Section A5 Effectiveness against target organisms and intended uses: Active substance IR3535®

Subsection (Annex Point)			Official use only
5.1	**Function (IIA5.1)**	Insect Repellent	
5.2	**Organism(s) to be controlled and products, organisms or objects to be protected (IIA5.2)**	-	
5.2.1	**Organism(s) to be controlled (IIA5.2)**	The following organisms are controlled (PT19): **Mosquitoes** Anopheles spec Aedes spec Culex spec Mansonia spec **Ticks** Ixodes spec **Lice** Pediculus spec **Flies** Stomoxys spec Simuliidae Tabanidae Musca spec Phlebotomus spec **Wasps** Pollistes spec **Bees** Apis spec	x
5.2.2	**Products, organisms or objects to be protected (IIA5.2)**	PT19: IR3535® is an insect repellent to protect humans from insects. It is applied to the human skin in diluted lotions or pump-sprays.	
5.3	**Effects on target organisms, and likely concentration at which the active substance will be used (IIA5.3)**	-	

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5.3.1	**Effects on target organisms (IIA5.3)**	The insects are repelled by the a.s. For details please refer to Table 5.3-1.	
5.3.2	**Likely concentrations at which the A.S. will be used (IIA5.3)**	IR3535® is mainly used at concentrations ranging from 10 to 20% in lotions and pump sprays. However, there are also products with higher or lower concentrations on the market. For details, please refer to Table B2/1 in Document IIIB, Section 2.	x
5.4	**Mode of action (including time delay) (IIA5.4)**	-	
5.4.1	**Mode of action**	Years of experience and several in vivo and in vitro efficacy tests performed with IR3535®, indicate that IR3535® mainly acts via the vapour phase. The mode of action of IR3535® is not a passive masking of an attracting odour of a victim, but an active repellent effect as insects avoid to enter regions with IR3535® vapours. The exact biochemical mode of action of insect repellents is not yet known (Doc. No. 392-004; Section A5.4.1/01). However, according to the cited document, it is known that DEET has an olfactory-based repellent effect. Based on the knowledge gained from the efficacy tests with IR3535® and the behaviour of the insects in these tests, it is most self-evident to assume that IR3535® has an olfactory-based effect. The applicant assumes that no additional information is necessary to cover this data requirement, as any further investigations would only be of interest for basic research and would not contribute to the assessment of the efficacy or of the hazards or of the safe use of IR3535® based biocidal products.	x
5.4.2	**Time delay**	The repellence action starts directly after application.	
5.5	**Field of use envisaged (IIA5.5)**		
	MG03: Pest control	Product Type 19	
	Further specification	Insect Repellent used in human hygiene products.	
5.6	**User (IIA5.6)**		
	Industrial	No	
	Professional	No	
	General public	Yes	
5.7	**Information on the occurrence or possible occurrence of the development of resistance and appropriate management strategies**		

Section A5 Effectiveness against target organisms and intended uses: Active substance IR3535®

	(IIA5.7)		
5.7.1	**Development of resistance**	Development of resistance is not known. Due to the repellent action of IR3535®, insects are repelled, but not killed. Therefore there is no selection pressure and no resistance can be developed, as explained in detail as follows: IR3535® is an insect repellent and not an insecticide. Resistance is typically developed if there is a selection pressure on a population of species, in such a way that individuals that are more tolerant against the substance in question do not die and can therefore reproduce. Unlike insecticides, IR3535® is not used to kill insects, but only to hinder them from entering areas where IR3535® has been applied. Generally, a repellent applied on human or animal skin hinders, e.g. blood sucking insects, from biting. One could argue that this effect constitutes a positive selection pressure, in such a way that the repelled insects may die of starvation and would therefore be removed from the population, so that insects, which are more tolerant, i.e. which are less repelled, would have a feeding advantage and would therefore be in favour for reproduction. Such a scenario would only be of relevance if the majority of potential hosts in an habitat of a population of insects was treated with an insect repellent, so that the insects would have severe problems to find hosts which are not treated with the repellent. Such a scenario is extremely unlikely, as the occurrence of insect repellent treated hosts in a habitat of a population of insects is only sporadic. In other words, the amount of blood not available for the insects, due to the protection by a repellent, is negligible compared to the overall amount of blood available from other sources.	x
5.7.2	**Management strategies**	Not relevant, as explained in 5.7.1.	
5.8	**Likely tonnage to be placed on the market per year (IIA5.8)**	**Confidential information:** Please refer to the "Confidential-Data file".	

Section A5 Effectiveness against target organisms and intended uses: Active substance IR3535®

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	[redacted]
Comments	[redacted]
Conclusion	[redacted]
	COMMENTS FROM ..
Date	Give date of comments submitted
Results and discussion	Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant´s summary and conclusion. Discuss if deviating from view of rapporteur member state
Conclusion	Discuss if deviating from view of rapporteur member state
Reliability	Discuss if deviating from view of rapporteur member state

Section A5 Effectiveness against target organisms and intended uses: Active substance IR3535®

Acceptability	Discuss if deviating from view of rapporteur member state
Remarks	

Table 5.3-1: Summary table of experimental data on the effectiveness of the active substance against target organisms at different fields of use envisaged, where applicable

Function	Field of use envisaged	Test substance	Test organism(s)	Test method / condition	Test results: effects, mode of action, resistance	Reference*)
Insect Repellent	PT19	Ethanolic solutions of the repellents in the following concentration were used: IR3535®: 10%, 15%, 20%, 30% DEET: 10%, 20%, 33%	*Aedes aegypti*	Alcoholic solutions of IR3535® (10%, 15%, 20%, 30%) and DEET (10%, 20%, 33%) were tested on arms of [redacted] humans (male and female) per formulation. An area of [redacted] was treated with [redacted] of the respective formulation. The rest of the arm was covered. The arm was held in the cage containing the mosquitoes directly after treatment and at hourly intervals for five minutes. The repellent action was assumed to be ended when two mosquitoes have sucked themselves full on the treated surface.	Mean value from 10 measurements per formulation for the time when at least two mosquitoes have sucked themselves full on the treated surface. Formulation / Repellent action /min IR3535® 10% 252 IR3535® 15% 351 IR3535® 20% 447 IR3535® 30% 456 DEET 10% 297 DEET 20% 343 DEET 33% 378	[redacted] 1981, Doc. No. 336-1901, Section point A5.3.1/01 End of study summary

Function	Field of use envisaged	Test substance	Test organism(s)	Test method / condition	Test results: effects, mode of action, resistance	Reference*)
Insect Repellent	PT19	Solutions of the repellents in the following concentration were used (vehicle is not stated, but it is most likely ethanol): IR3535®: 0.5%, 1.0%, 2.0%, 2.5%, 3.0%, 5.0% DEET: 0.5%, 1.0%, 2.5%, 5.0%	*Aedes albopictus*	Three tests on repellent action were undertaken according to the following principle: The left arms of humans were treated [redacted] with one formulation. Right arms remained untreated. Immediately and at hourly intervals the volunteers went into a bamboo thicket and stayed for 10 min. The numbers of bites by *Aedes albopictus* on arms (leg) were counted. [redacted] The purpose of a fourth test was to practically see the repellent efficacy, oily feel and odour of IR3535® in comparison with DEET.	In the first test the dosages of [redacted] of IR3535® and DEET completely inhibited biting by *Aedes albopictus*. On the non-treated arms 12 – 47 bites were noted. In the second test both a.s. at [redacted] dosage inhibited biting by *Aedes albopictus* up to [redacted] hours after treatment. IR3535® at [redacted] dosage gave 100% inhibition up to [redacted] hours after treatment, while DEET gave inhibition only at immediate time after treatment. After [redacted] hours for IR3535® at [redacted] concentration, 3 scars were noted on the treated arms, respectively. On the non-treated arms 15 – 41 bites were noted. In the third test (IR3535® [redacted]; DEET [redacted]) both a.s. completely inhibited biting up to [redacted] hours after treatment, except for one biting observed after 4 hours (arm, [redacted]). After [redacted] hours, between 1 to 5 scars were noted on the treated arms. On the non-treated arms (leg) 15 – 60 bites were noted. In the fourth test aerosols containing [redacted] of IR3535® and [redacted] of DEET were compared. The repellent efficacy of both formulations was comparable and oily feel of IR3535® disappeared earlier than that of DEET. As to the odour, IR3535® gave better results than DEET.	[redacted] 1989 – 1990, Doc. No. 336-1902, Section point A5.3.1/02 End of study summary

