Food and Agriculture Organization
of the United Nations

FAO SPECIFICATIONS AND EVALUATIONS FOR AGRICULTURAL PESTICIDES

HEXAZINONE

3-cyclohexyl-6-dimethylamino-1-methyl-1,3,5-triazine-2,4-(1*H*,3*H*)-dione

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DISCLAIMER[1]

FAO 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.

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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) - currently available as 3rd revision of the 1st edition (2016) - , 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 JMPM, 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 pesticide in accordance with chapters 4 to 9 of the "Manual on development and use of FAO and WHO specifications for pesticides".

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

FAO specifications developed 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.

* NOTE: PUBLICATIONS ARE AVAILABLE ON THE INTERNET AT (http://www.fao.org/agriculture/crops/core-themes/theme/pests/jmps/ps-new/en/) OR IN HARDCOPY FROM THE PLANT PROTECTION INFORMATION OFFICER.

PART ONE

SPECIFICATIONS

INFORMATION

HEXAZINONE

Common name:

hexazinone (E-ISO, (m)F-ISO, BSI, ANSI, WSSA)

Synonyms:

none

Chemical names:

IUPAC, 3-cyclohexyl-6-dimethylamino-1-methyl-1,3,5-triazine-2,4-(1*H*,3*H*)-dione.
CA, 3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4-(1*H*,3*H*)-dione.

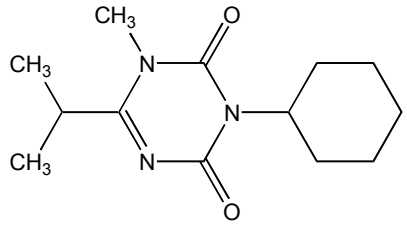
CAS No:

[51235-04-2]

CIPAC No:

374

Structural formula:



Molecular formula:

$C_{12}H_{20}N_4O_2$

Relative molecular mass:

252.31

Identity tests:

HPLC retention time, IR spectrum.

HEXAZINONE TECHNICAL MATERIAL

FAO Specification 374 / TC (December 2018*)

*This specification, which is PART ONE of this publication, is based on an evaluation of data submitted by the manufacturers whose names are listed in the evaluation reports (*374/2003, 374/2011 & 374/2018*). It should be applicable to relevant products of these manufacturers 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 the products of other manufacturers. The evaluation reports (*374/2003, 374/2011 & 374/2018*) as PART TWO form an integral part of this publication.*

1 Description

The material shall consist of hexazinone, together with related manufacturing impurities, in the form of white to light grey fine crystalline solid, and shall be free from visible extraneous matter and added modifying agents or stabilizers.

2 Active ingredient

2.1 **Identity tests** (CIPAC 374/TC/M-, CIPAC Handbook J, 2000, p.72, 2000)

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 **Hexazinone content** (CIPAC 374/TC/M-, CIPAC Handbook J, p.72, 2000)

The hexazinone content shall be declared (not less than 950 g/kg) and, when determined, the average measured content shall not be lower than the declared minimum content.

3 Relevant impurities

3.1 **Carbamic acid, ethyl ester** (ethyl carbamate) (Note 1)

Maximum: 0.05 g/kg (50 ppm).

Note 1 The analytical method for determination of ethyl carbamate in hexazinone TC is provided in Appendix 1.

* Specifications may be revised and/or additional evaluations may be undertaken. Ensure the use of current versions by checking at: http://www.fao.org/agriculture/crops/thematic-sitemap/theme/pests/jmps/ps-new/en

HEXAZINONE WATER DISPERSIBLE GRANULES

FAO Specification 374 / WG (December 2018*)

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 (374/2017). It should be applicable to WG 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 WG produced by other manufacturers. The evaluation report (374/2017), as PART TWO, forms an integral part of this publication.

1 Description

The material shall consist of a homogeneous mixture of technical hexazinone, complying with the requirements of the FAO specification 374/TC (December 2018), together with carriers and any other necessary formulants. It shall be in the form of white granules for application after disintegration and dispersion in water. The formulation shall be dry, free-flowing, essentially non-dusty, and free from visible extraneous matter and hard lumps.

2 Active ingredient

2.1 **Identity tests** (CIPAC 374/TC/M2, CIPAC Handbook J, p.72, 2000)

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 **Hexazinone content** (CIPAC 374/WG/M/3, CIPAC Handbook J, p.72, 2000)

The hexazinone content shall be declared (above 500 g/kg) and, when determined, the average content measured shall not differ from that declared by more than ± 25 g/kg.

3 Relevant impurities

3.1 **Carbamic acid, ethyl ester** (ethyl carbamate) (Note 1)

Maximum: 0.05 g/kg (50 ppm) of the hexazinone content found under 2.2.

* Specifications may be revised and/or additional evaluations may be undertaken. Ensure the use of current versions by checking at: http://www.fao.org/agriculture/crops/thematic-sitemap/theme/pests/jmps/ps-new/en

4 Physical properties

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

pH range: 6.0 to 9.0

4.2 **Wettability** (MT 53.3, CIPAC Handbook F, p. 164, 1995)

The formulation shall be completely wetted in 10 s in CIPAC Standard Water D, without swirling.

4.3 **Wet sieve test** (MT 185, CIPAC Handbook K, p. 149, 2003) (Note 2)

Maximum: 0.3 % retained on a 75 µm test sieve.

4.4 **Dispersibility** (MT 174, CIPAC Handbook F, p. 435, 1995)

Dispersibility: minimum 90 % after 1 minute of stirring.

4.5 **Suspensibility** (MT 184.1) (Notes 3, 4 & 5)

A minimum of 90 % shall be in suspension after 30 min in CIPAC Standard Water D at 30 ± 2°C.

4.6 **Persistent foam** (MT 47.3, CIPAC Handbook O, p. 177, 2017) (Note 6)

Maximum: 25 ml after 1 minute.

4.7 **Dustiness** (MT 171.1) (Notes 7 & 8)

Essentially non-dusty.

4.8 **Flowability** (MT172.1, CIPAC Handbook O, p. 187, 2017)

At least 99 % of the formulation shall pass through a 5 mm test sieve after 20 drops of the sieve.

4.9 **Attrition resistance** (MT 178.2, CIPAC Handbook K, p. 140, 2003)

Minimum: 98 % attrition resistance.

5 Storage stability

5.1 **Stability at elevated temperature** (MT 46.3, CIPAC Handbook J, p. 128, 2000)

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

- pH range (4.1),
- wet sieve test (4.3),
- dispersibility (4.4),
- suspensibility (4.5),
- dustiness (4.7),
- attrition resistance (4.8).

Note 1 The analytical method for determination of ethyl carbamate in hexazinone WG is provided in Appendix 2.

Note 2 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 3 MT 184.1 is the revised version of MT 184 and was adopted at the 2018 CIPAC Meeting in Panama. Prior to its publiction in an next Handbook, copies of the method can be obtained through https://www.cipac.org/index.php/methods-publications/pre-published-methods

Note 4 The formulation should be tested at the highest and lowest rates of use recommended by the supplier, provided this does not exceed the conditions given in method MT 184.1.

