WHO SPECIFICATIONS AND EVALUATIONS FOR PUBLIC HEALTH PESTICIDES

SPINOSAD

A mixture of spinosyn A,

(2*R*,3a*S*,5a*R*,5b*S*,9*S*,13*S*,14*R*,16a*S*,16b*R*)-2-(6-deoxy-2,3,4-tri-*O*-methyl-α-L-mannopyranosyloxy)-13-(4-dimethylamino-2,3,4,6-tetradeoxy-β-D-erythropyranosyloxy)-9-ethyl-2,3,3a,5a,6,7,9,10,11,12,13,14,15,16a,16b-hexadecahydro-14-methyl-1*H*-8-oxacyclododeca[*b*]*as*-indacene-7,15-dione,

and spinosyn D,

(2*R*,3a*S*,5a*R*,5b*S*,9*S*,13*S*,14*R*,16a*S*,16b*R*)-2-(6-deoxy-2,3,4-tri-*O*-methyl-α-L-mannopyranosyloxy)-13-(4-dimethylamino-2,3,4,6-tetradeoxy-β-D-erythropyranosyloxy)-9-ethyl-2,3,3a,5a,6,7,9,10,11,12,13,14,15,16a,16b-hexadecahydro-4,14-dimethyl-1*H*-8-oxacyclododeca[*b*]*as*-indacene-7,15-dione,

with spinosyns A:D proportions in the range 50:50 to 95:5

World Health Organization

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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[1] 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 "Manual on the development and use of FAO and WHO specifications for chemical pesticides." 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 8 of the above-mentioned manual.

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 above-mentioned manual and supported by other information sources. Evaluation reports include 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 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. Evaluations bear the date (year) of the meeting at which the recommendations were made by the JMPS.

[1] The publications are available on the Internet under the WHO Prequalification Unit – Vector Control Product Assessment Team (PQT/VCP) website, https://extranet.who.int/prequal/vector-control-products

PART ONE: SPECIFICATIONS

SPINOSAD

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Spinosad Information

ISO common name

Spinosad (BSI, E-ISO, ANSI), being a mixture of spinosyns A and D, with A:D proportions in the range 50:50 to 95:5

Synonyms

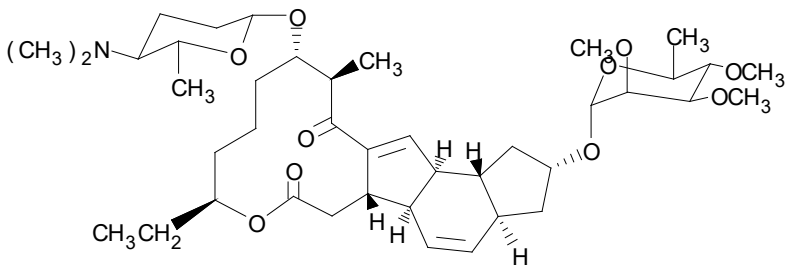
None

Chemical names

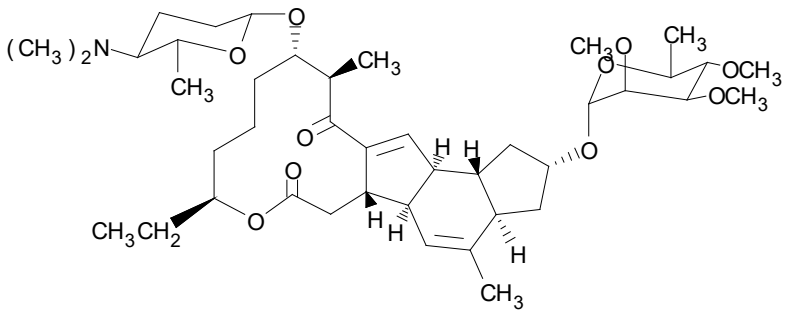
IUPAC A mixture of spinosyn A, (2*R*,3a*S*,5a*R*,5b*S*,9*S*,13*S*,14*R*,16a*S*,16b*R*)-2-(6-deoxy-2,3,4-tri-*O*-methyl-α-L-mannopyranosyloxy)-13-(4-dimethylamino-2,3,4,6-tetradeoxy-β-D-erythropyranosyloxy)-9-ethyl-2,3,3a,5a,6,7,9,10,11,12,13,14,15,16a,16b-hexadecahydro-14-methyl-1*H*-8-oxacyclododeca[*b*]*as*-indacene-7,15-dione, and spinosyn D, (2*R*,3a*S*,5a*R*,5b*S*,9*S*,13*S*,14*R*,16a*S*,16b*R*)-2-(6-deoxy-2,3,4-tri-*O*-methyl-α-L-mannopyranosyloxy)-13-(4-dimethylamino-2,3,4,6-tetradeoxy-β-D-erythropyranosyloxy)-9-ethyl-2,3,3a,5a,6,7,9,10,11,12,13,14,15,16a,16b-hexadecahydro-4,14-dimethyl-1*H*-8-oxacyclododeca[*b*]*as*-indacene-7,15-dione, with A:D proportions in the range 50:50 to 95:5

CA [2*R*-[2*R**,3a*S**,5a*R**,5b*S**,9*S**,13*S**(2*R**,5*S**,6*R**),14*R**,16a*S**,16b*R**]]-2-[(6-deoxy-2,3,4-tri-*O*-methyl-α-L-mannopyranosyl)oxy]-13-[[5-(dimethylamino)tetrahydro-6-methyl-2*H*-pyran-2-yl]oxy]-9-ethyl-2,3,3a,5a,5b,6,9,10,11,12,13,14,16a,16b-tetradecahydro-14-methyl-1*H*-*as*-indaceno(3,2-*d*)oxacyclododecin-7,15-dione (spinosyn A), mixture with [2S-[2*R**,3a*S**,5a*R**,5bR*,9R*,13R*(2S*,5R*,6S*),14S*,16aR*,16bR*]]-2-[(6-deoxy-2,3,4-tri-*O*-methyl-α-L-mannopyranosyl)oxy]-13-[[5-(dimethylamino)tetrahydro-6-methyl-2*H*-pyran-2-yl]oxy]-9-ethyl-2,3,3a,5a,5b,6,9,10,11,12,13,14,16a,16b-tetradecahydro-4,14-dimethyl-1*H*-*as*-indaceno(3,2-*d*)oxacyclododecin-7,15-dione (spinosyn D)

Structural formulae



spinosyn A



spinosyn D

Empirical formulae

spinosyn A: $C_{41}H_{65}NO_{10}$
spinosyn D: $C_{42}H_{67}NO_{10}$

Relative molecular mass

spinosyn A: 732.0
spinosyn D: 746.0

CAS Registry number

spinosyn A: 131929-60-7
spinosyn D: 131929-63-0

CIPAC number

636

EEC number

434-300-1

Identity tests

HPLC retention time, positive-ion ESI LC-MS.

Spinosad Technical Material

WHO Specification 636/TC (February 2007*)

*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 (*636/2005*). It should be applicable to TC produced by this manufacturer but it is not an endorsement of those products nor a guarantee that they comply with the specifications. The specification may not be appropriate for TC produced by other manufacturers. The evaluation report (*636/2005*), as PART TWO, forms an integral part of this publication.*

1 Description

The material shall consist of Spinosad together with related manufacturing impurities and shall be a grey/white to tan coloured powdery material, free from visible extraneous matter and added modifying agents.

2 Active ingredient

2.1 **Identity tests** (636/TC/(M)/2, CIPAC Handbook L, p.123, 2005)

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 **Spinosad content** (636/TC/(M)/3, CIPAC Handbook L, p.123, 2005)

The Spinosad (spinosyn A + spinosyn D) content shall be declared (not less than 850 g/kg) and, when determined, the average measured content shall not be lower than the declared minimum content.

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* Specifications may be revised and/or additional evaluations may be undertaken. Ensure the use of current versions by checking at the WHO Prequalification Unit – Vector Control Product Assessment Team (PQT/VCP) website: https://extranet.who.int/prequal/vector-control-products/specifications-new-procedure.

Spinosad Granules

WHO Specification 636/GR/1 (October 2024*)

*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 reports (*636/2005, 636/2012, 636/2024*). It should be applicable to relevant products of this manufacturer and those of any other formulators who use only TC from the evaluated source. The specification 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 who use TC from other sources. The evaluation reports (*636/2005, 636/2012, 636/2024*), as PART TWO, form an integral part of this publication.*

1 Description

The material shall consist of granules containing technical Spinosad, complying with the requirements of WHO specification 636/TC, together with suitable carriers and any other necessary formulants. The granules shall be free from visible extraneous matter and hard lumps, free-flowing, essentially non-dusty and intended for application by machine.

2 Active ingredient

2.1 **Identity tests** (636/GR/(M)/2, CIPAC Handbook L, p.126, 2005)

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 **Spinosad content** (636/GR/M/3, CIPAC Handbook O, p.146,2017)

The Spinosad (spinosyn A + spinosyn D) content shall be declared (g/kg) and, when determined, the average measured content shall not differ from that declared by more than the following tolerances:

Declared content, g/kg	Tolerance
up to 25 Note: the upper limit is included in the range	± 10% of the declared content

3 Physical properties

3.1 **Pour and tap density** (MT 186, CIPAC Handbook K, p.151, 2003)

Pour density: 0.47 to 0.61 g/ml.

Tap density: 0.52 to 0.66 g/ml.

* Specifications may be revised and/or additional evaluations may be undertaken. Ensure the use of current versions by checking at the WHO Prequalification Unit – Vector Control Product Assessment Team (PQT/VCP) website: https://extranet.who.int/prequal/vector-control-products/specifications-new-procedure.

3.2 **Nominal size range (MT 170, CIPAC Handbook F, p.420, 1995) (Note 1)**

Not less than 850 g/kg of the formulation shall be within the size range of 1100 to 1600 µm.

3.3 **Dustiness** (MT 171.1, CIPAC Handbook P, p.235, 2021)

Essentially non-dusty (Note 2).

3.4 **Attrition resistance** (MT 178.3) (Note 3)

Minimum attrition resistance: 98%.

4 Storage stability

4.1 **Stability at elevated temperature** (MT 46.4, CIPAC Handbook P, p.232, 2021)

After storage at 54 ± 2°C for 14 days, the determined average active ingredient content must not be lower than 95% relative to the determined average content found before storage (Note 4) and the formulation shall continue to comply with the clauses for:

- nominal size range (3.2),
- dustiness (3.3),
- attrition resistance (3.4).