Function	Field of use envisaged	Test substance	Test organism(s)	Test method / condition	Test results: effects, mode of action, resistance	Reference*)
Insect Repellent	PT19	Solutions of IR3535® in the following concentration were used (vehicle is not stated, but it is most likely ethanol): 20%, 20% + 9% Ethohexadiol; 30% Autan was used as a standard	*Aedes aegypti*	Evaluation of repellents on mice. • 60 starved female insects 5 to 10 days old per cage. One cage per treatment. • Mice: Ventral side of the mice was shaved. Four mice were tested per formulation. • Dosage: [redacted] on the ventral surface of the mouse (3cm x 5cm). • Exposure: Every hour during [redacted] hours each mouse was exposed 10 min. to the mosquitoes. • Non treated surface was covered with a plastic sheet (2cm x 5cm) which was removed after [redacted] hours.	The protection time based on the first bite on 4 treated mice can be summarised as follows: IR3535® 20%: up to 4 h IR3535® 20% + 9% Ethohexadiol: up to 3 h IR3535® 30%: up to 6 h [redacted] After [redacted] hours the plastic sheet was removed. All the mice independent of treatment were bitten by more than 5 mosquitoes on the non treated surface.	[redacted] 1992, Doc. No. 336-1903, Section point A5.3.1/03 End of study summary

Function	Field of use envisaged	Test substance	Test organism(s) Test method / condition	Test results: effects, mode of action, resistance	Reference*)
Insect Repellent	PT19	Ethanolic solutions of the repellents in the following concentration were used: IR3535® 20% DEET 20%	*Pediculus humanus* **Test Method and Conditions:** The escape effect obtained on body lice, *Pediculus humanus* in the presence of IR3535® was observed and compared to the results obtained with a reference repellent, DEET. Large paper sheets were marked with concentric circles of radii of 2, 4, 8, 16 and 32 cm. Square fabric pieces of 1cm x 1cm on which lice were placed at the start of the experiment. Tests: • IR3535®: lice are placed on a - **A**: fabric without repellent; - **B**: fabric with ethanol; - **C**: fabric with IR3535® ® 20% in ethanol. • IR3535®: - **A**: fabric without repellent + lice - **B**: (fabric + lice) then ethanol - **C**: (fabric + lice) then IR3535® 20% in ethanol. • DEET: - fabric with DEET 20% in ethanol + lice • DEET: - (fabric + lice) + DEET 20% in ethanol Test samples were placed in the middle circle on the paper and the distance travelled by the lice were measured after [redacted]	• The impulse to escape appears to come sooner with IR3535® than with DEET. After [redacted] of observation, 6% of the lice exposed are still on the fabric and 68% are over 32 cm away from the treated area. • With DEET, the reaction time of the lice is longer; there is virtually no movement during the first 30 sec. This starts in the period between [redacted] After [redacted] only 1% still remain on the fabric, and 87% of the lice exposed are over 32 cm away. • The curative effect which was attempted to obtain by treating the lice on their pieces of fabric with the same quantities of IR3535® and DEET lotions has yielded the following results: With IR3535® and DEET the lice do not move very much, probably due to a slight intoxication and perhaps because the insect has difficulty in locating the area to be avoided, since its sensorial organs have been treated. • As a general conclusion, it is considered that the two products have approximately the same performance, with DEET having a slight advantage in terms of overall repellent effect and with IR3535® having a faster action.	[redacted] 1993, Doc. No. 336-1904, Section point A5.3.1/04 End of study summary

<table>
<tr><th>Function</th><th>Field of use envisaged</th><th>Test substance</th><th>Test organism(s)</th><th>Test method / condition</th><th>Test results: effects, mode of action, resistance</th><th>Reference*)</th></tr>
<tr>
<td>Insect Repellent</td>
<td>PT19</td>
<td>Ethanolic solutions of the repellents in the following concentration were used:

IR3535® 20%
DEET 20%</td>
<td>Pediculus humanus capitis</td>
<td>Bioclinical in vivo trial to test the efficacy of repellent lotions (IR3535®) in order to prevent re-infestation of lice on humans after the use of a pediculicidal shampoo. [redacted]

Three parallel groups treated with a commercial anti-lice shampoo in one application of 2 shampooings for 3 min.:
1) Tested group: additionally treated with IR3535® [redacted]
2) Positive control group: additionally treated immediately with a reference product DEET [redacted].
3) Negative control group: not treated with any repellent
Prior to repellent application, the hair was brushed and washed with the anti-lice shampoo twice and lice were counted that were brushed off and found in rinsing water and drying towel. [redacted] The hair was brushed and the lice counted, noting whether they were small (the result of an inadequate anti-nit action of the shampoo) or large (the result of an inadequate repellent action or re-infestation in the case of the negative control group). The hair was washed and (small and large) lice were counted as on day 0.</td>
<td>
<table>
<tr><th></th><th>Number of cases</th><th colspan="4">Infection Day 0</th></tr>
<tr><th></th><th></th><th>Brushing</th><th>Rinsing</th><th>Drying</th><th>Total</th></tr>
<tr><td>IR3535®</td><td>20</td><td>45</td><td>63</td><td>412</td><td>520</td></tr>
<tr><td>DEET</td><td>20</td><td>41</td><td>89</td><td>401</td><td>531</td></tr>
<tr><td>Negative Control</td><td>20</td><td>64</td><td>54</td><td>385</td><td>493</td></tr>
<tr><th></th><th>Number of cases</th><th colspan="4">Infection Day 7</th></tr>
<tr><th></th><th></th><th>Brushing</th><th>Rinsing</th><th>Drying</th><th>Total</th></tr>
<tr><td>IR3535®</td><td>20</td><td>1</td><td>0</td><td>2</td><td>3</td></tr>
<tr><td>DEET</td><td>20</td><td>2</td><td>2</td><td>4</td><td>8</td></tr>
<tr><td>Negative Control</td><td>20</td><td>22</td><td>2</td><td>93</td><td>116</td></tr>
</table>
The lotion based on IR3535®, which was used [redacted] made it possible to prevent massive re-infestation in a highly infested environment (DEET showed similar results). An anti-lice or anti-nit action was not observed. Tolerance of volunteers was excellent.
</td>
<td>[redacted] 1993
Doc. No. 336-1905, Section point A5.3.1/05

End of study summary</td>
</tr>
</table>

Function	Field of use envisaged	Test substance	Test organism(s)	Test method / condition	Test results: effects, mode of action, resistance	Reference*)
Insect Repellent	PT19	IR3535® 67% blended with melissa (balm mint) oil IR3535® 67% blended with aromatic / silicone oils Thermal evaporation	*Musca domestica*	Tests to evaluate whether houseflies (*musca domestica*) are deterred from entering a room in which insect repellent is being released by thermal evaporation. Tests were performed in a chamber of 5.5 cubic metres (illuminated from a window) which stands in a room of approx. 20 cubic metres (dark except enlighted by light entering through the test chamber). The test chamber is supplied with an extraction fan to vent contaminated air. An evaporator device was plugged in 15 min. before start of the test to heat up the evaporator plate. The evaporation container of the respective formulation was fitted to the device. The access door and observation window were closed. [redacted] the houseflies were released from their cage into the outer room. [redacted] the observation windows were removed and the number of flies entering and leaving the test chamber counted during a 20 min. period. At the end of 20 min. the number of flies remaining in the chamber was recorded. Further counts [redacted] and as described above) were made after [redacted] hours.	In most test replicates a large number of flies entered the chamber during one or most of the evaluation periods. Many of these in replicates using repellent formulation followed arc shaped flight paths that crossed the threshold of the observation window opening and immediately returned to the outer room. In some cases flies that flew directly towards the illuminated outside window would reach it, hit it, and return to the observation window immediately. Relatively few flies remained in the treated room for a long period but if they did so, they mostly remained inactive standing on walls or the frame of either window. With IR3535® plus melissa oil, flies entering the chamber frequently appeared irritated by the vapour and spent time on cleaning their legs and faces; flies exposed to IR3535® plus aromatic/silicone oils spent less time grooming.	[redacted] 1995, Doc. No. 336-1906, Section point A5.3.1/06 End of study summary

Function	Field of use envisaged	Test substance	Test organism(s)	Test method / condition	Test results: effects, mode of action, resistance	Reference*)
Insect Repellent	PT19	Hydro alcoholic gel CARBOPOL = excipient A IR3535® 5% in A IR3535® 10% in A IR3535® 15% in A DEET 5% in A DEET 15% in A	*Pollistes galliens and Apis melifera*	The aim of this study was to evaluate the capture of wasps and bees (attracted by a mixture of water, honey and fruits) on a reference and placebo trap, compared to the captures on traps coated with the repellent to test. The study was conducted during [redacted] days, with a daily counting of the captures, this in outdoor conditions, frequently infested by these two species. The repellent was daily applied on the traps, except on that defined as the reference and placebo. The traps were placed in the immediate vicinity of an apiary, each trap being 50 cm apart. A statistical evaluation was done at the end of the study, in order to analyse the possible differences concerning the numbers.	*Reference trap*: 47 captures of wasps and 87 captures of bees are observed within [redacted] days. The daily average are 6.7 and 12.4 for these two species. *Placebo trap:* 52 captures for the wasps, 86 captures for the bees *Test article trap:* The best result obtained with the repellents seem to be those with IR3535® [redacted] (3 wasps, 6 bees; 0.4 and 0.9 daily av. respectively). The scores obtained with the compositions DEET [redacted] (19 wasps, 26 bees; 2.7 and 3.7 daily av. respectively) and IR3535® [redacted] (31 wasps, 51 bees; 4.4 and 7.3 daily av. respectively) seem to be less favourable, in spite of identical experimental conditions. The values for treated traps are practically 50% below those for the reference trap and the placebo trap. Therefore it is obvious that these 2 formulation show a significant repelling effect.	[redacted] 1995, Doc. No. 336-1907, Section point A5.3.1/07 End of study summary

