WHO SPECIFICATIONS AND EVALUATIONS

FOR PUBLIC HEALTH PESTICIDES

ETHYL BUTYLACETYLAMINOPROPIONATE*

also known as

IR3535®

3-(*N*-acetyl-*N*-butyl)aminopropionic acid ethyl ester

World Health Organization

* Ethyl butylacetylaminopropionate is the INCI common name but, for ease of reference, the trade name IR3535® may be used.

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Disclaimer[1]

WHO specifications are developed with the basic objective of promoting, as far as practicable, the manufacture, distribution and use of pesticides that meet basic quality requirements.

Compliance with the specifications does not constitute an endorsement or warranty of the fitness of a particular pesticide for a particular purpose, including its suitability for the control of any given pest, or its suitability for use in a particular area. Owing to the complexity of the problems involved, the suitability of pesticides for a particular purpose and the content of the labelling instructions must be decided at the national or provincial level.

Furthermore, pesticides which are manufactured to comply with these specifications are not exempted from any safety regulation or other legal or administrative provision applicable to their manufacture, sale, transportation, storage, handling, preparation and/or use.

WHO disclaims any and all liability for any injury, death, loss, damage or other prejudice of any kind that may be arise as a result of, or in connection with, the manufacture, sale, transportation, storage, handling, preparation and/or use of pesticides which are found, or are claimed, to have been manufactured to comply with these specifications.

Additionally, WHO wishes to alert users to the fact that improper storage, handling, preparation and/or use of pesticides can result in either a lowering or complete loss of safety and/or efficacy.

WHO is not responsible, and does not accept any liability, for the testing of pesticides for compliance with the specifications, nor for any methods recommended and/or used for testing compliance. As a result, WHO does not in any way warrant or represent that any pesticide claimed to comply with a WHO specification actually does so.

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[1] This disclaimer applies to all specifications published by WHO.

INTRODUCTION

WHO establishes and publishes specifications* for technical material and related formulations of public health pesticides with the objective that these specifications may be used to provide an international point of reference against which products can be judged either for regulatory purposes or in commercial dealings.

From 2002, the development of WHO specifications follows the **New Procedure**, described in the 1st edition of Manual for Development and Use of FAO and WHO Specifications for Pesticides (2002). This **New Procedure** follows a formal and transparent evaluation process. It describes the minimum data package, the procedure and evaluation applied by WHO and the experts of the "FAO/WHO Joint Meeting on Pesticide Specifications" (JMPS).

WHO Specifications now only apply to products for which the technical materials have been evaluated. Consequently, from the year 2002 onwards the publication of WHO specifications under the **New Procedure** has changed. Every specification consists now of two parts, namely the specifications and the evaluation report(s):

Part One: The Specification of the technical material and the related formulations of the pesticide in accordance with chapters 4 to 9 of the 1st edition of the "FAO/WHO Manual on Pesticide Specifications."

Part Two: The Evaluation Report(s) of the pesticide, reflecting the evaluation of the data package carried out by WHO and the JMPS. The data are provided by the manufacturer(s) according to the requirements of chapter 3 of the "FAO/WHO Manual on Pesticide Specifications" and supported by other information sources. The Evaluation Report includes the name(s) of the manufacturer(s) whose technical material has been evaluated. Evaluation reports on specifications developed subsequently to the original set of specifications are added in a chronological order to this report.

WHO specifications under the **New Procedure** do not necessarily apply to nominally similar products of other manufacturer(s), nor to those where the active ingredient is produced by other routes of manufacture. WHO has the possibility to extend the scope of the specifications to similar products but only when the JMPS has been satisfied that the additional products are equivalent to that which formed the basis of the reference specification.

Specifications bear the date (month and year) of publication of the current version. Dates of publication of the earlier versions, if any, are identified in a footnote. Evaluations bear the date (year) of the meeting at which the recommendations were made by the JMPS.

* Footnote: The publications are available on the Internet under (http://www.who.int/whopes/quality/en/).

PART ONE

SPECIFICATIONS

IR 3535

ETHYL BUTYLACETYLAMINOPROPIONATE (IR3535®)

INFORMATION

Common name

Ethyl butylacetylaminopropionate (INCI*)

Synonyms

IR3535®, AI 3-70763, EBAAP, ethyl 3-(*N*-butylacetamido)propionate, Merck 3535, OMS 3065

Chemical names

IUPAC: 3-(*N*-acetyl-*N*-butyl)aminopropionic acid ethyl ester

CA: beta-alanine, *N*-acetyl-*N*-butyl-, ethyl ester

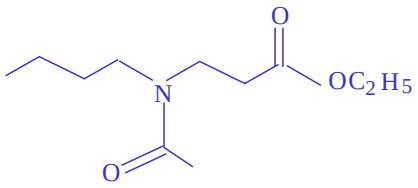
CAS Registry number

52304-36-6

CIPAC number

667

Structural formula



Empirical formula

$C_{11}H_{21}NO_3$

Relative molecular mass

215.3

* INCI is the International Nomenclature of Cosmetic Ingredients, a system developed by the European Cosmetic, Toiletry and Perfume Association (COLIPA).

