



Food and Agriculture Organization
of the United Nations

FAO SPECIFICATIONS AND EVALUATIONS FOR AGRICULTURAL PESTICIDES

CHLORPYRIFOS

O,O-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate

2020

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DISCLAIMER¹

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 that 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.

FAO 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, FAO 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.

FAO 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, FAO does not in any way warrant or represent that any pesticide claimed to comply with a FAO specification actually does so.

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 1999 onward, the development of FAO specifications follows the **New Procedure**, described first in the 5th edition of the "Manual on the development and use of FAO specifications for plant protection products" and later 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 JMPS, rather than the JMPS.]

FAO Specifications now only apply to products for which the technical materials have been evaluated. Consequently, from the year 1999 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. Evaluations bear the date (year) of the Meeting at which the recommendations were made by the JMPS.

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SPECIFICATIONS

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CHLORPYRIFOS

INFORMATION

ISO common name

Chlorpyrifos (E-ISO, BSI, ANSI, ESA)

Synonyms

Chlorpyriphos ([m] F-ISO, JMAF)

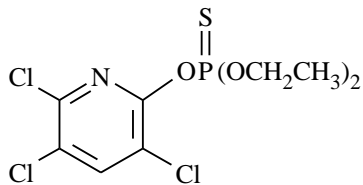
Chlorpyriphos-éthyl ([m] France)

Chemical names

IUPAC O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate

CA O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate

Structural formula



Molecular formula

C₉H₁₁Cl₃NO₃PS

Relative molecular mass

350.6

CAS Registry number

2912-88-2

CIPAC number

221

Identity tests

HPLC retention time, GC retention time, mass spectrum (from GC-MS)

CHLORPYRIFOS TECHNICAL MATERIAL

FAO Specification 221 / TC (February 2020)

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 (221/2002, 221/2004, 221/2006, 221/2008, 221/2014 & 221/2019). It should be applicable to TC produced by 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 TC produced by other manufacturers. The evaluation reports (221/2002, 221/2004, 221/2006, 221/2008, 221/2014 & 221/2019), as PART TWO, form an integral part of this publication.

1 Description

The material shall consist of chlorpyrifos together with related manufacturing impurities and, below its melting point (Note 1), shall be a white to tan coloured crystalline solid, free from visible extraneous matter and added modifying agents.

2 Active ingredient

2.1 Identity tests (221.b/TC/M/2, CIPAC Handbook 1C, p. 2028, 1985) (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 Chlorpyrifos content (221.b/TC/M/3, CIPAC Handbook 1C, p.2028, 1985)

The chlorpyrifos content shall be declared (not less than 970 g/kg) and, when determined, the average measured content shall not be lower than the declared minimum content.

3 Relevant impurities

3.1 Sulfotep (*O,O,O',O'*-tetraethyl dithiopyrophosphate, CAS No. 3689-24-5) (Note 3)

Maximum: 3 g/kg

3.2 Acetone insolubles (MT 27, CIPAC Handbook F, p. 88, 1995)

Maximum retained on a 45 µm test sieve: 5 g/kg

4 Physical properties

4.1 Acidity (MT 191, CIPAC Handbook L, p. 143, 2006)

Maximum acidity: 1 g/kg calculated as H₂SO₄

Note 1 The melting point should be in the range, 38-45°C.

Note 2 In addition to HPLC and GC retention times, the mass spectrum (obtained by GC-MS) provides good evidence of identity. The total ion current chromatogram and mass spectrum are shown in Figure 1.

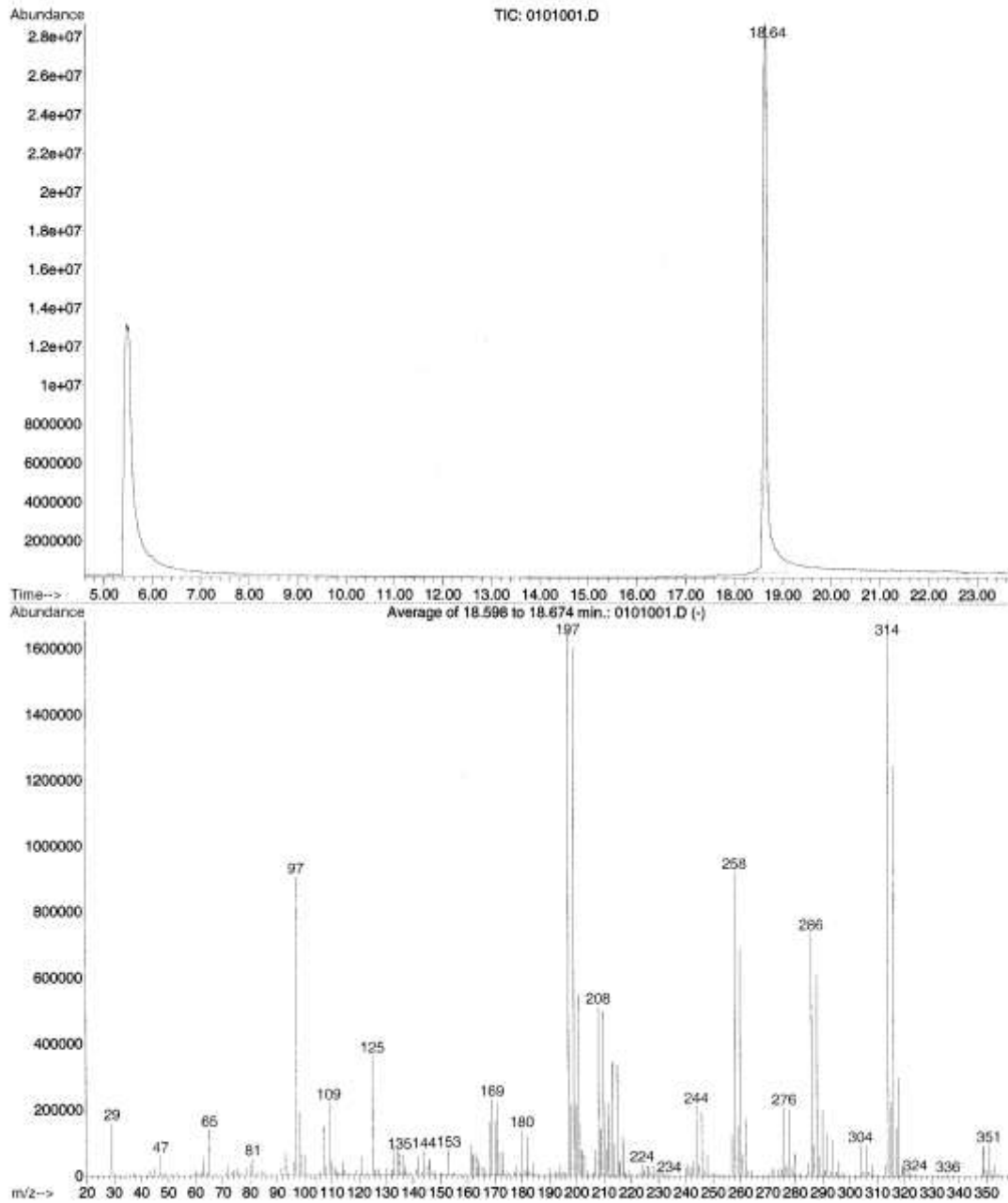


Figure 1. Total ion current chromatogram and mass spectrum of chlorpyrifos

Note 3 The methods for determination of sulfotep in technical and formulated chlorpyrifos are described in Appendix 1 of Evaluation 221/2004, in Part Two of this publication.

CHLORPYRIFOS EMULSIFIABLE CONCENTRATE

FAO Specification 221 / EC (February 2020)

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 (221/2002, 221/2004, 221/2006, 221/2008). It should be applicable to relevant products of these manufacturers, 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 (221/2002, 221/2004, 221/2006, 221/2008), as PART TWO, form an integral part of this publication.

1 Description

The material shall consist of technical chlorpyrifos (complying with the requirements of WHO specification 221/TC (February 2020) dissolved in suitable solvents together with any necessary formulants. It shall be in the form of a stable and homogeneous pale yellow to amber coloured liquid, free from visible suspended matter and sediment, to be applied as an emulsion after dilution in water.

2 Active ingredient

2.1 Identity tests (221.b/EC/M/2, CIPAC Handbook 1C, p. 2031, 1985) (Note 1)

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

2.2 Chlorpyrifos content (221.b/EC/M/3, CIPAC Handbook 1C, p. 2031, 1985)

The chlorpyrifos content shall be declared (g/kg or g/l at $20 \pm 2^\circ\text{C}$, Note 2) and, when determined, the average measured content shall not differ from that declared by more than the following amounts:

Declared content in g/kg or g/l at $20 \pm 2^\circ\text{C}$	Tolerance
Up to 100	$\pm 10\%$ of the declared content
above 100 up to 250	$\pm 6\%$ of the declared content
above 250 up to 500	$\pm 5\%$ of the declared content
<u>Note</u> in each range the upper limit is included	

3 Relevant impurities

3.1 Sulfotep (*O,O,O',O'*-tetraethyl dithiopyrophosphate, CAS No. 3689-24-5) (Note 3)

Maximum: 3 g/kg (0.3%) of the chlorpyrifos content found under clause 2.2.

4 Physical properties

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

The pH of a 1 % aqueous dispersion shall be 4.5 to 6.5.

4.2 Emulsion stability and re-emulsification (MT 36.3, CIPAC Handbook K, p. 137, 2003) (Note 4)

The formulation, when diluted at $30 \pm 2^\circ\text{C}$ 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: 2ml
2.0 h	'Cream', maximum: 2ml 'Oil', maximum: trace
24 h	Re-emulsification complete
24.5 h	'Cream', maximum: 2ml 'Oil', maximum: trace
<p><u>Note:</u> tests after 24 h are required only where the results at 2 h are in doubt.</p>	

4.3 Persistent foam (MT 47.3, CIPAC Handbook O, p. 177, 2017)

Maximum: 20 ml after 1 minute.

5 Storage stability

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

After storage at $0 \pm 2^\circ\text{C}$ for 7 days, the volume of solid or liquid which separates shall not be more than 0.3 ml.

5.2 Stability at elevated temperature (MT 46.4) (Note 5)

After storage at $54 \pm 2^\circ\text{C}$ for 14 days, the determined average active content shall not be lower than 95%, relative to the determined average content found under 2.2 before storage (Note 6), and the formulation shall continue to comply with the clauses for:

- pH range (4.1);
- emulsion stability/re-emulsification (4.2).

Note 1 In addition to HPLC and GC retention times, the mass spectrum (obtained by GC-MS) provides good evidence of identity. See Figure 1, attached to the specification for chlorpyrifos TC.

Note 2 In cases of dispute, the analytical results shall be calculated as g/kg.

Note 3 The methods for determination of sulfotep in technical and formulated chlorpyrifos are described in Appendix 1 of Evaluation 221/2004, in Part Two of this publication.

Note 4 Field dilution rates include concentrations below 5% but the MT 36.1 test has been shown to be indicative of performance over the recommended range of dilutions.

Note 5 MT 46.4 is the harmonized and updated accelerated storage method. It was presented and provisionally adopted at the 2019 CIPAC Meeting in Braunschweig. Prior to its publication in a next

Handbook, copies of the method can be obtained through
<https://www.cipac.org/index.php/methods-publications/pre-published-methods>

Note 6 Samples taken before and after this test may be analyzed concurrently to reduce analytical error.

CHLORPYRIFOS ULTRA LOW VOLUME LIQUID

FAO Specification 221 / UL (July 2020)

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 (221/2002, 221/2004, 221/2006, 221/2008 & 221/2020). It should be applicable to relevant products of these manufacturers, 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 (221/2002, 221/2004, 221/2006, 221/2008 & 221/2020), as PART TWO, form an integral part of this publication.

1 Description

The material shall consist of technical chlorpyrifos complying with the requirements of FAO specification 221/TC (February 2020), dissolved in suitable solvents together with any other necessary formulants. It shall be in the form of a stable and homogeneous pale yellow to brown coloured liquid, free from visible suspended matter and sediment, to be applied without dilution using appropriate hand-held or aerial ULV application equipment.

2 Active ingredient

2.1 Identity tests (221/UL/(M)/2, CIPAC Handbook K, p. 23, 2003) (Note 1)

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

2.2 Chlorpyrifos content (221/UL/(M)/3, CIPAC Handbook K, p. 23, 2003)

The chlorpyrifos content shall be declared (g/kg or g/l at $20 \pm 2^\circ\text{C}$, Note 2) and, when determined, the average measured content shall not differ from that declared by more than the following amounts:

Declared content in g/kg or g/l at $20 \pm 2^\circ\text{C}$	Tolerance
Up to 100	$\pm 10\%$ of the declared content
above 100 up to 250	$\pm 6\%$ of the declared content
above 250 up to 500	$\pm 5\%$ of the declared content
above 500	± 25 g/kg or g/l
Note in each range the upper limit is included	

3 Relevant impurities

3.1 Sulfotep (*O,O,O',O'*-tetraethyl dithiopyrophosphate, CAS No. 3689-24-5) (Note 3)

Maximum: 3 g/kg (0.3%) of the declared chlorpyrifos content found under clause 2.2.

4 Physical properties (Notes 4 and 5)

4.1 Acidity (MT 191, CIPAC Handbook L, p. 143, 2006)

Maximum acidity: 2 g/kg calculated as H_2SO_4

5 Storage stability

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

After storage at $0 \pm 2^\circ\text{C}$ for 7 days, the volume of solid or liquid which separates shall not be more than 0.3 ml.

5.2 Stability at elevated temperature (MT 46.4) (Note 6)

After storage at $54 \pm 2^\circ\text{C}$ for 14 days, the determined average active content shall not be lower than 95%, relative to the determined average content found under 2.2 before storage (Note 7), and the formulation shall continue to comply with the clause for:

- acidity (4.1).

Note 1 In addition to HPLC and GC retention times, the mass spectrum (obtained by GC-MS) provides good evidence of identity. See Figure 1, attached to the specification for chlorpyrifos TC.

Note 2 In cases of dispute, the analytical results shall be calculated as g/kg.

Note 3 The methods for determination of sulfotep in technical and formulated chlorpyrifos are described in Appendix 1 of Evaluation 221/2004, in Part Two of this publication.

Note 4 Viscosity can be critically important for successful application of a UL formulation but the requirements are dependent upon both the formulation and the application technique or equipment. For this reason, no clause is provided for kinematic viscosity.

Note 5 Loss of droplet mass by volatilization can be critical for UL formulations because, if the losses are significant, the proportion of the spray which drifts from the target, and the distance over which the drift occurs, is likely to increase. The volatilization and additional drift that occur in practice are dependent on the initial droplet size spectrum and the height through which droplets fall, the air temperature and wind speed. In addition, a degree of volatilization which may be unacceptable for one type of application may be of little or no consequence in another case. At present, no method is available to allow measurement of loss by volatilization to be related to the potential increase in drift and therefore no clause is provided for volatility.

Note 6 MT 46.4 is the harmonized and updated accelerated storage method. It was presented and provisionally adopted at the 2019 CIPAC Meeting in Braunschweig. Prior to its publication in a next Handbook, copies of the method can be obtained through <https://www.cipac.org/index.php/methods-publications/pre-published-methods>

Note 7 Samples taken before and after this test may be analyzed concurrently to reduce analytical error.