Function	Field of use envisaged	Test substance	Test organism(s)	Test method / condition	Test results: effects, mode of action, resistance	Reference*)
Insect Repellent	PT19	Alcoholic (50%) solutions of the repellents in the following concentration were used: IR3535® 10%, 20%, 30% Application rate: [redacted]	*Ixodes ricinus*	The shaved skin on rabbit backs was treated with a [redacted] IR3535® test formulation with the three different concentrations. After [redacted] of skin drying in air the female *Ixodes ricinus* were transferred to the rabbits. A group of [redacted] female ticks was used for each of the animals treated with [redacted] and [redacted] concentration (repeated three times) and for the animals treated with [redacted] concentration (repeated twice). The effectiveness of repellency action of examined solutions of insect repellent was determined by the numbers of ticks attacking the rabbit skin coated with substance after [redacted] hours. The protection time of this repellent was considered to be the time between treatment and the penetration of Ixodes ricinus females into rabbit skin. At the same time and under the same conditions the control was undertaken in which the ticks were transferred on the rabbit skin coated only with alcoholic solution without a. s.	IR3535® had a strong repellent action on Ixodes ricinus ticks. Laboratory assays with females showed that tested substance with [redacted] and [redacted] concentrations may protect from attack by the ticks through [redacted] hours. Insect repellent in these concentrations essentially decreased the attachment of *Ixodes ricinus* females also through nex [redacted] hours. The tested substance with [redacted] concentration repelled the females of examined species for [redacted] hours. After using the highest concentration the dead ticks appeared as early as after [redacted] and [redacted] hours.	[redacted] 1995, Doc. No. 336-1908, Section point A5.3.1/08 End of study summary

Function	Field of use envisaged	Test substance	Test organism(s)	Test method / condition	Test results: effects, mode of action, resistance	Reference*)
Insect epellent	PT19	Alcoholic (50%) solutions of the repellents in the following concentration were used: IR3535® 5%, 10%, 15% DEET 15%, 30%, 60%	*Ixodes scapularis*	Each of the formulations was evenly applied to 90 mm filter paper discs at the rate of [redacted] and allowed to dry for 10 min. before each was placed in an uncovered 90 mm petri dish. A clean disc of aluminium, 24 mm diameter x 0.05 mm thick, was placed in the centre of each treated paper. Prior to each assay, ticks were randomly selected and lightly anaesthetised with CO_2 and transferred to the aluminium disc at the centre of the prepared test arena. One minute was allowed to elapse for ticks to recover from anaesthetisation before timing began. At [redacted] min., the total number of ticks that left the aluminium disc and appeared moribund or dead were excluded from the assay and the starting N was reduced accordingly. One replicate of this assay consistent of one plate ([redacted] ticks) for each test substance at each concentration plus one control plate containing filter paper treated with ethanol only. The assay was replicated 5 times using all new materials (except for aluminium disc that were thoroughly cleaned in acetone) and new groups of ticks.	For each assay replicate, a percent repellency was calculated for each test substance/concentration/time interval as follows: Percent Repellency = 100 – (% ticks leaving disc in treatment /% ticks leaving disc in negative control) Ticks in untreated control plates crawled off the aluminium discs quickly and without apparent hesitation. At [redacted] min., a mean of 18% of ticks remained on the discs. In all plates containing repellent paper, ticks were seen to touch the treated paper and withdraw onto the aluminium discs. By [redacted] min., many ticks on the DEET treated plates were moribund or dead. Toxicity was not clearly evident in the IR3535® treated plates. IR3535® [redacted] repelled deer ticks at least as effectively as DEET[redacted] or [redacted] in these experiments.	[redacted] 1996, Doc. No. 336-1909, Section point A5.3.1/09 End of study summary

Function	Field of use envisaged	Test substance	Test organism(s)	Test method / condition Test results: effects, mode of action, resistance (first out of four pages for this study)	Reference*)
Insect Repellent	PT19	Ethanolic solutions of the repellents in the following concentration were used: IR3535® 5%, 10%, 15% DEET 15%, 30%´, 60%	*Ixodes scapularis*	**Test method and condition:** **Deer ticks, *in vitro*:** Filter paper discs were treated at a rate of [redacted] and aged for [redacted] min. before testing. At this time a smaller untreated filter paper disc with [redacted] ticks was placed on top of and in the middle of each treated disc. Discs were placed in separate petri dishes. The number of ticks leaving the untreated disc and moving onto the treated paper of each disc were counted after 3 min. and thereafter on an hourly basis. Data from treatments were compared similarly with ethanol (with no repellent) treated papers used as controls. Percent repellency at each time interval and for each treatment concentration is calculated as: % repellency = 100 (#on disc in control – #on treatment disc) / #on disc in control. **Results:** **Deer ticks, *in vitro*:** Calculated IR3535® repellency ranged from [redacted]. The calculated repellency of DEET even at the highest concentration (i. e.) did not prove against Ixodes scapularis nymphs. As a result no more testing was conducted using this method. **Remark by applicant:** The author states in the report: "No further testing of this species using this assay method was conducted because the first test had shown that this bioassay did not adequately reflect the range of repellency for either compund when compared to the middle index finger assay". That is why we regard this part of the study as not valid to substantiate a repellency claim against Ixodes. Contd.	[redacted] 1995, Doc. No. 336-1911, Section point A5.3.1/10

Function	Field of use envisaged	Test substance	Test organism(s)	Test method / condition Test results: effects, mode of action, resistance (second out of four pages for this study)	Reference*)
Insect Repellent	PT19	Ethanolic solutions of the repellents in the following concentration were used: IR3535® 5%, 10%, 15%, (30%) DEET 15%, 30%´, 60%	*Ixodes scapularis*	**Test method and condition:** **Deer ticks, *in vivo*:** The 1st and 3rd joint of the index fingers of volunteers were treated with the respective formulation. Pre-testing of each group of ticks to be used in a single test were accomplished by placing ticks on ethanol only treated 1st and 3rd joints of index finger of each hand. Ethanol was applied as the control at the rate of [redacted] Five deer ticks were used per test and replicated twice per time interval per person. The ticks were placed on the untreated 2nd joint of the index finger while the finger was held in a horizontal position to determine if normal host seeking behaviour is observed. The number of ticks crawling into the 1st or 3rd joint at 3 min. were recorded. If, in a group 80% (4 out of 5) failed to respond , that group was replaced by another group, tested in the same manner. The resulting pre-test data served as the control data for that group. After pre-testing, the 1st and 3rd index finger joint of one hand was treated with an appropriate concentration of IR3535® at a rate of [redacted] the same finger joints of the other hand was treated with amount of formulation of the DEET commercial standard. All the treatments were allowed to age for [redacted] min. and, after this time, a group of [redacted] ticks was placed on the untreated 2nd index finger joints each hand and held in a horizontal position. Ticks that moved to either treatment area were considered not to be repelled. The results were recorded after 3 min., at that time the ticks were removed and the evaluation was repeated with a 2nd group of ticks. All evaluations were repeated hourly for [redacted] hours (until failure of repellency for IR3535®, which will be denoted as < 90% repellency) in two successive tests. An entire trial, consisting of IR3535® and DEET at each of the individuals daily evaluations for each of the three concentration, was replicated three times by [redacted] human volunteers. The percent repellency at each time interval is calculated as: % repellency = 100 (#on pre test – #on treatment) / #on pre-test. Contd.	[redacted] 1995, Doc. No. 336-1911, Section point A5.3.1/10

Function	Field of use envisaged	Test substance	Test organism(s)	Test method / condition Test results: effects, mode of action, resistance (third out of four pages for this study)	Reference*)
Insect Repellent	PT19	Ethanolic solutions of the repellents in the following concentration were used: IR3535® 5%, 10%, 15% DEET 15%, 30%´, 60%	*Ixodes scapularis*	**Result:** **Deer ticks, *in vivo*:** General, < 90% repellency of deer ticks was noted for IR3535® at all concentrations [redacted] hour after treatment and continued to decline thereafter for [redacted] hours. As a result, all tick assays were conducted for a total of [redacted] hours each. At the time of treatment, IR3535® and DEET were not significantly different in repelling ticks at [redacted] and [redacted] compared with DEET at [redacted] and [redacted], respectively. Although IR3535® at [redacted] achieved > 93% repellency of deer ticks at this time period, repelleny of DEET at [redacted] was slightly but significantly greater that IR3535® . However, it should be mentioned that IR3535® tick repellency at [redacted] hour was not significantly different at the [redacted] and [redacted] IR3535® concentrations than [redacted] and [redacted] DEET, respectively nor was there a significant difference in tick repellency for [redacted] IR3535® compared with [redacted] DEET at [redacted] hours. At [redacted] hours after treatment both repellents gave < 80% repellency. Assays using [redacted] IR3535® in a limited test indicated repellency of ≥ 90% of deer ticks for [redacted] h at this concentration. However, caution is warranted in interpretation of these initial data. Due to a limited number of ticks left over from previous assays, only one test was conducted. Also, after [redacted] h of testing some ticks had to be reused from the previous morning's testing because not enough unexposed ticks were available on that day to use. Some of the reused ticks were observed to be sluggish in their movement on the untreated portion of the middle index finger, as a result, the data after this time does need to be replicated to assure accuracy of true repelleny of [redacted] IR3535®. Contd.	[redacted] 1995, Doc. No. 336-1911, Section point A5.3.1/10

