Section A6.1.1/01 Acute Toxicity
Annex Point IIA, VI.6.1.1 Oral, Rat, LD_{50}

[redacted]

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1997): Insect Repellent 3535 (Article Number 111887) – Acute Toxicity Study in Rats after oral Administration; [redacted]; Doc. No. 521-003 (unpublished)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Merck KGaA	
1.2.2	Companies with letter of access	No companies with Letter of Access	
1.2.3	Criteria for data protection	Data on existing a.s submitted for the first time for entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, EU Guideline 87/176/EEC (EEC, 1987) which is in compliance with OECD Guideline 401 (adopted in 1987, deleted in 2002).	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted]	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	[redacted]	
3.1.4	Description	[redacted]	
3.1.5	Stability	The dosing solution was prepared freshly prior to application.	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	[redacted]U	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	6 to 9 weeks 177 (164 – 189) g	
3.2.6	Number of animals per group	10/group (5 males and 5 females)	
3.2.7	Control animals	No, not necessary for this kind of study.	

Section A6.1.1/01 Acute Toxicity
Annex Point IIA, VI.6.1.1 Oral, Rat, LD_{50}

3.3	**Administration/ Exposure**	
3.3.1	Post-exposure period	15 days
3.3.2	Type	Stomach tube
3.3.3	Concentration	5000 mg/kg bw
3.3.4	Vehicle	Aqua demineralisata
3.3.5	Concentration in vehicle	250 g/L
3.3.6	Total volume applied	20 mL/kg bw
3.3.7	Controls	None, not necessary for this kind of study.
3.4	**Examinations**	[redacted]
3.5	**Method of determination of LD_{50}**	Limit test
3.6	**Further remarks**	None
		4 RESULTS AND DISCUSSION
4.1	**Clinical signs**	[redacted]
4.2	**Pathology**	[redacted]
4.3	**Other**	[redacted]
4.4	**LD_{50}**	> 5000 mg/kg bw
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The acute oral toxicity of IR3535® was investigated in one dose group of 10 rats (5/sex) in a limit test following guideline 87/176/EEC which is in compliance with OECD 401.
5.2	**Results and discussion**	All animals survived and had gained weight at study termination. Signs of toxicity as incomplete eyelid closure, salivation, and locomotor disturbance were seen 1 – 15 minutes after treatment and lasted up to day 2. There were no organ alterations detected at gross necropsy.
5.3	**Conclusion**	LD_{50} > 5000 mg/kg bw [redacted]
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	No

Section A6.1.1/01 Acute Toxicity
Annex Point IIA, VI.6.1.1 **Oral, Rat, LD_{50}**

	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]
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
	[redacted]
	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*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*
Results and discussion	*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*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.1.1/01 Acute Toxicity

Annex Point IIA, VI.6.1.1 **Oral, Rat, LD_{50}**

Table A6.1.1/01-1: Summary of Acute Oral Toxicity

Dose [mg/kg bw]	**Sex**	**Number of dead / number of investigated**	**Time of death**	**Observations** (number of animals affected)
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
LD_{50} value	>5000 mg/kg bw			

Section A6.1.2/01 Acute Toxicity
Annex Point IIA, VI.6.1.2 Dermal, Rat, Limit test

[redacted]

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		1 REFERENCE
1.1	**Reference**	[redacted] (1973): Acute Toxicity of BE 3535 after Local Application to 1/10 of the Body Surface of Rats; [redacted] Report No. not indicated, Doc. No. 522-003 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Merck KGaA
1.2.2	Companies with letter of access	No companies with Letters of Access
1.2.3	Criteria for data protection	Data on existing a.s submitted for the first time for entry into Annex I.
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	No, however, materials and methods used are comparable to current OECD 402 guideline (1987)
2.2	**GLP**	No, study was conducted prior to implementation of GLP
2.3	**Deviations**	Application duration was only 6 hours. It is not stated whether the body weight was determined. Initial body weight of the rats was lower than the recommended range of 200 – 300 g.
		3 MATERIALS AND METHODS
3.1	**Test material**	[redacted]
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2
3.1.3	Purity	[redacted]
3.1.4	Description	[redacted]
3.1.5	Stability	[redacted]
3.2	**Test Animals**	
3.2.1	Species	rat
3.2.2	Strain	Sprague-Dawley
3.2.3	Source	[redacted]
3.2.4	Sex	males and females
3.2.5	Age/weight at study initiation	The initial weight was between 100 and 106 g. The initial age of the males was 38 days, of the females 42 days.
3.2.6	Number of animals per group	5/sex/group
3.2.7	Control animals	no, not necessary for this kind of study

Section A6.1.2/01 Acute Toxicity
Annex Point IIA, VI.6.1.2 Dermal, Rat, Limit test

3.3	**Administration/ Exposure**	
3.3.1	Type	Dermal
3.3.2	Doses	6.35, 7.9, and 10.0 mL/kg bw (approximately equivalent to 6.35, 7.9, and 10.0 g/kg bw)
3.3.3	Post-exposure period	14 days
3.3.4	Area covered	4.5 x 5 cm^2 (approximately 1/10 of the body surface)
3.3.5	Occlusion	not indicated
3.3.6	Vehicle	no, not necessary because the test substance is a liquid and was applied undiluted
3.3.7	Concentration in vehicle	not applicable
3.3.8	Total volume applied	6.35, 7.9, and 10.0 mL/kg bw
3.3.9	Duration of exposure	6 hours, [redacted]
3.3.10	Removal of test substance	yes, with lukewarm (30°C) water
3.4	**Examinations**	[redacted]
3.5	**Method of determination of LD_{50}**	[redacted]
3.6	**Further remarks**	[redacted]
		4 RESULTS AND DISCUSSION
4.1	**Clinical signs**	[redacted]
4.2	**Pathology**	[redacted]
4.3	**Other**	[redacted]
4.4	**LD_{50}**	>10.0 mL/kg bw (equivalent to 10.0 g/kg bw)

Section A6.1.2/01 Acute Toxicity
Annex Point IIA, VI.6.1.2 Dermal, Rat, Limit test

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	IR3535® was tested for its acute dermal toxicity in the rat. Materials and methods used were comparable to those specified in OECD 402. Deviations are not considered to have negatively influenced the study. Five animals/sex/group were treated for 6 hours with 6.35, 7.9, and 10.0 mL/kg bw (ca. 6.35, 7.9, and 10.0 g/kg bw). After 6 hours, the test substance was removed with lukewarm water. Animals were observed for clinical sings, skin reactions were recorded. All animals were necropsied.
5.2	**Results and discussion**	None of the animals died or showed signs of systemic intolerance. There were no pathological findings. Draize grade 2 skin reactions (pronounced erythema) were noted in all dose groups. Erythemas recovered within 24 – 30 hours.
5.3	**Conclusion**	LD_{50} >10.0 mL/kg bw (equivalent to 10.0 g/kg bw) [redacted]
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	No

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]
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Section A6.1.2/01 Acute Toxicity

Annex Point IIA, VI.6.1.2 **Dermal, Rat, Limit test**

Table A6.1.2/01-1. Table for Acute Dermal Toxicity

Dose [mL/kg bw]	Number of dead / number of investigated	Time of death (range)	Observations
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
LD_{50} value	> 10.0 mL/kg bw		

Section A6.1.3/01 Acute Toxicity

Annex Point IIA, VI.6.1.3 Inhalation, Rat, LC_{50}

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1 REFERENCE

1.1	**Reference**	[redacted] (1995): Study on the Acute Inhalation Toxicity LC_{50} of Art. No. 111887 (Insekt-Repellent 3535) as a Liquid Aerosol in Rats (4-hour Exposure); [redacted]; Doc. No. 523-001 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Merck KGaA
1.2.2	Companies with letter of access	No companies with Letters of Access
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes, OECD guideline 403 US EPA/FIFRA § 81-3 UA EPA/TSCA 40CFR § 798.1150 EU Guidelines 92/69/EEC
2.2	**GLP**	Yes
2.3	**Deviations**	Yes, oxygen content and humidity not mentioned air changes 27 instead of 15

3 MATERIALS AND METHODS

3.1	**Test material**	[redacted]
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2
3.1.3	Description	[redacted]
3.1.4	Purity	[redacted]
3.1.5	Stability	[redacted]
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Wistar [redacted]
3.2.3	Source	[redacted]
3.2.4	Sex	male and female

Section A6.1.3/01 Acute Toxicity

Annex Point IIA, VI.6.1.3 Inhalation, Rat, LC_{50}

3.2.5	Age/weight at study initiation	Animals were about 8 to 9 weeks of age at study beginning. Mean male body weight was 267 g on the day of exposure, females weighed 197 g. The body weights did not vary more than 20% of the mean on the day of exposure.
3.2.6	Number of animals per group	5/sex/group
3.2.7	Control animals	no, not necessary for this kind of study
3.3	**Administration/ Exposure**	Inhalation
3.3.1	Post-exposure period	14 days
3.3.2	Concentrations	nominal: 16.3 mg/L (calculated from the amount of substance consumed and the air flow) target: 5 mg/L analytical (via GC): 5.07 +/- 0.93 mg/L (mean of 4 samples)
3.3.3	Particle size	MMAD: 1.3 µm GSD: 2.98 µm Respirable aerosol fraction: 98%
3.3.4	Type or preparation of particles	liquid aerosol
3.3.5	Type of exposure	nose-only
3.3.6	Vehicle	no, liquid test substance
3.3.7	Concentration in vehicle	not applicable
3.3.8	Duration of exposure	4 hours
3.3.9	Controls	not applicable
3.4	**Examinations**	[redacted]
3.5	**Method of determination of LC_{50}**	Limit test
3.6	**Further remarks**	[redacted]

Section A6.1.3/01 Acute Toxicity

Annex Point IIA, VI.6.1.3 Inhalation, Rat, LC_{50}

	4 RESULTS AND DISCUSSION	
4.1 Clinical signs	[redacted]	
4.2 Pathology	[redacted]	
4.3 Other	[redacted]	
4.4 LC_{50}	> 5.1 mg/L	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	The acute inhalation toxicity of IR3535® was investigated in Wistar rats (5/sex/group) in a limit test following OECD 403 guideline (4-hour exposure period). The MMAD was 1.3 µm, the GSD 2.98 µm.	
5.2 Results and discussion	All animals survived and had gained weight at study termination. Clinical signs observed mainly consisted of respiration changes (irregular, intermittent, accelerated), blood discharge of the nose, and piloerection. All clinical signs had cleared by day 7 of post-exposure. There were no findings at necropsy.	X
5.3 Conclusion	LC_{50} > 5.1 mg/L [redacted]	
5.3.1 Reliability	[redacted]	
5.3.2 Deficiencies	No	

Section A6.1.3/01 Acute Toxicity

Annex Point IIA, VI.6.1.3 **Inhalation, Rat, LC_{50}**

	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]
Materials and Methods	[redacted]
Results and discussion	[redacted]
	[redacted]
Conclusion	[redacted]
	[redacted]
	[redacted]
	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	Give date of comments submitted
Materials and Methods	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
Results and discussion	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
Acceptability	Discuss if deviating from view of rapporteur member state
Remarks	

Section A6.1.3/01 Acute Toxicity

Annex Point IIA, VI.6.1.3 **Inhalation, Rat, LC_{50}**

Table A6.1.3/01-1: Particle Size Distribution

Group number	Dose [mg/L]	Type of exposure	MMAD	GSD	Mass < 3 µm [%]
1	5.1	nose-only	1.3 µm	2.98 µm	77%

Table A6.1.3/01-2: Summary Acute Inhalation Toxicity

Group number	Dose [mg/L]	Type of exposure	Sex	Number of dead / number of investigated	Time of death [day]	Clinical signs (Number of animals affected, and time interval of duration of symptoms)	BW (gram) (mean±SD)
[redacted]							
LC_{50} value	>5.1 mg/L						

Section 6.1.4/01 Acute Toxicity
Annex Point IIA, VI.6.1.4 Eye Irritation, Rabbit

[redacted]

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		1 REFERENCE
1.1	**Reference**	[redacted] (1996): Insect Repellent 3535 (Article Number 111887) – Primary Eye Irritation Test in Rabbits; [redacted] Report No.: 40/12/96; Doc. No. 566-004 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Merck KGaA
1.2.2	Companies with letter of access	No Companies with Letters of Access
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I.
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, US EPA, Subdivision F: 81-4 (1989) which is comparable to OECD guideline 405
2.2	**GLP**	Yes
2.3	**Deviations**	None
		3 MATERIALS AND METHODS
3.1	**Test material**	[redacted]
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2
3.1.3	Purity	[redacted]
3.1.4	Description	[redacted]
3.1.5	Stability	[redacted]
3.2	**Test Animals**	
3.2.1	Species	Rabbit
3.2.2	Strain	Iva: NZW
3.2.3	Source	[redacted]
3.2.4	Sex	male and female
3.2.5	Age/weight at study initiation	Animals were about 37 to 38 weeks of age. Males weighed between 3.73 and 4.33 kg, females between 4.37 and 4.47 kg.
3.2.6	Number of animals per group	3/sex
3.2.7	Control animals	no, not necessary for this kind of study

Section 6.1.4/01 Acute Toxicity
Annex Point IIA, VI.6.1.4 Eye Irritation, Rabbit

3.3	**Administration/ Exposure**	
3.3.1	Preparation of test substance	undiluted, test substance is liquid
3.3.2	Amount of active substance instilled	0.1 mL
3.3.3	Exposure period	24 hours
3.3.4	Post-exposure period	15 days
3.4	**Examinations**	
3.4.1	Ophthalmoscopic examination	yes
3.4.2	Scoring system	Draize system (identical to scoring system given in OECD 405 (2002))
3.4.3	Examination time points	60 min, 24, 48, 72 hours; thereafter daily up to day 15.
3.4.4	Other investigations	determination of body weights prior to dosing, on days 5, 8, 11,and 15
3.5	**Further remarks**	None

4 RESULTS AND DISCUSSION

4.1	**Clinical signs**	[redacted]
4.2	**Average score**	
4.2.1	Cornea	[redacted]
4.2.2	Iris	[redacted]
4.2.3	Conjunctiva	
4.2.4	Redness	[redacted]
4.2.5	Chemosis	[redacted]
4.3	**Reversibility**	[redacted]
4.4	**Other**	[redacted]
4.5	**Overall result**	[redacted]

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	IR3535® was tested for eye irritation in rabbits according to OECD 405. IR3535® was instilled into the lower conjunctival sac of the left eye in 3 animals/sex. The right eye remained untreated and served as control. Twenty-four hours after instillation, the eye was washed with physiological saline. Eyes were examined by ophthalmoscope 60 minutes, 24, 48, and 72 hours after treatment and daily thereafter up to 15 days. At the end of the study period, eye irritation was additionally examined by instillation of fluorescein solution. Eyes were scored according to the system of Draize.