Note 1 Higher ratios increase the risk of segregation and adverse effects on the flow rate. This should be checked with the machine to be used. The purchaser should check that the nominal size range is suitable for his requirements because different size ranges may affect biological activity.

Note 2 The optical method of MT 171.1 usually shows good correlation with the gravimetric method and can, therefore, be used as an alternative where the equipment is available. Where the correlation is in doubt, it must be checked with the formulation to be tested. In case of dispute, the gravimetric method shall be used.

Note 3 The revised CIPAC method for attrition resistance (CIPAC/5321, MT 178.3) combining methods MT 178 and MT 178.2 into a single method was adopted as full CIPAC method in 2023. Prior to its publication in the next Handbook, the method can be obtained through the CIPAC prepublication scheme: https://www.cipac.org/index.php/m-p/pre-published-methods.

Note 4 Samples of the formulation taken before and after the accelerated storage stability test may be analysed concurrently after the test in order to reduce the analytical error.

Spinosad Extended Release Granules

WHO Interim Specification 636/GR/2 (October 2024*)

*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 reports (*636/2012, 636/2024*). 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, irrespective of the source of TC. The evaluation reports (*636/2012, 636/2024*), given in PART TWO, form an integral part of this publication.*

1 Description

The material shall consist of granules containing technical Spinosad, complying with the requirements of WHO specification 636/TC, in the form of irregular spherical light tan granules, together with suitable carriers and any other necessary formulants. The granules shall be dry, free from visible extraneous matter and hard lumps, free-flowing, essentially non-dusty and intended for application by machine (Note 1).

2 Active ingredient

2.1 **Identity tests** (636/GR/M/2, CIPAC Handbook O, p.146, 2017)

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 **Spinosad content** (636/GR/M/3, CIPAC Handbook O, p.146, 2017)

The Spinosad (spinosyn A + spinosyn D) content shall be declared (25 g/kg) and, when determined, the average content measured shall not differ from that declared by more than ± 25%.

3 Physical properties

3.1 **Pour and tap density** (MT 186, CIPAC Handbook K, p.151, 2003)

Pour density: 1.2 to 1.4 g/ml.

Tap density: 1.3 to 1.5 g/ml.

3.2 **Nominal size range** (MT 170, CIPAC Handbook F, p.420, 1995) (Note 2)

Not less than 850 g/kg of the formulation shall be within the nominal declared size range of 850 to 2000 μm.

3.3 **Dustiness** (MT 171.1, CIPAC Handbook P, p.235, 2021)

Essentially non-dusty (Note 3).

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* Specifications may be revised and/or additional evaluations may be undertaken. Ensure the use of current versions by checking at the WHO Prequalification Unit – Vector Control Product Assessment Team (PQT/VCP) website: https://extranet.who.int/prequal/vector-control-products/specifications-new-procedure.

3.4 **Attrition resistance** (MT 178.3) (Note 4)

Minimum attrition resistance: 98%.

4 Storage stability

4.1 Stability at elevated temperature (MT 46.4, CIPAC Handbook P, p.232, 2021)

After storage at 54 ± 2°C for 14 days, the determined average active ingredient content must not be lower than 95% relative to the determined average content found before storage (Note 5), and the formulation shall continue to comply with the clauses for:

- nominal size range (3.2),
- dustiness (3.3),
- attrition resistance (3.4).

Note 1 The granular formulation is an extended release product intended to be applied to habitats of vector larvae. As the catalogue of pesticide formulations does not provide an own designation for slow release granules other than coated granules, the designation “extended release GR” is used to allow a discrimination between the granules based on corn cob, floating on water and providing single-brood efficacy (FAO/WHO evaluation report 636/2005) and the current GR based on silica, sinking in water and designed to provide extended release of Spinosad over a longer time period and controlling multiple broods of the vectors.

Note 2 Higher ratios increase the risk of segregation and adverse effects on the flow rate. This should be checked with the machine to be used. The purchaser should check that the nominal size range is suitable for his requirements because different size ranges may affect biological activity.

Note 3 The optical method of MT 171.1 usually shows good correlation with the gravimetric method and can, therefore, be used as an alternative where the equipment is available. Where the correlation is in doubt, it must be checked with the formulation to be tested. In case of dispute, the gravimetric method shall be used.

Note 4 The revised CIPAC method for attrition resistance (CIPAC/5321, MT 178.3) combining methods MT 178 and MT 178.2 into a single method was adopted as full CIPAC method in 2023. Prior to its publication in the next Handbook, the method can be obtained through the CIPAC prepublication scheme: https://www.cipac.org/index.php/m-p/pre-published-methods.

Note 5 Samples of the formulation taken before and after the accelerated storage stability test may be analysed concurrently after the test in order to reduce the analytical error.

Spinosad Aqueous Suspension Concentrate

WHO Specification 636/SC (October 2024*)

*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 reports (*636/2005, 636/2024*). It should be applicable to relevant products of this manufacturer and those of any other formulators who use only TC from the evaluated source. The specification 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 who use TC from other sources. The evaluation reports (*636/2005, 636/2024*), as PART TWO, form an integral part of this publication.*

1 Description

The material shall consist of a suspension of fine particles of technical Spinosad complying with the requirements of WHO specification 636/TC in an aqueous phase together with suitable formulants. After gentle agitation, the material shall be homogeneous (Note 1) and suitable for further dilution in water.

2 Active ingredient

2.1 **Identity tests** (636/SC/(M)/2, CIPAC Handbook L, p.125, 2005)

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 **Spinosad content** (636/SC/(M)/3, CIPAC Handbook L, p.125, 2005)

The Spinosad (spinosyn A + spinosyn D) content shall be declared (g/kg or g/l at 20 ± 2ºC, Note 2) and, when determined, the average content measured shall not differ from that declared by more than the following tolerance:

Declared content, g/kg or g/l at 20 ± 2ºC	Tolerance
above 100 up to 250	± 6% of the declared content
above 250 up to 500	± 5% of the declared content
Note: the upper limit is included in each range	

3 Physical properties

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

pH range: 6.5 to 8.5.

3.2 **Pourability** (MT 148.2,) (Note 3)

Maximum "residue": 5%.

3.3 **Spontaneity of dispersion** (MT 160.1) (Notes 4 & 5)

* Specifications may be revised and/or additional evaluations may be undertaken. Ensure the use of current versions by checking at the WHO Prequalification Unit – Vector Control Product Assessment Team (PQT/VCP) website: https://extranet.who.int/prequal/vector-control-products/specifications-new-procedure.

Minimum: 75% after 5 minutes in CIPAC standard water D at 25 ± 5°C.

3.4 **Suspensibility** (MT 184.1, CIPAC Handbook P, p.245, 2021) (Note 5)

Minimum: 70% after 30 minutes in CIPAC standard water D at 25 ± 5°C.

3.5 **Wet sieve test** (MT 185.1,) (Note 6 & 7)

Maximum: 0.5% of the formulation shall be retained on a 75 µm test sieve.

3.6 **Persistent foam** (MT 47.3, CIPAC Handbook O, p. 177, 2017) (Note 8)

Maximum: 20 ml after 1 min.

4 Storage stability

4.1 **Stability at 0°C** (MT 39.3, CIPAC Handbook J, p.126, 2000)

After storage at 0 ± 2°C for 7 days, the formulation shall continue to comply with the clauses for:

- suspensibility (3.4);
- wet sieve test (3.5).

4.2 **Stability at elevated temperature** (MT 46.4, CIPAC Handbook P, p.232, 2021)

After storage at 54 ± 2°C for 14 days, the determined average active ingredient content must not be lower than 95% relative to the determined average content found before storage (Note 9), and the formulation shall continue to comply with the clauses for:

- pH range (3.1),
- pourability (3.2),
- spontaneity of dispersion (3.3),
- suspensibility (3.4),
- wet sieve test (3.5).

Note 1 Before sampling to verify the formulation quality, inspect the commercial container carefully. On standing, suspension concentrates usually develop a concentration gradient from the top to the bottom of the container. This may even result in the appearance of a clear liquid on the top and/or sediment on the bottom. Therefore, before sampling, homogenize the formulation according to the instructions given by the manufacturer or, in the absence of such instructions, by gentle shaking of the commercial container (for example, by inverting the closed container several times). Large containers must be opened and stirred adequately. After this procedure, the container should not contain a sticky layer of non-dispersed matter at the bottom. A suitable and simple method of checking for a non-dispersed sticky layer "cake" is by probing with a glass rod or similar device adapted to the size and shape of the container. All the physical and chemical tests must be carried out on a laboratory sample taken after the recommended homogenization procedure.

Note 2 Unless homogenization is carried out carefully, it is possible for the sample to become aerated. This can lead to errors in the determination of the mass per millilitre and in calculation of the active ingredient content (in g/l) if methods other than OECD 109 or CIPAC MT 3.3 are used. If the buyer requires both g/kg and g/l at 20°C, then in case of dispute, the analytical results shall be calculated as g/kg.

Note 3 The revised CIPAC method for pourability (CIPAC/5355, MT 148.2) combining methods MT 148 and MT 148.1 into a single method was adopted as provisional CIPAC method in 2024.

Prior to its publication in the next Handbook, the method can be obtained through the CIPAC prepublication scheme: https://www.cipac.org/index.php/m-p/pre-published-methods.

Note 4 The revised CIPAC method for spontaneity of dispersion of liquid formulations forming suspensions on dilution with water (CIPAC 5323, MT 160.1) was adopted as full CIPAC method in 2023. Prior to its publication in the next Handbook, the method can be obtained through the CIPAC prepublication scheme: https://www.cipac.org/index.php/m-p/pre-published-methods.

Note 5 Chemical assay is the only fully reliable method to measure the mass of active ingredient still in suspension. However, the simpler gravimetric method may be used on a routine basis provided that it has been shown to give equal results to those of chemical assay. In case of dispute, chemical assay shall be the referee method.

Note 6 This test detects coarse particles (for example, caused by crystal growth) or agglomerates (crust formation) or extraneous materials which could cause blockage of spray nozzles or filters in the spray tank.

Note 7 The revised CIPAC method for wet sieve test (CIPAC/5353, MT 185.1) combining methods MT 182 and MT 185 into a single method was adopted as full CIPAC method in 2024. Prior to its publication in the next Handbook, the method can be obtained through the CIPAC prepublication scheme: https://www.cipac.org/index.php/m-p/pre-published-methods.

Note 8 The mass of sample to be used in the test should correspond to the highest rate of use recommended by the supplier. The test is to be conducted in CIPAC standard water D at 25°C ± 5°C.

Note 9 Samples of the formulation taken before and after the accelerated storage stability test may be analysed concurrently after the test in order to reduce the analytical error.

