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THE ROYAL SOCIETY

Can anything be done to maintain the effectiveness of pyrethroid-impregnated bednets against malaria vectors?

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Pyrethroid-treated bednets are the most promising available method of controlling malaria in the tropical world. Every effort should be made to find methods of responding to, or preventing, the emergence of pyrethroid resistance in the *Anopheles* vectors. Some cases of such resistance are known, notably in *An. gambiae* in West Africa where the *kdr* type of resistance has been selected, probably because of the use of pyrethroids on cotton. Because pyrethroids are irritant to mosquitoes, laboratory studies on the impact of, and selection for, resistance need to be conducted with free-flying mosquitoes in conditions that are as realistic as possible. Such studies are beginning to suggest that, although there is cross-resistance to all pyrethroids, some treatments are less likely to select for resistance than others are. Organophosphate, carbamate and phenyl pyrazole insecticides have been tested as alternative treatments for nets or curtains. Attempts have been made to mix an insect growth regulator and a pyrethroid on netting to sterilize pyrethroid-resistant mosquitoes that are not killed after contact with the netting. There seems to be no easy solution to the problem of pyrethroid resistance management, but further research is urgently needed.

Keywords: *Anopheles*; malaria; pyrethroid resistance; insecticide-treated bednet; resistance management; organophosphates

1. INTRODUCTION

Malaria is by far the most important vector-borne disease, causing an estimated 300–500 million cases and 1.4–2.6 million deaths per year, 80–90% of them in Africa (WHO 1995). Pyrethroid-treated bednets have been shown in recent trials to have an important impact on (i) cases of malaria in China and India with low to moderate amounts of malaria transmission (e.g. Cheng *et al*. 1995; Jana-Kara *et al*. 1995), (ii) incidence of infection and/or prevalence of anaemia in areas of tropical Africa with moderate to intense malaria transmission (Stich *et al*. 1994; Snow *et al*. 1996; Curtis *et al*. 1998), (iii) hospital admissions in Kenya with severe malaria (Nevill *et al*. 1996), and (iv) all-cause child deaths in several parts of Africa (Alonso *et al*. 1993; D'Alessandro *et al*. 1995; Binka *et al*. 1996; Nevill *et al*. 1996).

In comparative trials with untreated nets, treated nets reduced malaria much more effectively (e.g. Jana-Kara *et al*. 1995) because the insecticide deposit reduces the chances of mosquitoes entering or biting through the net. Furthermore, where many people in a community use treated nets, these act as baited traps and kill a large proportion of the local mosquitoes before they can reach the age at which malaria parasites have reached maturity, thus reducing the malaria risk for the whole community.

Treated nets are more affordable and acceptable than house spraying (Kere & Kere 1992; Curtis *et al*. 1998), and it is extremely important that the early promise of the treated-net method is sustained. There are questions about long-term funding in very poor countries (Lengeler *et al*. 1996) and about whether the reduction of vector populations without eradication will leave vulnerable those human populations that have not acquired their normal anti-malaria immunity (Snow *et al*. 1997). However, the subject of this paper is the threat to sustainability of this method from pyrethroid resistance in the *Anopheles* vectors, which might blunt the effectiveness of the method to the low and generally cost-ineffective levels now seen with untreated nets.

2. OCCURRENCE OF PYRETHROID RESISTANCE IN *ANOPHELES* SPECIES

In several dipteran species, resistance to knockdown by DDT (*kdr*) confers positive cross-resistance to pyrethroids (e.g. Farnham 1973; Prasittisuk & Busvine 1977), and it was feared that already existing resistance to DDT in anophelines would make them preadapted to resisting pyrethroids. However, several cases of DDT resistance were found to be due to metabolic mechanisms, which would not be expected to affect susceptibility to pyrethroids (Hemingway *et al*. 1985), and DDT-resistant *An. gambiae* from Zanzibar showed no cross-resistance to permethrin (R. T. Rwegoshora, unpublished data). However, by 1988, resistance to pyrethroids in several anopheline species had already been reported to the World Health Organization (WHO) (see tabulation by Malcolm (1988)).