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CHLORPYRIFOS

FAO/WHO EVALUATION REPORT 221/2020

Recommendations

The Meeting recommended that:

- (i) The chlorpyrifos UL produced by Simonis B.V., the Netherland, should be considered as equivalent to the chlorpyrifos UL reference specification.
- (ii) The existing FAO specification for chlorpyrifos UL should be extended to encompass the product (240 g/L) from Simonis B.V., the Netherlands

Appraisal

The Meeting considered data submitted in 2020 by the company Simonis B.V (Simonis) for the determination of the equivalence of their UL formulation with a nominal content of 240 g/L to the reference specification 221 / UL (February 2020). This specification was published originally in 2004 and referred to the products from Dow AgroSciences and Makhteshim Chemical Works, it has been extended to the UL formulations from Cheminova and Gharda. Simonis utilizes technical chlorpyrifos from Gharda Chemicals Ltd. to produce its UL formulation. Chlorpyrifos TC produced by Gharda has been evaluated by JMPS and was deemed equivalent to the quality produced by the manufacturer of the reference (Dow Agroscience) in 2008. A formal letter was received from Gharda confirming that they supply their chlorpyrifos TC to Simonis.

Simonis submitted a study where 6 samples from 6 formulation batches, all produced in March 2020, had been tested for compliance with the published chlorpyrifos UL specification. The results allowed the conclusion, that all 6 samples entirely complied with the specification 221 / UL (February 2020). The content of the relevant impurity sulfotep is well below the limit of 3 g/kg of the declared chlorpyrifos content in 5 batches, in one batch the content is 2.74 g/kg, but still below the limit of 3 g/kg.

After storage at 0 °C for 7 days no separated solid or liquid was visible, the content of chlorpyrifos after 14 days at 54 °C decreased at ca. 1 %.

According to Section 3.2 of the Manual, the submitted data are considered sufficient to conclude, that the chlorpyrifos UL produced by Simonis should be considered as equivalent to the reference formulation.

ANNEX 1. REFERENCES

FAO/WHO Manual, 2016		2016	Manual on development and use of FAO and WHO specifications for pesticides, First edition -third revision http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/Specs/JMPS_Manual_2016/3rd_Amendment_JMPS_Manual.pdf
		2020	Final report Analysis of 6 samples as received and after accelerated storage Wallonie recherche CRA-W Report RE/20/U10/25102/2 version 01

CHLORPYRIFOS

FAO/WHO EVALUATION REPORT 221/2019

Recommendation

The Meeting recommended that

- (i) The chlorpyrifos TC as proposed by Zhejiang Xinnong Chemical Co., Ltd, China should be accepted as equivalent to the chlorpyrifos reference profile.
- (ii) The FAO specification for chlorpyrifos TC should be extended to encompass the technical material manufactured by Zhejiang Xinnong Chemical Co., Ltd.

Appraisal

The data for chlorpyrifos TC was provided by Zhejiang Xinnong Chemical Co., Ltd (Zhejiang Xinnong) in September 2018 and subsequently updated in April and December 2019, in support of an equivalence determination with the TC reference profile. The Meeting noted, that chlorpyrifos is an insecticide used in agriculture and public health and therefore has both FAO and WHO specifications. When contacted, Zhejiang Xinnong responded that they applied for an equivalence with the FAO specification for chlorpyrifos TC only. Therefore, the extension is for the FAO specification solely.

The data submitted were broadly in accordance with the requirements of the Manual on development and use of FAO and WHO specifications for pesticides (2016-third revision of the first edition) and complied with the existing FAO/WHO specifications for chlorpyrifos TC.

The proposer submitted a 'Certificate for Pesticide Registration' issued by the Argentinian SENASA¹ and dated 24 September 2014, which confirmed that chlorpyrifos TC manufactured by Zhejiang Xinnong was registered in Argentina with a declared minimum purity of 970 g/kg, which is consistent with the declared minimum purity in the data submitted to FAO.

The Meeting was provided with commercially confidential information on the manufacturing process, batch analysis data on chlorpyrifos and all impurities present at or above 1 g/kg and their manufacturing limits in the TC. Mass balances were 986.86 to 988.31 g/kg in the 5-batch data. The percentage of unknowns was lower than 13 g/kg. The declared minimum active ingredient content was the same as that of the published FAO and WHO specification (970 g/kg). The company confirmed that their product complies with the existing FAO TC specification.

Sulfotep (*O,O,O',O'*-tetraethyl dithiopyrophosphate), was found to be about 0.3 g/kg (detection limit =0.16 g/kg), while the maximum level of this relevant impurity was 3 g/kg in the specification. Acetone insolubles were determined according to the method MT 27, CIPAC F to be around 4 g/kg, while the limit set in the TC specification was maximum 5 g/kg. Both of the levels of sulfotep and acetone insolubles comply with the current FAO/WHO specifications.

The manufacturing process utilized by Zhejiang Xinnong is different to that used in the production of the reference TC. Zhejiang Xinnong's process involved the synthesis of an

¹ The Argentinian SENASA (Servicio Nacional de Sanidad Vegetal y Calidad Agroalimentaria) is the national authority for registration of pesticides in the country.

intermediate, which was used as a starting material in the process of the manufacturer of the reference. The other 2 steps were similar with that of the holder of the reference specification.

The analytical method for the active ingredient in technical material is based on a reversed phase HPLC on a C₈-column, with acetonitrile/water (80:20, v/v) as mobile phase and UV detection at 229 nm. The method was adequately validated. The identity of the active ingredient was confirmed by FTIR, HNMR and HPLC retention time.

The analytical method for the relevant impurity, sulfotep in technical material is based on GC-FID and external standard calibration. The method was properly validated. The identity of the impurity was confirmed by FTIR and GC-MS.

The analytical method for the impurities at or above 1 g/kg in technical material is based on GC-FID and external standard calibration. The method is properly validated on specificity, linearity, LOD/LOQ, reproducibility and system precision, repeatability and recovery. The identity of the impurity was confirmed by FTIR and GC-MS.

Test methods for determination of physico-chemical properties of the technical active ingredient were based on OECD, EPA and EEC guidelines.

The mutagenicity test in vitro in *Salmonella typhimurium* strains allowed the conclusion that chlorpyrifos TC produced by Zhejiang Xinnong did not induce reverse mutations in the tester strains. Yet, the Meeting noted some shortcomings in the study and deviations from the OECD guideline – e.g. the density of the bacteria before the start of the assay was not reported. Nevertheless, as cultures were freshly prepared and growth was demonstrated and the necessary density of 10⁹ cells/ml were achieved, it could be assumed that a substantial proportion of the cells were viable. The Meeting therefore conclude, that the deviation is not considered to invalidate the test.

The link between the batch analytical profile and the purity/impurity profile of the samples used in toxicity and physical-chemical property studies were not provided. The proposer submitted a statement to confirm that the toxicity study batch was in compliance with the specification, but no supporting data was provided.

The Meeting noted several issues with the data package. They included the following shortcomings:

- In their initial 5-batch analysis, the company did not use the CIPAC method for the determination of the content of chlorpyrifos in the TC. Upon request by the Meeting, the proposer later on submitted a suitable bridging study, that confirmed the results obtained with the in-house method.
- The method of analysis for determination of residues of the relevant impurity sulfotep was different from the peer validated method published in Annex 1, Chlorpyrifos evaluation report (221/2004). Again, upon request by the Meeting the proposer later on submitted a bridging study where the results of the in-house and peer-validated methods were compared. The results of that comparison showed, that the maximum limits for sulfotep in the chlorpyrifos TC were compliant with the published reference specification.
- Furthermore, an aromatic solvent is used in the last step of the manufacturing process, but possible residues of that solvent were not determined. Zhejiang Xinnong later provided a study on residue levels of that solvent in the finished TC. The results led to the conclusion, that the level of that solvent were below 1 g/kg. The Meeting concluded, that

residues of that solvent are too low to be relevant and are not required to be included in the 5 batch study.

- Trichloropyridinol is used as an intermediate in the last step of manufacture process but not analyzed and no maximum limit was proposed for this potential impurity. Also in that case, the company submitted a study for determination of that potential impurity in the TC. Levels found in the batches were below 1 g/kg. The Meeting concluded, that the low levels on trichloropyridinol did not qualify that impurity for being further considered.

Taking these additional studies and explanations into account, the Meeting concluded that Zhejiang Xinnong's chlorpyrifos TC was considered equivalent to the chlorpyrifos reference TC based on Tier-1 evaluation.

Furthermore, the Meeting recommended an editorial update of the chlorpyrifos formulation specifications in terms of reference to the latest versions of CIPAC MT methods like MT 47.3 for measuring persistent foam and for the new harmonized method for accelerated storage, MT 46.4.

**SUPPORTING INFORMATION
FOR
EVALUATION REPORT 221/2019**

Table 1 Chemical composition and properties of chlorpyrifos technical material

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 98.7 -98.8% and percentages of unknowns were 1.17 – 1.31%.		
Declared minimum chlorpyrifos content		970 g/kg		
Relevant impurities ≥ 1 g/kg and maximum limits for them		Sulfotep, maximum: 3 g/kg Acetone insoluble: 5 g/kg		
Relevant impurities < 1 g/kg and maximum limits for them:		none		
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	42.0 – 42.9 °C	98.7	OECD 102	SR-130064
Solubility in organic solvents	>250g/l in n-heptane, >250g/l in p-xylene, >250g/l in 1,2-dichloroethane, >250g/l in methanol, >250g/l in acetone and >250g/l in ethyl acetate at 19.9°C	98.7 ¹	CIPAC MT181	SR-130064

FORMULATIONS AND CO-FORMULATED ACTIVE INGREDIENTS

The current submission is for a TC only.

METHODS OF ANALYSIS AND TESTING

The analytical method for the active ingredient in technical material is CIPAC 221.b/TC/M- published in Handbook 1C. The identity of the active ingredient was confirmed by FTIR, ¹H-MR and GC-MS.

The analytical method for the relevant impurity, sulfotep, in TC relies is based on GC-FID or GC-MS (see Appendix 1). Initially, the 5-batch data package submitted by Zhejiang) Xinnong relied on in-house methods and were later on successfully bridged to the collaboratively validated CIPAC method for the content of chlorpyrifos in the TC and the peer-validated method for sulfotepp. The identity of the active ingredient was confirmed by FTIR and GC-MS.

Residues of one of the most common intermediates in the last step of the manufacturing process (see e.g. the monograph¹ on chlorpyrifos, ATSDR of CDC), trichloropyridinol, is based on GC-FID and external standard calibration. The method is properly validated on with a LOQ of 1.0 mg/L. Test methods for determination of physico-chemical properties of the technical active ingredient were based on OECD, EPA and EEC guidelines.

EXPRESSION OF THE ACTIVE INGREDIENT

The content of the active ingredient chlorpyrifos is expressed as chlorpyrifos.

¹ <https://www.atsdr.cdc.gov/ToxProfiles/tp84-c4.pdf>

ANNEX 1

HAZARD SUMMARY PROVIDED BY THE PROPOSER

Notes.

- (i) Zhejiang Xinnong provided written confirmation that the toxicological data included in the following summary were derived from chlorpyrifos having an impurity profile similar to those referred to in Table 1, above.
- (ii) The conclusions expressed in the summary below are those of the proposer, unless otherwise specified.

Table 2. Mutagenicity profile of technical chlorpyrifos based on an *in vitro* test

Species	Test	Purity g/kg	Conditions and guidelines	Result	Reference
<i>Salomonella typhimurium</i> Strains TA 1535 TA 97a TA 98 TA 100 TA 102	Bacterial reverse mutation assay	976	OECD Guidelines for Testing of Chemicals No. 471 Two independent experiments both with and without metabolic activation. The tester strains were exposed to test article in triplicate cultures at the dose of:1000 µg/plate, 300 µg/plate , 100 µg/plate , 30 µg/plate , 10 µg/plate .	Negative	R/13822/AME S/13

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	2016	Manual on development and use of FAO and WHO specifications for pesticides. 2016. 3 rd Revision of First Edition. FAO Plant Production and Protection Paper. Revised.
XN-003	Vlado Damnjanovic	2013	Study Title: Determination of active content and impurity profile of chlorpyrifos. Study/Report Number: Study XN-003. GLP. Test facility: Agrifor Scientific Pty Ltd., Sponsor: Zhejiang XinNong Chemical Co., Ltd., Unpublished.
SR-130064	WEN CHUN CHEN	2014	Study Title: Physical-chemical test of chlorpyrifos TC. Study/ Report Number: SR-130064 GLP Test facility: Demeter laboratory, Huikwang Corporation Sponsor: Zhejiang XinNong Chemical Co., Ltd., Unpublished.
R/13822/AMES/13		2013	Salomonella typhimurium, Reverse Mutation Assay Of Chlorpyrifos Technical 97.6%
NCW-2019-006	J. Zhang	2019	Preliminary Analysis of Active Ingredient and Sulfotep Impurity in 3-Batch Chlorpyrifos Tech.
BGP-2019-047	B. Yang	2020	Three Batches Limit Test of ... (an aromatic solvent) and 3,5,6-trichloropyridin-2-ol in Chlorpyrifos Technical

CHLORPYRIFOS

FAO/WHO EVALUATION REPORT 221/2014

Recommendations

The Meeting recommended the following:

- (i) that the chlorpyrifos TC proposed by Bharat Rasayan Limited, India (Bharat) be accepted as equivalent to the chlorpyrifos reference profile
- (ii) the existing FAO specification for chlorpyrifos TC should be extended to encompass the technical material manufactured by Bharat.
- (iii) the existing WHO specification for chlorpyrifos TC should be extended to encompass the technical material manufactured by Bharat.

Appraisal

The data packages for chlorpyrifos TC was provided in 2012 in support of an equivalence determination with the TC reference profile.

The data submitted were broadly in accordance with the requirements of the revised (second revision, November 2010) 1st edition of the Manual on development and use of FAO and WHO specifications for pesticides [FAO/WHO Manual, 2010] and supported the existing specification.

The confidential data on the manufacturing specification of chlorpyrifos and the 5-batch analysis results submitted to FAO and WHO are identical to those provided to India for registration purposes (Dr. S. Phogat, 26 May 2015). The impurities and QC limits for chlorpyrifos TC produced by Bharat agree exactly between the information submitted to FAO, to WHO and to the Indian authority.

The Meeting was provided with commercially confidential information on the manufacturing process and batch analysis data on chlorpyrifos and all impurities present at or above 1 g/kg and their manufacturing limits in the TC. Mass balances were 984.2 to 988.0 g/kg in the 5-batch data. The declared minimum active ingredient content was the same as that of the published FAO and WHO specification (970 g/kg). The company confirmed that their product complies with the existing TC specification.

Manufacturing limits for impurities identified in the technical material did not exceed the limits in the reference profile. Two new impurities were identified, both with manufacturing QC limits of 1 g/kg, with measured values in the 5-batches below the respective LOQs of the analytical methods used (< 0.33 g/kg and < 0.01 g/kg respectively).

The Meeting identified several issues with the data package. These points were:

- The company did not use the CIPAC Method for determination of content of chlorpyrifos in the TC
- Two new impurities that were not identified in the reference profile but one of the impurity was present at levels above 1 g/kg would at first glance render the TC material non-equivalent
- The data submitted in India for registration are apparently not identical to those submitted to FAO/WHO

The first point was addressed by Bharat by reanalyzing the batches using CIPAC method published in Handbook 1C. Bharat have provided data for the analysis of the chlorpyrifos content in the 5 batches of TC using the CIPAC method 221.b/TC/M-. The data show that

the TC from Bharat complies with the current specification for chlorpyrifos. Bharat also provided data for the analysis of sulfotep in the batches using the peer validated method referred to in the FAO and WHO specifications. These data also show the TC can comply with the current specification.

