SESSION II
Prophylaxis and Control (I)

**Chairs:** Terri L MEINKING (USA), Arezki IZRI (France) & Cuneyt BALCIOĞLU (Turkey)

**Plenary Lectures**

11.15 – 11.30  María I PICOLLO (Argentina). Management of pyrethroid-resistant head louse populations

11.30 – 11.45  Ian F BURGESS (UK). New target sites for pediculicides

11.45 – 12.00  Olivier CHOSIDOW (France). Role of oral ivermectin in the management of head, body and pubic lice

**Oral Presentations**

12.00 – 12.15  Jutta KLASEN, Birgit HABEDANK; Ralf BIALEK, Hermann FELDMEIER; T SCHÄFER, Rüdiger PANZER, Philipp LANG, Regina FÖLSTER-HOLST (Germany). First report on kdr-like mutations in human head louse populations in northern Germany: a comparison of genetic findings with clinical and laboratory trials on the effectiveness of pyrethrum and dimeticone pediculicides

12.15 – 12.30  Ariel C TOLOZA, María I PICOLLO (Argentina). The role of the monooxygenases from *Pediculus humanus capitis* in the detoxification of the compounds 1,8-cineole and DDVP

12.30 – 12.45  Johan LINDH (Sweden). Head louse resistance to malathion and permethrin in Sweden

**Poster Presentations**


H 03. İ. Cüneyt BALCIOĞLU, Özgür KURT, Kor YERELİ, Yusuf ÖZBEL, Kim LARSEN, Johan LINDH (Turkey, Denmark & Sweden). Investigation of permethrin resistance in head lice samples from Manisa province in Turkey

H 04. İ. Cüneyt BALCIOĞLU, Ozgur KURT, M Emin LIMONCU, Nogay GIRGINKARDESLER, Cemal BILAC, Tuba TABAK, Yusuf OZBEL, Kim S LARSEN (Turkey & Denmark). Therapeutic efficacy of frequent combing against head lice in school children
H 05. I. Cüneyt BALCIOĞLU, Özgür KURT, M. Emin LIMONCU, V. Özge ERMİŞ, Tuba TABAK, Tuba OYUR, Hasan MUSLU, Hakan KAVUR, Serhan GÖRGÜN, Nogay GIRGINKARDESLER, Cemal BİLAÇ, Yusuf OZBEL (Turkey). The efficacy of seasonal dry combing against head lice (*Pediculus humanus capitis*) in children from two schools in Manisa, Turkey

H 06. M. Emin LIMONCU, İ. Cüneyt BALCIOĞLU, Tuba OYUR, Gizem ZEYBEK, Ulvi ZEYBEK (Turkey). Investigation of pediculocidal activities of the volatile oil components of some medical plants raised in Turkey

H 07. V.M. BOWLES, L.D. SCHULZ (Australia). The development of a novel treatment for head lice in children

H 08. Claudia VASSENA, Anabella GALLARDO, Paola GONZÁLEZ AUDINO, Gastón MOUGABURE CUETO, Ariel TOLOZA, María I PICOLLO (Argentina). Adulticidal and ovicidal effect of experimental lotions based on Ivermectin

H 09. Paola GONZÁLEZ AUDINO, Gastón MOUGABURE CUETO, Claudia VASSENA, Ariel TOLOZA, Anabella GALLARDO, María I PICOLLO (Argentina). Comparative toxicity of oxygenated monoterpenoids in experimental hydro-alcoholic lotions: Correlation with physical parameters

H 10. Mark N BURGESS, Ian WHELAN, Elizabeth R BRUNTON, Ian F BURGESS (UK). Making nit removal easier by making them visible


H 12. Shirley C GORDON, K SHEPHERD (USA). Comparison study of three leading combs: A secondary data analysis

H 13. Kim S LARSEN, Johan LINDH (Denmark & Sweden). Misdiagnosis of head lice: 3 case stories


H 15. Gerald C COLES, Terri L MEINKING (UK & USA). In vitro activity of a coconut oil based shampoo

H 16. Anabella GALLARDO, María I PICOLLO, Gastón MOUGABURE CUETO (Argentina). Toxic interactions between the constituents of geranium essential oil in *Pediculus humanus capitis*

H 17. Anabella GALLARDO, María I PICOLLO, Gastón MOUGABURE CUETO (Argentina). Relationship between viscosity and pediculicide potency of mono-terpenoids in human lice.
Management of pyrethroid-resistant head louse populations