Note 5 Chemical assay is the only fully reliable method to measure the mass of active ingredient still in suspension. In case of dispute, chemical assay shall be the "referee method".

Note 6 The mass of sample to be used in the test should be specified at the highest rate recommended by the supplier. The test is to be conducted in CIPAC standard water D.

Note 7 The amended and corrected version of MT 171 - MT 171.1 - was adopted at the CIPAC Meeting in 2015. Prior to its publication in a next Handbook, the method can be downloaded from the CIPAC website http://www.cipac.org/index.php/methods-publications/errata

Note 8 Measurement of dustiness must be carried out on the sample "as received" and, where practicable, the sample should be taken from a newly opened container, because changes in the water content of samples may influence dustiness significantly. 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 9 Analysis of the formulation, before and after the storage stability test, should be carried out concurrently (i.e. after storage) to reduce analytical error.

PART TWO

EVALUATION REPORTS

HEXAZINONE

HEXAZINONE

FAO EVALUATION REPORT 374/2018

Recommendations

The Meeting recommended the following:

i) the change of manufacturer of the reference hexazinone TC from E.I. DuPont to Tessenderlo Kerley, Inc. should be noted by FAO.

ii) the updated and confirmed FAO specification for hexazinone TC submitted by Tessenderlo Kerley, Inc. should be adopted by FAO.

Appraisal

The Meeting noted, that in a press release[1] dated October 2015 Tessenderlo Kerley, Inc. (TKI), Phoenix (USA) announced the acquisition of hexazinone active ingredient and formulated products from E.I. Dupont. DuPont was up to then the holder of the reference FAO specification for hexazinone TC (FAO/WHO EVALUATION REPORT 374/2003).

As such a transition may rise some concerns on the continued validity of the FAO specification for hexazinone TC (see also FAO/WHO Manual, Section 2.7 on revision of specifications), TKI was contacted by FAO and a statement on the support of the reference specification and possible changes therein was requested.

TKI later on sent a written confirmation (TKI, 2018)[2] to FAO confirming the continued support for the FAO reference specification for hexazinone TC and submitted a set of quality control data on current production of hexazinone TC.

TKI explained, that the manufacturing site and -process for hexazinone were not affected by the transition from DuPont to to their company and had remained the same. The Meeting therefore considered the quality control data as sufficient to demonstrate, that the specification for hexazinone TC remained valid until further notice. The quality control data included, among other, the content of hexazinone in technical material which was well compliant with the minimum purity of 950 g/kg and the content of ethylcarbamate which was clearly below the maximum permitted level of 0.05 g/kg. The Meeting therefore concluded, that TKI should be noted as new holder of the reference specification and that hexazinone TC produced by TKI should be considered as the new reference specification.

[1] Cited after Agrow, accessible through https://agrow.agribusinessintelligence.informa.com/AG023164/TKI-to-acquire-DuPont-hexazinone-assets
[2] Letter from TKI, Mr. L. Castro, to FAO, Mme Yang, dated June 13 2018

HEXAZINONE

FAO EVALUATION REPORT 374/2017

Recommendations

The Meeting recommended that:

> the FAO specification for hexazinone WG submitted by Nutrichem Co. Ltd and as amended should be adopted by FAO.

Appraisal

The data for hexazinone WG were submitted by Nutrichem Co., Ltd (Nutrichem) in February 2015 in support of a new WG specification. At the moment only a FAO specification for TC is published (E.I. DuPont, extended in 2011 to Nutrichem).

The specifications for GR, WP, SP and SL formulations originally proposed by E.I. DuPont were not published at that time due to a lack of a peer validated method for the determination of the relevant impurity ethyl carbamate in the formulations.

Originally the water content, a pH range and the content of the relevant impurity, ethyl carbamate after after accelerated storage were specified by the company. The Meeting questioned the necessity of these clauses. The necessity of the clauses were reconsidered, and subsequently the clause for water content and the limit for ethyl carbamate after accelerated storage were removed by the company, the pH range however was confirmed to be required to safeguard the stability of hexazinone and was kept.

The values stated in the WG specification were justified by a study on a single batch sample. For several properties more stringent value could be derived from the study. The Meeting asked for further data from ongoing production. The company submitted these data for 6 batches produced over 18 month. The data demonstrated a constant product quality and the Meeting did not accept them as explanation. Later the company revised the specification and proposed more discriminatory limits, which were accepted by the Meeting.

In the CIPAC meeting in 2014 it was confirmed, that the existing method for the determination of hexazinone in WG formulations (374/WG/M/3) is applicable for Hexazinone WG manufactured by Nutrichem with a modification in standard and sample preparation, consisting of a twofold dilution.

The analytical method for determination of the relevant impurity ethyl carbamate is a HPLC-MS method, separation is achieved on a ZORBAX XDB C_8 reverse phase column with detection by a MS detector in SIM mode with extract ions of 90 and 112, and quantified by external standard.

The method was successfully peer validated by three independent laboratories. This was considered to well fulfil the requirements of the Manual and was accepted by the Meeting.

Following the decision of 2014, the methods for the TC and WG are provided as Appendices 1 and 2, respectively, to the Evaluation reports.

SUPPORTING INFORMATION
FOR
EVALUATION REPORT 374/2017

Formulations

The main formulation type is WG, other formulations available are SP, SL

Methods of analysis and testing

In 2014 it was confirmed that the existing CIPAC method for the determination of hexazinone in WG formulations (374/WG/M/3) is applicable for Hexazinone WG manufactured by Nutrichem with a modification in standard and sample preparation, consisting of a twofold dilution.

The analytical method for determination of the relevant impurity ethyl carbamate in the WG formulation is performed through HPLC-MS system, separated on a ZORBAX XDB C8 reverse phase column, detected by MS detector in SIM mode with extract ions of 90 and 112, and analyzed by comparing with external standard. The method is peer validated and provided in Appendix 2.

Containers and packaging

No special requirements for containers and packaging have been identified

Expression of the active ingredient

The active ingredient is expressed as hexazinone.

ANNEX 1

REFERENCES

NC-2013-105	Yue Wang 2014	Chemical and Physical Characterization of Hexazinone 75% WDG. Study NC-2013-105. Report NC-2013-105. GLP. Nutrichem Laboratory Co., Ltd., China. Unpublished.
NCW-2014-089	Yue Wang 2014	Peer Validation of Analytical Methodology for the Assay of Ethyl Carbamate in Hexazinone 75% WG (Method No.: NC2013105B) Study NCW-2014-089. Report NCW-2014-089. Non-GLP. Nutrichem Laboratory Co., Ltd., China.