Spinosad Bilayer Tablets for Direct Application

WHO Specification 636/DT/1 (October 2024*)

*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 reports (*636/2005, 636/2007, 636/2010, 636/2012, 636/2024*). It should be applicable to relevant products of this manufacturer and those of any other formulators who use only TC from the evaluated source. The specification 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 who use TC from other sources. The evaluation reports (*636/2005, 636/2007, 636/2010, 636/2012, 636/2024*), as PART TWO, form an integral part of this publication.*

1 Description (Note 1)

The material shall consist of two homogeneous layers of technical Spinosad complying with the requirements of WHO specification 636/TC together with carriers and any other necessary formulants. It shall be in the form of tablets for direct application. The formulation shall be of dry, unbroken tablets, free from visible extraneous matter.

2 Active ingredient

2.1 **Identity tests** (636/DT/M/2, CIPAC Handbook M, p.197, 2009) (Note 2)

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 **Spinosad content** (636/DT/M/3, CIPAC Handbook M, p.197, 2009) (Note 2)

The Spinosad (spinosyn A + spinosyn D) content shall be declared (74.8 g/kg) and, when determined, the average measured content shall not differ from that declared by more than ±10%.

2.3 **Tablet dose uniformity** (636/DT/M/3, CIPAC Handbook M, p.197, 2009) (Note 2)

The Spinosad (spinosyn A + spinosyn D) content, measured separately in 20 tablets, shall have a relative standard deviation (RSD) of not more than 10%.

3 Physical properties

3.1 **Tablet integrity** (Notes 2, 3 & 4)

No broken tablets observed on inspection.

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* Specifications may be revised and/or additional evaluations may be undertaken. Ensure the use of current versions by checking at the WHO Prequalification Unit – Vector Control Product Assessment Team (PQT/VCP) website: https://extranet.who.int/prequal/vector-control-products/specifications-new-procedure.

3.2 **Attrition of tablets** (MT 193, CIPAC Handbook O, p.204, 2017 (Notes 2 & 4)

Maximum degree of attrition: 2%.

4 Storage stability

4.1 **Stability at elevated temperature** (MT 46.4, CIPAC Handbook P, p.232, 2021)

After storage at 54 ± 2°C for 14 days without pressure (Note 5), the determined average active ingredient content must not be lower than 95% relative to the determined average content found before storage (Note 6) and the formulation shall continue to comply with the clauses for:

- tablet integrity (3.1),
- attrition of tablets (3.2).

Note 1 The tablets are round, 12 mm in diameter, approximately 6 mm in height, weighing approximately 1.34 g. The tablets consist of two layers. One contains an effervescent system with its water-soluble acid component present in excess, a combination intended to aid gentle dispersion in water after application and to provide a relatively quick initial release of the active ingredient into the water. The other layer provides a slower release of Spinosad to maintain an appropriate concentration over an extended period of time. The tablets are not intended for dispersion in water prior to application.

Note 2 For determination of identity (2.1) and Spinosad content (2.2), a sufficient quantity of entire tablets may be milled and thoroughly mixed to provide a homogeneous powder prior to weighing portions for analysis. Alternatively, the Spinosad content may be calculated as the average g/kg obtained from the analyses of 20 separate tablets (2.3), and identity may be confirmed using one or more individual tablets. Tablets to be analysed individually must also be crushed or milled to a fine powder prior to extraction.

To determine tablet integrity (3.1), carefully remove all 250 tablets from a pack and count those that are broken. Unbroken tablets (from test 3.1) should be used in the tests for degree of attrition (3.2) and storage stability (4.1).

Note 3 Visual observation only. At least 20 tablets should be inspected.

Note 4 Prior to testing, tablets should be retained within the moisture-barrier packaging to preserve the integrity of the effervescent system within the tablets.

Note 5 Without pressure means that the test is performed as specified by CIPAC MT 46.4, but no pressure is applied to the sample during its aging. Tablets are to be retained within the sealed moisture-barrier packaging throughout the 14 days of storage at 54°C.

Note 6 Samples of the formulation taken before and after the accelerated storage stability test may be analysed concurrently after the test in order to reduce the analytical error.

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Spinosad Monolayer Tablets for Direct Application

WHO Interim Specification 636/DT/2 (October 2024*)

*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 reports (*636/2012, 636/2024*). 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, irrespective of the source of TC. The evaluation reports (*636/2012, 636/2024*), given in PART TWO, form an integral part of this publication.*

1 Description

The material shall consist of a homogeneous mixture of technical Spinosad complying with the requirements of WHO specification 636/TC together with carriers and any other necessary formulants. It shall be in the form of round, white to light tan monolayer tablets with an average diameter of 25 mm for direct application. The formulation shall be of dry, unbroken tablets, free from visible extraneous matter (Note 1).

2 Active ingredient

2.1 **Identity tests** (636/DT/M/2, CIPAC Handbook M, p.197, 2009)

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 **Spinosad content** (636/DT/M/3, CIPAC Handbook M, p.197, 2009)

The Spinosad (spinosyn A + spinosyn D) content shall be declared (83.3 g/kg) and, when determined, the average measured content shall not differ from that declared by more than ±10%.

2.3 **Tablet dose uniformity** (636/DT/M/3, CIPAC Handbook M, p.197, 2009)

The Spinosad (spinosyn A + spinosyn D) content, measured separately in 20 tablets, shall have a relative standard deviation (RSD) of not more than 10%.

3 Physical properties (Note 1)

3.1 **Tablet integrity** (Notes 2 & 3)

No broken tablets observed on inspection.

* Specifications may be revised and/or additional evaluations may be undertaken. Ensure the use of current versions by checking at the WHO Prequalification Unit – Vector Control Product Assessment Team (PQT/VCP) website: https://extranet.who.int/prequal/vector-control-products/specifications-new-procedure.

3.2 **Attrition of tablets** (MT 193, CIPAC Handbook O, p.204, 2017 (Note 2 & 4)

Maximum degree of attrition: 2%.

4 Storage stability

4.1 **Stability at elevated temperature** (MT 46.4, CIPAC Handbook P, p.232, 2021)

After storage at 54 ± 2°C for 14 days without pressure (Note 4), the determined average active ingredient content must not be lower than 95% relative to the determined average content found before storage (Note 5) and the formulation shall continue to comply with the clauses for:

- tablet integrity (3.1),
- attrition of tablets (3.2).

Note 1 The tablets are round, 25 mm in diameter, approximately 6 mm in height, weighing approximately 6 g. The tablets consist of one layer only which provides a release of Spinosad over an extended period of time to maintain an appropriate concentration during typically 30 days. The tablets are not intended for dispersion in water prior to application.

Note 2 For determination of identity (2.1) and Spinosad content (2.2), a sufficient quantity of entire tablets may be milled and thoroughly mixed to provide a homogeneous powder, prior to weighing portions for analysis. Alternatively, the Spinosad content may be calculated as the average g/kg obtained from the analyses of 20 separate tablets (2.3) and identity may be confirmed using one or more individual tablets. Tablets to be analysed individually must also be crushed or milled to a fine powder, prior to extraction.

To determine tablet integrity (3.1), carefully remove all tablets from a pack and count those that are broken. Unbroken tablets (from test 3.1) should be used in the tests for degree of attrition (3.2) and storage stability (4.1).

Note 3 Visual observation only. At least 20 tablets should be inspected.

Note 4 Without pressure means that the test is performed as specified by CIPAC MT 46.4, but no pressure is applied to the sample during its aging. Tablets are to be retained within the commercial packaging throughout the 14 days storage at 54°C.

Note 5 Samples of the formulation taken before and after the accelerated storage stability test may be analysed concurrently after the test in order to reduce the analytical error.

Spinosad Emulsifiable Concentrate

WHO Specification 636/EC (October 2024*)

*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 reports (*636/2010, 636/2024*). It should be applicable to relevant products of this manufacturer, and those of any other formulators who use only TC from the evaluated source. The specification 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 who use TC from other sources. The evaluation reports (*636/2010, 636/2024*), as PART TWO, form an integral part of this publication.*

1 Description

The material shall consist of technical Spinosad, complying with the requirements of WHO specification 636/TC, in the form of a clear amber liquid, dissolved in suitable solvents together with any other necessary formulants. It shall generally be in the form of a stable homogeneous liquid, free from visible suspended matter and sediment, to be applied as an emulsion after dilution in water (Note 1).

2 Active ingredient (Note 2)

2.1 Identity tests (636/EC/M/2, CIPAC Handbook O, p.146, 2017)

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 Spinosad content (636/EC/M/3, CIPAC Handbook O, p.146, 2017)

The Spinosad (spinosyn A + spinosyn D) content shall be declared (206 g/kg or 240 g/L at 20°C ± 2°C, Note 3) and, when determined, the average content measured shall not differ from that declared by more than ±6%.

* Specifications may be revised and/or additional evaluations may be undertaken. Ensure the use of current versions by checking at the WHO Prequalification Unit – Vector Control Product Assessment Team (PQT/VCP) website: https://extranet.who.int/prequal/vector-control-products/specifications-new-procedure.

3 **Physical properties** (Note 2)

3.1 **Emulsion stability and re-emulsification** (MT 36.3, CIPAC Handbook K, p.137, 2003)

The formulation, when diluted at 25 ± 5°C at 1% v/v (Note 4) with CIPAC standard waters A and D, shall comply with the following.

Time after dilution	Limits of stability
0 h	Initial emulsification complete
0.5 h	"Cream", maximum: trace
2 h	"Cream", maximum: 1 ml "Free oil", maximum: 0 ml
24 h	Re-emulsification complete
24.5 h	"Cream", maximum: 1 ml "Free oil", maximum: 0 ml
Note: in applying MT 36.3, tests after 24 h are required only where results at 2 h are in doubt.	

3.2 **Persistent foam** (MT 47.3, CIPAC Handbook O, p. 177, 2017 (Note 5)

Maximum: 10 ml after 1 min.

4 **Storage stability**

4.1 **Stability at 0°C** (MT 39.3, CIPAC Handbook J, p.126, 2000)

After storage at 0 ± 2°C for 7 days, the volume of solid and/or liquid which separate shall not be more than 0.3 ml.

4.2 **Stability at elevated temperature** (MT 46.4, CIPAC Handbook P, p.232, 2021)

After storage at 54 ± 2°C for 14 days, the determined average active ingredient content must not be lower than 95% relative to the determined average content found before storage (Note 6), and the product shall continue to comply with the clauses for:

- emulsion stability and re-emulsification (3.1).