Function	Field of use envisaged	Test substance	Test organism(s)	Test results: effects, mode of action, resistance (fourth out of four pages for this study)	Reference*)
Insect Repellent	PT19	Ethanolic solutions of the repellents in the following concentration were used: IR3535® 5%, 10%, 15% DEET 15%, 30%´, 60%	*Stomoxys calcitrans*	**Test method:** **Stable flies:** The essays were conducted in 12x12x24 inch screened cages with each containing approximately 50 non-blood fed adult flies. One forearm of each human volunteer was treated with the appropriate concentration of each repellent. The other forearm of each volunteer was treated with the same rate of ethanol as the repellents to serve as control. Dilutions are applied at a rate of [redacted] The arms were placed in separate cages. The time to the first bite as well as the number of flies biting after 30 sec. exposure were recorded. All evaluations were repeated [redacted] hours or until failure of repellency for IR3535® , which will be denoted as < 90% repellency in two successive evaluations. An entire trial, consisting of daily treatments of IR3535® and DEET for each one of the three concentrations, was evaluated by [redacted] volunteers and replicated three times. At the completion of one test, the volunteers switched treatments so that neither person solely evaluates one repellent. The repellency at each time interval for all repellent treatments is calculated as: % repellency = 100 (#biting in control – #biting in treatment) / #biting in control. **Results:** **Stable flies:** Generally stable fly repellency of IR3535® was < 62% in all test, even at the time of treatment (t_0). Additionally, IR3535® was consistently lower in repellent properties compared with DEET. IR3535® was significantly lower in repellency at [redacted] h for [redacted] and [redacted] concentrations compared with paired DEET assays at [redacted] and [redacted] respectively. However, no significant differences in repellency were observed when IR3535® was tested at [redacted] and [redacted] compared to DEET at [redacted] and [redacted]. At [redacted] h after treatment, [redacted] DEET significantly repelled more biting stable flies that IR3535® at [redacted] and [redacted]. At [redacted] h, no significant difference was detected in stable fly repellency between IR3535® and DEET at any concentration. Generally fly repellency increased with increasing IR3535® concentration, with the exception of [redacted] and [redacted] concentrations. However, adding or subtracting the standard error with its appropriate mean for each of these concentrations revealed that mean repellency overlapped one another at each time interval. As a result, fly repellency for IR3535® at [redacted] and [redacted] concentrations were similar.	[redacted] 1995, Doc. No. 336-1911, Section point A5.3.1/10 End of study summary

Function	Field of use envisaged	Test substance	Test organism(s)	Test method / condition Test results: effects, mode of action, resistance (first out of four pages for this study)	Reference*)
Insect Repellent	PT19	Ethanolic solutions of the repellents in the following concentration were used: IR3535® 1.875%, 3.75%, 7.5%, 15%, 30% DEET 30%	*Simuliidae*	**Black flies:** In an in vitro assay, it is expected that the landing and probing rates on repellent treated membranes will be lower than those on untreated membranes or EtOH-treated membranes. Due to the nature of the in vitro assay used for black flies, it was decided that only probing rates would be used to indicate whether or not IR3535® was efficacious. Repellent testing on black flies was carried out at the [redacted]. Trials were conducted after the population peak for black flies. A piece of latex condom was used to simulate skin. In each test, membranes received one of five treatments: blank control, Ethanol control, 30% DEET and 30%, 15%, 7.5%, 3.75% and 1.875% IR3535®; [redacted] in all cases. Flies held in their collection vials, were placed on membranes that had been stretched over the surface of a 2'' x 4'' metal box, equipped with a heating element and thermostat to maintain a constant 37 °C temperature. **Results:** The % repellency data demonstrated that IR3535® was effective in reducing biting by at least 75% up to [redacted] hours post-application at all doses except 1.875%. As the test was started late in the season when sufficient numbers of flies were only available in the late afternoon/early evening, it was not feasible to do more rigorous time-course studies.	[redacted] 1995, Doc. No. 336-1912, Section point A5.3.1/11

Results of efficacy test of IR3535® against black flies in an in vitro assay	Test 1				Test 2				Test 3				Test 4				Test 5			
	IR3535® 30%	DEET 30%	Blank	Ethanol	IR3535® 15%	DEET 30%	Blank	Ethanol	IR3535® 7 5%	DEET 30%	Blank	Ethanol	IR3535® 3 75%	DEET 30%	Blank	Ethanol	IR3535® 1 875%	DEET 30%	Blank	Ethanol
Probing	1	0	25	26	3	3	36	34	0	0	14	7	1	1	6	7	4	0	8	0
Not probing	53	54	29	28	79	79	46	48	32	32	18	25	31	15	10	25	22	26	18	32
Total tested	54	54	54	54	82	82	82	82	32	32	32	32	32	16	16	32	26	26	26	32

End of table

<table>
<tr><th>Function</th><th>Field of use envisaged</th><th>Test substance</th><th>Test organism(s)</th><th>Test method / condition
Test results: effects, mode of action, resistance
(second out of four pages for this study)</th><th>Reference*)</th></tr>
<tr><td>Insect Repellent</td><td>PT19</td><td>Ethanolic solutions of the repellents in the following concentration were used:

IR3535®
0.9%, 1.875%, 3.75%, 7.5%, 15%, 30%, 50%, 80%

DEET 30%, 80%</td><td>Tabanidae</td><td>Deer flies: Generally, in an in vitro assay, it is expected that the landing and probing rates on repellent-treated membranes will be lower than on control membranes. Due to the nature of the in vitro assay used for deer flies, however, it was expected that only landing rates (or time spent on the membranes) would be used to indicate whether or not IR3535® was efficacious.
Membranes (Parafilm®) were heated on a metal slide warmer, equipped with a heating element and thermostat set at 40 °C. Flies were transferred from the scintillation vials and placed individually in a plexiglass cylinder (approx. 7.5 cm diameter and 15 cm high) which rested on a mirror. The top of the cylinder was covered with a fine mesh through which the deer flies could bite. The treated membranes were placed directly on top of the mesh. A light was trained from above to attract the flies to the membrane. The amount of time spent on the membrane and the incidence of probing was observed in the mirror. The total amount of time allowed for each fly was 2 min. Thus the data recorded for each fly were no., sec. spent on the membrane and whether or not the fly had probed.
Results:
<table><tr><th></th><th></th><th colspan="2">DEET</th><th colspan="8">IR3535®</th></tr><tr><th>Results of efficacy tests using IR3535® against deer flies</th><th>Ethanol</th><th>80%</th><th>30%</th><th>80%</th><th>50%</th><th>30%</th><th>15%</th><th>7.5%</th><th>3.75%</th><th>1.875%</th><th>0.9%</th></tr><tr><td>Probing</td><td>33</td><td>7</td><td>5</td><td>6</td><td>6</td><td>4</td><td>6</td><td>6</td><td>3</td><td>2</td><td>4</td></tr><tr><td>Not probing</td><td>146</td><td>90</td><td>79</td><td>43</td><td>43</td><td>78</td><td>76</td><td>34</td><td>37</td><td>8</td><td>6</td></tr><tr><td>Total tested</td><td>178</td><td>97</td><td>84</td><td>49</td><td>49</td><td>82</td><td>82</td><td>40</td><td>40</td><td>10</td><td>10</td></tr><tr><td>% probing</td><td>18.44</td><td>7.22</td><td>5.95</td><td>12.24</td><td>12.24</td><td>4.88</td><td>7.32</td><td>15.00</td><td>7.5</td><td>20.00</td><td>40.00</td></tr></table>Probing rates were very low. Only 18.4 % of deer flies probed on the Ethanol-treated membrane. There were 11 – 12% fewer probes on the DEET-treated membranes relative to the Ethanol control. IR3535® 30%, IR3535® 15% and IR3535® 3.75% were equally as efficacious in reducing the percentage of deer flies probing. Probing was highest (49%) in IR3535® 0.9% - treated membranes but this probing value is based on a sample size of only [redacted] individuals. For statistical analyses, therefore, the mean amount of time spent on the membranes was investigated.