The second point was addressed by a discussion of the level and identity of the two new impurities. One of these impurities is an organic solvent present at levels of a few hundred mg/kg. Earlier considerations of the consequences of the presence of low levels of this solvent in other TC materials had led to the conclusion, that residues of this compound do not significantly contribute to the hazard of this TC. A closer examination of the identity of the second impurity revealed, that is in fact a tautomer of a compound present at similar or higher levels in the reference profile.

The third issue was resolved by submitting a revised confidential data package to the Indian authority. A written confirmation was received (e-mail Dr. S. Phogat, 2015) that the manufacturing specifications is the same as submitted to FAO and WHO.

All issues being resolved, the Meeting concluded that the TC from Bharat can be considered as equivalent to the reference profile by Tier-1.

**SUPPORTING INFORMATION
FOR
EVALUATION REPORT 221/2014**

Table 2. Chemical composition and properties of chlorpyrifos 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 and WHO. Mass balances were 98.42 to 98.80 % and percentages of unknowns were 1.2 to 1.58 %.		
Declared minimum chlorpyrifos content		970 g/kg		
Relevant impurities ≥ 1 g/kg and maximum limits for them		O, O, O, O-tetraethyl dithiopyrophosphate (Sulfotep), Max. 3.0 g/kg		
Relevant impurities < 1 g/kg and maximum limits for them:		None		
Stabilisers or other additives and maximum limits for them:		None		
Parameter	Value and conditions	Purity %	Method reference	Study number
Melting temperature range	42.1 – 43.4 °C	97.54	Melting Point Apparatus CIPAC MT 2 Guideline: EEC A.1, OECD 102, U.S. EPA OPPTS 830.7200	IIBAT Study Number: 12221 Date: 01/10/2012
Solubility in organic solvents	at 20 ± 0.5 °C <ul style="list-style-type: none"> • Acetone: >1000 g/L • 1,2-Dichloroethane: >1000 g/L • Ethyl acetate: >1000 g/L • n-Heptane: >1000 g/L • Methanol: >500 g/L • Xylene: >1000 g/L 	97.54	<i>Guideline:</i> EEC A.6, OECD 105, U.S. EPA OPPTS 830.7840	Study Number: 12224 Date: 26/11/2012

ANNEX 1

HAZARD SUMMARY PROVIDED BY THE PROPOSER

Note: Bharat Rasayan provided written confirmation that the toxicological data included in the following summary were derived from chlorpyrifos having impurity profiles similar to those referred to in Table 2, above.

Table 3. Mutagenicity profile of the technical material based on *in vitro* tests

Species	Test	Conditions and guidelines	Result	Reference
<i>Salmonella typhimurium</i>	Bacterial reverse mutation test with five strains of <i>Salmonella typhimurium</i> TA 1535, TA 1537, TA 98, TA 100 and TA 102	<i>Guideline: OECD 471 / OPPTS 870.5100 with and without S9. Each concentration, including the negative, vehicle and positive controls was tested in triplicate. The test item was tested at the 0.313, 0.625, 1.25, 2.5 and 5 mg/plate concentrations,. Purity: 97.48%</i>	<i>Negative, Non mutagenic</i>	<i>Study Number: 3549,</i>

ANNEX 2. REFERENCES

Study number	Author(s)	year	Study title. Study identification number. Report identification number. GLP [if GLP]. Company conducting the study.
e-mail communication	Dr. S. Phogat, Central Insecticide Board & Registration Commission, Ministry of Agriculture, Govt. of India	2015	Confirmation of similarity of confidential data packages submitted to FAO and WHO and Central Insecticide Board & Registration Commission, received 26 May 2015
FAO/WHO Manual	FAO/WHO	2010	Manual on development and use of FAO and WHO specifications for pesticides. November 2010 – second revision of the First Edition.
Study number: 12383		2012	Title: Chemical Composition of Five Batches of Chlorpyrifos Technical Enforcement Analytical Methods for Chlorpyrifos TGAI, fulfilling the Requirements of OPPTS Guideline 830.1800, and EC Council Directive 94/37/EC Article 1.9 to 1.11 Study number: 12383, GLP Sponsor: M/s. Bharat Rasayan Limited, New Delhi, India
Study number 12221		2012	Title: Chlorpyrifos Technical: Laboratory Study of Melting point: Study number: 12221, GLP Sponsor: M/s. Bharat Rasayan Limited, New Delhi, India
Study number 12219		2012	Title: Chlorpyrifos Technical: Determination of Active Ingredient Content Study number: 12219, GLP Sponsor: M/s. Bharat Rasayan Limited, New Delhi, India
Study number 12224		2012	Title: Chlorpyrifos Technical: Laboratory Study of Solubility in Organic Solvents Study number: 12224, GLP Sponsor: M/s. Bharat Rasayan Limited, New Delhi, India
Study number 3549		2012	Title: Bacterial Reverse Mutation Assay with Chlorpyrifos Technical Study number: 3549, GLP Sponsor: M/s. Bharat Rasayan Limited, New Delhi, India

CHLORPYRIFOS

FAO/WHO EVALUATION REPORT 221/2008

Recommendations

The Meeting recommended that:

- (i) the existing FAO specifications for chlorpyrifos TC, EC and UL should be extended to encompass the corresponding products of Gharda Chemicals Limited;
- (ii) the existing WHO specifications for chlorpyrifos TC and EC should be extended to encompass the corresponding products of Gharda Chemicals Limited.

Appraisal

The Meeting considered data and information on chlorpyrifos, submitted by Gharda Chemicals Limited, in support of the extension of the existing (October 2004) FAO specifications for TC, EC and UL and the existing (October 2004) WHO specifications for TC and EC.

The Meeting was provided with data on the physico-chemical properties of pure chlorpyrifos. The purity of chlorpyrifos used to obtain the physico-chemical data was declared to be 98%. Chlorpyrifos is a solid substance with a melting point of 42-44°C. The octanol-water partition coefficient suggests that bioconcentration is high ($\log Pow = 5.0$ at 24.5°C). The vapour pressure is 1×10^{-3} Pa at 25°C.

The Meeting was also provided with confidential information on the manufacturing process, the manufacturing specification and 5-batch analysis data (conducted according to GLP on production scale batch samples) for chlorpyrifos and all detectable impurities. The proposer declared a minimum content of 980 g/kg for chlorpyrifos in the TC. The identity of the impurities at a level > 1 g/kg, not considered relevant, were provided. Mass balances were high with a range from 990 to 995 g/kg.

Sulfotep (*O,O,O',O'*-tetraethyl dithiopyrophosphate) was considered as a relevant impurity, although it was not detected in the five batches analysed (detection limit = 1 g/kg). The manufacturing limit given was 2 g/kg and 3 g/kg was proposed as the specification limit. Assuming that the acute oral LD_{50} of sulfotep is 5 mg/kg bw, that of chlorpyrifos is 229 mg/kg bw, and that the minimum content of the active ingredient is 970 g/kg, it can be calculated that the RelHaz is 45.80, and the MTIHaz, 2.42. The latter represents an increase by 242% of the acute toxicity of chlorpyrifos at the maximal theoretical sulfotep content in chlorpyrifos. This being in excess of 10%, the impurity is indeed relevant. The maximum limit for the impurity, Imp_{max} is $0.21 \sim 0.2\% = 2$ g/kg (Manual on development and use of FAO and WHO specifications for pesticides). This would be in line with the manufacturing QC limit of 2 g/kg, and in accordance with the proposed specification limit of 3 g/kg.

Acetone insolubles (according to the method MT 27 published in the CIPAC Handbook F) were also declared as relevant impurities with a maximum of 5 g/kg retained on a 45 μm test sieve. No information was provided or identified that would indicate that the other identified impurities are relevant.

As far as impurities are concerned, the reported limits were supported by the 5-batch analysis data submitted by Gharda Chemicals Limited. The 5-batch analysis report (dated

of 9 November 2006), conducted according to GLP principles, showed an impurities profile similar to the Dow Agrosiences reference profile and can be considered equivalent.

The toxicological and ecotoxicological studies presented in annex 1 were sufficiently described. Chlorpyrifos was evaluated by the WHO/IPCS and by the FAO/WHO JMPR in 1999. The estimated acceptable daily intake (ADI) for humans was set at 0 to 0.01 mg/kg bw and the acute reference dose (ARfD) at 0.1 mg/kg bw. The WHO/IPCS hazard classification of chlorpyrifos is "Moderately hazardous, Class II". Within the EU, according to the 19th adaptation to technical progress of Council Directive 67/548/EEC, chlorpyrifos is classified as Toxic by swallowing (T, R25), Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment (N, R50/53).

Chlorpyrifos manufactured by Gharda Chemicals Limited is approved in Australia. The chemistry and quality control data (manufacturing process, purity and impurities, declaration of composition (specifications), analytical results for five batches, analytical method details with validation data, storage stability, packaging details and toxicology data) submitted by Gharda Chemicals in support of the FAO and WHO specifications for chlorpyrifos are identical to those assessed by the APVMA (Australian Pesticides and Veterinary Medicines Authority) for approval of the new source of chlorpyrifos.

The analytical method for determination of the active ingredient content (including HPLC retention time identity test) was based on the CIPAC method 221.b. Chlorpyrifos was determined by reverse phase HPLC using UV detection at 300 nm and 1,4-dibromonaphthalene as internal standard. The same method was followed for determination of chlorpyrifos in the EC formulation. The methods used for determination of impurities were based on GC using the internal standard calibration and HPLC using the external standard calibration, which were validated as per SANCO/3030/99 rev. 4 working document and US EPA 540/9/82/018 guideline. The method for determination of the relevant impurity sulfotep was based on GC-FID using the external standard calibration. Test methods for determination of physico-chemical properties of technical active ingredient were based on OECD, EPA and EEC guidelines, and for the formulations were CIPAC methods, as indicated in the specifications.

The limits proposed for physical properties of the EC and UL formulations, and the methods for testing them, comply with the requirements of the FAO/WHO manual on pesticides specifications.

**SUPPORTING INFORMATION
FOR
EVALUATION REPORT 221/2008**

Physico-chemical properties of chlorpyrifos

Table 1. Physico-chemical properties of pure chlorpyrifos

Parameter	Value(s) and conditions	Purity %	Method reference	Reference
Vapour pressure	1.0 x 10 ⁻³ Pa at 25°C	98%	OECD 104	1 <i>C.CFO.038</i>
Melting point	Melting point = 42-44°C	98%	OECD 102	2 <i>C.CFO.035</i>
Solubility in water	0.39 mg/l at 19.5°C	98%	OECD 105	3 <i>C.CFO.037</i>
Octanol/water partition coefficient	log P _{ow} = 5.0 at 24.5°C	98%	OECD 117	4 <i>C.CFO.039</i>
Photolysis characteristics	(12 h light) Quantum yield : 1.18 x 10 ⁻² t _{1/2} (summer) : 4.2 days t _{1/2} (winter) : 9.7 days	98%	OPPTS 835.2210	5 <i>C.CFO.066</i>
Dissociation characteristics	Practically non dissociative by nature	98%	OECD 112	6 <i>C.CFO.067</i>

Table 2. Chemical composition and properties of chlorpyrifos 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 and WHO. Mass balances were 99.0-99.5 %.
Declared minimum chlorpyrifos content	980 g/kg
Relevant impurities ≥ 1 g/kg and maximum limits for them	Sulfotep (O,O,O',O'-tetraethyl dithiopyrophosphate, CAS No. 3689-24-5): maximum 3 g/kg
Relevant impurities < 1 g/kg and maximum limits for them	None
Stabilizers or other additives and maximum limits for them	None
Melting range of the TC	42-44°C

ANNEX 1
HAZARD SUMMARY PROVIDED BY THE PROPOSER

Note: Gharda Chemicals Limited provided written confirmation that the toxicological data included in the following summary were derived from chlorpyrifos having impurity profiles similar to those referred to in Table 2, above.

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

Species	Test	Duration and conditions or guideline adopted	Result	Reference
Rat (Sprague dawley)	Acute oral	OECD 401	LD ₅₀ = 229 mg/kg bw Purity: not available	7 <i>T.CFO.047</i>
Rat (Sprague dawley)	Acute dermal	OECD 402	LD ₅₀ > 2000 mg/kg bw Purity: not available	8 <i>T.CFO.048</i>
Rat (Sprague dawley)	Acute Inhalation	Gaitonde Committee guidelines	LC ₅₀ = 0.385 mg/L Purity: 99%	9 <i>T.CFO.098</i>
Rabbit, New Zealand white	Skin irritation	OECD 404	Non irritant Purity: not available	0 <i>T.CFO.049</i>
Rabbit, New Zealand white	Eye irritation	OECD 405	Mild irritant Purity: not available	11 <i>T.CFO.045</i>
Guinea pig	Skin sensitisation	OECD 406	Non sensitizer Purity: not available	12 <i>T.CFO.112</i>

Table B. Mutagenicity profile of the technical material based on in vitro and in vivo tests

Species	Test	Conditions and guidelines	Result	Reference
<i>Salomonella typhimurium</i>	Bacterial reverse mutation assay	OECD Guidelines for Testing of Chemicals No. 471 EEC Directive 9/831/EEC, Annex V (Directive 84/449/EEC) US Environmental Protection Agency Method Concentrations up to 5000 µg/plate Purity: 99%	non-mutagenic	13 <i>T.CPO.011</i>
Mice	Chromosomal aberration	OECD guideline 475 Low dose : 3.125 mg/kg bw Medium dose : 6.250 mg/kg bw High dose : 12.50 mg/kg bw Purity: 98%	NOEL = 6.25 mg/kg bw	14 <i>T.CPO.043</i>
Mouse	Micronuclei test	OECD guideline Low dose : 3.124 mg/kg bw Medium dose : 6.250 mg/kg bw High dose: 12.50 mg/kg bw Purity: 98%	Non mutagenic NOEL = 12.50 mg/kg bw	15 <i>T.CPO.042</i>

Table C. Ecotoxicology profile of the technical material

Species	Test	Duration and conditions or guidelines	Result	Reference
<i>Daphnia magna</i> (water flea)	Acute toxicity	48 h flow through observations at 24 and 48 h OECD procedure 202 EEC Methods for determination of ecotoxicity (Annex to Directive 92/69/EEC)	48 h EC ₅₀ = 0.16 µg/l	16 <i>T.CFO.061</i>
<i>Oncorhynchus mykiss</i> (Rainbow trout fish)	Acute toxicity	Under flow through exposure conditions for 96 hrs OECD procedure 203 EEC Methods for determination of ecotoxicity (Annex to Directive 92/69/EEC)	LC ₅₀ = 24 µg/l NOEL = 1.6 µg/l	17 <i>T.CFO.062</i>
<i>Lampito mauriti</i> (earthworm)	Acute toxicity	OECD guideline 207 (soil test method) in sterilized soil for 14 days	LC ₅₀ (14 d) = 78.0 ± 7.40 ppm (soil)	18 <i>T.CFO.051</i>
<i>Apis mellifera</i> (Honey bee)	Acute oral toxicity	Dosed and observed for 24, 48 h at 24-25°C United Kingdom Control of Pesticides Regulation 1986, EPA Pesticide Assessment Guidelines 1989 for Non-target pests EPPO guidelines 1992 for the Efficacy Evaluation of Plant Protection Products Guideline no. 170	LD ₅₀ (48 hrs) = Oral: 0.12 µg/bee	19 <i>T.CFO.054</i>
<i>Apis mellifera</i> (Honey bee)	Acute contact toxicity	Topical application, observed at 24, 48 h at 24-25°C United Kingdom Control of Pesticides Regulation 1986, EPA Pesticide Assessment Guidelines 1989 for Non-target pests EPPO guidelines 1992 for the Efficacy Evaluation of Plant Protection Products Guideline no. 170	LD ₅₀ (48 hrs) = Contact: 0.11 µg/bee	20 <i>T.CFO.054</i>
<i>Colinus virginianus</i> (Bobwhite quail)	Acute oral toxicity	Test levels : 0, 500, 1000, 2000 mg/kg EPA guidelines, Series 71 – Avian and Mammalian Testing	LD ₅₀ = 153 mg/kg bw	21 <i>T.CFO.046</i>

