

WHO SPECIFICATIONS AND EVALUATIONS FOR PUBLIC HEALTH PESTICIDES

MALATHION

S-1,2-bis(ethoxycarbonyl)ethyl O,O-dimethyl
phosphorodithioate



**World Health
Organization**

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Disclaimer¹

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

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

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

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

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

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

¹ This disclaimer applies to all specifications published by WHO.

INTRODUCTION

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

From 2002, the development of WHO specifications follows the **New Procedure**, described in the Manual for development and use of FAO and WHO specifications for chemical pesticides. This **New Procedure** follows a formal and transparent evaluation process. It describes the minimum data package, the procedure and evaluation applied by WHO and the experts of the “FAO/WHO Joint Meeting on Pesticide Specifications” (JMPS).

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

Part One: The Specification of the technical material and the related formulations of the pesticide in accordance with chapters 4 to 9 of the above-mentioned manual.

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

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

Specifications bear the date (month and year) of publication of the current version. Evaluations bear the date (year) of the meeting at which the recommendations were made by the JMPS.

* Footnote: The publications are available on the internet under the WHO Prequalification Unit – Vector Control Product Assessment Team (PQT/VCP) website, <https://extranet.who.int/pgweb/vector-control-products>

PART ONE
SPECIFICATIONS

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WHO SPECIFICATIONS FOR PUBLIC HEALTH PESTICIDES

MALATHION

INFORMATION

ISO common name: malathion (E-ISO, (m)F-ISO, ESA, BAN)

Synonyms: maldison, malathon, mercaptothion, mercaptotion, carbofos

Chemical name:

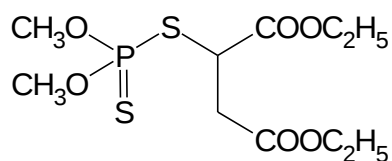
IUPAC: S-1,2-bis(ethoxycarbonyl)ethyl O,O-dimethyl phosphorodithioate

CA: butanedioic acid, [(dimethoxyphosphinothioyl)thio]-, diethyl ester

CAS No: 121-75-5

CIPAC No: 12

Structural formula:

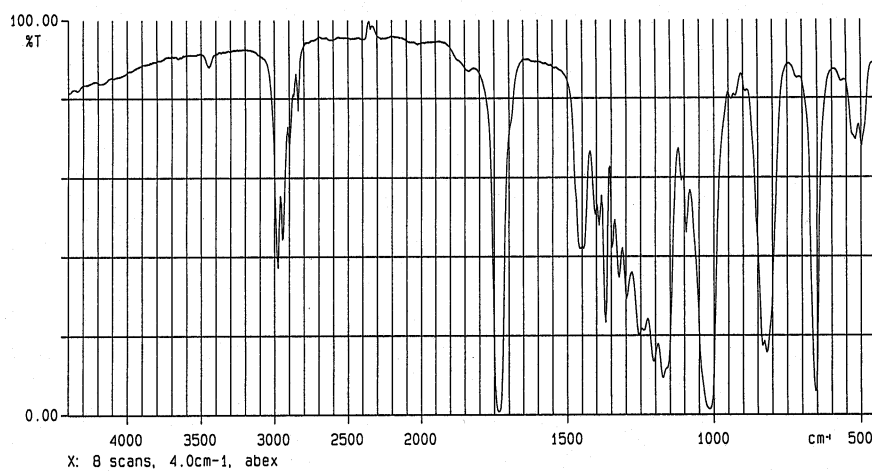


Molecular formula: C₁₀H₁₉O₆PS₂

Relative molecular mass:

330.36

Identity tests: GC retention time; infra-red spectrum (see below).



WHO SPECIFICATIONS FOR PUBLIC HEALTH PESTICIDES

MALATHION TECHNICAL MATERIAL

WHO Specification 12/TC (September 2022*)

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 (12/2003, 12/2020, 12/2022.1). 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 specification. The specification may not be appropriate for TC produced by other manufacturers. The evaluation reports (12/2003, 12/2020, 12/2022.1) as PART TWO forms an integral part of this publication.

1 Description

The material shall consist of malathion, together with related manufacturing impurities, and shall be a clear, colourless to light amber liquid with a characteristic odour and free from visible extraneous matter and added modifying agents, except odour modifying agents as required.

2 Active ingredient

2.1 Identity tests (12/TC/(M3)/2, CIPAC Handbook K, p.89, 2003)

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 Malathion content (12/TC/(M3)/3, CIPAC Handbook K, p.89, 2003)

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

3 Relevant impurities (Note 1)

3.1 Malaoxon (CAS No. 1634-78-2; butanedioic acid, (dimethoxyphosphino thioyl), diethyl ester)

Maximum: 1 g/kg.

3.2 Isomalathion (CAS No. 3344-12-5; succinic acid, mercaptodiethylester, S-ester with O,S-dimethyl phosphorodithioate)

Maximum: 4 g/kg.

3.3 MeOOSPS-triester (CAS No. 2953-29-9; phosphorodithioic acid, O,O,S-trimethyl ester).

Maximum: 15 g/kg.

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

3.4 **MeOOOPS-triester** (CAS No. 152-18-1; phosphorothioic acid, O,O,O-trimethyl ester)

Maximum: 5 g/kg.

4 **Physical properties**

4.1 **Acidity** (MT 31.2, CIPAC Handbook F, p.98, 1995)

Maximum: 2 g/kg, calculated as H₂SO₄.

Note 1 Methods for determination of the relevant impurities are described in Appendices 3, 4 and 5 to the evaluation, in Part 2 of this document. The methods correspond to Cheminova Analytical Method numbers: VAM 008-02 for malaoxon; VAM 005-03 for isomalathion; and VAM 206-01 for MeOOSPS-triester and MeOOOPS-triester.

WHO SPECIFICATIONS FOR PUBLIC HEALTH PESTICIDES

MALATHION DUSTABLE POWDER

WHO Specification 12/DP (September 2022*)

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 (12/2003, 12/2020). It should be applicable to relevant products of this manufacturer, and those of any other formulators who use only TC from the evaluated sources. 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 (12/2003, 12/2020) as PART TWO forms an integral part of this publication.

1 Description

The material shall consist of a homogeneous mixture of technical malathion, complying with the requirements of WHO specification 12/TC, together with carriers and any other necessary formulants (Note 1). It shall be in the form of a fine, free-flowing powder, free from visible extraneous matter and hard lumps.

2 Active ingredient

2.1 Identity tests (12/DP/(M3)/2, CIPAC Handbook K, p.93, 2003)

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 Malathion content (12/DP/(M3)/3, CIPAC Handbook K, p.93, 2003)

The malathion content shall be declared (g/kg) and, when determined, the average content measured shall not differ from that declared by more than the following tolerance.

Declared content in g/kg	Tolerance
above 25 up to 100	-10% to +25% of the declared content
<u>Note:</u> the upper limit is included in the range	

3 Relevant impurities (Note 2)

3.1 Malaoxon (CAS No. 1634-78-2; butanedioic acid, (dimethoxyphosphino thioyl), diethyl ester)

Maximum: 0.1% of the malathion content found under 2.2.

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

- 3.2 **Isomalathion** (CAS No. 3344-12-5; succinic acid, mercaptodiethylester, S-ester with O,S-dimethyl phosphorodithioate)

Maximum: 2.5% of the malathion content found under 2.2.

- 3.3 **MeOOSPS-triester** (CAS No. 2953-29-9; phosphorodithioic acid, O,O,S-trimethyl ester).

Maximum: 1.6% of the malathion content found under 2.2.

- 3.4 **MeOOOPS-triester** (CAS No. 152-18-1; phosphorothioic acid, O,O,O-trimethyl ester)

Maximum: 0.5% of the malathion content found under 2.2.

4 Physical properties

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

Maximum: 1 g/kg, calculated as H₂SO₄.

- 4.2 **Dry sieve test** (MT 59.1, CIPAC Handbook F, p.177, 1995)

Maximum: 5% retained on a 75 µm test sieve. Not more than (0.005 x X)% of the mass of the sample used for the determination shall be present as malathion in the residue on the sieve, where X is the malathion content (g/kg) found under 2.2 (Note 3).

5 Storage stability

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

After storage at 54 ± 2°C for 14 days, the determined average active ingredient content must not be lower than 85%, relative to the determined mean found before storage (Note 4) and the formulation shall continue to comply with the clauses for:

- malaoxon (3.1);
- isomalathion (3.2);
- MeOOSPS-triester (3.3);
- MeOOOPS-triester (3.4);
- acidity (4.1);
- dry sieve test (4.2).

Note 1 Odour modifying agents may be included so that the odour is not objectionable, if required for specific uses.

Note 2 Methods for determination of the relevant impurities are described in Appendices 3, 5 and 6 to the evaluation, in Part 2 of this document. The methods correspond to Cheminova Analytical Method numbers: VAM 208-01 for malaoxon; VAM 005-03 for isomalathion; and VAM 206-01 for MeOOSPS-triester and MeOOOPS-triester.

Note 3 For example, if the determined malathion content of the formulation is 40 g/kg and a 20 g sample is used in the test, then the amount of malathion in the residue on the sieve should not exceed 0.040 g, calculated from:

$$\frac{(0.005 \times 40) \times 20}{100} \text{ g}$$

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

WHO SPECIFICATIONS FOR PUBLIC HEALTH PESTICIDES

MALATHION ULTRA LOW VOLUME LIQUID

WHO Specification 12/UL (September 2022*)

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 (12/2003, 12/2020). It should be applicable to relevant products of this manufacturer, and those of any other formulators who use only TC from the evaluated sources. 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 (12/2003, 12/2020) as PART TWO forms an integral part of this publication.

1 Description

The material shall consist of technical malathion, complying with the requirements of WHO specification 12/TC together with any necessary formulators. It shall be in the form of a stable homogeneous liquid, free from visible suspended matter and sediment.

2 Active ingredient

2.1 Identity tests (12/EC/(M3)/2, CIPAC Handbook K, p.91, 2003)

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 Malathion content (12/EC/(M3)/3, CIPAC Handbook K, p.91, 2003)

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

3 Relevant impurities (Note 1)

3.1 Malaoxon (CAS No. 1634-78-2; butanedioic acid, (dimethoxyphosphino thioyl), diethyl ester)

Maximum: 0.1% of the malathion content found under 2.2.

3.2 Isomalathion (CAS No. 3344-12-5; succinic acid, mercaptodiethylester, S-ester with O,S-dimethyl phosphorodithioate)

Maximum: 0.4% of the malathion content found under 2.2.

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

3.3 **MeOOSPS-triester** (CAS No. 2953-29-9; phosphorodithioic acid, O,O,S-trimethyl ester)

Maximum: 1.6% of the malathion content found under 2.2.

3.4 **MeOOOPS-triester** (CAS No. 152-18-1; phosphorothioic acid, O,O,O-trimethyl ester)

Maximum: 0.5% of the malathion content found under 2.2.

4 **Physical properties**

4.1 **Acidity** (MT 31.2, CIPAC Handbook F, p.98, 1995)

Maximum: 2 g/kg, calculated as H₂SO₄.

5 **Storage stability**

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

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

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

After storage at 54 ± 2°C for 14 days, the determined average active ingredient content must not be lower than 950 g/kg (Note 2) and the formulation shall continue to comply with the clauses for:

- malaoxon (3.1);
- isomalathion (3.2);
- MeOOSPS-triester (3.3);
- MeOOOPS-triester (3.4);
- acidity (4.1).

Note 1 Methods for determination of the relevant impurities are described in Appendices 3, 4 and 5 to the evaluation, in Part 2 of this document. The methods correspond to Cheminova Analytical Method numbers: VAM 008-02 for malaoxon; VAM 005-03 for isomalathion; and VAM 206-01 for MeOOSPS-triester and MeOOOPS-triester. If formulants are incorporated into the UL, it may be necessary to use an alternative method to avoid interference. In this case, Cheminova Analytical Method number VAM 203-01 (Appendix 2) should be used.

Note 2 Samples of the formulation taken before and after the storage stability test may be analyzed together after the test in order to reduce the analytical error.

WHO SPECIFICATIONS FOR PUBLIC HEALTH PESTICIDES

MALATHION EMULSIFIABLE CONCENTRATE

WHO Specification 12/EC (September 2022*)

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 (12/2003, 12/2020). It should be applicable to relevant products of this manufacturer, and those of any other formulators who use only TC from the evaluated sources. The specification is not an endorsement of those products, nor a guarantee that they comply with the specification. The specification may not be appropriate for the products of other manufacturers who use TC from other sources. The evaluation report (12/2003, 12/2020) as PART TWO forms an integral part of this publication.

1 Description

The material shall consist of technical malathion complying with the requirements of WHO specification 12/TC, dissolved in suitable solvents (Note 1), together with any other necessary formulants. It shall be in the form of a stable homogeneous liquid, free from visible suspended matter and sediment, to be applied as an emulsion after dilution in water (Note 2).

