FAO SPECIFICATIONS AND EVALUATIONS FOR AGRICULTURAL PESTICIDES

CLOFENTEZINE

3,6-bis(2-chlorophenyl)-1,2,4,5-tetrazine

FOOD AND AGRICULTURE ORGANIZATION *of* THE UNITED NATIONS

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CLOFENTEZINE

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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.

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

INTRODUCTION

FAO establishes and publishes specifications* for technical material and related formulations of agricultural 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) and amended with the supplement of this manual (2006), which is available only on the internet through the FAO and WHO web sites. This **New Procedure** follows a formal and transparent evaluation process. It describes the minimum data package, the procedure and evaluation applied by FAO and the Experts of the FAO/WHO Joint Meeting on Pesticide Specifications (JMPS). [Note: prior to 2002, the Experts were of the FAO Panel of Experts on Pesticide Specifications, Registration Requirements, Application Standards and Prior Informed Consent, which now forms part of the JMPS, rather than the JMPS.]

FAO Specifications now only apply to products for which the technical materials have been evaluated. Consequently from the year 2000 onwards the publication of FAO 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 plant protection product in accordance with chapter 4, 5 and 6 of the 5th edition of the "Manual on the development and use of FAO specifications for plant protection products".

PART TWO: **The Evaluation Report(s)** of the plant protection product reflecting the evaluation of the data package carried out by FAO and the JMPS. The data are to be provided by the manufacturer(s) according to the requirements of Appendix A, annex 1 or 2 of the "Manual on the development and use of FAO specifications for plant protection products" 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.

FAO 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. FAO 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.

* NOTE: PUBLICATIONS ARE AVAILABLE ON THE INTERNET AT (http://www.fao.org/ag/agp/agpp/pesticid/)
OR IN HARDCOPY FROM THE PLANT PROTECTION INFORMATION OFFICER.

PART ONE

SPECIFICATIONS

CLOFENTEZINE

CLOFENTEZINE

INFORMATION

ISO common names

Clofentezine (ANSI, BSI, E-ISO, F-ISO)

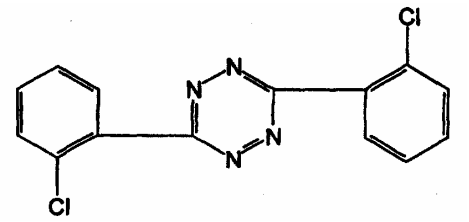
Synonyms

None

Chemical names

IUPAC 3,6-bis(2-chlorophenyl)-1,2,4,5-tetrazine
CA 3,6-bis(2-chlorophenyl)-1,2,4,5-tetrazine

Structural formula



Empirical formula

$C_{14}H_8Cl_2N_4$

Relative molecular mass

303.1

CAS Registry number

74115-24-5

CIPAC number

418

EEC Number

277-728-2

Identity tests

HPLC-UV (235 nm) retention time; IR spectrum

CLOFENTEZINE TECHNICAL MATERIAL

FAO Specification 418/TC (April 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 (*418/2006*). 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 (*418/2006*), as PART TWO, forms an integral part of this publication.*

1 Description

The material shall consist of clofentezine, together with related manufacturing impurities, and shall be a magenta crystalline solid, free from visible extraneous matter and added modifying agents or stabilizers.

2 Active ingredient

2.1 **Identity tests** (418/TC/M/2, CIPAC Handbook G, p.18, 1995)

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 **Clofentezine content** (418/TC/M/3, CIPAC Handbook G, p.18, 1995)

The clofentezine 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.

* Specifications may be revised and/or additional evaluations may be undertaken. Ensure the use of current versions by checking at: http://www.fao.org/ag/agp/agpp/pesticid/.

CLOFENTEZINE AQUEOUS SUSPENSION CONCENTRATE

FAO specification 418/SC (April 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 (*418/2006*). 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 report (*418/2006*), as PART TWO, forms an integral part of this publication.*

1 Description

The material shall consist of a suspension of fine particles of technical clofentezine, complying with the requirements of FAO specification 418/TC (April 2007), 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** (418/SC/M/2, CIPAC Handbook G, p.18, 1995)

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 **Clofentezine content** (418/SC/M/3, CIPAC Handbook G, p.18, 1995)

The clofentezine 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 tolerances:

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

3 Physical properties

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

The pH shall be in the range 6.0 to 7.5.