ANNEX 2. REFERENCES

Gharda document number or other references	Year	Title of report or publication details	
1	C.CFO.038	1996	Physical & chemical characteristics of Chlorpyrifos Determination – vapour pressure
2	C.CFO.035	1996	Physical & chemical characteristics of Chlorpyrifos Determination – Melting point
3	C.CFO.037	1996	Physical & chemical characteristics of Chlorpyrifos – Solubility in water
4	C.CFO.039	1996	Physical & chemical characteristics of Chlorpyrifos – n-Octanol/Water partition coefficient
5	C.CFO.066	2006	Physical & chemical characteristics of Chlorpyrifos – Direct photo transformation in water
6	C.CFO.067	2006	Physical & chemical characteristics of Chlorpyrifos – Determination of the dissociation constant
7	T.CFO.047	1997	Acute oral toxicity in Wistar rats
8	T.CFO.048	1997	Acute dermal toxicity study in Wistar rats
9	T.CFO.098	1997	Acute inhalation toxicity in Wistar rats
10	T.CFO.049	1997	Acute dermal irritation / corrosion study in New Zealand White Rabbits
11	T.CFO.045	1997	Acute eye irritation/corrosion study in New Zealand white rabbits
12	T.CFO.112	1997	Skin sensitisation in the Guinea pig
13	T.CPO.011	1991	Chlorpyrifos Tech. Bacterial Mutation Assay
14	T.CPO.043	1993	Mutagenicity study : Chromosomal aberration in mouse
15	T.CPO.042	1993	Mutagenicity study: Micronuclei Test in mouse
16	T.CFO.061	1997	Chlorpyrifos Technical : Acute toxicity to <i>Daphnia magna</i>
17	T.CFO.062	1997	Chlorpyrifos Technical : Acute toxicity to Rainbow Trout
18	Pesticide Manual		The Pesticide Manual 13 th Edn. – Chlorpyrifos - Crop Protection Publication
19	T.CFO.051	1993	Chlorpyrifos technical – Acute toxicity study in earthworm (<i>Lampito mauriti</i>)
20	T.CFO.054	1997	Chlorpyrifos Technical –Acute toxicity to honey bees (<i>Apis mellifera</i>)
21	T.CFO.046	1996	Chlorpyrifos technical – Acute toxicity (LD ₅₀) to Bobwhite quail

CHLORPYRIFOS

FAO/WHO EVALUATION REPORT 221/2006

Recommendations

The Meeting recommended that:-

- (ii) the existing FAO specifications for chlorpyrifos TC, EC and UL should be extended to encompass the corresponding products of Cheminova A/S;
- (ii) the existing WHO specifications for chlorpyrifos TC and EC should be extended to encompass the corresponding products of Cheminova A/S.

Appraisal

The Meeting considered data on chlorpyrifos, submitted by Cheminova A/S, in support of the extension of existing (October 2004) FAO specifications for TC, EC and UL and existing (October 2004) WHO specifications for TC and EC.

The Meeting was provided with confidential information on the manufacturing process and 5-batch analysis data on the content of active ingredient and impurities with manufacturing limits $\geq 1\text{g/kg}$. Mass balances were very high, 99.6-100.5%, with no reported unknowns. These data, and the corresponding hazard data (see Annex 1), were confirmed as similar in most respects to those submitted by the manufacturer for registration in the USA, although a complete comparison of impurity data was not possible in this case

One of the impurities did not occur in the reference profile and, on this basis, the Cheminova TC did not appear to be equivalent. However, from a comparison of the acute toxicology data, the Meeting agreed that the Cheminova TC is equivalent to the chlorpyrifos upon which the original specification is based. The company provided data confirming that the levels of sulfotep, chlorpyrifos-oxon and "iso-chlorpyrifos" (see FAO/WHO evaluation report 221/2002) did not increase during storage (Cheminova 1998).

The original Cheminova study on inhalation toxicity was difficult to compare with the FAO/WHO reference data, because the highest dose tested was relatively low. The manufacturer had submitted a replacement study of inhalation toxicity to the registration authority in the USA. The US EPA review (USEPA 2004) indicated that the new study supported comparability with the inhalation toxicity of the chlorpyrifos TC upon which the reference FAO/WHO hazard profile was based.

Cheminova confirmed that its chlorpyrifos products comply with the existing FAO and WHO specifications and that the analytical and physical test methods contained therein are applicable.

**SUPPORTING INFORMATION
FOR
EVALUATION REPORT 221/2006**

Physico-chemical properties of chlorpyrifos

Table 1. Chemical composition and properties of chlorpyrifos 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 and WHO. Mass balances were 996-1005 g/kg.
Declared minimum chlorpyrifos content	970 g/kg
Relevant impurities ≥ 1 g/kg and maximum limits for them	Sulfotep (O,O,O',O'-tetraethyl dithiopyrophosphate, CAS No. 3689-24-5): maximum 3 g/kg
Relevant impurities < 1 g/kg and maximum limits for them:	None
Stabilizers or other additives and maximum limits for them:	None
Melting temperature range	Melting point $42.6 \pm 0.1^\circ\text{C}$ (purity: 99.3% w/w) [reference 5 CYF]

Hazard summary

Chlorpyrifos was evaluated by the FAO/WHO JMPR for toxicology in 1972, 1977, 1982, and 1999, and for residues in 1972, 1974, 1975, 1977, 1981, 1982, 1983, 1989, 1995, 2000, and 2004. It is currently under review by the European Commission and the US EPA.

Containers and packaging

To avoid corrosion, containers of iron, steel, tin plate or copper should not be used unless lined with a suitable material.

ANNEX 1
HAZARD SUMMARY PROVIDED BY THE PROPOSER

Note: Cheminova A/S provided written confirmation that the toxicological data included in the following summary were derived from chlorpyrifos having impurity profiles similar to those referred to in Table 1, above.

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

Species	Test	Duration and conditions or guideline adopted, purity	Result	Reference
Rat, Sprague Dawley (m,f)	Acute oral	FIFRA 81-1; purity 99.3%	LD ₅₀ (m,f) = 320 mg/kg bw (260-393) LD ₅₀ (m) = 276 mg/kg bw (167-455) LD ₅₀ (f) = 350 mg/kg bw (285-429)	8 CYF
Rat, Sprague Dawley (m,f)	Acute dermal	FIFRA 81-2; purity 99.3%	LD ₅₀ >2000 mg/kg	9 CYF
Rat, Sprague Dawley (m,f)	Acute dermal	FIFRA 81-2; purity 98.5%	LD ₅₀ >2000 mg/kg	USEPA 2004
Rat, Sprague Dawley (m,f)	Acute inhalation	FIFRA 81-3; purity 99.3%	MLC (m,f) >36 mg/m ³ (32-40), no deaths.	10 CYF
Rat, Sprague-Dawley (m,f)	Acute inhalation	FIFRA 81-3; purity 97.8%	LC ₅₀ >5.22 mg/l	Cheminova 2004
Rabbit, New Zealand white (sex not stated)	Skin irritation	FIFRA 81-5; purity 99.3%	Mild irritant. No corrosive effects.	11 CYF
Rabbit, New Zealand white (sex not stated)	Eye irritation	FIFRA 81-4; purity 99.3%	Mild irritant (class 4, modified Kay & Calandra classification), all rabbits showed positive effects.	12 CYF
Guinea pig, albino Dunkin-Hartley (f)	Skin sensitization	FIFRA 81-6; purity 99.3%	Non-sensitizer.	13 CYF

ANNEX 2. REFERENCES

Cheminova document number or other reference	Year and title of report or publication details
5 CYF	1994. Chlorpyrifos Technical – Determination of Melting Point.
8 CYF	1994. Chlorpyrifos Technical: Acute Oral Toxicity Test in the Rat.
9 CYF	1994. Chlorpyrifos Technical: Acute Dermal Toxicity (Limit Test) in the Rat.
10 CYF	1994. Chlorpyrifos Technical: Acute Inhalation Toxicity Study Four-Hour Exposure (Nose Only) in the Rat.
11 CYF	1994. Chlorpyrifos Technical: Acute Dermal Irritation Test in the Rabbit.
12 CYF	1994. Chlorpyrifos Technical: Acute Eye Irritation Test in the Rabbit.
13 CYF	1994. Chlorpyrifos Technical: Magnusson & Kligman Maximisation Study in the Guinea Pig.
Cheminova 1998	1998. Chlorpyrifos_Storage_Stability_Study.pdf, sent to FAO 24 March 2007.
Cheminova 2004	2004. Chlorpyrifos_Acute_Inhalation_Study_2004.pdf, sent to FAO 24 March 2007.
USEPA 2004	2004. Data reviews for acute dermal toxicity and acute inhalation toxicity testing. 20060531154740354.pdf sent to FAO 24 March 2007.

CHLORPYRIFOS

EVALUATION REPORT 221/2004

Validation of analytical methods for determination of sulfotep

Explanation

The 2002 JMPS recommended adoption of specifications for chlorpyrifos TC and EC, subject to acceptable validation of the analytical method for determination of the relevant impurity, sulfotep. The specification limit accepted by the Meeting for sulfotep was 3 g/kg of chlorpyrifos.

In 2004, the results of a peer validation, conducted by Dow AgroSciences and Makhteshim Chemical Works, were reported to FAO and WHO (NAFST702). Five batches of TC (from 2 sources), 5 batches of EC (480 g/l) (from 2 sources) and spiked blank formulations were analyzed in two laboratories, using the GC-FID method described in Appendix 1.

A GC-MS method was also validated by Dow AgroSciences, for use as a confirmatory method and for the analysis of formulations which give interference by GC-FID. The formulation samples used for the peer validation (above) were analyzed, together with a UL (300 g/l) formulation which was analyzed as 5 replicates on 2 days. MS detection was by HP 5973 MSD in EI mode, with the ion at m/z 322 used for quantification and the ion at m/z 202 for confirmation.

Results

GC-FID. Linearity, precision and recovery data were similar from both laboratories. Recovery from formulation blanks and chlorpyrifos TC, spiked with sulfotep at 0.3-4.1 g/kg was in the range 81-115%, indicating good accuracy. Linearity over a similar range was good, with r^2 values >0.998 . Results from analysis of the TC and EC samples are given in Table 1. An apparent slight and variable bias between the two laboratories seemed to be within the uncertainty (i.e. accuracy + precision) expected for determinations at the low concentrations involved.

GC-MS. Recovery, linearity, and precision of the GC/MS method were evaluated by Dow AgroSciences for the determination of sulfotep in the TCs and ECs analyzed for peer validation of the GC-FID method. Precision was also assessed for the determination of sulfotep in the Dow AgroSciences 300 UL, because the GC-FID method may give unreliable results due to interference from formulants in this product. The GC-MS method also provides an important means for confirmation of identity and quantity of the impurity.

Recovery at 0.37-1.8 g/kg from the Makhteshim EC and a Dow AgroSciences UL formulation blank was 94.4-100.9% and 93.5-97.0%, respectively, indicating good accuracy. Linearity was also good, with $r^2 >0.9999$. Precision of analysis of the UL formulation analyzed over 2 days was good (RSD 3.2%, $n=10$). In terms of accuracy and precision of analysis of TC and EC samples, the GC-MS and GC-FID methods were very similar (Table 1).

Table 1. Determination of sulfotep in TC and EC formulations (n=5).

Lab	TC 1		TC 2		EC 1		EC 2	
	mean, g/kg	RSD%	mean, g/kg	RSD%	mean, g/kg	RSD%	mean, g/kg	RSD%
GC-FID								
DAS	2.50	11.6	0.14	2.9	1.28	11.7	0.07	1.4
MCW	1.95	9.2	0.11	0.0	1.12	11.6	0.07	14.3
GC-MS								
DAS	2.60	15.0	0.13	3.1	1.15	11.7	0.06	2.5

Recommendation

The Meeting recommended that the GC-FID method should be accepted by FAO and WHO as validated for use in support of the specifications and that the GC-MS method may be used as an alternative, or for confirmatory purposes.

Reference

NAFST702 2004. Collaborative method validation for the analysis of sulfotep in technical product and formulations containing chlorpyrifos.

Note: the methods for determination of sulfotep in technical and formulated chlorpyrifos are available from the Pesticide Management Group of the FAO Plant Protection Service or can be [downloaded here](#).

CHLORPYRIFOS

EVALUATION REPORT 221/2002

Explanation

The data for chlorpyrifos were evaluated for review of existing FAO tentative specifications for TC and EC (AGP: CP/208, 1984) and existing WHO full specifications for TC and EC (WHO/SIT/21.R3 and WHO/SIF/36.R3, 1999).

Chlorpyrifos is not under patent.

The use of chlorpyrifos in public health has been reviewed by WHO (WHO 1997). In addition, chlorpyrifos EC has been successfully evaluated by the WHO Pesticide Evaluation Scheme as a mosquito larvicide (WHO 1980).

Chlorpyrifos was evaluated by the FAO/WHO JMPR in 1972, 1974 (residues only), 1975 (residues only), 1977, 1981 (residues only), 1982, 1983 (residues only), 1995 (residues only), 1999 (periodic review of toxicology) and 2000 (periodic review of residues). At the time of review it was under review by the European Commission and the US EPA.

Draft specifications and supporting data were provided independently by Dow AgroSciences and Makhteshim Chemical Works, in 2002.

Uses

Chlorpyrifos is a non-systemic organophosphorus insecticide, acting as a cholinesterase inhibitor, with contact, stomach and respiratory action. It is used in agriculture, horticulture, viticulture and forestry, in a wide range of crops, and in disease vector (mosquito) control and other public health applications. It is used for control of Coleoptera, Diptera, Homoptera and Lepidoptera in soil or on foliage, household pests (Blattellidae, Muscidae, Isoptera), mosquitoes (larvae and adults), and pests in animal houses.

Identity of the active ingredient

ISO common name

Chlorpyrifos (E-ISO, accepted)

Chemical name(s)

IUPAC: O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate

CA: O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate

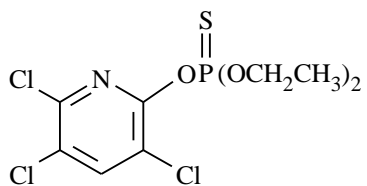
Synonyms

Chlorpyrifos (BSI, ANSI, ESA)

Chlorpyriphos ([m] F-ISO, JMAF)

Chlorpyriphos-éthyl ([m] France)

Structural formula



Molecular formula

C₉H₁₁Cl₃NO₃PS

Relative molecular mass

350.6

CAS Registry number

2912-88-2

CIPAC number

221

Identity tests

HPLC retention time (CIPAC 1C, 1985, 2028-2031), GC-MS.

