

## Controlled study of malathion and *d*-phenothrin lotions for *Pediculus humanus var capitis*-infested schoolchildren

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### Summary

Anecdotal reports have suggested that reduced efficacy of pediculicides against *Pediculus humanus capitis* could be related to resistance to treatments.

Ovicidal and pediculicidal activities of 0.5% malathion and 0.3% *d*-phenothrin lotions were tested in an experimental model of *P humanus capitis* grown on rabbits to ensure that the two treatments were pharmacologically equipotent. We then did a randomised controlled trial in which the lotions were administered to 193 *P humanus capitis*-infested schoolchildren (malathion, 95; *d*-phenothrin, 98). Success rate was defined as the absence of both live lice and viable nits. Before treatment, live lice were collected and subjected to a pediculicidal test. Pharmacological tests showed 100% killing of the rabbit-grown nits and lice after exposure to both pediculicides. On day 1 of the controlled trial, the success rate was 92% in the malathion group (95% CI, 0.86–0.97) and 40% in the *d*-phenothrin group (0.30–0.49) ( $p < 0.0001$ ); on day 7, it was 95% in the malathion group (0.90–0.99) and 39% in the *d*-phenothrin group (0.29–0.48,  $p < 0.0001$ ). Malathion was also significantly more active in the pediculicidal tests compared to *d*-phenothrin and control.

These results suggest an acquired resistance to *d*-phenothrin in the schoolchildren tested, since all other conditions of the administration of insecticides were standardised.

Lancet 1994; 344: 1724–27

### Introduction

Pediculosis capitis affects millions of children worldwide, especially those 5–14 years.<sup>1</sup> Acetylcholinesterase-inhibiting malathion, pyrethrin, and synthetic pyrethroid pediculicides, such as *d*-phenothrin, are widely used treatments.<sup>2,3</sup> However, an increasing rate of head-lice infestation has been reported in France, USA, Israel, and Turkey and could be related to a reduced efficacy of these treatments.<sup>4,5</sup> Several factors may influence the effectiveness of treatments, making interpretation of trial results difficult: ovicidal and pediculicidal activities of the pharmacological formulations, lotions eradicating head lice better than shampoos,<sup>6</sup> duration and quantity of the preparations applied, frequency of applications, and reinfestation. Development of clinical and/or parasitological resistance to insecticides has also been suspected of decreasing efficacy, but reports are anecdotal.<sup>5,10–13</sup> Since a pediculicide should be equally effective against live lice and viable nits, the efficacy of the treatments must be judged both clinically and experimentally.<sup>2,3</sup>

Our study aimed to compare the efficacies of malathion and *d*-phenothrin lotions. We first evaluated the ovicidal and pediculicidal activities of the lotions with *Pediculus humanus capitis* grown on rabbits to ensure that the two treatments were pharmacologically equipotent. Afterwards, we did a controlled trial including a parasitological test to assess resistance to the pediculicides.

### Patients and methods

#### Experimental rabbit model

0.5% malathion lotion (Prioderm, Sarget) and 0.3% *d*-phenothrin lotion (Item, Gandhour) were tested on *P humanus* bred on white New Zealand rabbits.<sup>14</sup> Experimentally-grown lice have never been exposed to pediculicides, thereby preventing them from developing drug resistance. The investigator who graded these tests was unaware of treatment assignments.

**Ovicidal test.** 130–160 nits, firmly fixed to their support, were immersed in each lotion. After 3 min of contact, nits were washed three times with tap water, allowed to dry at room temperature, then incubated at 28°C with relative humidity of 70–80%. Live-hatched first-instar lice, capable of feeding on blood, were counted. The other nits were nonviable. A group of untreated nits was used as a control.

**Pediculicidal test.** Two groups of lice of different ages (50 larval instar lice and 50 adults), fed four hours before the test, were immersed in each lotion. After 3 min of contact, the lice were washed with tap water and dried on filter paper. They were then incubated at 28°C with relative humidity of 70–80%. 24 hours later, the numbers of live and killed lice were recorded. A third group of lice (same number and same age), which were untreated but washed and dried, served as the control.