ETHYL BUTYLACETYLAMINOPROPIONATE (IR3535®)*
TECHNICAL MATERIAL

WHO specification 667/TC (February 2006**)

This specification, which is PART ONE of this publication, is based on an evaluation of data submitted by the manufacturer whose name is listed in the evaluation report (667/2005). It should be applicable to relevant products of this manufacturer but it is not an endorsement of those products, nor a guarantee that they comply with the specification. The specification may not be appropriate for the products of other manufacturers. The evaluation report 667/2005, as PART TWO, forms an integral part of this publication.

1 Description

The material shall consist of ethyl butylacetylaminopropionate (IR3535®) together with related manufacturing impurities, in the form of a colourless to slightly yellowish and almost odourless liquid, free from visible extraneous matter and added modifying agents.

2 Active ingredient

2.1 **Identity tests** (667/TC/M/2, CIPAC Handbook, Note 1)

The active ingredient shall comply with an identity test and, where the identity remains in doubt, shall comply with at least one additional test.

2.2 **Ethyl butylacetylaminopropionate (IR3535®) content** (667/TC/M/3, CIPAC Handbook, Note 1)

The ethyl butylacetylaminopropionate content shall be declared (not less than 980 g/kg) and, when determined, the average measured content shall not be lower than the declared minimum content.

3 Physical properties

3.1 **pH range** (MT 75.3, CIPAC Handbook J, p.131, 2000))

pH range of a 5% aqueous solution: 4.0 to 6.0.

Note 1 Methods for the identification and determination of ethyl butylacetylaminopropionate (IR3535®) content were adopted by CIPAC in 2005 but are not yet published in a Handbook. Prior to publication of the Handbook, copies of the methods may be obtained through the CIPAC website, http://www.cipac.org/prepubme.htm or from the CIPAC Secretary, Dr László Bura (mail to bura.laszlo@ntksz.ontsz.hu).

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* In the absence of an ISO common name, and for ease of reference, the proprietary code name IR3535® may be used instead of the INCI common name, ethyl butylacetylaminopropionate.

** Specifications may be revised and/or additional evaluations may be undertaken. Ensure the use of current versions by checking at: http://www.who.int/whopes/quality/en/.

PART TWO

EVALUATION REPORTS

ETHYL BUTYLACETYLAMINOPROPIONATE (IR3535®)

WHO SPECIFICATIONS AND EVALUATIONS FOR
PUBLIC HEALTH PESTICIDES

ETHYL BUTYLACETYLAMINOPROPIONATE (IR3535®)

EVALUATION REPORT 667/2005

Recommendations

The Meeting recommended that:
1) the existing interim specification for ethyl butylacetylaminopropionate (IR3535®) TC should be withdrawn;
2) the proposed specification for ethyl butylacetylaminopropionate (IR3535®) TC should be adopted by WHO.

Appraisal

The data for ethyl butylacetylaminopropionate (IR3535®) were evaluated for review of an interim WHO specification for the TC (WHO/IS/TC/667/2001). No specifications were submitted for the formulations, which include solids and a diverse range of liquid materials.

The draft specification and the supporting data were provided by Merck KGaA, Germany, in 2004. The active ingredient is not under patent.

IR3535® does not have an ISO common name and its INCI common name, ethyl butylacetylaminopropionate, is less convenient for general use than the proprietary name, IR3535®.

IR3535® is an insect repellent that is not under patent and has been evaluated by WHOPES for efficacy (WHO 2001). WHO reports on efficacy were not relevant to, nor used in, the present evaluation.

IR3535® is moderately volatile, moderately soluble in water, very soluble in a wide range of organic solvents but it is not classed as fat soluble. It does not absorb UV light at wavelengths >250 nm and therefore photolysis should not occur.

Confidential information on the manufacturing process, and the impurities present at or above 1 g/kg, was provided by the proposer. Mass balances were high, in the range 996-1015 g/kg, and unknowns did not exceed 2 g/kg. These data were confirmed as essentially similar to those submitted to the authorities for registration of IR3535® in Australia (APVMA 2005). The minimum content of active ingredient is 980 g/kg (average 997, minimum 988, maximum 999, n=93, 2-year period).

The existing interim specification identifies no relevant impurities. When the existing specification was developed, WHO/PCS considered that there was no evidence to suggest that the impurities are more toxic than the active ingredient. WHO/PCS opinion remained unchanged and the Meeting agreed that none of the impurities should be designated as relevant.

