PEER REVIEW HISTORY

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ARTICLE DETAILS

| TITLE (PROVISIONAL) | Prevention of head louse infestation: A randomised, double-blind, cross-over study of a novel concept product, 1% 1,2-octandiol spray versus placebo. |
|--------------------|---------------------------------------------------------------------------------------------------------------------------------|
| AUTHORS            | Burgess, Ian; Brunton, Elizabeth; French, Rebecca; Burgess, Nazma                                                              |

VERSION 1 - REVIEW

| REVIEWER           | Yana Vinogradova                                               |
|--------------------|-----------------------------------------------------------------|
| University of Nottingham |
| United Kingdom     |
| REVIEW RETURNED    | 20-Feb-2014                                                    |

| GENERAL COMMENTS   | The abstract states that each treatment was applied for 6 weeks. This information should be repeated in Methods. Methods The last sentence on page 8 states that patients infested during the trial were treated and, according to page 7, last paragraph, the treatment for elimination of lice takes at least a week. Presumably, therefore, patients after their first infestation should not be in the trial for at least a week as they were being treated. It is also not stated anywhere in Methods whether participants continued to use the spray after the first infestation (and any following treatment) or were removed from the analysis. It is not clear, therefore how infested/treated patients were handled in the analysis. Sample size calculations should give the estimated sample size before and after the allowance for drop-outs. Which statistical test was used in the calculations to estimate differences in outcome? Statistical analysis: Page 11: For outcome, analysis of a seven point ranking score is not appropriate. The outcome is the time to first infestation, therefore, a survival analysis with adjustment for cross-over design should be used. An example of such an adjustment can be found in Colleoni 2001 J Clin Oncol, pubmed id 21321298. Page 12: One of the end points, viz. the number of infestations that occurred during each 6-week treatment period, needs more explanation – which infestations were counted and whether they were new infestation or not yet treated previous infestations. The last paragraph of page 12 is very unclear in terms of the handling of drop outs, particularly with respect to assumed weekly infestation rates of 6% and 2% infestation rates mentioned in the sample size calculations on page 10. Outcomes should not be assumed and use of survival analysis with censoring might resolve this. This whole area needs reconsideration. Results Baseline: There should be descriptive statistics for the excluded group along with included placebo and treatment arms. Table 1 shows only the baselines only for the two treatments arms, but if |
randomisation was properly done, no systematic differences would be expected. Twenty-eight patients were, however, excluded from PP analysis – a large proportion of the original recruitment – so it would be interesting to see whether this group differed from the analysed sample.

Page 17: Information on how the infestation was assessed belongs to Methods rather than Results and should be moved.

Page 18: The dropped out patients should be either censored or removed from the analysis.

Table 2 and Figure 2 contain total numbers of lice found, which is not informative in a study about infestations. Perhaps showing the numbers of separate infestations would be more relevant.

Page 19: Mean numbers of confirmed infestations are not meaningful; perhaps proportions of infested children would better represent the results.

This paper describes a crossover RCT and reports the results. The trial is properly designed but the statistical analysis needs major revision.

REVIEWER

ARIEL C TOLOZA
Research at the Centro de Investigaciones de Plagas e Insecticidas (CONICET-UNIDEF)
BUENOS AIRES, ARGENTINA

REVIEW RETURNED

27-Feb-2014

GENERAL COMMENTS

The work is ready to be published.

One important observation is that this is the first study involving a non-repellent product intended to prevent head lice from establishing an infestation.

I have several observations/comments:
intro
lines 22-40. repellency effectiveness is limited but there are several studies indicating its effect (Insect repellents, ed. Debboun, Frances, Strickman). I agreed with the authors that the compounds that had well demonstrated action on mosquitoes might not had the same effect on head lice. The proposed model of the action of a repellent compound is that odorants enter the lymph through pore tubules in the cuticle, are solubilized and encapsuled by the odorant binding proteins (OBP), and transported to the olfactory receptors. When its pathway is inactivated by an odorant-degrading enzyme (ODE), the repellent effect is established. However, very few works were conducted in the head louse model.
Was the 1,2-octanediol tested on a repellent arena against head lice?
You mentioned that "Fourteen (22.2%) participants stated they averaged fewer than two hair washes per week". How frequently the another enrolled participants washed his/her hair?.