#### Controlled trial

**Enrollment of children.** The study protocol was approved by an appropriate institutional review committee and conducted in Paris elementary schools selected because of the high rate of infestation the year before. Children and their parents were

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Test	Malathion	d-phenothrin	Control
Ovicidal (nits)	100% (n=142)	100% (n=157)	23% (n=136)
Pediculicidal (lice)	100% (n=100)	100% (n=100)	0% (n=100)

Table 1: Rabbit-bred *P humanus*: ovicidal (% nonviable nits) and pediculicidal (% lice killed) activity of malathion and d-phenothrin lotions

unaware of the day the treatment would be given. Eligible children had to be *P humanus capitis*-infested, have live lice and/or more than 5 creamy white or yellow nits closer than 1.5 cm to the scalp (the previously reported definition of clinically viable nits<sup>15</sup>), and to have obtained parental written informed consent. A semi-quantitative evaluation of the infestation was made at the time of inclusion: live lice, 0 (absence), + (1-5), ++ (6-10), +++ (11-20), ++++ (>20). Viable nits (counted on the most severely infested hair clump): 0 (absence), + (1 viable nit every 1000 hairs), ++ (1 viable nit every 100 hairs), +++ (1 viable nit every 10 hairs), ++++ (1 viable nit on every hair). Children were excluded if they had any scalp infection.

**Design and procedures.** The study was preceded by a training period for physicians and health-care assistants. A psychologist explained the study to the teachers and the children, and distributed an information sheet for the parents written in French or appropriate foreign languages. Since each school class constitutes an environmental unit for *P humanus capitis* dissemination, the randomisation unit was the class and not the child; all infested children of the same class received the same lotion applied by a health-care assistant not involved in assessing the effect of treatment. This procedure assured a blind assessment of the endpoints. Randomisation was stratified by school. 0.5% malathion and 0.3% d-phenothrin lotions were from the same lots used in the experimental model. At the time of inclusion, day 0; after 24 hours, day 1; and 7 days later, day 7; children were examined by an investigator unaware of the lotion used. In another room, health-care assistants applied sufficient lotion to wet the scalp and base of the hair shaft; lotion remaining was kept until the end of the study, then measured to estimate the amount used. Lotion was applied once in the morning. Any other pediculicidal treatment was forbidden during the study. Since d-phenothrin has to be applied for at least 10 minutes and malathion for at least 6 hours,<sup>2</sup> we instructed the parents to rinse their children's hair with a standard non-treating shampoo (to remove the smell) only after 8-12 hours; we further instructed them to treat sheets, pillowcases, clothes, hats, soft toys, or other fomites with an appropriate pediculicidal powder. Children's contacts, eg, family members, received instructions to treat themselves with the same lotion as the child.

**Parasitological evaluation.** Activities of lotions were evaluated on human-grown lice in a hatching-nits test and a pediculicidal test.<sup>15,16</sup> Nits were collected on day 0 and, when infestation persisted, on day 1 and day 7; lice were collected on day 1. Hairs with at least 5 viable nits were removed with scissors. Lice were removed with a fine-tooth comb. Nits and lice were transferred to sterile Petri dishes, closed with self-adhesive tape, identified, and quickly transported to the parasitology laboratory. The parasitologist was unaware of the insecticide assignments for both tests.

**Hatching-nits test.** Nits were examined with a binocular microscope; empty or probably dead nits were discarded and plump nits transferred to another Petri dish. Dishes were incubated at 29°C with relative humidity of 70-80%, and examined twice daily for 10 days. Hatched nits were judged viable if the young lice were able to feed on the back of the hand of one of us (MI) in less than 20 minutes; the other nits were considered nonviable.

**Pediculicidal tests.** Lice were examined with a binocular microscope; dead, damaged, or less than fully mobile lice were discarded. Pediculicidal lotion was applied according to WHO recommendations to determine susceptibility.<sup>13,17</sup> This pediculicidal test was first validated by a preliminary study with

the experimental model of *P humanus capitis* bred on rabbits (unpublished data).

### End points

On day 1 and day 7, the absence of both live lice and parasitologically viable nits (hatching test) defined the success of treatment; the main end point of the trial was the success rate on day 7. Live lice and viable nits were also compared between groups.

### Analysis

Efficacy of malathion and d-phenothrin lotions is between 95-100%.<sup>2,18</sup> An occasional report suggested a lower efficacy of these treatments, therefore we chose a day 7 success rate of 90%. Sample size was estimated at 376 (188 in each group) on an assumption of benefit given by a success rate on day 7 of 85% with d-phenothrin and 95% with malathion, a type I error of 0.05, a type II error of 0.10, and a two-sided test. Given an infestation rate of 5-10%, screening of 8400 children was foreseen. One interim analysis was planned at a level of  $\alpha'=0.029$  to maintain an overall level of  $\alpha=0.05$ .