3.2 **Pourability** (MT 148.1, CIPAC Handbook F, p.348, 1995)

Maximum “residue”: 2%.

* Specifications may be revised and/or additional evaluations may be undertaken. Ensure the use of current versions by checking at: http://www.fao.org/ag/agp/agpp/pesticid/.

3.3 **Spontaneity of dispersion** (MT 160, CIPAC Handbook F, p.391, 1995) (Note 3)

A minimum of 85% of the clofentezine content found under 2.2 shall be in suspension after 5 min in CIPAC standard water D at 30 ± 2°C.

3.4 **Suspensibility** (MT 184, CIPAC Handbook K, p.142, 2003) (Note 3)

A minimum of 90% of the clofentezine content found under 2.2 shall be in suspension after 30 min in CIPAC standard water D at 30 ± 2°C.

3.5 **Wet sieve test** (MT 185, CIPAC Handbook K, p.148, 2003) (Note 4)

Maximum: 0.1% of the formulation shall be retained on a 75 µm test sieve.

3.6 **Persistent foam** (MT 47.2, CIPAC Handbook F, p.152, 1995) (Note 5)

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 46.3, CIPAC Handbook J, p.128, 2000)

After storage at 54 ± 2°C for 14 days, the determined average active ingredient content must not be lower than 98%, relative to the determined average content found before storage (Note 6) 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 of 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 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 Chemical assay is the only fully reliable method to measure the mass of active ingredient still in suspension. However, simpler methods such as gravimetric and solvent extraction determination may be used on a routine basis provided that these methods have been shown to give equal results to those of the chemical assay method. In case of dispute, the chemical method shall be the referee method.

Note 4 This test detects coarse particles (e.g. 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 5 The test should be carried out at the highest application concentration. 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 should be analyzed concurrently after the test in order to reduce the analytical error.

PART TWO

EVALUATION REPORTS

CLOFENTEZINE

CLOFENTEZINE

FAO/WHO EVALUATION REPORT 418/2006

Recommendation

The Meeting recommended that specifications proposed by Makhteshim Agan Industries Group for clofentezine TC and SC, as amended, should be adopted by FAO.

Appraisal

The meeting considered data on clofentezine, submitted by Makhteshim Agan Industries Group, in support of proposed new FAO specifications for clofentezine TC and SC.

Clofentezine is a magenta-coloured crystalline solid. It has very low solubility in water but is slightly to moderately soluble in organic solvents It undergoes hydrolysis, the rate of which increases with pH (over the range 4-9). It is susceptible to fairly rapid photolysis. Clofentezine has no acidic or basic properties of practical significance (it is an extremely weak base of estimated pKa = -1.68 but the very low water solubility prevents experimental observation of its protonation).

The meeting was provided with confidential information on the manufacturing process and manufacturing specifications for purity and impurities, which were supported by 5 batch analysis data. Mass balances were 98.9-100.5% and no unidentified impurities were detected. The Meeting questioned whether or not an additional impurity could occur but the manufacturer stated that its absence was ensured through control of the starting materials. A statement was provided by the UK Pesticides Safety Directorate confirming that the confidential data on the manufacturing process and declaration of composition submitted to the FAO were the same as those submitted to the national regulatory authority.

The Meeting agreed that none of the impurities should be designated as relevant.

A full CIPAC analytical method is available for determination of the clofentezine content of the TC and SC.

The proposed specifications for TC and SC were essentially in accordance with the requirements of the manual (FAO/WHO 2006). However, the Meeting questioned the proposed upper limit for the pH range of the SC formulation (7.5), because the supporting data indicated that rapid hydrolysis occurs at pH 8. The company stated that, although hydrolysis is rapid in water, in the SC formulation the active ingredient is stable throughout the proposed range. The Meeting therefore accepted the proposed limits for pH in the SC.

SUPPORTING INFORMATION
FOR
EVALUATION REPORT 418/2006

Uses

Clofentezine is an acaricide, which interferes with cell growth and differentiation during the final stages of embryonic and early larval development. It is used in agriculture for the protection of ornamentals, food and non-food crops in the field, orchards or under glass, against spider mites.