Section 6.1.4/01 Acute Toxicity
Annex Point IIA, VI.6.1.4 Eye Irritation, Rabbit

5.2	Results and discussion	The following observations were made: grade 1 opacity of the cornea from day 1 to day 7 in all animals, and the concerned areas of the cornea were grades 2 and 4. Irritation of the iris was not noted. The conjunctivae showed redness (grade 1 and 2), chemosis (grades 1 to 3), and discharge (grade 1 to 3). These irritations lasted from day 1 to day 7. Later on, no signs of irritation were noted. After instillation of a fluorescein solution on day 15, no abnormal findings were noted. [redacted]
5.3	Conclusion	According to Commission Directive 2001/59/EC the test material IR3535® is not irritant to the eye [redacted].
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	No

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]
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*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*
Results and discussion	*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*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.1.4/01-1: Results eye irritation and trigger value for R36 (Directive 67/548/EEC)

	Cornea						Iris						Conjunctiva-redness						Conjunctiva-chemosis					
Time / Rabbit	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]						[redacted]						[redacted]						[redacted]					
[redacted]	[redacted]						[redacted]						[redacted]						[redacted]					
[redacted]																								

Section A6.1.4/02 Acute Toxicity
Annex Point IIA, VI.6.1.4 Skin Irritation, Rabbit

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1 REFERENCE

Reference: [redacted] (1973): Local Tolerance Test of Different Preparations of BE 3767 and BE 3535 in Rabbits (Patch Test); [redacted] Study No.: not indicated; Doc. No. 565-002 (unpublished)

Data protection: Yes

1.1.1	Data owner	Merck KGaA
1.1.2	Companies with letter of access	No companies with Letters of Access
1.1.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

Guideline study: Yes,
Hazardous Substances, Part 191, Section 11, FDA, Washington 1965. Methods used are comparable to OECD 404.

GLP: No

Deviations: Yes (OECD 404),

- duration of treatment 24 hours instead of 4 hours
- test material was not used undiluted
- amount of test material administered is not indicated
- oedema formation was determined by other methods (thickness measurements) than recommended

3 MATERIALS AND METHODS

Test material: [redacted]

3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2
3.1.3	Purity	[redacted]
3.1.4	Description	[redacted]
3.1.5	Stability	[redacted]

Test Animals

3.1.6	Species	rabbits
3.1.7	Strain	New Zealand White
3.1.8	Source	[redacted]
3.1.9	Sex	both sexes
3.1.10	Age/weight at study initiation	2.3 – 2.8 kg age: not indicated
3.1.11	Number of animals	intact skin: 3/sex

Section A6.1.4/02 Acute Toxicity
Annex Point IIA, VI.6.1.4 Skin Irritation, Rabbit

	per group	scarified skin: 3/sex
3.1.12	Control animals	yes, semi-occlusive
Administration/ Exposure		Dermal
3.1.13	Application	
3.1.14	Preparation of test substance	10% test substance in 50% aqueous ethanol
3.1.15	Test site and Preparation of Test Site	3 animals per sex: shorn intact skin 3 animals per sex: shorn scarified skin application site (2.5 cm x 2.5 cm): between the fore and hind legs at the back of the animals.
3.1.16	Occlusion	yes
3.1.17	Vehicle	50% aqueous ethanol
3.1.18	Concentration in vehicle	10%
3.1.19	Total volume applied	not indicated
3.1.20	Removal of test substance	not indicated
3.1.21	Duration of exposure	24 hours
3.1.22	Post-exposure period	14 days
3.1.23	Controls	treatment of controls not indicated
Examinations		
3.1.24	Clinical signs	[redacted]
3.1.25	Dermal examination	[redacted]
3.1.26	scoring system	[redacted]
3.1.27	Examination time points	[redacted]
Further remarks		[redacted]

Section A6.1.4/02 Acute Toxicity
Annex Point IIA, VI.6.1.4 Skin Irritation, Rabbit

4 RESULTS AND DISCUSSION

Average score

4.1.1 Erythema [redacted]

4.1.2 Oedema [redacted]

Reversibility [redacted]

Other examinations [redacted]

Overall result [redacted]

5 APPLICANT'S SUMMARY AND CONCLUSION

Materials and methods

IR3535® was applied to the shaved intact or scarified back of New Zealand White rabbits. Three rabbits/sex were used each for the intact skin application and the scarified application. Concurrent control groups with intact or scarified skin were utilised (3/sex/group). IR3535® treatment solution consisted of a 10% dilution of IR3535® in 50% aqueous ethanol. Animals were treated for 24 hours under semi-occlusive conditions. Skin was examined for erythema according the Draize scoring system immediately after the 24 hour dosing period and daily thereafter. Oedema formation was monitored weekly by means of skin thickness determinations. Animals were observed for clinical signs over a post-exposure period of 14 days. Body weights and food consumption was monitored.

Results and discussion

The test material did not cause skin reactions. Oedema were not formed as indicated by the measured skin thickness which was comparable between the treatment and the control group. IR3535® is not irritant to skin.

[redacted]

Conclusion

IR3535® is not a skin irritant [redacted]

5.1.1 Reliability [redacted]

5.1.2 Deficiencies

Yes,

no data in tabulated form of body weights, and food consumption investigations for oedema formation were not conducted according to recommendations of the OECD 404 guideline

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]
Materials and Methods [redacted]

Section A6.1.4/02 Acute Toxicity

Annex Point IIA, VI.6.1.4 **Skin Irritation, Rabbit**

Results and discussion	[redacted]
Conclusion	[redacted]
	[redacted]
	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*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*
Results and discussion	*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*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.1.4/02-1: Results skin irritation (intact skin) after application of IR3535®

[redacted]			
	[redacted]		
	[redacted]		
[redacted]	[redacted]		
[redacted]	[redacted]		
	[redacted]		
[redacted]	[redacted]		
[redacted]			
[redacted]			

Table A6.1.4/02-2: Results skin irritation (scarified skin) after application of IR3535®

[redacted]			
	[redacted]		
	[redacted]		
[redacted]	[redacted]		
[redacted]	[redacted]		
	[redacted]		
[redacted]	[redacted]		
[redacted]			
[redacted]			

Table A6.1.4/02-3: Results skin irritation (intact skin) control

[redacted]			
	[redacted]		
	[redacted]		
[redacted]	[redacted]		
[redacted]	[redacted]		
	[redacted]		
[redacted]	[redacted]		
[redacted]			
[redacted]			

Table A6.1.4/02-4: Results skin irritation (scarified skin) control

[redacted]			
	[redacted]		
	[redacted]		
[redacted]	[redacted]		
[redacted]	[redacted]		
	[redacted]		
[redacted]	[redacted]		
[redacted]			
[redacted]			
[redacted]			

Section A6.1.4/03 Acute Toxicity
Annex Point IIA, VI.6.1.4 Skin Irritation, Rabbit

[redacted]

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] (1977): Topical Hazard Evaluation Program of Candidate Insect Repellent AI3-70763 3[N-n-Butyl-N-acetyl]aminopropionic acid-ethyl ester; [redacted] Doc. No. 581-002 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Merck KGaA
1.2.2	Companies with letter of access	No companies with Letters of Access
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes, Toxicology Division Procedural Guide, USAEHA, 1972. Methods used are comparable to OECD 404
2.2	**GLP**	No
2.3	**Deviations**	Yes (to OECD 404), • duration of treatment 24 hours instead of 4 hours • no individual or group mean scores for erythema and oedema given • scoring system not indicated • no information about clinical signs, body weight development, post-application period

3 MATERIALS AND METHODS

3.1	**Test material**	[redacted]
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2
3.1.3	Purity	[redacted]
3.1.4	Description	[redacted]
3.1.5	Stability	[redacted]
3.2	**Test Animals**	
3.2.1	Species	rabbits
3.2.2	Strain	New Zealand White

Section A6.1.4/03 Acute Toxicity
Annex Point IIA, VI.6.1.4 Skin Irritation, Rabbit

3.2.3	Source	not indicated
3.2.4	Sex	not indicated
3.2.5	Age/weight at study initiation	not indicated
3.2.6	Number of animals per group	intact skin: 6 rabbits scarified skin: 6 rabbits
3.2.7	Control animals	not necessary for this kind of study
3.3	**Administration/ Exposure**	Dermal
3.3.1	Application	
3.3.2	Preparation of test substance	undiluted
3.3.3	Test site and Preparation of Test Site	6 animals: shorn intact skin 6 animals: shorn abraded skin
3.3.4	Occlusion	not indicated in the report
3.3.5	Vehicle	none
3.3.6	Concentration in vehicle	not applicable
3.3.7	Total volume applied	0.5 mL
3.3.8	Removal of test substance	not indicated
3.3.9	Duration of exposure	24 hours
3.3.10	Post-exposure period	not indicated
3.3.11	Controls	controls were not used
3.4	**Examinations**	
3.4.1	Clinical signs	not indicated
3.4.2	Dermal examination	yes
3.4.3	scoring system	not indicated
3.4.4	Examination time points	not indicated
3.5	**Further remarks**	None

Section A6.1.4/03 Acute Toxicity
Annex Point IIA, VI.6.1.4 Skin Irritation, Rabbit

4 RESULTS AND DISCUSSION

4.1	**Average score**	
4.1.1	Erythema	[redacted]n
4.1.2	Oedema	[redacted]sion
4.2	**Reversibility**	[redacted]
4.3	**Other examinations**	[redacted]
4.4	**Overall result**	[redacted]

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	IR3535® was applied to the shaved intact or abraded of New Zealand White rabbits. Six rabbits were used each for the intact skin application and the scarified application. Animals were treated for 24 hours.
5.2	**Results and discussion**	The test material caused no primary irritation of the intact skin or the skin surrounding an abrasion.
5.3	**Conclusion**	IR3535® is not a skin irritant [redacted]
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	Yes, - no individual scores given - no information about clinical signs, body weight development, post-application period

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]
Materials and Methods	[redacted]
Results and discussion	[redacted]

Section A6.1.4/03 Acute Toxicity

Annex Point IIA, VI.6.1.4 **Skin Irritation, Rabbit**

Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*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*
Results and discussion	*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*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.1.4/04 Acute Toxicity
Annex Point IIA, VI.6.1.4 Skin Irritation, Human

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted] (1996): Test for skin irritation in humans, modified Duhring chamber test; [redacted] Doc. No. 565-004 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Merck KGaA
1.2.2	Companies with letter of access	No companies with Letters of Access
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I.
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, Duhring chamber test method according to Frosch & Kligman, 1979
2.2	**GLP**	No
2.3	**Deviations**	Not applicable
		3 MATERIALS AND METHODS
3.1	**Test material**	[redacted]
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2
3.1.3	Purity	[redacted]
3.1.4	Description	[redacted]
3.1.5	Stability	[redacted]
3.2	**Test Animals**	
3.2.1	Species	human
3.2.2	Strain	not applicable
3.2.3	Source	not indicated
3.2.4	Sex	both sexes
3.2.5	Age/weight at study initiation	Age: 28 – 52 years
3.2.6	Number of animals per group	10 volunteers/group
3.2.7	Control animals	yes, treated with water (negative control) or with 0.2% sodium dodecylsulfate (positive control)
3.3	**Administration/ Exposure**	Dermal
3.3.1	Application	

Section A6.1.4/04 Acute Toxicity
Annex Point IIA, VI.6.1.4 Skin Irritation, Human

3.3.1.1	Preparation of test substance	IR3535® was formulated as follows (solution B): 10% IR3535® 40% ethanol 50% demineralized water
3.3.1.2	Test site and Preparation of Test Site	volar side of the lower arm in the aluminium chambers
3.3.2	Occlusion	aluminium chamber (12 mm in diameter) containing appropriate filter papers was used
3.3.3	Vehicle	ethanol and water
3.3.4	Concentration in vehicle	10%
3.3.5	Total volume applied	0.05 mL
3.3.6	Removal of test substance	not indicated
3.3.7	Duration of exposure	day 1: 18 hours day 2 – 5: 6 hours (18 hours apart)
3.3.8	Post-exposure period	day 6 and 7: none day 8: scoring
3.3.9	Controls	yes, negative controls (receiving water) and positive controls (receiving 0.2% sodium dodecylsulfate)
3.4	**Examinations**	
3.4.1	Clinical signs	not indicated
3.4.2	Dermal examination	yes

Section A6.1.4/04 Acute Toxicity
Annex Point IIA, VI.6.1.4 Skin Irritation, Human

3.4.2.1	scoring system	[redacted]
3.4.2.2	Examination time points	[redacted]
3.5	**Further remarks**	[redacted]

Section A6.1.4/04 Acute Toxicity
Annex Point IIA, VI.6.1.4 Skin Irritation, Human

		4 RESULTS AND DISCUSSION
4.1	**Average score**	[redacted]
4.1.1	Erythema	[redacted]
4.1.2	Oedema	[redacted]
4.1.3	Desquamation	[redacted]
4.1.4	Fissures	[redacted]
4.2	**Reversibility**	[redacted]
4.3	**Other examinations**	[redacted]
4.4	**Overall result**	[redacted]
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	IR3535® (0.05 mL) formulated as 10% solution in water/ethanol was applied to the volar side of the lower arm to an area of 12 mm in diameter. On day 1, the test substance was applied for 18 hours, from days 2 to 5 for 6 hours (18 hours apart). On day 8, skin was scored by visual examination as well as by means of chromametry and measurement of the transepidermal water loss (TWL).
5.2	**Results and discussion**	The test material caused no erythema. Chromametry values and TWL values were comparable to the negative control and to the pre-dose values, thus, the test material was not irritating to the skin. The positive control showed the sensivity of the test system.
5.3	**Conclusion**	IR3535® is not considered to be a skin irritant [redacted]
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	No

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]
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]

Section A6.1.4/04 Acute Toxicity
Annex Point IIA, VI.6.1.4 Skin Irritation, Human

Remarks	-
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*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*
Results and discussion	*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*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.1.4/04-1: Results human skin irritation

[redacted]			
[redacted]			
[redacted]			
[redacted]			
[redacted]			
[redacted]			

5®

Section A6.1.4/05 Acute Toxicity
Annex Point IIA, VI.6.1.4 Phototoxicity

[redacted]

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted] (1986): Investigation for Phototoxic Potential with Insekt-Repellent 3535, Art.-Nr. 11887 in Albino Guinea Pigs; [redacted]; Doc. No. 565-003 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Merck KGaA
1.2.2	Companies with letter of access	No companies with Letters of Access
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I.
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Not applicable, a respective guideline is not available
2.2	**GLP**	yes
2.3	**Deviations**	Not applicable
		3 MATERIALS AND METHODS
3.1	**Test material**	[redacted]
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2
3.1.3	Purity	[redacted]
3.1.4	Description	[redacted]
3.1.5	Stability	[redacted]
3.2	**Test Animals**	
3.2.1	Species	guinea pig
3.2.2	Strain	Himalayan white spotted
3.2.3	Source	[redacted]
3.2.4	Sex	not indicated
3.2.5	Age/weight at study initiation	weight: 300-450 g age: approximately 8 weeks
3.2.6	Number of animals per group	A total of 10 animals were used in the main study. Four animals were used for determination of the highest non-irritating concentration.
3.2.7	Control animals	no
3.3	**Administration/ Exposure**	Dermal
3.3.1	Application	
3.3.1.1	Preparation of test substance	10% test substance in ethanol. To enhance skin penetration 2% DMSO were added to the test substance preparation.