Maria I PICOLLO

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In Argentina, the infestation with *Pediculus humanus capitis* is an endemic sanitary problem affecting 30% of school-aged children. Moreover, more than 90% of head louse populations have developed high levels of resistance to permethrin (28.6-71.4) and other pyrethroids. As a consequence, there had been a trend to the use of herbal remedies for the control of pyrethroid-resistant head lice. First studies demonstrated that the essential oils from natural aromatic plants from Argentina produced differential fumigant toxicity against head lice. Similarly, the terpenoid constituents of the effective essential oils, also demonstrated differential activity. Additionally, some whole oils resulted more active than any of its individual components. In order to improve the use of these natural compounds as pediculicides, we investigated the physiochemical parameters related with the activity of the monoterpenoids, as well as the toxic interactions of the components that affect the activity of the mix. A positive relationship was found between the fumigant efficacy of the compounds and the corresponding vapor pressures. The more volatile compounds were more effective fumigants against lice and also against their eggs, indicating that these compounds could be incorporated into formulations as potential pediculicides against both stages. Moreover, a clear correlation was established between the viscosity and the contact toxic effect. It was a non-linear relationship which demonstrated the highest pediculicide potency at a medium range of viscosities. This means that the pediculicial activity could be improve modifying the viscosity of the formulation. The individual components of Argentinean plants varied in their activity when they were evaluated alone or mixed (in the same natural ratio) with other components of the whole oil. For example, a clear synergism was established by topical application between pulegona and citral (the major components of *Aloysia citriodora*), and between citronelol, linalol and citronellol from the geranium oil. These interactions were also found in pediculicide formulations and lead the development of a commercial product based on the synergized monoterpenes. All this knowledge represents important tools to design novel strategies to improve the overall management of head louse infestations.

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New target sites for pediculicides

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Mechanical methods for eliminating ectoparasites, such as grooming, depend only upon the skill of the groomer and the structure of the device being used for their efficacy. The most important limitation of the method is the patience and tolerance of the one being groomed. Killing lice using neurotoxic insecticides was considered to be the best alternative approach and some experts thought it was not only simple but also virtually fool proof. Given the limitations of habitat involved and the relatively small numbers of parasites, it is remarkable that lice have not only been able to avoid extinction but also to develop levels of resistance that make them tolerant of almost any neurotoxic insecticide. Since most people prefer some kind of rapid elimination method there must be other ways to eliminate lice that are effective and well tolerated. For many years the concept of physically acting chemical treatments was dismissed as being an ideal of a fringe minority, mainly because the few options available were of limited effectiveness and in most cases cosmetically unacceptable. Anyone who has tried to wash petrolatum from hair testifies to this. However, since the beginning of this century there has been a trend to develop physically acting pediculicides, but what is “physically acting”? Each of these chemicals acts on a target site, much as insecticides, but in this case the action is not primarily physiological. Whether a material “asphyxiates”, disrupts water balance, dehydrates, strips cuticular lipid, or damages the physical integrity of the cuticle is sometimes open to interpretation. All of these methods will kill lice and some kill their eggs. However, we do not yet know how effective some are, whether they are truly physical in action, or whether lice may eventually develop resistance to them.

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Role of oral ivermectin in the management of head, body and pubic lice

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Ivermectin (IVM), a semi-synthetic derivative of the avermectin family of macrocyclic lactones, interrupts \( \gamma \)-aminobutyric acid-induced neurotransmission in invertebrates and has been used to treat onchocerciasis, lymphatic filariasis, helminthiases and ectoparasites, mainly scabies. In an uncontrolled study, a single, standard 200-\( \mu \)g/kg oral dose of IVM eradicated head-lice infestation in only 6/26 (23%) of the subjects. A multicenter, cluster-randomized, double-blind, controlled trial comparing oral IVM (400 \( \mu \)g/kg) to 0.5% malathion lotion (on days 1 and 8) was conducted on patients with live lice not eradicated by topical insecticides used 2–6 weeks before enrollment, i.e., “difficult-to-treat” patients. The cluster was the household. A total of 812 patients from 376 households were randomized. The primary end point was the absence of head lice on day 15. For the intention-to-treat population (ITT), 95.2% of ivermectin-group and 85% of malathion-group patients were louse-free on day 15 (difference: 10.2 percentage points, 95% confidence interval, 4.6–15.7; \( p<0.001 \)). For the per-protocol population (PP), 97.1% of ivermectin-group and 89.8% of malathion-group patients were louse-free on day 15 (difference: 7.3 percentage points; 95% confidence interval, 2.8–11.8, \( p = 0.002 \)). There were no significant differences in adverse events between the treatment groups. A recent randomized clinical trial (RCT) performed in an impoverished community in Brazil showed that mass treatment with IVM (200 \( \mu \)g/kg) reduced the incidence of infestation. Regarding body lice, an RCT was performed comparing IVM single dose (24 mg) with a placebo in 82 homeless people with pruritus. At day 14, in both ITT and PP populations, IVM was associated with absence of pruritus (OR = 4.38; 95%CI: 1.07 ; 17.77). IVM has been used by other groups for the control of pubic lice, including those localized in the eyelashes.
First report on kdr-like mutations in human head louse populations in northern Germany: a comparison of genetic findings with clinical and laboratory trials on the effectiveness of pyrethrum and dimeticone pediculicides