Identity

ISO common names

Clofentezine (ANSI, BSI, E-ISO, F-ISO)

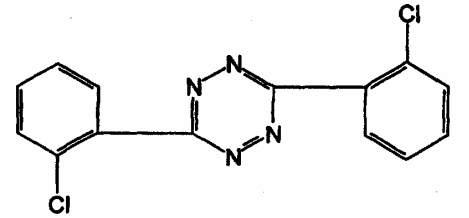
Synonyms

None

Chemical names

IUPAC 3,6-bis(2-chlorophenyl)-1,2,4,5-tetrazine
CA 3,6-bis(2-chlorophenyl)-1,2,4,5-tetrazine

Structural formula



Empirical formula

$C_{14}H_8Cl_2N_4$

Relative molecular mass

303.1

CAS Registry number

74115-24-5

CIPAC number

418

EEC Number

277-728-2

Identity tests

HPLC-UV (235 nm) retention time; IR spectrum

Physical and chemical properties

Table 1. Physicochemical properties of pure clofentezine

Characteristic	Value	Purity, %	Method	Reference
Vapour pressure	6.0×10^{-7} Pa at 20°C	99.3	EC Directive 92/69 A4	R-13285
Melting point	183.4°C (1); 182.1°C (2)	99.3	EC Directive 92/69 A1 Method (1): Differential Scanning Calorimetry (DSC) (2)	R-13283
Boiling point	Decomposes before boiling	99.3	EC Directive 92/69 A2	R-13283
Decomposition temperature	190-250°C	99.7	EC Directive 92/69 A1, OECD 102 (1995)	R-13283
Solubility in water at 22ºC	2.52 µg/l at pH 5 <2.0 µg/l at pH 7 <2.0 µg/l at pH 9	98.2, radio-purity	Batch equilibration with clofentezine/acetone (99:1)	R-12523
Octanol:water partition coefficient	Log P_{OW} = 4.1 at 40°C at pH 2, 7 and 9 Log P_{OW} = 4.09 at 25°C Log P_{OW} = 3.1 at 20°C	99.7 99.9 Not stated	EC Directive 92/69 A8, OECD 11 EC Method A8, L251 (1984), OECD 107, shake-flask method Shake-flask method	R-12519
Hydrolysis characteristics, half-life, days	10.3 at 22°C at pH 4.95 2.08 at 38°C at pH 4.95 1.43 at 22°C at pH 6.98 0.21 at 38°C at pH 6.98 0.70 at 10°C at pH 9.18 0.18 at 22°C at pH 9.18 1.1 at 25°C at pH 7 0.6 at 35°C at pH 7	>98.4 99.7	US EPA Subdiv N Ref. PB83-153973 Sec. 161-1 (1982) 92/69/EEC, C7; OECD 111	R-12520, R-13318
Photolysis characteristics, half-life, days, at pH 5.05	Natural sunlight: <7 d Dark control: >31 d Calculated values for 12/12h light/dark cycles: Lat. Spring Summer Fall 30°N 0.74 0.71 0.89 40°N 0.81 0.74 1.1 50°N 0.93 0.80 1.5	97.5, ^{14}C-radiopurity	In-house method similar to SETAC except natural light used; Calculation using GCSOLAR program	R-12521, R-13286
Dissociation characteristics	Non-acidic. Very weakly basic, with theoretically calculated pKa of -1.68 (Scifinder Registry), not experimentally measurable due to very low water solubility	-	-	R-16523

Table 2. Chemical composition and properties of clofentezine technical material (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 98.9–100.5%. No unidentified impurities were detected.
Declared minimum clofentezine 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
Stabilizers or other additives and maximum limits for them:	None
Melting or boiling temperature range	180-195°C, followed by decomposition in the temperature range 190-250°C

Hazard summary

Clofentezine has been evaluated by the FAO/WHO JMPR for toxicology (JMPR 1986b, 2005) and residues (JMPR 1986a, 1987, 1989, 1990 & 1992). It was scheduled for periodic re-evaluation by FAO/WHO JMPR (toxicology in 2005, residues in 2007). The 1986 JMPR estimated an ADI of 0-0.02 mg/kg bw/d, which was confirmed in 2005 (JMPR 2005). The 2005 JMPR concluded that it was unnecessary to estimate an ARfD, as "clofentezine has low acute toxicity and does not cause developmental toxicity or any other toxicological effect that would be elicited by a single exposure".