Contd.</td><td>[redacted]
1995, Doc. No. 336-1912, Section point A5.3.1/11</td></tr>
</table>

Function	Field of use envisaged	Test substance	Test organism(s)	Test results: effects, mode of action, resistance (third out of four pages for this study)	Reference*)
Insect Repellent	PT19	Ethanolic solutions of the repellents in the following concentration were used: IR3535® 0.9%, 1.875%, 3.75%, 7.5%, 15%, 30%, 50%, 80% DEET 30%, 80%	*Tabanidae*	**Results:** **Deer flies (contd.):** IR3535® / DEET Duration of time spent by deer flies on treated membranes: Ethanol; IR3535® 0.9%; 1.875%; 3.75%; 7.5%; 15%; 30%; 50%; 80%; DEET 30%; 80% N: 179; 10; 10; 40; 40; 82; 82; 49; 49; 84; 97 MEAN: 94.70; 101.10; 93.50; 88.43; 81.93; 55.55; 51.30; 46.76; 47.84; 63.93; 62.88 STDEV: 34.82; 34.20; 43.60; 37.88; 39.39; 44.26; 43.91; 39.98; 43.49; 43.19; 35.66 SEMEAN: 2.60; 10.80; 13.80; 5.99; 6.23; 4.89; 4.85; 5.71; 6.21; 4.71; 3.62 There is no significant difference in amount of time spent on the membrane among the ethanol, IR3535® 0.9%, IR3535® 1.875%, IR3535® 3.75 and IR3535® 7.5% treatments. In contrast the IR3535® 15%, 30%, 50% and 80% treatments significantly lower the amount of time pent on the membrane, relative to the "ethanol to IR3535® 7.5% group". There are no significant differences among the IR3535® 15% to IR3535® 80% group in terms of efficacy. However, it is interesting to note that IR3535® 15% is no different from DEET 30% or DEET 80%, but that the higher concentrations of IR3535® are significantly better than DEET at reducing the amount of time spent on the membranes.	[redacted] 1995, Doc. No. 336-1912, Section point A5.3.1/11

Contd.

<table>
<tr><th>Function</th><th>Field of use envisaged</th><th>Test substance</th><th>Test organism(s)</th><th>Test results: effects, mode of action, resistance
(fourth out of four pages for this study)</th><th>Reference*)</th></tr>
<tr><td>Insect Repellent</td><td>PT19</td><td>Ethanolic solutions of the repellents in the following concentration were used:

IR3535®
0.9%, 1.875%, 3.75%, 7.5%, 15%, 30%, 50%, 80%

DEET 30%, 80%</td><td>Stomoxys calcitrans</td><td>Stable flies: The experimental protocol was similar to that used in deer fly tests (this study). Wild stable flies of a colony held at [redacted] were used. Only an ethanol-treated membrane was used as a negative control. IR3535® (100%, 80%, 50%, 30% and 15%) and DEET 30% were tested. No „evaluation time interval“ trials, i. e., time course studies, were conducted. 12 Trials were conducted, always with DEET 30% and EtOH as a control. IR3535® was tested in two concentration per trial. See table for number of tests per concentraion. Data for each concentration of IR3535® were pooled. The average amount of time spent on the membranes was compared among treatments.
Results: IR3535® [redacted] is as effective as DEET [redacted]. IR3535® [redacted] and IR3535® [redacted] are significantly less effective than DEET [redacted], whereas IR3535® [redacted] and IR3535® [redacted] are significantly more effective than DEET [redacted] in reducing the amount of time spent on treated membranes.
<table>
<tr><th></th><th colspan="7">IR3535®</th></tr>
<tr><th>Duration of time spent by stable flies on treated membranes</th><th>Ethanol 12 tests</th><th>DEET 30% 12 tests</th><th>15% 4 tests</th><th>30% 6 tests</th><th>50% 6 tests</th><th>80% 4 tests</th><th>100% 4 tests</th></tr>
<tr><td>N</td><td>110</td><td>110</td><td>37</td><td>55</td><td>55</td><td>36</td><td>36</td></tr>
<tr><td>MEAN</td><td>102.75</td><td>37.25</td><td>77.65</td><td>52.02</td><td>33.13</td><td>24.14</td><td>9.81</td></tr>
<tr><td>STDEV</td><td>22.32</td><td>34.48</td><td>30.70</td><td>40.60</td><td>30.57</td><td>26.21</td><td>9.37</td></tr>
<tr><td>SEMEAN</td><td>2.13</td><td>3.29</td><td>5.05</td><td>5.47</td><td>4.12</td><td>4.37</td><td>1.56</td></tr>
</table>
</td><td>[redacted]
[redacted]
[redacted]
1995, Doc. No. 336-1912, Section point A5.3.1/11

End of study summary</td></tr>
</table>

Function	Field of use envisaged	Test substance	Test organism(s)	Test method/ condition	Test results: effects, mode of action, resistance	Reference*)
Insect Repellent	PT19	Ethanolic solutions of the repellents in the following concentration were used: IR3535® 20% DEET 20% Application rates: [redacted] (arm) [redacted] (leg)	*Aedes aegypti* *Culex quinquefasciatus* *Culex Tritaeniorhynchus* *Culex gelidus* *Mansonia dives* *Ma. Uniformis* *Ma. Annulata* *Ma. Annulifera* *Anopheles minimus* *An. Maculatus*	The insect repellents IR3535® and DEET were prepared as 20% solutions in absolute ethanol and evaluated for repellency against many mosquito species in Thailand under laboratory and field conditions using human subjects. In the laboratory [redacted] was applied per [redacted] of exposed area on a volunteers forearm [redacted], whereas in the field, volunteers legs (from knee to ankle, with a surface area of about 712 – 782 cm^2) were treated with [redacted] per exposed area [redacted].	In the laboratory both IR3535® and DEET showed similar repellency for [redacted] against Aedes aegypti, for [redacted] against Culex quinquefasciatus, and for [redacted] against Culex Tritaeniorhynchus, respectively. Under field conditions, both IR3535® and DEET provided a high degree of protection against various mosquito vectors ranging from 94 – 100% during the test periods. Both repellents provided a high level of protection for at least [redacted] against Aedes albopictus and for at least [redacted] against Culix gelidus, Cx. Tritaeniorhynchus, Cx. Quinquefasciatus, Mansonia dives, Ma. Uniformis, Ma. Annulata, Ma. Annulifera, Anopheles minimus and An. Maculatus. This study documents the potential of IR3535® for use as a topical treatment against a wide range of mosquito species belonging to several genera.	[redacted] 2001, Doc. No. 336-1913, Section point A5.3.1/12 End of study summary

Function	Field of use envisaged	Test substance	Test organism(s)	Test method/ condition	Test results: effects, mode of action, resistance	Reference*)
Insect Repellent	PT19	IR3535® 10% (referred to as EBAP) DEET 10% in diffetent matrizes containing: • AAC (acrylates/C_{10-30} alkyl/acrylate crosspolymer) • Triethanolamine • CCT (caprylic/capric trigliceride) • Isopropyl Myristate • Water	*Aedes aegypti*	This test was to evaluate the influence of different emulsifiers on the repellency effect of IR3535® and DEET. Emulsions of 10 % w/w repellent were tested against the biting laboratory bred mosquitoes *Aedes aegypti* by the method of Bueschner.	Generally, the repellency of the tested emulsions using DEET or IR3535® against *Aedes aegypti* was > 50% in all samples. The results of the biological efficiency demonstrated that emulsions containing IR3535® showed a better repellent action than samples with DEET.	R. Milutinovic, J. Milic, N. Stajkoviv and A. Cvetkovic 2000. Doc. No. 392-001, Section point A5.3.1/13 End of study summary

Function	Field of use envisaged	Test substance	Test organism(s)	Test method/ condition	Test results: effects, mode of action, resistance	Reference*)
Insect Repellent	PT19	0.1 / 0.3 / 0.6 / 0.8 mg a. s. /cm^2 of legs were diluted in 20 ml ethanol. This leads to the following concentration ranges: DEET 2 – 13% IR3535® 2 – 13% KBR 3023 2 – 13%	mainly: *Anopheles gambiae*	Synthetic insect repellents, IR3535® and KBR 3023 (also known as picaridin, or by the trade name Bayrepel®), were tested in Burkina Faso against mosquito vectors of disease to compare their relative efficacy and persistence profiles to those of DEET. Four groups of two persons each received each repellent and placebo in 4 x 4 latin square scheme. This scheme was repeated for each concentration.	Collection of >49000 mosquitoes (~95% belonging to the *Anopheles gambiae* complex) showed that after an exposure of 10 h, KBR 3023 produced the highest protection against anophelines, followed by DEET, then IR3535. The response of aedines was more variable. By fitting a logistic plane model 95% effective dosages (ED_{95}) were estimated for *An. gambiae* s.l., as well as a decay constant characterizing the exponential loss of repellent from the skin, with time. The ED_{95} values for DEET, IR3535®, and KBR 3023 were 94.3, 212.4, and 81.8 µ/cm^2 respectively. The decay constants were estimated at -0.241, -0.240, and -0.170 h^{-1} respectively. The corresponding estimates of half-life were 2.9, 2.9, and 4.1 h. Immunoenzymatic detection of the circumsporozoite protein (CSP) of *Plasmodium falciparum* in 842 *An. gambiae* s.l. showed that CSP-positive mosquitoes were equally frequent in treated and control subjects, indicating that the repellents could produce a reduction in the number of malaria infectious bites.	C. Costantini, A. Badolo, E. Ilboudo-Sanogo 2003. Doc. No. 392-002, Section point A5.3.1/14 End of study summary