Table 1 summarizes more recent published reports where pyrethroid resistance has apparently arisen as a result of selection by factors other than impregnated

Table 1. *Reports up until 1998 of cases of pyrethroid resistance in* Anopheles *spp. selected by factors other than use of impregnated bednets*

Anopheles spp.	country	reference(s)
albimanus	Guatemala	Beach *et al.* (1989)
sacharovi	Iraq	Akiyama (1996)
stephensi	India	Chakravorthy & Kalayasundaraman (1992)
stephensi	Dubai, UAE	Ladonni & Townson (1998), Vatandoost *et al.* (1998)
gambiae s.s.	Côte d'Ivoire and Burkina Faso	Elissa *et al.* (1993), Darriet *et al.* (1997), Martinez-Torres *et al.* (1998)

Table 2. *Results of tests for whether pyrethroid resistance had been selected by several years' use of impregnated bednets, as compared with where there had been no such use*

(Results from China and Tanzania are given in terms of median time for knockdown during exposure to nets treated with deltamethrin (25 mg m^{-2}) or permethrin (500 mg m^{-2}). The Kenyan results are presented as median time for mortality after timed exposures to paper impregnated with 0.25% permethrin.)

country	*Anopheles* spp.	KT_{50} or LT_{50} (min)				reference
China	years of net use	0	1	6	7	Kang *et al.* (1995)
(Hubei and Sichuan)	*sinensis*	11.0	11.6	11.3	8.7	
	anthropophagus	—	—	—	9.0	
Tanzania	years of net use	0		8		Curtis (1996)
(Tanga)	*gambiae*	10.1		12.2		
	funestus	7.2		7.3		
Kenya	years of net use	0	1	2	3	Vulule *et al.* (1996)
(Kisumu)	*gambiae*	13	31	28	36	

bednets. Resistance in *An. albimanus* in Guatemala was considered by Beach *et al.* (1989) to be connected with earlier selection by organophosphates (OPs). Earlier reports to WHO of resistance of *An. sacharovi* in Turkey and Syria have been extended by Akiyama (1996) to northern Iraq, but full susceptibility was found in anophelines from Baghdad and Basra. *An. stephensi* from Dubai (where much insecticide is used against nuisance insects) showed resistance to pyrethroids as well as to other insecticides (Ladonni & Townson 1998). The latter stock has been selected with permethrin in the laboratory to give a much higher level of resistance to a range of pyrethroids (as well as the 'near pyrethroid' etofenprox) and was used in the experiments with free-flying mosquitoes described below.

There have been several reports from Côte d'Ivoire and Burkina Faso of resistance in *An. gambiae s.s.* This is of the *kdr* type and involves cross-resistance to DDT, various pyrethroids and etofenprox (Elissa *et al.* 1993; Darriet *et al.* 1997; Martinez-Torres *et al.* 1998). The resistance gene can be detected by a method using the polymerase chain reaction and is thought to have been selected by pyrethroids used on cotton. Data will soon be published on laboratory and field tests of the effect of pyrethroid-impregnated netting on mosquitoes carrying this resistance gene (P. Guillet, personal communication).

Tests have been made at several sites in Sichuan and Hubei Provinces in China and in the village of Mng'aza in the Tanga Region of Tanzania. At the time of the tests, treated nets had been used in these places for 6–8 years and, in each country, comparisons were made with places where there had been no use of treated nets. As shown in table 2, no evidence of higher tolerance was found in the *Anopheles* spp. from the treated areas (Kang *et al.* 1995; Curtis 1996). In China the same testing method showed that strong resistance was being progressively selected during the time that a laboratory strain of *Culex quinquefasciatus* was maintained in a pyrethroid factory, presumably because of pollution of its environment by pyrethroid dust.

In Tanzania, recent tests (J. Myamba and C. Curtis, unpublished data) have included those in which larvae were collected close to villages, with or without long-term use of treated nets; the larvae were reared and the adults tested on the day of emergence. Such tests showed no evidence for resistance and, by using a standardized adult age for testing, avoided possible complications from the decline with age of pyrethroid tolerance which was found in both *An. stephensi* and *An. gambiae* (Hodjati & Curtis 1996). In addition, this avoided possible influences on the test results from any induction of tolerance that might arise by sub-lethal previous exposure of adult mosquitoes living in villages where many treated nets are in use.