The WHO hazard classification of clofentezine is: U, "unlikely to present acute hazard in normal use" (WHO 2006).

Clofentezine is currently under EU review, according to Council Directive 91/414/EEC (list 3a, UK as Rapporteur Member State). The review has thus far concluded (EU 2005), with respect to a tested formulation, that clofentezine should be classified as follows.

Human health effects: none.
Ecotoxicological effects: R52, harmful to aquatic organisms;
R53, may cause long-term adverse effects in the aquatic environment.

Clofentezine was evaluated by the US EPA (USEPA 1999) and classified (Makhteshim 2005) as follows.

Acute oral, dermal and inhalation toxicity: Category III
Acute eye and skin irritation: Category IV
Dermal sensitization hazard: Not applicable

With respect to potential carcinogenicity, the US EPA concluded that clofentezine is in Category C (possible human carcinogen) with Q_1 as 4.31×10^{-7} (well below the level of concern of 1×10^{-5}).

Formulations and co-formulated active ingredients

The main formulation type available is SC. These formulations are registered and sold in many countries throughout the world, as either 200 or 500 g/l. Clofentezine may be co-formulated with bifenthrin (Torant CL, Percut, containing 200 g/l clofentezine + 40 g/l bifenthrin).

Methods of analysis and testing

The analytical method for determination of the active ingredient (including identity tests), in TC and SC, is a full CIPAC method (CIPAC G). Clofentezine is determined by HPLC, using UV detection at 235 nm and internal standardization.

The methods for determination of impurities are based on HPLC, using UV detection and internal standardization, following calibration with authentic standards.

Test methods for determination of physico-chemical properties of the technical active ingredient were OECD, EPA, EC while those for the SC formulation were CIPAC, as indicated in the specification.

Physical properties

The physical properties, the methods for testing them and the limits proposed for the SC formulation, comply with the requirements of the FAO/WHO manual (FAO/WHO 2006).

Containers and packaging

No special requirements for containers and packaging have been identified.

Expression of the active ingredient

The active ingredient is expressed as clofentezine, in g/kg or g/l in the SC.

ANNEX 1

HAZARD SUMMARY PROVIDED BY THE PROPOSER

Note: Makhteshim Agan Industries Group provided written confirmation that the toxicological and ecotoxicological data included in the following summary were derived from clofentezine having impurity profiles similar to those referred to in Table 2, above.

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

Species	Test	Duration, conditions, guideline adopted, purity	Result	Reference
Rat (m,f)	Oral	Single oral dose (14-d), in-house method equivalent to 92/69/EEC B.1. Dose levels 0, 800, 1131, 1600, 2261, 3200 mg/kg. Purity 99.0%	LD_{50} >3200 mg/kg bw	R-12815
Rat (m,f)	Dermal	Single dermal dose (14-d), in-house method equivalent to 92/69/EEC B.3. Dose levels 0, 2100 mg/kg. Purity 99.3%	LD_{50} >2100 mg/kg bw	R-12631
Rat (m,f)	Inhalation	Single inhalation dose (14-d). US EPA (1978) 43, (163), 37336-37402 equivalent to 92/69/EEC B.2. Dose level 1.51 mg a.i./litre. Purity: 80 WP (77.6-82.4% w/w clofentezine)	LC_{50} >1.51 mg/l	R-12606
Guinea pig (f)	Skin irritation	Dosing for 24 h, in-house method equivalent to 92/69/EEC B.4. Dose 33.3 mg. Purity 99.1%	Not irritant	R-12600
Rabbit (f)	Eye irritation	US EPA, Subdivision F,81-4 (1982) equivalent to 92/69/EEC B.5. Dose 70 mg. Purity 99.3%	Not irritant	R-12824, R-12628
Guinea pig (f)	Skin sensitization	In-house method equivalent to 92/69/EEC B.6, M & K. Purity not stated	Not a skin sensitizer	R-12612