When the participants applied the tested product or the placebo (i.e.= before/after to attend classes, etc.)?.

Repellent products might last up to 6-8h, which is the period that kids are in the school. Why do you think that this active acts in a different way of a repellent compound?.
The work is ready to be published.

One important observation is that this is the first study involving a non-repellent product intended to prevent head lice from establishing an infestation.

**REVIEWER**  
Marina Eremeeva  
Georgia Southern University, Statesboro, USA

**REVIEW RETURNED**  
08-Apr-2014

**GENERAL COMMENTS**  
Although the authors applied suitable statistical methods for this report, I am not a statistician and thus cannot comment whether these were the best or most correct statistical tools for this particular analysis.

**VERSION 1 – AUTHOR RESPONSE**

Reviewer Name Yana Vinogradova

1. The abstract states that each treatment was applied for 6 weeks. This information should be repeated in Methods.

Response: This information is already supplied in the Methods under the sub-section entitled “Statistical analysis” where it is mentioned three times on Page 12. A new entry has been made in the sub-section entitled “Study medications” to clarify and emphasise the point.

Methods
2. The last sentence on page 8 states that patients infested during the trial were treated and, according to page 7, last paragraph, the treatment for elimination of lice takes at least a week. Presumably, therefore, patients after their first infestation should not be in the trial for at least a week as they were being treated. It is also not stated anywhere in Methods whether participants continued to use the spray after the first infestation (and any following treatment) or were removed from the analysis. It is not clear, therefore how infested/treated patients were handled in the analysis.

Response: We apologise that these points not been made clear in the text. The middle paragraph of the sub-section entitled “Study medications” has now been redrafted as follows to answer the points and make the procedures clearer:

“At enrolment, and at cross-over between using the different treatment sprays, we provided treatment to all participants to eliminate any lice already present, even if none were detected. For this we used dimeticone 4% liquid gel (Hedrin Once liquid gel, Thornton & Ross, UK) applied for 15 minutes before washing, with a repeat treatment after 7 days, which was not strictly necessary due to the high level of efficacy exhibited by the product [9] but the second application was a requirement of approval for the study by the MHRA assessor. We used the same product to treat infestations of participants and household members acquired during the course of the study. Participants who were found to have contracted an infestation at any point were not withdrawn. Treatments were applied by investigators. Participants who had been infested continued to use their designated spray during the period between applications of dimeticone liquid gel because the therapeutic product is non-residual and thus conferred no protective effect between treatments.”

3. Sample size calculations should give the estimated sample size before and after the allowance for
drop-outs.

Response: The sample size estimation summary given in the eponymous sub-section does give the estimation of sample size before allowance for drop out (64 participants) and the allowance for drop out of an additional four participants making 68 in total. This whole section is a much précised version of the sample size determination text provided in the protocol. We do not feel that there would be any benefit in expanding the manuscript text with the full wording (as provided below to demonstrate to you how this process was conducted) but, if you feel it appropriate, it could be possible to submit the whole protocol as a supplementary file.

“The study has been designed to detect superiority of the test product (Octanediol 1% Solution) over placebo in clinical use. It is known that octanediol 5% is effective to eliminate an established head louse infestation. However, it was also observed during the original in vitro studies of 1,2-octanediol that solutions containing 1% active material were able to kill lice, albeit more slowly, and inhibit them from laying eggs.

This suggests that regular use may prevent an infestation from developing in about 60%-70% of users over a period of a school term, whereas people at similarly high risk of infestation who take no action, or as in this study use placebo, might be expected to go through a school term without infestation in about 25%-35% of cases.