HEXAZINONE

FAO EVALUATION REPORT 374 / 2011

Recommendations

The Meeting recommended that:

(I) the existing FAO specification for hexazinone TC should be extended to encompass the product of Nutrichem Co., Ltd. China

Appraisal

The data for hexazinone were evaluated in support of the extension of the existing FAO specification 374/TC (2002), published in 2006. The reference specification and the supporting data were provided by E.I. DuPont de Nemours and Company, USA.

The Meeting was provided with confidential information on the manufacturing process, together with 5-batch analytical data and manufacturing specification for purity and all impurities ≥ 1 g/kg. Mass balances were in the range of 99.4 – 99.8 %. The confidential data presented are identical to those submitted for registration in China.

The Nutrichem TC data is in compliance with the existing specification. One of the impurities did not appear in the reference profile of impurities. Because the specified content of this impurity is less than 3 g/kg, equivalence could be assessed in a Tier-1 approach, which is essentially based on chemical evidence like comparison of manufacturing specifications and some *in vitro* mutagenicity data (cf. Section 3.2, FAO/WHO Manual, November 2010 - second revision of the First Edition). No *in vivo* acute toxicity data are available for the Nutrichem TC.

Nutrichem proposed an analytical method for the determination of hexazinone in TC that is similar but not identical to the published CIPAC method 374/TC/M/- (CIPAC Handbook J, p. 72). The Meeting requested, based on the requirements of the FAO/WHO Manual (collaboratively validated analytical method for the active ingredient, Section 3.1 C), a bridging study for the Nutrichem method and a confirmation that the CIPAC method is applicable to the TC. Nutrichem submitted the bridging study and re-examined the batches using the CIPAC-method. The results of both methods (CIPAC method and Nutrichem in-house) are in good agreement for all batches.

For the determination of the relevant impurity ethyl-carbamate Nutrichem proposed a determination by HPLC-MS, while the method available on the FAO homepage is by GC/FID. The Meeting requested a peer validation on the HPLC-MS method or the use of the published GC-FID method. Nutrichem submitted a peer validation based on three independent laboratories. This was considered to fulfill the requirements of the Manual and accepted by the Meeting. Both methods may be downloaded from the FAO AGP website using the link provided in Note 1 of the TC specification.

Based on the studies and documents submitted, the Meeting concluded that the hexazinone TC produced by Nutrichem is considered equivalent to that of the reference profile in Tier-1 of the equivalence process.

No copies of the studies on physical-chemical properties of hexazinone were submitted for evaluation.

SUPPORTING INFORMATION
FOR
EVALUATION REPORT 374 / 2011

Table 1. **Physico-chemical properties of pure hexazinone**

Parameter	Value(s) and conditions	Purity %	Method reference (and technique if the reference gives more than one)	Study number
Vapour pressure	3.6 x 10^{-5} Pa at 25°C (extrapolated)	98.54	OECD 104, by extrapolation, EPA Guideline 830.7950	207-2-11-0651
Melting point.	114.0~115.5 °C	98.54	EPA Guideline 830.7200	NC-2009-037
Temperature of decomposition	not available	-	-	-
Solubility in water	29.8 g/l at 20 ± 0.5 °C	98.54	EPA Guideline 830. 7840	NC-2009-037
Octanol/water partition coefficient	log P_{OW} = 1.95 at 26.5 °C	98.54	EPA Guideline 830. 7570	NC-2009-037
Hydrolysis characteristics	Half-life : 1 year at room temperature at pH 4, 7 and 9	98.54	OECD 111 and EPA Guideline 835. 2120	NC-2010-050
Photolysis characteristics	not available	-	-	-
Dissociation characteristics	pK_a = 1.8	98.54	EPA Guideline 830.7370	NC-2009-037
Solubility in organic solvents	617.5 g/l in methanol at 20 ± 0.5 °C 0.53 g/l in n-hexane at 20 ± 0.5 °C 134.7 g/l in n-octanol at 20 ± 0.5 °C	98.54	EPA Guideline 830. 7840	NC-2009-037

Table 2. **Chemical composition and properties of hexazinone technical materials (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 99.4 – 99.8 %. No unknowns were identified in the 5 batch analyses.
Declared minimum [a.i.] content	970 g/kg
Relevant impurities ≥ 1 g/kg and maximum limits for them	None
Relevant impurities < 1 g/kg and maximum limits for them:	Ethyl carbamate, 0.05 g/kg
Stabilisers or other additives and maximum limits for them:	None

Parameter	Value and conditions	Purity %	Method reference	Study number
Melting temperature range of the TC	114 - 115 °C	98.54	EPA Guideline 830.7200	NC-2009-037
Solubility in organic solvents	617.5 g/L in methanol 0.53 g/ L in n-hexane 134.7 g/L in n-octanol, all at 20 °C.	98.54	EPA Guideline 830.7840	NC-2009-037

FORMULATIONS

Nutrichem Co. Ltd. produces the TC and sells it to formulators.

METHODS OF ANALYSIS AND TESTING

The analytical method for the active ingredient (including identity tests) is the CIPAC method published in Handbook J. In addition, a reverse phase HPLC, using DAD detection at 265 nm and external standardisation was validated and used. The method for determination of the non-relevant impurities is based on GC-FID with internal standardization.

The analytical method for determination of the relevant impurity ethyl carbamate is based on HPLC-MS, with a chromatography on a ZORBAX XDB C_8 reverse phase column. Detection of ethyl carbamate was by in single ion monitoring (SIM) mode on m/z 90 and 112 respectively, and quantification with an external standard.

Test methods for determination of physico-chemical properties of the technical active ingredient were OECD/EPA, as indicated in Table 1.

CONTAINERS AND PACKAGING

No special requirements for containers and packaging have been identified.

EXPRESSION OF THE ACTIVE INGREDIENT

The content of hexazinone is expressed as hexazinone.

Annex 1

Hazard Summary Provided by the Proposer

Toxicological summaries

Notes.

(i) The proposer confirmed that the toxicological and ecotoxicological data included in the summary below were derived from hexazinone having impurity profiles similar to those referred to in the table above.

(ii) The conclusions expressed in the summary below are those of the proposer, unless otherwise specified.

Table 2. **Mutagenicity profile of technical hexazinone based on *in vivo* tests**

Species	Test	Purity %	Guideline, duration, doses and conditions	Result	Study number
S. typhimurium (strains TA1535, TA1537, TA98 & TA100) *E.Coli (strain WP2(pkM101))*	*in vitro* bacterial gene mutation assay	98.16 %	OECD Guideline 471 48 h Between 5.09 and 0.06 mg/plate With and without metabolic activation by S9	non mutagenic / non pro-mutagenic	B-01115

Note: According to the tiered equivalence approach and proposed Tier-1 equivalence, no data on ecotoxicity of technical hexazinone in was submitted.