Note 1 The formulation may show some sedimentation upon standing for longer time. The sediments stem from minor components of Spinosad, which have a tendency to crystallize. Before sampling, homogenize the formulation according to the instructions given by the manufacturer, or, in the absence of such instructions, gently shake the commercial container (for example, by inverting the closed container several times, large containers must be opened and stirred adequately).

Note 2 All the physical and chemical tests must be carried out on a laboratory sample taken after the recommended homogenization procedure.

Note 3 If the buyer requires both g/kg and g/l at 20°C, then in case of dispute the analytical results shall be calculated as g/kg.

Note 4 For ground application 1% v/v dilution represents the maximum dilution rate.

Note 5 The mass of sample to be used in the test should correspond to the highest rate of use recommended by the supplier (1% v/v dilution). The test is to be conducted in CIPAC standard water D.

Note 6 Samples of the formulation taken before and after the storage stability test may be analysed concurrently after the test in order to reduce the analytical error.

PART TWO: EVALUATION REPORTS FOR SPINOSAD

SPINOSAD

SPINOSAD
Evaluation Report 636/2024

Summary of Action

In May 2024, WHO was notified that Dow Agrosciences, LLC ("Dow") underwent an entity change and is now incorporated as Corteva Agriscience ("Corteva"). In this frame, the ownership of the data packages supporting the specifications for Spinosad Technical Material and Spinosad Suspension Concentrate, previously submitted by Dow Agrosciences, LLC, were transferred to Corteva Agriscience.

Additionally, the ownership of the data packages supporting the specifications for Spinosad Granules, Spinosad Extended Release Granules, Spinosad Bilayer Tablets for Direct Application, Spinosad Monolayer Tablets for Direct Application and Spinosad Emulsifiable Concentrate, which were jointly developed and submitted by Dow Agrosciences and Clarke Mosquito Control Products, Inc., were transferred from Dow Agrosciences to Clarke Mosquito Control Products, Inc.

Moreover, Clarke Mosquito Control Products Inc, the manufacturer of Spinosad granules, Spinosad extended release granules (GR), Spinosad bilayer tablets for direct application and Spinosad monolayer tablets for direct application (DT), notified WHO that currently there is no existent ability or success to demonstrate a uniform release of Spinosad in a quality laboratory setting, hence being unable to develop an appropriate standard test to measure directly the release properties of the GR or DT. Additionally, the release of Spinosad at a parts-per-billion (PPB) level is not easily analysed by routine analytical chromatography techniques.

Given that:

1. since 2012, no method is available to measure directly the slow release properties of the Spinosad extended release granules (GR) or the slow release properties of the Spinosad monolayer tablets for direct application (DT);
2. the release rate of active ingredient clause is not a mandatory clause for GR and a non-existent clause for DT; and
3. currently, no other solid formulations with slow release or extended-release vector control products that have WHO specifications have this requirement,

the Meeting requested the need of an identification test for QA purposes (i.e., related to the nature of the physical properties, extended release test or other appropriate identification solution) to differentiate between a granule/tablet formulation and an extended release granule/tablet formulation.

Moreover, Clarke Mosquito Control Products Inc notified WHO that the CIPAC methods referenced for attrition of tablets and stability at elevated temperature need to be updated in the Spinosad specifications according to the "Manual on the development and use of FAO and WHO specifications for chemical pesticides, Second Edition (2022)" and the last published CIPAC methods.

The Second Edition of the Manual included clauses for the flowability of these formulation types. In order for the specifications to be updated to include this clause, data on the flowability must be submitted by the applicant.

The Meeting also recommended to replace, in the WHO specifications for Spinosad formulations, as appropriate, the method for dustiness MT 171 by MT 171.1, the method for attrition resistance MT 178 by MT 178.3, the method for pourability MT 148.1 by MT 148.2, the method for spontaneity of dispersion MT 160 by MT 160.1, the method for suspensibility MT 184 by MT 184.1, the method for wet sieve test MT 185 by MT 185.1, the method for persistent foam MT 47.2 by MT 47.3.

These revised MT methods are considered to provide equivalent results to the previous version.

These changes will then be in alignment with the FAO 2021 specifications for Spinosad GR and SC.

With the clarification of the need for development of a method to differentiate between rapid and slow release formulations for the solid formulations types, it was determined that the interim classification of the specification should be retained. This then supersedes and replaces the conditions of the interim status presented in the evaluation report 636/2012.

SPINOSAD
Evaluation Report 636/2012

Recommendations

The Meeting recommended that time-limited interim specifications (until January 2017) for Spinosad extended release granules (GR) and Spinosad monolayer tablets for direct application (DT), proposed by Dow AgroSciences, as amended, should be adopted by WHO.

Appraisal

Spinosad extended release granules (GR)

Supporting data and draft specification for a new slow release formulation of Spinosad GR, jointly provided by Dow AgroSciences and Clarke Mosquito Control, were considered by the JMPS for development of a new WHO specification. Spinosad GR has been developed as mosquito larvicide by Clarke Mosquito Control and the specification, therefore, is limited to WHO. The product was successfully evaluated as larvicide by WHOPES and a recommendation for its use in public health was recently published (WHO 2013). The proposed specification for GR was broadly in agreement with the guidelines given in the Manual (FAO/WHO 2010).

Formulation type, description, content of active ingredient and analytical method

Spinosad is formulated as granules with 25 g/kg of the complex of active ingredients spinosyn A and D. The formulation is intended for direct application to mosquito larvae habitats. The granules are formulated with silica so that they do not float on the water, but sink to the ground and release the complex of active ingredient over prolonged period of time. Besides having a different nominal content as the other existing GR formulations (WHO specification 636/GR (February 2007)), the different aspect of the current formulation is expected to allow a fast and unambiguous visual differentiation from the other existing granules. As the catalogue of pesticide formulations (see Manual, Appendix E: CropLife International codes for technical and formulated pesticides) does not provide an own code for such a formulation type, the code for granules (GR) was chosen to designate this formulation and a Note added explaining the slow release properties of this product.

The CIPAC method for Spinosad is published in Handbook L and covers, beside the TC, SC and GR formulations. An extension of the scope (CIPAC/4847) of CIPAC method 636/GR/(M) for the determination of the Spinosad content of this new granules formulation (GR) was accepted as a provisional CIPAC method in 2012 and was promoted to a full CIPAC method in 2013. The tolerance for the difference of the measured to the declared content of Spinosad are with ± 25 % in line with the Manual for a nominal content of 25 g/kg in an inhomogeneous formulation.

Physical-chemical properties and storage stability

The clauses and limits in the physical-chemical subsection were mainly in agreement with the requirement of the Manual. In addition to standard clauses for a GR formulation, the company proposed to specify the flowability after storage under heat and pressure (MT 172). The Meeting considered this clause as not necessary and the company agreed to remove it. The size distribution of the granules is quite uniform with a minimum of 850 g/kg being in the size range between 850 to 2000 µm, the formulation is essentially non-dusty and minimum attrition is 98 %. After accelerated storage at 54°C for 2 weeks, the products still complies with size distribution, dustiness and attrition resistance.

As no method is currently available to measure directly the slow release properties of the granules, the Meeting proposed to publish the specification as a time-limited (for a 3 years period) interim specification and to invite the manufacturer to develop an appropriate standard test to measure directly the release properties of the GR.

The Meeting agreed also to update the existing specification for the granules as follows:
- Revision of the specification code to 636/GR/1 to distinguish the granules from the new extended release granules (636/GR/2).
- Update of the CIPAC method for nominal size range to MT 170 as the MT 58 is no longer supported by CIPAC.

Spinosad monolayer tablets for direct application (DT)

Supporting data and draft specification for a new slow release formulation of Spinosad DT, jointly provided by Dow AgroSciences and Clarke Mosquito Control in 2011, were considered by the JMPS for development of a new WHO specification. Spinosad DT has been developed as mosquito larvicide by Clarke Mosquito Control and the specification, therefore, is limited to WHO. The product was successfully evaluated as larvicide by WHOPES and recommendation for its use in public health was recently published (WHO 2013). The proposed specification for DT was broadly in agreement with the guidelines given in the Manual (FAO/WHO 2010).

Formulation type, description, content of active ingredient and analytical method

Spinosad is formulated as tablets for direct application with 83.3 g/kg of the complex of active ingredients, spinosyn A and D. The formulation is intended for direct application to mosquito larvae habitats. The tablets are formulated in such a way that they release the complex of active ingredient over prolonged period of time. As the formulation code for tablets for direct application covers quite a wide range of possible release characteristics for the active ingredient, a Note was added to explain the main characteristics of the product: a monolayer tablet providing extended release properties of the tablets. Besides having a higher nominal content as the other existing DT formulation having a published WHO specification (WHO 636/DT (September 2011)), the current formulation is a monolayer system without the effervescent part. The monolayer appearance and the different size and weight allow a fast and unambiguous visual identification of the two tablet types.

The CIPAC method for Spinosad DT, published in Handbook M, is applicable to this tablet type and concentration range of Spinosad as well. The tolerance for the difference of the measured to the declared content of Spinosad are with ±10 % in line

with the Manual for a nominal content of 83.8 g/kg. The tablet dose uniformity expressed as relative standard deviation when 20 tablets are analysed individually should be less than 10 % – an important factor to ensure efficacy as related to a single tablet.

Physical-chemical properties and storage stability

The clauses and limits in the physical-chemical subsection were essentially in agreement with the requirement of the Manual. No broken tablets should be present and degree of attrition as per the amended MT 193 should be lower than 2 %. The accelerated storage test needs to be carried out in the original packaging (therefore without compaction), and after storage at 54°C for 2 weeks, the product should still comply with content of Spinosad, tablet integrity and degree of attrition.

As no method is currently available to measure directly the slow release properties of the tablet, the Meeting proposed to publish the specification as a time-limited (for a 3 years period) interim specification and to invite the manufacturer to develop an appropriate standard test to measure directly the release properties of the DT.

The Meeting agreed also to revise the specification code of the other existing DT formulation to 636/DT/1 and to rename the title as "Spinosad bilayer tablets for direct application" in order to clearly distinguish it from the new monolayer tablets (636/DT/2).

Annex 1. References

Study number	Author(s)	Year	Study title. Study identification number. Report identification number. GLP [if GLP]. Company conducting the study
	FAO/WHO	2010	Manual on development and use of FAO and WHO specifications for pesticides. Second revision of the 1st edition. FAO, Rome and WHO, Geneva, November 2010 (internet publications).
	WHO	2013	Report of the Sixteenth WHOPES Working Group Meeting, WHO/HQ, Geneva, 22-30 July 2013. WHO, Geneva, document ISBN 978 92 4 150630 4 and WHO/HTM/NTD/WHOPES/2013.6

SPINOSAD
Evaluation Report 636/2010

Recommendations

The Meeting recommended that the specification for Spinosad EC, proposed by Dow AgroSciences, as amended, should be adopted by WHO.

