# Insecticide susceptibility status of bedbugs in London

# 2006

**Sponsor:** Greater London Pest Liaison Group

**Study start date:** October 2006
**Study completion date:** February 2007

**Principal personnel:** Richard Naylor, University of Sheffield
Clive Boase, the Pest Management Consultancy

## Certification

*This report represents a true and accurate record of all data obtained.*

Signed:  Date: 19 June 2007

Richard Naylor

Signed:  Date: 19 June 2007

Clive Boase

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# 1. Summary

The aims of this project were to determine the susceptibility to carbamate and pyrethroid insecticides of bedbugs from the London area, and to report the findings back to the Greater London Pest Liaison Group.

Ten strains of bedbugs (*Cimex lectularius*) were collected from infested residential premises across the London area, and cultured in the laboratory.

Filter papers were treated with the previously determined discriminating doses of bendiocarb and alphacypermethrin ($LC_{99}$s of 57 and 36 mg ai/$m^2$ respectively). Replicated batches of adult bedbugs from each field strain, together with a known susceptible strain, were exposed through tarsal contact to the treated filter papers, and mortality read after 48 hr exposure.

Close to 99% of the susceptible bedbugs died when exposed to the bendiocarb and alphacypermethrin. However across the ten field strains, mortality on bendiocarb showed a mean of 1.8% (range 0 – 16%), while on alphacypermethrin, the mean mortality across all strains was 0.8% (range 0 - 8%).

All London strains collected show a much reduced susceptibility to both bendiocarb and alphacypermethrin. It appears that resistance is widespread. It is suggested that treatment practices should be examined and modified as necessary, in order to maintain effective bedbug control.

## 2. Background

After several decades during which numbers of bedbugs in London were at a relatively low level, the numbers of infestations are now rising sharply. A study in 2006 by L. Richards (pers. comm.) found that data from 8 London local authorities showed an annual increase since 2000 in the number of requests from the public for bedbug treatment, of 24.7%.

Coupled with the increase in requests for service is an apparent increase in the proportion of call-backs, suggesting that the control measures applied are not fully effective. An initial study (Boase, '06) on 3 strains of bedbugs from Hampshire, Wales and London showed that all 3 strains had reduced susceptibility to bendiocarb and alphacypermethrin.

To understand the situation in London better, the Greater London Pest Liaison Group offered to fund a study to determine the insecticide susceptibility status of bedbugs from the London area.

## 3. Project objectives

The objectives of this project were to:

Use previously established discriminating doses for bendiocarb and alphacypermethrin, to determine the susceptibility status of 10 strains of bedbugs collected from the London area.

To report on the findings.

# 4. Methodology

### *4.1 Source and rearing of bedbug strains*

Prior to this study, discriminating doses for bendiocarb and alphacypermethrin were established using three susceptible strains of bedbugs:

Strain 1 was sourced from Cambridge Entomology in October 1999.

Strain 2 was sourced from Maryland Inc. USA in June 2003;

Strain 3 sourced from Bayer Environmental Science, Monheim, Germany in June 2006.

Table 1 Details on the 10 field strains of bedbugs:

| London postcode of location where strain collected | Type of premises | Date collected |
|---|---|---|
| NW3 | Residential property | 15/09/06 |
| KT12 | Residential property | 11/07/06 |
| W3 | Residential property | 18/08/06 |
| SE1 | Residential property | 02/08/06 |
| SW2 | Residential property | 15/07/06 |
| EN8 | Residential property | 11/07/06 |
| NW10 | Residential property | 21/07/06 |
| N6 | Residential property | 05/09/06 |
| SW14 | Residential property | 01/08/06 |
| E1 | Residential property | 23/10/06 |

The field strains were established from collections made in infested premises. The premises were simply those at which the resident had requested bedbug treatment. Sites were not selected as having particularly difficult-to-control problems. The numbers of individual insects on which the strains were based ranged from 30 to 150.

In the laboratory the bedbugs were offered rabbits as a blood source at weekly intervals. All procedures involving rabbits adhered to the UK's Animals (Scientific Procedures) Act 1986, and were covered by UK Home Office licenses. All rearing of bedbug strains was carried out in the insectaries at Sheffield University. Strains were not pressurised with insecticides in the laboratory.

### *4.2 Insecticides*

The insecticides tested were:

Bendiocarb (carbamate). Batch number: 332-25B (99% purity)

Alpha-cypermethrin (pyrethroid). Batch number: 337-134C (99% purity)

Technical grade insecticides were purchased from Greyhound Chromatography & Allied Chemicals Ltd, Birkenhead, UK.