2 Active ingredient

2.1 Identity tests (12/EC/(M3)/2, CIPAC Handbook K, p.91, 2003)

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 Malathion content (12/EC/(M3)/3, CIPAC Handbook K, p.91, 2003) (Note 3)

The malathion content shall be declared and, when determined, the average content measured shall not differ from that declared by more than the following tolerances:

Declared content in g/kg or g/l at 20 ± 2°C	Tolerance
above 250 up to 500 above 500	± 5% of the declared content ± 25 g/kg or g/l
<u>Note</u> in each range the upper limit is included.	

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

3 Relevant impurities (Note 4)

3.1 **Malaoxon** (CAS No. 1634-78-2; butanedioic acid, (dimethoxyphosphino thioyl), diethyl ester)

Maximum: 0.1% of the malathion content found under 2.2.

3.2 **Isomalathion** (CAS No. 3344-12-5; succinic acid, mercaptodiethylester, S-ester with O,S-dimethyl phosphorodithioate)

Maximum: 0.8% of the malathion content found under 2.2.

3.3 **MeOOSPS-triester** (CAS No. 2953-29-9; phosphorodithioic acid, O,O,S-trimethyl ester)

Maximum: 1.6% of the malathion content found under 2.2

3.4 **MeOOOPS-triester** (CAS No. 152-18-1; phosphorothioic acid, O,O,O-trimethyl ester)

Maximum: 0.5% of the malathion content found under 2.2.

4. Physical properties

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

Maximum: 2 g/kg calculated as H₂SO₄.

4.2 **Emulsion stability and re-emulsification** (MT 36.3, CIPAC Handbook K, p.137, 2003) (Note 5)

The formulation, when diluted at 30 ± 2°C with CIPAC Standard Waters A and D, shall comply with the following:

Time after dilution	Limits of stability, MT 36.3
0 h	Initial emulsification complete
0.5 h	"Cream", maximum: 2 ml
2.0 h	"Cream", maximum: 4 ml "Free oil", maximum: 0.5 ml
24 h	Re-emulsification complete
24.5 h	"Cream", maximum: 4 ml "Free oil", maximum: 0.5 ml
<u>Note:</u> tests after 24 h are required only where results at 2 h are in doubt.	

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

Maximum: 25 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 ± 2°C for 7 days, the volume of solid and/or liquid which separates shall not be more than 0.3 ml.

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

After storage at $54 \pm 2^{\circ}\text{C}$ for 14 days, the determined average active ingredient content must not be lower than:

- (i) 90% relative to the determined average content found before storage (Note 7), for products with a declared content of 500 g/kg or less;
- (ii) the determined average content found before storage (Note 7) minus 50 g/kg, for products with a declared content of more than 500 g/kg;

and the formulation shall continue to comply with the clauses for:

- malaoxon (3.1);
- isomalathion (3.2);
- MeOOSPS-triester (3.3);
- MeOOOPS-triester (3.4);
- acidity (4.1) (except that a maximum of 3 g/kg acidity is permitted);
- emulsion stability and re-emulsification (4.2).

Note 1 Caution: transesterification may occur if methanol or other short-chain alcohols are present in the solvent.

Note 2 Odour modifying agents may be included so that the odour is not objectionable, if required for specific uses.

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

Note 4 The method for determination of the relevant impurities is described in Appendix 2 to the evaluation, in Part 2 of this document. The method corresponds to Cheminova Analytical Method number: VAM 203-01.

Note 5 As outlined in CIPAC MT 36.3, the test concentrations should be based on those in the recommended directions for use supplied with the product. Where several concentrations are recommended, the highest and lowest concentrations within the scope of the method should be used.

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

Note 7 Samples of the formulation taken before and after the storage stability test may be analyzed together after the test in order to reduce the analytical error.

WHO SPECIFICATIONS FOR PUBLIC HEALTH PESTICIDES

MALATHION EMULSION, OIL IN WATER

WHO Specification 12/EW (September 2022*)

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 (12/2003, 12/2012, 12/2020, 12/2022.2). It should be applicable to relevant products of this manufacturer, and those of any other formulators who use only TC from the evaluated sources. 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 (12/2003, 12/2012, 12/2020 12/2022.2) as PART TWO forms an integral part of this publication.

1 Description

The formulation shall consist of an emulsion of technical malathion, complying with the requirements of WHO specification 12/TC, in an aqueous phase together with suitable formulants (Note 1). After gentle agitation, the formulation shall be homogeneous and suitable for dilution in water.

2 Active ingredient

2.1 Identity tests (12/EW/(M)/2, CIPAC Handbook K, p.92, 2003)

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 Malathion content (12/EW/(M)/3, CIPAC Handbook K, p.92, 2003) (Note 2)

The malathion content shall be declared (440 g/L) and, when determined, the average content measured shall not differ from that declared by more than 5%.

3. Relevant impurities (Note 3)

3.1 Malaoxon (CAS No. 1634-78-2; butanedioic acid, (dimethoxyphosphino thioyl), diethyl ester)

Maximum: 0.8% of the malathion content found under 2.2.

3.2 Isomalathion (CAS No. 3344-12-5; succinic acid, mercaptodiethylester, S-ester with O,S-dimethyl phosphorodithioate)

Maximum: 0.6% of the malathion content found under 2.2.

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

- 3.3 **MeOOSPS-triester** (CAS No. 2953-29-9; phosphorodithioic acid, O,O,S-trimethyl ester)

Maximum: 1.6% of the malathion content found under 2.2.

- 3.4 **MeOOOPS-triester** (CAS No. 152-18-1; phosphorothioic acid, O,O,O-trimethyl ester)

Maximum: 0.5% of the malathion content found under 2.2.

4 Physical properties

- 4.1 **pH range** (1% aqueous dilution) (MT 75.3, CIPAC Handbook J, p.131, 2000)

pH range: 2 to 5.

- 4.2 **Pourability** (MT 148.1, CIPAC Handbook J, p.133, 2000)

Maximum "residue": 5%.

- 4.3 **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, MT 36.3
0 h	Initial emulsification complete
0.5 h	"Cream", maximum: 2 ml
2.0 h	"Cream", maximum: 4 ml
	"Free oil", none
24 h	Re-emulsification complete
24.5 h	"Cream", maximum: 2 ml
	"Free oil", none
<u>Note:</u> tests after 24 h are required only where results at 2 h are in doubt.	

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

Maximum: 50 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, no separation of particulate or oily matter shall be visible after gentle agitation.

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

After storage at $54 \pm 2^{\circ}\text{C}$ for 14 days, the determined average active ingredient content must not be lower than 90% relative to the determined average content found before storage (Note 6) and the formulation shall continue to comply with the clauses for:

- malaoxon (3.1);
- isomalathion (3.2);
- MeOOSPS-triester (3.3);
- MeOOOPS-triester (3.4);
- pH range (4.1);
- emulsion stability and re-emulsification (4.3).

Note 1 Odour modifying agents may be included so that the odour is not objectionable, if required for specific uses.

Note 2 If the buyer requires both g/kg and g/l at 20°C , then in case of dispute the analytical results shall be calculated as g/kg.

Note 3 Methods for determination of the relevant impurities are described in Appendices 1, 3 and 5 to the evaluation, in Part 2 of this document. The methods correspond to Cheminova Analytical Method numbers: VAM 202-01 for malaoxon; VAM 005-03 for isomalathion; and VAM 206-01 for MeOOSPS-triester and MeOOOPS-triester.

Note 4 As outlined in CIPAC MT 36.3, the test concentrations should be based on those in the recommended directions for use supplied with the product. Where several concentrations are recommended, the highest and lowest concentrations within the scope of the method should be used.

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

Note 6 Samples of the formulation taken before and after the storage stability test should be analyzed together after the test in order to reduce the analytical error.

PART TWO
EVALUATION REPORTS

MALATHION

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WHO SPECIFICATIONS FOR PUBLIC HEALTH PESTICIDES

MALATHION

FAO/WHO EVALUATION REPORT 12/2022.2

Recommendations

The Meeting recommended the following:

- (i) The existing FAO specification for malathion EW should be extended to encompass the corresponding product of Tagros Chemicals India Private Limited.
- (ii) The existing WHO specification for malathion EW should be extended to encompass the corresponding product of Tagros Chemicals India Private Limited.

Appraisal

The Meeting considered data and information submitted in January 2019 by Tagros Chemicals India Private Limited (Tagros), in support of the extension of the existing FAO/WHO specification for malathion EW (Emulsion, oil in water). The data submitted by Tagros were 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).

Tagros provided a complete data package on the physical and chemical properties of their malathion EW formulation (440 g/L) to support the compliance with the existing FAO/WHO specification.

The company provided test results on a single batch of the EW formulation. However, Tagros indicated that the specification is representative of the quality of their EW by providing historical QC data (ten different batches). The stability of the EW after the accelerated storage test (MT 46.3) was demonstrated to be acceptable and no significant adverse effects of storage of the formulated product was observed in term of active ingredient content, pH, emulsion stability and re-emulsification. The Meeting noted that malaoxon was not detected (by ^{31}P NMR) before and after the accelerated storage, however the content of the other three impurities (isomalathion, MeOOSPS-triester and MeOOOPS-triester) increased after storage but their concentration levels remained below the maximum limit of the current FAO/WHO EW specification. Following the JMPS meeting of 2019 and given that malaoxon had not been determined in the historical QC data (ten different batches), analysis of at least three further batches for malaoxon content were requested. Additionally, the LOD or LOQ of the NMR method used for demonstrating the non-presence of malaoxon was requested as well as a justification on how malaoxon content in the EW formulation can be controlled if routine NMR analysis cannot be done. In response, the company provided results of analysis of malaoxon content in three batches using a validated HPLC-UV method (as regards the method LOD (0.2 g/kg) and LOQ (0.88 g/kg), specificity, linear range, accuracy, and precision).

After the request of the JMPS meeting of 2020, the company provided validation data from two laboratories of the HPLC method used for the analysis of the malaoxon in the EW formulation (peer validation). The method was validated in the two laboratories as

regards the LOD (0.038 g/kg in the first laboratory and 0.064 g/kg in the second laboratory) and LOQ (0.79 g/kg in the first laboratory and 0.36 g/kg in the second laboratory), specificity, linear range, accuracy and precision.

The validation data of both laboratories indicate that the HPLC method is suitable for use in the Tagros's malathion EW.

During storage at 0°C there was no layer separation and there was no significant physical change.

For the determination of malathion (before and after storage) in EW formulation the company used the official CIPAC method published in CIPAC Handbook K (CIPAC 12/EW/(M)/3, CIPAC Handbook K, p.92, 2003). The method relies on capillary GC with internal standardization with docosane and had been revalidated with respect to specificity, linearity, and precision. Methods used for determination of the relevant impurities are the ones published as appendices to the FAO/WHO specifications and had been re-validated with respect to specificity, linearity, and precision.

Tagros used the updated CIPAC methods for the determination of physical and chemical properties for their malathion EW. The test results for the physical and chemical properties of malathion EW formulation produced by Tagros demonstrated that the product complies with the existing FAO/WHO specification for malathion EW.

ANNEX 1 : REFERENCES

Study number	Author(s)	year	Study title. Study identification number. Report identification number. GLP [if GLP]. Company conducting the study.
7970	K. Dattatreya Chary	2018	Determination of pH of Malathion 44% EW. Study No. 7970. GLP. RCC Laboratories India Private Limited.
7971	K. Dattatreya Chary	2018	Determination of Pourability of Malathion 44% EW. Study No. 7971. GLP. RCC Laboratories India Private Limited.
7972	K. Dattatreya Chary	2018	Determination of emulsion stability of Malathion 44% EW. Study No. 7972. GLP. RCC Laboratories India Private Limited.
7973	K. Dattatreya Chary	2018	Determination of Foam Persistence of Malathion 44% EW. Study No: 7973. RCC Laboratories India Private Limited.
7974	K. Dattatreya Chary	2018	Low Temperature stability of Malathion 44% EW. Study No. 7974. GLP. RCC Laboratories India Private Limited.
7975	K. Dattatreya Chary	2018	Accelerated Storage Stability of Malathion 44% EW. Study No. 7975. GLP. RCC Laboratories India Private Limited.
MLT/MXN/38K19	Gopinath M.	2019	Determination of malaoxon content in three batches of malathion 44% EW. Study No. MLT/MXN/38K19. Tagros Chemicals India Private Limited.
10733	J. Ramesh	2021	Determination of Malaoxon content in Malathion 44% EW. Study No. 10733. RCC Laboratories India Private Limited.
G21116	Ananda Raju P.	2021	Method validation and analysis of malaoxon in Malathion 440 G/L EW. Study No. G21116. GLP. Eurofins Advinius Limited.

WHO SPECIFICATIONS FOR PUBLIC HEALTH PESTICIDES

MALATHION

FAO/WHO EVALUATION REPORT 12/2022.1

Recommendations

The Meeting recommended the following:

- (i) The malathion TC, as proposed by Tagros Chemicals India Private Limited, should be accepted as equivalent to the malathion reference profile.
- (ii) The existing FAO specification for malathion TC should be extended to encompass the material produced by Tagros Chemicals India Private Limited.
- (iii) The existing WHO specification for malathion TC should be extended to encompass the material produced by Tagros Chemicals India Private Limited.