Function	Field of use envisaged	Test substance	Test organism(s)	Test method/ condition	Test results: effects, mode of action, resistance	Reference*)
Insect Repellent	PT19	IR3535® 10% IR3535® 20% DEET 10% DEET 20% Aqueous cream and hydroalcoholic spray for each repellent and concentration. 4 Commercially available formulations with IR3535®: • Expedition insect repellent 20.07% a. s. • Bug Guard Plus with SPF30 sunscreen 7.5% a. s. • Bug Guard Plus with SPF15 sunscreen 7.5% a. s: • Bug Guard Plus 7.5% a. s.	*Aedes aegypti* *Culex quinquefasciatus*	Arm-in-cage laboratory evaluations of 2 proprietary formulations of the mosquito repellents IR3535 and N,N-diethyl-3-methylbenzamide (DEET; aqueous cream, hydroalcoholic spray) were made with 10 and 20% concentrations of each repellent. Also, 4 commercially available products containing IR3535® (Expedition insect repellent 20.07% active ingredient [AI], Bug Guard Plus with SPF30 sunscreen 7.5% AI, Bug Guard Plus with SPF15 sunscreen 7.5% AI, and Bug Guard Plus 7.5% AI) were tested. All comparisons were made on an equal formulation or concentration basis. Eight volunteers tested all formulations or products 3 times against laboratory-reared, Aedes aegypti and Culex quinquefasciatus mosquitoes (6-10 days old). Formulations were applied to a forearm at the rate of 0.002 g/cm2. The other forearm was not treated and served as a control. Elapsed time to 1st and 2nd consecutive bite was recorded.	Mean protection time (i.e., time to 1st bite) with proprietary formulations of IR3535® were comparable to those of DEET, with 20% concentrations providing greater protection against Ae. aegypti (3 h) and Cx. quinquefasciatus (6 h). Mean protection time for commercial products containing IR3535® ranged from nearly 90 to 170 min for Ae. aegypti and 3.5 to 6.5 h for Cx. quinquefasciatus. Mean time to the 2nd bite was similar to time to 1st bite for each mosquito species, product, and formulation.	J. E. Cilek, J. L. Petersen and C. E. Hallmon 2004. Doc. No. 392-003, Section point A5.3.1/15 End of study summary

Function	Field of use envisaged	Test substance	Test organism(s)	Test method/ condition	Test results: effects, mode of action, resistance	Reference*)
Insect Repellent	PT19	IR3535® 10% DEET 10% Hydroalcoholic spray for each repellent.	*Phlebotomus mascittii* *Phlebotomus duboscqi*	Arm-in-cage laboratory evaluations of 10% w/w ethanolic solutions of IR3535® and DEET. Two volunteers tested both products 5 times against laboratory-reared sand flies (2-15 days old). Sand flies were used only once per test, i.e. for each new series of tests, new sand flies were used. 1 g of the repellent solutions were applied to a forearm and the back of a hand (with a pump-spray device). Before applying the repellent solutions, ethanol abs. was applied as a negative control and showed no repellent action. The other forearm was not treated and served as a control. The test was stopped when the first sand fly made an attempt to bite. The experimental part of this study was performed in the year 2005 according to internal records of the applicant.	Mean protection time (i.e., time to 1st bite) against *P. duboscqi* was 5.9 h for both repellents. Mean protection time against *P. mascittii* was 10.4 h for IR3535® and 8.8 h for DEET.	T. J. Naucke, S. Lorentz, H.-W. Grünewald 2006. Doc. No. 392-005, Section point A5.3.1/16 End of study summary

Function	Field of use envisaged	Test substance	Test organism(s)	Test method/ condition	Test results: effects, mode of action, resistance	Reference*)
Insect Repellent	PT19	IR3535® 10% (Spray) IR3535® 10% (Lotion) IR3535® 15% (Spray) IR3535® 15% (Lotion) IR3535® 20% (Spray) Picaridin 10% (Lotion) Picaridin 20% (Spray)	*Aedes aegypti*	Ten volunteers tested all the products against *A. aegypti* in a field study. The samples were applied to forearms and remained on the skin for at least 2 h. Amount of repellent: 1.5 g of lotion or 1.0 g of spray per 600 cm² of skin (applied with glass pipettes and rubbed into skin). The other forearm was not treated and served as a control. The times to first, second and third bites were noted. The experimental part of this study was performed in February 2005, as stated in the report.	Mean protection time (1st bite) against *A. aegypti* was 322 to 410 min for all repellents. Mean protection time (2nd bite) for all repellents was 411 to 459 min and for the third bite 463 to 518 min. All products except IR3535® 10% (lotion) gave 95% protection against bites over 6 h. IR3535® 10% (lotion) provided 95% protection over 4 hours.	T. J. Naucke, R. Kröpke, G. Benner, J. Schulz, K. P. Wittern, A. Rose, U. Kröckel, H.-W. Grünewald 2007. Doc. No. 392-006, Section point A5.3.1/17 End of study summary

Function	Field of use envisaged	Test substance	Test organism(s)	Test method/ condition	Test results: effects, mode of action, resistance	Reference*)
Insect Repellent	PT19	IR3535® 15% (Spray) IR3535® 15% (Lotion 1) IR3535® 15% (Lotion 2) Picaridin 20% (Spray 1) Picaridin 20% (Spray 2)	*Aedes aegypti* *Anopheles darlingi* *Anopheles albitarsus* *Culex pedroi*	11 volunteers (10 male, 1 female) tested all the products on exposed legs in a field study. Samples were spread evenly over each leg from ankle to knee. Amount of repellent: 1.5 g of lotion or 1.0 g of spray per 600 cm² of skin. A 70% ethanol solution served as negative control. The times to first bites were noted. The experimental part of this study was performed in the year 2006 according to internal records of the applicant.	All tested samples provided lasting protection over several hours. There was no significant difference in protection times between the active substances. The mean protection time was between more than 200 and less than 450 minutes. Application form of IR3535® as a lotion resulted in extended protection time compared to IR3535® as a hydro-alcoholic spray.	R. Kröpke, G. Benner, J. Schulz, K. P. Wittern, A. Hill N, Beyer N 2007. Doc. No. 392-007, Section point A5.3.1/18 End of study summary

Appendix to Doc IIIA05: Response of Applicant to comments made by the RMS in April 2008.

Advice of the RMS - August 2008

1) General comments from RMS

Section A5 (concerning active substance) has been updated in 2006.
Studies provided in order to assess efficacy of the active substance (IR 3535® or ethyl butylacetylaminopropionate) are old . Studies were indeed conducted between 1981 and 1996, i.e. more than 20 years ago. More recent publications (2000-2004) are provided for efficacy of the active substance against mosquitoes. These publications however do not mention when the studies were conducted. This should be specified by the applicant.

Applicant's response:
Section A5 was not "updated" in 2006. By that date, it was submitted for the first time together with the complete dossier to the RMS Belgium for the purpose of the inclusion of IR3535® into Annex I to the BPD. There is only one study in the dossier which was indeed performed in 1981. This shows that IR3535® is in fact an old substance which has been successfully used in repellent products for more than two decades. The applicant is of the opinion that the study performed in 1981 is scientifically valid and that the fact that it is was performed more than 20 years ago cannot lead to the conclusion that it is invalid.

The applicant does not agree to the statement that the majority of the studies was performed more than 20 years ago. In fact, most studies were performed in the 1990s. More than 50% of the efficacy studies in the dossier were performed in 1995 or later, so that at the date of the submission of the dossier more than 50% of the studies were not older than 2 – 11 years.
With this post-submission, three new publications are submitted, for which study summaries are provided in Table 5.3-1 (last three summaries, highlighted in yellow). These studies confirm the results of earlier studies, proving the good repellent efficacy of IR3535® .