Physico-chemical properties of pure chlorpyrifos

Table 1. Physico-chemical properties of pure chlorpyrifos

Parameter	Value(s) and conditions	Purity %	Method reference
Vapour pressure	2.546 x 10 ⁻³ Pa (1.91 x 10 ⁻⁵ mm Hg) at 25°C (Dow)	99.8	OECD 104/EPA D (equivalent to EEC A4)
	1.033 x 10 ⁻³ Pa (7.75 x 10 ⁻⁶ mm Hg) at 20°C, by extrapolation (Dow) 6.8 x 10 ⁻⁴ Pa at 25°C, by extrapolation (Makhteshim)	>99	OECD 104, gas saturation
Melting point, boiling point and/or temperature of decomposition	Melting point: 42.0°C (Dow)	99.8	EEC Method A1, OECD 102
	Melting point: 41.0 to 43.2°C (Makhteshim)	>99 *	OECD 102 (Fisher-Johns)
	Boiling point: None observed up to 400°C (Dow) Decomposition temperature: major exotherm over range 201 - 265°C (-190 Joules/g), minor exotherm 340 – 390°C (-82 Joules/g) (Dow)	99.8 99.8	EEC Method A2 EEC Method A1/A2 – DSC
Solubility in water	0.941 mg/l at 20°C (pH unknown) (Dow)	99.8	EEC Method A6/OECD 105
	0.588 mg/l at 20°C (pH not stated) (Makhteshim)	98.2	OECD 105 flask method
Octanol/water partition coefficient	log K _{ow} = 4.7001 at 20°C (Dow)	>98	EEC Method A8
	log P _{ow} = 4.76 at 25°C (Makhteshim)	>99	OECD 107 shake flask method
Hydrolysis characteristics	Half life in buffers at 25°C pH 5: 72 days pH 7: 72 days pH 9: 16 days (Dow)	Mixture of ¹⁴ C and 99.8	EPA Sub. N 161-1
	Half life in buffers at 30°C pH 4.0: 72 days pH 7.0: 40 days pH 9.0: 24 days (Makhteshim)	>99.5 *	EPA test method CS5000
Photolysis characteristics	Direct photo-transformation was observed in buffer solutions and river waters, under both natural and artificial lighting conditions. Approximate 50% conversion after 30-40 days was observed. (Dow)	¹⁴ C and 99.8	EPA Sub. N 161-1
Dissociation characteristics	Does not dissociate. Not determinable by titration, spectrophotometric or conductimetric methods, due to very low water solubility (Dow)	99.8	OECD Guideline 112

* 2004 footnote. The manufacturer stated that these data for purity of chlorpyrifos were incorrect. For melting point the correct value was 98.2% (not >99%) and for hydrolysis rate it was 99.5% (not >99.5%).

Chemical composition and properties of chlorpyrifos technical material (TC)

Table 2. Chemical composition and properties of technical chlorpyrifos

Manufacturing process, maximum limits for impurities ≥ 1 g/kg, 5 batch analysis data	Confidential information supplied and held on file by FAO and WHO. Mass balances were: (i) 99.0 to 99.5%, with no unknowns detected, although certain impurities were not fully characterized (Dow); (ii) 98.8 to 99.5%, with no unknowns detected, although certain impurities were not fully characterized (Makhteshim).
Declared minimum chlorpyrifos content	970 g/kg.
Relevant impurities ≥ 1 g/kg and maximum limits for them	sulfotep, maximum 3 g/kg.
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 of the TC and/or TK	38 to 45 °C (Dow). 41.5 to 43.5 (Makhteshim). Decomposition starts at temperatures above 200°C (Dow). Decomposes above 160°C (Makhteshim).

Toxicological summaries

Notes.

- (i) The proposers confirmed that the toxicological and ecotoxicological data included in the summary below were derived from chlorpyrifos having impurity profiles similar to those referred to in the table above.
- (ii) The conclusions expressed in the summary below are those of the proposers, unless otherwise specified.

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

Species	Test	Duration and conditions or guideline adopted	Result
Male and female rats	Oral	OECD 401	LD ₅₀ = 223 mg/kg bw (Dow)
Rats (Charles River CD) male female	Oral	OECD 401	LD ₅₀ = 221 mg/kg bw LD ₅₀ = 144 mg/kg bw (Makhteshim)
Male and female rats	Dermal	Meets OECD 402	LD ₅₀ = >2000 mg/kg bw (Dow)
Male and female rabbits	Dermal	OECD 402	LD ₅₀ = >5000 mg/kg bw (Dow)
Male and female rabbits	Dermal	OECD 402, EPA subdivn F 163.81-2	LD ₅₀ = >2000 mg/kg bw (Makhteshim)
NZW rabbits	Inhalation	Meets OECD 403	LC ₅₀ = >200 mg/m ³ (Dow)
Rats male female	Inhalation	EPA subdivn F 163.81-3, 4 h	LC ₅₀ = >4070 mg/m ³ LC ₅₀ = 2890 mg/m ³ (Makhteshim)

Species	Test	Duration and conditions or guideline adopted	Result
NZW rabbits	Skin irritation	OECD 404	Slight irritation observed which did not persist. Irritancy was less than trigger levels of classification. (Dow)
NZW rabbits	Skin irritation	EPA subdvn F 163.81-5	Mild irritant to the skin (Makhteshim)
NZW rabbits	Eye irritation	OECD 405	Slight irritation observed which did not persist. Irritancy was less than trigger levels of classification (Dow)
NZW rabbits	Eye irritation	EPA subdvn F 163.81-4	Moderate irritant to the eye (Makhteshim)
Guinea pigs	Skin sensitization	OECD 406 and EPA 81-6	Non-sensitizer (Dow)
Guinea pigs	Skin sensitization	EPA subdvn F 163.81-6	Non-sensitizer (Makhteshim)

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

Note. The data in Table 4 were presented by Dow but additional data on sub-acute to chronic toxicity were also presented to WHO by Makhteshim for the purposes of the 1999 FAO/WHO JMPR evaluation of toxicology.

Species	Test	Duration and conditions or guideline adopted	Result
Rat, F-34	Short term toxicity	13 week, RBC ChE main target organ/adverse effect	NOAEL = 0.1 mg/kg bw/d LOAEL = 1 mg/kg bw/d
Mouse, CD-1	Short term toxicity	13 week, brain ChE, body weight	NOAEL = 0.7mg/kg bw/d, male NOAEL = 1.3 mg/kg bw/d, female LOAEL = 7.1 mg/kg bw/d, male LOAEL = 13.5 mg/kg bw/d, female
Dog, beagle	Short term toxicity	13 week, RBC ChE main target organ	NOAEL = 0.1 mg/kg bw/d LOAEL = 1 mg/kg bw/d
Rat, F-344	Long term toxicity	2 years	NOAEL = 0.1 mg/kg bw/d LOAEL = 1 mg/kg bw/d Not carcinogenic
Mouse, CD-1	Long term toxicity	18 months, RBC ChE]	NOAEL = 0.7mg/kg bw/d, male NOAEL = 0.7 mg/kg bw/d, female LOAEL = 6.1 mg/kg bw/d, male LOAEL = 6.6 mg/kg bw/d, female Not carcinogenic
Dog, beagle	Long term toxicity	1 to 2 years, RBC ChE	NOAEL = 0.1 mg/kg bw/d LOAEL = 1 mg/kg bw/d

Species	Test	Duration and conditions or guideline adopted	Result
Rat, CD	Reproductive toxicity	not stated	NOAEL = 0.1 mg/kg bw/d, dam LOAEL = 1 mg/kg bw/d, dam NOAEL = 1 mg/kg bw/d, litter LOAEL = 10 mg/kg bw/d, litter No reproductive effect
Rat, CD	Teratology	not stated	NOAEL = 0.1 mg/kg bw/d, dam LOAEL = 3 mg/kg bw/d, dam NOAEL = 15 mg/kg bw/d, litter LOAEL = no value given, litter Dam, cholinergic signs & ↓ bw. Litter, ↑ post-implant loss
Rat, F-344	Acute neurotoxicity	not stated	NOAEL = 10 mg/kg bw/d LOAEL = 50 mg/kg bw/d Minimal transient signs of toxicity No neuropathological alterations.
Rat, F-344	Sub-chronic neurotoxicity	not stated	NOAEL = 1 mg/kg bw/d LOAEL = 5 mg/kg bw/d Perineal soiling

The 1999 JMPR concluded that chlorpyrifos is unlikely to pose a carcinogenic risk to humans.

Table 5. Human studies of toxicity

Study	Species	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Main target organ/ adverse effect
Acute oral	Human	1.0	m = - f = 2.0	RBC ChE
Acute dermal	Human	5.0	-	RBC ChE
			5.0 (x20 12h exposures) 25.0 (x3 12h exposures)	Plasma ChE
Sub-acute oral	Human	0.1	-	None

- = not determined.

The 1999 JMPR reaffirmed an ADI of 0-0.01 mg/kg bw. This was on the basis of a NOAEL of 1 mg/kg bw per day for inhibition of brain acetylcholinesterase activity in studies in rats, mice and dogs, using a 100-fold safety factor, and on a NOAEL of 0.1 mg/kg bw per day for inhibition of erythrocyte acetylcholinesterase activity in the study of human subjects exposed for nine days, using a 10-fold safety factor.

In addition, the 1999 JMPR allocated an acute reference dose of 0.1 mg/kg bw. This was on the basis of a study in which human volunteers received a single oral dose of chlorpyrifos, a NOAEL of 1 mg/kg bw for inhibition of erythrocyte acetylcholinesterase activity, and incorporating a safety factor of 10.

Table 6. Mutagenicity profile of the technical material based on *in vitro* and *in vivo* tests

Note. The data in Table 6 were presented by Dow but additional data on mutagenicity were also presented to WHO by Makhteshim for the purposes of the 1999 FAO/WHO JMPR evaluation of toxicology.

Species	Test	Conditions	Result
<i>In vitro</i> tests			
<i>S. cerevisiae</i> D3	Rec assay	Not stated	Negative
<i>B. subtilis</i> H17, M45	Rec-assay	20, 100, 200, 500, 1000 or 2000 µg/plate in DMSO	Negative
<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	Reverse mutation ^a	10, 50, 100, 500, 1000 or 5000 µg/plate in DMSO	Negative ^a
<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	Reverse mutation ^a	1, 3.162, 10, 31.62 or 100 µg/plate in DMSO	Negative ^{a c}
<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	Reverse mutation ^a	30, 100, 300, 3000 or 10,000 µg/plate in DMSO	Negative ^a
<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	Reverse mutation	Not stated	Negative
<i>E.coli</i> WP2	Reverse mutation	Not stated	Negative
<i>E coli</i> and <i>Bacillus subtilis</i>	Relative toxicity	Not stated	Negative
Chinese hamster ovary cells	CHO/HGPRT forward mutation ^a	10, 20, 25, 30, 40 or 50 µM	Negative ^a
Chinese hamster ovary cells	CHO/HGPRT forward mutation ^a	5, 10, 25, 50 or 75 µg/ml (-S9; 16 h exposure) 5, 10, 20, 30, 40 or 50 µg/ml (-S9; 16 h exposure) 30, 50, 100, 300 or 1000 µg/ml (+S9) in DMSO	Negative ^{a g}
Human lymphocytes (Laz-007)	Sister Chromatid Exchange ^a	0.02, 0.2, 2 or 20 µg/ml in ethyl alcohol	Negative ^{a h}
Chinese hamster ovary cells	Chromosomal aberration ^a	0.975, 1.47, 2.93, 4.89, 9.75, 14.7, 29.3, 48.9, 97.5 or 147 µg/ml (-S9, 19 h exposure) 1.56, 3.12, 5.2, 10.4, 15.6, 31.2, 52, 104 or 156 µg/ml (-S9, 10 h exposure) 9.75, 14.7, 29.3, 48.9, 97.5, 147 or 293 µg/ml (+S9, 19 h exposure) 1, 1.5, 3, 5, 10, 15, 30, 50 or 100 µg/ml, and 2.95, 4.95, 9.85, 14.8, 29.6, 49.4, 98.5 or 296 µg/ml (+S9, 10 h exposure)	Negative ^{a i j}
Rat hepatocytes	Chromosomal aberration ^a	16.7, 50, 167, 500, 1667 or 5000 µg/ml (harvested after 24 h) and 5, 16.7, 50 or 167 µg/ml (harvested after 24 h and 48h) in DMSO	Negative ^{a k}
Chinese hamster ovary cells	Sister Chromatid Exchange	1, 10 or 100 µg/ml in acetone	Negative
Rat hepatocytes in DMSO	Unscheduled DNA synthesis	1, 3.16, 10, 31.6 or 100 µM	Negative

Species	Test	Conditions	Result
<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	Reverse mutation ^a	0, 1, 3.16, 10, 31.6 or 100 µg/plate in DMSO	Negative ^a
Chinese hamster ovary cells	CHO/HGPRT forward mutation ^a	0, 3.5, 7, 8.8, 10.5, 14 or 17.5 µg/ml in DMSO	Negative ^{a l}
Rat lymphocyte	Chromosomal aberration ^a	16.7 to 5000 µg/ml in DMSO	Negative ^{a m}
Rat hepatocyte	Unscheduled DNA synthesis	1, 3.16, 10, 31.6 or 100 µM in DMSO	Negative
<i>In vivo</i> tests			
Mouse (CD-ICR BR)	Micronucleus ^b (marrow cells)	0, 7, 22 or 70 mg/kg, PO in corn oil	Negative ^b
Chick embryos (Cornell K-strain eggs)	Chromosomal aberration ^b	1.11, 11.1, 111, 1110 or 2220 µg/embryo	Negative ^{b n}
Bovine blastocysts	Chromosomal aberration ^b	Not stated	Negative ^b
Mouse (CD-1 (ICR) BR)	Micronucleus ^b (marrow cells)	90 mg/kg, PO in corn oil	Negative ^b
Mouse (CD-1)	Micronucleus ^b (marrow cells)	0, 7, 22, 70 or 90 mg/kg, PO in corn oil	Negative ^b

Positive control substances were used in all assays and gave expected results.

DMSO = dimethyl sulfoxide

^a Both with and without metabolic activation.

^b With metabolic activation.

^c Cytotoxicity observed at 100µg/plate (TA 100, 1535, 1537, 1538) with precipitation. TA98 test material precipitation in the absence of toxicity.

^d Test material cytotoxic at 10µg/plate and above.

^e Dose-related increases in revertant frequency (2-fold or greater than controls) seen in all tester strains, ± S9. These increases were always lower than positive controls (by 2- to 50-fold) and were not statistically significant.

^f Increase in revertants at all dose levels, -S9, in TA1537 only. No dose-response relationship demonstrated.

^g Test material toxic at 50µg/ml in one assay -S9.

^h At 2 and 20µg/ml, the SCE frequency was statistically significantly different to controls, but was not double the control frequency.

ⁱ -S9: cytotoxic at highest doses. Increase in gaps only at 52µg/ml (10 h exposure). No increases in other aberrations.

^j +S9: cytotoxic at 15µg/ml (10 h incubation). In one 10 h assay, there was a significant increase in cells with aberrations (including gaps) at 3 and 10µg/ml. Incidence of aberrations (excluding gaps) not statistically significant and not dose-dependent. In repeat 10 h assay, no increase in incidence of aberrations.

^k Test material cytotoxic at 500µg/ml and above (-S9) and 167µg/ml and above (+S9) in the first assay. No mitotic index measurable at 50µg/ml (-S9) or 167µg/ml (±S9).

^l Test material precipitated at 10.5, 14.0 and 17.5µg/ml, ±S9.

^m Test material cytotoxic at 50µg/ml and above, ±S9.

ⁿ Increases in mortality at 1110 and 2220µg/embryo.

Chlorpyrifos was not genotoxic in a range of studies *in vitro* and *in vivo*. The 1999 JMPR concluded that chlorpyrifos is not genotoxic.

Table 7. Ecotoxicology profile of the technical material

Note. The data in Table 7 were presented by Dow but additional data on ecotoxicology were also presented to WHO by Makhteshim for the purposes of the 1999 FAO/WHO JMPR evaluation of toxicology.