The structure and size of this study are based on parameters that are not normally considered in clinical investigations. Unlike most clinical investigations, the participants in this study do not already have a treatable condition. The aim is to prevent a treatable condition but is unlike other “preventive” studies, e.g. vaccine trials, in that those are normally long-term population studies engaging large numbers of participants with a quite small potential for detectable failure overall.

In order to address a number of unknown factors related to the risk of infestation, estimations for this risk have been made based on a calculated overall risk for the population as a whole. For this a number of estimations were based on public domain information and experience obtained in earlier clinical studies (there are no published independent data of incidence or prevalence for this infestation available). The estimation for incidence of risk of infestation is as follows:

The number of units of head louse treatments sold annually in the UK is approximately 2.5 million. Therefore, the number of units of head louse treatments sold weekly is approximately 49,145. It was estimated that approximately 50% of these are for new infestations in children, i.e. the remaining 50% of units are for adults or infants or either for retreatment after failure to cure or are additional purchases as part of a 2x application treatment regimen. Therefore, the number of new cases of head louse infestation is actually approximately 24,572 per week. And as there are approximately 5.4 million children in the highest “at risk” age group in the UK (4-13 years) this means that approximately 0.455% of children become infested each week.

In order to relate these data to this study it is known that the child population of Cambridgeshire in the “at risk” age group is about 51,000, which means that approximately 232 cases of head louse infestation occur in the whole of Cambridgeshire each week.

Two approaches to the study size estimation were considered, a straightforward comparative study of two groups or a cross-over study using a survival analysis consideration, with each participant acting as his or her control. The arguments in favour of the cross-over model appear stronger in that smaller numbers of participants can be engaged, to minimise exposure to the new preparation, and it also offers internal controlling, with each participant acting as his or her control (with the assumption that the individual’s risk remains more or less constant over the relatively short time of the study). The risk factors for each individual are sufficiently unknown that randomisation alone may not wholly address any disparity in infestation risk due to social and family circumstances, especially in a relatively small
study cohort. Therefore, self-controlling for each individual is an attractive option to avoid any skew resulting from these unknown factors. By using a cross over study it would be possible to use a survival analysis approach in relation to time to first infestation (see Analytical Methods, below).

Calculations were made to investigate the possibility of using the cross-over estimations to limit participant numbers, for example to say 15 per group. However, when the “incidence” figures above were applied, it was found that it would be likely it would be impossible to make a reliable comparison between the product and placebo because of the possible low rate of infestation in the population as a whole.

In order to partially address the problems outlined above it is planned to mostly recruit from contacts, obtained from previous clinical studies, who have expressed an interest to take part in a study of this type. This group is known to be more at risk. Therefore, based on past study data relating to when people have contracted lice after study treatments, there is a reasonable expectation of an infestation rate of between 3 and 5 instances of reinfestation per year for each person in that group, i.e. a risk of about 3.6-4 possible reinfection events per week for the whole study population. This doesn’t mean that they will get lice but rather that they could come into contact with someone who does have them and lice could therefore be passed to them.

An estimation of sample size was made using conventional “survival analysis” calculations and the table below is taken from http://www.stattools.net/SSizSurvival_Pgm.php. The table of sample size for 2 survival ratios is applicable to the common situation where the probability of Type I error \( \alpha = 0.05 \) (95% confidence) and power = 0.8 (80% power). In conventional survival analysis terms it assumes that the sample size in each of the two arms is the same, which in this case is certainly correct because each individual participates in both treatment arms sequentially. The rows and columns represent the anticipated proportions of participants remaining louse free in each of the two treatment arms, and each cell is the total sample size (both treatment arms together) for that row/column combination. The shading covers the anticipated range of outcomes. These estimations of size that are proposed for this study appear to be at around the lower bound of population size required for the 30-35% difference in outcome between treatments necessary to detect superiority (range 62-90 participants) using a cross-over model, thus fulfilling the preferred aim of minimising the number of participants in the study.