As indicated above, a reduction in mean age of the local mosquito population is one of the achievable aims of community-wide introduction of treated nets. We would be interested to see age-standardized resistance tests in western Kenya where Vulule *et al.* (1994, 1996) reported a rise in permethrin tolerance of *An. gambiae* collected in the field as adults in four villages after one year of use of treated nets or curtains (table 2). There was no further rise after two more years' use. Such a pattern seems more

Table 3. *Numerical example to show the advantage, in a population, only part of which is exposed to an insecticide, if a dose can be used which kills all exposed heterozygotes (RS) for a resistance gene*

(Note that parents of the next generation are the sum of those not exposed plus those that survive exposure. Note that the rise in the frequency of R is far greater in case (ii) than in case (i).)

	RR	RS	SS	total	frequency of R
population at Hardy–Weinberg equilibrium	10	19 980	9 980 010	10 000 000	0.1%
10% unexposed to insecticide	1	1998	998 001	1 000 000	
(i) survivors of 90% exposure to dose that kills all RS and SS:	9	0	0	—	—
then parents of next generation:	10	1998	998 001	1 000 009	0.101%
(ii) survivors of 90% exposure to dose that kills 75% of SS and 50% of RS:	9	8991	2 245 502	—	—
then parents of next generation:	10	10 989	3 243 503	3 254 502	0.169%

consistent with a life-shortening or other phenotypic effect of extensive use of pyrethroids in the area rather than with selection for resistance genes, which, once started, might be expected to proceed progressively so long as pyrethroid exposure continued. However, there is no doubt that resistance genes were present in the population, as artificial selection by Vulule *et al.* (1994) produced a strain with unequivocal resistance, although not as strong as that in the selected strain derived from *An. stephensi* of Dubai origin.

3. POSSIBLE METHODS OF USING PYRETHROIDS TO MINIMIZE THE RISK OF RESISTANCE IN *ANOPHELES*

If there is any truth in the general belief that low doses select for resistance, this could be due to a failure of low doses to kill resistance heterozygotes, i.e. rendering of the resistance genetically dominant or partly dominant at low doses, in contrast to recessiveness at high doses. From the Hardy–Weinberg ratio, new resistance genes, when still rare, would almost entirely be heterozygous until they have been selected to a moderate frequency. Thus, it is the heterozygous response that is crucial in the field because if it is only the very small number of resistance homozygotes that survive exposure, these will almost certainly be far outnumbered by susceptibles that avoid exposure, e.g. in houses without nets or in feeding and resting places other than dwelling houses. Thus, selection favouring resistance homozygotes alone would raise the frequency of the resistance gene only extremely slowly in the important early stages of build up of resistance (table 3).

In determining whether heterozygotes for resistance genes are less easily killed than susceptible homozygotes, it is important that the tests are made in conditions that are as realistic as possible. This is especially so with pyrethroids, which have an irritant effect on insects. We attempted laboratory simulations of the situation in a tropical bedroom provided with a treated net by sitting under such a net with an arm against it and releasing female mosquitoes to fly freely in a mosquito-proof laboratory room. We did not release males, because only females are attracted to blood feed and therefore we assumed that there would be little contact of males with the nets and so virtually no selection for resistance in that sex. The females could be seen probing the net, and were often seen flying away and sometimes trying again. If they found the arm they might blood feed through the net but, with treated nets, many were found knocked down on the floor. We have so far been able to expose each of many replicate batches of mosquitoes for only half an hour, after which time the knocked-down and the still-active mosquitoes were collected and scored after 1 h and 24 h for delayed mortality or recovery from knockdown. Table 4*a* (based on Hodjati & Curtis (1997)) indicates that, with heterozygotes for the resistance of the selected Dubai strain of *An. stephensi*, there was a higher knockdown and mortality using a net treated with target doses of permethrin of 200 mg m^{-2} than with 500 mg m^{-2} (the actual doses were found by analysis to be somewhat less). On the lower dose there was 100% mortality after 24 h of both the heterozygotes and susceptible homozygotes, i.e. no selection for resistance can be expected. However, on the higher dose there was significantly lower mortality of the heterozygotes than the susceptibles. This apparently paradoxical lower mortality on the higher dose can be explained by the observed earlier take-off of mosquitoes irritated by netting with the higher dose, leading to shorter average exposure to the higher dose.