Species	Test	Duration and conditions	Result
House sparrow	Acute oral toxicity	US EPA 71-1	LD ₅₀ = 122 mg/kg
Mallard duck Bobwhite quail	Short term dietary toxicity	Meets OECD 205 Meets OECD 205	LC ₅₀ = 180 ppm LC ₅₀ = 423 ppm
Mallard duck Bobwhite quail	One generation reproductive toxicity	Not stated	NOEC = 25 ppm NOEC = 125 ppm
Roach	Acute toxicity	96 hours, conditions not stated	96 h LC ₅₀ 250 µg/l
Ide (Orfe)		96 hours, conditions not stated	96 h LC ₅₀ 10 µg/l
<i>Daphnia magna</i>		48 hours, conditions not stated	48 h LC ₅₀ 1.7 µg/l
Green alga (unspecified)		72 hours, conditions not stated	72 h EC ₅₀ 580 µg/l
Midge (unspecified)		10 days, conditions not stated	10 d EC ₅₀ 383 µg/kg sed.
Amphipod (unspecified)		10 days, conditions not stated	10 d EC ₅₀ 399 µg/kg sed
Rainbow trout <i>Daphnia magna</i>	Chronic toxicity	21 days, conditions not stated	21 d LC ₅₀ ca 2 µg/l NOEC 0.51 µg/l
		21 days, conditions not stated	21 d LC ₅₀ 0.06 µg/l NOEC 0.056 µg/l
Fathead minnow	Two-generation life-cycle	Not stated	NOEC 0.57 µg/l
Honey bees	Acute toxicity	Not stated	Oral LD ₅₀ 0.36 µg/bee Contact LD ₅₀ 0.07 µg/bee
Coccinellidae Carabidae Staphylinidae Tenebrionidae Neuroptera Spiders Hymenoptera	Topical application	Not stated	ca 0.3 µg/adult, 0.1 µg/larvae "Harmful" (IOBC) "Harmful" (IOBC) 96 h ED ₅₀ ca 60 µg/kg bw ca 0.02 µg/adult, 0.05 µg/larvae EC ₁₀₀ 720 ppm residue EC ₉₀ 10 ppm residue
Earthworms (unspecified) Technical 48%EC	Acute toxicity	14 days, conditions not stated	14 d LC ₅₀ 210 ppm
		14 days, conditions not stated	14 d LC ₅₀ 313 ppm

Species	Test	Duration and conditions	Result
Soil micro-organisms			
Effects of (unspecified) formulation on:			
Dehydrogenase activity		Not stated	Unaffected at 1.5 x field rate, transient inhibition at 5 x field rate
Respiration		Not stated	Unaffected at 5 x field rate
Nitrogen turnover		Not stated	Mainly unaffected at 5 x field rate

Chlorpyrifos is not persistent in soil and leaching is not significant. Therefore there is negligible risk to following crops or of groundwater contamination. Volatilization is a significant dissipative process in the environment although, once in the vapour phase, chlorpyrifos is short-lived. Transport to surface water *via* spray drift poses a risk to aquatic species, although the duration of exposure is brief because chlorpyrifos dissipates in microbially active natural water systems with a half-life of less than one week. The compound will also tend to migrate to sediment.

Health risks for avian and mammalian species following the consumption of treated vegetation and contaminated insects are considered to be low. The slightly higher long-term risk indicated for insectivorous species and birds grazing on treated grassland is not likely to arise due to:-

- (i) the high acute toxicity of chlorpyrifos to insects, preventing residue build-up in this food source;
- (ii) the generally low persistence of chlorpyrifos in the environment; and
- (iii) the rapid decline of residues in species forming the diet.

Aquatic organisms (fish and invertebrates) are potentially at risk, especially in the event of a direct overspray to static water bodies. However the introduction of buffer zones appropriate to each crop should ensure that aqueous concentrations remain below the environmentally acceptable concentration (EAC) of 1 µg/L, even in the event of multiple applications. Although chlorpyrifos is fat-soluble, the rates of biotransformation and excretion of metabolites largely mitigate bioconcentration. The risks to algal species, earthworms, soil micro-organisms and sewage bacteria are considered to be low, even in worst-case scenarios, without taking into consideration the rapid dissipation processes that occur in the environment.

Chlorpyrifos is extremely toxic to honeybees and beneficial insects. However, it has no growth inhibitory activity and the effects of treatment are relatively short-lived. Most beneficial insect populations recover quite rapidly and label restrictions on the time of application are intended to minimise the risks to honeybees.

Data from a number of field studies confirm that effects on earthworm population fecundity and viability are minimal at field use rates.

Soil microbiological processes are generally unaffected by field use of chlorpyrifos.

Chlorpyrifos was evaluated by the WHO/PCS and by the FAO/WHO JMPR in 1999. The estimated acceptable daily intake (ADI) for humans was set at 0 to 0.01 mg/kg bw and the

acute reference dose (acute RfD) at 0.1 mg/kg bw. The WHO/PCS hazard classification of chlorpyrifos is “Moderately hazardous, Class II”.

Formulations and co-formulated active ingredients

The main formulation types available are EC, CS and UL. Other formulation types are produced (GR, WP, WG, DP) for various markets. UL formulations are used principally for locust control.

Formulations of the same type but intended for use in agriculture or public health are not identical and should not be used interchangeably, but they share basic quality standards and thus product quality can be controlled through common specifications.

Chlorpyrifos may be formulated alone or co-formulated with other active ingredients, such as pyrethroid insecticides.

Chlorpyrifos formulations are registered and sold in many countries throughout the world.

Methods of analysis and testing

The analytical method for the active ingredient (including the HPLC retention time identity test) is a full CIPAC method (CIPAC 1C). Chlorpyrifos is determined by internal standard reversed-phase LC, using UV detection at 300 nm. The internal standard used is 1,4 dibromonaphthalene.

Extension of the CIPAC method to UL formulations was adopted as provisional by CIPAC in 2002, following submission of validation data by Dow. Validation data for extension of the CIPAC method to CS formulations were not provided. Validated methods for the determination of “free” chlorpyrifos and its “release rate” in the CS formulations were not available.

An alternative analytical method for chlorpyrifos, based on capillary GC-FID and external standardization is used routinely by Makhteshim. A variant of this, which employed octadecane as an internal standard, was also used and supported by validation data (Makhteshim report R-8197, 1995). Although these methods appear to be practical alternatives to the HPLC method, they have not been validated by collaborative study and the HPLC method must therefore be considered to be the referee method for support of the specifications.

The methods for determination of impurities were based on LC-UV or GC-FID, utilised according to the nature of the impurity.

The method of analysis for the relevant impurity, sulfotep, is based on GC-FID analysis with external standardization but has not yet been peer-validated*.

Test methods for determination of physico-chemical properties of the technical active ingredient were based on OECD, EPA and EEC procedures, while those for the formulations were CIPAC, as indicated in the specifications, or OECD, EPA or EEC.

* The method was peer validated in 2004, see evaluation report 221/2004.

Physical properties

The limits proposed for physical properties of the EC and UL formulations, and the methods for testing them, comply with the requirements of the FAO Manual (5th edition).

No methods or limits were provided for “free” chlorpyrifos, “release rate” and “freeze/thaw stability” of CS formulations.

Containers and packaging

No special requirements for containers and packaging have been identified.

Expression of the active ingredient

The active ingredient is expressed as chlorpyrifos, in g/kg (or alternatively for liquid formulated products, in g/l). In cases of dispute, the content is expressed as g/kg.

Appraisal

Chlorpyrifos is an active ingredient for which tentative FAO and full WHO specifications were accepted in 1984 and 1999, respectively. Review of the specifications was proposed independently by three companies. Chlorpyrifos is not subject to patent and has been widely used in agriculture and certain vector control and public health applications for many years.

Chlorpyrifos is a slightly volatile, non-systemic organophosphorus insecticide of low water-solubility, classified as fat-soluble by the FAO/WHO JMPR. Although hydrolysis and photolysis in solution is slow, it is of short persistence in animals, plants, soil and water and therefore bioaccumulation is unlikely.

The proposed minimum purity of the TC, 970 g/kg, is significantly higher than that of the 1984 FAO tentative specification, which was 940 ± 20 g/kg (in effect a minimum of 920 g/kg) and that of the 1999 WHO full specification, which was for a minimum of 920 g/kg.

Confidential information on the manufacturing processes and impurity profiles was provided by the two proposers. The impurity data presented by Dow and Makhteshim were essentially the same as those presented by the companies to the UK authorities for the purposes of registration.

The content of the impurity, sulfotep, was not limited by the 1984 FAO tentative specification and the 1999 WHO full specification. This compound has higher acute toxicity than chlorpyrifos, by oral, dermal and inhalation routes. The data from one proposer indicated that it can exceed 1 g/kg in the TC in current production, whereas data from the other proposer indicated that it does not (<0.8 g/kg). The meeting agreed that sulfotep is a relevant impurity.

Chlorpyrifos is a phosphorothionate and other compounds of this type are known to have the potential to isomerize in storage, to form a potentially more toxic S-alkyl isomer. A manufacturing limit of 5 g/kg for “*iso*-chlorpyrifos” (O,S-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate) was based on a hypothetical 100% yield from the maximum limit for the precursor impurity in the raw materials used to manufacture chlorpyrifos. Dow provided data for *iso*-chlorpyrifos to show that, although it increased in concentration during storage of chlorpyrifos in the presence of air and at very high temperatures (90-110°C), no increase was detectable during 10 days at 60°C. The higher temperatures are such that they can never be attained in storage practice and are unrealistically extreme as a test of accelerated storage stability. It is clear that *iso*-chlorpyrifos is unlikely to increase in

concentration during the CIPAC test at 54°C for 14 days and, for practical purposes, this is normally considered to be the most stringent test of storage stability. No acute toxicity data were available for *iso*-chlorpyrifos. Dow considered that its toxicity would be comparable to that of chlorpyrifos itself. The opinion of WHO/PCS was that *iso*-chlorpyrifos could be more toxic than chlorpyrifos but that it was unlikely that a significant increase in overall toxicity would occur even if *iso*-chlorpyrifos occurred at the highest concentration theoretically achievable (which is not attained in practice). Given the evidence that the concentration of *iso*-chlorpyrifos is normally <1 g/kg and does not increase during storage, the meeting concluded that it should not be regarded as a relevant impurity.

Phosphorothionates also generally have a tendency to undergo air oxidation during storage, to form the corresponding oxons. The oxons are usually more potent inhibitors of acetylcholinesterase, although they are not necessarily more toxic, because they may also be metabolized more rapidly. As in the case of *iso*-chlorpyrifos, a manufacturing limit of 2 g/kg for “chlorpyrifos oxon” (O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphate) was based on a hypothetical 100% yield from the maximum limit for the precursor impurity in the raw materials used to manufacture chlorpyrifos. Dow provided data to show that chlorpyrifos oxon can increase in concentration during storage of chlorpyrifos in the presence of air at very high temperatures (90-110°C) and it seems possible that, at these very high temperatures, a free radical mechanism is initiated. In contrast, no increase in the concentration of chlorpyrifos oxon was detectable during 10 days storage at 60°C and thus it is unlikely in practice that its concentration would increase during storage, or during the CIPAC test at 54°C for 14 days. Dow determined the acute oral LD₅₀ of chlorpyrifos oxon to be 300 mg/kg bw, indicating that the oxon is not more toxic than chlorpyrifos. On the basis of this evidence, the meeting concluded that chlorpyrifos oxon is not a relevant impurity.

On the basis of the most complete series of related data for toxicology, ecotoxicology and stability, the purity and impurity data presented by Dow were considered by the meeting to form the reference profile. The meeting concluded that the Makhteshim TC was equivalent to that of Dow.

The analytical method for chlorpyrifos is a full CIPAC method and an extension of the method to UL was adopted as provisional by CIPAC in 2002. The analytical method for the relevant impurity, sulfotep, has not yet been peer-validated.

The proposed specification for TC was in accordance with the requirements of the FAO Manual. A limit for water was specified in the previous FAO and WHO specifications but, because the water content is limited by its low affinity for chlorpyrifos, the clause is not included in the new specification.

The proposed specifications for EC and UL were also in accordance with the requirements of the Manual. The limit for acidity in the UL specification is higher than of the corresponding clause for the TC, due to the formulants. As it has been demonstrated that the concentration of sulfotep does not increase during storage, it is not necessary to determine its concentration after the test of stability at elevated temperature.

The meeting considered the problems of specifying kinematic viscosity and volatility in chlorpyrifos UL formulations, though recognising that the issues also applied to UL formulations of other pesticides. The appropriate kinematic viscosity is very dependent upon the application equipment used and therefore this parameter, though important, cannot be considered in isolation within the specification. The meeting concluded that the clause for kinematic viscosity should be removed from the specification but that its importance would be recognised by retention of a footnote to the specification. The

meeting also considered the specification of volatility. The impact of evaporation, mainly of solvent, from spray droplets inevitably increases the potential for spray drift and increases both the risks of deposition beyond the target area and lower than expected deposition on the target. However, loss by evaporation does not depend solely on the volatility of the solvent and drift does not depend solely upon droplet size. To a greater or lesser extent, both are also dependent upon air temperature, air movement, initial droplet size, and the sedimentation distance. So, for example, under otherwise identical conditions, loss by volatilization may be more important for an aerial application than for an equivalent ground-based application, because of the difference in the height of fall and the difference in air movement as the ground is approached. The meeting concluded that manufacturers should be urged to develop a meaningful general test for volatility, together with supporting data to enable the results of the test to be interpreted for a range of applications. In the absence of the test and supporting information, the meeting considered that it would be inappropriate and potentially misleading to include a clause and limit for volatility in specifications for UL formulations.

The meeting considered the need for inclusion in the specifications of a note cautioning against misuse, in that a specification applying to both agricultural and public health products should not be construed as a recommendation that an agricultural product could be used equally for public health purposes, or *vice versa*. Concern was expressed that some manufacturers charge much more for public health products that are notionally similar to those used in agriculture. The meeting acknowledged the issue but concluded that pesticides must be used in accordance with the label recommendations. The meeting agreed that the standard disclaimer, which prefaces all specifications, is sufficient to make this clear to those who use specifications.

Recommendations

The meeting recommended that the proposed specifications for TC and EC should be adopted by FAO and WHO, and that the proposed specification for UL should also be adopted by FAO, subject to satisfactory peer validation of the method for determination of the relevant impurity, sulfotep* (TC, EC, UL).

The meeting also recommended that suitable test methods and limits for clauses for active ingredient content; the relevant impurity, free active ingredient content; release rate; and freeze/thaw stability should be developed to support development of a specification for CS formulations of chlorpyrifos.

* The method was peer validated in 2004, see evaluation report 221/2004.

References

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Appendix 1

to FAO/WHO evaluation report 221/2004

Methods for the determination of sulfotep in chlorpyrifos TC, EC and UL

(adapted from Dow AgroSciences method DAS-AM-01-058)

Outline

Sulfotep (*O,O,O',O'*-tetraethyl dithiopyrophosphate, Cas No. 3689-24-5) is dissolved/extracted from the sample with *iso*-octane. In the case of EC and UL, the extract is shaken with water to remove water-solubles. The separated *iso*-octane is filtered and an aliquot is analyzed by GC-FID or GC-MS, using external standards of sulfotep in chlorpyrifos

Standard preparation

Stock solution. Weigh accurately, to 0.1 mg, approximately 30 mg of sulfotep analytical standard into a 10 ml volumetric flask. Dilute to volume with *iso*-octane at 20°C to produce a concentration of 3.0 µg/µl.