Analysis was on intention-to-treat. We used non-parametric tests (Fisher's test for binary parameters,  $\chi^2$  for categorical parameters and the Kruskal and Wallis test for continuous parameters) to compare treatment groups. Treatment comparison was adjusted for categorical covariates with the Mantel-Haenszel test. Since the randomisation unit was the schoolclass and not the child, a generalised regression model provided an appropriate test for comparing the two treatment groups. 95% CIs were computed for the main end point.

Louse survival from the pediculicidal test on human-grown *P humanus* were analysed with Kaplan-Meier estimates and log-rank tests.

Characteristic	Malathion (n=95)	d-phenothrin (n=98)
Age at randomisation (yr)	8.6 (1.6)	8.9 (1.6)
Sex—no of children (%)		
Male	31 (33)	41 (42)
Female	64 (67)	57 (58)
Home no (mean)		
Number of rooms	3.3 (1.2)	3.3 (1.8)
Length of hair—no of children (%)*		
Long	37 (39)	20 (21)
Mid-long	23 (24)	33 (34)
Short	35 (37)	44 (45)
Colour of hair—no of children (%)		
Blond	15 (16)	18 (18)
Brown	49 (52)	55 (56)
Red	4 (4)	4 (4)
Dark	27 (28)	21 (22)
Texture of hair—no of children (%)		
Straight	67 (71)	69 (70)
Curly	19 (20)	25 (26)
Frizzy/kinky	9 (9)	4 (4)
Pruritus—no of children (%)	54 (57)	65 (66)
Excoriations—no of children (%)	25 (26)	39 (40)
Evaluation of infestation		
Live lice—no of children (%)		
0	18 (19)	24 (24)
+	45 (47)	35 (36)
++	9 (9)	15 (15)
+++	12 (13)	15 (15)
++++	11 (12)	9 (9)
Viable nits—no of children (%)*		
0	19 (20)	8 (8)
+	32 (34)	41 (45)
++	22 (23)	24 (25)
+++	18 (19)	20 (21)
++++	4 (4)	4 (4)

The 2 groups were similar at baseline except for a significant difference for the length of hair ( $p=0.02$ ; chi-square). \*One value missing in the d-phenothrin group.

Table 2: Baseline characteristics of the *P humanus capitis*-infested schoolchildren assigned to receive malathion or d-phenothrin lotion\*

## Results

Table 1 compares insecticidal activities of malathion and *d*-phenothrin lotions assessed in the experimental model of rabbit-grown *P humanus*. There was complete ovicidal and pediculicidal activity (100% mortality of nits and lice after contact with either lotion).

The controlled trial took place January–May, 1992. 8353 children were screened; 279 were infested. Parental written informed consent was obtained for 193 children from 137 classes which were randomised to receive either malathion lotion or *d*-phenothrin lotion. The number of infested children in the classes was: 1 child in 93, 2 in 33, 3 in 10, and 4 in 1. 95 children received malathion lotion and 98 received *d*-phenothrin lotion. Baseline characteristics were similar except for a significant difference for the length of hair in the *d*-phenothrin group ( $p=0.02$ ;  $\chi^2$ ) (table 2). Based on the total volumes of insecticidal lotions remaining after treatment of 81 children in the malathion group and 82 children in the *d*-phenothrin group, amounts applied in the two groups were similar (mean 17.2 mL *vs* 16.2 mL). All but 7 children were seen at the scheduled visits: 3 missed the visit on day 1 (1 in the malathion group and 2 in the *d*-phenothrin group) and 4 others (1 in the malathion group and 3 in the *d*-phenothrin group), seen on day 1, were lost to follow-up by day 7; we were able to estimate the missing values based on the observations noted at the other visit.