Thus, contrary to the accepted doctrine, a lower dose of this insecticide runs less risk of selecting for resistance than does a higher dose. We have for a long time advocated the use of a lower dose on the grounds of economy, in view of our data showing that its performance against susceptible wild mosquitoes is as good as that of the higher dose (Lines *et al.* 1987; Curtis *et al.* 1996). The argument for the use of a lower dose is further strengthened by the above data illustrating the relative risks of selecting for resistance.

Table 4*b* shows the preliminary data of I. Kasumba (unpublished) on various doses of lambdacyhalothrin found by analysis to range between 1 and 13 mg m^{-2}. The highest of these doses is approximately what is now commonly used for net treatment; markedly higher doses tend to make net users sneeze (Njunwa *et al.* 1991). At each of the doses tested, the resistance heterozygotes showed a knockdown rate that was distinctly lower than that of susceptibles, i.e. one could expect selection for resistance at each of these doses. We have been advocating this or similar alpha-cyano pyrethroids, in preference to permethrin, because the former are effective even after

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Table 4. *Survival of susceptible (Beech stock) and heterozygous resistant (Beech × Dubai)* An. stephensi *after exposure to a human subject under a bednet with various alternative treatments (target dose and actual deposit found by gas liquid chromatography are indicated)*

(*a*) Data of Hodjati & Curtis (1997) with permethrin-treated nets and eight replicates for each genotype, each with 18–25 mosquitoes.

permethrin dose (mg m^{-2})		% survival 24 h after exposure		fitness of SS relative to RS (%)
target	actual	RS	SS	
0	0	100	100	100
200	147	0	0	—
500	470	23	5	22

(*b*) Data of I. Kasumba (unpublished) with various doses of lambdacyhalothrin. As indicated, some of the nets were washed and re-treated. Data are based on five replicate experiments with a total of *ca*. 90 mosquitoes tested on each net.

lambdacyhalothrin dose (mg m^{-2})		% still active 1 h after exposure		fitness of SS relative to RS (%)
target	actual	RS	SS	
0	0	93.6	85.1	90.9
3 (washed 1 ×)	1	75.8	42.6	56.2
3 (unwashed)	2	63.2	34.4	54.4
1 × 5 (washed 4 ×)	3	55.9	19.1	34.7
20 (unwashed)	13	28.5	12.5	43.9

washing nets and after treatment with much lower doses than are required where permethrin is used (Curtis *et al*. 1996). However, if there is confirmation of a reduced risk of selecting resistance when permethrin is used at a dose of 200 mg m^{-2}, then it would seem that we should make the latter the treatment of choice. This would be in line with the view urged by Roush (1989) that related compounds, to which there is cross-resistance, may nevertheless vary in the selective advantage that each gives to resistance genes, and the one giving the smallest selective advantage should be chosen. In our case, reverting to permethrin would require acceptance of a price in terms of higher treatment frequencies and costs in order to delay the onset of a resistance crisis. However, such a crisis would potentially be even more expensive in monetary terms and/or in lives lost from uncontrollable malaria.

4. POSSIBLE USE OF NON-PYRETHROIDS TO MANAGE PYRETHROID RESISTANCE IN *ANOPHELES*

Organophosphates (OPs) were tested on bednets by Brun & Sales (1976). Table 5 shows a summary of the more recent data of Miller *et al*. (1991), who compared bednets treated with pirimiphos methyl or pyrethroids in experimental huts. The OP performed as well as the pyrethroids in killing *An. gambiae* but not in protecting

Table 5. *Summary of data of Miller* et al. (1991) *on numbers of* An. gambiae *per night found to have entered, blood fed and died in experimental huts in The Gambia, in which human subjects slept under bednets with a standard pattern of holes to simulate a damaged net in domestic use*