### *4.3 Insecticide susceptibility assays*

The method of exposure of bedbugs to the insecticides followed that of Fletcher and Axtell (1993). Appropriate quantities of the insecticides were dissolved in acetone, and Whatman No. 2, 11 cm diameter filter paper were then treated with 1.2 ml dilutions of these insecticides. This was sufficient volume to totally wet the filter paper without runoff. Papers were allowed to dry for 24hr being used. Ten mixed-sex adult bedbugs were placed onto the filter paper and held in place using an inverted bottom of a Petri dish (9 cm diameter). Five replicate dishes of every treatment were used, making 50 insects exposed to each treatment. Mortality was determined at 48 h exposure. Insects were classed as 'dead' if they showed no signs of movement when turned on their backs. Twitching animals were scored as 'alive'.

For each test, five control replicates of 10 insects were also run, in which they were exposed to papers treated with acetone only.

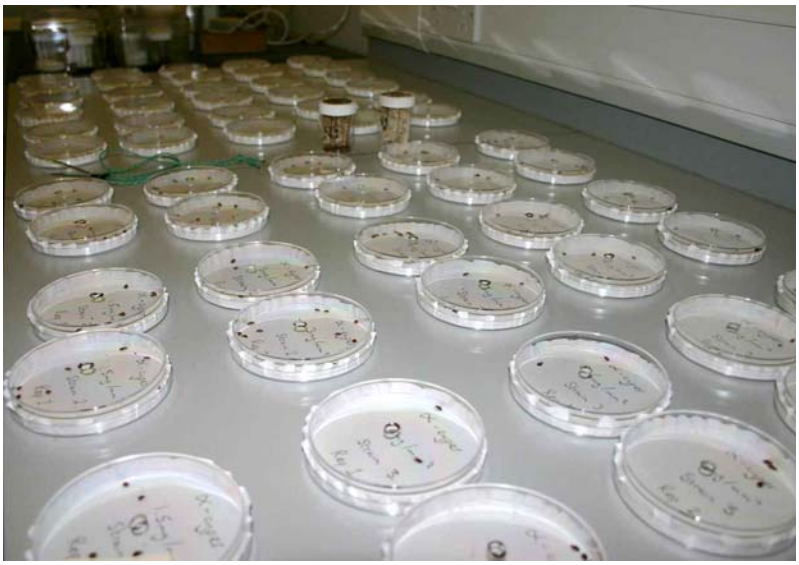
All Petri dishes were maintained at 26±1$^{o}$C, 70±2% R.H. and a 12:12 light : dark regime.

The discriminating doses in Table 2 below were established in an earlier study, using all three susceptible strains listed in 4.1 above. The same experimental technique was used as here, involving exposure of bedbugs to deposits of technical insecticide on filter paper.

Table 2 Bedbug insecticide discriminating doses used in this study

| Insecticide | Discriminating dose ($LC_{99}$) |
|---|---|
| Bendiocarb | 57 mg ai/m$^2$ |
| Alpha-cypermethrin | 36 mg ai/m$^2$ |

Fig 1: Experimental set-up showing Petri dishes of bedbugs on filter papers



## 5. Results & Discussion

All raw data are shown in Appendix I.

A summary of the data is shown in Table 3 below. No correction has been made for control mortality, as in every test this was 0%.

Table 3 Mortality of bedbugs on discriminating doses of bendiocarb and alphacypermethrin

| London postcode of location where strain collected | % mortality on discriminating dose of bendiocarb | % mortality on discriminating dose of alpha-cypermethrin |
|---|---|---|
| Susceptible laboratory strain (1) | 98.7 | 99.3 |
| NW3 | 0 | 0 |
| KT12 | 2 | 0 |
| W3 | 0 | 8 |
| SE1 | 16 | 0 |
| SW2 | 0 | 0 |
| EN8 | 0 | 0 |
| NW10 | 0 | 0 |
| N6 | 0 | 0 |
| SW14 | 0 | 0 |
| E1 | 0 | 0 |
| Untreated control (Using susceptible lab strain 1) | 0 | |

Comparing the mortality of the susceptible strain with that of the ten field strains when exposed to bendiocarb or to alpha-cypermethrin, it is clear that the field strains are much less susceptible to both. It is very likely that this reduced susceptibility is as a result of resistance to carbamates and pyrethroids in these two strains.

Although only one pyrethroid has been tested here, in general, resistance to one pyrethroid tends to indicate that the insects are resistant to most other pyrethroids.

This discriminating dose technique is designed to detect resistant strains, but does not provide an exact measure of the resistance factor. Work with three other UK bedbug field strains has previously shown that resistance factors for alphacypermethrin are >100x, while with bendiocarb they are in the range 10-100x to >100x.

