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Evidence for double resistance to permethrin and malathion in head lice

A.M.R.DOWNS, K.A.STAFFORD,* I.HARVEY† AND G.C.COLES*

*Departments of Dermatology, *Veterinary Science and †Public Health, Bristol University, Bristol, U.K.*

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Summary A rising prevalence of head lice among school children and rising sales of insecticides with anecdotal evidence of their treatment failure, led us to examine whether head lice in Bristol and Bath were resistant to the insecticides available for treating head lice. Ten schools in Bristol and Bath were visited to collect field samples of head lice. A comparison was made of the survival rates of fully sensitive laboratory reared body lice and field samples of head lice on insecticide exposure. To confirm the *in vitro* relevance of these tests we performed supervised treatments of affected subjects with malathion or permethrin. There were significant differences ($P < 10^{-6}$ Fishers exact test) between head and body lice survival for malathion and permethrin exposure, but not for carbaryl. There was an 87% failure rate for permethrin and a 64% failure rate for malathion with the topical treatment of a selected number of infested school children. We conclude that there is a high resistance to permethrin and malathion, but head lice remain fully sensitive to carbaryl. This is the first report of doubly resistant head lice. As permethrin, phenothrin (a very similar synthetic pyrethroid) or malathion are the active ingredients in all the over-the-counter head lice treatments in the U.K., then it is likely that head lice prevalence will continue to increase. The resistance against permethrin employed by the head louse is probably the *kdr* (knockdown resistance) mechanism, and an enzyme-mediated malathion-specific esterase is the likely mechanism against malathion.

Key words: head lice, insecticide resistance, organophosphates, synthetic pyrethroids

Sales of insecticides for the treatment of head lice in the UK have risen over the last 10 years, which may reflect both an increased prevalence of head lice and the poor efficacy of available treatments. The removal of 'nit nurses' and a change to group learning, which encourages head-to-head contact, may have contributed to an increase in infection rates, and with more lice being treated there is a greater risk of resistance developing.

Evidence is gathering that head lice resistance is occurring, certainly against synthetic pyrethroids[1–3] and possibly against organophosphates.[4] All previous *in vitro* studies have exposed samples of head lice to different concentrations of insecticide for between 16 and 24 h, or exposed the head lice to a single concentration of insecticide and measured the time until either 50% or 100% of the sample of insects are dead. The natural mortality of 10% a day, makes the significance of some of these tests difficult to interpret.[3,5] It is also hard to draw meaningful conclusions where *in vitro* studies can only examine the LD_{50} (drug dose achieving 50% mortality) compared with the LD_{100}.[3,5] With some *in vivo* trials, there has been a failure to compensate for re-infection of the study patients, as the cause for apparent treatment failure.[6] We evaluated the possibility of head lice insecticide resistance in Bristol and Bath, with *in vitro* and *in vivo* experiments using LD_{100} studies and inspection of treated children before re-infection was likely.

Correspondence: Dr A.M.R.Downs, Department of Dermatology, Bristol Royal Infirmary, Bristol BS2 8HW, U.K. Fax: 01179 282845

Subjects and methods

Insecticide impregnated filter papers were made by dipping standard Whatman type 1 filter papers into a range of insecticide concentrates as shown in table 1. Body lice were used as a test model for head lice, as both types of lice are *Pediculus humanus* subspecies.[7] There are no known laboratory or field strains of susceptible head lice. Fully insecticide sensitive laboratory bred

body lice[7] were used to establish an LD_{100} for permethrin, malathion and carbaryl and an LD_{75} for DDT, at 2 h exposure on the impregnated filter papers at 30 °C and 70% relative humidity (r.h.)—the optimum survival conditions for human lice[7]—based on a World Health Organization method for determining susceptibility to insecticides.[8] Death was taken as the absence of all movements or irreversible intoxication (paralysis or continuous tonic-clonic spasm). Control body lice were placed on untreated filter papers. Both freshly made and 3-month-old impregnated filter papers (stored in the dark at 4 °C) were compared for the insecticides permethrin, malathion, carbaryl and DDT.