Section A6.1.4/05 Acute Toxicity
Annex Point IIA, VI.6.1.4 Phototoxicity

3.3.1.2	Test site and Preparation of Test Site	Both flanks were shaved 2 hours prior to test article application. On each flank 5 test sites of 2 cm^2 size were marked on each flank.
3.3.2	Occlusion	no
3.3.3	Vehicle	ethanol
3.3.4	Concentration in vehicle	10% (the highest non-irritating concentration as determined in a pre-test using 4 naive animals) Four animals were treated with the undiluted test substance and 30, 10, 3, and 1% concentration in a suitable vehicle (0.025 mL/2 cm^2).
3.3.5	Total volume applied	0.025 mL of test substance dilution (10% in ethanol)
3.3.6	Removal of test substance	not indicated
3.3.7	Duration of exposure	not indicated
3.3.8	Post-exposure period	not indicated
3.3.9	Controls	Separate control animals were not used. Both flanks of the animals were shaved. Each flank consisted of 5 test sites. To four test sites IR3535® test dilution was applied, the fifth test site was treated with the known phototoxic substance 8-methoxypsoralen (0.1% in alcohol (not further specified)) i.e. the positive control.
3.4	**Examinations**	
3.4.1	Clinical signs	[redacted]
3.4.2	Dermal examination	[redacted]
3.4.2.1	scoring system	[redacted]
3.4.2.2	Examination time points	[redacted]
3.5	**Further remarks**	[redacted]

4 RESULTS AND DISCUSSION

Section A6.1.4/05 Acute Toxicity
Annex Point IIA, VI.6.1.4 Phototoxicity

4.1	**Average score**	[redacted]
4.1.1	Erythema	[redacted]
4.1.2	Oedema	[redacted]
4.2	**Reversibility**	[redacted]s
4.3	**Other examinations**	[redacted]
4.4	**Overall result**	[redacted]

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The phototoxic potential of IR3535® was investigated in 10 guinea pigs. A volume of 0.025 mL/2 cm^2 IR3535® (10% in ethanol) and the positive control 8-methoxypsoralen (0.1% in alcohol) were applied to both shaved flanks of the test animal to separate sites. Thirty minutes after application, the left flank was irradiated with UV-A (20 J/cm^2, known not to produce erythematogenic reactions). The right flank was not irradiated and served as irritation control. Four, 24 and 48 hours after application, skin reactions (erythema and oedema) were recorded.
5.2	**Results and discussion**	IR3535® caused grade 1 skin reactions in 1/10 animal at the 4 hour reading point. Twenty-four and 48 hours after application, no skin reactions induced by IR3535® were observed. At the positive control site, all animals showed grade 1 reactions after 4 hours. Skin reactions induced by the positive control became more severe (grade 2 to 3) after 24 and 48 hours. The positive control showed the sensivity of the test system.
5.3	**Conclusion**	IR3535® is not considered to be phototoxic.
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	No

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]
Materials and Methods	[redacted]
Results and discussion	[redacted]

Section A6.1.4/05 Acute Toxicity

Annex Point IIA, VI.6.1.4 **Phototoxicity**

Conclusion	[redacted]
	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*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*
Results and discussion	*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*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.1.4/05 Acute Toxicity

Annex Point IIA, VI.6.1.4 **Phototoxicity**

Table A6.1.4/05-1: Phototoxicity Testing - Results

	Left Flank irradiated						Right Flank non-irradiated					
	treated with IR3535®			treated with positive control[1)]			treated with IR3535®			treated with positive control[1)]		
Animal no.	4 hours	24 hours	48 hours	4 hours	24 hours	48 hours	4 hours	24 hours	48 hours	4 hours	24 hours	48 hours
[redacted]												
[redacted]												
[redacted]												
[redacted]												
[redacted]												
[redacted]												
[redacted]												
[redacted]												
[redacted]												
[redacted]												

1) 8-methoxypsoralen

Section A6.1.5/01 Skin sensitisation

Annex Point IIA, VI.6.1.5 Buehler method

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] (1997): Delayed Contact Hypersensitivity Study in Guinea Pigs; [redacted] Doc. No. 567-002 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Merck KGaA
1.2.2	Companies with letter of access	No companies with Letters of Access.
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes, US EPA, guideline 81-6 which is comparable to OECD 406
2.2	**GLP**	Yes
2.3	**Deviations**	Skin reactions were not performed according to the recommended scoring system given in OECD 406. However, the system used is comparable to the Magnusson-Kligman method given in OECD 406.

3 MATERIALS AND METHODS

3.1	**Test material**	[redacted]
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2
3.1.3	Purity	[redacted]
3.1.4	Description	[redacted]
3.1.5	Stability	[redacted]
3.1.5.1	Preparation of test substance for application	[redacted]
3.1.5.2	Pretest performed on irritant effects	[redacted]

Section A6.1.5/01 Skin sensitisation

Annex Point IIA, VI.6.1.5 Buehler method

3.2	**Test Animals**	
3.2.1	Species	Guinea Pig
3.2.2	Strain	Hartley
3.2.3	Source	[redacted]
3.2.4	Sex	both sexes
3.2.5	Age/weight at study initiation	Animals weighed between 348 and 482 g. Animals were about 8 weeks old.
3.2.6	Number of animals per group	20 test animals 10 naive control animals 5 positive control animals 8 pilot animals
3.2.7	Control animals	yes, positive and negative controls
3.3	**Administration/ Exposure**	
3.3.1	Induction schedule	day of start and once a week thereafter for two weeks (total of 3 induction applications), application interval 7 days
3.3.2	Way of Induction	topical, occluded, [redacted]
3.3.3	Concentrations used for induction	undiluted, test substance is a liquid
3.3.4	Challenge schedule	two weeks after the last induction application
3.3.5	Concentrations used for challenge	undiluted, test substance is a liquid
3.3.6	Re-challenge	no
3.3.7	Scoring schedule	24 and 48 hours after challenge
3.3.8	Removal of the test substance	not indicated
3.3.9	Positive control substance	alpha-Hexylcinnamaldehyde technical (85%) induction 2.5% in ethanol challenge: 2.5 and 5% in acetone

Section A6.1.5/01 Skin sensitisation

Annex Point IIA, VI.6.1.5 Buehler method

3.4	**Examinations**		
3.4.1	Pilot study	yes	
3.5	**Further remarks**	0.3 mL test substance were applied to each animal. Initial and final body weights were determined. A gross necropsy was performed on any animal that died.	X
		4 RESULTS AND DISCUSSION	
4.1	**Results of pilot studies**	[redacted]	
4.2	**Results of test**		
4.2.1	24 h after challenge	[redacted]	
4.2.2	48 h after challenge	[redacted]	
4.2.3	Other findings	[redacted]	
4.3	**Overall result**	[redacted]	

Section A6.1.5/01 Skin sensitisation

Annex Point IIA, VI.6.1.5 Buehler method

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The skin sensitising potential of IR3535® was investigated using the Buehler method. The study was conducted according to the provisions given in OECD 406.

Twenty treatment animals were induced three times (7 days apart) with the undiluted test substance. The test concentrations are based on a pilot study using naive animals. Two weeks after the last induction application, animals were challenged with the undiluted test substance. Positive control animals were treated with alpha-hexycinnamaldehyde in the same way as the treatment animals. The naive negative control animals were challenged concurrently either with the test substance or with the positive control.

5.2 Results and discussion

Following challenge with the undiluted test substance, there were no grade 1 skin reactions in either the treatment group or the naive negative control group. The incidence and severity of the skin reactions in the test group were comparable to those observed in the naive negative control group. The sensitivity of the test system was shown by the clear skin reactions produced in the positive control group treated with alpha-hexylcinnamaldehyde.

5.3 Conclusion

Under the conditions described in the report, IR3535® is not a skin sensitiser [redacted]

5.3.1 Reliability [redacted]

5.3.2 Deficiencies No

Section A6.1.5/01 Skin sensitisation

Annex Point IIA, VI.6.1.5 Buehler method

	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]
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	Give date of comments submitted
Materials and Methods	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
Results and discussion	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
Acceptability	Discuss if deviating from view of rapporteur member state
Remarks	

Table A6.1.5/01-1: Results sensitisation - using Buehler method

	time	application	Observations/Remarks[1)]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]

[redacted]

Section A6.1.5/02 Skin sensitisation

Annex Point IIA, VI.6.1.5 Photoallergenicity

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1986): Determination of Photoallergenicity with Insekt-Repellent 3535 (Art. Nr. 11887) in Albino Guinea Pig; [redacted]; Doc. No. 567-001 (unpublished)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Merck KGaA	
1.2.2	Companies with letter of access	No companies with Letters of Access.	
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Not applicable, a respective guideline is not available	
2.2	**GLP**	Yes	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted]	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	[redacted]	
3.1.4	Description	[redacted]	
3.1.5	Stability	[redacted]	
3.1.5.1	Preparation of test substance for application	10% in ethanol, 0.1 mL/8 cm^2	
3.1.5.2	Pretest performed on irritant effects	not indicated	

Section A6.1.5/02 Skin sensitisation

Annex Point IIA, VI.6.1.5 Photoallergenicity

3.2	**Test Animals**	
3.2.1	Species	Guinea Pig
3.2.2	Strain	Himalayan white
3.2.3	Source	[redacted]
3.2.4	Sex	not indicated
3.2.5	Age/weight at study initiation	Animals weighed between 300 and 450 g. Animals were about 8 weeks old.
3.2.6	Number of animals per group	10/group, total 3 groups including positive and negative control
3.2.7	Control animals	yes, positive and negative controls
3.3	**Administration/ Exposure**	
3.3.1	Induction schedule	0.1 mL of the test dilution / 8 cm^2 days 1, 3, 5, 8, and 10 followed by irradiation (10 J/cm^2 UV-A and 1.8 J/cm^2 UV-B) 30 minutes after application prior to first induction animals received 4 injections of FCA (1:1 in oleum olivae) each of 0.1 mL application site: nuchal area
3.3.2	Way of Induction	topical
3.3.3	Concentrations used for induction	10% in ethanol
3.3.4	Challenge schedule	On day 35 animals were challenged with 0.025 mL/2 cm^2 by topical application to the shaved flanks (right and left side). Thereafter, the left site of the animals was irradiated with 10 J/cm^2 UV-A light. The right side was not irradiated
3.3.5	Concentrations used for challenge	10% in ethanol
3.3.6	Re-challenge	no
3.3.7	Scoring schedule	24 and 48 hours after challenge
3.3.8	Removal of the test substance	not indicated
3.3.9	Positive control substance	3,3',4',5-tetrachlorosalicylanilide (TCSA) induction: 3% in acetone challenge: 0.1% in acetone
3.3.10	Negative control	Negative control animals were treated with FCA only during induction. The challenge application was performed as done with the experimental group.

Section A6.1.5/02 Skin sensitisation

Annex Point IIA, VI.6.1.5 Photoallergenicity

3.3.11	Light Source	UV-A: 320-400 nm, 10 J/cm^2 UV-B: 280-320 nm, 1.8 J/cm^2
3.4	**Examinations**	
3.4.1	Pilot study	not performed
3.5	**Further remarks**	None
		4 RESULTS AND DISCUSSION
4.1	**Results of pilot studies**	[redacted]
4.2	**Results of test**	
4.2.1	24 h after challenge	[redacted]
4.2.2	48 h after challenge	[redacted]
4.2.3	Other findings	[redacted]
4.3	**Overall result**	[redacted]

Section A6.1.5/02 Skin sensitisation

Annex Point IIA, VI.6.1.5 Photoallergenicity

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The photoallergic potential of IR3535® was investigated using guinea pigs. There is no guideline available for such a kind of study. Ten guinea pigs per group were injected with 4 injections of FCA (0.1 mL) on day 1. Thereafter, animals were induced with either IR3535® (10% in ethanol), the positive control 3,3',4',5-tetrachlorosalicylanilide (3% in acetone), or remained untreated (negative control). Thirty minutes after induction, the animals were irradiated with UV-A and UV-B light. The induction applications were repeated on days 3, 5, 8, and 10. On day 35, animals were challenged. Therefore, IR3535® was applied to the flanks of the test and negative control animals. The positive control group was treated with 0.1% 3,3',4',5-tetrachlorosalicylanilide in acetone. The left flank of the animals was irradiated with UV-A light, the right site was not irradiated. Twenty-four and 48 hours after challenge, skin reactions were graded.
5.2	**Results and discussion**	There were no skin reactions observed neither 24 nor 48 hours after challenge application with 10% IR3535® and subsequent UV-irradiation. The same result was obtained in the negative control animals. In the positive control animals, grade 1 to 2 were observed on the left side in all animals. Grade 1 skin reactions were observed on the right (non-irradiated site) in 8/10 animals at both reading points.
5.3	**Conclusion**	Under the conditions described in the report, IR3535® does not posses photoallergic potential.
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	No

Section A6.1.5/02 Skin sensitisation

Annex Point IIA, VI.6.1.5 Photoallergenicity

	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]
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	Give date of comments submitted
Materials and Methods	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
Results and discussion	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
Acceptability	Discuss if deviating from view of rapporteur member state
Remarks	

Table A6.1.5/01-1: Results of Photoallergenicity Testing

	time	application	Observations/Remarks
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]

Section A6.2/01 Toxicokinetics in mammals
Annex Point IIA, VI.6.2 **Rat, gavage and i.v.**

[redacted]

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1 REFERENCE

1.1	**Reference**	[redacted] (1996): Synthesis and in vivo – Stability of a ^{14}C-Labelled Material; Institute of Pharmacokinetics and Metabolism, [redacted] Doc. No. 414-001 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Merck KGaA
1.2.2	Companies with letter of access	No companies with Letters of Access
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No, the intention of this study was to investigate whether ^{14}C IR3535® is stable in the rat organism and in a cream preparation.
2.2	**GLP**	No, not necessary preliminary experiments
2.3	**Deviations**	Not applicable

3 MATERIALS AND METHODS

3.1	**Test material**	[redacted] N-[1-^{14}C]acetyl-3-n-butylaminopropionate
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	[redacted]
3.1.3	Purity	[redacted]
3.1.4	Description	[redacted]
3.1.5	Stability	[redacted]

Section A6.2/01 Toxicokinetics in mammals
Annex Point IIA, VI.6.2 Rat, gavage and i.v.

