

# Lethal and behavioural effects of three synthetic repellents (DEET, IR3535 and KBR 3023) on *Aedes aegypti* mosquitoes in laboratory assays

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**Abstract.** The knock-down, mortality and ‘irritancy’ effects of three synthetic repellents (DEET, IR3535 and KBR 3023) on *Aedes aegypti* (L) (Diptera: Culicidae) were evaluated in the laboratory in the absence of animal bait. Filter paper tests were carried out to assess the knock-down effect (KD<sub>t</sub><sub>50</sub> and KD<sub>t</sub><sub>95</sub>) and mortality (LC<sub>50</sub> and LC<sub>95</sub>) induced by each repellent. ‘Irritancy’ tests were carried out to compare the flight response (time to first take-off, or FT) to increasing concentrations of repellents (2–7%) and at five distances from the treated surface (0–40 mm). DEET had an insecticidal effect (KD<sub>t</sub><sub>50</sub> = 9.7 min at 7%; CL<sub>50</sub> = 1165 mg/m<sup>2</sup>), whereas IR3535 and KBR 3023 did not. Relative to an untreated control, IR3535 was an irritant (relative irritancy or RI > 1) at doses of 5% and 7% (RI = 17.7 and 9.9, respectively), whereas DEET was an irritant at lower concentrations (RI = 12.3 at 2% DEET). KBR 3023 was the weakest irritant over the same range of concentrations (RI<sub>max</sub> = 3.6 at 6%). DEET was more of an irritant (RI<sub>20</sub> = 9.4) than IR3535 (RI<sub>20</sub> = 2.9) over a range of distances (0–20 mm), and KBR 3023 was not an irritant unless mosquitoes made contact with the treated surface. All three repellents had a significant effect on mosquitoes, but DEET exhibited a more complex mode of action than the others due to its insecticidal properties. The repellents do not behave as a single class of compounds with a common mode of action, but most probably affect different physiological systems in insects. The physiological and molecular mechanisms of repellents, especially DEET, should be investigated to ensure a better use of these molecules for skin applications and/or for treating materials against mosquitoes.

**Key words.** *Aedes aegypti*, DEET, IR3535, KBR 3023, irritancy, knock-down effect, mortality, repellent.

## Introduction

The use of repellents is among the oldest methods of personal protection against mosquito bites (Curtis *et al.*, 1991; Seyoum *et al.*, 2002). Over the last 50 years, synthetic repellents such as dimethylphtalat (DMP), ethyl hexanediol (EHD) and diethyl-m-toluamid (DEET) have been developed. The latter is still the most commonly used repellent worldwide (Fradin, 1998), despite concerns over its possible toxicity to pregnant women and children (Koren *et al.*, 2003).

In recent years new formulations have been developed, such as IR3535 [ethyl 3-(N-buthylacetylaminopropionate)], which has been approved in the U.S.A. for skin applications (World Health Organization, 2001a). This repellent conferred more than 90% protection for 6 h against *Anopheles gambiae* Giles and *An. funestus* Giles (Marchio, 1996). Nevertheless, IR3535 applied on human skin is less persistent than DEET against *Anopheles* mosquitoes (Costantini *et al.*, 2004). Another repellent, KBR 3023 (Bayrepel or 2-(2-hydroxyethyl)-1-piperidine-carboxylic acid 1-methylpropyl ester) shows equal or higher

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efficacy than DEET against *Aedes aegypti* (L), *Ae. albopictus* (Skuse) and *An. gambiae* (Yap *et al.*, 1998; Badolo *et al.*, 2004), and has a favourable toxicological profile (W.H.O., 2001b; Nentwig *et al.*, 2002).

There is a lack of standardized experimental protocols for comparing the performances of repellents. The behavioural response of mosquitoes to repellents also varies according to the species considered (Curtis *et al.*, 1987; Badolo *et al.*, 2004). Most studies record the protection time and/or the number of bites after application to human skin (Schreck, 1977). Few studies report the effect of repellents on insects in the absence of animal bait (Moss, 1996; Xue *et al.*, 2003) and such investigations are essential to understanding their impact on insect physiology.