This finding confirms earlier pilot studies (Boase, ‘06) in which 3 strains of bedbugs from around the UK were found to be similarly resistant. In addition, broadly similar studies on bedbug field strains in the USA have also recently shown a high level of resistance to pyrethroids (Romero, 2006).

It is also interesting that all 10 strains show similar levels of resistance. It would appear that the pyrethroid and carbamate resistance in bedbugs is not confined to a few isolated pockets, but may have appeared years ago and has now become widely and uniformly distributed.

Considering the implications of these results for practical pest control, resistance found in this kind of test does not automatically imply that bedbugs cannot be killed in practice. Nonetheless, resistance is definitely present, and does need to be taken into account when planning bedbug control work. The results show that there is a risk that single treatments with these active ingredients may not be fully effective.

Feedback from practical bedbug control does indicate that formulated insecticides at commercial dosages can often still achieve useful kill of bedbugs, and that a sequence of treatments involving different classes of active ingredient such as insect growth regulators, may give much better efficacy.

## 6. Recommendations

The current resurgence of bedbugs is substantially increasing the workload of those involved in pest control. Given that the resurgence appears to be linked to reduced susceptibility to common insecticides, it is proposed that this topic is investigated further, in order to identify ways of improving the effectiveness of bedbug control.

## 7. References

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## 8. Authors' contact details:

Richard Naylor
Sheffield University
Phone: 0114 222 0080
email: r.naylor@sheffield.ac.uk

Clive Boase
the Pest Management Consultancy
phone: 01440 706127
email: clive@pest-management.com

# Appendix I – Raw Data

| Treatment | Strain postcode | Rep | Dead males | Dead females | Total dead | Percentage mortality | Strain percentage mortality |
|---|---|---|---|---|---|---|---|
| Bendiocarb | Susceptible strain | Total of all tests | 74/75 | 74/75 | 148/150 | 98.7 | 98.7 |
| Bendiocarb | NW3 | 1 | 0 | 0 | 0 | 0 | |
| Bendiocarb | NW3 | 2 | 0 | 0 | 0 | 0 | |
| Bendiocarb | NW3 | 3 | 0 | 0 | 0 | 0 | |
| Bendiocarb | NW3 | 4 | 0 | 0 | 0 | 0 | |
| Bendiocarb | NW3 | 5 | 0 | 0 | 0 | 0 | 0 |
| Bendiocarb | KT12 | 1 | 0 | 0 | 0 | 0 | |
| Bendiocarb | KT12 | 2 | 0 | 0 | 0 | 0 | |
| Bendiocarb | KT12 | 3 | 1 | 0 | 1 | 10 | |
| Bendiocarb | KT12 | 4 | 0 | 0 | 0 | 0 | |
| Bendiocarb | KT12 | 5 | 0 | 0 | 0 | 0 | 2 |
| Bendiocarb | W3 | 1 | 0 | 0 | 0 | 0 | |
| Bendiocarb | W3 | 2 | 0 | 0 | 0 | 0 | |
| Bendiocarb | W3 | 3 | 0 | 0 | 0 | 0 | |
| Bendiocarb | W3 | 4 | 0 | 0 | 0 | 0 | |
| Bendiocarb | W3 | 5 | 0 | 0 | 0 | 0 | 0 |
| Bendiocarb | SE1 | 1 | 1 | 1 | 2 | 20 | |
| Bendiocarb | SE1 | 2 | 0 | 1 | 1 | 10 | |
| Bendiocarb | SE1 | 3 | 1 | 0 | 1 | 10 | |
| Bendiocarb | SE1 | 4 | 1 | 0 | 1 | 10 | |
| Bendiocarb | SE1 | 5 | 2 | 1 | 3 | 30 | 16 |
| Bendiocarb | SW2 | 1 | 0 | 0 | 0 | 0 | |
| Bendiocarb | SW2 | 2 | 0 | 0 | 0 | 0 | |
| Bendiocarb | SW2 | 3 | 0 | 0 | 0 | 0 | |
| Bendiocarb | SW2 | 4 | 0 | 0 | 0 | 0 | |
| Bendiocarb | SW2 | 5 | 0 | 0 | 0 | 0 | 0 |
| Bendiocarb | EN8 | 1 | 0 | 0 | 0 | 0 | |
| Bendiocarb | EN8 | 2 | 0 | 0 | 0 | 0 | |
| Bendiocarb | EN8 | 3 | 0 | 0 | 0 | 0 | |
| Bendiocarb | EN8 | 4 | 0 | 0 | 0 | 0 | |
| Bendiocarb | EN8 | 5 | 0 | 0 | 0 | 0 | 0 |
| Bendiocarb | NW10 | 1 | 0 | 0 | 0 | 0 | |
| Bendiocarb | NW10 | 2 | 0 | 0 | 0 | 0 | |
| Bendiocarb | NW10 | 3 | 0 | 0 | 0 | 0 | |
| Bendiocarb | NW10 | 4 | 0 | 0 | 0 | 0 | |
| Bendiocarb | NW10 | 5 | 0 | 0 | 0 | 0 | 0 |