3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Wistar Hsd/Win: WV
3.2.3	Source	not indicated
3.2.4	Sex	male
3.2.5	Age/weight at study initiation	The animals weighed around 220 g. The age at study initiation is not indicated.
3.2.6	Number of animals per group	2 animals for i.v. treatment 2 animals for oral gavage treatment
3.3	**Administration/ Exposure**	Oral and i.v.
3.3.1	Dosing regime	oral single dose of radiolabelled IR3535® at 0.2 mg/animal (0.37 MBq/animals) equivalent to 0.82 mg/kg bw and 0.91 mg/kg bw i.v. single dose of radiolabelled IR3535® at 0.2 mg/animal (0.33 mBq/animal) equivalent to 0.90 mg/kg bw
3.3.2	Type	i.v. and gavage
3.3.3	Vehicle	60% aqueous polyethylene glycol
3.3.4	Concentration in vehicle	Not indicated in the report
3.3.5	Total volume applied	Not indicated in the report
3.3.6	Controls	Not necessary for this type of study
3.4	**Examinations**	
3.4.1	Excretion balance	i.v. and oral: Collection of: $^{14}CO_2$ in exhaled air, excretion with urine and faeces (each over 72 hours), residual radio-activity (after 72 hours)
3.4.2	Body fluids sampled	none
3.4.3	Tissues sampled	not performed
3.5	**Statistics**	not performed
3.6	**Further remarks**	None

Section A6.2/01 Toxicokinetics in mammals

Annex Point IIA, VI.6.2 **Rat, gavage and i.v.**

4 RESULTS AND DISCUSSION

4.1	**Absorption and excretion balance**	[redacted]
4.2	**Tissue distribution**	[redacted]
4.3	**Metabolites**	[redacted]
4.4	**Absorption**	[redacted]

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	IR3535® was applied to two groups of male rats each containing 2 animals. The oral group was treated with a single dose of 0.8-0.9 mg/kg bw/day, the i.v. group with a single dose of 0.9 mg/kg bw/day IR3535®. Directly after dosing, animals were placed in metabolism cages and exhaled air, urine, and faeces were collected for 72 hours and examined for radioactivity. After 72 hours, the animals were sacrificed and the residual radioactivity was determined.
5.2	**Results and discussion**	The majority of the administered radioactivity was excreted via urine (79-89% of the dose). A smaller amount was recovered in faeces (6-14% of the dose), 0.4-0.6% of the dose were exhaled. Only 1.5-3.2% of the dose were recovered in residual carcass. The total recoveries were between 94 and 98% of the administered dose. The excretion routes were identical after oral and i.v. application. IR3535® was rapidly and completely absorbed from the GIT.
5.3	**Conclusion**	IR3535® was rapidly and completely absorbed from the GIT. Excretion of IR3535® was also rapid and complete within 72 hours. The main excretion route was urine (79-89% of the dose) followed by faeces (6-14% of the dose) and exhaled air (0.4-0.6% of the dose).
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	No

Section A6.2/01 Toxicokinetics in mammals

Annex Point IIA, VI.6.2 **Rat, gavage and i.v.**

	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]
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*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*
Results and discussion	*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*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.2/01-1: Excretion balance of IR3535® 72 hours after dosing

		Radioactivity excreted [% of administered dose]				
Dose	Sex	Urine	Faeces	Exhaled Air	Residual radioactivity	Recovery
single oral dose						
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
single i.v. dose						
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

Section A6.2/02 Toxicokinetic and metabolism in mammals
Annex Point IIA, VI.6.2 Rat & rabbit, dermal & intravenous, single dosing

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	1 REFERENCE	
1.1	**Reference**	[redacted] (1996): Insect Repellent 3535 (Art. No. 111887): Pharmacokinetic and Metabolism Study after Intravenous and Dermal Application of the ^{14}C-labelled Compound to Male Rats and Rabbits; [redacted] Doc. No. 512-001 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Merck KGaA
1.2.2	Companies with letter of access	No companies with Letter of Access.
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I.
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, EPA guideline Series 85-1 (October 1982) and 85-3 (July 1993)
2.2	**GLP**	Yes
2.3	**Deviations**	No
	3 MATERIALS AND METHODS	
3.1	**Test material**	(a) radiolabelled 14C-IR3535® (b) unlabelled IR3535®
3.1.1	Lot/Batch number	(a) [redacted] (b) [redacted]
3.1.2	Specification	(a) [redacted] (b) As given in section 2
3.1.3	Purity	(a) [redacted] (b) [redacted]
3.1.4	Description	(a) [redacted] (b) [redacted]
3.1.5	Stability	[redacted]

Section A6.2/02 Toxicokinetic and metabolism in mammals
Annex Point IIA, VI.6.2 Rat & rabbit, dermal & intravenous, single dosing

3.2	**Test Animals**	
3.2.1	Species	(a) Rat (b) Rabbit
3.2.2	Strain	(a) Wistar, [redacted] (b) New Zealand White: [redacted]
3.2.3	Source	[redacted]
3.2.4	Sex	male
3.2.5	Age/weight at study initiation	(a) Rats weighed between 191 and 217 g on the day of treatment and were 7 – 9 weeks of age (b) Rabbits weighed ca. 1.7 – 1.9 kg on the day of treatment and were about 3 months old
3.2.6	Number of animals per group	Group A:10 male rats and 2 male rabbits Group B: 8 male rats and 2 male rabbits
3.3	**Administration/ Exposure**	Dermal and i.v.
3.3.1	Dosing regime	Group A: single i.v. dose rats : 15.6 mg/kg bw (nominal), 15.7 mg/kg bw (actual) rabbits : 1.6 mg/kg bw (nominal), 1.5 mg/kg bw (actual) Group B : single dermal dose rats :253 mg/kg bw (nominal), 239 mg/kg bw (actual) rabbits :25.3 mg/kg bw (nominal), 26.9 mg/kg bw (actual)
3.3.2	Type	Group A: tail vein (rats), ear vein (rabbits) Group B: dermally to intact, shaved skin, 4 cm^2 (occlusive: rats adhesive bandage, rabbits whole body stocking)
3.3.3	Vehicle	Group A: 0.9% NaCl Group B: none, IR3535® is liquid
3.3.4	Concentration in vehicle	Group A: 1.04 mg ^{14}C-IR3535®/mL Group B: not applicable
3.3.5	Total volume applied	Group A: rats: 0.1-0.4 mL/100 g body weight rabbits: 0.4 – 2.0 mL/kg body weight Group B: rats: 0.05-0.4 mL/4 cm^2 rabbits: 0.05-0.4 mL/4 cm^2
3.3.6	Controls	Not necessary for this type of study

Section A6.2/02 **Toxicokinetic and metabolism in mammals**

Annex Point IIA, VI.6.2 **Rat & rabbit, dermal & intravenous, single dosing**

3.4	**Examinations**	
3.4.1	Excretion routes	[redacted]
3.4.2	Body fluids sampled	[redacted]
3.4.3	Tissues sampled	[redacted]
3.4.4	Metabolism	[redacted]
3.5	**Statistics**	[redacted]
3.6	**Further remarks**	[redacted]

Section A6.2/02 **Toxicokinetic and metabolism in mammals**
Annex Point IIA, VI.6.2 **Rat & rabbit, dermal & intravenous, single dosing**

4 RESULTS AND DISCUSSION

4.1 Excretion balance

[redacted]

4.2 Toxicokinetic

[redacted]

4.3 Dermal absorption

[redacted]

4.4 Tissue distribution

[redacted]

4.5 Metabolites

[redacted]

Section A6.2/02 Toxicokinetic and metabolism in mammals
Annex Point IIA, VI.6.2 Rat & rabbit, dermal & intravenous, single dosing

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The study was conducted according to EPA guideline 85-1 and 85-3.

The plasma levels and excretion of ^{14}C-IR3535® from plasma was investigated during 96 hours after single i.v. administration and during 24 hours after single dermal application to male rats and rabbits. Furthermore, metabolite pattern in plasma was determined in both species at selected time intervals.

Ten male rats and 2 male rabbits were treated by a single i.v. dose (15.7 and 1.5 mg/kg bw, respectively). Two rats were sacrificed scheduled after 0.5, 1, 2, 4, and 96 hours to obtain blood samples. At the same time points, blood was obtained from the ear vein of the 2 rabbits. The excretion of radioactivity via urine and faeces was determined in the two rats scheduled for sacrifice after 96 hours and in the two rabbits which were also sacrificed after 96 hours.
Eight male rats and 2 male rabbits were treated with a single dermal dose of 239 mg/kg bw and 26.9 mg/kg bw, respectively. Blood was obtained after 1, 4, 8, and 24 hours from 2 rats which were sacrificed. At the same time points blood was obtained from the rabbits which were sacrificed after 24 hours. The excretion of radioactivity was studied for 24 hours in the animals scheduled for the 24 hour sacrifice.

Radioactivity in urine and faeces was determined. The radioactivity in plasma and the metabolic profile were determined after precipitation of proteins after 0.5, 1, 2, and 4 hours (i.v. dose) and after 1, 4, 8, and 24 hours (dermal dose).

Based on the determined radioactivity in plasma, the kinetic profile of IR3535® after single i.v. and dermal was calculated.

5.2 Results and discussion

After a single i.v. dose, radioactivity elimination from plasma followed a first-order kinetic. Excretion was very fast as indicated by the low calculated half-live (0.5 and 0.7 hours in rats and rabbits, respectively). Accordingly, after 96 hours, elimination of radioactivity via urine and faeces was virtually complete (89.6 to 95.9% of the dose and 2.1 to 16.8% of the dose, respectively). The majority of radioactivity had been excreted within the first 24 hours after dosing.

After a single dermal dose, the highest concentration of total radioactivity in plasma was reached after 8 hours in rats and after 4 hours in rabbits. Thereafter, radioactivity declined. Based on the ratio of AUC after dermal and i.v. application, similar amounts of radioactivity were absorbed in the rat (18% of the dose) and in rabbits (27% of the dose). Based on the 24 h excretion of radioactivity in urine and faeces, dermal penetration rates account for 8% in rats and to about 18-26% in rabbits.

In all examined plasma pools no parent compound was detected. Almost exclusively the carboxylic acid of IR3535®, N-acetyl-N-butyl-3-aminopropionic acid, was determined indicating that IR3535® is rapidly and completely hydrolysed at the ester moiety in both species.

No relevant differences were found in the metabolism and toxicokinetic profile of IR3535® in both species.

5.3 Conclusion

see results and discussion

5.3.1 Reliability

[redacted]

Section A6.2/02 Toxicokinetic and metabolism in mammals
Annex Point IIA, VI.6.2 **Rat & rabbit, dermal & intravenous, single dosing**

5.3.2 Deficiencies No

	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]
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
	[redacted]
Reliability	[redacted]
Acceptability	A[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*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*
Results and discussion	*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*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.2/02-1: Excretion balance of IR3535® after dosing (Groups A and B)

		Radioactivity excreted [% of administered dose]			
Group A –single i.v.					
Group B –single dermal					

Table A6.2/02-2: Plasma level of IR3535® in pooled samples at different time points after dosing (Groups A and B)

		Radioactivity plasma [µg equivalents/g plasma[1)]]	
Group A –single i.v.			
		[redacted]	[redacted]
Time after dosing [hours]	Sex		
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]		[redacted]	[redacted]
Group B –single dermal			
		[redacted]	[redacted]
Time after dosing [hours]	Sex		
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]		[redacted]	[redacted]

[redacted]

Table A6.2/02-3: Percentage dermally absorbed IR3535® in rats and rabbits calculated from AUC values

	Species			
	Rats		Rabbits	
Route	i.v.	dermal	i.v.	dermal
[redacted]				

[redacted]

Table A6.2/02-4: Metabolite Pattern in plasma

		Species			
		Rats		Rabbits	
Route	time point [hours]	i.v.	dermal	i.v.	dermal
[redacted]					

[redacted]

Section A6.2/03 Toxicokinetic in mammals
Annex Point IIA, VI.6.2 Rat dermal, single dosing