As a follow up of the RMS's request for specification of study dates, the applicant has recently tried to contact the authors of the respective studies:
- The author of the publication provided as Doc. No. 336-1913 confirmed by e-mail that the experimental part was performed in 2000. We herewith post-submit the publication to which we have attached the e-mail from the author under the same Document number (Doc. No. 336-1913; Section point A5.3.1/12). Please exchange the old document not containing the e-mail information concerning experimental dates for the new document in the electronic and paper version of the dossier.
- The author of the publication provided as Doc. No. 392-003 confirmed by e-mail that the experimental part was performed from February 26, 2003 through July 17, 2003. We herewith post-submit the publication to which we have attached the e-mail from the author under the same Document number (Doc. No. 392-003; Section point A5.3.1/15). Please exchange the old document not containing the e-mail information concerning experimental dates for the new document in the electronic and paper version of the dossier.
- The dates of the performance of the studies described in the publications provided as Doc. Nos. 392-001 and 392-002 and could not be retrieved. Unfortunately, e-mail sent to authors were not answered. These publications are from 2000 and 2003, respectively. The applicant assumes that the studies were performed not earlier than 1 to 2 years before the publications. The reason for the RMS's focus on the dates of the performance of the studies, seems to be the idea that the older the reports the higher the chance that in the meantime development of resistance may have occurred, so that old studies may not be representative anymore. The applicant is of the opinion that the date of the performance is not relevant to assess the quality of the results of the studies, as the development of resistance against IR3535® is extremely unlikely, if not impossible. Please refer also to comment related to the "resistance" question, which is included in this post-submission (see below).

Advice of RMS concerning applicant's response to general comments

It seems that RMS can accept the arguments of the applicants. More than 50% of the studies were indeed not older than 2 – 11 years at time of the submission of the dossier (2006).
Studies concerning efficacy of IR3535® against mosquitoes (Anopheles, Aedes, Culex and Mansonia spec) were initially conducted between 1981 and 2003. In the answer to comments made by the RMS in April 2008, applicant provides two new studies on efficacy of IR3535® against mosquitoes (Anopheles, Aedes and Culex spec.). These studies (Naucke, 2007 and Kropke, 2007) confirm the repellent efficacy of IR3535® against mosquitoes.
Studies concerning efficacy of IR3535® against flies (Stomoxys, Simuliidae, Tabanidae, Musca spec) were conducted in 1995. In the answer to comments made by the RMS in April 2008, applicant provides one new study on efficacy of IR3535® against flies (Phlebotomus). This study (Naucke, 2006) confirms the repellent efficacy of IR3535® against flies.

It thus seems that development of resistance or loss of efficacy or acquired tolerance to the active compound did not appear in the course of time, at least for mosquitoes and flies.
Studies concerning efficacy of IR3535® against ticks (Ixodes spec) were conducted in 1995-96.
Studies concerning efficacy of IR3535® against lice (Pediculus spec) were conducted in 1993.
Study concerning efficacy of IR3535® against wasps (Pollistes spec) and bees (Apis spec) was conducted in 1995.
These studies, conducted between 1993 and 1996 show repellent effect of IR3535® against ticks, lice, wasps and bees. Since development of resistance or loss of efficacy or acquired tolerance to the active compound did not appear in the course of time, for mosquitoes and flies, it could be assumed that the same was observed for ticks, lice, wasps and bees.
It must also be underlined that the major use of insects repellents is obviously use against mosquitoes. For these insects, repellent efficacy of IR3535® has been recently confirmed.

2) Comments from RMS on section 5.1.
Applicant only mentions "repellent action" however, Doc N°336-1904 mentions a slight intoxication in Pediculus humanus. In the same way, Doc 336-1906 mentions inactivity in Musca domestica and Doc 336-1908 refers to dead ticks . These effects, other than repellent action, suggest that a direct toxic action can occur. This point should be discussed by the applicant.

Applicant's response on potential toxic effects:
The effects which went beyond repellent action as observed in the cited studies are unlikely to occur under real life use conditions under which insects have the chance to avoid areas where IR3535®-vapours are present. Under the conditions of the tests the insects could not avoid the treated areas. Detailed comments are given below.

Applicant's response to RMS comments on Doc. No. 336-1904, Section point A5.3.1/04:
A slight intoxication was observed when the lice (*Pediculus humanus*) were first placed on the fabric which was then treated with the repellent solutions. This test was performed to investigate whether there is a curative effect of the repellents, i.e. whether insects which are already present on a surface, would be expelled when the area where they are present is treated with a repellent. The applicant wants to emphasise that the efficacy claim for IR3535® is only a repellent claim. No other effects are claimed, neither an insecticidal nor a therapeutic pediculicidal effect. The part of the study, dealing with curative effects was only summarised in the dossier for the sake of completeness, but not to add an additional claim. IR3535® based biocidal products should only be used to repel insects, i.e. they should not be used to directly treat insects as done in one part of the study. As already described in the study summary, the insects came in direct contact with the repellent solutions, because the fabric where the insects were present was directly treated with repellent solutions. This leads to much higher exposure of the insects compared to typical use conditions. Furthermore, the insects, since their sensorial organs had been directly treated, are likely to have had difficulties in locating areas with lower repellent concentrations. Consequently, they were disoriented and did not move away from the fabric.

Applicant's response to RMS comments on Doc. No. 336-1906, Section point A5.3.1/06:
This test shows the intrinsic properties of IR3535® to exhibit a repellent action against houseflies. Some flies remained inactive inside a chamber flooded with blended repellent vapours. This effect can be explained by the fact that the flies did not feel a concentration gradient of the repellent, along which

they could escape the unwanted odours. It can be assumed that the repellent was more or less present all over the chamber. Under real life use conditions, when the repellent product is applied on human skin, it evaporates slowly over a time period of a few hours and the flies would easily find the direction to escape the vapours, i.e. away from the treated skin. It should also be pointed out that those flies which appeared to be irritated were exposed to a blend of repellent and melissa oil, i.e. this effect must be attributed to the melissa oil and not to the active substance IR3535® as such behaviour was not observed when melissa oil was absent.

Applicant's response to RMS comments on Doc. No. 336-1908, Section point A5.3.1/08:
In this test, rabbit skin was treated with ethanolic IR3535® at concentrations of 10%, 20% and 30% active substance. The ticks were directly placed on the treated skin. In the highest dose (30% IR3535®), after 4 hours, 2 ticks out of 20 died. The ticks which were directly placed on the skin, were obviously not interested in attacking the skin, i.e. they did not notice that they were sitting on skin. Under real life conditions, the ticks would not try to reach treated skin because they would not notice it due to the effect of the repellent. If the ticks got accidentally in contact with treated skin, they would not penetrate it, as was shown in the test. In the latter case, if a tick remained sitting on the skin for 4 hours there might be a 10% chance that the tick died, if the skin was treated with a 30% IR3535® solution. At 10 and 20%, which are both concentrations which are praxis relevant, no toxic effects were found. In addition, a mortality of 2 in 20 (10%) as observed with 30% solution is too weak an effect as to be able to attribute insecticidal properties to IR3535®.

Advice of RMS concerning applicant's response to comments on section 5.1. (function)

Arguments as provided by the applicant seem to be acceptable. The RMS thus agree with the claim "insect repellent" as function for the active substance IR3535®.

3) Comments from RMS on section 5.2.1 (Organisms to be controlled)
No efficacy studies are provided with the active substance (IR 3535®) against Ctenocephalides spec. These organisms should be deleted. In application form, it is mentioned that IR 3535® can be applied directly on human or animal skin. Since no studies were conducted on animal skin, this field of use should not be claimed.

Applicant's response:
The applicant agrees that no efficacy studies are provided for Ctenocephalides spec. and that this claim should therefore be removed.
With respect to the comment on the application on human or animal skin, the applicant comments as follows:

- The applicant applies for IR3535® to be used in PT19. According to the first review regulation (Commission Regulation 1896/2000), PT19 consists of the sub groups PT19.01 and PT19.02. The official name of PT 19.01 is "Repellents applied directly on human or animal skin". The use of IR3535® described in detail in the dossier, is the application of IR3535® on human skin. This use belongs to PT19 and to the sub group PT19.01 and consequently, in the application form, this PT sub group is cited.
- It is the understanding of the applicant that the purpose of the Annex I dossier and especially of the Document IIIA, is to describe the intrinsic properties of the active substance. The efficacy studies provided in the dossier prove the intrinsic properties of IR3535® to have a repellent action against various types of insects.

- The repellent efficacy was tested in *in vitro* as well as in *in vivo* tests performed with humans, rabbits and mice. As IR3535® acts via the vapour phase (please also refer to the comment on the mode of action question) and consequently the repellent action of IR3535® does not depend on the surface onto which a IR3535® based biocidal product is applied, as long as the active substance can evaporate.
- The fact that for the purpose of the Annex I Dossier, no animal specific studies were provided cannot lead to a restriction that only uses on human skin should be allowed. It is the understanding of the applicant that specific label claims (human, animal or other) should be addressed at the product authorisation stage on the national level. Please note, that the applicant is not a supplier of IR3535®-based biocidal products, but only produces and sells the active substance to formulators. It is the responsibility of the formulators, based on an Annex I listing of IR3535®, to register their own proprietary biocidal products with their specific label claims, if necessary, substantiated with specific efficacy tests and specific risk assessments.