Appraisal

The Meeting considered data and supporting information submitted in January 2019 by Tagros Chemicals India Private Limited (Tagros), for the determination of the equivalence of their technical material with the specification for malathion TC (FAO/WHO specification 12/TC). The data submitted were 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). The reference specification and supporting data for malathion were provided by Cheminova and the FAO/WHO specifications had been published in 2002 (under the new procedures) and then revised in 2013 for malathion DP, EC, UL and EW on basis of data provided by Cheminova. The acquisition by FMC International of the former proposer and holder of the FAO/WHO reference specifications for malathion TC (Cheminova) was noted by FAO/WHO in the evaluation report 12/2020. As declared by FMC, no significant changes in the production process had occurred and FMC ensured the continued support for malathion TC and its formulations.

Malathion is the ISO common name for diethyl (dimethoxythiophosphorylthio)succinate or S-1,2-bis(ethoxycarbonyl)ethyl O,O-dimethyl phosphorodithioate (IUPAC). The technical material is a racemic mixture of *R*-malathion [butanedioic acid, [(dimethoxyphosphinothioyl)thio]-, diethyl ester (2*R*)-(9CI)] and *S*-malathion [butanedioic acid, [(dimethoxyphosphinothioyl)thio]-, diethyl ester (2*S*)-(9CI)].

Based on the existing FAO/WHO specification, four impurities have been identified as relevant with maximum levels as follows: 1 g/kg for malaoxon (CAS No: 1634-78-2), 4 g/kg for isomalathion (CAS No: 3344-12-5), 15 g/kg for the MeOOSPS-triester (CAS No: 2953-29-9) and 5 g/kg for the MeOOOPS-triester (CAS No: 152-18-1).

The toxicity of malathion was evaluated by the FAO/WHO JMPR in 1963, 1966, 1997, 2003 and 2016. In 2016 JMPR reaffirmed the ADI of 0–0.3 mg/kg body weight (for the sum of malathion and malaoxon, adjusted for its potency, and expressed as malathion) based on the NOAEL of 500 ppm (equal to 29 mg/kg bw per day) and the ARfD of 2 mg/kg body weight (for sum of malathion and malaoxon, adjusted for its potency, and expressed as malathion) based on the NOAEL of 15 mg/kg bw. It was also concluded

that its metabolite malaoxon is approximately 30-fold more toxic than malathion. Both the ADI and ARfD are established for the sum of malathion and malaoxon (corrected for its potency), expressed as parent malathion. The other metabolites of malathion considered by the JMPR Meeting of 2016 are less potent than the parent compound and therefore would be covered by the ADI and ARfD for malathion. JMPR concluded that malathion is unlikely to be genotoxic at anticipated dietary exposures, is not teratogenic and is not immunotoxic.

The Meeting was provided with a detailed description of the manufacturing process of the technical material along with the specifications of the raw materials, the 5-batch analysis data for malathion and all impurities $\geq 1\text{g/kg}$ and their manufacturing limits in the TC. The 5-batch analysis study was performed according to GLP guidelines. The manufacturing process utilized by Tagros is similar to that from Cheminova (now FMC) and supported the 5-batch data.

Mass balances in the 5-batch analysis data ranged from 992.01 g/kg to 994.57 g/kg. The minimum purity of malathion in the TC is 950 g/kg and complies with the existing specification. The percentage of the unknowns ranged from 5.4 g to 8.0 g/kg (based on the submitted 5-batches) and was considered acceptable by the Meeting. The specified maximum limits for the four relevant impurities comply with the current maximum FAO/WHO specification limits. The impurity profiles for the reference source and the new source are different given that the new source contains new impurities specified at quite high levels. The applicant provided in 2021 further toxicological data to address their relevance and the Meeting concluded that since none of these impurities are of the “oxon” form, it could be reasoned that they are non-relevant.

As noticed by the JMPS in 2019, in the screening chromatograms three unknown impurities had been detected (at level below 1 g/kg) and the proposer had been requested to demonstrate the non-relevance of these three unknown impurities. The proposer provided information on the identity/chemical structure of these impurities and toxicological data to support their claimed non-relevance. One of these impurities was considered as non-relevant (based on FAO/WHO toxicological assessment). For the rest two, the proposer provided in 2021 further toxicological data to address their relevance. Additionally, the applicant provided quantification data about these two impurities. The Meeting concluded that at the determined levels both are not considered as relevant.

Malathion in TC was determined using the CIPAC method 12/TC/M(3)/3, published in CIPAC Handbook K. According to this CIPAC method, malathion is determined by GC-FID and internal standardisation with docosane. Despite not being required, the CIPAC method used for malathion content in the TC was re-validated by measuring specificity, selectivity, linearity of detector response and linear range, precision, intermediate precision, robustness and accuracy.

Isomalathion and malaoxon in Tagros' malathion TC were determined using the HPLC method provided in the current FAO/WHO specifications in Appendices 3 and 4 respectively. The method has been re-validated as regards the specificity, selectivity, linearity of detector response and linear range, precision, intermediate precision, robustness, accuracy, LOD and LOQ. The LOD for these impurities was calculated from the linear regression constants and the LOQ was the lowest fortification level at which the method was validated with acceptable results for accuracy and intermediate precision (recovery and RSD). The validated LOQ for isomalathion is 0.84 g/kg and for malaoxon is 0.42 g/kg.

MeOOOPS-triester and MeOOSPS-triester in Tagros' malathion TC were determined by the GC-FID external standardization method provided in the current FAO/WHO specifications in Appendix 5. The method has been re-validated as regards the specificity, selectivity, linearity of detector response and linear range, precision, intermediate precision, robustness, accuracy, LOD and LOQ. The LOD for these impurities was calculated from the linear regression constants and the LOQ was the lowest fortification level at which the method was validated with acceptable results for accuracy and intermediate precision (recovery and RSD). The validated LOQ for MeOOOPS-triester is 3.25 g/kg and for MeOOSPS-triester is 5 g/kg.

All the other identified organic impurities were determined by HPLC-UV and an inorganic impurity was determined by a suitable titration method.

A screening HPLC-DAD method with a scan range from 190 to 400 nm has been submitted for the identification of all the impurities in the 5-batches of malathion TC.

The identity of malathion and its impurities in the five batches of malathion TC were confirmed by ¹H-NMR, mass spectra (GC-MS or LC-MS), IR spectrum and GC/HPLC retention time matching.

The proposer provided mutagenicity data (Ames test according to OECD 471 guidelines). The mutagenicity study was conducted using TA 98, TA 100, TA1 535 and TA 1537 strains of *Salmonella typhimurium* and WP2uvrA (pKM101) strain of *Escherichia coli*. The results of the GLP study allowed the conclusion that the Tagros' malathion TC is not mutagenic in *Salmonella typhimurium* strains up to the OECD 471 recommended top dose of 5000 µg/plate under the conditions of testing employed. The Meeting noted that the mutagenicity study was conducted on one of the 5 batches in the 5-batch analysis study.

The Meeting concluded that the purity/impurity and mutagenicity profile of the malathion TC produced by Tagros indicated Tier 1 equivalence with the reference profile supporting the existing FAO/WHO specification 12/TC).

**SUPPORTING INFORMATION
FOR
EVALUATION REPORT 12/2022.1**

Uses

Malathion is a non-systemic organophosphorus insecticide, with contact and stomach action. It is used in agriculture to control a wide range sucking and chewing insect pests in a variety of field crops, fruits and vegetables. Malathion can also be used for insect control on livestock, in stables and on stored products. It is widely used in public health, including the eradication of malaria, dengue and other vector borne diseases. It is also widely used in control of locusts and grasshoppers.

Table 1. Chemical composition and properties of malathion 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 99.20 – 99.46 % and percentage of unknowns were 0.54– 0.80 %.		
Declared minimum malathion content		950 g/kg		
Relevant impurities ≥ 1 g/kg and maximum limits for them		Malaoxon (CAS No. 1634-78-2; butanedioic acid, (dimethoxyphosphino thioyl), diethyl ester). Maximum: 1 g/kg. Isomalathion (CAS No. 3344-12-5; succinic acid, mercaptodiethylester, S-ester with O,S-dimethyl phosphorodithioate) Maximum: 4 g/kg. MeOOSPS-triester (CAS No. 2953-29-9; phosphorodithioic acid, O,O,S-trimethyl ester). Maximum: 15 g/kg. MeOOOPS-triester (CAS No. 152-18-1; phosphorothioic acid O,O,O-trimethyl ester) Maximum: 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	Not relevant for liquid products	-	-	-

ANNEX 1

HAZARD SUMMARY PROVIDED BY THE PROPOSER

Toxicological summaries

Notes.

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

Table A. Mutagenicity profile of malathion technical material based on *in vitro* tests

Species	Test	Purity %	Guideline, duration, doses and conditions	Result	Study number
<i>Salmonella typhimurium</i> strains TA 98, TA 100, TA 1535 and TA 1537 and WP2uvrAm(pKM101) strain of <i>Escherichia coli</i>	Bacterial reverse mutation test (Ames test)	95.25 % w/w	OECD 471 16, 50, 158, 500, 1581 and 5000 µg/plate	Non-mutagenic	Study No. G17043

ANNEX 2 : REFERENCES

Study number	Author(s)	Year	Study title. Study identification number. Report identification number. GLP [if GLP]. Company conducting the study.
G17044	Ravinanth Gogineni	2019	Five Batch Analysis of Malathion Technical. Study No.: G17044. GLP. Eurofins Advinius Limited.
G17043	Divyashree K.	2018	Malathion Technical: Bacterial Reverse Mutation Test. Report No.: G17043. GLP. Eurofins Advinius Limited.
FR001225_27	J V Cotterill and C Ridgway	2019	<i>In silico</i> assessment of genotoxicity of two impurities of malathion. Project No. FR001225_27. Fera Science Ltd.
FR02225_06	J V Cotterill and S Jones	2020	<i>In silico</i> assessment of carcinogenicity, mutagenicity and reprotoxicity of three impurities of malathion. Project No. FR02225_06. Fera Science Ltd.
QSAR-0015/20	Gustavo D. C. Gustinelli	2020	Quantitative Structure-Activity Relationships (QSAR) Study Evaluation of the Cholinesterase Inhibition Potential of Malathion and Impurities. Study No. QSAR-0015/20. SCI-AGRO.
QSAR-0016/20	Gustavo D. C. Gustinelli	2020	Quantitative Structure-Activity Relationships (QSAR) Study. Evaluation of the Genetic Toxicity and Cholinesterase Inhibition Potentials of Malathion and Impurity O,O,S-trimethyl phosphorothioate. Study No. QSAR-0016/20. SCI-AGRO.
119043	Margerison S.	2020	Notice of approval of an active constituent (malathion from Tagros Chemicals India Private Limited). Australian Pesticides and Veterinary Medicines Authority (APVMA). 28 January 2020.
G24168	Smitha B. Shetty	2021	Analysis of O,O,S-Trimethylthiophosphate (malathion impurity) Study No. G24168. GLP. Eurofins Advinius Limited.

WHO SPECIFICATIONS FOR PUBLIC HEALTH PESTICIDES

MALATHION

FAO/WHO EVALUATION REPORT 12/2020

Recommendations

The Meeting recommended the following:

- (i) The acquisition of the former proposer and holder of the FAO and WHO reference specifications for malathion TC, DP, UL, EC and EW - Cheminova A/S, Denmark - by FMC International should be noted by FAO and WHO.
- (ii) The FAO specification for malathion UL should be corrected with respect of expression of the active ingredient content and appropriate tolerance range.
- (iii) The FAO and WHO specifications for malathion TC, DP, UL, EC and EW should be editorially revised to refer to the current CIPAC methods.

Appraisal

The reference specifications for malathion TC, DP, EC and UL had been proposed by Cheminova A/S in 2002 and published by FAO and WHO in 2004. The FAO Specification for malathion EW published in 2004 was later extended to WHO in 2012. The Meeting noted, that the malathion specifications published in 2004 were qualified for a review as outlined by the JMPS in 2019 and 2020, and in addition the acquisition of Cheminova by FMC Corporation (FMC) in 2014¹ further underpinned the necessity of a review of the published specifications and evaluations.

FAO and WHO, in agreement with the provisions of the Manual, Section 3.6 (Prioritization criteria for review of specifications) contacted FMC with a request to confirm that with the acquisition of Cheminova, no changes in the manufacturing process had taken place and that FMC ensure the continued support for the malathion reference specifications for TC, DP, UL, EC and EW.

FMC Corporation confirmed in writing² that malathion TC is still produced by Cheminova, now a subsidiary of FMC with the same process, that no significant changes in the production process had taken place that would merit a revision of the confidential data package and that FMC ensure the continued support for malathion TC and formulations.