Appraisal

Supporting data and draft specifications for Spinosad EC, jointly provided by Dow AgroSciences and Clarke Mosquito Control, were considered by the JMPS for development of a new WHO specification. Spinosad EC has been developed as mosquito larvicide by Clarke Mosquito Control and the specification is therefore limited to WHO. The product was successfully evaluated as larvicide by WHOPES and recently a recommendation for its use in public health was published (WHO, 2011).

Spinosad is formulated as an emulsifiable concentrate (EC) with 240 g/L or 20.6 % of the complex of active ingredients spinosyn A and D. The formulation is intended for dilution with water in the spray tank at a recommended rate of 2 fl oz per 20 gallons which corresponds to 59.15 ml of the formulated product in approximately 75.7 L of water.

The proposed specification for EC was broadly in agreement with the guidelines given in the Manual (FAO/WHO 2006). The only non-standard clause - the requirement for mixing the EC formulation prior to use or sampling for quality control purposes - was discussed by the Meeting. The companies explained that the formulation may show some faint sedimentation upon standing. Some minor spinosyn compounds, which stem from the fermentation process, are prone to crystallization. These minor components however can be easily redissolved upon stirring. The mixing process ensures a homogeneous formulation without sediments ready for application. The Meeting agreed to include the information in a Note to the description clause, drawing the attention of the reader to the fact that a homogeneous formulation like an EC must be homogenized before sampling for chemical and physical-chemical testing.

The method extension for the analysis of Spinosad in EC formulations was presented at the 2010 CIPAC Meeting in Slovenia and adopted as full CIPAC method in 2011. The identity tests are the same as for the TC, published in Handbook L (retention times of spinosyn A and D, respectively, in HPLC and ESI spectra in LC-MS). The Meeting noted that the EC is not based on mineral oil, which would not dissolve the spinosyns, but on a semipolar water immiscible continuous phase, which is miscible with methanol as the extraction solvent. Complete dissolution of the EC is essential for a good recovery, therefore the homogeneity of the resulting solution should be carefully checked in each EC formulation to be analysed.

The tolerances for the average content of Spinosad are with ±6 % in line with the Manual for a nominal content of 240 g/L. The company proposed to include the lower concentration range of 25 to 100 g/L as well, explaining that it would cover future developments of ECs having lower content. As WHO specifications are linked to WHOPES recommendation, which refers to the tested concentration of 20.6 % or 240 g/L, the concentration of 240 g/L only was included.

The clauses for relevant impurities and acidity/alkalinity were omitted, because Spinosad does not contain relevant impurities nor are produced during storage nor is sensitive to rapid hydrolysis catalysed by lower or higher pH values. The accelerated storage test was done according to CIPAC MT 46.3 for liquid formulations.

The Meeting agreed also to change the method for determination of the degree of attrition in the specification for the DT formulation and to adapt the clause according to the DT specification guideline of the November 2010 – second revision of the first edition of the FAO/WHO Manual and the recommended revised CIPAC method MT 193.

Annex 1. References

Study number	Author(s)	Year	Study title. Study identification number. Report identification number. GLP [if GLP]. Company conducting the study
	FAO/WHO	2006	Manual on development and use of FAO and WHO specifications for pesticides. March 2006 revision of the 1st edition. FAO, Rome and WHO, Geneva, March 2006 (internet publications).
	FAO/WHO	2010	Manual on development and use of FAO and WHO specifications for pesticides. Second revision of the 1st edition. FAO, Rome and WHO, Geneva, November 2010 (internet publications).
	WHO	2011	Report of the 14th WHOPES Working Group Meeting, WHO/HQ, Geneva, 11-15 April 2011. WHO, Geneva, document to press.

SPINOSAD
Evaluation Report 636/2007

Recommendations

The Meeting recommended that the specification for Spinosad DT, proposed by Dow AgroSciences, as amended, should be adopted by WHO, subject to satisfactory evaluation of these products in public health applications by WHOPES[1].

Appraisal

Supporting data and draft specifications for Spinosad DT, provided by Dow AgroSciences, were considered by the JMPS for development of a new WHO specification. Spinosad DT is intended only for mosquito larviciding and the specification is, therefore, restricted to WHO.

Each tablet consists of two homogenous horizontal layers of technical Spinosad. An upper layer consists of technical Spinosad in an effervescent system providing fast release of active ingredient upon application to water, whereas the lower layer is formulated to dissolve in water gradually over time. The tablets are vacuum-packed in a resealable moisture-barrier pouch.

The proposed specification for DT was broadly in agreement with the guidelines given in the manual[2]. The manufacturer presented supporting information on the release of Spinosad from the DT formulation into 200-litre water volumes, showing an initial fast release due to the dissolution of the effervescent layer followed by a slow release of additional active ingredient over prolonged time from the second layer. The Meeting considered the necessity for a release rate clause. However, instead of such a clause, a brief description of the dissolution characteristics was added in Note 1 of the specification, providing information on the two-phase delivery of Spinosad to treated water.

The method of analysis of Spinosad DT was accepted as a full CIPAC method in 2007, with the identity tests being the same as those for the TC, published in CIPAC Handbook L. The tolerance for the average content of Spinosad is with ±10 % in line with the manual for a nominal content of 74.8 g/kg. The manufacturer proposed a maximum 10% relative standard deviation for the tablet dose uniformity, which was accepted by the Meeting.

Clauses for relevant impurities and acidity/alkalinity were unnecessary. There are no relevant impurities in Spinosad and, due to the effervescent system, a clause for acidity/alkalinity is not meaningful. The accelerated storage test used was CIPAC MT 46.3, sub-method 2 (no pressure applied). This was accepted by the Meeting, as tablets may suffer from pressure exerted and the subsequent tests of physical properties after storage (tablet integrity and attrition resistance) are prone to artefact formation. As the tablets are vacuum-packed within a robust flexible pouch (containing 250 tablets) which minimizes movement and potential compression of tablets, this was

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[1] The WHOPES Working Group reviewed and recommended Spinosad 7.48% DT for mosquito larviciding in 2007.

[2] Manual on development and use of FAO and WHO specifications for pesticides. March 2006 revision of the 1st edition. Available only on the internet at http://www.fao.org/ag/agp/agpp/pesticid/ and http://www.who.int/whopes/quality/.

considered acceptable. With this form of packaging, and with one half of the tablet containing an effervescent system to initiate disintegration after application to water, the clause and limit for tablet integrity were considered to be sufficient without the need for the specification to include a test of tablet hardness.

SPINOSAD
Evaluation Report 636/2005

Recommendations

The Meeting recommended that:

(i) the specifications for spinosad TC, SC and GR, proposed by Dow AgroSciences, should be adopted by FAO;

(ii) the specifications for spinosad TC, SC and GR, proposed by Dow AgroSciences, should be adopted by WHO, subject to satisfactory evaluation of these products in public health applications by WHOPES*.

Appraisal

The Meeting considered data and draft specifications for spinosad, submitted by Dow AgroSciences in 2004. Spinosad is a macrocyclic lactone insecticide that had not previously been the subject of a WHO or FAO specification. The data submitted were in accordance with the requirements of the manual (FAO/WHO 2002) and supported the proposed FAO and WHO specifications for TC, SC and GR.

The spinosad toxicology was evaluated by the FAO/WHO JMPR in 2001 (JMPR 2001). Spinosad residues data were evaluated by the FAO/WHO JMPR in 2001 (JMPR, 2001) and there are currently several Codex maximum residue limits (MRLs) for spinosad. Spinosad was reviewed and approved by the U.S. EPA in 1997 and subsequent regulatory reviews and approvals have occurred in more than 60 countries including Australia, Brazil, Canada, India, Japan, New Zealand and South Africa. Spinosad has been under evaluation by the European Commission as a new active substance since 2000 and EU member state evaluations and provisional approvals have occurred in the Netherlands, Spain and the UK. Spinosad SC and GR formulations are under development as mosquito larvicides and are currently being evaluated by WHOPES, with a report expected in 2006.

Spinosad is under patent in some countries (Australia, Japan, UK), until December 2009, and in the country of technical product manufacture (USA), until March 2015.

The ISO common name, spinosad, denotes an insecticide consisting of two components, called spinosyns A and D (which may be referred to simply as A and D, below). The spinosyns are produced by a soil bacterium, *Saccharopolyspora spinosa,* belonging to the group Actinomycetes, a large group of gram-positive filamentous or branching bacilli.

Spinosad is produced in a fermentation process, where it is obtained by extraction and purification of the whole broth. Spinosyns A and D are present in the isolated spinosad, in proportions of 65-95% and 5-35%, respectively, together with traces of spinosyn-related compounds and other materials derived from the fermentation and purification process. The specified proportions of spinosyns A and D in spinosad are in agreement with the definition of the ISO common name.

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* The WHOPES Working Group reviewed and recommended spinosad 12% SC and 0.5% GR for mosquito larviciding in 2006.

The two main spinosyns, A and D, are closely related structurally and represent more than 85% of technical spinosad and are responsible for most of its insecticidal activity. They differ only in the presence of an additional methyl group attached to the bridging carbon of the indacene moiety in spinosyn D. Spinosyns A and D are relatively high molecular weight compounds (732 and 746, respectively). The additional methyl group has a significant effect on certain properties and many of the physico-chemical data were generated using separated and purified A and D.

Spinosyns A and D have very low vapour pressures, making them essentially non-volatile. Spinosyns A and D are weak bases, with pK_as of 8.1 and 7.9, respectively. Spinosyn A has a rather low, and pH-dependent, water solubility (290 mg/l at pH 5), with that of D even lower (29 mg/l at pH 5). As may be expected for weak bases, the water solubility decreases with increasing pH in both cases. The octanol/water partition coefficient is also pH-dependent, 2.8 and 3.2 at pH 7, expressed as log P K_{ow} for A and D, respectively, with increasing log P K_{ow} with increasing pH. Both spinosyns are resistant to hydrolysis in sterile, buffered water, with no detectable hydrolysis at pH 5 and increasing but very slow hydrolysis at pH 7 and 9. Aqueous photolysis of A and D at pH 7 was rapid with a half-life of less than one day.