Jutta KLASEN¹, Birgit HABEDANK¹; Ralf BIALEK², Hermann FELDMEIER³; T SCHÄFER⁵, Rüdiger PANZER⁴, Philipp LANG⁴, Regina FÖLSTER-HOLST⁴

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In recent years a growing number of failures in head lice treatments have been reported possibly due to resistance to pyrethrum and pyrethroids. Resistance is thought to be mediated by a recessive gene mutation known as the "knockdown" resistance gene (kdr). We investigated the prevalence of head lice infestation in children from kindergartens and primary schools in northern Germany, the prevalence of kdr-mutation in head-lice DNA collected from the infested children and the effectiveness of pyrethrum- and dimeticone-based head louse products in clinical and laboratory trials. The prevalence of head lice infection in children 3 aged to 19 years was 3.7% (95% CI 2.6 – 4.7%). Prevalence in girls was 2.3 x higher than in boys. Prevalence increased along with age and was highest in the 7-9 years age group (7.7%, 95% CI 5.2-10.1%). All analyzed lice (n=67) had a mutation in the kdr-gene. All mutations were homozygous. In a second study we treated 87 infested children with different head louse treatment products: A: 0.3% Pyrethrum (n=27), B: 92% dimeticone (n=35) and C: 100% dimeticone (n=25). Between 89 and 96% of the patients could be cured after two treatments according to the label claims within 8-10 days. There was no significant difference between the efficacies of the 3 products, although the first part of the study revealed 100% prevalence of the kdr-like mutations. The results reinforced the good laboratory results of all three products against colony reared sensitive body lice. The impact of the kdr-like mutation for the susceptibility of head lice against pyrethrum has to be discussed.

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The role of the monooxygenases from *Pediculus humanus capitis* in the detoxification of the compounds 1,8-cineole and DDVP

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During past decades, chemical control against the head louse *Pediculus humanus capitis* De Geer, has been based in the application of products containing permethrin. The repetitive overuse of pediculicides has resulted in the development of high levels of resistance to one or more of these products worldwide. Nowadays, there is a trend in the use of alternative insecticides like the essential oils obtained from aromatic plants. In an afford to find effective tools to reduce pyrethroid-resistant head lice populations, we studied the synergistic effect of the piperonyl butoxide (PBO) on the pediculicidal activity of the 1,8-cineole and the organophosphate DDVP. The head lice population used in our study had a resistant ratio of 40. Moreover, part of this resistance is due to the increased enzymatic activity of the cytochrome P<sub>450</sub>. The methodology employed here consisted of the topical application of the PBO on lice, followed by the exposition to the vapor phase of the compounds. The addition of PBO increased significantly the toxicity of either 1,8-cineole or DDVP. The present study indicates the importance of the microsomal monooxygenases (cytochrome P<sub>450</sub>) in the degradation of the monoterpenes like the 1,8-cineole. This suggests that a possible combination of monoterpenes or phytochemicals with PBO into a formulation might provide a better control to insecticide-resistant populations of head lice.

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Head louse resistance to malathion and permethrin in Sweden

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In Sweden, according to sales record, treatment of head lice with pesticides is very common. The most common pesticide used is malathion and during the last 5 years an increasing number of treatment failures have been reported. However, no scientific investigation has been pursued. Here, we present data of malathion resistant head lice in the area of Stockholm, Sweden. Live lice were collected and malathion resistance was measured following the WHO protocol. After the measurements, lice were kept in 96% alcohol at -20ºC until genomic DNA was isolated. Detection of permethrin resistance was performed by sequencing the kdr gene. The methods of choice were improved PCR and sequencing. In short, the use of M13-flanked specific primers, Ampli Taq gold fast enzyme and BigDyeTerminator v1.1 enabled us to develop an initial PCR, which was ready within 25 min and sequencing reactions within 30 min. Purification steps were performed by dilutions and ethanol precipitation. Since the newly introduced sequences for the sequencing reaction are M13 reverse and forward, the same stock solutions could be used in all reactions. The percentage of permethrin resistance lice in Stockholm, Sweden will be presented.