The Meeting further noted that some editorial updates for the TC and formulation specifications were necessary to bring them in line with the most recent versions of the specification templates in the Manual, in particular with the latest versions of CIPAC MT methods. These revised MT methods are deemed to provide equivalent results as the previous versions, so all limits in the clauses affected remained the same as for the previous versions. FMC reviewed the proposed editorial updates and agreed with the majority of proposed changes. They include:

¹ Press release by FMC cited after <https://www.croplife.com/crop-inputs/fmc-announces-cheminova-takeover/>

² e-mail of FMC International, R. McKenna to FAO received on 30 November 2020.

- Replacement of determination of acidity using MT 31.2 by MT 191 for the DP, EC and EW ;
- Replacement of the accelerated storage test using MT 46.3 with MT 46.4 ;
- Correction and adaptation of some footnotes.

The reason to keep MT 31.2.1 for the TC and UL is, that the scope of MT 191 is to determine acidity in formulations that - generally speaking - are expected to contain surfactants. However, FMC explained that for the UL formulation MT 31.2 is better suited, as the UL does not contain surfactants - they are not required for the application of the formulation by ULV equipment (see below). MT 31.2.1 provides detailed instructions for the determination of acidity in a TC after dilution with acetone. This method is therefore deemed to be better suited for a TC and the UL. The Meeting agreed with the proposition of FMC and concluded that acidity in malathion UL should be determined using MT 31.2.1, despite the fact that the UL template in the 2016 Manual refers to MT 191.

The most significant change made was the expression of the active ingredient content in the FAO UL formulation - the version of 2013 specified in clause 2.2:

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

When contacted, FMC explained the following: malathion UL as end use product is effectively the technical material (TC) - repackaged without addition of co-formulants / solvents. As the Manual requires different provisions to express declared content for a TC – i.e. "not less than 950 g/kg" – compared to the UL, i.e. with a concentration range (above 500 g/kg) which is in line with the proposed wording for agricultural use products given in the Manual, the conversion of the TC into a UL, and the way the concentrations are expressed, cannot entirely reflect this transition - it is a more conceptual one than a classical formulation process. The latter would mean in most cases the addition of some solvents etc. which is not the case with this UL. The Meeting also noted, that the WHO specification for malathion UL shows a declared content of "not less than 950 g/kg".

The same issues had been discussed at the 2002 JMPs Meeting and covered in the evaluation report (36/2002), where it was noted:

"In the proposed UL specification, the active ingredient content is expressed as a minimum (950 g/kg) only, instead of the standard expression ($>500 \pm 25$ g/kg). This is because the UL is, in effect, freshly prepared TC (which must comply with the requirements of a formulation, including storage stability). "

Noting this, the Meeting concluded that the range of active ingredient content in the FAO UL specification should be set to "above 500 g/kg or g/L" and the appropriate tolerance range - ± 25 g/kg or g/L - included, in accordance with the standard wording for a formulation given in the Manual. However, the above explanation was added to explain the background of that change made, due to the nature of the UL, in this case being effectively the TC repackaged without additional inerts. In contrast, the WHO specification for malathion UL should be kept to reflect a declared content of minimum 950 g/kg, as this end use product has been approved for public health use.

WHO SPECIFICATIONS FOR PUBLIC HEALTH PESTICIDES

MALATHION

FAO/WHO EVALUATION REPORT 12/2012

Recommendations

The Meeting recommended the following:

- (i) The FAO specification for malathion EW, as amended, should be extended to WHO.
- (ii) The FAO and WHO specifications for malathion TC, DP, UL, EC and EW should be revised to refer to the current CIPAC methods.

Appraisal

The Meeting considered data and information submitted by Cheminova A/S (Denmark) in support of the extension to WHO of the existing FAO specification for malathion EW.

The malathion EW under consideration for public health use is a 440 g/L formulation. This product was tested and evaluated by WHOPES and received a recommendation in 2012 for outdoor space spraying as either thermal or cold fogging for the control of mosquitoes. The manufacturer confirmed that the product is the same as that for which the FAO specification was developed and published in 2004. Four relevant impurities (malaoxon, isomalathion, MeOOSPS-triester and MeOOOPS-triester) were identified in the specifications of malathion.

A full CIPAC method for the determination of malathion is now published in Handbook K (CIPAC 12/EW/(M)/2, CIPAC Handbook K, p.92, 2003). The method relies on capillary GC and replaces the former packed column method published in Handbook 1B. Methods for determination of relevant impurities are published as appendices to the WHO specifications. The Meeting agreed to publish the methods for relevant impurities also in the FAO specifications.

The limits specified in the FAO specification for malathion EW are acceptable for inclusion in the WHO specification. Nevertheless, for emulsion stability, the CIPAC method MT 36.3 has to be referred instead of MT 36.1.1 and MT 36.2. Data provided by Cheminova using MT 36.3 showed that limits of the specification are acceptable. Moreover, Cheminova provided data on the pH of the diluted formulation and not of the undiluted formulation supporting a range of 2 to 5.

For malathion identity and content in the FAO and WHO specifications for TC, DP, UL and EC, the Meeting agreed to refer to the CIPAC methods as published in the CIPAC Handbook K. The Meeting agreed also to update the reference to the CIPAC method for emulsion stability in the FAO and WHO specifications for malathion EC (MT 36.3 instead of MT 36.1.1) and the CIPAC method for persistent foam (MT 47.3 instead of MT 47.2) in the FAO and WHO specifications for malathion EC and EW, as well as to revise some footnotes to be in line with the specification guidelines of the November 2010 – second revision of the first edition of the FAO/WHO Manual and the current CIPAC methods.

WHO SPECIFICATIONS FOR PUBLIC HEALTH PESTICIDES

MALATHION

EVALUATION REPORT 12/2003

Explanation

The data and draft specifications for malathion were considered for the review of existing specifications. Existing FAO specifications for malathion TC, DP, WP, OL and EC were developed in 1998 (FAO, 1988), whereas existing WHO specifications for malathion TC, DP, WP and EC were developed in 1999 (WHO, 1999). FAO specifications were proposed for malathion TC, DP, UL, EC and EW. WHO specifications were proposed for malathion TC, DP, UL and EC.

Malathion is not under patent.

Malathion was evaluated by the FAO/WHO JMPR for toxicology in 1965, 1966, and 1997, and for residues in 1966, 1968, 1969, 1970, 1973, 1975, 1977, 1984, 1999 and 2000.

Malathion is currently under evaluation and review by the European Commission, the US EPA, the UK¹ and Denmark.

Draft specifications and supporting data were provided by Cheminova A/S in 2002.

Uses

Malathion is a non-systemic organophosphorus insecticide, with contact and stomach action. It is used in agriculture to control a wide range of sucking and chewing insect pests in a variety of field crops, fruits and vegetables. Malathion can also be used for insect control on livestock, in stables and on stored products. It is widely used in public health, including the eradication of malaria, dengue and other vector-borne diseases. It is also widely used in control of locusts and grasshoppers.

Identity of the active ingredient

ISO common name: malathion (E-ISO, (m)F-ISO, ESA, BAN)

Synonyms: maldison, malathon, mercaptothion, mercaptotion, carbofos

Chemical name:

IUPAC: S-1,2-bis(ethoxycarbonyl)ethyl O,O-dimethyl phosphorodithioate

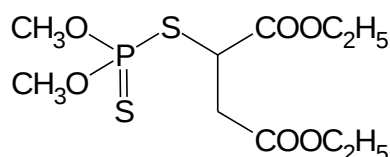
CA: butanedioic acid, [(dimethoxyphosphinothioyl)thio]-, diethyl ester

CAS No: 121-75-5

¹ 2004 footnote. The manufacturer noted that the UK review had been completed and that a review of malathion had been initiated in Australia.

CIPAC No: 12

Structural formula:



Molecular formula: $C_{10}H_{19}O_6PS_2$

Relative molecular mass:

330.36

Identity tests: GC retention time; infra-red spectrum.

Physical and chemical properties of malathion

Table 1. Physico-chemical properties of pure malathion.

Parameter	Value(s) and conditions	Purity %	Method	Reference																
Vapour pressure	25°C: 4.5 ± 1.1 x 10 ⁻⁴ Pa, 3.4 ± 0.82 x 10 ⁻⁶ mm Hg. 35°C: 3.1 ± 0.84 x 10 ⁻³ Pa, 2.3 ± 0.63 x 10 ⁻⁵ mm Hg. 45°C: 1.9 ± 0.47 x 10 ⁻² Pa, 1.4 ± 0.35 x 10 ⁻⁴ mm Hg.	98.9	US EPA D63-9	Teeter and Blasberg, 1988																
Boiling point	No value could be determined due to decomposition above approx. 174°C.	99.1	EEC A2	Cuthbert and Mullee, 2001																
Melting point	Melting point: below -20°C.	99.1	EEC A1	Cuthbert and Mullee, 2001																
Temperature of decomposition	Onset of decomposition at 174°C at 100 kPa.	99.1	EEC A2	Cuthbert and Mullee, 2001																
Solubility in water	148 mg/l at 25 ± 1°C	[¹⁴ C]-malathion radiochemical purity: 98.4	US EPA D63-8	Kabler, 1989																
Octanol/water partition coefficient	log K _{ow} = 2.7 at 25°C	[¹⁴ C]-malathion radiochemical purity: 98	US EPA D63-11	Mangels, 1987																
Hydrolysis characteristics	Measurements at 20 mg/l in aqueous buffers, 0.65% acetonitrile, 25 ± 1°C for 28 days (note 1). <table><tr><td>pH</td><td>Half-life</td><td>Rate constant</td></tr><tr><td>5</td><td>107 days</td><td>0.0065 day⁻¹</td></tr><tr><td>7</td><td>149 hours</td><td>0.00463 hours⁻¹</td></tr><tr><td>9</td><td>11.8 hours</td><td>0.0587 hours⁻¹</td></tr></table>	pH	Half-life	Rate constant	5	107 days	0.0065 day ⁻¹	7	149 hours	0.00463 hours ⁻¹	9	11.8 hours	0.0587 hours ⁻¹	[¹⁴ C]-malathion radiochemical purity >98	US-EPA N161-1	Teeter, 1988				
pH	Half-life	Rate constant																		
5	107 days	0.0065 day ⁻¹																		
7	149 hours	0.00463 hours ⁻¹																		
9	11.8 hours	0.0587 hours ⁻¹																		
Photolysis characteristics	Photolysis with xenon lamp at pH 4, 9.5 mg/l in methanol + buffer, at 25°C for 30 days continuous exposure (note 2). <table><tr><td></td><td>% remaining after 30 days</td><td>Rate constant</td><td>Half-life</td></tr><tr><td>Sensitised (acetone)</td><td>70.4%</td><td>0.00972 day⁻¹</td><td>71 days</td></tr><tr><td>Non-sensitised</td><td>78.7%</td><td>0.00707 day⁻¹</td><td>98 days</td></tr><tr><td>Dark controls</td><td>~90%</td><td></td><td></td></tr></table>		% remaining after 30 days	Rate constant	Half-life	Sensitised (acetone)	70.4%	0.00972 day ⁻¹	71 days	Non-sensitised	78.7%	0.00707 day ⁻¹	98 days	Dark controls	~90%			[¹⁴ C]-malathion (radiochemical purity 94.9; unlabelled malathion purity 98.4	US-EPA N161-2	Carpenter, 1990
	% remaining after 30 days	Rate constant	Half-life																	
Sensitised (acetone)	70.4%	0.00972 day ⁻¹	71 days																	
Non-sensitised	78.7%	0.00707 day ⁻¹	98 days																	
Dark controls	~90%																			

Parameter	Value(s) and conditions	Purity %	Method	Reference
Dissociation characteristics	Does not dissociate in water (as expected from the chemical structure)	-	-	Friis, 1988

Note 1. Malathion hydrolysis products were detected as follows:

Hydrolysis product	Product as % applied dose		
	pH 5	pH 7	pH 9
malathion monoester	1.8%	23.3%	40.0%
ethyl hydrogen fumarate	0.6%	18.7%	36.1%
diethyl thiosuccinate		23.3%	10.4%
malathion dicarboxylic acid		3.7%	3.1%

Note 2. Malathion photolysis products were the malathion half-acids and a compound identified in the report as *S*-(1,2-dicarbethoxy)ethyl-*O*-methylhydrogen phosphorodithioate, which was interpreted to be:

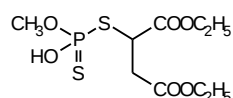


Table 2. Chemical composition and properties of malathion technical material (TC).

Note: maximum limits for impurities take into account the levels produced at manufacture and the increased concentrations generated during 2 years at 20°C.

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 99.0-99.7% (mean 99.3%)
Declared minimum malathion content	950 g/kg
Relevant impurities ≥ 1 g/kg and maximum limits for them:	
Malaoxon CAS No: 1634-78-2 CAS name: butanedioic acid, (dimethoxyphosphinothioyl)-, diethyl ester	1 g/kg
Isomalathion CAS No: 3344-12-5 CAS name: succinic acid, mercaptodiethylester, <i>S</i> -ester with <i>O,S</i> -dimethyl phosphorodithioate	4 g/kg
MeOOSPS-triester CAS No: 2953-29-9 CAS name: phosphorodithioic acid, <i>O,O,S</i> -trimethyl ester	15 g/kg
MeOOOPS-triester CAS No: 152-18-1 CAS name: phosphorothioic acid, <i>O,O,O</i> -trimethyl ester	5 g/kg
Relevant impurities < 1 g/kg and maximum limits for them:	None
Stabilisers or other additives and maximum limits for them:	None
Melting point	Below -20°C (malathion purity 99.1%)
Boiling point	Not determined due to decomposition.