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

Species	Test	Duration, conditions, guideline adopted, purity	Result	Reference
Rat (m,f)	Oral	17-d, in-house method equivalent to guideline 92/69/EEC B.7, range finding study. Purity >99%, dose levels 0, 5, 20,80, 320,1280 mg/kg	NOEL = 5 mg/kg bw/d LOEL = no adverse effects at any dose.	R-12565
Rat (m,f)	Oral dietary	90-day, In-house method equivalent to guideline 2001/59/EC B.26. Purity >99.1%, dose levels 0, 40, 400, 4000 ppm (m: 0, 2.65, 26.2, 265; f: 0, 2.91, 29.3, 292 mg/kg)	NOEL = 40 ppm (2.81 mg/kg bw/d) LOEL = 400 ppm (27.8 mg/kg bw/d)	R-12605
Mouse (m,f)	Oral dietary	90-day, In-house method equivalent to guideline 2001/59/EC B.26. Purity >99.0%, dose levels 0, 200, 1000, 5000 ppm (m: 0, 30.3, 151.4, 757.1; m: 0, 35.2, 176.5, 884.9 mg/kg)	NOEL = 200 ppm (30.3 mg/kg bw/d) LOEL = 1000 ppm (151.4 mg/kg bw/d)	R-12609A

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

Species	Test	Duration, conditions, guideline adopted, purity	Result	Reference
Dog (m,f)	Oral dietary	90-day, In-house method equivalent to guideline 2001/59/EC B.26. Purity >99.7%, dose levels 0, 3200, 8000, 20000 ppm	NOEL = none defined LOEL = 3200 ppm (123.8 mg/kg bw/d)	R-12603
Dog (m,f)	Oral dietary	1-year, In-house method equivalent to guideline 2001/59/EC B.27. Purity 98.2%, dose levels 0, 50, 1000, 20000 ppm (m: 0, 1.75, 33.2, 692.6; f: 0, 1.70, 38.8, 719.1 mg/kg)	NOEL = 50 ppm (1.72 mg/kg bw/d) LOEL = 1000 ppm (35 mg/kg bw/d	R-12621A
Rat (m,f)	Feeding, carcinogenicity	2-year, in-house method according to US EPA proposed guidelines, subpart F, 40 CFR, part 163 (1978 & 1982) and OECD, section 4 (May 1981). Purity 97.2%, dose levels 0, 10, 40, 400 ppm (m: 0, 0.43, 1.72, 17.3; m: 0, 0.55, 2.18, 22.1 mg/kg)	NOAEL (toxicity) = 40 ppm (1.95 mg/kg bw/d) NOAEL (carcinogenicity) = 400 ppm (19.7 mg/kg bw/d) No evidence of carcinogenicity	R-12623A
Mouse (m,f)	Feeding, carcinogenicity	2-year, in-house method according to guidelines EPA/FIFRA (1978) equivalent to method 87/302/EEC B.32. Purity 98.7%, dose levels 0, 50, 500, 5000 ppm (m: 0, 5.0, 50.7, 543.4; m: 0, 5.3, 56.9, 557.1 mg/kg)	NOAEL (toxicity) = 50 ppm (5 mg/kg bw/d) NOAEL (carcinogenicity) = 5000 ppm (550 mg/kg bw/d) No evidence of carcinogenicity	R-12624A
Rat (m,f)	Feeding, 2 generation reproduction	In-life phase: 1-year, in-house method equivalent to method 87/302/EEC B.35. Purity 97.9-99.3%, dose levels 0, 4, 40, 400 ppm (ca. 0, 0.4, 4, 40 mg/kg)	NOAEL (reproductive) = 400 ppm (40 mg/kg bw/d) NOAEL (developmental) = 40 ppm (4 mg/kg bw/d) No evidence of reproductive toxicity at any dose level	R-12620A
Rat (m,f)	Teratogenicity and developmental toxicity	Dosing phase: about 1 month, in-house method equivalent to method 87/302/EEC B.31. Purity 100%, dose levels 0, 320, 1280, 3200 mg/kg	NOAEL (reproductive) = 3200 mg/kg bw/d NOAEL (developmental) = 3200 mg/kg bw/d No evidence of teratogenicity at any dose level	R-12610A
Rabbit	Teratogenicity and developmental toxicity	In-life phase: about 2 months, in-house method equivalent to method 87/302/EEC B.31. Purity 98.5%, dose levels 0, 250, 1000, 3000 mg/kg	NOAEL (reproductive) = 3000 mg/kg bw/d NOAEL (developmental) = 1000 mg/kg bw/d No evidence of teratogenicity at any dose level	R-12613A