Annex 2
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. November 2010 - second revision of the First Edition. FAO Plant Production and Protection Paper: http://www.fao.org/agriculture/crops/core-themes/theme/pests/pm/jmps/manual/it/
	FAO	2006	FAO SPECIFICATIONS AND EVALUATIONS FOR AGRICULTURAL PESTICIDES Hexazinone
NC-2009-037		2009	Chemical and Physical Characterization of Hexazinone TGAI: Color, Physical State, Odor, Stability, Oxidation/Reduction, pH, UV-Vis, Melting Point, Density, Dissociation Constant, Partition Coefficient and Water Solubility.GLP. Nutrichem Laboratory Co., Ltd. Unpublished.
NC-2010-050		2010	Determination of hydrolysis rate of Hexazinone TGAI. GLP. Nutrichem Laboratory Co., Ltd. Unpublished
B-01115		2010	BACTERIAL REVERSE MUTATION TEST – Hexazinone. GLP.. Unpublished
NC-2009-038	Hongxia Li	2009	Preliminary Analysis and Enforcement Analytical Method of Hexazinone TGAI. NC-2009-038. Report NC-2009-038. GLP. Nutrichem Laboratory Co., Ltd., China. Unpublished.
207-2-11-0651		2010	Vapour Pressure of Hexazinone. Study207-2-11-0651. Report 207-2-11-0651. GLP. Jai Research Foundation, India. Unpublished.
NC-2011-075	Hongxia Li	2011	Preliminary Analysis of Active Ingredient in hexazinone TGAI – 5-batch Analysis of Active Ingredient in Hexazinone TGAI with CIPAC Method. GLP. Nutrichem Laboratory Co., Ltd., China. Unpublished.
NC2009038C	Yue Wang		Peer Validation of Analytical Methodology for the Assay of Ethyl Carbamate in Hexazinone TGAI, Nutrichem Laboratory Co., Ltd.

HEXAZINONE

FAO/WHO EVALUATION REPORT 374/2003

Explanation

The data for hexazinone were evaluated for review of existing FAO specifications for the technical material (374/TC/S/F), granules (374/GR/S/F), water dispersible granules (374/WG/S/F), water soluble powders (374/SP/S/F) and soluble concentrates (374/SL/S/F), which were developed in 1998 under the old procedure (AGP: CP/364).

Hexazinone had not been evaluated by the FAO/WHO JMPR or by WHO/PCS. A complete review by the United States EPA (Registration Eligibility Decision) was completed in 1994. A subsequent review (Tolerance Reassessment Eligibility Decision) was completed in 2002 by the US EPA.

The draft FAO specification and supporting data were provided by E. I. du Pont de Nemours and Company in September 2002.

Uses

Hexazinone is a herbicide that inhibits photosynthesis. It is used in sugarcane, alfalfa and in non-crop weed control.

Identity

ISO common name:

hexazinone

Chemical name(s)

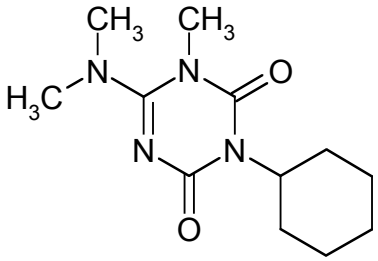
IUPAC: 3-cyclohexyl-6-dimethylamino-1-methyl-1,3,5-triazine-2,4(1*H*,3*H*)-dione

CA: 3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1*H*,3*H*)-dione

Synonyms or code names:

DPX-A3674

Structural formula:



Molecular formula:

$C_{12}H_{20}N_4O_2$

Relative molecular mass:

252.3 g/mol

CAS Registry number:

51235-04-2

CIPAC number:

374

Identity tests:

HPLC retention time and IR spectrum.

Physico-chemical properties

Table 1. Physico-chemical properties of pure hexazinone.

Parameter	Value(s) and conditions	Purity %	Method reference
Vapour pressure	<1.33 x 10^{-5} Pa at 25°C	99.7%	OECD 104 (gas saturation method using thermal evolution analyzer)
Melting point, boiling point and/or temperature of decomposition	Melting point: 113-115°C Boiling point: not available Decomposition temperature: not available	98.0%	OECD 102
Solubility in water	29.8 g/l at 25°C at pH 7	>99.9%	OECD 105, CIPAC MT 157, OTS CG-1500
Solubility in organic solvents	acetone, 792 g/l at 20°C chloroform, 3880 g/l at 20°C dimethylformamide, 836 g/l at 20°C methanol, 2650 g/l at 20°C toluene, 386 g/l at 20°C hexane, 3 g/l at 20°C	not stated	not stated
Partition coefficient n-octanol/water	log P_{OW} = 1.2 at 25°C and pH 7	97.2%	OECD 107, OTS CG-1400 HPLC collection LSC analysis method
Hydrolysis characteristics	No detectable hydrolysis after 30 days at 25°C and pH 5, 7 or 9	>97% radiochemical purity	EPA Pesticide Assesment Guidelines 161-1 (1982)
Photolysis characteristics	Photolytically stable at pH 7 and 25°C	>96% radiochemical purity	EPA/600-3-82-022 (1982)
Dissociation characteristics	pKa ~ 2.2	>95%	OECD 112 OTS CG-1200

Table 2. Chemical composition and properties of hexazinone 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 983–997 g/kg.
Declared minimum hexazinone content	950 g/kg (see Note)
Relevant impurities ≥ 1 g/kg and maximum limits for them	None.
Relevant impurities < 1 g/kg and maximum limits for them:	Carbamic acid, ethyl ester <50 mg/kg.
Stabilisers or other additives and maximum limits for them:	None.
Melting or boiling temperature range	113–115°C

Note: the minimum purity declared by the proposer was 957.5 g/kg but the Meeting agreed that a value of 950 g/kg should be adopted, because the analytical method is not sufficiently precise to support the more accurate value.

Toxicological summaries

Notes.

(i) The proposer confirmed that most of the toxicological and ecotoxicological data included in the summary below were derived from hexazinone having impurity profiles similar to those referred to in the table above.

(ii) The conclusions expressed in the summary below are those of the proposer, unless otherwise specified.

Table 3. Toxicology profile of the hexazinone technical material, based on acute toxicity, dermal, irritation and sensitization.

Species	Test	Duration and conditions or guideline adopted, a.i. form and purity	Result
Rat	Oral	USEPA Pesticide Assessment Guidelines Subdivision F, 81-1 TC (>98 %)	LD_{50} = 1200 mg/kg bw (USEPA MRID 41235004)[1]
Rat	Inhalation	EEC Annex V Method B2 & OECD 403 SP (90%)	LC_{50} >5 mg/l (USEPA MRID No. 41756701)[1]
Rabbit	Dermal	OECD 402. EEC Annex V (B3) USEPA 81-2	LD_{50} > 5000 mg/kg bw (not review by the EPA)
Rabbit	Skin irritation	Occluded exposure for 24h. Observation for 24h. (1973) TC + surfactants (purity not stated)	Slightly irritating (USEPA MRID No. 00104974)[1]
Rabbit	Eye irritation	USEPA (1982) Pesticide Registration Guidelines. TC (>95%)	Severe irritant (USEPA MRID No. 00106003)[1]
Guinea pig	Skin sensitization	Buehler method TC (>99%)	Not a sensitizer (USEPA MRID No. 41235005)[1]

[1] US EPA MRIDs: United States Environmental Protection Agency Master Record Identification. Studies showing MRIDs indicate official acceptance of study result in both the EPA Reregistration Eligibility Decision (1994) and Tolerance Reassessment Eligibility Decision (2002).