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**POSTER PRESENTATIONS**

**H 02.**

**Efficacy of the LouseBuster™ – a novel device for treating head lice**

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Human head lice (*Pediculus humanus capitis*) occur worldwide and infest millions of children and adults every year. Head lice infestations (*Pediculosis*) are psychologically stressful, physically irritating, and their bites can lead to secondary infection. *Pediculosis* is one of the leading causes of K-6 school absence. The prevalence of head lice in many countries is increasing rapidly because of resistance to the chemicals used in many head lice treatments. We tested the efficacy of an alternative method for controlling head lice - the LouseBuster™ - a custom built, FDA cleared, medical device designed to kill head lice and their eggs using controlled, heated air that is directed to the scalp and hair's roots. We used a sample of 56 infested subjects to evaluate the efficacy of the LouseBuster™. We compared the viability of lice and eggs on randomly assigned pre- and post-treatment halves of each subject's scalp. We also compared the efficacy of the device in the hands of experienced versus novice operators. Mortality of lice and eggs was 94.8% following treatment by experienced operators. Novice operators were able to use the device effectively after a short training session; their results did not differ significantly from those of experienced operators. No adverse events were associated with treatment. The LouseBuster™ is extremely efficacious in killing lice and their eggs. The use of heated air is appealing because it is a fast, safe, non-chemical treatment. Furthermore, head lice are unlikely to evolve resistance to desiccation, the apparent mode of action.

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Investigation of permethrin resistance in head lice samples from Manisa province in Turkey

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Infestation caused by human head louse (Pediculus humanus capitis) is one of the most important infestations of childhood, especially after the detection of head lice strains that are resistant to one or more of the insecticides available in the market. Recently, it was found out that the mutations in sodium channels, T9291 and L932F were related to permethrin resistance in head lice. This is probably the first study about permethrin resistance in head lice samples collected in Turkey. A total of 100 head lice samples were collected from primary school children in Manisa province and kept at minus 20 degrees Celsius and 96% alcohol until genomic DNA from the lice were isolated in Sweden. Permethrin resistance was discovered using a novel method for FAST PCR and fast sequencing of the kdr gene. Using M13-flanked specific primers, Amplitaq gold fast enzyme and BigDyeTerminator v1:1, an initial PCR was followed by a sequencing procedure which took 25 and 30 minutes. Purification steps were performed by dilutions and ethanol precipitation. The initial assessments indicated that permethrin resistance was present among the head lice samples from Manisa. We plan to expand this investigation to all Turkey with more samples from different geographic regions to reveal the latest stage of permethrin resistance in Turkey.

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H 04.
Therapeutic efficacy of frequent combing against head lice in school children

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As part of the treatment regime for head lice, wet combing (combing out the lice in wet hair with the use of a conditioner) is often mentioned as a kind of golden standard. However, there is actually limited support documenting the efficacy of this method compared to the advantages of combing dry hair (dry combing). Furthermore, in some countries it is recommended to comb every second day for two weeks although the effect of this frequency of combing has never been tested. In the present study, we therefore aimed to assess the efficacy of the PDC lice comb for wet and dry combing every second day for two weeks. A total of 69 children infested with head lice were included the study. They were assigned into two groups, dry combing (34 children) and wet combing (35 children) methods and the combing was applied every second days for two weeks. The specifications (sex, age etc) of the children in both groups were similar. The ages of the children were between 6 and 15 and in both groups the majority (62.2%) was between 10 and 13. In both groups, most of the children were girls (65.2%). Only 12 (17.4%) of 69 children had received head lice treatment previously. No significant difference was found in the specifications (such as sex (χ²: 0.356), age, length of hair (χ²: 0.658), hair thickness (χ²: 0.439), hair type (χ²: 0.223), etc), as well as infestation level (χ²: 0.476) of the children in both groups. During the initial combing, 59.4%, 27.5% and 13% of the children had light, moderate and heavy infestation according to the criteria in our questionnaire, respectively. The average number of adult lice found at day 0 and day 14 were 3.31±2.8 and 0.85±1.37, respectively, while the average number of nymphs was 0.25±0.74 and 0.41±0.82 in day 0 and day 14, respectively. A significant difference was found in the total number of adults (p=0.000) but there was no significant difference in the total number of nymphs (p>0.005) at the beginning and at the end of the study. At the end of the two week study period, no lice were found in 50.0% of the children in the dry combing group and in 58.1% of the children in the wet combing group. There was no significant difference (χ²: 0.527) between the two groups but combing was easier in the wet combing group, especially in the children who had long hair, according to the researcher’s observations. In conclusion, our data showed that wet combing, as well as dry combing has a limited value in the treatment of head lice even when combing every second day for two weeks. One of the reasons for this might be the very high re-infestation level that was observed in the present study. <kim@ksl.dk>
The efficacy of seasonal dry combing for head lice (*Pediculus humanus capitis*) in children from Manisa province, Turkey