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	1 REFERENCE	
1.1	**Reference**	[redacted] (1996): Insect Repellent 3535 (Art. No. 111887): Dermal Absorption and Pharmacokinetic Study on Various Organs and Tissues of Male Rats and Excretion Pattern of Radioactivity after Single Dermal Administration of the ^{14}C-Labelled Compound; [redacted]; Doc. No. 511-001 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Merck KGaA
1.2.2	Companies with letter of access	No companies with Letter of Access.
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I.
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, EPA guideline Series 85-3 (August 1994)
2.2	**GLP**	Yes
2.3	**Deviations**	No
	3 MATERIALS AND METHODS	
3.1	**Test material**	(a) radiolabelled ^{14}C-IR3535® (b) unlabelled IR3535® (ethyl-3-(N-butylacetamido)-propionate) (c) blank cream: 10-07/L, 10-05/L, and 10-06/L (commercial formulation vehicle)
3.1.1	Lot/Batch number	(a) [redacted] (b) [redacted] (c) [redacted]
3.1.2	Specification	(a) [redacted] (b) As given in section 2 (c) [redacted]
3.1.3	Purity	(a) [redacted] (b) [redacted] (c) [redacted]
3.1.4	Description	(a) [redacted] (b) [redacted] (c) [redacted]

Section A6.2/03 Toxicokinetic in mammals
Annex Point IIA, VI.6.2 Rat dermal, single dosing

3.1.5	Stability	[redacted]
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Wistar, [redacted]
3.2.3	Source	[redacted]
3.2.4	Sex	male
3.2.5	Age/weight at study initiation	Rats weighed between 170 to 220 g one day prior to treatment with ^{14}C-IR3535®. Animals were 7 to 9 weeks old at beginning of acclimatisation (at least 5 days).
3.2.6	Number of animals per group	28 animals/group
3.3	**Administration/ Exposure**	Animals were anaesthetised during application
3.3.1	Dosing regime	Low dose: 0.01 mg/cm^2, 0.524 mg/kg bw, 0.1 mg/rat Mid dose: 0.1 mg/cm^2, 5.475 mg/kg bw, 1 mg/rat High dose: 1.0 mg/cm^2, 50.64 mg/kg bw, 10 mg/rat Four animals per group were sacrificed after 0.5, 1, 2, 4, 10, 24, and 72 hours after application. The maximum duration of administration was 24 hours. Skin was washed three times with soap solution and once with water prior to sacrifice or after 24 hours as appropriate by means of gauze patches. The radioactivity was determined.
3.3.2	Type	Dermal, 10 cm^2 (back and shoulders), occlusive
3.3.3	Vehicle	Low dose: 10-07/L cream formulation Mid dose: 10-05/L cream formulation High dose: 10-06/L cream formulation
3.3.4	Concentration in vehicle	Low dose: 0.1 % IR3535® Mid dose: 1.0 % IR3535® High dose: 10 % IR3535®
3.3.5	Total volume applied	100 mg cream formulation/10 cm^2
3.3.6	Controls	Not necessary for this type of study

Section A6.2/03 **Toxicokinetic in mammals**

Annex Point IIA, VI.6.2 **Rat dermal, single dosing**

3.4	**Examinations**	
3.4.1	Excretion routes	[redacted]
3.4.2	Body fluids sampled	[redacted]
3.4.3	Tissues sampled	[redacted]
3.4.4	Metabolism	[redacted]
3.5	**Statistics**	[redacted]
3.6	**Further remarks**	[redacted]

Section A6.2/03 **Toxicokinetic in mammals**
Annex Point IIA, VI.6.2 **Rat dermal, single dosing**

4 RESULTS AND DISCUSSION

4.1 Excretion balance

[redacted]

4.2 Toxicokinetic

[redacted]

Section A6.2/03 **Toxicokinetic in mammals**

Annex Point IIA, VI.6.2 **Rat dermal, single dosing**

4.3 Dermal absorption

[redacted]

Section A6.2/03 **Toxicokinetic in mammals**

Annex Point IIA, VI.6.2 **Rat dermal, single dosing**

4.4 Tissue distribution

[redacted]

4.5 Metabolites

[redacted]

Section A6.2/03 Toxicokinetic in mammals
Annex Point IIA, VI.6.2 Rat dermal, single dosing

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The study was conducted according to EPA guideline 85-3.

The excretion and tissue distribution of IR3535® was investigated in male Wistar rats after single dermal application at dose levels of 0.524, 5.475, and 50.64 mg/kg bw corresponding to 0.1 %, 1 %, and 10 % IR3535® in the dosing cream formulation. Each group consisted of 28 male animals. After 0.5, 1, 2, 4, 10, 24, and 72 hours 4 males/group were sacrificed and radioactivity in liver, kidney, GIT, treated and untreated skin as well as carcass was determined. The excreted radioactivity in urine and faeces up to the sacrifice time point was also measured. IR3535® was applied up to the sacrifice time point except for the animals designated for the 72 hours termination time point which were treated for 24 hours under occlusive conditions. Directly before sacrifice or after 24 hours the treated skin was washed three times with soap solution and once with water. The radioactivity in the skin wash and the bandages was also determined.

5.2 Results and discussion

Total recoveries for all dose levels at all time points ranged from 86.34 % to 103.13 % of the applied dose.
Most radioactivity (at least 50 % of the applied dose) was washed off. In the low dose group, the dermal absorption of the radioactivity increased to 25 % of the applied dose after 10 hours and remained constant thereafter. In the mid and high dose group a plateau after 24 hours for dermal absorption was also determined at approximately 40 % and 36 % of the applied dose, respectively.
Excretion of the absorbed radioactivity was fast and essentially complete after 24 hours. Most absorbed radioactivity was excreted via urine approx. 20 %, 32 %, and 32 % of the dose within 24 hours after dosing at the low, mid, and high dose level, respectively. Radioactivity excreted via faeces was much lower max. 3 %.
IR3535® was distributed evenly over the body. Peak blood concentration was determined to be 0.5 hours after treatment. Radioactivity found in the carcass and tissues thereby including blood but excluding treated skin was highest after 1 hour (10-12.5 % of the dose at the low and mid dose level) and 4.4 % of the dose at the high dose level. Thereafter, radioactivity steadily decreased to approx. 1.5-1.6 % of the dose after 72 hours indicating that IR3535® has no potential for bioaccumulation. Highest amounts of radioactivity were found in the application site, the excretion organs kidney and liver as well as in the carcass. Seventy-two hours after dosing, remaining radioactivity in the animals was low.

5.3 Conclusion

The appropriate dermal penetration rate to be used in the human health risk assessment are considered to be 20 % for a formulation containing approx. 10 % IR3535®.

This conclusion is based on an exposure duration of 10 hours. Further the amount of radioactivity was not considered to be absorbed because there were no indications that the amount of radioactivity located in skin is bioavailable.

5.3.1 Reliability [redacted]

5.3.2 Deficiencies No

Section A6.2/03 Toxicokinetic in mammals

Annex Point IIA, VI.6.2 **Rat dermal, single dosing**

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]
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Section A6.2/03 Toxicokinetic in mammals

Annex Point IIA, VI.6.2 **Rat dermal, single dosing**

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*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*
Results and discussion	*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*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.2/03-1: Excretion balance of IR3535®

		Radioactivity excreted [% of administered dose]						
Time interval [hour]	Sex	Urine[1)]	Faeces	Total excreted	treated skin	carcass and tissues[2)]	skin wash and bandages	Recovery
Low dose (0.524 mg/kg bw, 0.1 % IR3535®)								
[redacted]								
Mid dose (5.475 mg/kg bw/daykg bw, 1.0 % IR3535®)								
[redacted]								
High dose (50.64 mg/kg bw, 10 % IR3535®)								
[redacted]								

[redacted]

Table A6.2/03-2: Tissue Distribution in µg parent equivalent/g tissue[1)] (expressed as % of dose)

Time point [hours]	0.5	1	2	4	10	24	72
Low dose (0.524 mg/kg bw, 0.1 % IR3535®)							
[redacted]							
[redacted]							
[redacted]							
[redacted]							
[redacted]							
[redacted]							
[redacted]							
[redacted]							
Mid dose (5.475 mg/kg bw, 1.0 % IR3535®)							
[redacted]							
[redacted]							
[redacted]							
[redacted]							
[redacted]							
[redacted]							
[redacted]							
[redacted]							

Table A6.2/03-2: Tissue Distribution in µg parent equivalent/g tissue[1)] (expressed as % of dose)

Time point [hours]	0.5	1	2	4	10	24	72
High dose (50.64 mg/kg bw, 10 % IR3535®)							
[redacted]							
[redacted]							
[redacted]							
[redacted]							
[redacted]							
[redacted]							
[redacted]							
[redacted]							

[redacted]

Table A6.2/03-3: Dermal penetration rates including radioactivity in skin

Sacrifice time point [hours]	0.5	1	2	4	10	24	72
Low dose (0.524 mg/kg bw, 0.1 % IR3535®)							
[redacted]							
Mid dose (5.475 mg/kg bw, 1.0 % IR3535®)							
[redacted]							
High dose (50.64 mg/kg bw, 10 % IR3535®)							
[redacted]							

[redacted]

Table A6.2/03-4: Dermal penetration rates excluding radioactivity in skin

Sacrifice time point [hours]	0.5	1	2	4	10	24	72
Low dose (0.524 mg/kg bw, 0.1 % IR3535®)							
[redacted]							
Mid dose (5.475 mg/kg bw, 1.0 % IR3535®)							
[redacted]							
High dose (50.64 mg/kg bw, 10 % IR3535®)							
[redacted]							

Section A6.2/04 Toxicokinetic in mammals
Annex Point IIA, VI.6.2 Rat dermal, single dosing

[redacted]

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	1 REFERENCE	
1.1	**Reference**	[redacted] (1996): Insect Repellent 3535 (Art. no. 111887): Bioretention Study in Male Rats after Single Dermal Administration of the ^{14}C-Labelled Compound at a Dose Level of 1.0 mg/cm^2; [redacted]; Doc. No. 511-002 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Merck KGaA
1.2.2	Companies with letter of access	No companies with Letter of Access.
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I.
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, EPA guideline Series 85-3: Section (f) (4) – Organ/Tissue Evaluation, August 30, 1994
2.2	**GLP**	Yes
2.3	**Deviations**	No
	3 MATERIALS AND METHODS	
3.1	**Test material**	(a) radiolabelled 14C-IR3535® (b) blank cream: 10-06/L (commercial formulation vehicle)
3.1.1	Lot/Batch number	(a) [redacted] (b) [redacted]
3.1.2	Specification	(a) [redacted] (b) [redacted]
3.1.3	Purity	(a) [redacted] (b) [redacted]
3.1.4	Description	(a) [redacted] (b) [redacted]

Section A6.2/04 Toxicokinetic in mammals
Annex Point IIA, VI.6.2 Rat dermal, single dosing

3.1.5	Stability	[redacted]
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	(a) Wistar, [redacted] (b) [redacted]
3.2.3	Source	[redacted]
3.2.4	Sex	male
3.2.5	Age/weight at study initiation	Rats weighed between 175 to 210 g one day prior to treatment with ^{14}C-IR3535®. Animals were about 7 of age at beginning of acclimatisation (5 to 6 days).
3.2.6	Number of animals per group	(a) 10 animals (b) 10 animals
3.3	**Administration/ Exposure**	Animals were anaesthetised during application
3.3.1	Dosing regime	1.0 mg/cm^2, 53.35 mg/kg bw, 10.3 mg/rat Two animals each were sacrificed after 1, 4, 8, 24, and 72 hours after application. The maximum duration of administration was 24 hours. Skin was washed three times with soap solution and once with water prior to sacrifice or after 24 hours as appropriate by means of gauze patches. The radioactivity was determined.
3.3.2	Type	Dermal, 10 cm^2 (back and shoulders), semiocclusive (except one hour sacrifice animals)
3.3.3	Vehicle	10-06/L cream formulation
3.3.4	Concentration in vehicle	10% IR3535®
3.3.5	Total volume applied	100 mg cream formulation/10 cm^2
3.3.6	Controls	Not necessary for this type of study

Section A6.2/04 Toxicokinetic in mammals

Annex Point IIA, VI.6.2 **Rat dermal, single dosing**

3.4	**Examinations**	
3.4.1	Excretion routes	[redacted]
3.4.2	Body fluids sampled	[redacted]
3.4.3	Tissues sampled	[redacted]
3.4.4	Metabolism	[redacted]
3.5	**Statistics**	[redacted]
3.6	**Further remarks**	[redacted]

Section A6.2/04 **Toxicokinetic in mammals**
Annex Point IIA, VI.6.2 **Rat dermal, single dosing**

4 RESULTS AND DISCUSSION

4.1 Excretion balance

[redacted]

4.2 Toxicokinetic

[redacted]

4.3 Dermal absorption

[redacted]

4.4 Tissue distribution

[redacted]

4.5 Metabolites

[redacted]

Section A6.2/04 Toxicokinetic in mammals

Annex Point IIA, VI.6.2 Rat dermal, single dosing

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The study was conducted according to EPA guideline 85-3.

The excretion from blood and tissue distribution of IR3535® was investigated in male rats (pigmented and non-pigmented) after single dermal application at dose levels of 53.35 mg/kg bw corresponding to 10% IR3535® in the dosing cream formulation. After 1, 4, 8, 24, and 72 hours 2 pigmented and 2 non-pigmented animals were sacrificed at each time point and radioactivity in organs and tissues including treated skin as well as blood was determined. IR3535® was applied under semiocclusive conditions up to the sacrifice time point except for the animals designated for the 72 hours termination time point which were treated for 24 hours. Directly before sacrifice or after 24 hours the treated skin was washed three times with soap solution and once with water. The radioactivity in the skin wash and the bandages was also determined. The dermal absorption was calculated.

5.2 Results and discussion

The present study showed that after 1, 4, 8, and 24 hour application of IR3535® in a cream formulation about 13, 30, 30, and 50% of the applied dose, respectively, were absorbed. Absorption after 24 hour application and further recovery period of 48 hours was calculated to be about 60% of the applied dose. Except for samples taken from application site and from liver, radioactivity was highest in blood (0.15% of the dose) and kidney (0.19% of the dose) at the 1 hour sampling point with rapid decreases thereafter. In liver and skin about 0.41-0.54% and 2.9-3.3% of the applied dose, respectively, were found after 1-24 hours. Radioactivity thereafter decreased to 0.12 and 0.81% of the dose, respectively. Radioactivity found in the other organs were low (max. 0.02% of the applied dose).

5.3 Conclusion

In conclusion, about 50% of the applied dose were absorbed within 24 hours of application. Radioactivity levels in treated skin and organs/tissues decreased from 24 to 72 hours indicating efficient elimination. Based on these time interval, the elimination half-lives were about 24 to 48 hours. At 72 hours, radioactivity at well quantifiable levels were found only in samples from the liver and the treated skin. No differences between pigmented and non-pigmented rats were observed.