Toxicological summaries

Some of the toxicological and ecotoxicological data, included in Tables 3, 4, 5 and 6, below, were derived from malathion having impurity profiles similar to those referred to in Table 2, above. However, some studies were performed using batches of malathion TC with a content of active ingredient below the currently declared minimum content of 950 g/kg. The technical active ingredient used in such studies

was Cythion Technical - a Cyanamid product, which is no longer produced. In addition, other studies were performed using batches of malathion TC that had impurity profiles and malathion contents which were unknown to the proposer, Cheminova A/S. In the following tables, Cythion is Cyanamid's trade name for malathion and Fyfanon is Cheminova's trade name for malathion.

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

Note: conclusions are those of the JMPR, where JMPR reviewed the study; in other cases the results are the conclusions of the study author.

Species	Test	Duration and conditions or guideline adopted	Result	Purity	Ref.
Acute Toxicology					
Rat M/F	Acute oral LD ₅₀ study in albino rats	EPA 81-1	Males: LD ₅₀ = 1768 mg/kg bw Females: LD ₅₀ = 1539 mg/kg bw	Malathion (Cythion) technical purity 94.6%	Fischer, 1991a
Rat M/F	Acute oral LD ₅₀ study in albino rats	EPA 81-1	Males: LD ₅₀ = 6156 mg/kg bw Females: LD ₅₀ = 4061 mg/kg bw	Malathion (Fyfanon) technical, purity 96.8%	Fischer, 1991b JMPR, 1997
Rat M/F	Acute oral LD ₅₀ in rats	No reference to guideline in the report. Study protocol similar to that in Annex II to Commission Directive 92/69/EEC	Combined males and females, LD ₅₀ = 5000 ± 385 mg/kg bw	Malathion (Fyfanon) technical purity not specified.	Terrell, 1978 JMPR, 1997
Rat M/F	Acute oral LD ₅₀ in rats	No reference to guideline in the report. Study protocol similar to that in Annex II to Commission Directive 92/69/EEC	Males: LD ₅₀ = 3800 mg/kg bw Female: LD ₅₀ = 4400 mg/kg bw	Malathion technical (Fyfanon) purity not specified Stored for 1 year at 5°C prior to test.	Terrell, 1979a
Rat M/F	Acute oral LD ₅₀ in rats	No reference to guideline in the report. Study protocol similar to that in Annex II to Commission Directive 92/69/EEC	Males: LD ₅₀ = 3200 mg/kg bw Females: LD ₅₀ = 3700 mg/kg bw	Malathion technical (Fyfanon) purity not specified Stored for 1 year at 20-25°C prior to test.	Terrell, 1979b
Rat M/F	Acute oral toxicity to rats	US-EPA Pesticide Assessment Guidelines, Subdivision F, 81-1(40 CFR Part 158/FIFRA)	Males: LD ₅₀ = 5400 mg/kg bw Females: LD ₅₀ = 5700 mg/kg bw	Malathion technical (Fyfanon) purity 96-98%. Isomalathion content <0.1%	Kynoch, 1986a JMPR, 1997
Rabbit M/F	Acute dermal LD ₅₀ on New Zealand albino rabbits	No guideline	Combined males and females group LD ₅₀ = 8790 ± 480 mg/kg bw	Malathion technical (Fyfanon), purity not specified.	Parke, 1978

Species	Test	Duration and conditions or guideline adopted	Result	Purity	Ref.
Rat M/F	Acute dermal toxicity to rats	US-EPA Pesticide Assessment Guidelines, Subdivision F, #81-2 (40 CFR 158/FIFRA)	Combined males and females group: Dermal LD ₅₀ >2000 mg/kg bw	Malathion technical (Fyfanon), purity 96-98%	Kynoch, 1986b JMPR, 1997
Rat M/F	Acute inhalation toxicity in rats, 4-hour exposure	No reference to guideline in this report, but the study was carried out according to US-EPA Pesticide Assessment Guidelines, Subdivision F, 81-3 (40 CFR 158/FIFRA)	Combined males and females group: LC ₅₀ >5.2 mg/l air	Malathion technical (Fyfanon), purity 96-98%	Jackson, 1986 JMPR, 1997
Rabbit M	Irritant effects on rabbit skin	US-EPA Pesticide Assessment Guidelines, Subdivision F, 81-5 (40 CFR part 158/FIFRA)	A single semi-occluded application to intact rabbit skin for 4 hours showed slight to well-defined transient dermal reactions.	Malathion technical (Fyfanon), purity 96-98%	Liggett and Parcell, 1985a JMPR, 1997
Rabbit M	Irritant effects on the rabbit eye	US-EPA Pesticide Assessment Guidelines, Subdivision F, 81-4 (40 CFR part 158/FIFRA)	Mild, reversible conjunctival reactions.	Malathion technical (Fyfanon), purity 96-98%	Liggett and Parcell, 1985b JMPR, 1997
Guinea pig F	Delayed contact hypersensitivity in the guinea pig	US-EPA Pesticide Assessment Guidelines, Subdivision F, # 81-6 (40 CFR part 158/FIFRA)	No evidence of delayed contact hypersensitivity was found.	Malathion technical (Fyfanon), purity 96-98%	Kynoch and Smith, 1986 JMPR, 1997
Rat M/F	Acute neurotoxicity in rats	US-EPA test guidelines for neurotoxicity screening battery, series 81-8, March 1991.	Dosing at 500, 1000 and 2000 mg/kg bw. No NOAEL, clinical signs occurred in all groups.	Malathion technical (Fyfanon) purity 96.4%	Lamb, 1994a JMPR, 1997
Rat M/F	Acute oral toxicity to rats	US-EPA test guidelines, OPPTS 870.1100 (1998), OECD 401	Combined males and females group: LD ₅₀ = 1857 (1677 to 2057) mg/kg bw	Malathion technical (Fyfanon). Test material 96.2% pure and contained 0.44% isomalathion (by spiking and analysis).	Moore, 2002
Rat M/F	Acute oral toxicity to rats	US-EPA test guidelines, OPPTS 870.1100 (1998), OECD 401 (1987)	Males: LD ₅₀ = 2687 (2122 to 3471) mg/kg bw Females: LD ₅₀ = 2098 (1608 to 2550) mg/kg bw	Malathion technical (Fyfanon). Purity 96%, isomalathion content 0.2%	Moore, 2003

Species	Test	Duration and conditions or guideline adopted	Result	Purity	Ref.
Rat M/F	Acute dermal	US-EPA test guidelines, OPPTS 870.1200 (1998), OECD 402 (1987)	Males and females Dermal LD ₅₀ = 2000 mg/kg bw	Malathion technical (Fyfanon). Test material 96.2% pure and contained 0.44% isomalathion (by spiking and analysis).	Bollen, 2003a
Rat M/F	Acute inhalation, 4 hours, nose-only exposure	US-EPA test guidelines, OPPTS 870.1300 (1998), OECD 403 (1981)	Males and females LC ₅₀ > 5.20 mg/l air	Malathion technical (Fyfanon). Test material 96.2% pure and contained 0.44% isomalathion (by spiking and analysis).	Decker <i>et al.</i> 2003
Rabbit M/F	Dermal irritant effects	US-EPA test guidelines, OPPTS 870.2500 (1998), OECD 404 (2002)	A single semi-occlusive application to intact rabbit skin for 4 hours produced no skin reactions	Malathion technical (Fyfanon). Test material 96.2% pure and contained 0.44% isomalathion (by spiking and analysis).	Bollen, 2003b
Rabbit F	Irritant effects on the rabbit eye	US-EPA test guidelines, OPPTS 870.2400 (1998), OECD 405 (2002)	Mild reversible conjunctival reaction. Marked signs of corneal and conjunctival irritation were observed. At 24 hours, no abnormalities were observed.	Malathion technical (Fyfanon). Test material 96.2% pure and contained 0.44% isomalathion (by spiking and analysis).	Bollen, 2003c
Guinea pig F	Delayed contact hypersensitivity	US-EPA test guidelines, OPPTS 870.2600 (1998), OECD 406 (1992)	In a guinea pig maximization test, delayed hypersensitivity was seen in 8 out of 19 animals	Malathion technical (Fyfanon). Test material 96.2% pure and contained 0.44% isomalathion (by spiking and analysis).	Bollen, 2003d
Mouse	Local lymph node assay	US-EPA test guidelines, OPPTS 870.2600 (1998), OECD 429 (2002)	In a murine local lymph node assay, malathion was found to be a non-sensitizer when tested at concentrations of up to 100% (undiluted)	Malathion technical (Fyfanon). Test material 96.2% pure and contained 0.44% isomalathion (by spiking and analysis).	Wang-Fan, 2003

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

Note: conclusions are those of the JMPR, where JMPR reviewed the study; in other cases the results are the conclusions of the study author.

Species	Test	Duration and conditions or guideline adopted	Result	Purity	Ref.
Short term studies (sub-chronic)					
Rat M/F	A 28-day dietary study in rats	OECD 407	NOAEL = 500 ppm (52 mg/kg bw/day)	Malathion technical (Fyfanon), purity 96.4%	Daly, 1993a JMPR, 1997
Dog M/F	A 28-day oral study in Beagle dogs.	No reference to guideline in the report. The study followed a protocol similar to that described in Annex II to Commission Directive 87/302/EEC of November 1987	Dosing at 125, 250 and 500 mg/kg bw/day. Clinical symptoms were noted at all dose levels. A NOEL or NOAEL could not be established.	Malathion technical (Cythion), purity 92.4%	Fischer <i>et al.</i> , 1988 JMPR, 1997
Rat M/F	A 3-month dietary study in rat	FIFRA, Subdivision F, Test Guideline #82-1 and OECD Health Effects Testing Guideline #408	NOAEL = 500 ppm (34 mg/kg bw/day)	Malathion technical (Fyfanon), purity 96.4%	Daly, 1993b JMPR, 1997
Dog M/F	One-year dietary study in Beagle dogs	US-EPA Pesticide Assessment Guidelines, Subdivision F, # 83-1 (40 CFR part 158/FIFRA)	NOAEL = 125 mg/kg bw/day	Malathion technical (Cythion), purity 95%	Schellenberger and Billups, 1987 JMPR, 1997
Rabbit M/F	21-day dermal toxicity study in rabbits	US-EPA Pesticide Assessment Guidelines, Subdivision F#82-2	NOAEL = 300 mg/kg bw/day	Malathion technical (Cythion), purity 94%	Moreno, 1989 JMPR, 1997
Rat M/F	A 13-week (6 hours a day, 5 days a week) whole body inhalation study in rat	US-EPA Pesticide Assessment Guidelines, Subdivision F, 82-4 (40 CFR part 158/FIFRA). Doses 0.1, 0.45, 2.0 mg/l	NOAEL (cholinesterase inhibition) = 0.1 mg/l. An overall NOAEL could not be established due to histopathological findings in the respiratory system at all dose levels.	Malathion technical (Fyfanon), purity 96.4%	Beattie, 1994
Chronic toxicity					
Rat M/F	A 24-month dietary toxicity and oncogenicity study in rat	US-EPA Pesticide Assessment Guidelines, Subdivision F, 83-5 (40 CFR part 158/FIFRA)	NOAEL = 500 ppm (29 mg/kg bw/day)	Malathion technical (Fyfanon), purity 96.4%	Daly, 1996 JMPR, 1997
Rat M/F	A 24-month dietary toxicity and oncogenicity study in rat	The study was conducted before modern guidelines were established.	Overall NOAEL: 100 ppm (equivalent to 5 mg/kg bw/day)	Malathion technical (Cythion), purity 92.1%	Rucci <i>et al.</i> , 1980 JMPR, 1997