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Infestation caused by the human head louse, *Pediculus humanus capitis*, is one of the most common infestations of school children in Turkey. The head lice are generally detected by parents or local family physicians when children have been complaining, e.g., of continuous itching of the scalp. The aim of the present study was to screen the same population of school children in two villages of Manisa province, namely Osmancalı and Maldan, at regular time periods. In the school of Osmancalı village, there are almost 400 registered children, aged between 6-15 years, coming from 20 small neighboring villages. In the second school, in Maldan village, 130 children, mainly living in this remote community and also from a few adjacent farms were included in the study. The children in both schools were screened for head lice four times using a PDC louse comb during the months of May and November of 2008 and 2009. The total number of examinations were 2,091: 1,565 in Osmancalı and 526 in Maldan). The data were analysed SPSS® v.15 program using CHAID and logistic regression analysis. The infestation rate of girls was significantly higher than that of boys (p<0.001) and enrollment to school in Osmancalı was a significant risk factor for boys (p=0.002). In addition, periodic examinations and treatments lowered the infestation rates among girls significantly (p=0.001). We conclude that periodic examination of school children for head lice is essential for the control of this common infestation.

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The human head louse, *Pediculus humanus capitis*, has recently developed resistance to many commercially available insecticides. This has resulted in the search for new pediculicides based on herbal products. The aim of this study was to assess the in vitro pediculocidal activities of volatile oils, extracted from 10 (I count 9) medical plants cultivated in Turkey, (*Rosa damascena*, *Pelargonium graveolens*, *Lavandula angustifolia*, *Salvia triloba*, *Rosmarinus officinalis*, *Citrus bergamia*, *Cymbopogon nardus*, *Citrus limonum* and *Cymbopogon flexuosus*). Head lice were collected from school children in the city of Manisa and kept at 27°C and 50% RH. Ten adults and ten nymphs were placed separately in Petri dishes containing human hair and the volatile oil was dropped onto the lice. The mobility of lice and the activity of their intestinal tract were observed for 24 hours. The time of death was defined as the moment when lice stopped moving and no peristaltic movements of the intestinal tract could be observed. The results were analyzed with SPSS v.15. The volatile oils of *Rosmarinus officinalis* (2 chemotypes) was found to be the most effective of all oils examined.

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The development of a novel treatment for head lice in children

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Infestation with head lice, Pediculus humanus capitis, is the second most common communicable disease behind the common cold. Control is centred on the use of several classes of insecticides, however the development of resistance, the requirement for two treatments which leads to issues around compliance, coupled with the lack of ovicidal efficacy has meant that new and improved products are needed. We have been working on developing a new product for the control of head lice based on the inhibition of metalloproteinases. Metalloproteinases perform a number of important roles in insect biology including egg hatching, moulting and digestion. Initial studies focussed on the ability of these compounds to inhibit egg hatching where it was shown that these compounds were potently ovicidal in an in vitro assay against body lice eggs. Further research with one of these inhibitors resulted in a formulation that was also able to kill the nymph and adult stages of lice following a 10 minute application. The compound has been formulated into a cosmetically acceptable preparation and progressed through an extensive preclinical toxicological program including a Phase 1 safety study in humans. The product, named DeOvo™ was recently evaluated in a Phase 2a study where it was demonstrated to be safe and well tolerated. Preliminary efficacy results were also assessed to determine if the in vitro data was reproducible following the application of the product to infested adult subjects. Using an ex vivo surrogate measure of efficacy post treatment, data was obtained to indicate that the product was both ovicidal and lousicidal following a single, brief exposure. Planning is now in progress to determine efficacy of the product following its application on children. The aim of this work is to develop a safe and effective, single treatment for the control of head lice.
H 08.
**Adulticidal and ovicidal effect of experimental lotions based on Ivermectin**

