DETECTION AND MANAGEMENT OF PYRETHROID RESISTANCE IN RELATION TO THE USE OF IMPREGNATED BEDNETS AGAINST MALARIA VECTORS

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Malaria is by far the most important vector borne disease. Latest estimates are of 300–500 million clinical episodes and 1.4–2.6 million deaths due to malaria each year, 80–90% of these being in tropical Africa (WHO, 1995). The most important practical advance against malaria in recent decades has been the introduction of pyrethroid impregnated bednets, which, for the price of treatment of a relatively small surface area, place a quick acting insecticide (with low human toxicity) directly in the path of the host-seeking mosquito. Operational programmes in China involving deltamethrin treatment of several million nets per year have lowered numbers of recorded malaria cases by about 75% over 3 years (Cheng *et al.*, 1995). In a series of trials supported by W.H.O. in Africa, child mortality from all causes has been reduced by between 17 and 63% as a result of the introduction of permethrin impregnated nets (Alonso *et al.*, 1991, D'Alessandro *et al.*, 1995a; Nevill *et al.*, 1996; Binka *et al.*, 1996).

The use of pyrethroid impregnated nets will certainly become more widespread in coming years. It is to be hoped that much of this expansion will be via publicly funded health services with subsidies to enable the rural poor (the main sufferers from malaria) to benefit. However, in towns people who can afford to do so will probably purchase nets and insecticide with which to impregnate them, with the intention of avoiding vector borne disease as well as nuisance mosquitoes, bedbugs and lice.

It is important to try to assess the immediacy of the threat of pyrethroid resistance to the continued effectiveness of impregnated nets and whether there are any means of delaying the appearance of resistance which are consistent with continuing to enjoy the benefits of impregnated nets. Some have argued that resistance is so obviously such an immediate threat that only untreated nets should be used, despite the fact that it has been shown that these are not so effective in preventing malaria under tropical village conditions as are treated nets (Jana-Kara *et al.*, 1994; D'Alessandro *et al.*, 1995b). This view seems an extreme form of the not uncommon ideology which justifies the non-use of an available pesticide to avoid the evil of resistance. Put in this way it should surely be obvious that there is no point in preserving susceptibility to a class of pesticides if they are never going to be used. The converse extreme view would be that one might as well use up the resource of susceptibility now. In answer to the question "shouldn't some susceptibility be left for posterity?" the retort might be made: "what has posterity ever done for me?". Ideally there would be some way of both benefiting from, and conserving, the resource of susceptibility. The conventional wisdom is that this can be done by a pre-planned rotation of unrelated compounds. However, in my opinion it has never been demonstrated by a controlled experiment that there is any advantage in the long term between such a pre-planned rotation and simply switching between compounds when forced to do so by the appearance of resistance and enabled to do so by the regression towards susceptibility (Curtis *et al.*, 1993).

In either case it is highly desirable that a safe, acceptable and effective alternative to reliance on pyrethroids alone is found for net treatment. DDT was used in the Second World War for bednet impregnation. DDT resistance in anophelines generally depends on a glutathione S-transferase mechanism which does not give cross resistance to pyrethroids (Hemingway *et al.*, 1985; Malcolm, 1988). However, Li *et al.* (1987) have shown that DDT is inferior in effectiveness and persistence on nets compared with pyrethroids. Among organophosphates, pirimiphos methyl has been tested on bednets and found less effective in preventing mosquito feeding and it also makes the net unpleasantly sticky (Miller *et al.*, 1991).

Proceedings of the Second International Conference on Urban Pests.
K.B. Wildey (editor). 1996

Bendiocarb is considered too toxic to use on bednets but acceptable for use on curtains. However, it does not persist as well as pyrethroids (Curtis *et al.*, 1996) and, in a direct comparison of bendiocarb or pyrethroid impregnated curtains, the latter were significantly more effective against the *Culex* vectors of filariasis in Sri Lanka (Weerasooriya *et al.*, 1996).

The different available pyrethroids do not seem to provide a solution to the problem. A strain of *Anopheles stephensi* from Dubai, selected for resistance to permethrin showed almost exactly the same resistance ratio for lambda-cyhalothrin, when assessed by observing time for knockdown in contact with an impregnated net (Table 1; based on Akhtar *et al.,* submitted). Surprisingly, however, a mixture of these pyrethroids gave slightly but significantly more rapid knockdown than either pure compound. Etofenprox is described as a "near pyrethroid" but is resisted by the Dubai strain to a similar degree as is permethrin (Curtis, 1993).

The Insect Growth Regulators pyriproxyfen and triflumuron have been reported to cause sterility in adult female insects (Langley *et al.*, 1988; Howard and Wall, 1995). These IGRs are very specific to insects and safe for close contact with humans. We are therefore interested in the possibility of mixing an IGR with a pyrethroid on nets. The intention is that any individual mosquito, which rests for long enough to pick up what would normally be a lethal dose of the pyrethroid, will also pick up a sterilising dose of the IGR. Thus a pyrethroid resistant individual which is not killed by this dose would be prevented from passing on its resistance genes and selection for resistance by the nets would thus be prevented. Miller (1994 and unpub.) tested a series of doses of pyriproxyfen with permethrin on nets against the Dubai strain of *An.stephensi* in a realistic manner by sitting under the treated nets with her arm against the net, allowing mosquitoes to bite through the net and pick up both compounds while biting. She was able to achieve over 90% sterility of the surviving mosquitoes in this way. However, the dose of pyriproxyfen required was such that it would greatly increase the cost of net impregnation. We are hoping for a better cost-effectiveness ratio with triflumuron, but it is clear that any scheme of this kind would add to the cost of net treatment. Programme organisers would therefore need very convincing experimental data to persuade them to accept extra costs now, to avoid or delay a disastrous outbreak of resistance at some unspecified time in the future.