Sample size table for 2 hazard ratios in survival analysis

| Hazard Ratio | 0.05 | 0.10 | 0.15 | 0.20 | 0.25 | 0.30 | 0.35 | 0.40 | 0.45 | 0.50 | 0.55 | 0.60 | 0.65 | 0.70 | 0.75 |
|--------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| 0.10         | 496  |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| 0.15         | 173  | 963  |      |      |      |      |      |      |      |      |      |      |      |      |      |
| 0.20         | 99   | 295  | 1414 |      |      |      |      |      |      |      |      |      |      |      |      |
| 0.25         | 69   | 155  | 406  | 1826 |      |      |      |      |      |      |      |      |      |      |      |
| 0.30         | 53   | 100  | 203  | 504  | 2186 |      |      |      |      |      |      |      |      |      |      |
| 0.35         | 43   | 73   | 127  | 245  | 588  | 2486 |      |      |      |      |      |      |      |      |      |
| 0.40         | 36   | 57   | 90   | 149  | 280  | 656  | 2723 |      |      |      |      |      |      |      |      |
| 0.45         | 32   | 47   | 68   | 103  | 167  | 307  | 2894 |      |      |      |      |      |      |      |      |
| 0.50         | 28   | 39   | 54   | 77   | 114  | 181  | 326  | 743  | 2997 |      |      |      |      |      |      |
| 0.55         | 26   | 30   | 38   | 49   | 65   | 88   | 126  | 195  | 343  | 761  | 3032 |      |      |      |      |
| 0.60         | 24   | 30   | 38   | 49   | 65   | 88   | 126  | 195  | 343  | 761  | 3032 |      |      |      |      |
| 0.65         | 22   | 27   | 33   | 41   | 52   | 67   | 90   | 128  | 195  | 339  | 744  | 2897 |      |      |      |
| 0.70         | 21   | 25   | 30   | 36   | 43   | 54   | 69   | 91   | 127  | 192  | 328  | 711  | 2727 |      |      |
| 0.75         | 20   | 23   | 27   | 31   | 37   | 44   | 54   | 68   | 89   | 123  | 183  | 310  | 660  | 2490 |      |
| 0.80         | 19   | 22   | 24   | 28   | 32   | 37   | 44   | 54   | 66   | 86   | 116  | 171  | 284  | 592  | 2186 |
| 0.85         | 18   | 20   | 23   | 25   | 28   | 32   | 37   | 43   | 52   | 63   | 80   | 107  | 154  | 250  | 508  |
| 0.90         | 18   | 19   | 21   | 23   | 26   | 28   | 32   | 36   | 42   | 49   | 59   | 73   | 95   | 133  | 209  |
| 0.95         | 17   | 19   | 20   | 21   | 23   | 25   | 28   | 31   | 34   | 39   | 45   | 53   | 64   | 81   | 108  |
For a randomise-by-individual approach, assuming a confidence level of 95% and a power of 80%, and anticipating the expected non-infestation rates to be 65% for Octanediol 1% Solution and 30% for the placebo, the required sample size is therefore estimated as 34 subjects per treatment. However, this study will operate a “hybrid” randomization in that only one person from each household will be randomized (index case) and all others in the house will be offered treatment when infestation arises but will continue to potentially act as a source of reinfestation. This approach reduces possible confounding issues of enrolling all members, in which some households will have all members on one intervention but others will have a mixture of active and placebo interventions. Therefore, for each participant on each arm of the cross-over the familial risk of reinfestation should be similar in this respect.