Table 4. Toxicology profile of hexazinone technical material based on repeated administration (sub-acute to chronic).

Species	Test	Duration and conditions or guideline adopted, a.i. form and purity	Result
Male and female rat (ChR-CD)	Oral 90-day feeding study	90 days (1973) TC (purity not stated)	NOEL = 1000 ppm (50 mg/kg) LOEL = 5000 ppm (250 mg/kg) (USEPA MRID No. 00104977)[1]
Dog (Beagle)	Oral 90-day feeding study	90 days (1973) TC (97.5%)	NOEL = 1000 ppm (25 mg/kg) LOEL = 5000 ppm (125 mg/kg) (USEPA MRID No. 00114484)[1]
Rabbit	21-day dermal toxicity	OECD 410 TC (>98.1%)	NOEL > 1000 mg/kg/day (USEPA MRID No. 4230900)[1]
Male and female rat (Crl:CD®BR)	Oral 2-year chronic tox. and carcinogenicity	Oral 2-year feeding (1975) WP (90% a.i.)	NOEL = 200 ppm (10 mg/kg) LOEL = 1000 ppm (50 mg/kg) (USEPA MRID No. 00108638)[1]
Male and female mouse (CD-1)	Oral 24-month carcinogenicity feeding study	Oral 2-year feeding (1981) TC (>95%)	NOEL = 200 ppm (28 and 34 mg/kg/day respectively for males and females). Considered by USEPA "not classifiable as to human carcinogenicity" (USEPA MRIDs 0079203, 41359301, 4250930)[1]
Male and female rat (Sprague-Dawley)	Two-generation reproduction study	US EPA Subdivision F 83-4 (1982) TC (>98%)	NOEL = 200 ppm (10 mg/kg/day) LOEL = 2000 ppm (100 mg/kg /day) (USEPA MRID No. 4206650)[1]
Female rat (Sprague-Dawley)	Developmental toxicity study	US EPA (1982) TC (94.2%)	NOEL = 100 mg/kg/day LOEL = 400 mg/kg/day (USEPA MRID No. 40397501)[1]

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

Species	Test	Conditions, a.i. purity	Result
Salmonella typhimurium (strains TA1535, TA1537, TA1538, TA98 & TA100)	*In vitro* bacterial gene mutation assay	Absence and presence of S9 metabolic activation TC (95%)	Negative for mutagenic activity (USEPA MRID No. 00098982)[1]
Chinese hamster ovary (CHO)cells	*In vitro* mammalian cytogenetics assay	Absence and presence of an S9 metabolic activation USEPA 870.5375 (1996), EEC 92/69 method B.10 (1992) TC (95%)	Positive for structural chromosome aberrations without activation. Negative for aberrations with activation (USEPA MRID No. 00130709)[1]
Chinese hamster ovary (CHO) cells	*In vitro* mammalian cell gene mutation assay	Absence and presence of S9 activation USEPA 84-2 (1982), EEC 87/302 part B (1987) hexazinone (95%)	Negative for CHO/HGRPT gene mutations (USEPA MRID No. 00076956)[1]

[1] USEPA MRIDs: United States Environmental Protection Agency Master Record Identification. Studies showing MRIDs indicate official acceptance of study result in both the EPA Reregistration Eligibility Decision (1994) and Tolerance Reassessment Eligibility Decision (2002).

Species	Test	Conditions, a.i. purity	Result
Rat primary hepatocytes (Sprague-Dawley)	*In vitro* unscheduled DNA synthesis (UDS)	U.S. EPA Pesticide Assessment Guidelines, Subdivision F, 83-1 TC (95%)	Negative for UDS (USEPA MRID No. 00130708)[1]
Male and female rat (Sprague-Dawley)	*In vivo* bone marrow cytogenetic assay	Oral gavage dosing TC (95%)	Negative for the induction of structural chromosome aberrations in bone marrow cells (USEPA MRID No. 00131355)[1]

Table 6. Ecotoxicology profile of hexazinone technical material.

Species	Test	Duration and conditions, a.i. purity	Result
Daphnia magna (Water flea)	Acute 48-hour static toxicity	EPA Method (1976) TC (95%)	EC_{50} = 151.6 ppm (USEPA MRID No. 00116269)[1]
Daphnia magna (Water flea)	Life cycle and reproductive test	U.S. EPA Pesticide Assessment Guideline; Subdivision E, 72-4 TC (>98%)	LOEC = 81 mg/l NOEC = 29 mg/l MATC = 48.5 mg/l (USEPA MRID Nos. 00078041, 41406002)[1]
Selenastrum capricornutum (Green alga)	120-hour effect on growth and growth rate	U. S. EPA Guidelines 122-2, 123-2. EPA Method 560/6-82-002, EC-22 TC (>98%)	EC_{50} = 7 ppb (USEPA MRID No. 41287001)[1]
Anabaena flos-aquae (Blue-green alga)	120-hour effect on growth and growth rate	U.S. EPA Non-Target Aquatic Plant Studies, Pesticides Assessment Guidelines, Subdivision J, 122-2, 123-2 (1982) TC (>98%)	EC_{50} = 0.21 ppm (USEPA MRID No. 43302701)[1]
Lemna gibba G3	14-day influence on growth and reproduction	EPA Method 600/9-78-018 TC (>98%)	EC_{50} = 37.4 ppb (USEPA MRID No. 43225101)[1]
Skeletonema costatum	120-hour effect on growth and growth rate	EPA Method 600/9-78-010 (1978) TC (>98%)	EC_{50} = 12 ppb (USEPA MRID No. 43225102)[1]
Navicula pelliculosa	120-hour effect on growth and growth rate	U.S. EPA Non-Target Aquatic Plant Studies, Pesticides Assessment Guidelines, Subdivision J, 122-2, 123-2 (1982) TC (>98%)	EC_{50} = 12 ppb (USEPA MRID No. 43302701)[1]
Crassostrea virginica (Eastern oyster)	48-hour developmental toxicity	EPA Method 660/3-75-009 TC (95%)	EC_{50} > 320 ppm (USEPA MRID No. 00047164)[1]
Palaemonetes pugio (Grass shrimp)	96-hour toxicity	EPA Method 660/3-75-009 TC (95%)	LC_{50} = 78 ppm (USEPA MRID No. 00047164)[1]

[1] USEPA MRIDs: United States Environmental Protection Agency Master Record Identification. Studies showing MRIDs indicate official acceptance of study result in both the EPA Reregistration Eligibility Decision (1994) and Tolerance Reassessment Eligibility Decision (2002).