| Treatment | Strain postcode | Rep | Dead males | Dead females | Total dead | Percentage mortality | Strain percentage mortality |
|---|---|---|---|---|---|---|---|
| Bendiocarb | SW14 | 1 | 0 | 0 | 0 | 0 | |
| Bendiocarb | SW14 | 2 | 0 | 0 | 0 | 0 | |
| Bendiocarb | SW14 | 3 | 0 | 0 | 0 | 0 | |
| Bendiocarb | SW14 | 4 | 0 | 0 | 0 | 0 | |
| Bendiocarb | SW14 | 5 | 0 | 0 | 0 | 0 | 0 |
| Bendiocarb | N6 | 1 | 0 | 0 | 0 | 0 | |
| Bendiocarb | N6 | 2 | 0 | 0 | 0 | 0 | |
| Bendiocarb | N6 | 3 | 0 | 0 | 0 | 0 | |
| Bendiocarb | N6 | 4 | 0 | 0 | 0 | 0 | |
| Bendiocarb | N6 | 5 | 0 | 0 | 0 | 0 | 0 |
| Bendiocarb | E1 | 1 | 0 | 0 | 0 | 0 | |
| Bendiocarb | E1 | 2 | 0 | 0 | 0 | 0 | |
| Bendiocarb | E1 | 3 | 0 | 0 | 0 | 0 | |
| Bendiocarb | E1 | 4 | 0 | 0 | 0 | 0 | |
| Bendiocarb | E1 | 5 | 0 | 0 | 0 | 0 | 0 |
| Untreated | Susceptible lab strain 1 | Total of all tests | 0/75 | 0/75 | 0/150 | 0 | 0 |

| Treatment | Strain postcode | Rep | Dead males | Dead females | Total dead | Percentage mortality | Strain percentage mortality |
|---|---|---|---|---|---|---|---|
| Alpha-cypermethrin | Susceptible strain (1) | Total of all tests | 75/75 | 74/75 | 149/150 | 99.3 | 99.3 |
| Alpha-cyper | NW3 | 1 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | NW3 | 2 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | NW3 | 3 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | NW3 | 4 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | NW3 | 5 | 0 | 0 | 0 | 0 | 0 |
| Alpha-cyper | KT12 | 1 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | KT12 | 2 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | KT12 | 3 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | KT12 | 4 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | KT12 | 5 | 0 | 0 | 0 | 0 | 0 |
| Alpha-cyper | W3 | 1 | 1 | 0 | 1 | 10 | |
| Alpha-cyper | W3 | 2 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | W3 | 3 | 1 | 0 | 1 | 10 | |
| Alpha-cyper | W3 | 4 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | W3 | 5 | 1 | 1 | 2 | 20 | 8 |
| Alpha-cyper | SE1 | 1 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | SE1 | 2 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | SE1 | 3 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | SE1 | 4 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | SE1 | 5 | 0 | 0 | 0 | 0 | 0 |
| Alpha-cyper | SW2 | 1 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | SW2 | 2 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | SW2 | 3 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | SW2 | 4 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | SW2 | 5 | 0 | 0 | 0 | 0 | 0 |
| Alpha-cyper | EN8 | 1 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | EN8 | 2 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | EN8 | 3 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | EN8 | 4 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | EN8 | 5 | 0 | 0 | 0 | 0 | 0 |
| Alpha-cyper | NW10 | 1 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | NW10 | 2 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | NW10 | 3 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | NW10 | 4 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | NW10 | 5 | 0 | 0 | 0 | 0 | 0 |
| Alpha-cyper | N6 | 1 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | N6 | 2 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | N6 | 3 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | N6 | 4 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | N6 | 5 | 0 | 0 | 0 | 0 | 0 |

| Treatment | Strain postcode | Rep | Dead males | Dead females | Total dead | Percentage mortality | Strain percentage mortality |
|---|---|---|---|---|---|---|---|
| Alpha-cyper | SW14 | 1 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | SW14 | 2 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | SW14 | 3 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | SW14 | 4 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | SW14 | 5 | 0 | 0 | 0 | 0 | 0 |
| Alpha-cyper | E1 | 1 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | E1 | 2 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | E1 | 3 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | E1 | 4 | 0 | 0 | 0 | 0 | |
| Alpha-cyper | E1 | 5 | 0 | 0 | 0 | 0 | 0 |
| Untreated | Susceptible lab strain 1 | Total of all tests | 0/75 | 0/75 | 0/150 | 0 | 0 |