Ten schools in Bristol and Bath were visited (total number of pupils = 3300) following permission granted by the South & West Research Ethics Committee. Schools were randomly chosen following consent from the individual head teachers. Consent for examining and treating children was obtained from parents, and the local school nurse was present during all examinations. Head lice were collected from school children (aged 5–11) using a fine-toothed lice comb. Live adult head lice were pooled to provide sufficient test numbers, randomized and exposed to impregnated filter papers at three concentrations of insecticides above and below LD_{100} for permethrin, carbaryl and malathion and equal to LD_{75} for DDT. The filter papers had been prewarmed and remained in an airtight 32-L temperature controlled portable incubator set at 30 °C and 70% r.h. using a beaker containing 34 g of potassium hydroxide in 100 mL of distilled water.[9] Control head lice were placed on untreated filter papers.

Two of the over-the-counter products for head lice were tested by the primary author (AMRD) on a group of school children (aged 7–9 years) from two of the 10 schools, all of whom had an infestation with live adult head lice. One per cent aqueous malathion (Derbac-M®, Seton-Scholl Healthcare plc, Tubiton House, Oldham, U.K.) or aqueous permethrin (1% Lyclear®, creme rinse, Warner Lambert UK Ltd, Lambert Court, Eastleigh, U.K.) was applied to the dry hair, massaged into the scalp, combed through and washed out 20 h later with a normal shampoo. Permethrin and malathion were left on the same length of time to standardize the protocol. The children's hair was re-examined 48 or 72 h after initial insecticide application, using a fine-toothed lice comb to identify live adult head lice. A head lice infestation was defined as the presence of one or more live adult lice. It is recognized that pediculicides are not 100% ovicidal[2,10] and for this reason the presence of nymphs was not taken as evidence of treatment failure.

Results

There was no difference in potency between 3-month-old and freshly prepared filter papers, establishing the long-term stability of these insecticides on filter paper at low concentrations when stored in the dark at 4 °C. Consistent percentage mortality results were obtained from the body lice and cat fleas (*Ctenocephalides felis*) on impregnated filter paper exposure (results not shown), which validates the reproducibility of this method of preparing filter papers. Ninety-five per cent of the school population was examined. Table 1 shows the combined head lice mortality for Bristol and Bath. There is a significant difference ($P < 10^{-6}$ Fishers exact test) between head and body lice survival for malathion, permethrin and DDT, but not for carbaryl.

For the treatment of Bristol school children with permethrin, 100 children were examined from three classrooms. Sixteen children were infested: 38% (six of 16) were heavily infested (≥ 10 adult lice) and 62% (10 of 16) had a lighter infestation (< 10 adult lice). Following treatment, 13% (two of 15) had no head lice (both had had light insect loads), and of the remaining 87% (13 of 15), 20% (three of 13) had a partial decrease in insect load, and 62% (10 of 13) remained unaltered. For the treatment of Bath school children with malathion, 65 children were examined from three classrooms. Fourteen children were infested; all with a light insect load. Following treatment, 36% (five of 14) had no head lice, and 64% (nine of 14) remained unaltered.

Discussion

The high survival rate of head lice on treated individuals suggests resistance to the local treatments used (permethrin in Bristol and malathion in Bath). The high survival rates on impregnated filter papers to both insecticides infers doubly resistant head lice in both areas. We suspect that this is not just a regional phenomenon. National Health Service prescriptions for England and Wales between 1990 and 1995 for all products with a licence for use against head lice have risen 3·7-fold, from just over 400 000 prescriptions to 1 500 000.[11] National sales data from head lice product manufacturers show a similar trend over the last 10 years and presumably reflect a rising head lice population.[11] The excessive chronic application of head lice treatments increases the chances of developing

Table 1. Total number of dead and alive lice for susceptible body lice, combined Bristol schools head lice and combined Bath schools head lice at three insecticide concentrations $\geq LD_{100}$ for susceptible body lice (permethrin, malathion, carbaryl) and at LD_{75} for DDT. All control lice (not shown) remained alive

