by modification in the sodium channel sensitivity affecting binding of certain insecticides. Although knockdown time can be a good indicator for susceptibility and early detection of some forms of insecticide resistance (Chandre et al. 1999),

it is more appropriate to assess lethal effects of therapeutic agents, rather than behavioral end points.

A 2- to 18-h exposure to insecticides in the above-mentioned protocols (Downs et al. 1999, Pollack et al. 1999, Zeichner 1999) may greatly exceed treatment duration, thereby diminishing clinical correlation. Depending on the insecticide's site of action and penetration capabilities from the impregnated disc, insecticidal effects may, or may not, be fully achieved within the designated time frame. An overlooked complicating factor is the ability of lice to assume an apparent moribund state, before later resurrounding from seemingly death. It has been well proven that insects have the ability to recover from insecticidal poisoning (Bloomquist and Miller 1986b). In short, insects are less dependent than mammals for continuous nervous control of respiration and circulation. The exact point of death is not readily defined. With efficacy testing in head lice, the end point must reflect irreversible morbidity or death. Thus, death cannot be simply defined as lack of motor activity on probing (Pollack et al. 1999), or irreversible intoxication defined as paralysis or continuous tonic-clonic spasm (Downs et al. 1999), because even cessation of normal body functions such as physical movements and gut peristalsis need to be documented for several hours to confirm expiration (Meinking et al. 1986).

Understanding that head lice can appear dead temporarily yet resurrect within a few hours partially explains the many claims of successful cures with alternative treatments including petroleum jelly, hair pomades, olive oil, mayonnaise, solid vegetable shortening, vinegar, mineral oil, and essential oils from health food stores (Meinking et al. 1999). Such products slow the movements of adult lice and may allow them to be more easily combed out of their scalps, but these substances are not lethal to lice (Meinking et al. 1999). For example, after placing an adult louse in petroleum jelly for 1 h, rinsing it off with water, and then drying it on filter paper, the louse will retain full motor function within 10 m.

For consideration is an *in vitro* pediculicidal test in which head lice are exposed to insecticides for similar duration, concentration, and exposure imitating routine clinical usage. Such a test would be reproducible and its results would reflect results obtained in clinical trials. In short, the standardized model of insecticidal killing would be merely a modification of the Meinking et al. (Meinking et al. 1986) detailed original protocol. For example with testing of a specific medicated shampoo, lice would be immersed into the same dilution and for the same time as clinical protocol dictates. Lice would then be transferred into a container of water simulating the rinsing of medication from the hair, before being placed on a cloth disc dampened with filter water. Lice activity would be monitored continuously both visually and by light microscopy for the first hour, followed by 30-m vigils thereafter. Even if all motor and respiratory function cease, monitoring should continue optimally for 24 h. (Of note, lice mortality after 24 to 36 h due to physiologic desiccation is normal.)

Ovicidal activity, which may be more relevant than pediculicidal function, should also be tested with a standardized protocol, and again would follow the model developed by Meinking (1986). First, one must examine nits using light microscopy to prove an intact larva exists. The features that point to a viable egg are existence of tan to brown oval egg capsule often with a visible eye spot on the developing larva, with an intact operculum. Hatched eggs are clear to white clinically, and by light microscopy, an empty shell is noted with a ruptured operculum (Meinking et al. 1986, Meinking 1999). Because eggs are laid close to the base of hair follicles, the distal ends of the hairs can be attached to small adhesive labels to facilitate transport of eggs to test solutions of insecticides for designated time intervals, followed by rinsing in tap water, and then air dried at ambient temperature (Meinking et al. 1986). The eggs are then incubated in the dark and then observed after 2 wk. Ovicidal activity is determined by the number of hatched eggs counted after that time duration.

Because controlled clinical trials are costly, time-consuming, and not always feasible, reliable *in vitro* pediculicidal and ovicidal tests of the various lice therapies are of paramount importance. Given the number of anecdotal and market-driven reported studies, a standardized protocol is essential for comparative analysis. As stated, evaluations based on adaptations of WHO guidelines are not ideal for the needs of the general public, whereas a protocol that reflects clinical exposure to insecticides in clinical usage is clearly preferable. Adhering to Meinking's proposed testing serves as a just analysis of the various anti-lice agents in a reproducible manner that genuinely reflects expected results in clinical trials.

References Cited

- Bloomquist, J. R., and T. A. Miller. 1986a. Sodium channel neurotoxins as probes of the knockdown resistance mechanism. *Neurotoxicology* 7: 217-224.
- Bloomquist, J. R., and T. A. Miller. 1986b. Neural correlates of flight activation and escape behavior in horseflies covering from pyrethroid poisoning. *Arch. Insect Biochem. Physiol.* 3: 551-559.
- Burkhart, C. G., and C. N. Burkhart. 1998. An assessment of topical and oral prescription and over-the-counter treatments for head lice. *J. Am. Acad. Dermatol.* 38: 979-982.
- Chandre, F., F. Darrier, L. Manga, M. Akogbeto, O. Faye, J. Mouchet, and P. Guillet. 1999. Status of pyrethroid resistance in *Anopheles gambiae sensu lato*. *Bull. WHO* 77: 230-234.
- Downs, A.M.R., K. A. Stafford, I. Harvey, and G. C. Coles. 1999. Evidence for double resistance to permethrin and malathion in head lice. *Br. J. Dermatol.* 141: 508-511.
- Filer, J. 1996. Egyptian bookshelf diseases. University of Texas Press, Austin, TX.
- Meinking, T. L., C. G. Burkhart, and C. N. Burkhart. 1999. Ectoparasitic diseases in dermatology: reassessment of scabies and pediculosis, pp. 67-108. *In* W. James [ed.], *Advances in dermatology*, vol. 15. Mosby, St. Louis, MO.
- Meinking, T. L. 1999. Infestations, pp. 78-118. *In* J. P. Callen [ed.], *Current problems in dermatology*, vol 11. Mosby, St. Louis, MO.
- Meinking, T. L., D. Taplin, D. C. Kalter, and M. W. Eberle. 1986. Comparative efficacy of treatments for pediculosis capitis infestations. *Arch. Dermatol.* 122: 267-271.
- Pollack, R. J., A. Kiszewski, P. Armstrong, C. Hahn, N. Wolfe, H. A. Rahman, K. Laserson, S. R. Telford, and A. Spielman. 1999. Differential permethrin susceptibility of head lice sampled in the United States and Borneo. *Arch. Pediatr. Adolesc. Med.* 153: 969-973.
- Rao, T. R. 1958. Resistance tests for lice, pp. 180-181. *In* Report on WHO, PAHO Seminar on susceptibility of insects to insecticide. Pan Am Sanitary Bureau, Regional Office of WHO, Washington, DC.
- [WHO] World Health Organization. 1976. Resistance of vectors and reservoirs of disease to pesticides. 22nd Report of the WHO Expert Committee on Insecticides. Technical Report Series 585. WHO, Geneva.
- [WHO] World Health Organization. 1981. Instructions for determining the susceptibility or resistance of body lice and head lice to insecticides. WHO/VBC/81.808.
- Zeichner, B. C. 1999. Baseline susceptibility of a laboratory strain of *Pediculus humanus humanus* (Anoplura: Pediculidae) using a modified World Health Organization testing protocol. *J. Med. Entomol.* 36: 903-905.

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