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The high levels of pyrethroid resistance in the majority of the lice populations in Buenos Aires, Argentina, challenges us to assess pediculicidal activity of Ivermectin, a drug used for a variety of parasitic diseases due to its high activity and its safety on humans. We investigated the adulticidal and ovicidal activity of Ivermectin technical and experimental hydro-alcoholic lotions based on this insecticide against permethrin-resistant head lice. Children aged 3-13 were examined for head lice, which were removed by a fine-toothed metal comb. After collection, lice were maintained at 0.5°C and 70-80% RH in the dark for a maximum of 18 hrs before their use in toxicological bioassays. Insecticidal activity was assessed by topical application on adults and nits. The Lethal Dose 50% for Ivermectin and permethrin showed that Ivermectin was 60 times more effective than permethrin on both adults and nits. The effectiveness of experimental lotions was evaluated by the immersion method. Batches of 10 adults were submerged for 2 minutes in 1 ml of insecticide lotions, and washed with 100 ml of water. Mortality was recorded after 18 hrs. The ovicidal activity of lotions was evaluated by 10 min immersion of batches of 10 eggs fixed to glass slide, washed in 50 ml water and dried over filter paper. Treated eggs were maintained at 28°C - 75% RH until hatching of untreated eggs. The Lethal Concentration 50% showed that the lotions with ivermectin are more effective than those with permethrin on both adults and nits, indicating that are a good alternative for commercial head lice products.

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Comparative toxicity of oxygenated monoterpenoids in experimental hydro-alcoholic lotions: Correlation with physical parameters

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The use of botanical compounds such as essential oils has recently become of great interest as a natural mean of pest control because of its repellent, ovicidal, adulticidal and feeding inhibition activity against various insect species including head lice. Essential oils are the steam-distillable fraction of plant tissues which are often responsible for a plant's distinctive scent, odour or taste. These oils are of rather complex composition, generally consisting of low molecular weight monoterpenes and related phenols. Due to the variability of their constitution the effects may not be reliable, safe and reproducible. Thus, it was our objective to test and compare the efficacy of pure oxygenated monoterpenoids which are main ingredients of essential oils that have proved good biological activity. We used pulegon and citral, components of Aloysia citrodora, and geraniol, citronelol, eugenol, mentol and linalool, components of geranium oil. The toxic activities were evaluated by the immersion method of head lice in hydro-alcoholic solutions. We tested ethanolic solutions from 0 to 70% and monoterpenoid concentrations from 0.05 to 10%. As end point we evaluated both inactivation and mortality of head lice. We found that citronelol and geraniol showed the highest mortality (>60%). Pulegone, linalool, mentol and citral produced inactivation between 42 and 55%, and mortality between 34 and 53%. A simple linear regression analyses showed statistically significant relationships between the toxic effects and viscosity of the monoterpenoids (p<0.05) but no with their log P.

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H 10.
Making nit removal easier by making them visible

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Anyone who has ever dealt with head lice knows that nits remain attached to hairs for months after hatching. They also know that viable eggs blend in with the hair making detection hard. But in some places “no nit” policies mean you must remove every eggshell to ensure the child can go back to school. So if you could see the eggs and nits more easily it should make removal quicker and more reliable, so if a contrasting colour could bind to the eggshell it would make detection and removal easier. The louse egg/nit has two components: a shell probably made from the polysaccharide chitin, and a glue-like material that holds the shell in place on the hair made from linear protein lamellae. Both are highly resistant to chemical or physical breakdown. Staining either or both of these presents a challenge because a product to be applied to children’s heads needs to be: safe, non-staining on skin or hair, non-persistent (decolorize in light or pH change), easy and cheap to formulate. We investigated a large selection of food dyes, as they have already been tested for safe use with humans and from these we focussed on how the dye stained the louse egg. Some were able to only stain the glue while others were only able to stain the eggshell or part of the eggshell. Some did not stain at all. For delivery we identified a gel as the most practical method for application and removal, which was cosmetically more acceptable than existing commercial preparations.

