Arena Tests with Piperonal, A New Louse Repellent

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ABSTRACT
Piperonal, once used to kill lice in Australian hospitals, was acclaimed as an effective pediculicide (Corlott, 1925) by the standards of the day. It is unusual in also exhibiting a repellent action against lice, a property only recently realised. A new, easy to use, low-fragrance, pump action spray, which incorporates 2% piperonal, was tested in the laboratory using clothing lice in an arena test and was found to exhibit consistently high repellency after half an hour, dropping only slightly after 24 hours. A well known multi-purpose insect repellent, diethyltoluamide (DEET), was then tested against piperonal. A 2% solution of piperonal was found to be almost twice as effective as a 50% solution of DEET.

In arena tests using lice with the tip segments of both antennae removed, no behavioural differences or statistically significant differences from a random distribution could be found between untreated and Rappell-treated areas. This strongly suggests that sense organ(s) on the tip of the antenna are necessary for detection of the repellent.

Although there can be no strict correlation between results in the laboratory and potential efficacy in the field, materials such as insecticides found to be effective in the laboratory have been found to be equally effective in the field. Furthermore, what with the threat of resistance to head lice insecticides, ineffectual treatment and the lack of contact tracing, a repellent would be of obvious use in the control of head lice.

INTRODUCTION
The best method of protecting people from any blood-sucking insect is to destroy the insect. Often this is impossible or impractical. However, a high degree of protection can be obtained by the proper use of an effective insect repellent.

None of our modern repellents are ideal. They may have some odour, feel oily, soften paints or plastics, need to be applied in high doses or at high concentrations and may be effective for only a few hours. Even the powerful multi-purpose insect repellent, diethyltoluamide (DEET) is known to cause harmful side effects above a concentration of 50% (Lancet, 1988) although high strength preparations are still in general use. Therefore, it is not surprising that any new insect repellent would meet with scepticism and caution from health professionals.

Head lice constitute an infectious disease (pediculosis capitis) well within our powers to conquer. However, nearly 3 million units of head lice and scabies treatments are supplied annually in the UK, much of it being used prophylactically or inappropriately (Burgess, 1992) and infection levels still remain unacceptable. As yet, the public are still often poorly informed as to the most proficient treatments. Insecticidal shampoos, ineffectual as treatments, are frequently employed by the public in the hope of preventing lice (Maunder, 1993). Thus an efficient, pleasant, easy to use, long-lasting head lice repellent would lower the level of abuse of pesticides and may even reduce infection rates or, when correctly used after treatment with insecticide, limit re-infections while contact tracing is being carried out.

Rappell (Charwell Pharmaceuticals), a new head lice repellent available from pharmacists, is applied to the hair daily as a fine mist spray. The active ingredient is 2% piperonal, a natural material produced by plants, presumably as a repellent against harmful insects. This relatively non-toxic, non-volatile compound has long been used extensively in cosmetics and in vanilla and cherry flavourings and has an excellent safety record (Merck Index, 1989).

Dechier (1947) described a repellent as 'a chemical that causes insects to make orientated movements away from its source' but the repellent nature of piperonal has not, until recently, been described. However, it has now been reported that, in the laboratory, a high degree of repellency of clothing lice (Pediculus humanus humanus) to treated hair and filter paper discs (arena test) has been observed (Burgess, 1993). The present tests shed further light on the behaviour of lice to piperonal. The theory that piperonal provokes a negative behavioural response mediated by antennal receptors, causing lice to avoid treated areas (Rappell Information Leaflet), can also be examined by using the arena test.

MATERIALS AND METHOD
Head lice (Pediculus humanus capitis) cannot live on other parts of the body or off the body. However, human clothing lice (Pediculus humanus humanus) can be grown in large numbers in laboratory colonies and provide the best available model.

Arena tests, first developed by Wigglesworth (1941), are based on lice distributing randomly in an inert environment. This random distribution does not occur when an
an attractant or repellent is present. The test is quick, simple and does not require elaborate analysis.

For each test paper, the control or test substance was dissolved as necessary in isopropanol, and 50μl of the solution was applied as evenly as possible to one half of a filter paper disc (5.5cm in diameter). Two test papers were prepared for each solution. The papers were then dried in a flow of moving air for 30 minutes. The effect of this drying was to concentrate the active material so that the essential difference between the papers lay in the absolute quantities of active material which were evenly distributed upon them. Rappell itself contains 30% alcohol, which likewise evaporates away after application. Each paper was then placed in the centre of a 10cm glass Petri dish. In the case of the 24 hour test, the test papers were left at room temperature for a further 23½ hours in open Petri dishes.

5-7 hours after engorgement, 10 young adult female lice (which are thought to be important in initiating infection), were placed in the centre of the disc and the Petri dish was covered. The dishes were then placed in an incubator at 30 ± 2°C and approximately 50% humidity.

After 5 minutes, which allowed time for the lice to deaggregate and distribute randomly, the lice on the treated side were counted. The dishes were then re-examined after each of a further 4 incubation periods of 2 minutes. Any lice found off the filter paper were excluded from the total sample number but were placed back on the filter paper to be counted at the next inspection. 5 repeats of this test were completed on the same day, where possible.