Assessment of whether such extra costs really are a reasonable investment would be helped by knowledge of whether and how quickly widespread use of pyrethroid impregnated nets has already selected for resistance.

Vulule *et al.* (1994) reported a significant rise in LT_{50} in *An.gambiae* after a year's use of impregnated nets or curtains in four Kenyan villages. However, they have found no further rise after two more years of use (Vulule *et al.*, 1996). They were able to select artificially in the laboratory for enhanced resistance and kindly provided us with this stock. Hodjati and Curtis (1996) showed that, with this selected stock, a susceptible *An.gambiae* or the Dubai stock of *An.stephensi*, older mosquitoes were less tolerant of pyrethroids than newly emerged individuals. Since the tests of Vulule *et al.* were done with adult mosquitoes collected in the villages and since widespread use of impregnated nets reduces the mean age of village mosquito populations (Magesa *et al.*, 1991; Vulule *et al.*, 1996), it is at least theoretically possible that apparent genetical resistance was actually due to the physiological effect of the populations being, on average, younger. The test of this hypothesis would be to compare newly emerged mosquitoes from pupae from the netted and control villages.

Table 1. Data of Akhtar *et al.* (submitted) on median knockdown times (KT_{50}) of pyrethroid resistant and susceptible strains of *An.stephensi* made to walk on netting impregnated with permethrin, lambdacyhalothrin or mixtures of these two compounds.

Impregnation doses:					
mg permethrin/sq m:	500	375	250	125	0
mg lambda-cyhalothrin/sq m:	0	2.5	5	7.5	10
KT_{50} (mins + secs):					
Dubai (resistant):	42'30"	35'56"	35'37"	37'30"	44'04"
India (susceptible):	14'22"	10'04"	10'00"	9'57"	14'22"
resistance ratio:	2.96	3.57	3.56	3.76	3.07

Table 2. Data of Kang *et al.* (1995) on median knockdown time (KT_{50}, in mins and secs) on netting impregnated with 15 mg deltamethrin/sq m of (a) *An.sinensis* from townships in China with varying durations of use of deltamethrin impregnated bednets, (b) *Culex quinquefasciatus* kept in a pyrethroid factory for varying durations:

(a)	Years of bednet treatment:	0	1	6	7
	KT_{50} of *An.sinensis*:	11'00"	11'36"	11'18"	8'42"
(b)	Years of factory maintenance:	0	0.25	1.5	2.5
	KT_{50} of *Cx quinquefasciatus*:	17'12"	52'00"	>120'	>120'

Table 3. Median knockdown time (KT_{50}, in mins and secs) on netting impregnated with 500 mg permethrin/sq m of the two malaria vector species in Tanzanian villages with 8 years use of permethrin impregnated nets or no known use of pyrethroids.

	8 yrs use	No known use
An.gambiae s.s.	10'05"	12'15"
An.funestus	7'12"	7'20"

Meanwhile two studies have been carried out in areas where net impregnation has been kept up since 1987 - in the large operation in China, mentioned above, and in a small Tanzanian village. In China, no difference was found between the time for knockdown in contact with treated nets of *An.sinensis* from the operational area in Sichuan, from areas in Hubei Province where net impregnation has been taken up more recently or from the outskirts of the city of Wuhan where there is no malaria and hence no use of deltamethrin treated nets (Table 2a, based on Kang *et al.*, 1995). Data on the more dangerous vector, *An.anthropophagus*, mainly using the W.H.O. discriminating dosage of 0.025% deltamethrin, also gave no evidence for resistance. Both testing methods were well able to detect resistance in a *Culex quinquefasciatus* strain (Table 2b) which had been maintained in a laboratory in a factory where a volatile pyrethroid was handled, which presumably created sufficient air pollution to act as an unintentional agent of selection.

In the small Tanzanian village of Mng'aza, permethrin impregnated polyester bednets were given to almost all families in 1987 (Njunwa et al., 1991) and in 1990 these were replaced by durable polyethylene nets (T.Wilkes, unpub.). The nets have been re-impregnated every 6 months. A recent survey showed that 55% of people still have, and use, their nets. Comparison of the time for knockdown of the two most efficient African malaria vectors, *An.gambiae s.s.* and *An.funestus* from this village and from a village about 10 km away, in which there has been no known use of pyrethroids, showed no difference in susceptibility between the villages (Table 3). However, there were indications of faster knockdown in *An.funestus* than *An.gambiae* (Table 3). Mng'aza is a small village with neighbouring villages 1–2 km away. There is evidence from mark-release-recapture of appreciable immigration from those villages into Mng'aza (Njunwa, 1993). The pyrethroid resistance known in *An.stephensi* is almost completely recessive (Curtis *et al.*, 1990). This situation fits the conditions for "recessive resistance with refugia" considered by Georghiou and Taylor (1977) for delaying the emergence of resistance. We are now expanding usage of impregnated nets in the villages neighbouring Mng'aza (hitherto the supposed "refugia") and it will be important to determine whether this allows pyrethroid tolerance to evolve.

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