Species	Test	Duration and conditions or guideline adopted	Result	Purity	Ref.
Mouse M/F	18-month dietary oncogenicity study in mice	US-EPA Pesticide Assessment Guidelines, Subdivision F, 83-2 (40 CFR part 158/FIFRA)	Overall NOAEL: 800 ppm (equivalent to 140 mg/kg bw/day)	Malathion technical (Fyfanon), purity 96.4%	Slauter, 1994 JMPR, 1997
Reproduction and developmental toxicity					
Rat M/F	Two-generation (two-litters) reproduction study in rats	US-EPA Pesticide Assessment Guidelines, Subdivision F, # 83-4 (40 CFR part 158/FIFRA)	NOAEL for reproduction and non-reproductions toxicity in parental animals: 7500 ppm (equivalent to 600mg/kg bw/day) NOAEL for developmental toxicity: 1700 ppm (equivalent to 130 mg/kg bw/day)	Malathion technical (Cythion), purity 94%	Schroeder, 1990 JMPR, 1997
Rat M/F	Developmental toxicity (embryo-fetal toxicity and teratogenicity) pilot study in rats	No reference to test guideline. Study protocol similar to that described in Annex II to Commission Directive 87/302/EEC of November 1987, but significant deviations	NOAEL, maternal toxicity: <300 mg/kg bw/day NOAEL, developmental toxicity: >1000 mg/kg bw/day	Malathion technical (Cythion), purity: 94%	Lochry, 1988
Rat M/F	Developmental toxicity study in rats	US-EPA Pesticide Assessment Guidelines, Subdivision F, # 83-3	NOAEL, maternal toxicity: 400 mg/kg bw/day NOAEL, teratogenicity: >800 mg/kg bw/day	Malathion technical (Cythion), purity: 94%	Lochry, 1989 JMPR, 1997
Rabbit M/F	Range-finding teratology study in rabbits	No reference to test guideline. Study protocol similar to that described in Annex II to Commission Directive 87/302/EEC of November 1987, but significant deviations	NOAEL, maternal toxicity: 100 mg/kg bw/day NOAEL, developmental toxicity: >400 mg/kg bw/day	Malathion technical (Cythion), purity 92.4%	Siglin <i>et al.</i> , 1985a
Rabbit M/F	Teratology study in rabbits	No reference to test guideline. Study protocol similar to that described in Annex II to Commission Directive 87/302/EEC of November 1987	NOAEL, maternal toxicity: 25 mg/kg bw/day NOAEL, developmental toxicity: >100 mg/kg bw/day	Malathion technical (Cythion), purity 92.4%	Siglin <i>et al.</i> , 1985b JMPR, 1997
Neurotoxicity studies					
Hens	42-Day neurotoxicity study in hens	US-EPA Pesticide Assessment Guidelines, Subdivision F, 81-7 (40 CFR part 158/FIFRA)	All dosed animals showed clinical symptoms. Malathion does not induce acute delayed neurotoxicity in hens.	Malathion technical (Cythion), purity 93.6%	Fletcher, 1989 JMPR, 1997

Species	Test	Duration and conditions or guideline adopted	Result	Purity	Ref.
Rat MF	Sub-chronic (13-week) neurotoxicity study in rats	US-EPA Pesticide Assessment Guidelines, Subdivision F, # 82-1(40 CFR part 158/FIFRA)	Overall NOEL was 5000 ppm (equiv 350 mg/kg bw/day)	Malathion technical (Fyfanon), purity 96.4%	Lamb, 1994b JMPR, 1997

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

The results are the conclusions of the JMPR where JMPR has reviewed the study; in other cases the results are the conclusions of the study author.

Species	Test	Duration and conditions or guideline adopted	Result	Purity	Ref.
Genotoxicity Studies					
<i>in vitro</i> <i>Salmonella typhimurium</i>	Bacterial microsome mutagenicity test	OECD 471 (1984) and US-EPA TSCA Guidelines, Final Rules FR 50, No 188 (1985)	Malathion was not mutagenic in presence or absence of metabolic activity.	Malathion technical (Cythion), purity 95.2%	Traul, 1987 JMPR, 1997
<i>in vitro</i> rat primary hepatocytes	Unscheduled DNA synthesis	US-EPA (FIFRA) 84-4	Malathion tested negative in the UDS assay.	Malathion technical (Cythion), purity 94%	Pant, 1989 JMPR, 1997
<i>in vitro</i> human lymphocytes	Mammalian chromosome aberration test	OECD 473 (1997)	Malathion technical was clastogenic in the absence of metabolic activation, at a test concentration that caused moderate toxicity	Malathion technical (Fyfanon), purity 96%	Edwards, 2001a
<i>In vitro</i> mouse lymphoma cells (L5187Y).	Mammalian cell gene mutation test	OECD 476 (1997)	Malathion caused a dose-related response on cloning efficacy, growth rate and mutation frequency. There were statistically significant increases in mutation frequency at the upper dose levels. The positive findings were associated with marked cytotoxicity.	Malathion technical (Fyfanon), purity 96%	Edwards, 2001b
<i>In vivo</i> rat bone marrow cells	Chromosome aberration	OECD 475 (1981) US-EPA 84-2 (TSCA Test Guideline, 40 CFR 798.5375 (1985)	Malathion did not induce chromosome aberrations in the bone marrow of rats.	Malathion technical (Cythion), purity 94.0%	Gudi, 1990 JMPR, 1997
<i>In vivo</i> rat hepatocytes	<i>In vivo</i> unscheduled DNA synthesis (UDS)	OECD 486 (1997)	Malathion was not genotoxic in the DNA-repair assay, at dose levels up to 2000 mg/kg body weight	Malathion technical (Fyfanon), purity 96.0%	Meerts, 2002

Table 6. Ecotoxicology profile of technical malathion.

Species	Test	Duration and conditions	Result	Purity	Reference
Fish					
<i>Cyprinodon variegatus</i> (Sheepshead minnow)	Acute toxicity, flow-through system: US EPA, EPA-660/3-75-009, April 1979, minor deviations	96 hours at 21 to 23°C	24 h LC ₅₀ = 0.043 mg/l 48 h LC ₅₀ = 0.043 mg/l 96 h LC ₅₀ = 0.040 mg/l NOEC = 0.018 mg/l	Malathion (Cythion), technical, purity 94%	Bowman, 1989
<i>Lepomis macrochirus</i> (Bluegill sunfish)	Acute toxicity, flow-through system: OECD 203	96 hours at 23 ± 1°C	24 h LC ₅₀ = 0.12 mg/l 48 h LC ₅₀ = 0.12 mg/l 72 h LC ₅₀ = 0.076 mg/l 96 h LC ₅₀ = 0.054 mg/l NOEC = 0.032 mg/l	Malathion (Fyfanon) technical, purity 96.9%	Gries and Purghart, 2001a
<i>Oncorhynchus mykiss</i> (Rainbow trout)	Acute toxicity, flow-through system: OECD 203	96 hours at 16 ± 1°C	24-h LC ₅₀ = 0.41 mg/l 48 h LC ₅₀ = 0.37 mg/l 72-h LC ₅₀ = 0.27 mg/l 96-h LC ₅₀ = 0.18 mg/l NOEC = 0.091 mg/l	Malathion technical (Fyfanon), purity 96.9%	Gries and Purghart, 2001b
<i>Cyprinus carpio</i> (Common carp)	Acute toxicity, flow-through system: OECD 203	96 hours at 23 ± 1°C	96 h LC ₅₀ = >10 mg/l NOEC = 1 mg/l	Malathion technical (Fyfanon), purity 96.9%	Gries and van der Kolk, 2002
<i>Gasterosteus aculeatus</i> (Three-spined stickleback)	Acute toxicity, flow-through system: OECD 203	96 hours at 10 ± 2°C	96 h LC ₅₀ = 0.022 mg/l NOEC = 0.005 mg/l	Malathion technical (Fyfanon), purity 96.9%	Gries <i>et al.</i> , 2002b
<i>Pimephales promelas</i> (Fathead minnow)	Acute toxicity, flow-through system: OECD 203	96 hours at 23 ± 1°C	96 h LC ₅₀ = >8.0 mg/l NOEC = 0.98 mg/l	Malathion technical (Fyfanon), purity 96.9%	Gries <i>et al.</i> , 2002a
<i>Oncorhynchus mykiss</i> (Rainbow trout) fertilised embryos, <8 hours.	Early life stage, flow through system: US-EPA (FIFRA) E 72-4	Exposure time 97 days at 7.8 to 13.6°C	NOEC = 0.021 mg/l	Malathion technical (Cythion), purity 94%	Cohle, 1989
Daphnia					
<i>Daphnia magna</i> (Daphnids, water flea) <24 hours old	Chronic toxicity, flow-through system: OECD 202-I	48 hours at 20 ± 1°C	48 h EC ₅₀ = 0.72 µg/l. NOEC = 0.21 µg/l	Malathion technical (Fyfanon), purity 96.9%	Gries and Purghart, 2001c
<i>Daphnia magna</i> (Daphnids, water flea) <24 hours old	Chronic toxicity, flow-through system: OECD 202, May 1981	21 days at 21 to 22°C	NOEC = 0.06 µg/l	Malathion technical (Cythion), purity 94%	Blakemore and Burgess, 1990

Species	Test	Duration and conditions	Result	Purity	Reference
Algae					
<i>Selenastrum capricornutum</i> (Green alga, unicellular)	Effect on growth, static water: OECD 201, June 1984	72 hours at 22 to 23°C	24 h E_rC_{50} = 12.6 mg/l. E_bC_{50} = 8.73 mg/l. 48 h E_rC_{50} = 12.7 mg/l. E_bC_{50} = 5.16 mg/l. 72 h E_rC_{50} = 13.0 mg/l. E_bC_{50} = 4.06 mg/l. NOEC (72 h) = 2.30 mg/l (growth rate) NOEC (72 h) = 0.811 mg/l (biomass production)	Malathion technical (Fyfanon), purity 96.4%	Jenkins, 1993
Birds					
<i>Colinus virginianus</i> (Bobwhite quail)	Acute oral toxicity, US EPA FIFRA § 163.71-1	single dose, 14 days observation	Acute oral LD_{50} , = 359 mg/kg bw NOEL = 195 mg/kg bw	Malathion technical (Fyfanon), purity 96.0%	Rodgers, 2002
<i>Colinus virginianus</i> (Bobwhite quail), <i>Anas platyrhynchos</i> (Mallard duck) <i>Coturnix japonica</i> (Japanese quail) <i>Phasianus colchicus</i> (Ring-necked pheasants)	Short-term dietary toxicity	5 days, in feed + 3 days post-dosing observation	Bobwhite quail: LC_{50} = 3497 ppm diet Mallard duck: LC_{50} = >5000 ppm diet Japanese quail: LC_{50} = 2962 ppm diet Ring-necked pheasant: LC_{50} = 2639 ppm diet	Malathion technical, purity 95%	Hill <i>et al.</i> , 1975
<i>Colinus virginianus</i> (Bobwhite quail)	Sub-chronic toxicity and reproduction: OECD 206; US EPA (FIFRA) E 71-4	21 weeks dosing, average 23°C and 70% relative humidity	NOEC = 350 ppm (reproductive parameters), NOEC = 110 ppm (sub-chronic toxicity)	Malathion technical (Fyfanon), purity 96.4%	Beavers <i>et al.</i> 1995
<i>Colinus virginianus</i> (Bobwhite quail)	Sub-chronic toxicity and reproduction: US EPA (FIFRA) E 71-4	21 weeks dosing, average min-max 19-21°C, 34-81% relative humidity	NOEC = 300 ppm diet (highest concentration tested)	Malathion technical (Cythion), purity 94.0%	Pedersen, 1989
<i>Anas platyrhynchos</i> (Mallard duck)	Sub-chronic toxicity and reproduction: US EPA (FIFRA) E 71-4	20 weeks dosing, average 16°C and 88% relative humidity	NOEC = 1200 ppm diet (reproductive parameters)	Malathion technical (Cythion), purity 94.0%	Pedersen and Fletcher, 1993
Bees					
<i>Apis mellifera</i> (Worker honey bee)	Acute toxicity	Single dose	acute contact LD_{50} , = 0.27 µg/bee acute oral LD_{50} , = 0.38 µg/bee	Malathion technical purity >95%	Stevenso n, 1978

Earthworms					
<i>Eisenia foetida foetida</i> (Earthworm)	Acute toxicity: OECD 207, April 1984	14 days exposure, 18.5 to 22°C, pH 7.5 to 7.8	14 day LC ₅₀ = 613 mg/kg soil NOEC = 246 mg/kg soil	Malathion technical (Fyfanon), purity 96.2%	Wüthrich, 1991

Malathion was evaluated by the WHO/IPCS and the FAO/WHO JMPR in 1963, 1965, 1966, 1968, 1969, 1970, 1973, 1975, 1977, 1984, 1997, 1999 and 2000. The ADI for malathion is 0-0.3 mg/kg bw (JMPR, 1997).

The IPCS hazard classification of malathion is Class III (slightly hazardous).

Potentially relevant manufacturing impurities

JSCI (2000) reported on the influence of impurities in malathion on its toxicity. Some impurities are toxic in their own right and at least one of them strongly potentiates the toxicity of malathion. In mammals, the relative safety of malathion, compared with many other cholinesterase inhibitors, has been attributed to the rapid hydrolytic degradation by carboxylesterases. Impurities that inhibit carboxylesterase activity have the ability to potentiate the toxicity of malathion and therefore it is important to control the impurity profile of malathion products.

Jensen (2001) listed six impurities which, because of their toxicological properties, should be considered in this context. Jensen (2000) provided a brief summary of the toxicities of the compounds, which are:

- isomalathion, CAS 2244-12-5)
- malaaxon, CAS 1634-78-2
- MeOSSPO-triester, CAS 22608-53-3
- MeOOSPO-triester, CAS 152-20-5
- MeOOOPS-triester, CAS 152-18-1
- MeOOSPS-triester, CAS 2953-29-9

Isomalathion (rat oral LD₅₀ 113 mg/kg bw, and a potentiator of malathion-induced toxicity) and malaaxon (rat oral LD₅₀ 215 mg/kg bw) are more toxic than malathion. Their manufacturing limits are 1 g/kg or greater and therefore they were proposed as relevant impurities.