This estimate of the likely numbers in relation to application of the Octanediol 1% Solution active material for 6 weeks followed by placebo for 6 weeks assumes that the potential rate of contact and reinfestation is more or less linear so that, where it was estimated from past data that a likely reinfestation rate would be approximately 4 per week for the whole population, the possible number of new cases would be 24 over the 6 weeks of one half term, or 12 cases per treatment if neither had any effect. However, if the test preparation is effective this number of cases could be reduced by approximately 60% resulting in 5 cases. This difference offers essentially similar powering to that of a parallel group calculation, although the statistical significance would be reduced overall if the reinfestation rate were to be no greater than 4 per week."

4. Which statistical test was used in the calculations to estimate differences in outcome?

Response: As stated in the sub-section “Statistical analysis”, “Binary data were analysed using the McNemar test and counts and ranked data using the Wilcoxon signed rank test for paired data.” This approach was approved by the ethics committee statistician and the two MHRA statisticians on recommendation of the statistical consultant engaged by the sponsor.

5. Statistical analysis:
Page 11: For outcome, analysis of a seven point ranking score is not appropriate. The outcome is the time to first infestation, therefore, a survival analysis with adjustment for cross-over design should be used. An example of such an adjustment can be found in Colleoni 2001 J Clin Oncol, pubmed id 21321298.

Response: It is true that the primary outcome was the time to first infestation. However, unlike the study cited in your comment there was no censoring and no selective cross-over. All cross-over events were timed and therefore essentially contemporaneous with all participants completing both 6 week periods of the study arms. As a result it was inappropriate to consider time-varied weighting. However, it was necessary to have some measure for evaluation of the time to first infestation, which was why the seven point ranking score was used as a means of achieving weighting appropriate to the study design. This was discussed at some length with the statisticians from the competent authorities and agreed as one acceptable way forward.

6. Page 12: One of the end points, viz. the number of infestations that occurred during each 6-week treatment period, needs more explanation – which infestations were counted and whether they were new infestation or not yet treated previous infestations.

Response: Apologies for this lack of clarity. The second paragraph has now been modified to, “Other endpoint analyses included whether infestation occurred at any time, how many new infestations occurred during each 6 weeks treatment period, and the number and types of adverse events.”
7. The last paragraph of page 12 is very unclear in terms of the handling of drop outs, particularly with respect to assumed weekly infestation rates of 6% and 2% infestation rates mentioned in the sample size calculations on page 10. Outcomes should not be assumed and use of survival analysis with censoring might resolve this. This whole area needs reconsideration.

Response: Again, apologies for the lack of clarity due to attempting an economy of words. This paragraph has now been redrafted to provide a fuller explanation of the planned approach. The important point is that the expectation was realistically for a low dropout rate based on knowledge of the community, although unexpected events could not be accounted for. Had a high dropout rate occurred it would have been necessary to address this specifically and change the approach to analysis but as there were only two dropouts overall our approach was ultimately justified. Nevertheless the redrafting providing more explanation is as follows:

“We performed analyses on both the intention to treat (ITT) and per-protocol (PP) groups. Prior to commencement we anticipated some drop outs, mostly during the second 6 week period of treatment. In order to address this problem, if it arose, we planned analyses to allow for drop out by making an assumption that this would be due to infestation. Thus for analysis of drop out we assumed that an infestation had occurred the first week a follow up was not possible. If this were to happen in the first treatment period, so there were no data for the second period, we made the assumption that the same response would have occurred in both 6 week periods. However, based on previous experience in this community, we also anticipated that drop out, were it to occur, would arise at a very low rate that would not require censoring or other specific measures to address the issue in the analyses.”

8. Results

Baseline: There should be descriptive statistics for the excluded group along with included placebo and treatment arms. Table 1 shows only the baselines only for the two treatments arms, but if randomisation was properly done, no systematic differences would be expected. Twenty-eight patients were, however, excluded from PP analysis – a large proportion of the original recruitment so it would be interesting to see whether this group differed from the analysed sample.