5.3.1 Reliability [redacted]

5.3.2 Deficiencies No

Section A6.2/04 Toxicokinetic in mammals

Annex Point IIA, VI.6.2 **Rat dermal, single dosing**

	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]
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*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*
Results and discussion	*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*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.2/04-1: Radioactivity in Bandages and Skin Washes

			Radioactivity [% of administered dose]		
Time interval [hour]	Pigmented	Number of animals	Bandage	Skin Wash	Absorbed[1)]
[redacted]					

Table A6.2/04-2: Tissue Distribution in % of applied dose[1)]

Time point [hours]	1[2)]	4	8	24	72
[redacted]					

Section A6.2/05 Toxicokinetic in mammals
Annex Point IIA, VI.6.2 Rat dermal, multiple dosing

[redacted]

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1 REFERENCE

1.1	**Reference**	[redacted] (1996): Insect Repellent 3535 (Art. No. 111887): 28-Day Toxicokinetic Study with Dermal Application to Rats; [redacted] Doc. No. 532-005 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Merck KGaA
1.2.2	Companies with letter of access	No companies with Letter of Access.
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes, Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, Paragraph 85-1: "Metabolism study"; US. EPA, November 1984 OECD 410 (1981): Repeated-Dose Dermal Toxicity Directive 92/69EEC, B. 9 (1992): Repeated Dose Toxicity - Dermal
2.2	**GLP**	Yes
2.3	**Deviations**	Yes, A control group was not used, food consumption was not monitored, haematology and clinical chemistry analysis as well as gross and histopathology were not performed as recommended by OECD 410. However, as this is a range-finding study, these deviations are not considered to have influenced the quality of the study.

3 MATERIALS AND METHODS

3.1	**Test material**	[redacted]
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in Section 2
3.1.3	Purity	[redacted]
3.1.4	Description	[redacted]
3.1.5	Stability	[redacted]
3.2	**Test Animals**	
3.2.1	Species	Rat

Section A6.2/05 Toxicokinetic in mammals
Annex Point IIA, VI.6.2 Rat dermal, multiple dosing

3.2.2	Strain	[redacted]
3.2.3	Source	[redacted]
3.2.4	Sex	male and female
3.2.5	Age/weight at study initiation	Male rats weighed between 195.6 and 216.6 g at acclimatisation, females between 193.6 and 212.7 g. Males were about 8 weeks at delivery, females about 10 weeks. Animals were acclimatised for one week under laboratory conditions.
3.2.6	Number of animals per group	12/sex/group
3.3	**Administration/ Exposure**	Dermal
3.3.1	Dosing regime	100, 1000, 3000 mg/kg bw/day
3.3.2	Duration of treatment	28 days
3.3.3	Frequency of exposure	6 hours per day 7 days per week
3.3.4	Post-exposure period	none
3.3.5	Area covered	25 cm^2
3.3.6	Occlusion	yes
3.3.7	Vehicle	Insect Repellent Cream (W/O) [redacted]
3.3.8	Concentration in vehicle	2, 20, and 60%
3.3.9	Total volume applied	5 mL/kg bw
3.3.10	Removal of test substance	yes, the application site was washed with lukewarm tap water and dried with paper towel
3.3.11	Controls	no, not considered necessary for this range-finding study
3.4	**Examinations**	
3.4.1	Observations	
3.4.2	Clinical signs	yes, daily
3.4.3	Mortality	yes, daily
3.4.4	Body weight	yes, weekly
3.4.5	Food consumption	no, not considered necessary for this range-finding study
3.4.6	Water consumption	no, not required
3.4.7	Ophthalmoscopic examination	no, not required
3.4.8	Haematology	no, not considered necessary for this range-finding study

Section A6.2/05 Toxicokinetic in mammals
Annex Point IIA, VI.6.2 Rat dermal, multiple dosing

3.4.9	Clinical Chemistry	no, not considered necessary for this range-finding study
3.4.10	Urinalysis	no, not required
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	no, not considered necessary for this range-finding study
3.5.2	Gross and histopathology	no, not considered necessary for this range-finding study
3.6	**Other examinations**	None
3.7	**Statistics**	Not performed
3.8	**Toxicokinetics**	
3.8.1	Excretion routes	[redacted]
3.8.2	Body fluids sampled	[redacted]
3.8.3	Tissues sampled	[redacted]
3.8.4	Metabolism	[redacted]

Section A6.2/05 **Toxicokinetic in mammals**
Annex Point IIA, VI.6.2 **Rat dermal, multiple dosing**

4 RESULTS AND DISCUSSION

4.1 Observations

4.1.1 Clinical signs [redacted]

4.1.2 Mortality [redacted]

4.1.3 Dermal observations [redacted]

4.2 Body weight gain [redacted]

4.3 Food consumption [redacted]

4.4 Ophthalmoscopic examination [redacted]

4.5 Blood analysis

4.5.1 Haematology [redacted]

4.5.2 Clinical chemistry [redacted]

4.5.3 Urinalysis [redacted]

4.6 Sacrifice and pathology

4.6.1 Organ weights [redacted]

4.6.2 Gross and histopathology [redacted]

4.7 Other [redacted]

4.8 Excretion balance [redacted]

4.9 Toxicokinetic [redacted]

4.10 Dermal absorption [redacted]

4.11 Tissue distribution [redacted]

4.12 Metabolites [redacted]

Section A6.2/05 Toxicokinetic in mammals
Annex Point IIA, VI.6.2 Rat dermal, multiple dosing

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The study was conducted according to EPA guideline 85-3 (metabolism part) and followed OECD 410. The intention of the study to find appropriate dose levels for a subsequent 90-day dermal toxicity study and to investigate the metabolism of IR3535® in blood after repeated dosing.

IR3535® was administered at daily doses of 100, 1000, and 3000 mg/kg bw/day for 28 days to groups of 12 rats/sex/group (6 hours per day, 7 days/week). For toxicokinetic examinations blood was collected on test days 3 and 28 (prior to treatment, 1, 3, and 6 hours after application of the test substance). Blood was centrifuged and the resulting plasma was analysed for the presence of metabolites.

5.2 Results and discussion

There were no treatment-related deaths during the course of the study. Clinical signs indicative for systemic toxicity were not observed. Body weight gain was within the expected range for animals of this age indicating no systemic toxicity of IR3535®. The most frequent skin reactions in animals of all groups included very slight to slight patchy erythema and scaling. The incidence, persistence and severity of these changes were dose-dependent. The carboxylic metabolite of IR3535® was found in plasma of all treated animals sampled 1, 3, and 6 hours after dosing with peak concentration 1 hour after dosing in the low and mid dose group and after 1 – 3 hours after dosing in the high dose group. The concentrations of the carboxylic metabolite increased with increasing doses of IR3535®. Higher concentrations were found on day 3 when compared to day 28.

5.3 Conclusion

Topical application of IR3535® at dose levels of 100, 1000, and 3000 mg/kg bw/day over a period of 28 days elicited minimal to slight local skin reactions. There was no evidence of systemic toxicity and the dose levels were considered to be appropriate for the subsequent 90-day study dermal toxicity study. IR3535® was hydrolysed at its ester moiety to yield the respective carboxylic acid.

5.3.1 Reliability [redacted]

5.3.2 Deficiencies No

Section A6.2/05 Toxicokinetic in mammals

Annex Point IIA, VI.6.2 **Rat dermal, multiple dosing**

		Evaluation by Competent Authorities
		Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
3.9		4 EVALUATION BY RAPPORTEUR MEMBER STATE
4.1	Date	[redacted]
4.2	Materials and Methods	[redacted]
4.3	Results and discussion	[redacted]
4.4	Conclusion	[redacted]
4.5	Reliability	[redacted]
4.6	Acceptability	[redacted]
4.7	Remarks	[redacted]
4.8		5 COMMENTS FROM ...
5.1	Date	*Give date of comments submitted*
5.2	Materials and Methods	*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*
5.3	Results and discussion	*Discuss if deviating from view of rapporteur member state*
5.4	Conclusion	*Discuss if deviating from view of rapporteur member state*
5.5	Reliability	*Discuss if deviating from view of rapporteur member state*
5.6	Acceptability	*Discuss if deviating from view of rapporteur member state*
5.7	Remarks	

Table A6.2/05-1: Concentration of Carboxylic Metabolite of IR3535® in Rat Plasma

Dose group [mg/kg bw/day]	Sex	Concentration of Carboxylic Metabolite [µg/mL]			
		Pre-dose	1 hour post-dose	3 hours post-dose	6 hours post-dose
Day 3					
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]		[redacted]	[redacted]	[redacted]	[redacted]
[redacted]		[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]		[redacted]	[redacted]	[redacted]	[redacted]
[redacted]		[redacted]	[redacted]	[redacted]	[redacted]
Day 28					
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]		[redacted]	[redacted]	[redacted]	[redacted]
[redacted]		[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]		[redacted]	[redacted]	[redacted]	[redacted]
[redacted]		[redacted]	[redacted]	[redacted]	[redacted]

Table A6.2/05-2: Skin Reactions

Skin reaction (maximal possible grade)*1)	Sex	Skin Reactions: % of affected animals *2) (median value of the highest individual daily grade)		
		[dose level [mg/kg bw/day]		
		100	1000	3000
[redacted]				
[redacted]				

Section A6.2/06 Metabolism *in vitro*
Annex Point IIA, VI.6.2 Rat and human hepatocytes

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1 REFERENCE

1.1	**Reference**	[redacted] (1996): Insect Repellent 3535 (Art. No. 111887) In vitro Metabolism in Hepatocytes of Rat and Man; [redacted]; Doc. No. 514-001 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Merck KGaA
1.2.2	Companies with letter of access	No companies with Letter of Access.
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes, EPA guideline Series 85-1
2.2	**GLP**	No, not required for this kind of study
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	(a) radiolabelled IR3535® ethyl-N-[1-14C]acetyl-3-N-n-butylaminopropionate (b) unlabelled IR3535®
3.1.1	Lot/Batch number	(a) [redacted] (b) [redacted]
3.1.2	Specification	(a) [redacted] (b) As given in section 2
3.1.3	Purity	(a) [redacted] (b) [redacted]
3.1.4	Description	(a) [redacted] (b) [redacted]
3.1.5	Stability	[redacted]

Section A6.2/06 Metabolism *in vitro*

Annex Point IIA, VI.6.2 **Rat and human hepatocytes**

3.2	**Test Animals**	*in vitro* study, rat and human hepatocytes
3.2.1	Species	(a) rat
		(b) human
3.2.2	Strain	(a) Lewis
		(b) not applicable
3.2.3	Source	(a) not indicated
		(b) 62 year old, male patient undergoing hepatic resections because of liver metastasis
3.2.4	Sex	(a) male
		(b) male
3.2.5	Age/weight at study initiation	(a) The age of the animals is not indicated in the report. Animals weighed between 200 and 230 g.
		(b) 62 year old
3.2.6	Number of animals per group	not applicable, *in vitro* study
3.3	**Administration/ Exposure**	IR3535® was applied to the isolated hepatocytes on culture day 4. Cells were incubated for 2, 4, 8, and 24 hours. Afterwards, the cell medium was removed by centrifugation and subject of metabolite analysis. The medium removed 2 hours after application was used for HPLC-MS/MS experiments. The hepatocytes were homogenised and the resulting organic phase was used for metabolite identification.
3.3.1	Dosing regime	1 µg IR3535® per mL cell medium ; 1% (v/v) ethanol in medium
3.3.2	Type	The test substance was added to the cell medium
3.3.3	Vehicle	ethanol
3.3.4	Concentration in vehicle	stock solution: 0.1 mg/mL (25.5 µCi/mL)
3.3.5	Total volume applied	20 µL of the stock solution
3.3.6	Controls	Yes, the stability of IR3535® in the absence of hepatocytes was investigated in the medium.
3.4	**Examinations**	
3.4.1	Excretion routes	[redacted]
3.4.2	Body fluids sampled	[redacted]
3.4.3	Tissues sampled	[redacted]
3.5	**Statistics**	[redacted]
3.6	**Further remarks**	[redacted]

Section A6.2/06 Metabolism *in vitro*

Annex Point IIA, VI.6.2 **Rat and human hepatocytes**

4 RESULTS AND DISCUSSION

4.1	**Excretion balance**	[redacted]
4.2	**Tissue distribution**	[redacted]
4.3	**Metabolites**	[redacted]
4.4	**Absorption**	[redacted]

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The study was conducted according to EPA guideline 85-1. Rat and human hepatocytes immobilised in collagen were incubated with ^{14}C-labelled IR3535® for 2, 4, 8, and 24 hours at a concentration of 1 µg/mL. A separate culture was prepared for each time point. Metabolite patterns were evaluated by gradient HPLC and radioactivity detection. Metabolite structures were confirmed by comparison with reference standards and LC-MS/MS. After incubation time, aliquots of the medium were used directly for metabolite identification. Hepatocytes were homogenised in acetone. After centrifugation, the organic phase was separated from the precipitate. The remaining radioactivity in the precipitate was determined after combustion.
5.2	**Results and discussion**	The only metabolite identified in rat and human hepatocytes and in the respective cultivation medium at each investigated time point was N-acetyl-N-butyl-3-aminopropionic acid, the acid of IR3535®. In rat samples a further minor peak was found, however, it could not be identified due to the small peak size. The parent compound IR3535® was not detected in all samples indicating that IR3535® was completely hydrolysed to the respective carboxylic acid (N-acetyl-N-butyl-3-aminopropionic acid). The metabolic pathway of IR3535® consists of hydrolysis of the ethyl ester moiety resulting in the respective carboxylic acid. The results of the experiments indicate that the metabolism of IR3535® in rat and man is identical. Thus, the rat is the appropriate species for investigating the toxicokinetic and toxicological profile of IR3535®.
5.3	**Conclusion**	see Results and Discussion
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	No

Section A6.2/06 Metabolism *in vitro*

Annex Point IIA, VI.6.2 **Rat and human hepatocytes**

	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]
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*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*
Results and discussion	*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*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.2/07 Toxicokinetic
Annex Point IIA, VI.6.2 Oral Rabbit

[redacted]