Since the pioneering work of Hocking (1971), Khan & Maibach (1972) and Browne (1977), there have been few studies on how repellents affect insect behaviour or prevent them from finding and biting their hosts. According to Davis (1985) and Boeckh *et al.*, (1996), DEET acts on antennal chemoreceptors by inhibiting the response to host odours. A comparative study carried out on 16 repellents in spray formulations (including DEET) also showed that repellents induce temporary knock-down (KD) and paralysis, which, in extreme cases, causes death (Xue *et al.*, 2003). In addition, Pennetier *et al.* (2005a) demonstrated a high level of synergy between DEET and propoxur (carbamate) in terms of KD effect and mortality against susceptible and pyrethroid-resistant *Ae. aegypti* mosquitoes. All these findings emphasize the need to further investigate the performances of repellents without any host to better appreciate the sensory and/or behavioural mechanisms associated with attraction and repulsion in insects.

The knock-down, mortality and irritancy effects of three synthetic repellents (DEET, IR3535 and KBR 3023) on *Ae. aegypti* were evaluated in the absence of a host. Standardized filter paper tests were carried out to assess the degree of knock-down and mortality induced by each repellent. The irritancy, defined as time taken to first take-off flight, of each repellent was measured over a range of concentrations (2–7%) and at a range of distances (0–40 mm).

## Materials and methods

### Mosquitoes

The insecticide-susceptible 'Bora Bora' strain of *Ae. aegypti*, originally from French Polynesia, was used for the bioassays. This strain has been maintained for more than 20 years under laboratory conditions (27 ± 2 °C and 80% RH).

### Repellents

Technical grade DEET (Sigma-Aldrich, St Quentin Fallavier, France), IR3535 (Merck, Strasbourg, France) and KBR 3023 (Bayer, Leverkusen, Germany) were used. The repellents were tested at increasing logarithmic concentrations of 2–7% active ingredient in acetone, with silicone oil as the carrier. Filter papers were dosed by dripping 2 mL of the mixture evenly over the paper (W.H.O., 1998).

### Assays

Owing to the high volatility of the repellents (Hoffman & Miller, 2002), the treated papers were tested 1 h after impregnation and each test lasted 60 min. Concentrations were expressed as weight per weight percentage of active ingredient in silicone oil.

Knock-down and mortality resulting from tarsal contact with treated filter paper were measured using W.H.O. test kits (cylinder tests; W.H.O., 1998) at 27 ± 2 °C and 80 ± 10% RH. Batches of 25 non-blood-fed females, 2–5 days old, were introduced into holding tubes and kept for a settling period of 60 min. They were then transferred to the exposure tube, which was held vertically under subdued light for 60 min. The number of knocked-down mosquitoes at the bottom of the tubes was recorded every 10 min. Mortality was recorded 24 h after exposure and corrected using the formula described by Abbott (1925). Data were analysed by the log-probit method according to Finney (1971) using Probit software (Raymond *et al.*, 1997). Times to 50% and 95% knock-down (KD<sub>t50</sub> and KD<sub>t95</sub>, respectively) and 95% confidence limits were estimated by Probit software. Each solution was tested four times and each test was replicated three times with different batches of insects to take into account interbatch variability.

Irritancy was tested with non-blood-fed, 2–5-day-old females, which were introduced individually into plastic cones fitted with treated filter paper. Subdued lighting and air temperature (28 ± 2 °C) were maintained during the test according to Hodjati & Curtis (1999). After a settling period of 60 s, the time to first take-off (FT) of each mosquito was recorded (Mouchet *et al.*, 1961). Mosquitoes which did not take off at least once during a period of 256 s were discarded. A simple program using the internal clock of a laptop computer was developed to run this test and analyse the data. Cumulative frequencies were used to calculate the time it took 50% and 95% of the mosquitoes to take-off (FT<sub>50</sub> and FT<sub>95</sub>) using the Probit software.

These observations were repeated over a range of distances from the treated surface (0 mm, 10 mm, 20 mm and 40 mm), using the concentration that induced the highest irritancy at 0 mm. The cones were held in position by two rectangular plates of Plexiglas® to keep them at the required distance from the treated papers. Each test was replicated five times.

The relative irritancy (RI) was estimated according to the distance (Dx) by the following formula:

$$RI_{(Dx)} = \frac{FT_{50}(Dx) \text{ of control}}{FT_{50}(Dx) \text{ of repellent}}$$

For a given distance, an RI > 1 indicates that the repellent provided higher irritancy than the control.