Response: We currently have descriptive statistics for the ITT group, as shown in Table 1, and also descriptive statistics for the PP group, which are shown in a table at the end of this document. From this it can be seen that there was little difference in demographic characteristics between the ITT and PP groups. It could be possible to extract data from the two sources to produce a similar table for the excluded group but I really do wonder whether this would have any benefit as it is unlikely to expose any significant characteristic, based on the comparisons between the other data sets.

9. Page 17: Information on how the infestation was assessed belongs to Methods rather than Results and should be moved.

Response: This has now been done.

10. Page 18: The dropped out patients should be either censored or removed from the analysis.

Response: The wording referring to the two drop outs has been removed from the text. Because of the way the analyses were performed these two had no practical effect on the outcome data and they were censored in developing the Kaplan-Meier plots. They were only mentioned in the text as part of the narrative as part of our process of being wholly transparent about management of data but, if keeping that information in the text is confusing or misleading, it is better to remove it and avoid any misunderstanding. Therefore the first paragraph describing analysis of the ITT population now reads:

“We found a total of 32 confirmed infestations in 20 participants, which broke down as 12 people
(19.0%) infested when using octanediol and 17 people (27.0%) when using placebo, three people using placebo caught lice on two separate occasions (Table 2, Figure 2). Infestations occurred in 3 participants when using octanediol but not placebo, in 8 participants when using placebo but not octanediol, and in 9 participants when using both. In this group, the infestation occurred earlier with placebo than with octanediol in 7 participants, earlier with octanediol than placebo in just one, and in another the infestations occurred after the same time interval on both treatments (Table 2).

11. Table 2 and Figure 2 contain total numbers of lice found, which is not informative in a study about infestations. Perhaps showing the numbers of separate infestations would be more relevant.

Response: Both Table 2 and Figure 2 do show the number of separate infestations. Each entry in Table 2 is representative of a single infestation, as defined in the protocol for the study. The numbers in Table 2 show the number of lice recovered from each infestation by the investigator. These data were included to show that apart from a difference in the number of infestations there was also a difference in “intensity” of each infestation, as demonstrated by the number of lice recovered, i.e. these figures were included for the purpose of showing that the treatment, even if not wholly effective to kill all lice invading the head, did have some effect to limit the numbers of lice capable of establishing.

In order to answer the point more fully and to clarify this presentation of data I have added two extra data lines to the table showing the number of infestation on any one day and the cumulative total of infestations over each period of treatment. I do not think this improves the information but if editorially you think this improves the clarity please keep it in whereas if you think it confuses the issue, rather than clarifies it, we would be pleased if you would delete the added lines.

As to Figure 2 the same point applies. The number of infestations is given for each assessment time point, and this is clearly marked in the legend within the image, together with the number of lice. If the number of lice is removed the point of the image is somewhat lost and would thereby make such a graphical image unnecessary. Therefore we do not believe that any change is necessary to this figure.

12. Page 19: Mean numbers of confirmed infestations are not meaningful; perhaps proportions of infested children would better represent the results.

Response: Yes, we agree. The reference to mean numbers of infestations was taken directly from the statistician’s report but we also find it hard to relate to actual numbers on the ground. That section of text has now been replaced with information of percentages together with some other analytical data previously overlooked during manuscript preparation showing that one treatment arm resulting in a outcome with greater significance than the other:

“The comparison of rate of confirmed infestations, based on the 8 people infested only while using placebo, versus the three infested only while using octanediol, did not show a significant difference (p = 0.2266). Overall 19.7% of participants were found to be infested while using octanediol compared with 27.9% of those using placebo. Among these we found a significant (p = 0.0453) advantage to 1% octanediol in relation to the primary outcome of time to first infestation in the group randomised to receive placebo first then octanediol. We found no advantage in the group receiving octanediol followed by placebo.”

13. This paper describes a crossover RCT and reports the results. The trial is properly designed but the statistical analysis needs major revision.
Response: Thank you for your comments, we found them helpful and reflective to make improvements to the text.

Reviewer Name ARIEL C TOLOZA

The work is ready to be published.