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1 REFERENCE

1.1	**Reference**	[redacted] (1997): Insect Repellent 3535 (Article Number 111887) Investigatory Study T 9400 with Oral Administration to Himalayan and New Zealand White Rabbits; [redacted] Doc. No. 531-003 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Merck KGaA
1.2.2	Companies with letter of access	No companies with Letter of Access.
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No, investigatory study to investigate differences between Himalayan and New Zealand White rabbits
2.2	**GLP**	No, not required for this kind of study
2.3	**Deviations**	Not applicable

3 MATERIALS AND METHODS

3.1	**Test material**	[redacted]
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2
3.1.3	Purity	[redacted]
3.1.4	Description	[redacted]
3.1.5	Stability	[redacted]

Section A6.2/07 Toxicokinetic
Annex Point IIA, VI.6.2 Oral Rabbit

3.2	**Test Animals**	
3.2.1	Species	rabbit
3.2.2	Strain	(a) Himalayan (b) New Zealand White
3.2.3	Source	(a) [redacted] (b) [redacted]
3.2.4	Sex	(a) female (b) female
3.2.5	Age/weight at study initiation	(a) 2.17 – 2.36 kg; 168 – 171 days (b) 2.97 – 3.64 kg; 185 – 193 days
3.2.6	Number of animals per group	3 per strain
3.3	**Administration/ Exposure**	IR3535® was applied to the animals by oral gavage once daily. The required volume of the test substance was drawn up into a syringe. Demineralised water was added to a total volume of 1 mL. This solution was applied to the animals. To ensure that the total dosing solution reaches the stomach, 5 mL demineralised water were additionally applied thereafter. Animals were dosed from day 0 to day 10.
3.3.1	Dosing regime	0.6 mL/kg bw/day (600 mg/kg bw/day)
3.3.2	Type	gavage
3.3.3	Vehicle	demineralised water
3.3.4	Concentration in vehicle	not applicable; see above
3.3.5	Total volume applied	6 mL/kg bw
3.3.6	Controls	No, not required for this kind of study
3.4	**Examinations**	
3.4.1	Excretion routes	[redacted]
3.4.2	Body fluids sampled	[redacted]
3.4.3	Tissues sampled	[redacted]
3.5	**Statistics**	[redacted]
3.6	**Further remarks**	[redacted]

Section A6.2/07 Toxicokinetic
Annex Point IIA, VI.6.2 Oral Rabbit

4 RESULTS AND DISCUSSION

4.1 Excretion balance [redacted]

4.2 Tissue distribution [redacted]

4.3 Metabolites [redacted]

4.4 Absorption [redacted]

4.5 Systemic toxicity [redacted]

4.6 Toxicokinetics [redacted]

Section A6.2/07 Toxicokinetic
Annex Point IIA, VI.6.2 Oral Rabbit

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The intention of this study was to examine whether there are differences in the peak plasma concentration and the time to peak between Himalayan and New Zealand White rabbits at identical dose levels under the same dosing regime.

Therefore, 3 rabbits per strain were treated via gavage with IR3535® with 0.6 mL/kg bw/day (600 mg/kg bw/day) for 10 days. Peak plasma concentrations and time to peak were determined on day 1 and on day 10 of dosing thereby utilising the IR3535® metabolite N-acetyl-N-butyl-3-aminopropionic acid as marker due to the absence of the parent compound in plasma as shown in a previous study (van Dijk, 1996, Doc. No. 512-001, Document IIIA, Section 6, 6.2/02). Additionally, animals were examined daily for clinical signs and mortality. Body weights were determined daily. At termination, all animals were examined by gross pathology. Liver, stomach, small and large intestine as well as kidneys were examined histologically.

5.2 Results and discussion

All rabbits survived. There were no clinical signs observed. All animals except one lost weight after the first dose. Thereafter, animals gained weight. The total mean body weight gain was comparable between the strains. At necropsy, one Himalayan rabbit showed gastric mucous membrane haemorrhages in the stomach. The histological findings consisted of focal regeneration in the stomach (pars muscularis), haemorrhages in the mucous membrane, and atrophy of the mucous membrane in 1/3, 1/3, and 2/3 Himalayan rabbits, respectively. Atrophy of the mucous membrane was also observed in one New Zealand White rabbit. Peak plasma concentrations were reached between 0.5 and 1 hour. Neither of the two strains showed any IR3535® metabolite, N-acetyl-N-butyl-3-aminopropionic acid, 24 hours after dosing, indicating a rapid excretion of the test material. There were no differences in plasma half life between the examined rabbit strains. The AUC-values were comparable.

5.3 Conclusion

There are no differences in the absorption profile of IR3535® from the GIT between the tested Himalayan and New Zealand white rabbit strain.

5.3.1 Reliability [redacted]

5.3.2 Deficiencies No

Section A6.2/07 Toxicokinetic
Annex Point IIA, VI.6.2 Oral Rabbit

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]
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*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*
Results and discussion	*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*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.2/07-1 Histological findings in animals treated with 0.6 mL/kg bw/day

	Himalayan rabbits	New Zealand White rabbits
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

Table A6.2/07-2 Plasma concentration [µg/mL] of N-acetyl-N-butyl-3-aminopropionic acid in animals treated with 0.6 mL/kg bw/day

day 1	Himalayan rabbits Mean [µg/mL]	New Zealand White rabbits Mean [µg/mL]
[redacted]		
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

Section A6.2/07 Toxicokinetic

Annex Point IIA, VI.6.2 **Oral Rabbit**

[redacted]		
[redacted]		
[redacted]		
[redacted]		
[redacted]		
[redacted]		
[redacted]		
[redacted]		
[redacted]		
[redacted]		
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[redacted]		
[redacted]		
[redacted]		
[redacted]		

[redacted]

Section A6.2/08
Annex Point IIA, VI.6.2

Percutaneous absorption through viable human skin membranes *(in vitro)*

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1 REFERENCE

1.1	**Reference**	[redacted] (2002): In vitro percutaneous absorption with IR3535 through viable human skin membranes; [redacted] Doc. No. 511-003 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Merck KGaA
1.2.2	Companies with letter of access	No companies with Letter of Access.
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Draft OECD guideline for the testing of chemicals (dermal delivery and percutaneous absorption: *in vitro* method, 1996); ECETOC recommendations (1993); report of ECVAM workshop 13 (1996); COLIPA guideline for cosmetic ingredients: percutaneous penetration and absorption (1995)
2.2	**GLP**	Yes
2.3	**Deviations**	Deviations according draft OECD guideline for the testing of chemicals (dermal delivery and percutaneous absorption: *in vitro* method, 1996): Information on solubility properties of the test substance in the receptor phase was not presented.

3 MATERIALS AND METHODS

3.1	**Test material**	a) Radiolabelled IR3535® (^{14}C-IR3535®) for spiking of 10-05/L formulations containing already 15 % (b) or 30 % (c) IR3535®, respectively. b) IR3535® formulation 15 % c) IR3535® formulation 30 % The resulting formulations are: d) IR3535® formulation 15% plus radiolabelled IR3535® e) IR3535® formulation 30% plus radiolabelled IR3535®
3.1.1	Lot/Batch number	a) [redacted] b) [redacted] c) [redacted] d) [redacted] e) [redacted]
3.1.2	Specification	a) [redacted] b) [redacted] c) [redacted] d) [redacted] e) [redacted]

Section A6.2/08 Percutaneous absorption through viable human skin membranes *(in vitro)*

Annex Point IIA, VI.6.2

3.1.3	Purity	a) [redacted] b), c), d) e) [redacted]
3.1.4	Description	a) [redacted] b), c) [redacted] d), e) [redacted]
3.1.5	Radiolabelling	[redacted]
3.1.6	Stability	[redacted]
3.2	**Test organ**	Skin
3.2.1	Species and strain of donor	Human, Caucasian
3.2.2	Number, sex and age of donors	1 donor, female, 41 years old
3.2.3	Gaining of test organ	The donated skin [redacted]) was received after abdominal surgery [redacted]
3.2.4	Handling of the test organ after surgery	The transportation of the skin to the laboratory was carried out within 1 hour of dissection, while the skin was kept on ice in a plastic container. Immediately after arrival, the subcutaneous fat and part of the dermis was removed and the thickness of the skin membranes was measured. Subsequently, the skin membranes were stored at 2-10 °C under sterile conditions for 19 hours prior placing them into the two compartment model.
3.2.5	Description and preparation of test organ	The study was performed on skin membranes of 0.55 ± 0.04 mm thickness and a permeability coefficient (Kp) of less than 2.5×10^{-3} cm/h for tritiated water. The skin membranes were glued to sterile glass rings (internal area 0.64 cm^2) and transferred to 6-well plates which allow the contact to the receptor fluid (two-compartment model).

Section A6.2/08 Percutaneous absorption through viable human skin membranes *(in vitro)*

Annex Point IIA, VI.6.2

3.2.5.1	Receptor fluid	DMEM and HAM F12 culture medium (3:1) supplemented with EGF (10 µg/L), hydrocortisone (400 µg/L), gentamicin (50 mg/L), and Foetal Calf Serum (10 %, v/v).
3.2.5.2	Plates	6-well plates on Netwell insert (200 µm mesh). The 6-well plates were placed in a humidified incubator gassed with 5 % CO_2 and 40 %O_2 at 32 °C. To obtain a homogenous distribution of the receptor fluid the 6-well plates were rocked on a platform ca. 9 times per minute.
3.2.5.3	Skin integrity	Before applying IR3535® in formulations, the skin integrity was assessed by determining the permeability coefficient (Kp) of tritiated water (37.0 MBq/g): after an equilibration period of approximately 1 hour, the inner side of the glass ring was dried under sterile conditions and 200 µL saline containing tritium water (38.0 kBq/mL) was applied in each glass ring. Samples of receptor fluid (200 µL) were collected at 1.0, 2.0, and 3.0 hours after application. Subsequently, tritium water remaining at the application site was removed with a sterile gauze swab. Skin membranes with a Kp below the cut-off value of 2.5×10^{-3} cm/hour were selected for the study.
3.2.5.4	Skin viability	Skin viability was evaluated by measurements of lactate in the receptor fluid (sampled at 4, 8, 12, 20, and 24 hours) on a Hitachi 911 centrifugal analysator, using a Boehringer reagent kit.
3.3	**Administration/ Exposure**	The dose samples was applied topically to the skin membranes as ingredient of 10-05/L formulations as a single application or a multiple dose.
3.3.1	Type of administration	Dermal *in vitro*
3.3.2	Preparation of dose samples	The dose samples were all prepared by adding radiolabelled IR3535® (a) to the formations (b and c) and then placing on a roller platform at 37 C for approximately 1 hour. Prior to the application to the skin membranes, the samples were kept at room temperature for approximately 1 hour.
3.3.3	Dosing regime	Group A: d), single (0 h): 1.63 mg/ cm^2 Group B: d), multiple (0, 4, 8 h): 4.02 mg/ cm^2 Group C: e), single (0 h): 1.70 mg/ cm^2 Group D: e), multiple (0, 4, 8 h): 9.99 mg/ cm^2
3.3.4	Number of replicates	4 replicates for group A, B, C, and D
3.3.5	Duration of treatment	24 hours
3.3.6	Post-exposure period	No
3.3.7	Area covered	0.64 cm^2
3.3.8	Occlusion	No

Section A6.2/08
Annex Point IIA, VI.6.2

Percutaneous absorption through viable human skin membranes *(in vitro)*

3.3.9	Vehicle	Ingredients of the formulations b) and c), except IR3535®: Dow Corning 3225C: 15 % Dow Corning 345: 10.0 % Gilugel SIL 15.0 % Euxyl K100: 0.20 % Water: Ad 100.00 %
3.3.10	Concentration in vehicle	IR3535®: 15 %, 30 %
3.3.11	Total volume applied	Not indicated.
3.3.12	Removal of test substance	Yes: Not during the application, but after 24 hours after the first treatment when all samples of the receptor fluid were taken. The removal of the test substance was performed on all skin samples of group A, B, C, and D to determine the total recovery of the test substance (see 3.4.1)
3.3.13	Controls	Testosterone was used as reference substance (group E). A single dose of 10 µl of non-radiolabelled testosterone plus radiolabelled [4-^{14}C]testosterone in ethanol (2.28 MBq/mL) was applied. The total dose was 16.1 µg/cm^2. The skin viability was evaluated by measurement of lactate in the receptor fluid (group F). No substances were applied.

Section A6.2/08 Percutaneous absorption through viable human skin membranes *(in vitro)*

Annex Point IIA, VI.6.2

3.4 Examinations

3.4.1 Sampling

[redacted]

3.4.2 Tape stripping

[redacted]

3.4.3 Skin washing

[redacted]

3.4.4 Preparation of samples for analysing

[redacted]

3.4.5 Analyser for radioactivity

[redacted]

3.5 Statistics

[redacted]

3.6 Further remarks

[redacted]

Section A6.2/08

Annex Point IIA, VI.6.2

Percutaneous absorption through viable human skin membranes *(in vitro)*

4 RESULTS AND DISCUSSION

4.1 Penetration through viable skin

[redacted]

4.2 Test material retained in skin membranes

[redacted]

4.3 Stripped tapes

[redacted]

4.4 Skin washings

[redacted]

4.5 Ring washings

[redacted]

4.6 Recovery

[redacted]

Section A6.2/08
Annex Point IIA, VI.6.2

Percutaneous absorption through viable human skin membranes *(in vitro)*

4.7 Toxicokinetics

[redacted]

4.8 Metabolites

[redacted]

Section A6.2/08
Annex Point IIA, VI.6.2

Percutaneous absorption through viable human skin membranes *(in vitro)*

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The intention of this study was to examine the *in vitro* percutaneous absorption of IR3535® through fresh viable human skin membranes following the respective draft OECD guideline for the testing of chemicals (dermal delivery and percutaneous absorption: *in vitro* method, 1996).

Therefore, IR3535® formulated as 15% and 30% in a cream and spiked with radiolabelled IR3535® was applied to human skin samples of one female human donor. Each formulation was applied either as single dose or as a multiple doses (3 times at 0, 4 and 8 h, 4 replicates each).

The skin acts as two-compartment model: The dermal side of the skin was in contact with the receptor fluid, while the stratum corneum, exposed to air, was applied with IR3535®. The receptor fluid was sampled after 4, 8, 12, 20 and 24 hours and the cumulative penetration (0-4h, 0-8h, 0-12h, 0-20h and 0-24h) was investigated by radioactivity measurements.