Species	Test	Duration and conditions, a.i. purity	Result
Uca pugilator (Fiddler crab)	96-hour toxicity	EPA Method 660/3-75-009 TC (95%)	LC_{50} >1000 ppm (USEPA MRID No. 00047164)[1]
Apis mellifera (Honey bee)	48-hour acute contact toxicity	U.S. EPA Non-Target Insect Data; Pesticides Assessment Guidelines 141-1 TC (98%)	LD_{50} and NOEL >100 µg/bee (USEPA MRID No. 41216502)[1]
Colinus virginianus (Bobwhite quail)	Acute oral toxicity	Wildlife International Method (1978) TC (96.8%)	LD_{50} >2251 mg ai/kg bw (USEPA MRID No. 00073988)[1]
Colinus virginianus (Bobwhite quail chicks)	8-day acute dietary study	EPA Protocol (1973) TC (97.5%)	LC_{50} >5000 ppm (USEPA MRID No. 00107878)[1]
Anas platyrhynchos (Mallard ducklings)	8-day acute dietary study	EPA Protocol (1973) TC (97.5%)	LC_{50} >5000 ppm (USEPA MRID No. 00104981)[1]
Oncorhynchus mykiss (Rainbow trout)	Acute 96-hour static toxicity	APHA Standard Method (1970) TC (97.5%)	LC_{50} >320 ppm (USEPA MRID No. 00104980)[1]
Lepomis macrochirus (Bluegill sunfish)	Acute 96-hour static toxicity	APHA Standard Method (1970) TC (97.5%)	LC_{50} >370 ppm (USEPA MRID 00104980)[1]
Pimephales promelas (Fathead minnow)	Acute 96-hour static toxicity	APHA Standard Method (1970) TC (97.5%)	LC_{50} = 274 ppm (USEPA MRID No. 00104980)[1]
Pimephales promelas (Fathead minnow)	Fish early life stage toxicity	EPA Method 540/0-86-136 (1986) (98%)	MATC = 24.6 ppm NOEL = 17 mg/l LOEL = 35.5 mg/l (USEPA MRID No. 41406001)[1]

Hexazinone is of low acute toxicity by oral, dermal or inhalation routes. It is slightly irritating to skin and a severe eye irritant but is not a skin sensitizer. Hexazinone has not been classified as a human carcinogen and is not mutagenic. Hexazinone has not been evaluated by the FAO/WHO JMPR.

Hexazinone has not been evaluated formally by WHO/PCS but has been classified according to WHO hazard rating as Class III, slightly hazardous (WHO 2002).

Formulations

The main formulation types available are water dispersible granules (WG), water soluble powders (SP), granules (GR) and soluble concentrates (SL). These formulations are registered and sold in many countries throughout the world.

[1] USEPA MRIDs: United States Environmental Protection Agency Master Record Identification. Studies showing MRIDs indicate official acceptance of study result in both the EPA Reregistration Eligibility Decision (1994) and Tolerance Reassessment Eligibility Decision (2002).

Methods of analysis and testing

Hexazinone is determined by reversed-phase HPLC, using water (pH 3)/acetonitrile (1+1, v/v) as eluent and UV detection at 254 nm. The method and an additional identity test were published in CIPAC Handbook J, p. 72, 2000. The manufacturer confirmed that the CIPAC WG method is suitable for analysis of the GR formulation, without modification.

Impurities were determined by GC-FID. The relevant impurity, ethyl carbamate, was quantified using two techniques: headspace with GC-FID and GC-MS. DuPont method A3674.300 (R), for the determination of ethyl carbamate in hexazinone TC and formulations had not been peer-validated[*].

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

Physical properties

The physical properties, the methods for testing them and the limits proposed for the WG, SP, GR and SL formulations, comply with the requirements of the FAO/WHO Manual (FAO/WHO 2002).

Containers and packaging

There are no special requirements for containers or packaging but both the technical material and the formulated products are severe eye irritants and the formulated products should be labelled in accordance with local/national guidelines.

Expression of the active ingredient

The active ingredient is expressed as hexazinone.

Appraisal

The data considered by the Meeting were submitted in support of a review of existing FAO specification for TC, GR, WP, SP and SL.

Hexazinone is a broad-spectrum herbicide, which can be applied pre- or post-emergence, to control a range of important grass and broad-leaf weeds in sugar cane, forestry, alfalfa, pastures, vegetation management and various other crops, such as blueberries and pineapples.

Hexazinone is a solid of low volatility, moderately solubility in water and very soluble in organic solvents, except those of very low polarity. It is stable to hydrolysis (pH 4-9) and photolysis and weakly basic.

Information was submitted on the manufacturing process, together with batch analysis and manufacturing specifications data. In batch analyses, accountability was good (983–997

[*] Du Pont 2005: Hexazinone (DPX-A3674) Determination of Ethyl Carbamate (IN-08387) in Hexazinone Technical Headspace Gas Chromatographic (GC) Trace Level Method (Method No. A3674.160.01.ST)

g/kg). Confirmation was received from the U.S. EPA that the information submitted on the manufacturing process and batch analytical data were the same as those submitted for registration in the USA.

The proposer stated that carbamic acid ethyl ester (ethyl carbamate) should be a relevant impurity, on the basis it may be formed as a trace impurity during the manufacturing process. The International Agency for Research on Cancer (IARC) classifies carbamic acid, ethyl ester, as a carcinogen (class 2A, probably carcinogenic to humans) and the U.S. EPA also considers this impurity to be of toxicological concern. The opinion of WHO/PCS supported these positions and the Meeting therefore agreed that ethyl carbamate should be included as a relevant impurity in the specifications, with a limit of 50 mg/kg. The Meeting agreed that no other impurity should be considered relevant.

The Meeting considered the specification requirements for minimum purity of the TC because, on the basis of the historical production records, the proposer had requested a limit of 957.5 g/kg. This figure also represented the minimum purity declared in support of the EPA evaluation and to national regulatory agencies in other countries. However, the quoted reproducibility (R) of the CIPAC method is 22 g/kg at 998 g/kg and thus the analytical method probably has insufficient precision to support such an accurately specified limit. The Meeting therefore agreed that the specified minimum should be 950 g/kg.

The proposer was asked to justify the manufacturing specification limits for certain impurities and stated that they were set on the basis of experience of the manufacturing process. The Meeting accepted the explanation.

Analytical methods for determination of hexazinone in the TC and formulations are full CIPAC methods, with the exception of the GR. The manufacturer stated that the CIPAC method for WG is applicable to GR without modification.

A method was provided for determination of the ethyl carbamate impurity in the technical material and formulated products but this had not been subjected to independent laboratory validation.

Draft specifications were submitted for TC, SP, WG, GR and SL. Clauses for water content and pH range were originally included but the Meeting and proposer agreed that these were unnecessary, because the water content was effectively controlled by other clauses, and because neither the active ingredient not formulations would be adversely affected by the pH.