IR3535® has neither acidic nor basic properties, so the pH of 4.7 produced by a 5% solution of pure active ingredient in CO_2-free water presumably resulted from acidic impurities. In a 3-month period in which water was not rate limiting (5% aqueous

solution), IR3535® was <0.2% degraded at 2-6ºC, about 10% degraded at 20-25ºC and about 80% degraded at 40ºC. Acid-catalyzed hydrolysis is known to occur and, although no data were provided, presumably alkaline hydrolysis can also occur. Although IR3535® is hydrolyzed only slowly, even at 40ºC, it is acid-catalyzed and therefore control of acid content is important for stability of IR3535®. The existing interim specification for TC includes a clause for pH range (4-6, 5% solution) The manufacturing specification (Merck 1999) also provides a limit of 1 g/kg acidity, calculated as acetic acid. Acetic acid (pK_a 4.7), as an hydrolysis product, is presumably the impurity which determines the pH of IR3535® aqueous solutions. If so, specification of a pH range of 4-6 provides a rather better means of controlling acidity than limiting it to 1 g/kg and also ensures that alkaline hydrolysis cannot occur. The Meeting therefore agreed that the clause for pH range is both necessary and appropriate.

Data provided on the toxicity of IR3535® indicate that the main hazards relate to eye and skin irritation. The associated risks were consequently evaluated by WHO/PCS for development of the existing specification. The skin irritation observed in animal experiments was mild and had not been observed in humans. As eye exposure can be prevented, the irritation risks were considered acceptable by WHO/PCS.

No long-term toxicity or carcinogenicity studies were reported. In reviewing data for the existing interim specification, WHO/PCS considered that the consistently negative findings in genotoxicity testing, together with the apparently innocuous chemical structure, make it unlikely that IR3535® is carcinogenic to humans. At that time (2001), WHO/PCS indicated that, considering the direct and potentially long-term dermal exposure resulting from intended use of the chemical, it would be advisable for the manufacturer to conduct a long-term carcinogenicity assay in rodents. The manufacturer stated that the extensive data package available shows that IR3535® does not pose the risk of carcinogenicity. The manufacturer also stated that in the company's submission for EU review, taking into account the negative results of genotoxicity and mutagenicity studies and in the interests of animal welfare, a carcinogencity study will not be conducted but that a sub-chronic toxicity study in a second animal species (non-rodent) is being conducted, for completion at the end of 2005 (Merck 2004a).

The overall WHO/PCS secretariat view, supported by registration acceptance decisions in the USA, Australia and elsewhere, was that the intended use of IR3535® as an insect repellent does not pose undue risks to the users.

No data were provided on the ecotoxicity of IR3535®. The manufacturer stated that the active ingredient is unlikely to pose risks to the environment in normal use. The WHO/PCS concurred with this view, as the intended use is unlikely to lead to release of the chemical in the environment.

Physical and toxicological test methods followed internationally recognised protocols.

The analytical method for determination of the active ingredient, in TC only, was adopted by CIPAC in 2005. The manufacturer produces no formulations but IR3535® is formulated by several hundred other companies throughout the world, each producing 3-10 different formulations (such as lotions, pump-sprays, aerosols, roll-ons, sticks, powders, gels, creams, wet-wipes). The formulants and co-formulated active ingredients (allantoin, bisabolol, film-forming agents, anti-ageing actives, polyglycols, UV filters, and many others) are different in each of >1000

formulations. There is no “typical” formulation and the manufacturer believed that collaborative study of the method on any particular formulation could give an inappropriate indication of method performance to be expected with other formulations (Merck 2004b). The Meeting accepted this argument.

Identity tests utilizing GC relative retention time and IR spectrum were considered acceptable but the Meeting noted that refractive index is likely to be of limited value as an identity test.

The draft specification for TC was in accordance with the requirements of the manual (FAO/WHO 2002).

SUPPORTING INFORMATION

FOR

EVALUATION REPORT 667/2005

Uses

IR3535 is an insect repellent for application to human skin and clothing in public health applications, to repel biting arthropods such as mosquitoes, flies and ticks (WHO 2001). The efficacy of IR3535 was independently evaluated by Cilek *et al*. (2004).

Identity

INCI name: ethyl butylacetylaminopropionate

Synonyms: IR3535®, AI 3-70763, EBAAP, Merck 3535,
ethyl 3-(*N*-butylacetamido)propionate

Chemical names

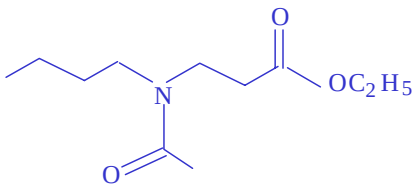
IUPAC: 3-(*N*-acetyl-*N*-butyl)aminopropionic acid ethyl ester

CA: *beta*-alanine, *N*-acetyl-*N*-butyl-, ethyl ester

CAS No: 52304-36-6

CIPAC number *667*

Structural formula:



Molecular formula: $C_{11}H_{21}NO_3$

Relative molecular mass: 215,3

Identity tests: GC (relative retention time), IR spectrum (Merck 1996b)