The Meeting was provided with commercially confidential information on the manufacturing process and 7-batch analysis data on purity and all impurities ≥1 g/kg. The Meeting noted that, although technical spinosad is of biological origin, the unaccountable fraction was 20 g/kg or less in all batches and that the data supported the proposed minimum active ingredient content of 850 g/kg. These data were confirmed as identical to those submitted for registration in Switzerland. One of the 7 batches, with a slightly higher content of D and an average content of the minor spinosyns, was utilized for the toxicity testing.

The Meeting agreed with the manufacturer that none of the impurities should be considered as relevant.

Analytical methods to determine spinosyns A and D in TC, SC and GR were adopted by CIPAC in 2005. Spinosyns A and D are determined by reversed-phase HPLC with a methanol/acetonitrile/water/acetic acid mobile phase and UV detection. The identity test is based on HPLC-separation of spinosyns A and D and detection by positive ion ESI-MS. The test is highly specific, involving comparison of the retention times of A and D in the HPLC-chromatogram, together with the mass spectra of A and D, showing proton- and sodium adducts and fragmentation.

Draft specifications were submitted for spinosad TC, SC and GR.

At the time of the meeting, the general distinction between TC and TK was still under discussion with industry, although a cut-off value for purity of 900 g/kg had been used as one criterion by the JMPS. The distinction is important because TK specifications have an upper limit for active ingredient content and TC specifications do not. The rationale has been to encourage production of TCs with the highest possible purity, because the maximum possible increase in hazard due to the active ingredient cannot exceed 10% (taken to represent a negligible increase), whereas the consequent proportional reduction in impurity levels may be very significant. This approach cannot be adopted for TK, because the maximum increase in hazard due active ingredient could exceed the 10% threshold.

On this basis, therefore, the proposed minimum content of spinosad in the technical grade active ingredient (850 g/kg) might be considered to represent a TK. The Meeting

noted that the hazards presented by spinosyns A and D are similar and therefore potential changes in their proportions do not affect the decision as to whether technical spinosad is a TC or a TK. Taking into account the manufacturing process, the nature of the impurities and the minimum content of the active ingredient, the Meeting considered that it was not necessary to introduce an upper limit for spinosad content and agreed that, exceptionally, technical spinosad should be considered to be a TC, rather than a TK.

The proportions of spinosyns A and D in technical spinosad TC were confirmed to be in agreement with the ISO definition of the spinosad common name and therefore it was not necessary to introduce a clause specifying the range of ratios.

The proposed specification for SC conformed to the guideline presented in the manual (FAO/WHO 2002) and was supported by the data held by the registration authorities in Switzerland.

The proposed specification for GR differed from the guideline given in the manual, in that the ±10% tolerance for a.i. content was narrower than the ±15% maximum. The manufacturer confirmed the proposed tolerance of ±10% for the 10 g/kg GR formulation was always met in practice and the Meeting agreed to accept it.

Supporting Information
For
Evaluation Report 636/2005

Uses

Spinosad is an insecticide, used for the control of caterpillars, thrips, beetle and fly pests in a range of fruit and vegetable crops, ornamentals, turf, and stored grains. Spinosad has contact activity on all life stages of insects, including eggs, larvae and adults. Eggs must be sprayed directly but larvae and adults can be effectively dosed through contact with treated surfaces. Spinosad is most effective when ingested. Foliar applications are not highly systemic, although trans-laminar activity is evident in certain vegetable crops and ornamental plants. Spinosad acts by altering the function of nicotinic- and GABA-gated ion channels of insect nervous systems but it does not interact with known binding sites for other nicotinic- or GABA-agonistic insecticides. It is used in agriculture, horticulture, forestry, and public health against a wide range of insects including thrips, Mediterranean fruit fly, olive fruit fly, codling moth, caterpillars, leaf miners, Colorado beetle and potato worm (Sparks *et al*. 1998).

Identity of the active ingredient

ISO common name

Spinosad (BSI, E-ISO, ANSI), being a mixture of spinosyns A and D, with A:D proportions in the range 50:50 to 95:5

Synonyms

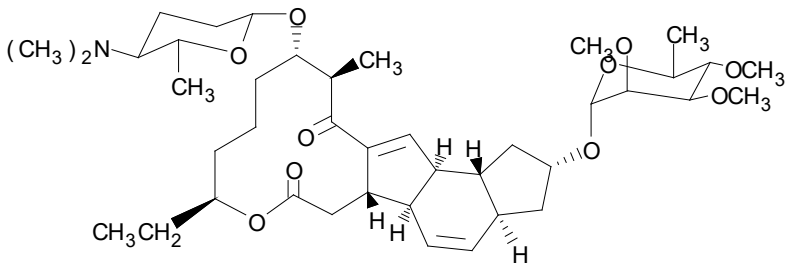
None

Chemical names

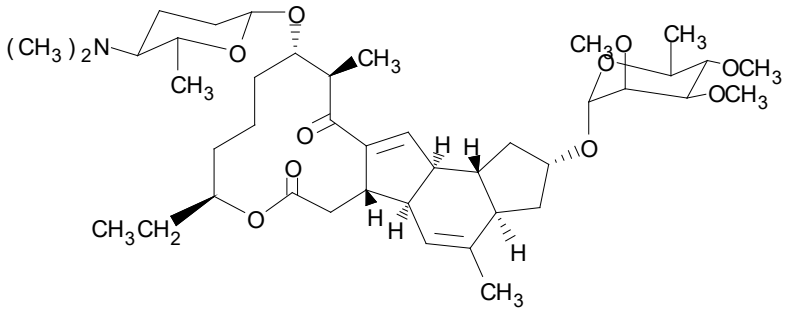
IUPAC A mixture of spinosyn A, (2*R*,3a*S*,5a*R*,5b*S*,9*S*,13*S*,14*R*,16a*S*,16b*R*)-2-(6-deoxy-2,3,4-tri-*O*-methyl-α-L-mannopyranosyloxy)-13-(4-dimethylamino-2,3,4,6-tetradeoxy-β-D-erythropyranosyloxy)-9-ethyl-2,3,3a,5a,6,7,9,10,11,12,13,14,15,16a,16b-hexadecahydro-14-methyl-1*H*-8-oxacyclododeca[*b*]*as*-indacene-7,15-dione, and spinosyn D, (2*R*,3a*S*,5a*R*,5b*S*,9*S*,13*S*,14*R*,16a*S*,16b*R*)-2-(6-deoxy-2,3,4-tri-*O*-methyl-α-L-mannopyranosyloxy)-13-(4-dimethylamino-2,3,4,6-tetradeoxy-β-D-erythropyranosyloxy)-9-ethyl-2,3,3a,5a,6,7,9,10,11,12,13,14,15,16a,16b-hexadecahydro-4,14-dimethyl-1*H*-8-oxacyclododeca[*b*]*as*-indacene-7,15-dione, with A:D proportions in the range 50:50 to 95:5

CA [2*R*-[2*R**,3a*S**,5a*R**,5b*S**,9*S**,13*S**(2*R**,5*S**,6*R**),14*R**,16a*S**,16b*R**]]-2-[(6-deoxy-2,3,4-tri-*O*-methyl-α-L-mannopyranosyl)oxy]-13-[[5-(dimethylamino)tetrahydro-6-methyl-2*H*-pyran-2-yl]oxy]-9-ethyl-2,3,3a,5a,5b,6,9,10,11,12,13,14,16a,16b-tetradecahydro-14-methyl-1*H*-*as*-indaceno(3,2-*d*)oxacyclododecin-7,15-dione (spinosyn A), mixture with [2S-[2*R**,3a*S**,5a*R**,5bR*,9R*,13R*(2S*,5R*,6S*),14S*,16aR*,16bR*]]-2-[(6-deoxy-2,3,4-tri-*O*-methyl-α-L-mannopyranosyl)oxy]-13-[[5-(dimethylamino)tetrahydro-6-methyl-2*H*-pyran-2-yl]oxy]-9-ethyl-2,3,3a,5a,5b,6,9,10,11,12,13,14,16a,16b-tetradecahydro-4,14-dimethyl-1*H*-*as*-indaceno(3,2-*d*)oxacyclododecin-7,15-dione (spinosyn D)

Structural formulae



spinosyn A



spinosyn D

Empirical formulae

spinosyn A: $C_{41}H_{65}NO_{10}$
spinosyn D: $C_{42}H_{67}NO_{10}$

Relative molecular mass

spinosyn A: 732.0
spinosyn D: 746.0

CAS Registry number

spinosyn A: 131929-60-7
spinosyn D: 131929-63-0

CIPAC number

636

EEC number

434-300-1

Identity tests

HPLC retention time, positive-ion ESI LC-MS.

Physico-chemical properties of spinosad

Table 1. Physico-chemical properties of pure spinosad

Parameter	Value(s) and conditions	Purity %	Method	References
Vapour pressure, at 25ºC	Spinosyn A 3.0 x 10^{-8} Pa Spinosyn D 2.0 x 10^{-8} Pa	99.9 >99	OECD No. 104 EEC method A4, Knudsen-effusion/weight loss method	DAS A01, DAS A36
Melting point	Spinosyn A 84 to 99.5ºC Spinosyn D 161.5 to 170ºC Spinosyn A + D 110 to 123ºC	98.3 98.0 88.0 (A+D)	OECD No. 102 EEC method A1	DAS A03
Temperature of decomposition	Decomposition start temperature: 172ºC , 92% weight loss during heating to 400ºC	88.0 (A+D)	Thermal analysis	DAS A18
Solubility in water, at 20°C	Spinosyn A 290 mg/l at pH 5 235 mg/l at pH 7 16 mg/l at pH 9 Spinosyn D 28.7 mg/l at pH 5 0.332 mg/l at pH 7 0.053 mg/l at pH 9	98.3 99.9 99.8	OECD No. 105: flask method column elution method column elution method	DAS A20, DAS A37
Octanol/water partition coefficient, at 23°C	Spinosyn A Log P K_{ow} = 2.78 at pH 5 Log P K_{ow} = 4.01 at pH 7 Log P K_{ow} = 5.16 at pH 9 Spinosyn D Log P K_{ow} = 3.23 at pH 5 Log P K_{ow} = 4.53 at pH 7 Log P K_{ow} = 5.21 at pH 9	97.0 98.0	EPA/FIFRA subdiv. D 63.11, shake flask method	DAS A08, DAS A47
Hydrolysis characteristics, at 25°C	Spinosyn A No hydrolysis at pH 5 Half-life = 648 d. at pH 7 Half-life = 200 d. at pH 9 Spinosyn D No hydrolysis at pH 5 and 7 Half-life = 259 d. at pH 9	99.9 99.9	FIFRA guideline 161-1	DAS K05
Photolysis characteristics	Spinosyn A Half-life in dilute aqueous buffer calculated as 0.96 d. in summer sunlight (June-July, Greenfield, Indiana, 39.8°N) Spinosyn D Half-life in dilute aqueous buffer calculated as 0.84 d. in summer sunlight (June-July, Greenfield, Indiana, 39.8°N).	94.7 93.6	FIFRA Guideline 161-2	DAS K06

Table 1. Physico-chemical properties of pure spinosad

Parameter	Value(s) and conditions	Purity %	Method	References
Dissociation characteristics, at 20°C	Spinosyn A pKa = 8.1 Ka = 7.94 x 10^{-9} Spinosyn D pKa = 7.87 Ka = 1.35 x 10^{-8}	97.0 97.0	OECD guideline 112, capillary electrophoresis method	DAS A04, DAS A07

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

Manufacturing process, maximum limits for impurities ≥ 1 g/kg, 5 batch analysis data	Confidential information supplied and held on file by FAO. Mass balances were 98.0-101.6%, maximum percentage of unknowns was 0.3%.
Declared minimum spinosad content	850 g/kg (spinosyn A + spinosyn D)
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
Melting temperature of the TC	110 to 123ºC, (spinosyn A + spinosyn D)

BACKGROUND INFORMATION ON TOXICOLOGY/ECOTOXICOLOGY

Dow AgroSciences confirmed that the toxicological and ecotoxicological data included in Annex 1, below, were derived from spinosad having impurity profiles similar to those referred to in Table 2, above.