The Meeting questioned the designation of the WG (water dispersible granule) specification, on the basis that the relatively high water solubility of hexazinone could make it an SG (water soluble granule). The proposer stated that, at the highest formulation concentrations recommended in the spray tank, the active ingredient is partly in solution and partly in suspension. The Meeting therefore agreed that the specification should be for a WG.

The Meeting questioned the proposed limit of 3% for the degree of dissolution test for the SP but the proposer confirmed that the small amount of residual material had not been found to pose difficulties over many years of practical use in the field. The existing specification did not comply with current requirements for SP (FAO/WHO 2002) but, using approximately similar tests, the specification limit was 2%. The Meeting accepted the proposed limit.

Subject to provision of suitable concentration ranges for active ingredient content of the formulations, the Meeting agreed that all other specification clauses fulfilled the requirements of the Manual.

Hexazinone has not been evaluated by the FAO/WHO JMPR or IPCS but it is of low acute toxicity by oral, dermal or inhalation routes and is classified by WHO as slightly hazardous. It has not been classified as a human carcinogen and is not mutagenic. Hexazinone is not a skin sensitizer but it is slightly irritating to skin and both hexazinone and its formulated products are classified as severe eye irritants. The Meeting agreed that appropriate labelling should be used on packaging, although this requirement is not part of the specifications development process.

Recommendations

The Meeting recommended that:

- the existing FAO specifications for hexazinone TC, GR, WG, SP and SL should be withdrawn when the revised specifications are published;
- the proposed specifications for hexazinone TC, GR, WG, SP and SL, as amended, should be adopted by FAO, subject to satisfactory peer validation of the method for determination of ethyl carbamate impurity*;
- although not a requirement of the FAO specifications, hexazinone products should be labelled as severe eye irritants.

References

Du Pont 2005 Hexazinone (DPX-A3674) Determination of Ethyl Carbamate (IN-08387) in Hexazinone Technical Headspace Gas Chromatographic (GC) Trace Level Method (Method No. A3674.160.01.ST)

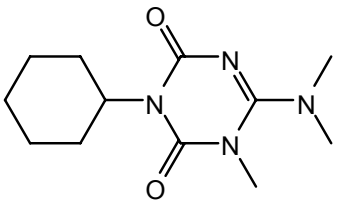
FAO/WHO 2002 Manual on development and use of FAO and WHO specifications for pesticides, 1st edition. FAO plant production and protection paper 173. FAO, Rome, 2002.

WHO 2002 The WHO recommended classification of pesticides by hazard and guidelines to classification, 2000-2002. WHO, Geneva, 2002.

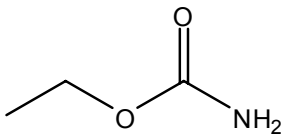
*Note added in editing, 2012: Acceptable independent laboratory validation was provided subsequently (Du Pont 2005) for the TC.

Appendix 1 Analytical Method of Hexazinone TGAI for Ethyl Carbamate (NO. NC2009038C)

1. Introduction

Test Item:	**Hexazinone TGAI**
Classification:	Herbicide
Active Ingredient:	Hexazinone
Empirical Formula:	$C_{12}H_{20}N_4O_2$
Molecular Weight:	252.3
CAS Register Number:	51235-04-2
IUPAC Name:	3-cyclohexyl-6-dimethylamino-1-methyl-1,3,5-triazine-2,4-(1H,3H)-dione
Structure Formula:	

Impurity Information		
Impurity Name	**Empirical Formula**	**Structure**

Impurity I	$C_3H_7NO_2$	

2. Summary

Limit Test of Impurity I in Hexazinone TGAI is performed through HPLC-MSD system, separated on a ZORBAX XDB C8 reverse phase column, detected by MS detector in SIM mode with extract ion of 90 and 112, and analyzed by comparing with external standard.

3. Determination of Impurity I in Hexazinone TGAI

3.1 Apparatus

Test system: HPLC-MSD 1100 system (Agilent Company).

Glassware: Volumetric;

Balance: Mettler Toledo XS 205;

Vials: 2 ml volume.

3.2 Reagents

Impurity I standard: known purity;

Hexazinone TGAI for analysis:

Acetic acid: Analytical grade;

Acetonitrile: HPLC grade;

Water: Redistilled;

4. Experimental Conditions for Chromatography

The following conditions have been established using an Agilent 1100 HPLC/MSD system. Chromatographic conditions may be changed to obtain satisfactory performance with other instruments provided adequate resolution and sensitivity are achieved.

Equipment: Agilent HPLC-MSD 1100 system.

Column: ZORBAX C8 150 x 4.6 mm, 5 micron

Mobile phase:

Time (min)	Acetonitrile (%)	Acetic acid aqueous solution (pH = 2.8 - 3.2)
0	10	90
3	10	90
10	90	10

Column Temperature: 30℃

Sample size injected: 5.0 µl

Stop time: 10.0 min

Post time: 5 min

Drying gas flow N_2: 11.0 L/min,

Drying Gas Temperature: 350 ^{0}C

Ionization Mode: API-ES

Polarity: Positive

Neb Press: 45 psig

Capillary voltage: 4000 V

Fragmentor: 70 V for SIM run

Gain EMV: 1.0

Actual Dwell: 289

SIM ions: 90 and 112 m/Z

Retention Time: **Impurity I:** *ca 3.0* min

5 Limit test of Impurity I

5.1 Preparation of stock Impurity I standard solutions

Accurately weigh about 10 mg analytical standard grade Impurity I into a 100 ml volumetric flask, dissolve and make to volume with acetonitrile.

5.2 Preparation of Impurity I LOQ standard solution

Prepare the Impurity I standard solution at acceptable LOQ concentration obtained from the method validation of LOQ by diluting certain amount stock Impurity I standard solution 5.1 with acetonitrile.

5.3 Preparation of sample solution

Prepare in duplicate per batch.

Approximately 100 mg of Hexazinone TGAI is accurately weighed into a 10 mL volumetric flask, dissolved and made to volume with acetonitrile. The flask is mixed thoroughly.

Another approximately 100 mg of Hexazinone TGAI with same batch is accurately weighed into a 10 mL volumetric flask, dissolved and made to volume with Impurity I LOQ standard solution 5.2. The flask is mixed thoroughly.

5.4 Preparation of blank solution

An acetonitrile blank solvent solution is prepared.

5.5 Analysis

When the instrument gets to equilibrium, inject the blank solution. Then, inject Impurity I LOQ standard solution and sample solutions in the following sequence: Impurity I LOQ standard solution, twice non-spiked sample solution, twice spiked sample solution, Impurity I LOQ standard solution. Determine the area of the Impurity I peak.