## Results

### Toxicity study

DEET showed a dose-dependant effect for both KD and mortality (probit regression lines,  $P > 0.05$ ; Table 1, Figs 1 and 2). The percentages of KD mosquitoes after 60 min (KD<sub>60</sub>) were 66%

**Table 1.** The physiological effects of DEET, IR3535 and KBR 3023 on *Aedes aegypti* mosquitoes.

Knock-down effect				Mortality			Irritancy			Irritancy at distance				
Dose	n	KDt <sub>50</sub> (min) (CI 95%)	KDt <sub>95</sub> (min) (CI 95%)	LC <sub>50</sub> (%)	LC <sub>95</sub> (%)	Dose	n	FT <sub>50</sub> (s) (CI 95%)	FT <sub>95</sub> (s) (CI 95%)	Dose	Dist. (mm)	n	FT <sub>50</sub> (s) (CI 95%)	FT <sub>95</sub> (s) (CI 95%)
<b>DEET</b>														
2%	399	-	-	-	-	2%	86	19.7 (16.0–24.2)	477.0 (305.4–850.1)	2%	2	78	23.8 (17.8–31.8)	249.7 (138.4–465.4)
3%	395	31.7 (31.1–32.3)	49.3 (47.9–50.8)	3.2 (3.1–3.3)	7.2 (6.7–7.7)	3%	68	73.1 (52.1–112.2)	> 1000	3%	10	75	35.4 (30.6–41.0)	191.6 (148.1–266.1)
5%	363	20.5 (15.5–27.0)	32.3 (22.0–50.3)	-	-	5%	-	-	-	5%	20	63	68.0 (55.0–86.4)	676.7 (403.1–1516.5)
7%	402	9.7 (8.8–10.6)	13.9 (11.9–16.2)	-	-	7%	-	-	-	7%	40	51	210.4 (138.2–405.3)	> 1000
<b>R3535</b>														
3%	-	-	-	-	-	3%	64	80.1 (62.8–108.1)	> 1000	3%	2	65	45.1 (36.4–57.1)	639.1 (398.0–110.1)
4%	-	-	-	-	-	4%	97	22.1 (18.9–25.8)	224.9 (168.0–322.6)	4%	10	60	79.0 (64.6–100.0)	628.7 (405.4–1173.3)
5%	499	-	-	-	-	5%	100	13.7 (11.8–15.8)	124.9 (96.3–171.6)	5%	20	53	184.7 (114.3–386.4)	> 1000
6%	493	-	-	-	-	6%	101	17.7 (15.2–20.6)	176.8 (134.1–247.8)	6%	40	57	138.6 (98.1–225.2)	> 1000
7%	388	-	-	-	-	7%	86	24.4 (20.3–29.5)	385.0 (262.2–628.1)	7%	2	51	268.3 (155.8–674.1)	> 1000
<b>KBR 3023</b>														
4%	286	-	-	-	-	4%	57	368.2 (181.1–1238.7)	> 1000	4%	10	52	403.9 (219.8–1326.9)	> 1000
5%	297	-	-	-	-	5%	60	109.8 (75.7–180.9)	> 1000	5%	20	-	-	-
6%	280	-	-	-	-	6%	65	66.8 (49.0–98.0)	> 1000	6%	40	-	-	-
7%	318	-	-	-	-	7%	59	208.4 (133.4–414.0)	> 1000	7%	2	-	-	-

and 99% for concentrations of 2% and 3% DEET, respectively (Fig. 1). Observed mortalities ranged from 14% to 96% (Fig. 2).

Conversely, no probit-regression line could be fitted for IR3535 and KBR 3023 for KD effect or mortality. Indeed, only 50% of mosquitoes were knocked-down after 60 min exposure to a 7% concentration of IR3535 or KBR 3023 (Fig. 1). Little or no mortality was observed for these two repellents at the concentrations tested.

#### Irritancy study

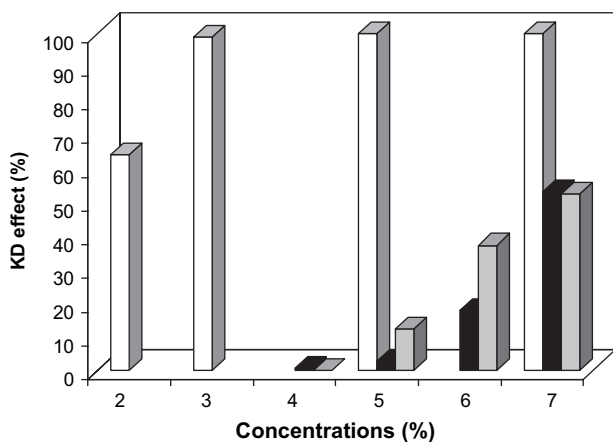
The  $FT_{50}$  and  $FT_{95}$  estimated for each repellent are shown in Table 1. All repellents caused significant irritancy to mosquitoes ( $RI > 1$ ,  $P < 0.05$ ) (Fig. 3). The  $FT_{50}$  of DEET was 19.7 s at 2% (Table 1). At 3% the irritancy of DEET significantly decreased, probably because of knock-down effect on the mosquitoes. No probit regression lines could be determined at 5% and 7%. The irritant property of IR3535 increased over concentrations of 3–5% and decreased at higher concentrations (Table 1), probably due to knock-down (Fig. 1). Low irritancy was found for KBR 3023 over all concentrations tested.