MeOSSPO-triester (reported LD₅₀ 26 mg/kg bw) and MeOOSPO-triester (reported LD₅₀ 47 mg/kg bw) are also more toxic than malathion but neither of them had been detected in the technical malathion manufactured by Cheminova. In addition, they were not generated during storage for 2 years at 20°C. Therefore they were not proposed as relevant impurities in the malathion manufactured as described in the present data submission.

MeOOOPS-triester (reported rat oral LD₅₀ 562 mg/kg bw) and MeOOSPS-triester (reported rat oral LD₅₀ 628 mg/kg bw, and a potentiator of malathion induced toxicity) occur at levels of approximately 2 and 12 g/kg respectively in the technical malathion manufactured by Cheminova. Levels of these impurities do not increase during 20°C storage for 2 years. MeOOSPS-triester and MeOOOPS-triester were both proposed as relevant impurities.

Formulations

The main formulation types available are:

- Dustable powders, DP,
- Ultra-low volume liquids, UL,
- Emulsifiable concentrates, EC,
- Emulsions, oil in water, EW.

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

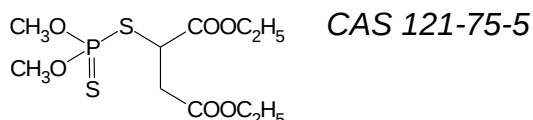
Methods of analysis and testing

With a range of impurities and the active ingredient to be determined in malathion products, a single analytical method cannot be used for all combinations and a range of methods has been developed.

Table 7. Chemical analytical methods for active ingredient (including identity tests) in malathion technical material and formulations

Analyte	Product	Method Code	Method
Malathion	TC EC EW DP	VAM 001-02	GC
Malaoxon	TC	VAM 008-02	HPLC
Malaoxon	DP	VAM 208-01	HPLC
Malaoxon	EW	VAM 202-01	³¹ P-NMR spectroscopy
Malaoxon	EC	VAM 203-01	³¹ P-NMR spectroscopy
Isomalathion	TC, EW, DP	VAM 005-03	HPLC
Isomalathion	EC	VAM 203-01	³¹ P-NMR spectroscopy
MeOOSPS-triester	TC	VAM 006-02	GC
MeOOSPS-triester	TC EW DP	VAM 206-01	GC
MeOOSPS-triester	EC	VAM 203-01	³¹ P-NMR spectroscopy
MeOOOPS-triester	EC	VAM 203-01	³¹ P-NMR spectroscopy

Malathion in TC, EC, EW, DP

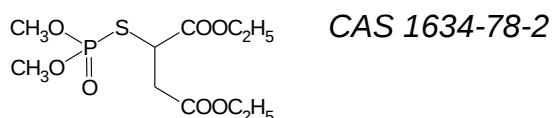


Malathion is determined by gas chromatography (GC) using a non-polar capillary column and FID, with quantification by internal standardization (Sørensen, 2002a). It is the same as Method VAM 001-02 (Knold, 2002a).

The method was collaboratively tested, shown to be suitable for malathion TC, EC, EW and DP (Sørensen, 2002b) and adopted by CIPAC in 2002 (CIPAC, 2002).

IR is used as an identity test for malathion TC, EC, EW and DP (Sørensen, 2002a).

Malaoxon in TC, EC, EW, DP



Malaoxon in the TC is determined by reversed phase liquid chromatography (HPLC), using a C18 column and UV detection with quantification by external standard

(Method VAM 008-02, Hinz, 2001a). The absorption at 215 nm is used to determine malaoxon.

In Method VAM 208-01 for DP formulations, the sample portion is sonicated with acetonitrile prior to HPLC analysis (Hinz, 2002c). The signal at 230 nm is used to determine malaoxon.

Method VAM 202-01 uses ^{31}P -NMR spectroscopy to measure malaoxon content in EW formulations, because simpler detection techniques are subject to interference from formulants in these liquid formulations. A portion of the test formulation is dissolved in 10% deuterated acetone in acetone and the molar ratio between malaoxon and malathion is determined by ^{31}P -NMR spectroscopy. The malaoxon content of the formulation is then calculated from the malathion content determined by GC (Hald, 2002a). Method VAM 203-01 is a similar procedure, but with sample dissolved in deuterated chloroform, applied to EC formulations (Hald, 2002c).

Validation data for malaoxon in malathion technical material and formulations are summarized in Table 8.

Table 8. Validation data for malaoxon in malathion TC and formulations.

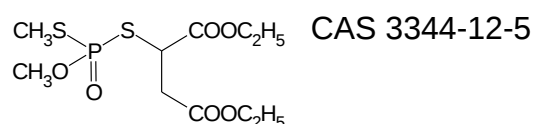
Substrate	Test (note 1)	Result (note 2)
Method VAM 008-02 (HPLC). Validation: Hinz, 2001b.		
TC	Linearity	Acceptable linearity over test range, 7-53 mg/kg in injection solution.
	Specificity	Interference did not occur from the following compounds, with retention times as shown: MeOOSPO-triester 3.4 min, MeOSSPO-triester 4.3 min, diethyl maleate 9.5 min, MeOOOPS-triester 9.6 min, mixed ester of malathion 9.9 min, malaoxon 10.8 min, malathion 13.8 min.
	Precision (of recovery)	$S_r = 0.022$ and 0.017 at malaoxon levels of 0.041% and 0.16% of the ai respectively ($n=5$).
	LOQ	0.04% of the ai.
	Recovery	Mean recovery ($n=5$) 105% at 0.041% of the ai and 106% at 0.16% of the ai.
Method VAM 008-02 (HPLC). Validation: Duff, 2002b.		
TC	Linearity	Acceptable linearity over test range 0.05 - 1.0% of the ai.
	Specificity	Some interference from unidentified material in TC.
	Precision (of recovery)	$S_r = 0.094$, 0.033 and 0.020 at malaoxon levels of 0.05% , 0.3% and 1% of the ai respectively ($n=5$).
	LOQ	0.05% of the ai.
	Recovery	Mean recovery ($n=5$) 119% at 1% of the ai, 111% at 0.3% of the ai, 89% at 0.05% of the ai.
Method VAM 208-01 (HPLC). Validation: Hinz, 2002d.		
DP	Linearity	Acceptable linearity over test range, 2-54 mg/kg in injection solution.
	Specificity	A blank DP formulation did not cause interference with malaoxon determination.
	Precision (of recovery)	$S_r = 0.051$ and 0.031 at malaoxon levels of 0.0032% and 0.013% of a "DP placebo" respectively ($n=5$).
	LOQ	0.08% of the ai.
	Recovery	Mean recovery ($n=5$) 100% at 0.0032% and 96% at 0.013% of a "DP placebo" (equivalent to 0.08% and 0.33% of the ai for a 4% DP).

Substrate	Test (note 1)	Result (note 2)	
Method VAM 202-01 (³¹ P NMR). Validation: Hald, 2002b.			
EW	Linearity	Acceptable linearity over test range 0.14-2.3% of the ai and 0.58-11% of the ai in two test formulations.	
	Specificity	No overlap in response from malaoxon at 0.029 g/l or other impurities in fresh samples or samples stored for 2-years.	
	Precision	S _r = 0.062, 0.019, 0.046, 0.029 and 0.026 at malaoxon levels of 0.25%, 0.58%, 1.2% 1.4% and 6.7% of the ai respectively (n=5).	
	LOQ	0.25% of the ai	
	Recovery	Mean recovery (n=5) 100% at 0.25% of the ai, 99% at 1.2% of the ai, 98% at 1.4% of the ai, 99% at 6.7% of the ai.	
Method VAM 203-01 (³¹ P NMR). Validation: Hald, 2002d.			
EC	Linearity	Acceptable linearity over test range 0.06-1.5% of the ai (tests on 3 formulations).	
	Specificity	No overlap in responses from malaoxon and from other impurities.	
	Precision	S _r (n=5)	malaoxon as % of the ai
		0.12	0.06%
		0.058	0.10%
		0.10	0.11%
		0.020	0.34%
0.015		0.58%	
0.020	0.62%		
LOQ	0.06% of the ai.		
Recovery	Mean (5 replicates) recovery (3 samples × 2 fortification levels) 93-98% at malaoxon levels of 0.06-0.62% of the ai.		

Note 1: LOQ: lowest concentration tested at which an acceptable mean recovery and relative standard deviation are obtained.

Note 2: S_r is relative standard deviation.

Isomalathion in TC, EC, EW, DP



Isomalathion is determined by reversed phase liquid chromatography (HPLC), using an ODS2 column and UV detection with quantification by external standard (Method VAM 005-03, Petersen 2001a). The absorption at 200 nm is used for measurement of the isomalathion concentration, while the absorption at 225 nm is used to check for the presence of interfering compounds.

TC, EW or DP samples are prepared by weighing into a bottle and mixing with 75% v/v acetonitrile/water. In the case of EW and DP, dissolution is assisted by ultrasonication. The solutions are centrifuged, if necessary, and the clear solution is ready for HPLC analysis.

Method VAM 203-01 uses ³¹P-NMR spectroscopy to measure the isomalathion content of EC formulations. A portion of the test formulation is dissolved in deuterated chloroform and the molar ratio between isomalathion and malathion is determined by ³¹P-NMR spectroscopy. The isomalathion content in the formulation is then calculated from the malathion content determined by GC (Hald, 2002c).

Validation data for isomalathion in malathion technical material and formulations are summarized in Table 9.

Table 9. Validation data for isomalathion in malathion TC and formulations.

Substrate	Test (note 1)	Result (note 2)
Method VAM 005-03 (HPLC). Validation: Petersen, 2001b.		
TC	Linearity	Acceptable linearity over test range, 7.6-182 mg/kg in injection solution
	Specificity	Isomalathion was separated from the active ingredient, malathion. It was also separated from diethyl fumarate, diethyl methylthiosuccinate and MeOOSPS-triester.
	Precision	$S_r = 0.0144$ and 0.0254 at isomalathion levels of 0.078% and 0.047% of the ai respectively ($n=5$).
	LOQ	0.03% of the ai.
	Recovery	Mean recovery ($n=5$) 120% at 0.03% of the ai and 104% at 0.54% of the ai.
EW 92 g/l	Precision	$S_r = 0.043$ and 0.087 at isomalathion levels of 0.12% and 0.11% of the ai respectively ($n=4$) (note 3).
	LOQ	0.13% of the ai.
	Recovery	Mean recovery ($n=5$) 100.2% at 0.13% of the ai and 102% at 0.55% of the ai.
DP 4%	Precision	$S_r = 0.090$ and 0.115 at isomalathion levels of 0.36% and 0.40% of the ai respectively ($n=5$).
	LOQ	0.025% of the ai
	Recovery	Mean recovery ($n=5$) 95% at 0.024% of the ai and ($n=4$) 96% at 0.50% of the ai (note 4).
Method VAM 005-03 (HPLC). Validation: Duff, 2002a.		
TC	Linearity	Acceptable linearity over test range, 15-300 mg/kg in injection solution.
	Specificity	Not tested.
	Precision	$S_r = 0.0113$ at isomalathion level of 0.15% of the ai ($n=5$).
	LOQ	0.05% of the ai.
	Recovery	Mean recovery ($n=5$) 99.6% at 0.05% of the ai and 104% at 1.0% of the ai.
EW 92 g/l	Precision	$S_r = 0.086$ at isomalathion level of 0.05% of the ai ($n=5$).
	LOQ	0.2% of the ai.
	Recovery	Mean recovery ($n=5$) 189% at 0.05% of the ai, 98% at 0.2% of the ai and 109% at 1.0% of the ai.
DP 4%	Precision	$S_r = 0.0137$ at isomalathion level of 0.62% of the ai ($n=5$).
	LOQ	0.05% of the ai.
	Recovery	Mean recovery ($n=5$) 83% at 0.05% of the ai and 121% at 1.0% of the ai.
Method VAM 203-01 (^{31}P NMR). Validation: Hald, 2002d.		
EC	Linearity	Acceptable linearity over test range 0.06 - 2.3% of the ai (tests on 3 formulations).
	Specificity	No overlap in responses from isomalathion and from other impurities.
	Precision	$S_r = 0.051$, 0.056 and 0.031 at isomalathion levels of 0.17% , 0.20% and 0.30% of the ai respectively ($n=5$).
	LOQ	0.16% of the ai.
	Recovery	Mean (5 replicates) recovery (3 samples $\times$ 2 fortification levels) 95 - 114% at isomalathion levels of 0.16 - 1.0% of the ai.

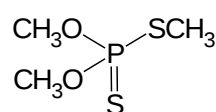
Note 1: LOQ: lowest concentration tested at which an acceptable mean recovery and relative standard deviation are obtained.

Note 2: S_r is relative standard deviation.

Note 3: In each of the EW precision tests, 1 of the 5 values was treated as an outlier and the mean was calculated on the remaining 4.

