

Clinical trial

## International guidelines for clinical trials with pediculicides

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### Introduction

The head louse is an obligate parasite that spends its entire life on its human host. Head lice feed exclusively on blood, and humans are the only known hosts. Typically, head lice transmit to a new host only when there is head-to-head contact. Head lice are found in most ethnic groups at all socioeconomic levels. The number of children per family, degree of crowding, amount of sharing of beds, local customs, number of social contacts, standard of healthcare, and socioeconomic status all contribute to the epidemiology of head louse infestations. Girls tend to be more frequently infested than boys after four years of age. Children between 3 and 14 years old are most frequently infested, although infants, adults, and the elderly can be infested as well. The most characteristic symptom of pediculosis capitis is pruritus of the scalp, which may begin 1–4 weeks after the initial infestation; however, subsequent infestations may result in itching

### Abstract

Pediculosis capitis, infestation with head lice, is common in all human societies. Chemical pediculicides are often used to control head louse infestations, particularly in wealthy communities. A significant number of different protocols have been used to test the efficacy and safety of pediculicides in clinical trials; this constrains scientific comparison of the evidence for efficacy of the different pediculicides. Here we recommend protocols for clinical trials of the efficacy and safety of single-, two-, and three-treatment interventions.

within a day. The itch–scratch cycle can result in secondary bacterial infection, leading to impetigo and pyoderma. Swelling of the local lymph nodes and fever is rare in developed countries but can be common in poor countries. Generalized prurigo-like allergic dermatitis due to antigens from lice has also been reported.<sup>1–3</sup> The direct and indirect cost of head louse infestation in the USA has been estimated to be \$1 billion annually.<sup>4</sup>

A significant number of different protocols have been used to test the efficacy of pediculicides in clinical trials. This constrains scientific comparison of the evidence for efficacy of the different pediculicides. The Cochrane review of clinical trials with pediculicides criticized the protocols of almost all of the 71 studies reviewed<sup>5</sup> but was withdrawn thereafter. Since 2004, 30 clinical trials of pediculicides have been registered at the US National Institute of Health (<http://clinicaltrials.gov/>) and a further 16 with the International Clinical Trials Registry Platform (<http://www.controlled-trials.com/isrctn/>); yet a standard

protocol for such clinical trials does not exist. This issue was the topic of a workshop at the Fourth International Congress on Phthiraptera, Cappadocia, Turkey, in 2010 (<http://www.icp4.org/>). This workshop and the subsequent discussion led to protocols that we recommend here for clinical trials on the efficacy and safety of single-treatment, two-treatment, and three-treatment interventions.

## Recommended protocols

### Assessment of the safety and efficacy of a single-treatment intervention, i.e. for claims that one treatment with the pediculicide will kill all lice and eggs so that the subject is louse-free

*Day 0 – treatment.* The outcome of the treatment can be assessed by visual examination of the hair and scalp and/or post-treatment water-rinsing of the hair into a plastic container, followed by straining of the water through a “flour-sack” towel.

*Day 1 – assessment by thorough visual examination (defined below).* If nymphs and adults are not found during the visual examination, the hair should be examined by dry- or wet-combing (defined below). Lice caught by the comb should be discarded.

*Day 10 – final assessment by visual examination and if negative then by either dry- or wet-combing.* Wet-combing is invariably more sensitive for the detection of light infections than dry-combing. Thus, wet-combing is recommended for the final assessment. The final assessment may be on Day 11 if the subject is not available on Day 10.

Up to four optional dry-combing assessments, with visual examination, may be added from Day 2 to Day 9. Because the aim of this type of protocol is to identify whether or not a product is successful using a single application of product, finding lice at any of these assessments indicates treatment failure.

We note that pediculicides that kill all lice and eggs on the scalp are easy to use and therefore highly desirable. Accordingly, pharmaceutical companies should aim to make pediculicides that will cure infections with a single treatment.

### Assessment of the efficacy and safety of a two-treatment intervention, i.e. for claims that two treatments with the pediculicide will kill all lice and eggs so that the subject is louse-free

*Day 0 – treatment 1.* The outcome of the treatment can be assessed by visual examination of the hair and scalp, and/or post-treatment water-rinsing of the hair into a plastic container, followed by straining of the water through a “flour-sack” towel.