Advice of RMS concerning applicant's response to comments on section 5.2.1. (organisms to be controlled)

As required by the RMS, Ctenocephaloides felis spec. have been deleted from the list of organisms to be controlled.
RMS underlines that, as active substance, IR3535® is actually considered as insect repellent to protect humans from insects. It is applied to human skin in diluted lotions or pump-sprays. New information should be supplied to support specific label claims (on animal or other) at product authorisation stage.

4) Comments from RMS on 5.3.2.(concentrations at which a.s. will be used)
According to the applicant, IR3535® is to be used at concentrations ranging from 10 to < 20% in lotions and pump sprays. Among the efficacy studies provided in the dossier , only those considering such concentrations can be considered. In Doc N°336-1906, the used concentrations are obviously too high. In study N° 336-1902, the used concentrations are obviously too low. The application form also mentions that the products containing the active substance are formulated as alcoholic solution. In doc N°336-1902, doc N°336-1903, doc N°336-1906 and doc 392-001, vehicles are not mentioned or do not seem to be alcohol. This should be discussed by the applicant.

Applicant's response to RMS comments on the concentration range at which the a.s. is to be used:
The applicant agrees that the statement

"*IR3535® is to be used at concentrations ranging from 10 to < 20% in lotions and pump sprays. This concentration range has shown to be efficacious."*

as given in Section A5.3.2 is misleading and herewith wants to withdraw this statement for the following reason: During the preparation of the dossier, the applicant collected typical concentration data from his clients which formulate IR3535® into biocidal products. The above statement is based on only limited information on typical concentrations of IR3535® in biocidal products and has unfortunately not been updated, after the applicant received and evaluated the data the clients had provided. From table B2/1 provided in Document IIIB, Section 2, which summarises the information on the concentrations at which the active substance is used in biocidal products, it becomes obvious that the range 10 – 20% is most typical, but there are also

products on the market which contain less than 10% or more than 20% IR3535®. Based on a statistical evaluation (75th percentile method), the applicant defined a model formulation containing 15% IR3535®, which was used as a representative product in Document IIIB as well as in the risk assessments. The applicant wants to replace the above statement by the following: "*IR3535® is mainly used at concentrations ranging from 10 to 20% in lotions and pump sprays. However, there are also products with higher or lower concentrations on the market. For details, please refer to Table B2/1 in Document IIIB, Section 2.*"

Applicant's response to RMS comments on Doc. No. 336-1906, Section point A5.3.1/06:
The concentration used in this study was 67% IR3535®. This concentration is indeed much higher than typical concentrations of IR3535® in biocidal products. However, the study is an in vitro study in which the active substance is actively thermally evaporated. As already outlined in the comment of the behaviour of the flies (see above), the setup of the study is not representative for a real life situation. Nevertheless, in agreement with the purpose of the Annex I inclusion dossier, this test proves the intrinsic property of IR3535® to have a repellent action against house flies. The efficacy under real life conditions as a housefly repellent to be applied to human skin should be discussed at the biocidal product authorisation stage after Annex I inclusion of the active substance. Consequently, the applicant considers the active substance concentration used in this test to be of minor relevance.

Applicant's response to RMS comments on Doc. No. 336-1902, Section point A5.3.1/02:
The concentrations tested were 0.5% to 5.0% IR3535®. This test was performed under field conditions and even low concentrations of IR3535® have shown to be efficacious against Aedes albopictus. There is no reason to expect that higher concentrations of IR3535® would be less efficacious. The applicant sees no reason, why this study should not be considered. It shows that IR3535® has remarkable efficacy against Aedes albopictus, under field conditions, even at low concentrations. The fact that the products on the market typically contain higher IR3535® concentrations, cannot lead to the conclusion that this study is invalid to prove that IR3535® is efficacious against Aedes albopictus.

Advice of RMS concerning applicant's response to comments on section 5.3.2. (concentrations at which a.s. will be used)
Since most of the efficacy studies were conducted with IR3535® at concentrations between 10 to 20% and since on 30 typical IR3535®-based products on the European market, 29 show IR3535® concentrations between 10 to 20% (see Table B2/1), it seems that the sentence ""IR3535® is mainly used at concentrations ranging from 10 to 20% in lotions and pump sprays" should be sufficient.

5) Comments from RMS on section 5.4.1 (Mode of action)
Mode of action of IR 3535® needs to be fully described (odour, specific action on behaviour? …)

Applicant's response to RMS comments to Section point A5.4.1:
In the dossier the following statement was provided: "*Insects are repelled from skin treated with IR3535®. No details on the modes of action are available.*" In the following the applicant provides additional / more detailed information on the mode of action, as far as it is known. The above statement should be replaced by the following:
"Years of experience and several in vivo and in vitro efficacy tests performed with IR3535®, indicate that IR3535® mainly acts via the vapour phase. The mode of action of IR3535® is not a passive masking of an attracting odour of a victim, but an active repellent effect as insects avoid to enter regions with IR3535® vapours. The exact biochemical mode of action of insect repellents is not yet known (Doc. No. 392-004; Section A5.4.1/01). However, according

to the cited document, it is known that DEET has an olfactory-based repellent effect. Based on the knowledge gained from the efficacy tests with IR3535® and the behaviour of the insects in these tests, it is most self-evident to assume that IR3535® has a similar mode of action as DEET, i.e. an olfactory-based effect. The applicant assumes that no additional information is necessary to cover this data requirement, as any further investigations would only be of interest for basic research and would not contribute to the assessment of the efficacy or of the hazards or of the safe use of IR3535® based biocidal products."

Advice of RMS concerning applicant's response to comments on section 5.4.1 (Mode of action)

Arguments as provided by the applicant, i.e. olfactory-based repellent effect, seem to be acceptable.

6) Comments from RMS on section 5.7. (Resistance)

Since only old efficacy studies are provided, this point would require extensive explanation. Development of resistance or loss of efficacy or acquired tolerance to the active compound should be extensively discussed by the applicant. Old efficacy studies could be considered only if resistance or loss of efficacy or acquired tolerance to the active compound did not develop among time. Other toxic actions than repellent are obviously observed with the active compound (see comments on point 5.1). Relevancy and impact of these effects on selection pressure need to be discussed by the applicant.

Applicant's response to RMS comments to Section point A5.7:

The applicant is of the opinion that the development of resistance against IR3535® is extremely unlikely, if not even impossible, as explained in the following.

IR3535® is an insect repellent and not an insecticide. Resistance is typically developed if there is a selection pressure on a population of species, in such a way that individuals that are more tolerant against the substance in question do not die and can therefore reproduce. Unlike insecticides, IR3535® is not used to kill insects, but only to hinder them from entering areas where IR3535® has been applied. Generally, a repellent applied on human or animal skin hinders, e.g. blood sucking insects, from biting. One could argue that this effect constitutes a positive selection pressure, in such a way that the repelled insects may die of starvation and would therefore be removed from the population, so that insects, which are more tolerant, i.e. which are less repelled, would have a feeding advantage and would therefore be in favour for reproduction. Such a scenario would only be of relevance if the majority of potential hosts in an habitat of a population of insects was treated with an insect repellent, so that the insects would have severe problems to find hosts which are not treated with the repellent. Such a scenario is extremely unlikely, as the occurrence of insect repellent treated hosts in a habitat of a population of insects is only sporadic. In other words, the amount of blood not available for the insects, due to the protection by a repellent, is negligible compared to the overall amount of blood available from other sources.

Advice of RMS concerning applicant's response to comments on section 5.7. (Resistance)

Arguments as provided by the applicant seem to be acceptable. See also "Advice of RMS concerning applicant's response to general comments"

7) Comments from RMS on section 5.8. (Likely tonnage to be placed on the market per year)
Confidential information was not provided.

Applicant's response to RMS comments to Section point A5.8:
The application agrees that the information on the tonnage was not provided in Section A5.
A confidential version of the amended Doc IIIA Section A5 is provided with this post-submission.
Please note that the information on the annual tonnage was already provided in Document IIB, Chapter 8.3.3 in the 2006 dossier in the context of the environmental risk assessments. The applicant herewith claims confidentiality on any tonnage information.

Advice of RMS concerning applicant's response to comments on section 5.8. (Likely tonnage to be placed on the market per year)

RMS has no remarks.

8) Comments from RMS on summary tables (see below)
Specific studies will be considered after answer of the applicant to the comments concerning the different sections.

Applicant's comment:
No comment.

Advice of RMS concerning summary tables

All studies have been considered. See "Advice of RMS concerning applicant's response to general comments". RMS agrees with the list of organisms to be controlled by IR3535® claimed by the applicant.

Mosquitoes
Anopheles spec
Aedes spec
Culex spec
Mansonia spec

Ticks
Ixodes spec

Lice
Pediculus spec

Flies
Stomoxys spec
Simuliidae
Tabanidae
Musca spec
Phlebotomus spec

Wasps Pollistes spec

Bees Apis spec

RMS underlines that the major use of insects repellents is obviously use against mosquitoes. For these insects, repellent efficacy of IR3535 has been recently confirmed.