One important observation is that this is the first study involving a non-repellent product intended to prevent head lice from establishing an infestation.

I have several observations/comments:
intro
1. lines 22-40. repellency effectiveness is limited but there are several studies indicating its effect (Insect repellents, ed. Debboun, Frances, Strickman). I agreed with the authors that the compounds that had well demonstrated action on mosquitoes might not had the same effect on head lice. The proposed model of the action of a repellent compound is that odorants enter the lymph through pore tubules in the cuticle, are solubilized and encapsuled by the odorant binding proteins (OBP), and transported to the olfactory receptors. When its pathway is inactivated by an odorant-degrading enzyme (ODE), the repellent effect is established. However, very few works were conducted in the head louse model.

Response: I am not sure whether this is just a comment or whether it is supposed to request some editorial response in the manuscript. Nothing in this comment regarding the mode of action of repellents at the physiological level impacts on our statement that conventional repellents developed for use against flying insects by their very nature may have little effect on a crawling insect that, as far as we are aware, employs no form of “host seeking” in the conventional sense, as applied to mosquitoes and other biting flying insects.

2. Was the 1,2-octanediol tested on a repellent arena against head lice?

Response: No investigation for possible repellent effect was conducted during the development of this specific product. However, 1,2-octanediol was subjected to a wide range of activity investigations by the original developer of the intellectual property covering its use for control and management of insects and other ectoparasites and, as far as we are aware, no study gave any indication that the chemical possess any repellent effects against any arthropod.

3. You mentioned that "Fourteen (22.2%) participants stated they averaged fewer than two hair washes per week". How frecuently the another enrolled participants washed his/her hair?

Response: Other participants stated at enrolment that they washed their hair with the following frequencies: 1-2 times (1); twice (19); 2-3 times (6); 3 times (8); 3-4 times (6); 4 times (1); 4-5 times (1); 4-6 times (1); 7 times (6). We did not include these data for two reasons. First they were so diverse and secondly few of the participants were consistent in what they actually did while participating in the study. So the initial statement only partially reflected actual practice.

4. When the participants applied the tested product or the placebo (i.e.= before/after to attend classes, etc.)?

Response: The sprays were applied after hair washing and partially drying the hair, as stated in the first paragraph of the sub-section “Study medications”. In many cases this was in the evening but some participants did apply the product in the morning.
5. Repellent products might last up to 6-8h, which is the period that kids are in the school. Why do you think that this active acts in a different way of a repellent compound?

Response: The active material is non-volatile so from the perspective of the conventional concept of “longevity” that has been widely applied to other protective chemicals such as repellents it did not matter when the product was applied because it would remain on the hair until washed out. We were not concerned only with children attending school. A considerable risk of infestation also applies outside of school, which is why a residual chemical effect was considered advantageous. This active substance has been shown in three published clinical studies, together with extensive in vitro work, to have a killing effect on lice. The therapeutic dose product sold as Hedrin Treat & Go contains 5% of the active substance in order to achieve a rapid kill. The product under investigation with 1% active was demonstrated to have an activity to kill lice ex vivo but at a slower rate than that found for the therapeutic entity. This attrition effect was the concept behind the development of the product.

Reviewer Name Marina Eremeeva

Institution and Country Georgia Southern University, Statesboro, USA
Please state any competing interests or state 'None declared': None declared

1. Although the authors applied suitable statistical methods for this report, I am not a statistician and thus cannot comment whether these were the best or most correct statistical tools for this particular analysis.

Response: Thank you for this comment. The proposed statistical procedures were extensively examined by a consultant statistician appointed by the sponsor and reviewed and revised according to the comments and suggestions of three independent statisticians, one from the research ethics committee and two from the competent authority, the MHRA. As a result we believe that the tools used were appropriate, although there will always be variations of opinion and additional investigative tools could be used but for the primary outcome analysis we believe this has been the best option for a simple answer.