*Day 1 – assessment by thorough visual examination.* If nymphs and adults are not found during the visual exami-

nation, the hair should be dry-combed. Lice caught by the comb should be left in the hair. Lice that survived this treatment might conceivably die during the next 10 days as a result of the intoxication, but it is likely that they would continue to lay eggs.

*Day 7 – treatment 2.* Delegates at the workshop at the Fourth International Congress on the Phthiraptera could not agree on the ideal day for the second treatment, i.e. Day 7, 8, 9, or 10 (see “Notes on egg-hatching & egg-laying in lice...”). Certainly, most lice hatch on or just before Day 7, but some lice may hatch on Days 8–10, particularly in cool climates. However, there are no persuasive data on the proportion of head lice that hatch *in vivo* on Days 8–10. Accordingly, clinical trial investigators need to choose the day of the second treatment.

*Day 10 – final assessment by first visual examination and if negative by either dry- or wet-combing.* The final assessment may be on Day 11 if the subject is not available on Day 10.

Up to four optional dry-combing assessments, with visual examination, may be added from Day 2 to Day 9. The combing should be stopped as soon as lice are found to reduce the therapeutic effect of the dry-combing; lice caught by the comb should be discarded.

### Assessment of the efficacy and safety of a three-treatment intervention, i.e. for claims that three treatments with the pediculicide will kill all lice and eggs so that the subject is louse-free

*Day 0 – treatment 1*

*Day 1 – assessment by thorough visual examination*

If nymphs and adults are not found during the visual examination, the hair should be dry-combed; lice caught by the comb should be left in the hair.

*Day 7 – treatment 2*

*Day 14 – treatment 3*

A third treatment, on Day 14, will kill all lice that hatched from eggs after the first and second treatments.

*Day 15 – final assessment by visual examination and if negative also by dry- or wet-combing*

Wet-combing is invariably more sensitive for the detection of light infections than dry-combing. Thus, wet-combing is recommended for the final assessment. The final assessment may be on Day 16 if the subject is not available on Day 15.

Up to four optional visual examination and/or dry-combing assessments may be added from Day 2 to Day 13. This dry-combing should stop as soon as lice are found, to reduce the therapeutic effect of the dry-combing; lice caught by the comb should be left in the hair. An alternative protocol is treatment on Days 0, 5, and 10. The primary aim of the treatment on Day 5 in that case is to minimize the number of lice on the subject

until the last treatment on Day 10. One advantage of the Day 0, 7, 14 protocol over the Day 0, 5, 10 protocol is that the Day 0, 7, 14 protocol is slightly easier for parents and subjects to follow as each treatment is on the same day of the week in three consecutive weeks.

#### Notes on egg-hatching and egg-laying in lice, and the timing of the second treatment

In three experiments, with a total of 847 newly-laid eggs of the head louse, Nuttall<sup>6</sup> found that eggs kept on the wrist or neck of a person hatched as follows: 0.5% after 5 days; 6.3% after 6 days; 62.9% after 7 days; 27.4% after 8 days; 2.8% after 9 days; and 0.1% after 10 days. Lang incubated 3200 head louse eggs at different temperatures, and found that at  $36 \pm 2$  °C (97 °F) the eggs hatched in 5–6 days, at  $31 \pm 2$  °C the eggs hatched in 6–11 days, whereas at  $27 \pm 2$  °C (81 °F) it took 9–16 days for the eggs to hatch.<sup>7</sup> Takano-Lee *et al.*<sup>8</sup> incubated 434 head louse eggs at  $29 \pm 2$  °C (84 °F) and found that lice hatched after 7–11 days. Bailey *et al.*<sup>9</sup> found that head louse eggs incubated either against the body or artificially at 31 °C (88 °F), and 70–80% RH hatched within nine days. So it is difficult to know when exactly the last egg will hatch, especially taking into consideration the different amounts of hair on the head, and thus different humidities, the external temperatures in different geo-climatic areas, the fact that lice in warm countries may lay eggs longer distances from the scalp (T.L. Meinking, unpublished data), and the observation that eggs treated with pediculicides hatch earlier than untreated eggs (T.L. Meinking and J. Burgess, unpublished data). Accordingly, there is not an ideal protocol for a clinical trial of pediculicides from the perspective of when the last egg will hatch. Taking into consideration the above, we suggest that in single-treatment protocol the assessment should be completed by Day 10. In two-treatment protocols, the second treatment should be applied on Days 7, 8, 9, or 10 and the final assessment on Day 10; whereas in the three-treatment protocols, there are two options: (i) either the second and third treatments on Days 5 and 10; or (ii) the second and third treatments on Days 7 and 14. Although a regime with the second and third treatments at weekly intervals (i.e. treatments on Days 0, 7, 14) is easier for parents to remember than a regime with treatments on Days 0, 5, and 10, the Day 0, 5, and 10 regime has the advantage of finishing the intervention sooner and thus, presumably, curing the infestation sooner than the regime with treatments on Days 0, 7, and 14. We suggest that pharmaceutical companies make clear, both on the outside of the box as well as on the Patient Information Leaflet, the recommended interval for the second and third applications of treatment. The final assessment should be 1–2 days after the last treatment; any additional days will only be further