Calibration standard. Transfer, accurately, 100 µl of stock solution (300 µg sulfotep) into a 10 ml volumetric flask, containing approximately 0.1 g of chlorpyrifos analytical standard and dilute to volume with *iso*-octane at 20°C. Mix thoroughly, to produce a solution containing chlorpyrifos at 10 µg/µl and sulfotep at 30 ng/µl, a concentration equivalent to sulfotep at 3 g/kg of chlorpyrifos). It is also appropriate to confirm that the chlorpyrifos analytical standard being used does not contain any sulfotep peaks. Where sulfotep is present in the chlorpyrifos analytical standard then appropriate correction factors should be made.

If required to demonstrate linearity of detector response, or to determine a wider range of impurity concentrations, additionally prepare calibration standards containing the same concentration of chlorpyrifos but with higher and lower concentrations of sulfotep.

Sample preparation

TC. Weigh, to the nearest 0.1 mg, 0.3 g of the TC into an appropriately sized screw top vial. Using a suitable dispenser, accurately add 29.7 ml *iso*-octane and mix thoroughly to dissolve the material. This is to produce an *iso*-octane solution containing chlorpyrifos at approximately 10 µg/µl.

EC or UL. Accurately weigh (to the nearest 0.1 mg) sufficient sample material to contain approximately 0.3 g chlorpyrifos into an appropriately sized screw top vial. Using suitable dispensers, add 10 ml distilled water and, accurately, (30 – x) ml *iso*-octane (where x = quantity of sample weighed, to the nearest 0.1 g) to the sample, cap the vial and shake the mixture vigorously for 20 min. This is to produce an *iso*-octane solution/extract of the sample, containing chlorpyrifos at approximately 10 µg/µl.

Centrifuge the mixture in a bench-top centrifuge at about 2000 rpm for 5 min to ensure good separation of the layers, carefully remove an aliquot of the top (*iso*-octane) layer with a pasteur pipette and filter it through a 25 mm diameter syringe filter (fitted with a GF/0.45 µm GHP membrane) into a vial for GC analysis.

Determination

Inject aliquots of calibration standard solution until the peak areas from successive injections differ by no more than 2%. Inject the sample extracts and calibration solutions in the sequence: calibration, sample 1, sample 2, calibration, sample 1, sample 2, calibration, etc. Ensure that peak areas from successive injections of the calibration standards do not differ by more than 2%.

Instrumentation and conditions (typical)

GC-FID procedure

Instrument	HP 6890 gas chromatograph or equivalent
Column	HP-1 (100% dimethylpolysiloxane), 30 m x 0.53 mm x 2.65µm film
Carrier gas	Nitrogen
Detector	Flame ionization detector at 275°C
Detector gases	Air 300 ml/min
	Hydrogen 30 ml/min
	Nitrogen 25 m/min
Carrier flow	8 ml/min
Injection temperature	220°C
Oven temperature program	70°C for 0.3 minutes, ramp 10°C/min to 110°C, hold 0.1 min, ramp 12°C/min to 180°C, hold 3 min, ramp 12°C/min to 260°C, hold 5 min.
Injection volume	1 µl splitless
Equilibration time	3 min
Total run time	25 min
Typical retention time, sulfotep	15.8 min
Integration	peak area

GC/MSD procedure

Instrument	HP 6890 gas chromatograph or equivalent
Column	DB-35MS (35% phenyl, 65% dimethylpolysiloxane), 30 m x 0.25 mm x 0.25 µm film
Carrier gas	Helium
Detector	HP 5973 Mass Selective Detector or equivalent
	Ionisation mode Electron Impact (EI)
	Ions monitored (SIM)
	Quantification m/z 322
	Qualifier m/z 202
	Source temperature 280°C
Carrier flow	1.2 ml/min
Injection temperature	220°C
Oven temperature program	70°C for 0.3 min, ramp 10°C/min to 110°C, hold 0.1 min, ramp 12°C/min to 180°C, hold 3 min, ramp 12°C/min to 260°C, hold 5 min
Injection volume	1 µl, splitless
Equilibration time	3 min
Total run time	25 min
Typical retention time, sulfotep	14.7 min
Integration	peak area

Calculations

1. Calculate the concentration (mg/ml) of sulfotep in the calibration solution, using the following formula.

$$C(i, \text{cal}) = \frac{[W_i \times (P/100)]}{10 \text{ ml}} \times \frac{0.10 \text{ ml}}{10 \text{ ml}}$$

where:

- $C(i, \text{cal})$ = concentration (mg/ml) of sulfotep in the calibration solution;
- W_i = weight (mg) of sulfotep in the stock solution;
- P = purity of sulfotep standard, %;
- 10 ml = volume of stock solution prepared;
- 0.10 ml = volume of stock solution added to the calibration solution;
- 10 ml = volume of calibration solution prepared.

2. Calculate the response factor (RF) for sulfotep, using the following formula.

$$RF(i) = \frac{C(i, \text{cal})}{\text{Area}(i, \text{cal})}$$

where:

- $RF(i)$ = response factor of sulfotep;
- $C(i, \text{cal})$ = concentration (mg/ml) of sulfotep in the calibration solution;
- $\text{Area}(i, \text{cal})$ = peak area obtained for sulfotep in the calibration solution.

3. Calculate concentration (g/kg) of sulfotep in the samples, using the following formula.

$$\text{sulfotep g/kg} = \frac{(\text{Area}(\text{sample}) \times RF(i))}{(W(\text{sample})/25\text{ml})} \times 1000$$

where:

- $RF(i)$ = response factor of the sulfotep in calibration solution;
- $\text{Area}(\text{sample})$ = peak area obtained for sulfotep in the sample solution;
- $W(\text{sample})$ = sample weight in mg;
- 25 ml = volume of sample solution prepared.

4. Calculate the sulfotep concentration in formulations as g/kg of chlorpyrifos, using the following formula.

$$\text{sulfotep, g/kg of chlorpyrifos} = \frac{\text{sulfotep, g/kg}}{\text{chlorpyrifos, g/kg}} \times 1000$$

where:

- sulfotep, g/kg = concentration of sulfotep in the formulation;
 - chlorpyrifos, g/kg = concentration of chlorpyrifos in the formulation.
-

Method DAS-AM-01-058

Sulfotepp impurities in Chlorpyrifos EC formulations

A. ABSTRACT

Method DAS-AM-01-058 has been validated for the analysis of the relevant impurity sulfotepp in chlorpyrifos EC formulations. Ranges of Chlorpyrifos EC products containing 8 different surfactants were evaluated.

Linearity, system and method precision, accuracy, and interferences were assessed and found to be acceptable.

Sulfotepp is determined using gas chromatography with an HP-1 megabore column and FID detection. Quantification is by peak area measurements using an external standard calculation.

The linearity of the method has been determined in the range 6 to 202 $\mu\text{g/mL}$ and is demonstrated by an R^2 of 0.998 for sulfotepp.

Method precision was determined by replicate analyses of one batch of chlorpyrifos EC. The precision at the 95 % confidence level was calculated as ± 2.0 %.

System precision was determined by replicate injection of a precision sample solution. The precision at the 95 % confidence level was calculated as ± 2.9 %.

Recoveries were assessed over the range 0.05 to 0.39 %w/w sulfotepp in the formulation. All recoveries for sulfotepp were within the limits 70-120% with an average recovery of 102.5%.

The method is applicable to the determination of sulfotepp at concentrations of approximately 0.05 to 0.39 % by weight in various chlorpyrifos formulations containing a range of surfactants.

B. SUMMARY AND INTRODUCTION

(1) Scope

A method has been validated for the determination of sulfotepp in chlorpyrifos EC formulations. Linearity, system precision, method precision, interferences, and accuracy were assessed. The procedure followed was based on Dow AgroSciences (NZ) Ltd, SOP/FORMS/04, "Validation of Methods of Analysis for Formulations".

(2) Principles of the analytical procedure

Sulfotepp concentrations are determined using gas chromatography, with splitless injection, nitrogen as the carrier gas and flame ionisation detection. Quantitation was by peak area measurement using external standard calculations.

C. MATERIALS AND METHODS

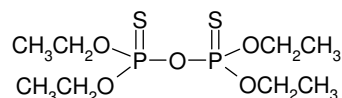
(1) Equipment

- i) Balance: capable of weighing to 0.1 mg, Mettler AT261 or equivalent
- ii) Agilent 6890 gas chromatography system consisting of a split/splitless injector, flame ionisation detector (FID), autosampler, and HP Chemstation (Rev. A.06.03 [509]) chromatographic software. Supplied by Agilent Technologies, Wellington, New Zealand, or equivalent.
- iii) Column: 30m x 0.53mm i.d capillary column coated with 2.65 µm film thickness of HP-1 (cross-linked dimethylpolysiloxane)
- iv) Dispensors: capable of dispensing a volume of 24.4 mL, Brand Dispensette 5-50 mL, or equivalent
- v) Normal laboratory volumetric glassware
- vi) Disposable syringes: 3 ml volume, Luer lock, available from Biolab, Auckland, New Zealand, or equivalent
- vii) Syringe filters: 25 mm, 0.45 µm pore size, GF/0.45 µm GHP Membrane, available from Life Technologies, Auckland, New Zealand

(2) Reagents and Standards:

(i) Reference Items:

Reference Item:	Sulfotepp analytical standard
Chemical Name:	O,O,O',O'-tetraethylthiodiphosphate
Molecular Structure:	



Molecular Weight:	322 g/mol
Reference Number:	TSN 101227

Assay: 98.6 %
Recertification Date: 16th August 2005
Storage Conditions: Freezer

- (ii) Solvents
Isooctane, Mallinckrodt HPLC grade, or equivalent.
- (3) Analytical Procedure:
- a) Preparation of calibration solutions:
- i) Prepare a sulfotepp stock solution by weighing (and recording to the nearest 0.1 mg) approximately 35 mg of sulfotepp analytical standard into a 10 mL volumetric flask. Dissolve in and dilute to volume with isooctane at 20°C. Sonication may be used to aid in dissolution.
 - ii) Prepare the calibration solution by transferring 60 µL of the stock solution into a 10 mL volumetric flask containing approximately 0.28 grams of chlorpyrifos Analytical Standard. Dissolve in and dilute to volume with isooctane at 20°C and analyse using the conditions given in Section 4.
- b) Preparation of sample solutions:
- i) Weigh and record (to the nearest 0.0001g) approximately 0.63 g of chlorpyrifos EC formulation into an appropriately sized sample glass vial.
 - ii) Add 10 mL of distilled water and 24.4 mL of isooctane to the sample using dispensers and shake vigorously for 20 minutes.
 - iii) Centrifuge at 2000 rpm for 5 minutes and filter an aliquot of the top organic layer of the prepared solution through a 0.45 µm syringe filter: 25 mm diameter with GF/0.45 µm GHP membrane directly into an autosampler vial.
 - iv) Analyze single aliquots of each solution using the conditions given in Section 4.
- (4) Instrumentation
- (i) Description
- An Agilent 6890 gas chromatography system consisting of a split/splitless injector, flame ionisation detector (FID), autosampler, and HP Chemstation (Rev.A.06.03 [509]) chromatographic software was used for chromatographic analyses during this method validation.
- (ii) Chromatographic Conditions
- The following chromatographic conditions were used for the method validations:

Instrument	HP 6890 Gas Chromatograph
Column:	HP-1, 30 m x 0.53 mm x 2.65µm film
Carrier gas	Nitrogen
Detector	Flame Ionisation Detector at 275 °C
Detector Gases - Air	300 mL/minute
- Hydrogen	30 mL/minute
- Nitrogen	25 mL/minute
Carrier flow	8 mL/min
Injection temperature	220°C
Oven temperature program	70 °C for 0.3 minutes, ramp 10 °C / minute to 110 °C, hold 0.1 minutes, ramp 12 °C / minute to 180 °C , hold 3 minutes, ramp 12 °C / minute to 260 °C, hold 5 minutes
Injection volume	1 µL
Equilibration time	3 minutes
Total run time	25 minutes
Typical Retention Time	
Sulfotepp	15.8 minutes
Quantitation	peak area

(iii) Analysis Procedures

Inject a single aliquot of the calibration solution and calculate the response factor using the equations given in section 8.

Inject a maximum of four sample solutions and calculate the weight % sulfotepp in the samples using the equations given in section 8.

(5) Interferences:

(i) Solvents

Isooctane and isooctane after water extraction were filtered through the 0.45 µm filter and showed no interference at the retention time of interest.

(ii) Labware

All glassware was washed using standard dishwashing liquid, rinsed with acetone and then dried. Glassware washed using this procedure was screened for interference as part of the isooctane check as described in (i) above.

(iii) Inerts Components

If the aqueous partition stage of sample preparation is not used then some surfactant inerts may show interfering peaks at or close to the retention time of sulfotepp on chromatographic system. The water extraction stage removed any surfactant interfering peaks and still yielded acceptable recoveries.

This method has been validated for Dow AgroSciences Chlorpyrifos EC formulations containing proprietary surfactants and is therefore only suitable for

these formulations without further validation of specificity. Contact Dow AgroSciences to confirm suitability.

(6) Confirmatory techniques:

Retention time comparisons between sulfotepp in standard and sample solutions was used as analyte confirmation for the method validation.

(7) Time Required for analysis:

A run time of 25 minutes is sufficient for the analysis of the compound of interest.

(8) Methods of Calculation

(i) Calculation of concentration (mg /mL) of sulfotepp in the calibration solution:

$$C(i, cal) = \frac{[W_i \times (P/100)]}{10\text{mL}} \times \frac{0.06\text{ mL}}{10\text{ mL}}$$

where

$C_{(i,cal)}$ = the concentration (mg/ml) of sulfotepp in the calibration solution

W_i = weight (mg) of sulfotepp in the stock solution

P = Purity of the impurity standard, expressed as a percentage

10 mL = the volume of stock solution prepared

0.06 mL = the volume of stock solution added to the calibration solution

10 mL = the volume of calibration solution prepared

(ii) Calculation of external standard response factor (RF) for sulfotepp:

$$RF(i) = \frac{C(i, cal)}{Area(i, cal)}$$

where

$RF_{(i)}$ = response factor of sulfotepp

$C_{(i,cal)}$ = the concentration (mg/ml) of sulfotepp in the calibration solution

$Area_{(i,cal)}$ = peak area obtained for sulfotepp in the calibration solution

(iii) Calculation of weight % concentration of sulfotepp in the samples:

$$\% w/w = \frac{(Area(sample) \times RF(i))}{(W(sample)/25\text{mL})} \times 100\%$$

where

$\% w/w$ = the weight percent of sulfotepp in the sample

$RF_{(i)}$ = response factor of the sulfotepp in calibration solution

$Area_{(sample)}$ = peak area obtained for sulfotepp in the sample solution

$W_{(sample)}$ = sample weight in mg

25 mL = the volume of sample solution prepared

D. RESULTS AND DISCUSSION

Results reported here are calculated by spreadsheet and may vary slightly from those calculated by hand.

(1) Accuracy

Accuracy was assessed by analysis of eleven recovery samples over the formulation concentration range 0.05 % to 0.39 %. All recoveries were within the limits of 70-120 %. The combined average recovery was 102.5 % for the eleven preparations. Recovery data is listed in Table III.

Three recovery samples were prepared using an inert mix of EC (1) and chlorpyrifos analytical standard, TSN 100227, fortified with a solution of sulfotepp in isooctane at levels ranging from 0.05 % to 0.16 %. Five recovery samples were prepared using an inert mix of 480EC(1) and chlorpyrifos technical fortified with a solution of sulfotepp in isooctane at levels ranging from 0.21 % to 0.39 %. Three recovery samples were prepared using an inert mix of 480EC (2) (containing Surfactants known to give interference peaks) and chlorpyrifos analytical standard fortified with a solution of sulfotepp in isooctane at levels ranging from 0.05 % to 0.15 %. The samples were prepared for analysis according to the sample preparation details in section C above.