Spinosad was evaluated for toxicology by the FAO/WHO JMPR in 2001. The JMPR concluded that spinosad has low acute toxicity. In studies with repeated doses, no acute toxicological alerts were observed that might indicate the need for establishing an acute reference dose (acute RfD). An ADI of 0–0.02 mg/kg bw was established on the basis of a NOAEL of 2.4 mg/kg bw per day in a 2-year study of toxicity and carcinogenicity in rats (Bond *et al*. 1995b, 1996d) and a 100-fold safety factor. The Swiss authorities assigned an ADI of 0-0.02 mg/kg bw/d, based on a NOEL of 2.4 mg/kg bw/d in the two year study on rats. This range is in agreement with the ADI assigned by the JMPR. The JMPR concluded that it was not necessary to assign an acute reference dose.

Maximum residue limits for spinosad have been set in Switzerland for a range of agricultural commodities. Estimated dietary intakes, based on typical food baskets, indicate that exposure of the population is expected to be well below the ADI.

The WHO hazard classification of spinosad is U, unlikely to present acute hazard in normal use (WHO 2004).

FORMULATIONS

The main formulation types available are suspension concentrates (SC) at 120 to 480 g spinosad/l, wettable powders (WP), water dispersible granules (WG), and

granules for direct application (GR). Spinosad may be co-formulated with other insecticide active ingredients.

The formulations are registered and sold in more than 60 countries throughout the world including the USA, Australia, Brazil, Canada, India, Japan, New Zealand, Switzerland and South Africa. Spinosad has been under EU evaluation as a new active substance since 2000, and meanwhile member state evaluations and provisional approvals have been granted in a number of EU countries including Italy, Netherlands, Spain, and the UK.

Methods of analysis and testing

Analytical methods for the identification and determination of spinosad content were adopted by CIPAC in 2005. The spinosad content (sum of spinosyns A and D) is determined by reversed-phase HPLC, using UV detection at 280 nm and external standardization. Definitive identification is by positive-ion ESI LC-MS, as no other technique is sufficiently specific.

Methods for the determination of impurities are based on reversed-phase HPLC with UV detection.

Test methods for determination of physico-chemical properties of technical spinosad were OECD/EC, while those for the formulations were CIPAC as indicated in the specifications.

Physical properties

The physical properties of the SC and GR formulations, the test methods and specification limits proposed, comply with the requirements of the manual (FAO/WHO 2002).

CONTAINERS AND PACKAGING

No special requirements for containers and packaging have been identified.

EXPRESSION OF ACTIVE INGREDIENT

The active ingredient is expressed as spinosad, which is the sum of spinosyn A + spinosyn D, in g/kg or g/l at 20 ± 2ºC.

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 spinosad having impurity profiles similar to those referred to in Table 2, above.

Table A. Toxicology profile of the spinosad technical material*, based on acute toxicity, irritation and sensitization

Species	Test	Duration and conditions	Result	References
Rat, m & f	Acute oral	OECD guideline 401 acute oral toxicity, 1987	$LD_{50} \geq 3738$ mg/kg bw (m) $LD_{50} > 5000$ mg/kg bw (f)	DAS B01, DAS B16
Mouse, m & f	Acute oral	OECD guideline 401 acute oral toxicity, 1987	LD_{50} >5000 mg/kg bw (m & f))	DAS B01, DAS B16
Rabbit, m & f	Acute dermal	OECD guideline 402 acute dermal toxicity, 1987	LD_{50} >5000 mg/kg bw (m & f)	DAS B07
Rat, m & f	Acute inhalation	EC test guideline (EC method B.2 acute toxicity (inhalation), 1984	LD_{50} >5.18 mg/l/4h	DAS B04
Rabbit, m & f	Skin irritation	OECD guideline 404 acute dermal irritation/corrosion, 1987	No irritation	DAS B05, DAS B30
Rabbit, m & f	Eye irritation	EC method B.5 acute toxicity (eye irritation), 1992	Mild transient irritation	DAS B09, DAS B32
Guinea pig, m	Skin sensitization	OECD guideline 406 skin sensitization, 1987, Buehler test	No sensitization	DAS B28
Guinea pig, f	Skin sensitization	EC test guideline (method B.6 skin sensitisation, 1996, Magnussen & Kligman test	No sensitization	DAS B33

* The spinosad TC used for the toxicity studies contained 771 g/kg A and 122 g/kg D, which was considered typical for spinosad, in terms of the ratio of spinosyns A and D and the content of other compounds.

Table B. Toxicology profile* of spinosad technical material based on repeated administration (sub-acute to chronic)**

Species	Test	Duration and conditions	Result	References
Rabbit, m & f	21-d dermal	OECD 410	NOAEL = 1000 mg/kg bw/d	DAS D05
Rat, m & f	14-d inhalation, 15-d recovery	OECD 412	NOAEL = 9.5 mg/m^3	DAS D22
Rat, m & f	13-week oral	OECD 408	NOAEL = 8.6 mg/kg bw/d LOAEL = 42.7 mg/kg bw/d	DAS D02
Rat, m & f	13-week oral	OECD 408	NOAEL = 7.7 mg/kg bw/d LOAEL = 39.1 mg/kg bw/d	DAS D20
Dog, m & f	13-week oral	OECD 409	NOAEL = 4.89 mg/kg bw/d LOAEL = 9.73 mg/kg bw/d	DAS D10
Mouse, m & f	3-month oral	OECD 408	NOAEL = 7.5 mg/kg bw/d LOAEL = 22.5 mg/kg bw/d	DAS D12
Dog, m & f	12-month oral	OECD 452	NOAEL = 2.68 mg/kg bw/d LOAEL = 8.22 mg/kg bw/d	DAS D03
Mouse, m & f	18-month oral, combined chronic toxicity and carcinogenicity	OECD 451	NOAEL = 11.4 mg/kg bw/d LOAEL = 32.7 mg/kg bw/d No carcinogenic potential	DAS I02, DAS I01, DAS I04, DAS I06
Rat, m & f	2-year oral, combined chronic toxicity and carcinogenicity	OECD 453	NOAEL = 2.4 mg/kg bw/d LOAEL = 11.4 mg/kg bw/d No carcinogenic potential	DAS I03, DAS I05
Rat	2-generation reproductive study	OECD 416	NOAEL = 10 mg/kg bw/d Reproduction NOAEL = 100 mg/kg bw/d	DAS F01
Rat	Teratogenicity	OECD 414	Maternal NOAEL = 50 mg/kg bw/d Developmental NOAEL = 200 mg/kg bw/d No teratogenic potential	DAS F03
Rabbit	Teratogenicity	OECD 414	Maternal NOAEL = 10 mg/kg bw/d Developmental NOAEL = 50 mg/kg bw/d No teratogenic potential	DAS F05
Rat, m & f	Neurotoxicity	OECD 424	No evidence of neurotoxicity in acute, sub-chronic and chronic studies	DAS B24, DAS I10, DAS D04

In addition to the data presented in Table B, the manufacturer provided data from a 28-day oral toxicity study in rats, in which the toxicity of a spinosyn A + D mixture was compared with that of spinosyn A (96.2%) and spinosyn D (93.0%). The mixture,

* In addition to the data presented, the toxicity of a spinosyn A + D mixture was compared with that of spinosyn A (96.2%) and spinosyn D (93.0%). Spinosyn A and spinosyn D were found to display similar toxicity in mammalian systems, with spinosyn A being slightly more toxic than spinosyn D at equivalent (expressed as mg/kg bw/d) dose levels (DAS D09).

** The spinosad TC used for the toxicity studies contained 771 g/kg A and 122 g/kg D, which was considered typical for spinosad, in terms of the ratio of spinosyns A and D and the content of other compounds.

spinosyn A and spinosyn D were found to display similar toxicity in mammalian systems, with spinosyn A being slightly more toxic than spinosyn D at equivalent dose levels (expressed as mg/kg bw/d.)

Table C. Mutagenicity profile of spinosad technical material* based on *in vitro* and *in vivo* tests

Species	Test	Conditions	Result	Reference
S. typhimurium TA98, TA100, TA1535,TA1537 and *E. coli* WP2uvrA	Ames test, pre-incubation *in vitro* , plate incorporation *in vitro,* OECD 471	50 to 5000 µg/plate	Negative	DAS E06
Mouse lymphoma cells, L5178Y	Mammalian cells *in vitro*, gene mutations, TK assay, OECD 476	1 to 50 µg/ml	Negative	DAS E04
Chinese hamster ovary (CHO-WB_L) cells	mammalian cells *in vitro*, cytogenic assay, OECD 473	20 to 100 µg/ml	Negative	DAS E01
Rat hepatocytes	mammalian cells *in vitro*, unscheduled DNA synthesis, OECD 482	0.1 to 5 µg/ml	Negative	DAS E02
Mouse	*In vitro* micronucleus test, OECD 474	2 daily oral doses: 500, 1000, 2000 mg/kg bw; sacrifice at 24 h after last dose	Negative	DAS E03

Based on these results, spinosad was considered to be non-genotoxic.