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

Species	Test system	Conditions, purity	Result	Reference
Salmonella typhimurium (TA 1535, TA 1537, TA 1538, TA 98 and TA 100)	*In vitro,* Ames test, reverse mutation assay	10 µg, 33 µg, 100 µg, 330 µg, 1 mg and 3.3 mg per plate ±S-9 mix. Purity not stated	Negative	R-12564
Mammalian cell line (CHO)	*In vitro*, chromosomal aberrations, mammalian cytogenicity	0.4, 2 and 4 µg/ml ± metabolic S-9 activation. Purity 99.6%	Negative	R-12635
LS1787 cells heterozygous at the thymidine kinase (TK ±) locus	*In vitro*, mouse lymphoma, mammalian all gene mutation	15, 30, 70, 100 and 128 µg/ml without S-9 mix; 2, 10, 30, 80 and 125 µg/ml with S-9 mix. Purity 98.4%	Negative	R-12615
Mouse bone marrow cells	*In vivo*, micronuclei induction, mouse micronucleus	Acute oral administration at 8000 mg/kg. Purity 99.6%	Negative	R-12636
Mouse bone marrow cells	*In vivo*, micronuclei induction, mouse micronucleus	Two oral administrations of 800, 1600 and 3200 mg/kg. Purity 99.6%	Negative	R-18737
Yeast, *Saccharomyces cerevisiae* strain D-7	*In vitro*, mitotic gene conversion & recombination	12.5, 25, 50, 100 and 200 µg/ml, ± S-9 mix. Purity 98.4%	Negative	R-12619
Strains H17 (Rec +) and M45 (Rec -) of *Bacillus subtilis*	*In vitro,* rec-assay, DNA damage	156, 313, 625, 1250 and 2500 µg/ disk without S-9 mix; 78, 156, 313, 625 and 1250 µg/ disk with S-9 mix. Purity not stated	Negative	R-12633
Rats, male CD Sprague-Dawley (30 in total)	*In vivo*, dominant lethal mutation test	Diet of 0, 4, 40 or 400 ppm for 10 weeks followed by pairing each with two females for 14 days. Purity 98.1%	Negative	R-12617

Table D. Ecotoxicology profile of clofentezine technical material

Species	Test	Duration and conditions, purity	Result	Reference
Salmo gairdneri (Oncorhynchus mykiss), rainbow trout	Acute toxicity	US EPA-660/3-75-009 (1975) equivalent to method 92/69/EEC C.1: 96-h, semi-static. Purity 99%, dose levels 0.039-0.005 mg/l.	LC_{50} >LOS*	R-12643
Salmo gairdneri (Oncorhynchus mykiss), rainbow trout	Acute toxicity	In-house method equivalent to method 92/69/EEC C.1: 96-h, continuous flow. Purity 98.6%, dose levels >0.0146 mg/l.	LC_{50} >LOS*	R-12664

∗ LOS = limit of solubility, i.e. <0.002-0.00252 mg/l.