Physico-chemical properties of IR3535®

Table 1. Physico-chemical properties of pure IR3535®

Parameter	Value(s) and conditions	Purity %	Method	Reference
Vapour pressure:	0.15 ± 0.01 Pa at 20°C	99.8	OECD no.104; EEC A.4; EPA D § 63-9	183634
Melting point	Liquid at room temperature	99.8	-	183612
Boiling point	Estimated metastable boiling point at 1 atm, slightly below 300°C. About 110ºC at 0.02 kPa	99.8	EPA D § 63-6; EEC A.2	183612
Temperature of decomposition	141°C (TC)	99.8	EPA D § 63-6; EEC A.2	183612
Solubility in water:	70 ± 3 g/l at 20°C non-buffered	99.8	OECD no.105; EEC A.6; EPA D § 63-8	183645

Table 1. Physico-chemical properties of pure IR3535®

Parameter	Value(s) and conditions	Purity %	Method	Reference
Solubility in organic solvents at room temperature:	*n*-heptane, >1000 g/l dichloromethane, >1000 g/l ethyl acetate, >1000 g/l *p*-xylene, >1000 g/l acetone, >1000 g/l methanol, 865 g/	99.8	EPA D § 63-8	183735
Octanol / water partition coefficient:	log P_{OW} = 1.7 at 23°C unbuffered	99.8	OECD no.117; EEC A.8; EPA D § 63-11	183656
Hydrolysis characteristics:	In 3 months, 5% w/w solutions in non-buffered water (initial solution pH 4.7, initial free acid <0.1 g/kg IR3535) showed no measurable (<0.2%) degradation in a refrigerator (+2 to +6°C), about 10% degradation at room temperature (20-25°C), and about 80% degradation at 40°C. Hydrolysis of the ester is acid catalyzed.	not reported	Not reported. Measurements made by HPLC with external standardization.	Merck 1996a
Storage stability	A sample was found to contain 998 g/kg before and after storage for 14 d at 54 ± 2ºC.	99.8%	CIPAC MT 46; EPA D § 63-13	183757
Photolysis characteristics:	No UV absorption >250 nm and not subject to direct photolysis	99.8	OECD draft; EPA Subdivision D Sec. 63-13; EU Directive 95/36/EC	184433
Dissociation characteristics:	None. 5% solution in non-buffered CO_2-free water was pH 4.7	99.8	CIPAC MT 75; EPA D § 63-12	183702
Flash point	159°C	99.8	DIN EN 22719; EEC A.9; EPA D § 63-15	183667
Mass per millilitre	998 g/l at 20 ± 0.5°C	99.8	EEC A.3; OECD 109; EPA D § 63-7	183623

Table 2. Chemical composition and properties of technical IR3535® (TC)

Manufacturing process, maximum limits for impurities ≥ 1 g/kg, 5 batch analysis data.	Confidential information supplied and held on file by WHO. Mass balances were 99.6-101.5% and percentages of unknowns were 0.16-0.21% (183746).
Declared minimum IR3535 content:	980 g/kg
Relevant impurities ≥ 1 g/kg and maximum limits for them:	None
Relevant impurities < 1 g/kg and maximum limits for them:	None
Stabilisers or other additives and maximum limits for them:	None

Table 2. Chemical composition and properties of technical IR3535® (TC)

Melting or boiling temperature range	Estimated metastable boiling point, slightly below 300°C. Not stable above about 141°C.

Toxicological summaries

IR3535 was reviewed by US EPA in 1999 (Reg. No. 70759). It was evaluated for efficacy by the WHOPES programme and has also been evaluated by WHO/PCS (WHO 2001). It has been registered for use in many countries, most recently in Russia (September 2004) and P.R. China (November 2004) and data have been provided for evaluation under the EU Biocidal Products Directive (submission number 257-835-0), for review in 2006.

No data were available on the ecotoxicology profile of IR3535®. The Proposer noted that national authorities do not request such data because this active ingredient is unlikely to pollute the environment by direct use.

The WHO hazard classification for IR3535® is Class U: unlikely to present acute hazard in normal use (WHO 2004). The use pattern makes it unlikely that ethyl butylacetylaminopropionate will be evaluated by FAO/WHO JMPR. The compound has been assessed according to EU Directives and allocated the following risk phrases:

Xi Irritant.
R 36 Irritating to eyes.
S 26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

Formulations

The main formulation types are lotions, creams, milks, gels, sprays, roll-ons and powders. These formulations are not prepared by the manufacturer of IR3535® but they are registered and sold in many countries throughout the world.

Methods of analysis

The analytical method for identification and determination of IR3535® is based on gas chromatography using a flame ionization detector and internal standardization using methyl undecanoate. The method was adopted, with provisional status, by CIPAC in 2005.

Identification is by GC relative retention time and IR spectrum. The manufacturer indicated that refractive index may also be used.

Impurity profile data were generated using a similar method as for determination of the active ingredient but the TC was injected directly without dilution and quantification was by area percent. The material accountability study was performed according to the US Environmental Protection Agency's, Pesticide Assessment Guidelines, Subdivision D; Series 62.

Physical properties

Test methods used to determine the physical properties of technical active ingredient were OECD, EPA, EU and DIN.