opportunity for reinfestation. When an assessment is 1–2 days after the first (or second) treatment, it should be assumed that any first-stage nymphs found in the hair, hatched from eggs in the hair, whereas any second and third nymphal stages and adult lice should be assumed to have survived the treatment. The possibility that these lice are the result of a reinfestation is small, especially when using insecticides that have some residual activity as residual activity should preclude reinfestation in the first 24 hours after treatment. There is not an ideal treatment regimen that is convenient and 100% certain of cure. Indeed, to guarantee cure, a pediculicide might have to be used four times, e.g. on Days 0, 5, 8, and 10. However, subjects would then be exposed too often to pediculicides, and the treatment would be very laborious and troublesome. Further, recommending four treatments implies that the product is less effective than competitors that recommend fewer treatments; thus, companies are unlikely to recommend a regime of four treatments. During the 10 d of intervention, the chances of eggs developing into a new generation of egg-laying females are small. As reviewed above, the time required for development from hatching to adult is 7–10 days. Providing a male louse is present, the pre-oviposition period (i.e. the time from the last moult to the laying of the first egg) is at least one day.<sup>10</sup> However, as for the eggs, the development of nymphs and adults also depends on several parameters, such as the temperature and humidity. In a series of experiments where nymphs were kept on the lower extremities of a volunteer, the first moult occurred after three days, the second moult after five days, and the third moult after eight days.<sup>8</sup> For a pediculicide to be acceptable, all crawling stages of lice should be killed by the first treatment. Otherwise, surviving lice will either continue laying eggs or, following further development, will soon be laying eggs when they reach adulthood. Indeed, even if another one or two treatments are applied, there will be always some eggs that have the potential to hatch later and repopulate the head of the treated individual.

#### Glossary and protocol notes

##### *Assessment of reinfestation*

Reinfestation is the transmission of lice to subjects during the clinical trial, which confounds assessment of efficacy. Investigators may argue that lice found in the hair during the trial, or at the final assessment, result from reinfestation. However, such arguments must be described comprehensively and the life-stage of putative migrants reported.

##### *Assessors*

Detecting head lice is not a trivial task, and thus only people with much experience should be engaged in clini-

cal trials. Investigators should document, comprehensively, the experience of assessors.

#### *Blinding*

Often it is impossible or impractical for the applicator of the pediculicide treatment to be blinded to the type of pediculicide because the smell and/or consistency and color of pediculicides differ. Blinding of the assessor is, however, possible and practical. Blinding of the assessor is absolutely critical if assessments of efficacy are to be scientific. The investigator should document, comprehensively, the measures aimed at blinding the assessor.

#### *Combs and combing*

Although we do not recommend any particular detection comb, investigators must make every effort to not injure lice during combing by: (i) detangling the hair with a broad-tooth comb before dry-combing; and (ii) removing lice from the comb after each and every passage of the comb through the hair. Combs with a gap of 2 mm and having tips with blunt parallel-sided teeth are preferred. If metal combs are used, they should be profiled and machine-set, and teeth should be short (about 12–15 mm long) to ensure the pins of metal teeth remain straight throughout their length and to minimize flex. Teeth should be robust enough to be rigid, i.e. minimum of flexibility when they are drawn through the hair.