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H 11.
A comparison of three louse combs for removal of louse eggs and nits from human hair

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There are many combs available, all claiming to remove lice and/or louse eggs from the hair, but few have been objectively tested. Combs vary considerably in the type and quality of materials used in manufacture and some were apparently designed without knowledge of either lice or their eggs. We made an objective comparison of three combs, two with steel pin teeth, with different tooth gaps, and one with plastic, square faced teeth using a slip peel tester to draw individual louse eggs or nits (hatched eggshells) between the teeth of combs fixed to the rolling platen of the device. The method provides a consistent pull on each hair and eggshell aligned consistently with the face of the comb. We found, to our surprise, that the ‘PDC’ plastic comb with a tooth gap of 0.23 mm was consistently (but not significantly) more effective to remove louse eggs and empty eggshells. Both ‘MPC’ pin combs were effective for removing unhatched eggs, although a tooth gap of 0.1 mm was non-significantly less effective than one of 0.15 mm. For removal of empty eggshells the plastic comb was significantly (p = 0.007) more effective than the 0.1 mm pin comb and non-significantly better than the other comb. It appears the structure of empty eggshells is sufficiently flexible when subjected to pressure they can slip between rounded metal teeth. As this type of tooth is commonly found on durable, so called, “nit combs” this observation may explain why nit removal can be so difficult.

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H 12. Comparison study of three leading combs: A secondary data analysis

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Fine tooth combs are commonly used as part of a head lice treatment regime. Head lice eggs and nits are firmly attached to the hair shaft making them difficult to remove. Failure to remove viable eggs may result in re-infestation. The visual presence of eggs or nits may be seen as a stigmatizing characteristic. The purpose of this study was to compare three leading nit combs ( Terminator, Licemeister and Rid) by evaluating their overall effectiveness in removing lice and their eggs. The efficacy of 3 fine tooth combs was evaluated. The hair of 100 participants (ages 3 to 50 years) was divided into two sections sagitally. The terminator comb was randomly assigned to one half of the hair and one of the other combs was randomly assigned to the second half of the hair. For each side of the head, the assigned comb was used to comb through the hair from root to tip for 20 strokes. The lice and eggs from the combings were counted and compared. Assessment of the hair and scalp revealed the visual presence of nits or eggs in 100% of the participants with 36% having 100-199 nits/eggs, 42% having 200-500 nits/eggs and 22% having greater than 500 nits/eggs. All three combs proved effective in removing lice with no statistically significant differences between them. The Terminator comb removed more eggs and nits than both the Licemeister and the Rid combs and the Licemeister comb removed more eggs and nits than the Rid comb. Overall the combs were equally effective in removing lice. The Terminator comb was more effective in removing eggs and nits. Further research is needed to determine the influence of factors such as egg/nit load, hair texture, and hair length on comb efficacy.

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H 13.
Misdiagnosis of head lice: 3 case stories

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A correct diagnosis of head lice is the basis for carrying out a treatment or not. It is well known that observing dead or empty eggs in the hair often makes parents believe that an active infestation is present, although these eggs are often just signs of a former infestation. However, sometimes treatments are carried out due to the misdiagnosis of finding live eggs of other parasites, or due to the presence of live insects that are mistaken for head lice. The present paper presents three cases of such misdiagnosis of head lice. In two very similar cases, one from Sweden and one from Switzerland, the finding of Psocoptera specimens (also known as booklice or barklice) in the hair led the infested persons to conclude that the problem was head lice. Both cases were in many ways identical: 1) Only the mothers in both families were found to be infested; 2) The Psocoptera infestations reoccurred several times over a period of several months. During this period of time, the infested persons had repeated treatments with pediculicides; 3) In both cases, a general practitioner confirmed to the patient that the problem was, in fact, head lice; 4) After some time, the Psocoptera disappeared without any identification of the route or source of infestation. The last case of misdiagnosis, also from Sweden, is a finding of eggs glued to the hair of a young boy. The eggs were thought to derive from head lice and treatment aimed at the eradication of head lice was pursued. Two months later, the boy still suffered pain and parts of the scalp started to swell. A picture of the eggs was sent to an entomologist who could distinguish the eggs as belonging to a warble fly. After closer examination, the eggs were identified as having originated from the reindeer warble fly (Hypoderma (Oedemagenena) tarandi). Proper treatment could then be given (Ivermectin).