	No. of body lice tested		Total no. of Bristol head lice tested		Total no. of Bath head lice tested	
	Dead	Alive	Dead	Alive	Dead	Alive
Malathion						
0·1 g/100 mL isopropanol	56	4	3*	75*	9*	120*
0·2	70	0	17*	53*	17*	106*
0·4	60	0	40*	32*	36*	92*
Permethrin						
0·025 g/100 mL isopropanol	57	3	4*	69*	14*	130*
0·05	70	0	7*	58*	12*	128*
0·1	60	0	12*	52*	22*	103*
Carbaryl						
0·8 g/100 mL isopropanol	62	8	61	3	129	6
1·6	70	0	70	5	140	2
3·2	70	0	77	3	140	2
DDT						
2 g/100 mL isopropanol	30	10	0*	32*		

* $P < 10^{-6}$ (Fisher's exact test); other differences not significant.

resistance to these products. Both malathion and permethrin are available over-the-counter as well as on prescription, so there are no safeguards to prevent unrestrained and indiscriminate use of either product. Carbaryl, in the U.K., is a prescription only medication. Its limited use may be a reason why we have not seen any resistance develop to this product.

Electrophysiological studies on the housefly show that synthetic pyrethroids have two effects on insect nervous tissue.[12] Type I effects cause hyperexcitability and uncoordinated movements, and are not related to mortality. Type II effects cause convulsions and paralysis, and are related to mortality. Our *in vitro* and *in vivo* studies confirm this observation in head lice. Permethrin exhibits type I effects, but at higher concentrations it exhibits type II effects.[13] It has been shown in mosquitoes,[14] and houseflies[15] that DDT has the same mode of action as synthetic pyrethroids, and that DDT resistance and pyrethroid resistance are due to an altered insecticide binding site within insect nerves (the so-called *kdr* resistance). As we have shown that the head lice are resistant to both permethrin and DDT, this suggests that the resistant mechanism in head lice is due to *kdr* resistance. We were unable to obtain an LD_{100} for DDT. This may have been due to DDT binding strongly to the filter paper and only a small amount entering the head louse.

Further studies on houseflies[13] have shown that pyrethroid toxicity is inversely related to temperature. The portable incubator allowed the filter papers and head lice to remain at a constant temperature.

Both malathion (an organophosphate) and carbaryl (a carbamate) irreversibly bind to acetylcholinesterase at the same binding site, preventing its function and causing spastic paralysis and death.[16] As head lice remain fully sensitive to carbaryl, it suggests that the malathion resistance is not due to an altered acetylcholinesterase binding site. It is likely that a specific malathion resistance mechanism is in operation. This probably means that head lice are not resistant to other organophosphates. Some authorities suggest that 5% is more appropriate for resistance testing for malathion, but we used up to 0·4% because it corresponded to a concentration that was beyond the LD_{100} for the susceptible body lice and a clear difference was observed.

Only 7–8-year-old pupils were assessed for topical treatment as they were found to be the most heavily infested age group. Permethrin was used to treat Bristol pupils and malathion was used for Bath pupils in order to adhere to the local prescribing advice for pediculicides. The study was therefore only single blind. Only 20% of infested school children had parental consent for treatment. Siblings and other family members were not treated so the potential for re-infestation was high.

However, following the conventional application of a topical insecticide, there is insecticide impregnation of the scalp and hair shafts. This allows a lethal dose of insecticide to be delivered to any newly hatched nymphs or refugee lice from another person for many days if not weeks.[17,18] The adult head lice that we found 48 and 72 h later on the scalp of the permethrin or malathion treated children must be resistant to these products. Given that lice can be difficult to find in hair with low levels of infection, then it is very likely that our results are an underestimate of the problem.

This study confirms the suspicion that head lice are resistant to the over-the-counter treatments available in the U.K. which contain malathion or permethrin and is in keeping with the types of resistance which have developed in other insect species[19] due to chronic pesticide use.

The Department of Health in the U.K. acknowledges that carbaryl has a mutagenic potential,[20] and should continue to have restricted use only. Novel insecticides, such as fipronil[21] and imidacloprid[22] which are effective flea pesticides, require a rapid evaluation for introduction into the market to provide new safe products for the chemical control of head lice.

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