* The spinosad TC used for the toxicity studies contained 771 g/kg A and 122 g/kg D, which was considered typical for spinosad, in terms of the ratio of spinosyns A and D and the content of other compounds.

Table D. Ecotoxicology profile* of spinosad technical material** or formulated product

Species	Test	Duration and conditions	Result	Reference
Daphnia magna (water flea)	Acute toxicity, static	48 h, FIFRA 72-2 & OECD 202 Part 1 (20 ± 2°C)	EC_{50} >1.0 mg as/l	DAS J38
Daphnia magna (water flea)	Acute toxicity, static, formulation 480SC	48 h, OECD 202 Part 1 (20 ± 2°C)	EC_{50} = 9.1 mg as/l	DAS MJ06
Daphnia magna (water flea)	Chronic toxicity	21 d, FIFRA 72-4 & OECD 202 Part 2 (20 ± 2°C)	NOEC = 0.0012 mg as/l (flow through) NOEC = 0.0080 mg as/l (semi-static)	DAS J15
Chironomus riparius (midge)	Chronic toxicity, static	25 d, OECD 219 (20 ± 0.5°C)	NOEC = 0.0016 mg as/l	DAS J51
Oncorhynchus mykiss (rainbow trout)	Acute toxicity, static	96 h, FIFRA 72-1 & OECD 203 ,12.5 ± 0.5°C	LC_{50} = 27 mg as/l	DAS J06
Lepomis macrochirus (bluegill sunfish)	Acute toxicity, static	96 h, FIFRA 72-1 & OECD 203 (21-22.1°C)	LC_{50} = 5.94 mg as/l	DAS J27
Cyprinus carpio	Acute toxicity, flow through	96 h, FIFRA 72-1 & OECD 203 (24.5-25.5°C)	LC_{50} = 4 mg as/l	DAS J05
Cyprinus carpio	Acute toxicity, static	96 h, OECD 203 (22 ± 2°C), 480 g/l SC	LC_{50} >49 mg as/l	DAS MJ16
Oncorhynchus mykiss (rainbow trout)	Early life-stage toxicity, flow through	80 day, FIFRA 72-4(a) & OECD 210 (12 ± 2°C)	NOEC = 0.5 mg as/l	DAS J12
Navicula pelliculosa (alga)	Static water	120 h, FIFRA 123-2 & OECD 201 (22 ± 1°C)	EC_{50} = 0.079 mg as/l	DAS J19
Navicula pelliculosa (alga)	Static water, formulation 480SC	120 h, OECD 201 (22 ± 1°C)	EC_{50} = 0.35 mg as/l	DAS MJ17
Anabaena flos-aquae (alga)	Static water	120 h, FIFRA 123-2 & OECD 201 (24 ± 2°C)	EC_{50} = 6.1 mg as/l	DAS J17
Selenastrum capricornutum (alga)	Static water	72 h, FIFRA 123-2 & OECD 201 (24 ± 2°C)	EC_{50} = 56 mg as/l	DAS J30
Lemna gibba (higher plant)	Static water	14 d, FIFRA 123-2 & OECD 221 (25.3 ± 0.15°C)	EC_{50} = 6.6 mg/l	DAS J16
Eisenia foetida (earthworm)	Acute toxicity	14 d, 20 ± 2°C	LC_{50} >970 mg as/kg dry soil	DAS J21

* Data were also provided on the effects of spinosad on non-target insects, including larvae of the hoverfly *Episyrphus balteatus* (DAS MJ25), the foliar-active predator *Chrysoperla carnea* (DAS MJ24), the parasitoid wasp *Aphidius colemani* (DAS MJ22) and the carabid beetle *Poecilus cupreus* (DAS MJ23).

** The spinosad TC used for the toxicity studies contained 771 g/kg A and 122 g/kg D, which was considered typical for spinosad, in terms of the ratio of spinosyns A and D and the content of other compounds.

Table D. Ecotoxicology profile* of spinosad technical material or formulated product**

Species	Test	Duration and conditions	Result	Reference
Apis mellifera (honey bee)	Oral exposure	OECD 213	LD_{50} = 0.057 µg/bee (spinosad) LD_{50} = 0.049 µg as/bee (480SC)	DAS J47
Apis mellifera (honey bee)	Contact exposure	OECD 214	LD_{50} = 0.0036 µg/bee (spinosad) LD_{50} = 0.050 µg as/bee (480SC)	DAS J20
Apis mellifera (honey bee)	Acute oral	EPPO 170	LD_{50} = 0.0057 µg/bee (spinosad) LD_{50} = 0.049 µg as/bee (480SC)	DAS MJ14
Colinus virginianus (bobwhite quail)	Acute oral toxicity	14 d, FIFRA 71-1	LD_{50} >2000 mg/kg bw	DAS J24
Colinus virginianus (bobwhite quail)	Short-term dietary toxicity	5 d, FIFRA 71-2 & OECD 205, 88% A+D	LC_{50} >5253 mg as/kg diet	DAS J26
Colinus virginianus (bobwhite quail)	Reproduction study	21 week, FIFRA 71-4(a) & OECD 206	NOEC = 550 mg/kg diet	DAS J01
Anas platyrhynchos (mallard duck)	Acute oral toxicity	14 d, FIFRA 71-1	LD_{50} >2000 mg/kg bw	DAS J23
Anas platyrhynchos (mallard duck)	Short-term dietary toxicity	5 d, FIFRA 71-2 & OECD 205	LC_{50} >5156 mg as/kg diet	DAS J25
Anas platyrhynchos (mallard duck)	Reproduction study	21 week, FIFRA 71-4(b) & OECD 206	NOEC = 550 mg/kg diet	DAS J02

The mode of action of spinosad is via activation of the nicotinic acetylcholine receptor, combined with effects on the GABA-receptor, leading to neuromuscular fatigue and paralysis in sensitive insect pests. None of the tests on mammals showed any evidence of symptoms reflecting the mode of action in target insects.

Annex 2. References

Dow AgroSciences document number	Year and title of report
DAS A01	1991. Vapour Pressure of Compound 232105 measured by the Knudsen-Effusion/Weight Loss Method.
DAS A03	1994. Series 63: Physical and Chemical Characteristics of the Technical Grade of Active Ingredient XDE-105.
DAS A04	1994. Determination of the Dissociation Constant of LY-232105.
DAS A07	1994. Determination of the Dissociation Constant of XDE-105 Factor D.
DAS A08	1994. Octanol/Water Partition Coefficient Determinations of Compound 232105.
DAS A18	1997. Thermogravimetric Analysis of Spinosad and Evolved Gas Analysis by Gas Chromatography/Mass Spectrometry.
DAS A20	1993. Solubility of Compound 232105 in pH = 9 Buffer Solution for Registration.
DAS A36	1991. Vapour Pressure of Compound 275043 Measured by the Knudsen-Effusion/Weight Loss Method.
DAS A37	1994. Solubility of Compound 275043 in Water and Buffer Solutions of pH = 5, 7, and 9 for Registration.
DAS A47	1994. Octanol/Water Partition Coefficient Determinations of Compound 275043.
DAS B01	1994. XDE-105: Acute Oral Toxicity Study in Fischer 344 Rats and CD-1 Mice.
DAS B04	1992. The Acute Inhalation Toxicity in the Fischer 344 Rat of Technical XDE-105.
DAS B05	1994. XDE-105: Primary Dermal Irritation Study in New Zealand White Rabbits.
DAS B07	1994. XDE-105: Acute Dermal Toxicity Study in New Zealand White Rabbits.
DAS B09	1994. XDE-105: Primary Eye Irritation Study in New Zealand White Rabbits.
DAS B16	1996. DE-105: Acute Oral Toxicity Study in Fischer 344 Rats and CD-1 Mice.
DAS B24	1994. XDE-105: Acute Neurotoxicity Study in Fischer 344 Rats
DAS B28	1996. A Skin Sensitization Study of DE-105 in Guinea Pigs (maximisation Test).
DAS B30	1999. Spinosad (Spinosyn A & D, 50:50 Mixture): Acute Dermal Irritation Study in New Zealand White Rabbits.
DAS B32	1999. Spinosad (Spinosyn A & D, 50:50 Mixture): Acute Eye Irritation Study in New Zealand White Rabbits.
DAS B33	1999. Spinosad (Spinosyn A & D, 50:50 Mixture): Dermal Sensitisation Potential Study in Hartley Albino Guinea Pigs.
DAS D02	1994. XDE-105: 13-week Dietary Toxicity and 4-week Recovery Studies in Fischer 344 Rats.
DAS D03	1995. XDE-105: 12 Month Oral Chronic Toxicity Study in Dogs.
DAS D04	1993. XDE-105: 13-Week Dietary Toxicity 4-week Recovery and 13-week Neurotoxicity Studies in Fischer 344 Rats (Neurotoxicity Portion).
DAS D05	1994. XDE-105: Probe and 21-day Repeated Dose Dermal Toxicity Study in New Zealand White Rabbits.
DAS D09	1994. XDE-105: Factor A and Factor D:28-day Dietary Toxicity Study in Fischer 344 Rats.
DAS D10	1994. XDE-105: 13-Week Oral Subchronic Toxicity Study in Dogs.
DAS D12	1992. Subchronic Toxicity Study in CD-1 Mice Administered XDE-105 in the Diet for 3 Months.
DAS D20	1999. Spinosad (50% Spinosyn A and 50% Spinosyn D): 13-Week Dietary Toxicity Study in Fischer Rats.
DAS D22	1999. Spinosad technical (DE-105): 14-day Nose only Aerosol Inhalation Toxicity and 2-week Recovery studies in Fischer 344 Rats.
DAS E01	1992. The Effect of XDE-105 on the In Vitro Induction of Chromosome Aberrations in Chinese Hamster Ovary Cells.

DAS E02	1992. The Effect of XDE-105 on the Induction of Unscheduled DNA Synthesis in Primary Cultures of Adult Rat Hepatocytes.
DAS E03	1992. The Effect of XDE-105 on the In Vivo Induction of Micronuclei in Bone Marrow of ICR Mice.
DAS E04	1992. The Effect of XDE-105 on the Induction of Forward Mutation at the Thymidine Kinase Locus of L5178Y Mouse Lymphoma Cells.
DAS E06	1996. Mutagenicity Test on XDE-105 in the Salmonella - Escherichia coli /Mammalian Microsome Reverse Mutation Assay Preincubation Method with a Confirmatory Assay).
DAS F01	1994. XDE-105: Two Generation Dietary Reproduction Study in Sprague-Dawley Rats.
DAS F03	1993. XDE-105: Oral Gavage Teratology Study in Sprague-Dawley Rats.
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