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H 14.
Parental views of a coconut oil emulsion shampoo

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To establish parents’ views on a new coconut oil based shampoo for the control of head lice, an advertisement was placed in a mass circulation national newspaper inviting parents to apply for a free bottle of shampoo and then to complete a questionnaire and return it to the University of Bristol. Families had to have at least one child with head lice. Of 286 questionnaires sent out 70% were returned encompassing 525 children. Of these 404 were reported to be infested and 58% of these were claimed to have ‘heavy’ lice burdens. Peak age for infestation was 6-8 years. Following treatment 56% stated that there was a 100% clearance rate with 43% stating most of the lice had gone. Sixty one per cent described it as excellent, 28% good and 8% OK. The shampoo and lice control was liked by 86% and 90% would recommend it to a neighbour. Forty per cent of the respondents said that they had tried phenothrin and it did not work, 25% permethrin, and 30% malathion which similarly did not work. This is not unexpected given the widespread nature of insecticide resistance in head lice in the UK. Other products tried unsuccessfully included vinegar, vodka, mayonnaise and fabric conditioner! Whilst results in this unsupervised trial with the coconut oil emulsion were less than obtained under supervised parental application (94 and 100% clearance) it is clearly a product liked by most families.

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In vitro activity of a coconut oil based shampoo

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A novel formulation of a fractionated coconut based shampoo was tested in vitro against body lice reared on rabbits and three populations of head lice. An artificially reared population was maintained in Amherst, Massachusetts, while the other two were obtained from children in Miami and Panama, Central America. Shampoo was poured over lice with stirring or they were placed on fabric impregnated with shampoo for a known length of time and then the shampoo was removed by rinsing on a fine sieve or fabric. The body lice stopped both body movement and gut peristalsis very quickly and all tested (20) were dead when examined after 15 or 20 minutes exposure. With the artificially reared head lice most appeared dead immediately and after 3 hours 63 were dead and 14 showed slight movement. Many of the dead lice appeared shrivelled. In Miami 46 lice of all stages were dead within 10 mins. In Panama, all lice (60) were dead within 15 mins and they were showing signs of cuticle dehydration. Many of the dead lice appeared shrivelled. The results indicated that the shampoo is highly effective in killing lice but suggests that the cultured lice may be more resilient than those from human heads. Prof. John Clark kindly supplied the cultured lice. Supported by BioSafe Technologies. 
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H 16. Toxic interactions between the constituents of geranium essential oil in *Pediculus humanus capitis*

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In the absence of effectiveness of conventional insecticides due to the evolution of insecticide resistance, investigations were focused to the development of selective human louse control alternatives. Plant essential oils and its constituents have been proposed as an alternative for insect control. Previous works evaluated the acute toxicity of different essential oils and its constituents against head lice, and demonstrated that the activity of essential oils is not just explained by the simple addition of the activities of its constituents, suggesting toxic interactions. The aim of this work was to determine the occurrence of toxic interactions between the components of the geranium essential oil. Bioassays were conducted using females of *Pediculus humanus capitis* (head lice). Head lice were collected from heads of infested children (6-12 yr old). We tested the toxic activity of the geranium essential oil, its major constituents, and different artificial mixtures of those constituents. Acute toxicity of compounds was determined by application of 0.1 µl of acetone solution of evaluated compounds on each insect’s abdomen. Mortality was measured 18 h after treatment. Citronellol showed the highest activity meanwhile citronellyl-fomate showed the lowest. The activity of geranium essential oil compared with those of artificial mixtures of individual constituents, demonstrated the occurrence of toxic interaction.

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H 17. 
Relationship between viscosity and pediculicide potency of monoterpenoids in human lice

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Insect cuticle is the first barrier that insecticides should penetrate in order to produce toxic effects. The penetration rate of these compounds mainly depends on its physico-chemical properties such as molecular size, polarity, vapor pressure and viscosity. The goal of this work was to determine weather there is a relationship between viscosity and pediculicide potency of some monoterpenoids in human lice. The study was carried out on Pediculus humanus humanus males and P. humanus capitis males and females. The Lethal Dose 50% (LD50) of the monoterpenoids was determined by the topical application of 0.1 µl acetone solutions of tested compounds on each insect's abdomen. Mortality was measured 18 hs after treatment. The viscosity of the compounds was established using a modified Ostwald viscosimeter. The relationship between viscosity and potency was evaluated by means of regression analysis. The results of these analyses showed a clear correlation between the viscosity and toxic effect of the evaluated monoterpenoids. It was a non-linear relationship which demonstrated low potency at the lowest and highest viscosity values, and the highest pediculicide potency at medium range of viscosities. 
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