#### *Comparator*

Many medical products regulators require a comparator product to be used in clinical studies. Regardless of the regulators, however, a comparator pediculicide is highly desirable because the efficacy data obtained from using comparators help to evaluate the efficacy of the test formulation and thereby reduce the risk of bias by the assessor and other trial personnel.

#### *Compliance*

Trials should be conducted in compliance with the World Medical Association Declaration of Helsinki; the requirements of the appropriate National Statement on Ethical Conduct in Research Involving Humans; ICH E6 Guidance for the Industry; Good Clinical Practice: Consolidated Guidance; the National Privacy Principles; and relevant State/Territory laws. The trial activities should be approved by a registered Medical Research Ethics Committee specializing in pediatric applications.

#### *Consent*

The subject or his/her legally acceptable guardian should give written informed consent before entering the trial. The investigator should give each subject or his/her

legally acceptable guardian full and adequate written information regarding the trial's objectives, procedures, and possible risks. The subject's rights in the efficacy trial must be clearly described, including an explanation that their participation in the trial is voluntary and that they may withdraw from the study at any time without penalty. The investigator should ensure that the Ethics Committee-approved versions of the consent form(s) were used, and that the original signed Informed Consent Form for each subject is available for review. Once the trial has concluded, the Informed Consent Forms should be stored along with trial data.

#### *Criteria for evaluation of efficacy*

Efficacy should be expressed as the "louse-free rate". Often, visual examination is sufficient to determine if live lice are present, especially in cases of heavy infestation. In such cases, dry-combing or wet-combing is not needed to confirm the presence of live lice. Visual inspection alone, however, is insufficient to declare a subject as "louse-free". It has been reported that combing is four to five times more effective in finding lice on the scalp than visual examination.<sup>11,12</sup>

#### *Criteria for evaluation of safety (tolerance)*

Subjects should be interviewed on-site about adverse effects during and immediately following the application of a pediculicide as well as just before the next scheduled assessment or treatment. The incidence and severity of adverse events should be compared between treatment groups. Reports on adverse events should be written immediately at the first observation of an adverse event. Adverse events should be followed up on subsequent days. A distinction should be made between objective observations (seen by the examiners and/or a dermatologist) and subjective observations (reported by a subject or his/her parents).

#### *Demographics*

Demographic details including gender, subject age, hair color (black, blonde, brown, red), hair length (short, medium, long), and hair type (straight, wavy, curly) for each treatment group should be collected and compared among treatment groups.

#### *Dry-combing*

Combing from scalp to the hair tips using a designated plastic or metal-toothed head lice comb (tooth gap  $\leq 0.3$  mm with rigid teeth) without water, shampoo or hair conditioner. Every part of the hair should be combed six times. The hair may be detangled with a wide-gap comb or regular brush before dry-combing. Live

head lice caught by the comb should be left in the hair to reduce the degree of intervention of the dry-combing. The hair may be lightly sprayed with water to dampen the hair and thus reduce static electricity.

#### *Effective pediculicide*

We propose that a louse-free rate of  $90 \pm 3\%$  is efficacious.

#### *Exclusion criteria*

Individuals should be excluded from a clinical trial of pediculicides if they have: (i) a history of allergies or adverse reactions to head lice products or the components of the specific products being tested; (ii) received treatment with any head louse product in the last seven days prior to participation in a trial; or (iii) scalp disease. We see no reason to exclude subjects whose hair has been dyed.

#### *Ex vivo assessments*

Tests conducted in parallel with a clinical study using head lice and their eggs collected from individual subjects. In these tests, the pre-treatment and post-treatment mortality rates are compared, for example, Barker and Altman.<sup>13</sup> *Ex vivo* assessments are very informative and thus desirable. Ideally, *ex vivo* assessments should use additional subjects, i.e. subjects who are treated in parallel but are not randomized participants in a clinical trial.

#### *Inclusion criteria*

(i) Individuals having at least five live lice and five apparently live eggs should be included in the trial; and (ii) a written informed consent by the subject or the subject's parent/guardian should be obtained.

#### *Intent-to-treat (ITT) population*

All of the subjects who were randomized and treated at least once with a given pediculicide. The ITT population is usually the primary population for determination of safety and efficacy.