On day 1, the success rate was 92% in the malathion group and 40% in the *d*-phenothrin group ( $p<0.0001$ ; Fisher's test and generalised regression model) (table 3). On day 7, malathion's success rate was 95% and that of the *d*-phenothrin group was 39% ( $p<0.0001$ ). Adjusting for the length of hair and the distribution of live lice and viable nits in the comparison of treatment groups did not modify the results ( $p<0.0001$ ; Mantel-Haenszel test). On day 7, live lice were found in 6 malathion-treated children and in 58 *d*-phenothrin-treated children. Among the 87 malathion-treated children considered to be cured on day 1, 4 (5%) were not cured on day 7 and, among the 8 children considered not to be cured on day 1, 1 (13%) remained infested on day 7. Among the 39 *d*-phenothrin-treated group considered to be cured on day 1, 14 (36%) were not cured on day 7, and among the 59 children considered not to be cured on day 7, 46 (78%) remained infested on day 7. No side effects were reported. Interim

Day	Malathion	<i>d</i> -phenothrin	<i>p</i>
1	92% (0.86–0.97)	40% (0.30–0.49)	<0.0001
7	95% (0.90–0.99)	39% (0.29–0.48)	<0.0001

% (95% CI).

Table 3: Success rates in schoolchildren assigned to receive malathion or *d*-phenothrin lotion

Test	Malathion (n=357)	<i>d</i> -phenothrin (n=416)	Control (n=320)
Dead at			
1 h	357 (100%)	40 (9.6%)	22 (6.9%)
2 h	–	12 (2.9%)	9 (2.8%)
24 h	–	282 (67.8%)	245 (76.6%)
Total	357 (100%)	334 (80.3%)	276 (86.3%)
Alive at			
24 h	0	82 (19.7%)	44 (13.7%)

Table 4: Pediculicidal test (survival distribution) at 3 different times after application of malathion, *d*-phenothrin, or no treatment to live lice collected from the heads of infested schoolchildren

results led to the decision to stop the study early, in accordance with the protocol ( $\alpha'=0.029$ ).

Results of the parasitological tests were used to compare the survival distributions of live lice collected from the heads of schoolchildren and subjected to malathion, *d*-phenothrin, or no treatment (control group) in the laboratory (table 4). One hour after application, 100% of the lice had been killed by malathion and 9.6% by *d*-phenothrin as compared with 6.9% of the control. Two hours after application, *d*-phenothrin had killed 12.5% of the lice, while 9.7% of the control lice were dead. 24 hours after application, 80.3% of the *d*-phenothrin-treated lice were dead *vs* 86.3% of the control. Survival distributions were significantly different between the three groups ( $p<0.001$ ; log-rank test), with the survival distribution of the malathion group being different from both *d*-phenothrin and control groups ( $p<0.001$ ; log-rank test). No difference was found between *d*-phenothrin and the control group ( $p=0.33$ ; log-rank test).

## Discussion

We found that a single application of malathion was more effective, evaluated as the percentage of children without both live lice and parasitologically viable nits, than *d*-phenothrin. As eggs need about a week to hatch, the main end point of the trial was set at day 7. Before the trial, we hypothesised that a 10% difference between the two insecticides would be clinically relevant. Indeed, malathion showed a better ovicidal efficacy, as indicated by the success rate on day 7 (95% *vs* 39%) but also a better pediculicidal efficacy, as indicated by the success rate on day 1 (92% *vs* 40%), regardless of the extent of infestation. Moreover, patients cured on day 1 in the *d*-phenothrin group had a 36% probability of having a relapse on day 7, while 95% of the patients cured on day 1 in the malathion group remained cured on day 7. Because viable nits are sometimes difficult to recognise clinically, we did a nit-hatching test to identify parasitologically viable nits,<sup>15</sup> before any insecticidal treatment was applied. The results confirmed the clinical observation that malathion was better, killing all the live lice, regardless of the duration of the application, unlike *d*-phenothrin and untreated controls.

Several factors may influence the efficacy of pediculicidal treatments. Before starting the trial, we confirmed that the commercially available malathion and *d*-phenothrin lotions were equally effective in eradicating nits and head lice bred on rabbits. We used a lotion preparation, which is the best for eradicating head lice.<sup>9</sup> The characteristics of both groups were similar for the duration of application and volume of lotion used, number of rooms at home that can be considered as a measure of contact or transmission, and hair colour and texture. *D*-phenothrin was applied for at least 8 hours which could have increased the efficacy of this treatment, since the usual duration of pyrethrin and pyrethroid application is 10 to 60 minutes.<sup>2,3</sup> The distribution of live lice and viable nits and, consequently, the extent of lice infestation was also similar in both groups. Although the two groups differed for the length of hair at the time of inclusion, the conclusions remained unchanged when the analysis was adjusted for this parameter. Moreover, no direct correlation between the length of hair and lice infestation rates has been shown.<sup>19</sup> Reinfestation was not a

bias in our trial, as the study was controlled and the parents were instructed to treat all the family members and the fomites at the same time as the index case.