(Means in the same column with different superscript letters differ at the 95% level of significance.)

net treatment	entered hut	blood fed	died
untreated	41.5^{a}	11.1^{a}	17.1^{a}
permethrin	16.2^{b}	4.5^{b}	11.1^{b}
lambda-cyhalothrin	33.8^{a}	5.3^{b}	30.5^{c}
pirimiphos-methyl	48.5^{a}	10.5^{a}	48.0^{d}

sleepers from being bitten, presumably because it lacks the irritancy of pyrethroids, which drives mosquitoes away after short contact or, with some formulations, even deters them from entering houses. The pirimiphos methyl formulation used caused stickiness of the net so that it attracted dirt, but we anticipate that the active ingredient could be reformulated to avoid this problem.

Different examples of resistance have been reported with either positive (e.g. Beach *et al*. 1989) or negative (Kurtak *et al*. 1987) cross-resistance between OPs and pyrethroids. The former situation would make OPs useless as a substitute for pyrethroids but the latter would make them an attractive means of actively driving pyrethroid resistance out of a population. The data of J. H. Kolaczinski (table 6) showed that, in *An. stephensi*, selection for resistance either to permethrin or malathion produced either no change or slight positive cross-resistance to the other compound. In *Cx quinquefasciatus*, however, selection for permethrin resistance had a negative effect on malathion tolerance. It remains to be seen how the *An. gambiae* populations in West Africa with pyrethroid resistance will respond to an organophosphate such as pirimiphos methyl on netting. Even if this has as good an insecticidal effect as shown in table 5 for a susceptible West African *An. gambiae*, and even though the toxicological profile of this compound is reassuring (Williams & White 1994), we anticipate some prejudice against the large-scale introduction of any OP for bedroom use in view of press coverage about the harmful effects of OP sheep dips to farmers and of OPs as a possible cause of the Gulf War syndrome.

The carbamate bendiocarb is considered safe enough for use on curtains but not on bednets. Tests against wild Tanzanian mosquitoes entering experimental huts showed that performance of bendiocarb-treated curtains was as good as pyrethroid-treated curtains (Curtis *et al*. 1996), However, in houses in Sri Lanka, pyrethroid-treated curtains performed significantly better than bendiocarb-treated curtains against a filariasis vector population of *Cx quinquefasciatus* (Weerasooriya *et al*. 1996).

The phenyl pyrazole fipronil has been tested on netting in the laboratory against anophelines, including the pyrethroid-resistant Dubai strain of *An. stephensi*. The netting was insecticidal but the time taken to kill the mosquitoes was so much delayed, compared with the action of pyrethroids (R. Williams and J. Kolaczinski, unpublished data), that it seems unlikely that it would assist in the

Table 6. *LT_{50} values (min) for different strains of mosquitoes selected with papers impregnated with 0.25% permethrin or 5% malathion in WHO insecticide-resistance test kits*

(Numbers in bold typeface indicate levels of resistance achieved by laboratory selection.)

species	strain	LT_{50} to 0.25% permethrin	LT_{50} to 5% malathion	cross-resistance to non-selecting compound
An. stephensi	Beech (susc.)	10.5	21.9	—
	Dubai	**486.2**	67.7	positive
	St Mal	9.4	**685.7**	none
Cx quinquefasciatus	PelSS (susc.)	51.7	42.7	—
	Muheza	**235.3**	29.5	negative
	Quinq	22.9	174.6	—
	Quinq (selected 19 generations)	**195.1**	36.4	negative[a]

[a]Compared with parental Quinq strain.