Test scores were summed, as were the total number of lice sampled, and the control was checked for a random distribution by using a Chi-square test with Yate's correction. The 5 repeats were summed to produce a percentage repellency value using the following formula:

\[
\% \text{Repellency} = \left( \frac{\Sigma \text{treated control} - \Sigma \text{treated test}}{\Sigma \text{control sample}} \right) \times 100
\]

This formula, adapted from Schreck (1977), was originally used to evaluate new compounds, as repellents, for crawling arthropods such as ticks, fleas and mites. If there were no repellent effect whatsoever, the lice would be expected to be found in approximately equal numbers on each side of the boundary. The percentage repellency describes the degree of variation from this equality.

The fifth antennal segments were removed from another group of adult female lice, under a dissection microscope using a very fine blade. Tests were then repeated as before except that the lice were given approximately 20 hours to recover, so that they were used 24 hours after their last feed. The lice were then watched in order to determine whether the repellent response was abolished. A control sample, consisting of placebo Rappell, made without the active ingredient, was also employed.

Another test was devised to find out whether repellents have any effect on the choice of egg laying site. This used 9cm diameter filter paper circles, each torn, not cut, into a square and bisected by a pencil line into 2 triangles. The rough edges normally form an attractive site for the laying of eggs. One half of each paper was impregnated with 200μl of either placebo or Rappell and the papers were dried for half an hour. A batch of 20 young adult females and 20 young adult males were incubated on the papers over a 24 hour period. The eggs laid were then counted. These tests were repeated over 5 days and egg counts summed for each type of area.

### RESULTS

A repellency value, for clothing lice, to treated filter discs was obtained for a 10% solution of DEET and for 10% piperonal. A high repellency of 95.8% was obtained for piperonal but only 42.9% of lice rested, were repelled by DEET. On the next day, a 2% solution of piperonal was found to be almost twice as effective (81.4% repellency) as a 50% solution of DEET against lice (41.0% repellency) (Table 1).

When Rappell treated papers were left exposed for half an hour or 24 hours, a slight drop in repellency was obtained, the repellency value of lice reducing from 87.9% to 77.3% (Table 2).

Clothing lice which were starved for 24 hours, exhibited a high repellency of 84.1% to piperonal treated areas. However, lice without antennal tips which were also starved for 24 hours
TABLE 3
COMPARISON OF RESPONSE TO RAPPELL FROM LICE WITH AND WITHOUT ANTENNAL TIPS

<table>
<thead>
<tr>
<th>Test Solutions</th>
<th>Σ Treated Side</th>
<th>Σ Sample</th>
<th>% Repellency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Placebo)</td>
<td>259</td>
<td>481</td>
<td>*</td>
</tr>
<tr>
<td>Rappell (lice without tips)</td>
<td>42</td>
<td>491</td>
<td>84.1%</td>
</tr>
<tr>
<td>Rappell (lice)</td>
<td>248</td>
<td>483</td>
<td>**</td>
</tr>
</tbody>
</table>

\[ * X^2 = 2.85, P > 0.05 \]
\[ ** X^2 = 0.17, P > 0.05 \]

were found to distribute randomly on the Rappell treated filter papers as did the control group on the placebo treated papers (Table 3).

When a comparison of the number of eggs laid over 24 hours on Rappell and placebo treated areas was made, a very highly significant result was obtained (\( X^2 = 163, P > 0.01 \)). Almost without exception the lice refused to lay eggs on Rappell treated areas.

**DISCUSSION**

This type of arena test has been developed to evaluate new compounds as repellents. It is designed to take advantage of the fact that in general lice will crawl around the edges of a paper, which is thought to simulate a hair or a seam. The results shown in Tables 1 and 3 demonstrate piperonal to be an effective lice repellent, under these test conditions. Conversely, the widely used insect repellent DEET had little effect, even at a concentration of 50%.

Behavioural changes of lice, on approaching a piperonal treated zone, were very noticeable. Some stopped and either reversed or turned away. Others turned and followed the line between zones, then veered off into the untreated zone. Yet others would continue until they returned to the non-treated side, occasionally accelerating dramatically. Those spending any great time on the treated zone became disoriented and increased their rate of turning. Further evidence of piperonal’s repellency was shown by the fact that given a choice, females would not lay on Rappell treated areas during a 24 hour period, this being a very highly significant difference.

The fifth antennal segment of lice contains many sensory organs including several peg organs and 2 pore organs of, as yet, unassigned function. Since lice only seem to respond to piperonal when with intact antennae, it seems likely that one or more of these organs are responsible for the detection of the repellent. Starved lice will still respond to piperonal. Touch is not necessary to produce an avoidance reaction as, if a crystal of pure piperonal is placed to one side of an arena test, lice will move to the opposite side. If the crystal is then moved near the lice, they will again move to the opposite side (R Irwin, personal communication). Furthermore, lice will even avoid, without contact, a Rappell treated cocktail stick placed in their path, but not an untreated stick. Thus, piperonal is likely to be working as an olfactory repellent. At present we can describe the behavioural response to piperonal but not why lice react to it.

The persistence of effectiveness is of great importance as many substances which might repel lice only work immediately after application. For example, alcohol can scarcely be said to be a useful repellent. However, arena tests using filter papers treated and left for 24 hours in low light still exhibited a very high degree of repellency to lice by Rappell. Indeed, young adult females avoided laying eggs on Rappell created areas during the whole of a 24 hour exposure period.

The above are preliminary results of tests with piperonal and, as yet, are still to be correlated with results in the field. Further laboratory tests are required to give a more comprehensive idea of the mode of action of piperonal against human lice.

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**REFERENCES**