#### Distance-dependant irritancy study

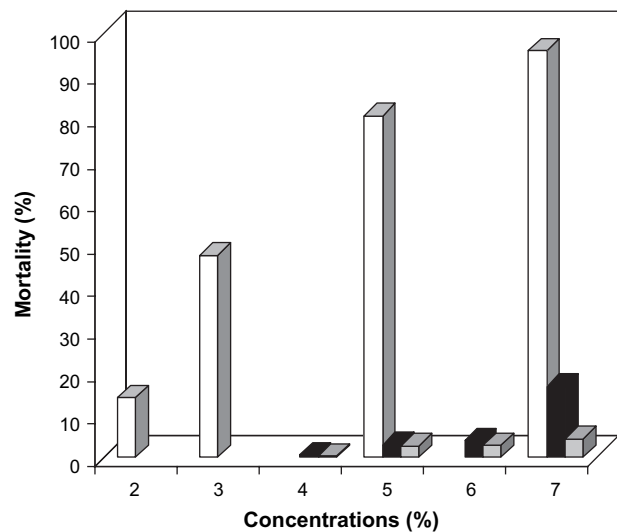
Only the most irritant concentrations of DEET (2%), IR3535 (5%) and KBR 3023 (6%) were used for this part of the study (Fig. 3). The  $FT_{50}$  and  $RI$  values for each repellent and each distance are shown in Table 1 and Fig. 4. The irritancy of DEET was significant up to 40 mm from the treated filter papers ( $RI_{40} = 3.2$ ). The irritant effects of IR3535 decreased sharply at 2 mm ( $RI_{02} = 4.6$ ) but remained constant until 40 mm ( $RI_{40} = 4.9$ ). KBR 3023 caused no significant amount of irritancy without tarsal contact.

## Discussion

The repellents DEET, IR3535 and KBR 3023 differed in their effects on *Ae. aegypti* in terms of knock-down, mortality and



**Fig. 1.** Comparison of knock-down effect (60 min) induced by DEET (white columns), IR3535 (black columns) and KBR3023 (grey columns) against *Aedes aegypti*.



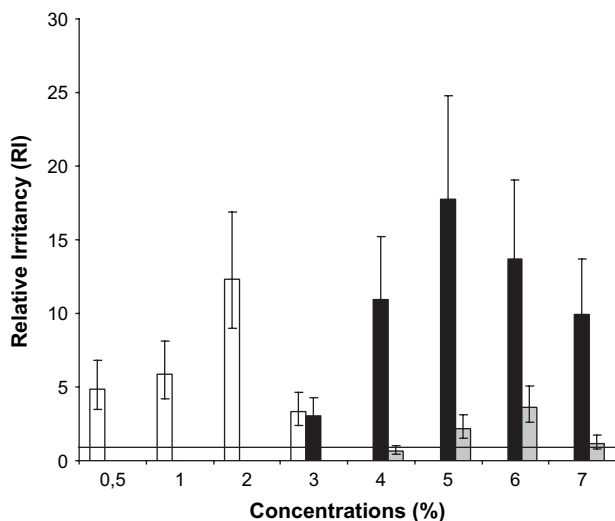
**Fig. 2.** Toxicity of DEET (white columns), IR3535 (black columns) and KBR3023 (grey columns) against *Aedes aegypti*.

irritancy. Most notable was the insecticidal effect of DEET that was not observed with IR3535 and KBR3023. The three types of response can be summarized as follows: KBR3023 had no obvious effect on mosquito behaviour and caused no KD or mortality; IR3535 had a significant effect on flight behaviour leading to some irritancy, but caused no KD or mortality; DEET caused mosquitoes to take-off significantly more quickly than the control mosquitoes, even at distances of 40 mm from the treated surface, and also caused a significant amount of KD and mortality.