Table 7. *Tests of effects of insect growth regulators, which were picked up by tarsal contact with netting, on fecundity/fertility of female* Anopheles stephensi

(*a*) Data of J. E. Miller (1994, unpublished) on the effect of pyriproxyfen on fecundity during contact with either a polyester bednet or an Olyset net which incorporates permethrin into polyethylene fibre. Numbers of mosquitoes in parentheses.

		pyriproxifen-treated net		
Anopheles stephensi strain	mean no. of eggs laid after use of a net untreated with pyriproxifen	pyriproxifen dose (g m^{-2})	net with permethrin?	mean no. of eggs laid
Beech (pyrethroid susceptible)	84.3 (15)	0.5	no	56.1 (26)
	77.9 (10)	1.0	no	47.2 (15)
Dubai (pyrethroid resistant)	78.6 (37)	1.0	no	51.2 (22)
	69.1 (32)	1.0	yes	51.8 (36)

(*b*) Data of J. H. Kolaczinski (unpublished) on mean numbers of eggs laid after feeding for 5 min through untreated netting and netting treated with 1% triflumuron (numbers of mosquitoes tested in parentheses). There was a significant difference between numbers of eggs laid but not between % hatch.

Anopheles gambiae strain	untreated net	triflumuron-treated net
Kwale (pyrethroid susceptible)	75.4 (39)	53.1 (23)

personal protection of sleepers under torn nets in the way that pyrethroids do.

To sustain the control of the *Simulium* vectors, the Onchocerciasis Control Programme in West Africa successfully switches between different non-residual larvicides in rivers in response to detection of resistance to the preferred compound, temephos, and to seasonal changes in flow rate which limit the acceptability of some compounds to certain seasons (Hougard *et al.* 1993). This situation, where all residues are rapidly swept away, contrasts with a bednet treatment operation in which any attempt to switch insecticide when resistance was detected, or to operate a pre-planned policy of rotation, would be complicated by the persistence for a year or more of residues of insecticides applied earlier. Thus, mixtures of decaying and freshly applied residues with unpredictable selective effects would exist for long periods. In fact, however, mixtures of appropriate dosages of unrelated compounds may have better prospects of managing resistance effectively than do rotations (Mani 1985; Curtis *et al.* 1993; Barnes *et al.* 1995). Carefully chosen mixtures of several antibiotics (with directly observed consumption of the drugs to ensure patient compliance and meticulous hygienic practices) are recognized as the only way to control multi-drug resistance in outbreaks of tuberculosis, leprosy and other bacterial diseases.

We have been trying for several years to devise an appropriate mixture to use for bednet impregnation. The principal underlying the use of a mixture is that, so long as the resistance genes to each component are independent, rare and at linkage equilibrium, almost all resistant individuals would be only resistant to one of the components and would be killed by the other component provided that it was at the required dosage. As with the homozygotes in table 3, double-resistant combinations are assumed to be very rare compared with the proportion of the population that can be realistically expected to escape any selection. Among the ways that these assumptions can break down would be the occurrence of a mutant

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conferring positive cross-resistance. It seems unlikely that one could exclude this possibility for a pyrethroid–OP mixture nor, perhaps, for a pyrethroid–carbamate mixture.

As an alternative, we have tried to exploit the reported female-sterilizing properties of some insect growth regulators (IGRs) (Howard & Wall 1995). The intention is that one would apply a pyrethroid–IGR mixture to the nets in a community so that any pyrethroid-resistant mosquito, which made prolonged contact with the net and avoided being killed, would have simultaneously picked up enough of the IGR so as to be sterilized and thus unable to pass on its pyrethroid resistance genes.

Table 7*a* summarizes the data of Miller (1994, unpublished) demonstrating a considerable reduction in fecundity in pyrethroid-susceptible and pyrethroid-resistant *An. stephensi* that had attempted to feed on human subjects through nets carrying residues of the IGR pyriproxyfen. However, the infecundity was not complete even at a pyriproxyfen dosage that would be unaffordably high. More recently, J. H. Kolaczinski (unpublished data) has obtained similar results with the IGR triflumuron (table 7*b*).

No doubt this does not exhaust the possibilities of the use of IGR–pyrethroid mixtures for resistance and we understand that J. Bisset and M. Rodriguez in Cuba, with the encouragement of the WHO Tropical Diseases Research Programme, are embarking on a further quest for an effective IGR–pyrethroid mixture for use against Latin American anophelines. We wish them luck, as finding an effective means of preventing pyrethroid resistance in malaria vectors seems to us the most important practical current task in medical entomology.

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