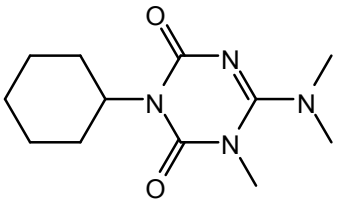
5.6 Result

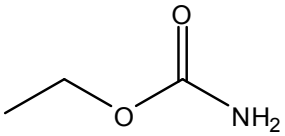
Compare Peak Area of Impurity I with LOQ standard solution. The corrected area in spiked samples is calculated as the subtraction of peak area in the spiked sample with the average peak area of the 2 nearest injections of the impurity I LOQ standard solution.

If both the area in TGAI and the corrected area in spiked sample are less than the area of LOQ standard solution, the content of Impurity I in the Hexazinone TGAI is given a limit test result of < LOQ (equivalent to W/W in Test Item). Otherwise, the quantitation method for Impurity I will be developed.

Appendix 2: Analytical Method for Determination Ethyl Carbamate in Hexazinone WG (Method No. NC2013105B)

1. Introduction

Test Item:	**Hexazinone WG**
Classification:	Herbicide
Active Ingredient:	Hexazinone
Empirical Formula:	$C_{12}H_{20}N_4O_2$
Molecular Weight:	252.3
CAS Register Number:	51235-04-2
IUPAC Name:	3-cyclohexyl-6-dimethylamino-1-methyl-1,3,5-triazine-2,4-(1H,3H)-dione
Structure Formula:	

Impurity Information		
Impurity Name	**Empirical Formula**	**Structure**
Impurity I	$C_3H_7NO_2$	

2. Summary

Limit Test of Impurity I in Hexazinone WG is performed through HPLC-MSD system, separated on a ZORBAX XDB C8 reverse phase column, detected by MS detector in SIM mode with extract ion of 90 and 112, and analyzed by comparing with external standard.

3. Determination of Impurity I in Hexazinone WG

3.1 Apparatus

Test system: Agilent 1200 LC/ G6120A MSD system or equivalence.

Glassware: Volumetric;

Balance: Mettler Toledo XS 205;

Vials: 2 ml volume.

3.2 Reagents

Impurity I standard: known purity;

Hexazinone WG for analysis:

Acetic acid: Analytical grade;

Acetonitrile: HPLC grade;

Water: Redistilled;

4. Experimental Conditions for Chromatography

The following conditions have been established using an LC-MSD system system. Chromatographic conditions may be changed to obtain satisfactory performance with other instruments provided adequate resolution and sensitivity are achieved.

Equipment: HPLC-MSD system.

Column: ZORBAX C8 150 x 4.6 mm, 5 micron

Mobile phase:

Time (min)	Acetonitrile (%)	Acetic acid aqueous solution (pH = 3.0)
0	10	90
3	10	90
10	90	10

Column Temperature: 30℃

Sample size injected: 5.0 µl

Stop time: 10.0 min

Post time: 5 min

Drying gas flow N_2: 11.0 L/min,

Drying Gas Temperature: 350 ^{0}C

Ionization Mode: API-ES

Polarity: Positive

Capillary voltage: 4000 V

Fragmentor: 70 V for SIM run

SIM ions: 90 and 112 m/Z

Retention Time: **Impurity I:** *ca 3.0* min

5 Limit test of Impurity I

5.1 Preparation of stock Impurity I standard solutions

Approximate 50 mg of analytical standard grade ethyl carbamate is accurately weighed into a 100 mL volumetric flask dissolved the flask was filled to line with the mixed solvent of acetonitrile and water with the volume ratio of 1:1 and mixed thoroughly to prepare the first stock ethyl carbamate standard solution. Transfer 10 mL first stock standard solution into a 100 mL volumetric flask. The flask was filled to the line with mixed solvent and mixed thoroughly as second stock standard solution.

5.2 Preparation of Impurity I standard solution

Prepare Ethyl Carbamate standard solution by transferring suitable amount of above second stock standard solution into a 10 ml volumetric flask, the flask is filled to line with mixed solvent of acetonitrile and water with the volume ratio of 1:1 and mixed well. The concentration of Ethyl Carbamate standard solution should be in the range of linearity determined by method validation assay.

5.3 Preparation of Ethyl Carbamate Quality Control (QC) solution

Prepare stock ethyl carbamate standard solutions as 5.1 and then prepare the ethyl carbamate quality control solution according to the same operation as the ethyl carbamate standard solution 5.2.

5.4 Preparation of sample solution

Prepare in duplicate per batch.

Approximate 1330 mg of Hexazinone WG is accurately weighed into a 10 mL volumetric flask, dissolved and made to volume with acetonitrile and water with the volume ratio of 1:1. The flask is mixed thoroughly.

5.5 Preparation of blank solution

Acetonitrile and water with the volume ratio of 1:1 is mixed to prepare blank solution of solvent.

5.6 Analysis

When the instrument gets to equilibrium, inject the blank solution. Then, inject standard solution, quality control solution and sample solutions in the following sequence: standard solution, QC solution, four times sample solution, standard solution. Determine the area ratio of the Hexazinone peak to internal standard solution.

Content of Impurity (ethyl carbamate %) = (R/R')x(W'/W) xρx100

Where:

W '- Mass of the related ethyl carbamate standard in the standard solution (mg);

R - Area of the ethyl carbamate peak in Test Item chromatograms;

W- Mass of Test Item (mg);

R'- Area of the ethyl carbamate peak in two chromatograms of the standard solution injections bracketing the sample injection;

ρ - Purity of the related ethyl carbamate standard (%)

Recovery of QC (ethyl carbamate %) = (Measured mass for QA/ W) x 100 = (R/R') x (W'/W) x 100

Where:

W '- Mass of the related ethyl carbamate standard in the standard solution (mg);

R - Area of the ethyl carbamate peak in QC chromatograms;

W- Mass of related ethyl carbamate standard in the QC standard solution (mg);

R'- Area ratio of the ethyl carbamate peak to internal standard peak in two chromatograms of the standard solution injections bracketing the sample injection; (the last QC injection is calculated using the area of the nearest one standard injection);

5. Limit Test of Impurity I

5.1 Preparation of Impurity I Standard Solution

Prepare ethyl carbamate standard solution by diluting above second stock ethyl carbamate standard solution at LOQ level determined by method validation assay.

5.2 Preparation of Sample Solution

The preparation is the same as that in section 4.4.

5.3 Preparation of Blank Solution

The preparation is the same as that in section 4.6.

5.4 Analysis

When the instrument gets to equilibrium, inject the blank solution. Then, inject ethyl carbamate standard solution and sample solutions in the following sequence: ethyl carbamate standard solution, four times sample solution, standard solution. Respectively determine the area of the ethyl carbamate peak.

5.5 Result

Compare peak area of ethyl carbamate in the test item and in standard solution. If the area in test item is less than the area of ethyl carbamate at the LOQ concentration in standard solution, the content of ethyl carbamate in the Hexazinone 75% WG is given a limit test result of < LOQ (equivalent to W/W in test item).