#### *In vitro assessments*

Performed to test the efficacy of pediculicides against active stages of laboratory strains of the body louse and its eggs and may be helpful in the development of new pediculicidal formulations. However, efficacy against the body louse *in vitro* may not be predictive of field results for head lice.<sup>14</sup>

#### *Life-stages of head lice*

Nymphal instar 1 (n1), nymphal instar 2 (n2), nymphal instar 3 (n3), adult, egg (live egg). The word "nit" is

reserved for empty egg shells, dead eggs, or a partly hatched egg (with dead-lice; still birth).<sup>1</sup>

#### *Louse-free rate*

The proportion of subjects on whom no live head lice (adults or nymphs) are found when the hair of subjects is examined by a specified method at a specified time point.

#### *Number and timing of assessments*

Subjects that present after their nominated assessment day may still be assessed, but the likelihood of reinfestation must be considered.

#### *Per-protocol (PP) population: criteria for assessing a subject as per protocol*

(i) The subject complies with all inclusion and exclusion protocol requirements; (ii) the subject provides signed informed consent authorization; (iii) treatments and assessments are administered on the specified days as per the protocol; (iv) the subject's Case Report Form is complete to enable a valid assessment of efficacy and safety; (v) the subject has not used any other head lice products during the trial or in the week preceding the trial; (vi) the subject has not used any other head lice products other than those specified in the protocol during the trial; and (vii) the subject has not used a head lice comb during the trial.

#### *Phase I, II and III trials*

The protocols described here are for Phase III trials. Phase I and II trials are highly desirable, sometimes vital, to the design of a sound Phase III trial, particularly for new or substantially modified formulations.

#### *Randomization*

Eligible subjects should be randomly assigned to receive one of the designated head lice treatments by a computer-generated code using blocked randomization, i.e. groups with a size pertinent to the size of the study, the number of interventions under investigation, and blinding. A good source for a randomization sequence that would provide adequate blinding is <http://www.randomization.com>.

#### *Removal of subjects from therapy or assessment*

Grounds for subject withdrawal from the trial should include: (i) parent/guardian or subject consent is withdrawn; (ii) the Investigator/Site Physician decides that a child's continued involvement in the study is not in the child's best interest; (iii) an adverse event that precludes further participation in the trial; (iv) subject noncompliance or major protocol violation that, in the opinion of the Investigator or Sponsor, necessitates subject



withdrawal; or (v) subject does not wish to be treated, i.e. subject assent withdrawn.

#### *Sibling and caregivers (e.g. parents)*

The siblings of the subjects in the trial, as well as the other family members and caregivers, should, if at all possible, be examined and, if infested, be simultaneously treated with the same pediculicide as the subject, to reduce the confounding effect of transmission of lice to subjects during the trial.

#### *Treatment*

Application of pediculicide to the hair of subjects as per the manufacturer's instructions or application of some other form of intervention, e.g. physical removal, physically disruptive device, or in the case of an investigational treatment, as per protocol approved by a suitably qualified ethics committee/IRB (and preferably registered at ClinicalTrials.gov or similar public online, trial register).

#### *Visual examination*

Examination of the hair and scalp, assisted by parting of the hair with the help of a comb, fingers, or hairdressers "stick", to determine whether or not live lice and viable eggs are present.

#### *Wet-combing*

Commercial hair-conditioner or other suitable lubricant (e.g. olive oil, silicone fluid, etc.) is applied liberally to the hair, and the hair detangled with a regular wide-toothed comb or brush. The hair is then combed with a plastic or metal-toothed louse comb. Every part of the hair is combed six times, starting from the scalp and down to the hair tips. During combing, the comb is wiped onto a white tissue, and the wipes are examined for lice. After combing, the conditioner is rinsed or towelled from the hair as desired by the subject. Wet-combing is a powerful detection technique to determine the final infestation status of a subject at the end of an efficacy trial, because the conditioner traps the lice in a viscous film, making it less likely for lice to avoid detection. Thus, the likelihood that a subject will be incorrectly categorized as louse-free, when in fact a low-grade infestation still exists, is substantially reduced compared with dry-combing. Wet-combing should only be used at the completion of the trial.

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