Containers and packaging

The active ingredient and its formulations should be stored in high density polyethylene containers.

Expression of the active ingredient

The active ingredient is expressed as ethyl butylacetylaminopropionate (IR3535®).

ANNEX 1

HAZARD SUMMARY PROVIDED BY THE PROPOSER

Note: The proposer provided written confirmation that the toxicological and ecotoxicological data included in the following summary were derived from IR3535® having impurity profiles similar to those referred to in Table 2, above.

Table A. Toxicology profile of technical IR3535®, based on acute toxicity, irritation and sensitization

Species	Test	Duration and conditions or guideline adopted	Result	Reference
Rat, Wistar (5 males, 5 females)	oral	87/176/EEC	LD_{50} >5000 mg/kg bw	T14215
Rat, Sprague-Dawley (5 males, 5 females per dose group)	oral	Undiluted test material applied at 7.9, 10.0, 12.6, 15.9 and 20.0 ml/kg	LD_{50} = 14.0 ml/kg	Merck 1973a
Rat, Wistar (6 males per dose level)	oral	Undiluted test material applied by stomach tube; 20-30 ml/kg at 2 ml/kg dose intervals.	LD_{50} = 24 ml/kg	Merck 1981e
Dog, mongrel (1 male, 1 female per dose group)	oral	Undiluted material by gavage at 1, 2, 4 and 8 g/kg bw followed by 10 ml water	All animals survived; doses of 2 g/kg bw or more induced vomiting after 30-60 minutes followed by salivation after 20-30 minutes.	Merck 1981a
Rat, Sprague-Dawley (5 males, 5 females per dose level)	dermal	Undiluted test material applied to shaven dorsal skin (6 h exposure; recovery period 14 d); doses 6.35, 7.9, 10.00 ml/kg.*	Up to 10 ml/kg bw no systemic reactions observed; erythemas at all dose levels, one rat had an erythema of grade 2.	Merck 1973b
Mouse, NMRI (5 males, 5 females per dose level)	dermal	Undiluted test material applied to shaven dorsal skin (6 h exposure; recovery period 14 d); doses 6.35, 7.9, 10.00 ml/kg.*	Up to 10 ml/kg bw no systemic reactions observed; erythemas observed at all doses.	Merck 1981b
Dog, beagle (1 male, 1 female per dose level)	dermal	Undiluted test material applied to shaven dorsal skin (6 h exposure; recovery period 14 d); doses 6.35, 7.9 and 10.00 ml/kg.*	Up to 10 ml/kg bw no systemic reactions observed; local erythemas observed at all doses.	Merck 1981c
Rat (5 males, 5 females)	inhalation	EPA Guideline No. 81-3; OECD Guideline 403	LC_{50} > 5.1 mg/l	13I0189/957012
Rabbit, New Zealand White (3 males, 3 females)	skin irritation	10% solution (in 50% aqueous ethanol) applied to shaven intact or scarified dorsal skin; 24 h exposure, 14 d recovery period.	No reactions of local or systemic intolerance.	Merck 1973c

Table A. Toxicology profile of technical IR3535®, based on acute toxicity, irritation and sensitization

Species	Test	Duration and conditions or guideline adopted	Result	Reference
Human volunteers (30)**	skin irritation	Closed epicutaneous irritation test using 15% a.i. in aqueous alcohol.	No skin reactions were observed.	Merck 1979
Human volunteers (10)**	skin irritation	Repetitive exposure test using 15% a.i. in aqueous alcohol; 3 wk at twice/wk (induction phase); 12 d break; then 7th application (challenge).	No toxic or allergic reactions.	Merck 1979
Guinea pig, Himalayan white spotted albino (10)	phototoxic potential	10% solution (+ 2% DMSO) in ethanol applied to shaven flanks.	No erythema or oedema observed at UV-exposed and unexposed sites.	061773
Guinea pig, Himalayan white spotted albino (10)	photo-allergenic potential	10% solution in ethanol applied to shaven nuchal area.	No erythema or oedema observed at UV-exposed and unexposed sites.	061762
Rabbit, New Zealand White (6)	photochemical skin study	25% solution in 95% (aqueous) ethanol applied to intact skin and exposed to UV light.	No photochemical reactions were observed.	51-0014-77
Rabbit (males, females)	eye irritation	EPA Guideline No. 81-4. Purity 98.9% (GC assay).	Undiluted active ingredient classified as irritant to the eye.	40/12/96
Rabbit (5)	eye irritation	10% solution in olive oil. Purity not recorded.	Erythema and swelling completely receded after 24 and 72 h, respectively.	Merck 1972a
Rabbit (2 groups of 3 animals each)	eye irritation	Single treatment with undiluted test material. Eyes either rinsed or not rinsed. Purity not recorded.	Intolerance reactions observed which receded more quickly in group with rinsed eyes.	Merck 1972b
Rabbit, albino (2 groups of 4 animals each)	eye irritation	15% aqueous alcoholic solution. Eyes either rinsed or not rinsed. Purity not recorded.	Intolerance reactions observed which receded more quickly in group with rinsed eyes.	Merck 1979
Rabbit, albino (6)	eye irritation	20% liquid in *iso*-propyl palmitate. Purity not recorded.	After 48 h complete recession of mild irritation.	Merck 1980
Rabbit, New Zealand White (6)	eye irritation	0.1 ml undiluted TC applied to one eye of each rabbit. Purity not recorded.	Mild injury to cornea and conjunctiva.	51-0014-77