At the end of the experiment the remaining test substance on the application site was removed by means of one cotton swab soaked in 50% hand soap/water and two cotton swabs soaked with water. Afterwards, the application site was tape stripped and then the skin membranes were digested.

Finally, the total recovery of radioactivity were investigated by separate measurements for radioactivity of all compartments (receptor compartment, skin tissue, tape strips, glass ring, and cotton swaps).

All radioactivity measurements were performed by a scintillation counter.

5.2 Results and discussion

<u>Penetration after 8 hours:</u>

After single application of IR3535® 21.7 and 28.9 % of the applied dose had penetrated for the 15 and 30 % formulation, respectively.

After multiple application of IR3535® 21.4 and 11.3 % of the applied dose had penetrated for the 15 and 30 % formulation, respectively.

<u>Penetration after 24 hours:</u>

If a single dose of IR3535® was applied, the percentage of the dose percutaneously penetrating through skin 24 hours after application was higher compared to multiple dosing.

Maximal penetration of 67 % of the dose was found for single application of 30 % IR3535®.

Maximal penetration of 43 % of the dose was found for multiple application of 15 % IR3535®.

The amount retained in the skin membranes was small and nearly identical in all samples (about 4 % of the dose).

The steady state was reached after about 1 hour (single application) or about 3 hours (multiple application).

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Annex Point IIA, VI.6.2

Percutaneous absorption through viable human skin membranes *(in vitro)*

5.3	**Conclusion**	IR3535® penetrated through viable human skin: The skin delivery (receptor fluid recovery plus skin residue) was about 70 % after an exposure of 24 hours at maximum (single dose, 30 % IR3535®). After 8 hours exposure the dermal penetration was between 11.3 % (multiple dose, 30 % IR3535®) and 28.9 % (single dose, 30 % IR3535®).
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	No

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]
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*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*
Results and discussion	*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*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.2/08-1 Percutaneous penetration *in vitro* of IR3535®

Group	Group size (replicates)	Test substance (radioactive concentration)	Total mean dose [mg/cm^2]	Application at [hour]	Sampling of receptor fluid (2 × 500 µL) at [hour]	Penetration within 8 hours*1) [% of the dose]	Penetration within 24 hours*1) [% of the dose]	Flux constant*1) [mg.cm^{-2}.h^{-1}]	Kp value*1) [cm.h^{-1}]	Lag time*1) [h]
[redacted]										
[redacted]										
[redacted]										
[redacted]										
[redacted]										
[redacted]										

[redacted]

Table A6.2/08-2 Recovery of IR3535® in human skin, % of dose

Group	Receptor fluid and cell wash*1)	Tape strips*1)	Skin rinse*1)	Ring*1)	Skin membrane (tissue)*1)	Total recovery*1)
[redacted]						

[redacted]

Table A6.2/08-3 Skin viability assessment: Lactate concentration in receptor fluid

Time	Lactate (U/L)*5)
[redacted]	

[redacted]

Table A6.2/08-4 Percutaneous penetration *in vitro* of IR3535® in human skin, % of applied dose

Time	Group A 15%, single*1)	Group B 15%, multiple*1)	Group C 30%, single*1)	Group D 30%, multiple*1)
[redacted]				

[redacted]

Section A6.2/09 Toxicokinetic and metabolism in humans

Annex Point IIA, VI.6.2

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		1 REFERENCE
1.1	**Reference**	[redacted] (2010) Biotransformation and toxicokinetics of IR3535® in humans after dermal exposure, [redacted] (unpublished report)
1.2	**Data protection**	Yes
1.1.1	Data owner	Merck KGaA
1.1.2	Companies with letter of access	none
1.1.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I.
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	No
2.2	**GLP**	No The study director, [redacted], is an acknowledged expert in the field of toxicokinetics and metabolism with a wide experience in the performance of kinetic studies in humans [redacted] is not GLP certified. However, the Institute follows internal quality guidelines (SOPs) and the study was conducted taking into account the basic principles of GLP. The study was inspected once by the GLP unit of the Sponsor at 6 July 2010.
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	
3.1.1	Lot/Batch number	a) [redacted] b) [redacted]
3.1.2	Specification	a) IR3535®, as given in section 2 b) Formulation EUS26-15 (pump spray) containing 20% IR3535®
3.1.3	Purity	a) [redacted] b) [redacted]
3.1.4	Description	a) [redacted] b) [redacted]
3.1.5	Stability	a) [redacted] b) [redacted]
3.1.6	Radiolabelling	[redacted].
3.2	**Test Subjects**	
3.2.1	Species	Human volunteers
3.2.2	Sex	Male and female

Section A6.2/09 Toxicokinetic and metabolism in humans

Annex Point IIA, VI.6.2

3.2.3	Age/weight at study initiation	Males: 20-24 years / 70-83 kg Females: 25-32 years / 50-62 kg
3.2.4	Number of subjects per group	5 subjects/sex
3.3	**Administration/ Exposure**	
3.3.1	Dosing regime	Single application of approx. 3 grams of a formulation containing 20% IR3535®.
3.3.2	Type	Single dermal application, exposed areas: legs, arms, face, neck, hands, feet (ca. 50% of the body surface). Subjects took a shower after 12 hours of exposure.
3.3.3	Vehicle	Formulation EUS26-15 consists of: [redacted]
3.3.4	Concentration in vehicle	20%
3.3.5	Total volume applied	Approx. 3 grams/person
3.3.6	Controls	The plasma of each volunteer was analyzed for IR3535® and IR3535®-free acid 24 hours prior dermal exposure. Neither the parent compound nor its only metabolite was detected in the samples.
3.4	**Examinations**	
3.4.1	Excretion routes	[redacted]
3.4.2	Body fluids sampled	[redacted]
3.4.3	Tissues sampled	[redacted]
3.5	**Statistics**	[redacted]

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3.6 Further remarks

[redacted]

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4 RESULTS AND DISCUSSION

4.1 Excretion balance

[redacted]

4.2 Tissue distribution

[redacted]

4.3 Metabolites

[redacted]

4.4 Absorption

[redacted]

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of this study was to determine extent of absorption and kinetics of excretion of IR3535® in humans after dermal application.

The toxicokinetic behaviour of IR3535® in humans was determined in five male and five female volunteers after application of a repellent formulation containing 20% IR3535®. Approx. 3 grams of the formulation were applied once to hands, arms, legs, feet, face and neck of each volunteer (ca. 50% of total body area). All volunteers showered 12 hours after application of the formulation. Urine and blood samples were taken at predetermined time points (urine: -1, 4, 8, 12, 16, 24, 36, 48 hours after application; plasma: -24, 0, 0.5, 1, 1.5, 2, 4, 6, 8, and 24 hours after application). The concentrations of IR3535® **1** and IR3535®-free acid **2** in urine and plasma were determined by LC-MS/MS.

AUC values were calculated using the software Kinetica v. 4.4.1. Absorption rates were calculated based on total amounts of IR3535® and IR3535®-free acid in the urine over 48 hours.

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5.2	**Results and discussion**	Concentrations of IR3535® and IR3535®-free acid in plasma: In plasma, concentrations of the parent compound IR3535® were at or below the limit of quantification (0.037 µmol/L). IR3535®-free acid peaked in plasma samples 2 – 6 hours after dermal application (see Figure A6.2/09-2). Cmax mean values for the free acid were 5.7 µmol/L in males, 3.0 µmol/L in females and 4.2 µmol/L in all volunteers. Mean AUC values for IR3535®-free acid were 41.6, 24.5 and 33.9 µmolxL^{-1}xh in males, females and all subjects, respectively. Concentrations of IR3535® and IR3535®-free acid in urine: In urine samples of all volunteers, both IR3535® and IR3535®-free acid were detected, however, only trace amounts of the parent compound IR3535® were found. Concentrations of IR3535®-free acid were several thousand-fold higher than the parent compound and peaked at the first two sampling points (4 h and 8 h after dermal application, see Fig. A6.2/09-2). Overall, IR3535® and IR3535®-free acid excreted with urine over 48 h represented 13.3 ± 3.05 % of the dose applied. Since IR3535® is rapidly and extensively metabolized and IR3535®-free acid has a low molecular weight and high water solubility, it is expected that urinary excretion of IR3535®-free acid and IR3535® represents the total extent of absorption of IR3535® in humans.
5.3	**Conclusion**	Based on the results of this study the dermal penetration rate of IR3535® is 13.3% after application of a typical market formulation under use conditions.
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	No

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]
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

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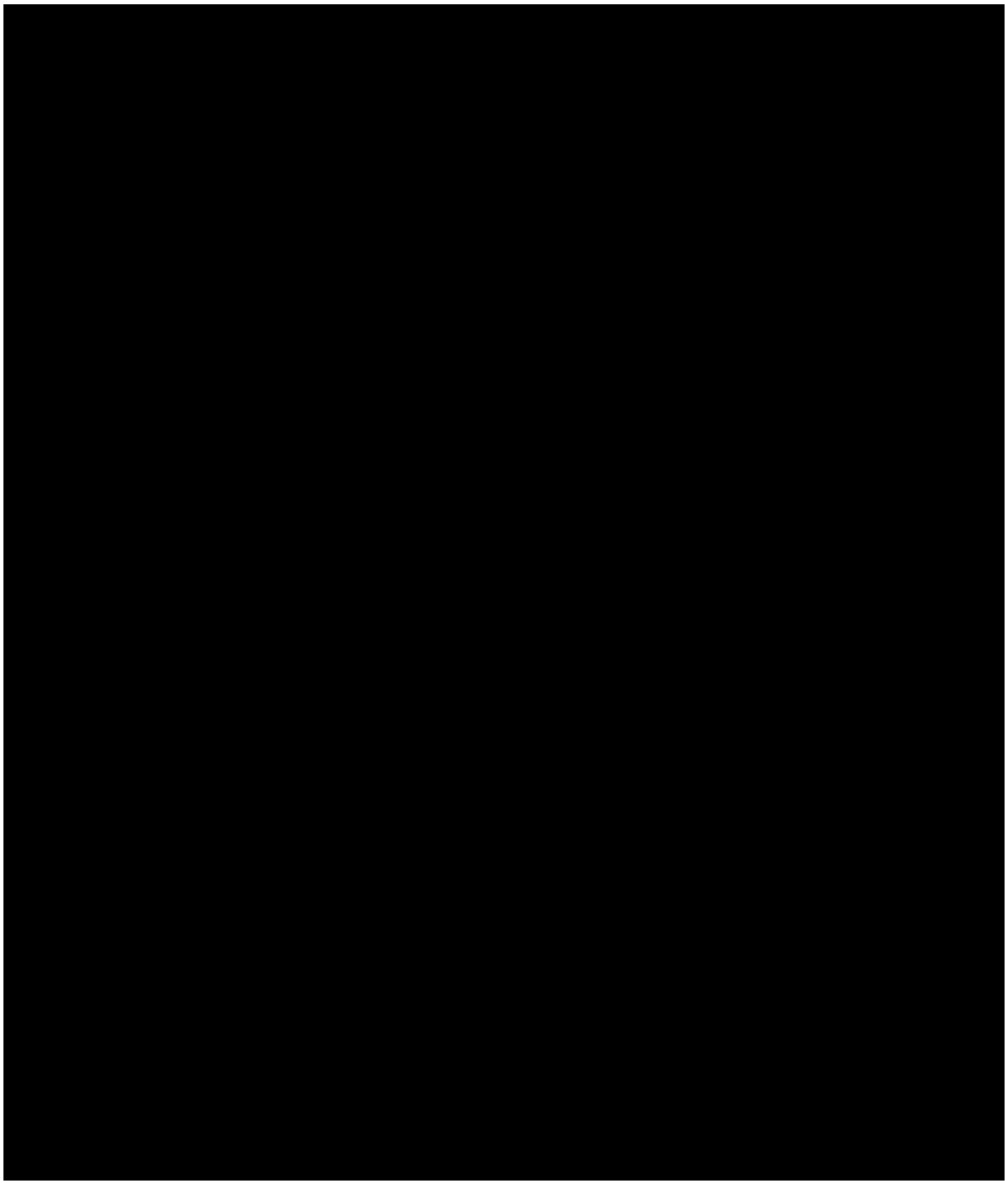
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating 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*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.2/09-1: Calculation of the absorption rate based on urinary excretion

Volunteers[3)]	Sex	Dose of formulation received [g]	Amount of IR3535[1)] applied [µmol]	IR3535 in urine [µmol]	IR3535-free acid[2)] in urine [µmol]	Total Recovery in urine [µmol]	Absorption [%]
[redacted]							



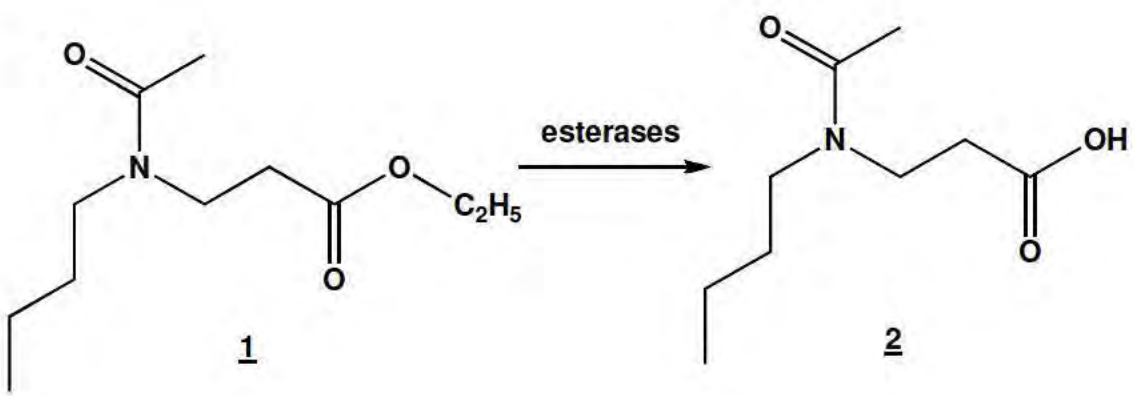


Figure A6.2/09-3: Biotransformation of IR3535® 1 to IR3535®-free acid 2 in mammals. 2 is the only known metabolite of 1 in mammals.