(2) Precision

System precision was measured by injection of the same sample ten times. The measured system precision was calculated using the Students T-Test at the 95% confidence level as ± 2.9 %. System precision data is listed in Table I.

Method precision was measured by preparation and injection of ten samples on one day, and five samples on the following day using the conditions described in section C above. The measured method precision was calculated using the Students T test at the 95% confidence level as ± 2.0 %. Method precision data is listed in Table II.

The Students T-Test is listed below for analysis of 15 samples:

Precision = 2.15 x Relative Standard Deviation

(3) Linearity

The detector response was found to be linear for sulfotepp in the following concentration ranges:

	Linear Range
Sulfotepp	6 $\mu\text{g/mL}$ to 202 $\mu\text{g/mL}$

Linearity samples were prepared by dilution of a stock calibration solution prepared in isooctane to contain approximately 200 $\mu\text{g/mL}$ sulfotepp. Linearity data for sulfotepp is presented in Figure 1.

(4) Limits of Detection and Quantification

The validated limit of quantification for this method was 0.05 % for sulfotepp in the formulation. This limit is determined from the linearity range analysed, in conjunction with accuracy and precision results discussed in this section.

(5) Limitations

Only the range of Dow AgroSciences inerts has been screened for interferenceS using this method. Caution needs to be taken when analysing products where the inerts are unknown and may produce interferant peaks. In these cases, and where the sulfotepp level is determined to have exceeded the specification maximum, it is recommended that a confirmatory technique is used, e.g. GC-MSD.

Where surfactant inerts are known, and can be checked for intereference peaks, then the need for a water extraction clean-up stage can be eliminated.

E. CONCLUSIONS

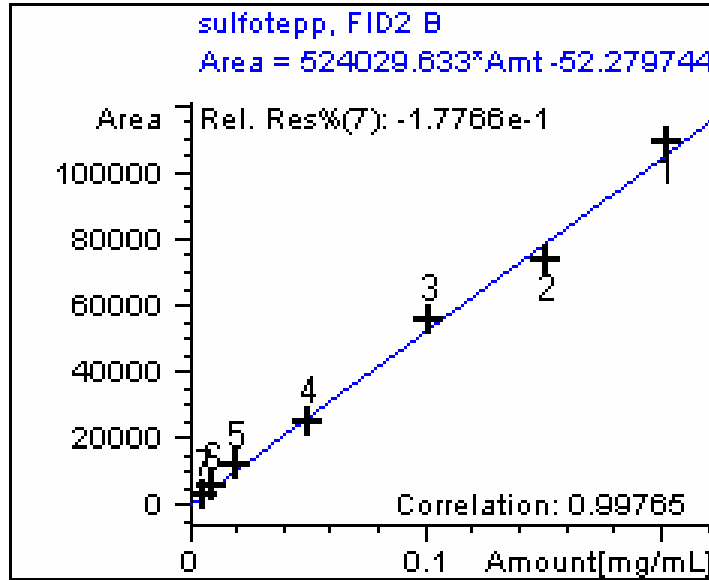
A gas chromatographic method using peak area quantitation and external standard calculation was validated for the analysis of sulfotepp in chlorpyrifos EC formulations containing proprietary inert components.. Linearity, system and method precision, accuracy, and interferences were assessed and found to be acceptable.

The statistical methods used were means, standard deviations, relative standard deviations, and t-test.

F TABLES AND FIGURES

(1) Linearity

Figure 1: Detector Linearity of Sulfotepp by FID



(2) Precision

Table I – System Precision Data

Injection Number	Analysis Result (% w/w)
Inj 1	0.113
Inj 2	0.111
Inj 3	0.114
Inj 4	0.113
Inj 5	0.112
Inj 6	0.111
Inj 7	0.115
Inj 8	0.114
Inj 9	0.114
Inj 10	0.115
Average	0.113
Std Dev	0.0015
RSD (%) ¹	1.3
Precision ²	± 2.9

1. Relative standard deviation (RSD) calculated by dividing the standard deviation by the average and multiplying by 100.
2. Precision calculated by multiplying the RSD by 2.26 (2.26 the students T-test for analysis of 10 samples at the 95% confidence level)

Table II. Method Precision Data

Sample Number	Analysis Result (% w/w)
Day 1	
Sample 1	0.113
Sample 2	0.113
Sample 3	0.113
Sample 4	0.112
Sample 5	0.113
Sample 6*	0.113
Sample 7*	0.113
Sample 8	0.114
Sample 9	0.115
Sample 10	0.112
Day 2	
Sample 11	0.112
Sample 12	0.112
Sample 13	0.112
Sample 14	0.111
Sample 15	0.111
Average	0.113
Std Dev ¹	0.0011
RSD (%) ²	0.94
Precision ³	± 2.0

1. Standard Deviation calculated using the (n-1) standard deviation.
2. Relative standard deviation (RSD) calculated by dividing the standard deviation by the average and multiplying by 100.
3. Precision calculated by multiplying the RSD by 2.15 (2.15 the students T-test for analysis of 15 samples at the 95% confidence level)

Table III. Method Recovery Data

Sample ID	Theoretical Concentration (% w/w)	Measured Concentration (% w/w)	Recovery (%)
R4A (EC1)	0.0534 ¹	0.0579	108.4
R5A (EC1)	0.1053 ¹	0.1057	100.4
R6A (EC1)	0.1556 ¹	0.1540	99.0
R7 (EC1)	0.2098 ²	0.2059	98.1
R8 (EC1)	0.2392 ²	0.2402	100.4
R9 (EC1)	0.3298 ²	0.3284	99.6
R10 (EC1)	0.3591 ²	0.3571	99.4
R11 (EC1)	0.3896 ²	0.3913	100.4
R14 (EC2)	0.0510 ¹	0.0581	113.9
R15 (EC2)	0.1022 ¹	0.1045	102.3
R16 (EC2)	0.1541 ¹	0.1622	105.3
		Average	102.5
		Std Dev	4.9
		RSD (%)	4.7

1. Theoretical concentration calculated by dividing mass of sulfotepp added to sample by the total sample weight of inerts, chlorpyrifos analytical standard and sulfotepp.
2. Theoretical concentration calculated by dividing mass of sulfotepp added to sample plus the mass of sulfotepp in the aliquot of chlorpyrifos technical weighed, by the total sample weight of inerts, chlorpyrifos technical and sulfotepp.

H ANALYTICAL METHOD SUMMARY

Sulfotepp in Chlorpyrifos EC Formulations DAS-AM-01-058

1. Standard Preparation

i) Stock solution:

Accurately weigh, to 0.1mg, approximately 0.035 g of sulfotepp analytical standard into a 10 mL volumetric flask. Dilute to volume with isooctane at 20 °C.

ii) Calibration Standard:

Pipette 60 uL of stock solution into a 10 mL volumetric flask containing approximately 0.28 g of chlorpyrifos analytical standard and dilute to volume with isooctane at 20 °C. Mix thoroughly.

Calculate the exact concentrations of impurities as detailed in section 4 (i).

2. Sample Preparation

i) *Formulations where surfactant inerts can be checked to confirm no interferences*

Accurately weigh, to the nearest 0.1mg, approximately 0.63 g of chlorpyrifos EC formulation technical into an 11 dram or other appropriately sized, screw top vial.

Add 24.4 mL of isocotane to the sample using a dispenser and shake to mix.

Filter an aliquot of the prepared solution through a 0.45 um syringe filter, 25 mm diameter with GF/0.45 um GHP Membrane.

ii) *Formulations containing interferant surfactants (or where the the surfactants are unknown)*

Accurately weigh, to the nearest 0.1mg, approximately 0.63 g of chlorpyrifos EC formulation technical into an 11 dram or other appropriately sized, screw top vial.

Add 10 mL of distilled water and 24.4 mL of isocotane to the sample using dispensers and shake vigorously for 20 minutes.

Centrifuge at 2000 rpm for 5 minute and filter an aliquot of the top layer of the prepared solution through a 0.45 um syringe filter, 25 mm diameter with GF/0.45 um GHP Membrane.

3. Instrumentation and Conditions

Instrument	HP 6890 Gas Chromatograph
Column:	HP-1, 30 m x 0.53 mm x 2.65µm film
Carrier gas	Nitrogen
Detector	Flame Ionisation Detector at 275 °C
Detector Gases - Air	300 mL/minute
- Hydrogen	30 mL/minute
- Nitrogen	25 mL/minute
Carrier flow	8 mL/min
Injection temperature	220°C
Oven temperature program	70 °C for 0.3 minutes, ramp 10 °C / minute to 110 °C, hold 0.1 minutes, ramp 12 °C / minute to 180 °C , hold 3 minutes, ramp 12 °C / minute to 260 °C, hold 5 minutes
Injection volume	1 µL
Equilibration time	3 minutes
Total run time	25 minutes
Typical Retention Time	
Sulfotepp	15.8 minutes
Quantitation	peak area

4. Calibration

- (i) Calculation of concentration (mg /mL) of sulfotepp in the calibration solution:

$$C_{(i,cal)} = \frac{[W_i \times (P/100)]}{10\text{mL}} \times \frac{0.06 \text{ mL}}{10 \text{ mL}}$$

where

$C_{(i,cal)}$ =	the concentration (mg/ml) of sulfotepp in the calibration solution
W_i =	weight (mg) of sulfotepp in the stock solution
P =	Purity of the impurity standard, expressed as a percentage
10 mL =	the volume of stock solution prepared
0.06 mL =	the volume of stock solution added to the calibration solution
10 mL =	the volume of calibration solution prepared

(ii) Calculation of external standard response factor (RF) for sulfotepp:

$$RF(i) = \frac{C(i, cal)}{Area(i, cal)}$$

where

$RF_{(i)}$ = response factor of sulfotepp
 $C_{(i, cal)}$ = the concentration (mg/ml) of sulfotepp in the calibration solution
 $Area_{(i, cal)}$ = peak area obtained for sulfotepp in the calibration solution

5. Sample Analysis

The response factor of the standard injection before the samples is calculated and then applied to the samples. **A maximum of four samples may be run and quantitated against each standard injection.**

Calculation of weight % concentration of sulfotepp in the samples:

$$\% w/w = \frac{(Area(sample) \times RF(i))}{(W(sample)/25mL)} \times 100\%$$

where

$\% w/w$ = the weight percent of sulfotepp in the sample
 $RF_{(i)}$ = response factor of the sulfotepp in calibration solution
 $Area_{(sample)}$ = peak area obtained for sulfotepp in the sample solution
 $W_{(sample)}$ = sample weight in mg
25 mL = the volume of sample solution prepared

6. Method Precision

Data obtained using this procedure indicates relative method precision values at the 95% confidence level (Table II) of.

Analyte	Precision
Sulfotepp	± 2.0 %

This data was obtained from analysis of a sample ten times on day one (including five sample preparations using the water extraction step) and five times on day two.

7. Accuracy

Analysis of sulfotepp added to samples of chlorpyrifos analytical standard or chlorpyrifos technical and inerts of 480EC(1) or 480EC(2), gave the following mean recovery level (Table III):

Concentration Range	Accuracy
0.05–0.39 %w/w	102.5 %

8. Linearity

The detector response was found to be linear for sulfotepp in the following concentration range:

	Linear Range
Sulfotepp	6 -202 ug/mL

Figure 1. Chromatogram of Standard Solution

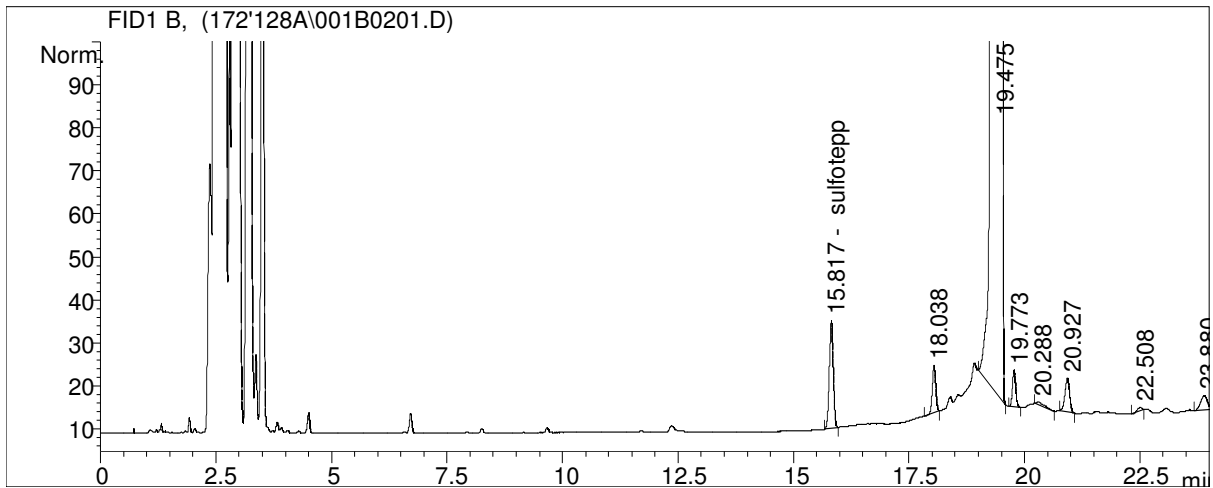


Figure 2. Chromatogram of Formulation without Water Extraction Step

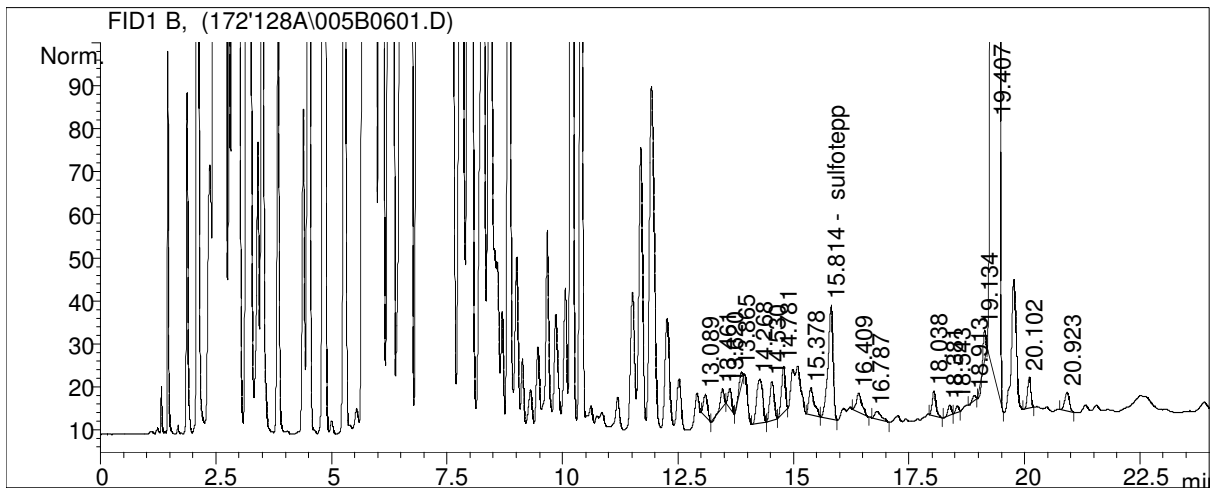


Figure 3. Chromatogram of Formulation with water extraction step included.