Table A. Toxicology profile of technical IR3535®, based on acute toxicity, irritation and sensitization

Species	Test	Duration and conditions or guideline adopted	Result	Reference
Guinea pig, Hartley (male, female, total of 43)	skin sensitization	EPA Guideline No. 81-6 (Buehler technique)	No sensitization potential.	96-8304-21
Guinea pig, Hartley (10)	skin sensitization	Intra-dermal injection of 0.1 ml 0.1% suspension in propylene glycol/saline	No sensitization potential.	51-0014-77

* Dosage levels for dermal exposure of mice, rats and dogs were identical on a body weight basis.

** WHO and FAO discourage human volunteer studies on pesticides and the JMPS does not normally include such data in evaluations. However, IR3535® is not a pesticide but an insect repellent, intended specifically for use on humans. Inclusion of the data was considered to be appropriate in this special case.

Table B. Toxicology profile of technical IR3535®, based on repeated administration (sub-acute to chronic)

Species	Test	Duration and conditions or guideline adopted	Result	Reference
Rat, Sprague-Dawley (15 males, 15 females per dose group)	4-week oral feeding	900 or 2700 mg/kg bw daily.	LOEL >2700 mg/kg bw	Merck 1974a
Dog, beagle (3 males and 3 females per dose group)	4-week oral feeding	100 or 1800 mg/kg bw daily by gavage in 1% aqueous methyl-hydroxyethyl cellulose gel.	Lowest toxic dose assumed to be >1800 mg/kg bw, although some test material perhaps lost by vomiting at this dose. Vomiting started 30 min after dosing on 2-7 d/wk.	Merck 1981d
Rabbit, New Zealand White (3 males and 3 females per dose group)	4-week oral feeding	500 or 1500 mg/kg bw daily by gavage in 1% aqueous carboxy-ethylcellulose gel.	Lowest toxic dose assumed to be between 500 and 1500 mg/kg bw per day. 1500 mg/kg led to deeper breathing and unrest for short period after dosing. Food consumption and body weight gain significantly reduced. No other effects.	Merck 1974b
Rabbit, New Zealand White	2-week oral feeding	EPA Guideline No. 83-3	Slight inhibition of food consumption and body weight gain at 600 mg/kg/day.	149022

Table B. Toxicology profile of technical IR3535®, based on repeated administration (sub-acute to chronic)

Species	Test	Duration and conditions or guideline adopted	Result	Reference
Rabbit, New Zealand White (3 males and 3 females per dose group)	4-week dermal application	1/10 of body surface treated with 3.33% solution, or 10.00 and 33.33% solution/suspensions, in 1% aqueous methylhydroxyethyl-cellulose gel.	LOEL assumed to be >33.33% concentration .	Merck 1974c
Rat, Wistar (10 males and 10 females per dose group)	90-day dermal	100, 1000 or 3000 mg/kg, daily, by occlusive dermal application for 13 wk. Applications in water/oil cream formulated from Dow-Corning 322C, Gilugel SIL 5, Dow-Corning 345 and Euxyl K 100.	NOEL = 3000 mg/kg/day.	Merck 1996c
Rat, Wistar (25 males and 25 females)	2-generation toxicity	EPA guideline No. 83-4; 100, 300 or 1000 mg/kg daily in water by gavage.	NOAEL = 300 mg/kg/day LOAEL = 1000 mg/kg/day.	T 9381
Rat, Sprague-Dawley (20 + 20 as control)	Embryo-foetal toxicity – oral (pilot study)	1800 mg/kg bw by gavage daily, from 6th to 15th day of pregnancy, in 1% aqueous methyl hydroxyethyl cellulose gel.	Lowest toxic dose for dams assumed to be about 1800 mg/kg bw and for foetuses above that dose. No indication of teratogenicity.	Merck 1975a
Rabbit, New Zealand White (10 + 10 as control)	Embryo-foetal toxicity – oral (pilot study)	1500 mg/kg bw by gavage daily, from 6th to the 18th day of pregnancy, in 1% aqueous methyl hydroxyethyl cellulose gel.	Lowest toxic dose for dams assumed to be slightly below 1500 mg/kg bw and for foetuses above that dose. No indication of teratogenicity.	Merck 1975b
Rabbit, New Zealand White (6 per dose group)	Dose-range finding developmental toxicity study	50, 100, 300, 600 or 1000 mg/kg per day, in 1% aqueous carboxy methylcellulose gel, by gavage in single daily doses from days 7-19 of gestation.	No test-related deaths or abortions at any dose. No external malformations or developmental variations observed in foetuses. Body weight gain and food consumption inhibited at 600 and 1000 mg/kg. No other effects.	149020
Rabbit, New Zealand White (20 per dose group)	Developmental toxicity study	100, 300 or 600 mg/kg/day. EPA Guideline No. 83-3	NOAEL for maternal toxicity = 300 mg/kg/day. NOAEL for developmental toxicity = 600 mg/kg/day.	149021