The lack of an effect of KBR 3023 on mosquito take-off behaviour suggests that it may not 'repel' insects (defined as causing orientation away from the source by Klowden [1996]), and is consistent with observations that it may interfere with blood-feeding (Boeckh *et al.*, 1996; Barnard *et al.*, 2002; Costantini *et al.*, 2004; Badolo *et al.*, 2004) and/or host seeking. Boeckh *et al.* (1996) showed that KBR 3023 may inhibit host attraction by altering antennal olfactory receptors in *Ae. aegypti*. Little is known about the mode of action of IR3535, but irritant effects such as those as observed in this study may be caused by neurotoxicity, according to Haynes (1988).

Although the exact mode of action of DEET is unknown, there is evidence that it has an effect on lactic acid chemoreceptors that are responsible for detecting kairomones associated with host seeking (Dogan *et al.*, 1999; Kline *et al.*, 2003). A recent electrophysiological study performed on DUM neurone (dorsal unpaired median neurones) of the American cockroach (*Periplaneta americana*) demonstrated that DEET induced a strong neurotoxic effect in insects (Pennetier *et al.*, 2005b) by disrupting calcium equilibrium in the nerve cells (Lapied *et al.*, 2006). These results probably explain the high mortality and knock-down effect observed with DEET on many insect species, including mosquitoes, of public health importance.

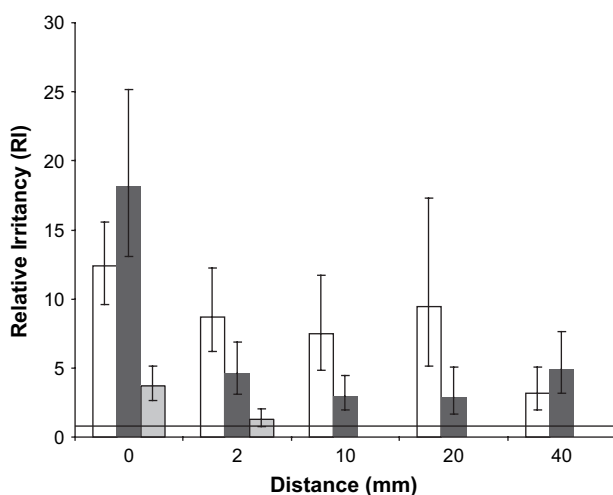
The findings of the present study are consistent with the conclusions of Davis (1985), who emphasized the fact that repellents do not behave as a single class of compounds with a common mode of action but most probably act on different



**Fig. 3.** Relative Irritancy (RI) induced by DEET (white columns), IR3535 (black columns) and KBR3023 (grey columns) on *Aedes aegypti*.

physiological systems. The term 'repellent' therefore can refer to molecules that may alter the functioning of sensory motor systems and/or have neurotoxic effects. Further investigations are now required to determine the exact mode of action of repellents and their physiological target sites in order to provide chemists with a rational guide for the development of more effective insect repellents that are safer in terms of public health.

Although DEET caused relatively high mortality and the knock-down effects were similar to what might be expected with pyrethroids under similar conditions, it cannot be classified as an operational insecticide because the concentrations required to kill mosquitoes are much greater than those usually recommended for pyrethroids. Deltamethrin, for example, is generally used to impregnate papers at only 0.05% (91 mg/m<sup>2</sup>),



**Fig. 4.** Irritancy of DEET (white columns), IR3535 (black columns) and KBR3023 (grey columns) with increasing distances on *Aedes aegypti*.

compared with 7% for DEET (2500 mg/m<sup>2</sup>) used in the present study. However, some authors emphasize the fact that repellent-impregnated bednets may have an effective role in malaria prevention (Curtis *et al.*, 1987; Badolo *et al.*, 2004). A field study in Burkina Faso has shown that KBR- and DEET-treated nets reduced blood-feeding rates of both *An. gambiae* and *Ae. aegypti* for more than 2 months (Badolo, 2004). Impregnation of materials greatly increases the persistence of repellents and allows the use of higher concentrations than those recommended for application to human skin (Rozendaal, 1997). In view of the spread of pyrethroid resistance in mosquitoes that have bearing on public health, (Chandre *et al.*, 1999; Brengues *et al.*, 2003), combining a repellent with insecticides on materials may have potential for vector control and the management of insecticide resistance.

### Acknowledgements

We are very grateful to Sigma-Aldrich (St Quentin Fallavier, France), Bayer (Leverkusen, Germany) and Merck (Strasbourg, France) for providing the technical grades of DEET, KBR3023 and IR3535.

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Accepted 4 April 2006

First published online 25 July 2006