Ovicidal and pediculicidal activities of the formulation and also important. Preliminary pharmacological tests on rabbit-bred lice and nits indicated that malathion and *d*-phenothrin had excellent activities. Because *d*-phenothrin is a synthetic pyrethroid with a greater specific pediculicidal activity than natural pyrethrins,<sup>2</sup> we did not use a mixture containing piperonyl butoxide, a synergist for pyrethrins. Commercially available products were chosen to evaluate the efficacy of insecticides on live lice. Although the results differed somewhat, the pediculicidal test on human-grown head lice reflected the conclusions of the clinical trial. A similar model using freshly collected head lice has previously been described and could represent an alternative approach.<sup>16</sup>

The repeated use of one pediculicide on a louse population is likely to select resistant lice.<sup>20,21</sup> *P humanus capitis* resistance has already been reported. Reports of resistance to DDT and lindane led to the introduction of carbaryl, malathion, pyrethrins, and synthetic pyrethroids.<sup>10,13,22</sup> Resistance to these new insecticides has also been reported but remains anecdotal.<sup>11-13</sup> We found malathion and *d*-phenothrin to be equipotent in an experimental model of *P humanus* bred on rabbits. Although this species differs in its feeding habits, lifestyle, and appearance from human head lice, the model was thought to be predictive of the pediculicidal activity in human louse infestation,<sup>14,16</sup> although these experimental lice were never exposed to pediculicides other than during testing and thus their development of resistance was prevented. It appears that results in laboratory-raised lice must not be predictive of results in children with pediculosis capitis.

Our results suggest the emergence of acquired resistance to this treatment by the tested head lice, since conditions of insecticide administration were standardised. In the UK, a trial showed a slightly greater efficacy of a 0.2% *d*-phenothrin shampoo vs a 0.5% malathion lotion,<sup>18</sup> a discrepancy that could reflect the over-use of pediculicides selecting resistant head-lice, especially in countries where pediculicides have been used for the past 10-20 years.<sup>13</sup> Malathion was marketed in France in 1983, whereas it entered the British market in 1971,<sup>2</sup> unlike the pyrethrins and synthetic pyrethroids that were developed in France 15 years ago. Moreover, the latter are widely used in many countries because they are safe, photostable, cosmetically more acceptable, easy to use, and may be prophylactic.<sup>2,3</sup> Shampoos expose lice to a short contact time and a low concentration of insecticide which may favour resistance to treatment.<sup>23,24</sup>

Regardless of the mechanism of resistance, physicians and pharmacists should be made aware of possible variations in the efficacy of pediculicidal preparations, and should advise non-responders to switch to another type, for example from pyrethrins or pyrethroids to malathion. The possibility of incomplete ovicidal activity may also lead to a standard recommendation of a second treatment after 7-10 days. New formulations and concentrations of insecticidal products, such as 1% permethrin or an association of pyrethrins or pyrethroids with malathion, would perhaps achieve better control of head-lice infestation.<sup>3</sup> In the UK, the policy of alternating the use of malathion and carbaryl every three years has been thought to minimise the risk of developing resistance.<sup>25</sup>

We think that it is always advisable to introduce new insecticides within this public-health policy to reduce the risk further. A regional survey based on a periodic pediculicidal test could also help to select the best pediculicide to eradicate *P humanus capitis*.

We thank J M Alexandre, Y Benin, L Blum, N Bravard, J Brice, C Brière, M Carnec, C Caulin, A Chauvin, J Choïn, L Chosidow, C Cotel, F Ficat, L Fonteneau, C Gervais-Philippe, S Guiguen, J C Guillaume, S Herson, B Hubert, J Jacobson, T Kalo, M F Kogut, Y Layer, Y Leclerc, A Mansion, C Muziot, C Paclot, F Poli, R Pomarede, M D Riou, M Robert, G Rykner, V Saada, N Sade, E Serna, A Serra for help in the study. We are indebted to all the children we visited at school and their parents, to the medical staff, social workers, teachers and school principals.

This work was supported by grants from the Direction Générale de la Santé and the Direction de la Pharmacie et du Médicament, Ministère de la Santé, the Délégation à la Recherche Clinique, Assistance Publique-Hôpitaux de Paris (911409), and the Institut National de la Santé et de la Recherche Médicale (INSERM) (CNEP 91CN51). Sarget Drug Company provided malathion (Prioderm) free of charge.

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