Table B. Toxicology profile of technical IR3535®, based on repeated administration (sub-acute to chronic)

Species	Test	Duration and conditions or guideline adopted	Result	Reference
Rabbit, Himalayan (15 + 15 control)	Developmental toxicity study	100, 300 or 1000 mg/kg per day in water by gavage in a single daily dose from days 6 to 19 of gestation.	High- and mid-dose levels produced significant maternal toxicity and were embryotoxic. LOEL = 0.1 ml/kg	T9382

Table 5. Mutagenicity profile of IR3535® technical material, based on *in vitro* and *in vivo* tests

Species	Test	Conditions	Result	Reference
Salmonella typhimurium & *Escherichia coli*	Ames test (*in vitro*)	150, 300, 600, 1200, 2500 and 5000 µg/plate using *Salmonella typhimurium* TA 100, TA 98, TA 1535, TA 1537, TA 1538 and *Escherichia coli* WP2 uvrA.	No mutagenic activity with and without addition of S-9 as the metabolizing system.	4/141/80
Salmonella typhimurium & *Escherichia coli*	Ames test (*in vitro*)	EPA Guidelines No. 84-1, 84-2; 5-5000 µg/plate tested using *Salmonella typhimurium* TA 98, TA 100, TA 102, TA 1535, TA 1537and *Escherichia coli* WP2 uvrA pkM101.	No mutagenic activity with and without addition of S-9 as the metabolizing system.	40/53/96
CHO cells	Chromosomal aberration test (*in vitro*)	EPA Guideline No. 84-2; cultures of CHO cells treated with 250, 500, 750, 1000, 1500, 2000, 2500 and 3000 µg/ml for approx. 28 h without metabolic activation and 500, 750, 1000, 1500, 2000, 2500, 3000 4000 and 5000 µg/ml for 3 h with metabolic activation.	No chromosomal aberrations in CHO cells without metabolic activation. With metabolic activation the test was positive at the two highest dose levels but these doses also produced cytotoxic effects.	17982-0-437
CHO cells	HGPRT test (*in vitro*)	0 to 4.2 µl/ml without metabolic activation and 0 to 8.0 µl/ml with metabolic activation were tested.	No mutations were induced at the HGPRT locus in CHO cells.	82/144
V 79 cells	HGPRT test (*in vitro*)	EPA Guideline No. 84-2; various concentrations up to 5000 µg/ml tested.	No mutations observed in the presence or absence of an exogenous metabolizing system.	128

Table 5. Mutagenicity profile of IR3535® technical material, based on *in vitro* and *in vivo* tests

Species	Test	Conditions	Result	Reference
Mouse (5 males and 5 females)	Micronucleus test (*in vivo*)	EPA Guideline No. 84-2; dose levels of 475, 950 and 1900 mg/kg were tested.	Up to 1900 mg/kg – a dose equivalent to approx. 73 % of the LD_{50} – no micronuclei in the polychromatic erythrocytes were induced.	221/12-1052