Table D. Ecotoxicology profile of clofentezine technical material

Lepomis macrochirus, bluegill fish	Acute toxicity	In-house method equivalent to method 92/69/EEC C.1: 96-hours, continuous flow. Purity 99.8%, dose levels >0.25 mg/l.	LC_{50} >LOS*	R-12649
Oncorhynchus mykiss, rainbow trout	Early life stage	Method equivalent to OECD 210 (1992); 97-days. Purity 99.5%, dose level 0.007 mg/l.	NOEC ≥ LOS*	R-12681
Lepomis macrochirus, bluegill fish	Bio-accumulation	US EPA 560/B-82_002 (1982), equivalent to OECD 305E. Purity 99.2%, dose level 0.033 mg/l.	BCF = 248	R-12666, R-12671
Daphnia magna, water flea	Acute toxicity	Complied with 92/69/EEC C.2; OECD 202 part I (1984); US EPA guideline EG1 31:5007-31:5009; US EPA-540/9-85-005; 48-h, static. Purity 99.8%, dose level 0.00145 mg/l.	EC_{50} > LOS*	R-12670
Daphnia magna, water flea	Acute toxicity	US EPA -660/3-75-009 (1975); US EPA draft 43 (132) (1978); 48-h, static. Purity 99.0%, dose level 0.08 mg/l.	EC_{50} > LOS*	R-15417
Daphnia magna, water flea	Chronic toxicity	OECD 202, part II (1984): USEPA – 540/9-86-141, about 1-month, flow-through. Purity 99.8%, dose level 0.025 mg/l.	NOEC ≥ LOS*	R-12679
Scenedesmus subspicatus, green alga	Acute toxicity	Dutch draft standard method NEN6505,6506 (1979) equivalent to method 92/69/EEC C.3. (=OECD 201, 1984). Purity not stated, dose levels >LOS.	EC_{50} ≥ LOS*	R-12642
Chironomus riparius	Chronic	BBA part VI, 2-2 (1995) Static+sediment. Purity >98.7%, dose levels 0.0625, 0.125, 0.25, 0.5, 1.0 mg/l.	NOEC = 0.5 mg/l	R-13278
Mallard duck	Acute toxicity	US EPA No132 § 163-71-1 (1978): single oral gavage. Purity 99 %, dose level 3000 mg/kg.	LD_{50} >3000 mg/kg	R-12646
Bobwhite quail	Acute toxicity	US EPA No132 § 163-71-1 (1978): single oral gavage. Purity not stated, dose level 7500 mg/kg.	LD_{50} >7500 mg/kg	R-12648
Mallard duck	Sub-acute dietary toxicity	US EPA No132 § 163-71-2 (1978): 3-d pre-treatment, 5-d treatment period, 3-d post-treatment period. Purity 99.3%, dose levels 2353, 3361, 4802, 6860, 9800, 14000, 20000 ppm.	LC_{50} >20000 ppm, equivalent to 3617 mg/kg bw/day	R-12647

Table D. Ecotoxicology profile of clofentezine technical material

Bobwhite quail	Sub-acute dietary toxicity	US EPA No132 § 163-71-2 (1978): 3-d pre-treatment, 5-d treatment period, 3-d post-treatment period. Purity 99.3%, dose levels 2353, 3361, 4802, 6860, 9800, 14000, 20000 ppm.	LC_{50} >20000 ppm, equivalent to 3448 mg/kg bw/day	R-12645
Mallard duck	Dietary reproduction	US EPA No132 § 163-71-4 (1982): 22 weeks. Purity 98.9%, dose levels 0, 30, 90, 270 ppm.	NOEC = 270 ppm, equivalent to 39.2 mg/kg bw/day	R-12680
Bobwhite quail	Dietary reproduction	US EPA No132 § 163-71-4 (1982): 22 weeks. Purity 98.9%, dose levels 0, 30, 90, 270 ppm.	NOAEC[1] = 90 ppm, equivalent to 6.6 mg/kg bw/day	R-12682
Honey bee	Acute contact	EPPO guideline no. 170 (1992). SC 43% a.i., dose levels 0, 98.2, 196.5 µg formulation/bee.	LD_{50} >196.5 µg product/bee, >84.5µg a.i./bee	R-13290
Honey bee	Acute oral	48 h. EPPO guideline no. 170 (1992). SC 43% a.i., dose levels 0, 7.5, 60.3, 587.4 6 µg formulation/bee.	LC_{50} >587.6 µg product/bee or >252.6 µg a.i./bee	R-13289

[1] This value represents the ecotoxicologically relevant NOAEC. Statistically non-significant effects on embryo viability at 90 ppm were counteracted by increased survival of young birds. Effects at LOEC (270 ppm) related to hatching rate, chick bodyweight and survival.

Annex 2. References

Makhteshim document number or other reference	Year and title of report or publication details
CIPAC G	Collaborative International Pesticides Analytical Council (CIPAC), 1995. Methods 418/TC/M and 418/SC/M, Handbook G, p.18 *et seq*.
EU 2005	Draft Assessment report on clofentezine. Report and Proposed Decision of the United Kingdom made to the European Commission under Article 8(1) of 91/414/EEC, August 2005.
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Makhteshim document number or other reference	Year and title of report or publication details
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R-12664	1986. Determination of the acute toxicity of [14C]-Clofentezine to rainbow trout (Salmo gairdneri) using a dynamic test system.
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