ANNEX 2. REFERENCES

Merck document No.	Year and title or published reference
061762	1986. Determination of Photoallergenicity with Insekt-Repellent 3535 (Art. Nr. 11887) in Albino Guinea Pig.
061773	1986. Investigation for Phototoxic Potential with Insekt-Repellent 3535, Art.-Nr. 11887 in Albino Guinea Pigs.
128	1996. Insect Repellent 3535 (Art. No. 111887) Mammalian Cell (V79) Gene Mutation Test.
13I0189/957012	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).
149020	1996. A Dose Range-finding Developmental Toxicity Study of IR 3535 in Rabbits.
149021	1997. A Developmental Toxicity Study of IR 3535 in Rabbits.
149022	1997. A two-week repeated dose toxicity study of IR3535 in non-pregnant rabbits.
17982-0-437	1996. Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells with and without Exogenous Metabolic Activation.
183612	1996. Determination of the boiling temperature of insect repellent 3535 (TGAI).
183623	1996. Determination of the density (liquid) of insect repellent 3535 (TGAI).
183634	1996. Determination of the vapour pressure of insect repellent 3535 (TGAI).
183645	1997. Determination of the water solubility of insect repellent 3535 (TGAI).
183656	1996. Determination of the partition coefficient (n-octanol/water) of insect repellent 3535 (TGAI).
183667	1996. Determination of the flash point of insect repellent 3535 (TGAI).
183702	1996. Determination of the pH of an aqueous dispersion of insect repellent 3535 (TGAI).
183735	1996. Determination of the solubility of insect repellent 3535 (TGAI) in 6 organic solvents.
183746	1997. Determination of the purity and impurities of insect repellent 3535 (TGAI).
183757	1996. Determination of the accelerated storage stability of insect repellent 3535 (TGAI) by heating.
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221/12-1052	1996. Insect Repellent IR 3535 (Art. No. 111887): Induction of Micronuclei in Bone Marrow of Treated Mice.
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40/12/96	1996. Insect Repellent 3535 (Article Number 111887) – Primary Eye Irritation Test in Rabbits.
40/53/96	1996. Insect Repellent 3535 (Art. No. 111887) – Bacterial Mutagenicity Assay, Salmonella typhimurium and Escherichia coli.
51-0014-77	1977. Topical Hazard Evaluation Program of Candidate Insect Repellent AI3-70763 3[N-n-Butyl-N-acetyl] aminopropionic acid-ethyl ester.
82/144	1982. An Investigation into the Possible Induction of Mutations at the HGPRT-Locus of Chinese Hamster Ovary Cells by “Insekt-Repellent 3535”.
96-8304-21	1997. Delayed Contact Hypersensitivity Study in Guinea Pigs.
APVMA 2005	2005. Comparison of manufacturing process information and batch analyses for ethyl butylacetylaminopropionate active constituent (IR 3535) submitted to WHO with the information submitted to APVMA.
Cilek *et al*. 2004	Cilek J.E., Petersen J.L. and Hallmon C.F., 2004. Comparative efficacy of IR3535 and deet as repellents against adult *Aedes aegypti* and Culex *quinquefasciatus*. *J. Amer. Mosquito Control Assoc.*, 20 (3), 299-304, 2004.

Merck document No.	Year and title or published reference
FAO/WHO 2002	Manual on development and use of FAO and WHO specifications for pesticides, 1st edition. FAO plant production and protection paper 173. FAO, Rome, 2002.
Merck 1972a	1972. Tolerance test of repellents BE 3304 and BE 3535 on mucous membranes.
Merck 1972b	1972. Tolerance test of repellents BE 3535 (purified) on mucous membranes of the rabbit eyes.
Merck 1973a	1973. Acute Toxicity of BE 3535 after oral administration to rats.
Merck 1973b	1973. Acute Toxicity of BE 3535 after Local Application to 1/10 of the Body Surface of Rats.
Merck 1973c	1973. Local Tolerance Test of Different Preparations of BE 3767 and BE 3535 in Rabbits (Patch Test).
Merck 1974a	1974. 4 week toxicity of BE 3535 in Sprague-Dawley rats after administration in the diet.
Merck 1974b	1974. 4 week toxicity of the repellent BE 3535 in New Zealand White rabbits after administration by gavage.
Merck 1974c	1974. Local and general (systemic) tolerance test of BE 3535 with 4 week application to the dorsal skin of NZW rabbits.
Merck 1975a	1975. The Effect of BE 3535 on the Pregnant Rat and the Fetus after Administration by Gavage (Pilot Study with 1 Dose Level).
Merck 1975b	1975. The Effect of BE 3535 on the Pregnant New Zealand White Rabbit and the Fetus after Administration by Gavage (Pilot Study with 1 Dose Level).
Merck 1979	1979. Medical Expert Opinion. Hamburg.
Merck 1980	1980. Expert report on the tolerance of insect repellents when applied to mucous membranes.
Merck 1981a	1981. Acute oral toxicity of BE 3535 after administration to mongrel dogs.
Merck 1981b	1981. Acute Toxicity of BE 3535 after Local Application to 1/10 of the Body Surface of Mice (pilot study).
Merck 1981c	1981. Acute Toxicity of BE 3535 after Local Application to 1/10 of the Body Surface of beagle dogs (pilot study).
Merck 1981d	1981. 4 week toxicity of the repellent BE 3535 in beagle dogs after administration by gavage.
Merck 1981e	1981. Acute Toxicity of insect repellent No. 127.
Merck 1996a	1996. Insect repellent 3535, stability in water.
Merck 1996b	Validation: Identification by IR spectrum. Analytical Laboratory Gernsheim. 11.09.1996. Merck KGaA, Darmstadt. Unpublished.
Merck 1996c	1996. Insect Repellent 3535 (Art. No. 111887) 90-day Subchronic Toxicity Study with Dermal Application to Rats.
Merck 1999	1999. Insect repellent 3535, monograph.
Merck 2004a	2004. JMPS evaluation of data on IR3535.
Merck 2004b	2004. IR3535 method, collaborative trial.
T14215	1997. Insect Repellent 3535 (Article Number 111887) – Acute Toxicity Study in Rats after oral Administration.
T9381	1997. Insect Repellent 3535 (Article Number 111887) – 2-Generation Study with Oral Administration to Rats.
T9382	1996. Insect Repellent 3535 (Article Number 111887) – Developmental toxicity study with oral administration to rabbits.