Note 4: One recovery value of 81.2% for isomalathion in DP was treated as an outlier and not included in the mean.

MeOOSPS-triester, in TC, EC, EW, DP



CAS 2953-29-9

MeOOSPS-triester in technical malathion is determined by gas chromatography (GC), using a non-polar capillary column and FID with quantification by an external standard (Method VAM 006-02: Sørensen, 2002c, Knold, 2002c). A portion of test material is weighed into a sample bottle and diluted with acetonitrile, ready for GC analysis. Method VAM 206-01 is essentially the same method which was applied to technical malathion and EW and DP formulations (Knold, 2002f).

Method VAM 203-01 uses ^{31}P -NMR spectroscopy to measure MeOOSPS-triester content in EC formulations. A portion of the test formulation is dissolved in deuterated chloroform and the molar ratio between MeOOSPS-triester and malathion is determined by ^{31}P -NMR spectroscopy. The MeOOSPS-triester content in the formulation is then calculated from the malathion content determined by GC (Hald, 2002c).

Validation data for MeOOSPS-triester in malathion technical material and formulations are summarised in Table 10.

Table 10. Validation data for MeOOSPS-triester in malathion TC and formulations.

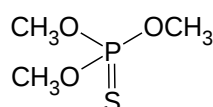
Substrate	Test (note 1)	Result (note 2)
Method VAM 006-02 (GC). Validation: Knold, 2002b.		
TC	Linearity	Acceptable linearity over test range, 72-297 mg/kg in injection solution (n=3).
	Specificity	Absence of interference from malathion and 8 impurities was demonstrated.
	Precision	$S_r = 0.009, 0.004$ and 0.006 at MeOOSPS-triester levels of 0.87%, 1.01% and 1.17% of the ai respectively (n=5).
	LOQ	0.1% of the ai.
	Recovery	mean recovery (n=5) 104% at 0.105% of the ai and 102% at 1.49% of the ai.
Method VAM 203-01 (^{31}P NMR). Validation: Hald, 2002d.		
EC	Linearity	Acceptable linearity over test range 0.06-4.5% of the ai (tests on 3 formulations).
	Specificity	No overlap in responses from MeOOSPS-triester and from other impurities, except for a peak from a minor impurity that can be separated by careful integration.
	Precision	$S_r = 0.005, 0.008$ and 0.006 at MeOOSPS-triester levels of 0.87%, 0.94% and 0.97% of the ai respectively (n=5).
	LOQ	0.14% of the ai.
	Recovery	mean (5 rep analyses) recovery (3 samples $\times$ 2 fortification levels) 94-109% at MeOOSPS-triester levels of 0.14-3.3% of the ai.
Method VAM 206-01 (GC). Validation: Knold, 2002g.		
TC	Linearity	Acceptable linearity over test range, 10-388 mg/l in injection solution (n=5).
	Precision	$S_r = 0.002-0.013$ at MeOOSPS-triester levels of 0.91-1.2% in the TC (n=5).

	LOQ	0.08% of the ai.
	Recovery from malathion standard	Mean recovery (n=5) 100% and 106% at 0.084% and 1.9% respectively of the ai.
EW	Precision	$S_r = 0.002-0.006$ at MeOOSPS-triester levels near 1% in the EW (n=5).
	LOQ	0.006% of the EW product.
	Recovery	Mean recovery (n=5) 103% and 104% at 0.006% and 0.13%, respectively, in an "EW placebo".
DP	Precision	$S_r = 0.006-0.016$ at MeOOSPS-triester levels of 0.43-0.57% in the DP product (n=5).
	LOQ	0.003% of the DP product.
	Recovery	Mean recovery (n=5) 99% and 102% at 0.003% and 0.06%, respectively, in a "DP placebo".
Method VAM 206-01 (GC). Validation: Cooney, 2002.		
TC	Linearity	Acceptable linearity over test range, 0.6-202 mg/l in injection solution (n=5).
	Specificity	No interference was apparent when chromatograms of reference standard MeOOSPS-triester were compared with chromatograms of malathion reference standard.
	Precision (of recovery)	$S_r = 0.0082$ and 0.0059 at MeOOSPS-triester levels of 0.1% and 1.5% of the ai, respectively (n=5).
	LOQ	0.1% of the ai
	Recovery	Mean recovery (n=5) 107% at 0.1% of the ai and 100% at 1.5% of the ai.
EW	Specificity	No interference was apparent when chromatograms of reference standard MeOOSPS-triester were compared with chromatograms of an "EW placebo".
	Precision (of recov tests)	$S_r = 0.068$ and 0.021 at MeOOSPS-triester levels of 0.07% and 1.1% of the ai respectively (n=5)
	LOQ	0.07% of the ai
	Recovery	mean recovery (n=5) 89% at 0.07% of the ai and 89% at 1.1% of the ai.
DP	Specificity	no interference was apparent when chromatograms of reference standard MeOOSPS-triester were compared with chromatograms of a "DP placebo".
	Precision (of recovery)	$S_r = 0.0095$ and 0.0077 at MeOOSPS-triester levels of 0.075% and 1.25% of the ai respectively (n=5).
	LOQ	0.075% of the ai.
	Recovery	mean recovery (n=5) 100% at 0.075% of the ai and 99% at 1.25% of the ai.

Note 1: LOQ: lowest concentration tested at which an acceptable mean recovery and relative standard deviation are obtained.

Note 2: S_r is relative standard deviation.

MeOOOPS-triester, in TC, EW, DP



CAS 152-18-1

See also Addendum 1 of this evaluation.

MeOOOPS-triester is determined by gas chromatography (GC), using a non-polar capillary column and FID with quantification by an external standard (Method VAM

006-02: Sørensen, 2002c, Knold, 2002c). A portion of test material is weighed into a sample bottle and diluted with acetonitrile, ready for GC analysis.

MeOOOPS-triester in technical malathion and in EW and DP formulations is determined by gas chromatography (GC), using a non-polar capillary column and FID with quantification by an external standard (Method VAM 206-01: Knold, 2002f).

Validation data for MeOOOPS-triester in malathion technical material are summarised in Table 11.

Table 11. Validation data for MeOOOPS-triester in malathion TC and formulations.

Substrate	Test (note 1)	Result (note 2)
Method VAM 006-02 (GC). Validation: Knold, 2002b.		
TC	Linearity	Acceptable linearity over test range, 16-66 mg/kg in injection solution (n=3).
	Specificity	Absence of interference from malathion and 8 impurities was demonstrated.
	Precision	$S_r = 0.010, 0.005$ and 0.006 at MeOOOPS-triester levels of 0.20%, 0.20% and 0.24% of the ai respectively (n=5).
	LOQ	0.05% of the ai.
	Recovery	Mean recovery (n=5) 101% at 0.046% of the ai and 100% at 0.39% of the ai.
Method VAM 206-01 (GC). Validation: Knold, 2002g.		
TC	Linearity	Acceptable linearity over test range, 3.2-118 mg/l in injection solution (n=5).
	Precision	$S_r = 0.003-0.025$ at MeOOOPS-triester levels of 0.21-0.25% in the TC (n=5).
	LOQ	0.04% of the ai.
	Recovery from malathion standard	mean recovery (n=5) 99% and 107% at 0.041% and 0.59% respectively of the ai.
EW	Precision	$S_r = 0.004-0.007$ at MeOOOPS-triester levels near 0.019% in the EW (n=5).
	LOQ	0.003% of the EW product.
	Recovery	Mean recovery (n=5) 102% and 108% at 0.003% and 0.04%, respectively, in an "EW placebo".
DP	Precision	$S_r = 0.025-0.034$ at MeOOOPS-triester levels of 0.0049-0.0066% in the DP product (n=5).
	LOQ	0.002% of the DP product.
	Recovery	Mean recovery (n=5) 100% and 102% at 0.002% and 0.02%, respectively, in a "DP placebo".
Method VAM 206-01 (GC). Validation: Cooney, 2002.		
TC	Linearity	Acceptable linearity over test range, 1.0-207 mg/l in injection solution (n=5).
	Specificity	No interference was apparent when chromatograms of reference standard MeOOOPS-triester were compared with chromatograms of malathion reference standard.
	Precision (of recov tests)	$S_r = 0.0088$ and 0.011 at MeOOOPS-triester levels of 0.05% and 0.5% of the ai respectively (n=5).
	LOQ	0.05% of the ai.
	Recovery	Mean recovery (n=5) 101% at 0.05% of the ai and 100% at 0.5% of the ai.

Substrate	Test (note 1)	Result (note 2)
EW	Specificity	no interference was apparent when chromatograms of reference standard MeOOOPS-triester were compared with chromatograms of an "EW placebo".
	Precision (of recovery)	$S_r = 0.012$ and 0.0045 at MeOOOPS-triester levels of 0.03% and 0.4% of the ai respectively ($n=5$).
	LOQ	0.03% of the ai.
	Recovery	Mean recovery ($n=5$) 92% at 0.03% of the ai and 90% at 0.4% of the ai.
DP	Specificity	No interference was apparent when chromatograms of reference standard MeOOOPS-triester were compared with chromatograms of a "DP placebo".
	Precision (of recovery)	$S_r = 0.024$ and 0.0043 at MeOOOPS-triester levels of 0.05% and 0.5% of the ai respectively ($n=5$).
	LOQ	0.05% of the ai.
	Recovery	mean recovery ($n=5$) 104% at 0.05% of the ai and 100% at 0.5% of the ai.

Note 1: LOQ: lowest concentration tested at which an acceptable mean recovery and relative standard deviation are obtained.

Note 2: S_r is relative standard deviation.

Methods for impurities were subjected to within-laboratory validation and for impurities in the TC and some formulations were also subjected to independent laboratory validation (Table 12, see also Addendum 1 for further information regarding determination of the MeOOOPS-triester).

Table 12. Summary of validation of methods for determination of impurities in malathion TC and formulations.

Analyte	Product	Method code	Method	LOQ (% of ai)	Validation
Isomalathion	TC	VAM 005-03	HPLC	0.03	independent lab.
Isomalathion	EW	VAM 005-03	HPLC	0.13	independent lab.
Isomalathion	EC	VAM 203-01	^{31}P -NMR spectroscopy	0.16	within lab.
Isomalathion	DP	VAM 005-03	HPLC	0.025	independent lab.
Malaoxon	TC	VAM 008-02	HPLC	0.04	independent lab.
Malaoxon	EW	VAM 202-01	^{31}P -NMR spectroscopy	0.25	within lab.
Malaoxon	EC	VAM 203-01	^{31}P -NMR spectroscopy	0.06	within lab.
Malaoxon	DP	VAM 208-01	HPLC	0.08	within lab.
MeOOSPS-triester	TC	VAM 006-02	GC	0.1	within lab.
MeOOSPS-triester	TC	VAM 206-01	GC	0.1	independent lab.
MeOOSPS-triester	EW	VAM 206-01	GC	0.07	independent lab.
MeOOSPS-triester	EC	VAM 203-01	^{31}P -NMR spectroscopy	0.14	within lab.
MeOOSPS-triester	DP	VAM 206-01	GC	0.075	independent lab.
MeOOOPS-triester	TC	VAM 006-02	GC	0.05	within lab.
MeOOOPS-triester	TC	VAM 206-01	GC	0.05	independent lab.
MeOOOPS-triester	EW	VAM 206-01	GC	0.03	independent lab.
MeOOOPS-triester	DP	VAM 206-01	GC	0.05	independent lab.

Physical and chemical properties

The physical properties, the methods for testing them and the limits proposed for the specifications for TC, DP, UL, EC and EW comply with the requirements of the WHO/FAO Manual (1st edition), with the following two exceptions.

In the proposed DP specification, the clause for malathion content (2.2), included a tolerance range for declared contents above 25 g/kg up to 100 g/kg of -10% to +25% of the declared content, instead of the usual $\pm 10\%$. The proposed +25% tolerance reflected an overage required to offset a significant degradation that may occur in freshly formulated material.

In the proposed UL specification, the active ingredient content is expressed as a minimum (950 g/kg) only, instead of the standard expression ($>500 \pm 25$ g/kg). This is because the UL is, in effect, freshly prepared TC (which must comply with the requirements of a formulation, including storage stability).

Storage stability at elevated temperature

Samples of technical malathion, EC, EW and DP were subjected to storage at 54°C ($\pm 2^\circ\text{C}$), in compliance with CIPAC MT 46.3.1, and were analyzed for content of active ingredient and impurities. The results are summarised in Table 12.

Table 12. Storage stability data for malathion TC and formulations held at 54°C for 14 days.

Compound	Before storage	After storage	Method	Reference
	Impurities as % of malathion content			
EC, 450 g/L, batch 31N				Knold, 2003b
Malathion	439 g/kg	430 g/kg	VAM 001-01	
MeOOSPS-triester	1.01%	0.98%	VAM 203-01	
MeOSSPO-triester	<0.1%	<0.1%	AM 461	
MeOOOPS-triester	0.19%	0.20%	AM 461	
Isomalathion	0.20%	0.28%	VAM 203-01	
MeOOSPO-triester	<0.1%	<0.1%	AM 461	
Malaoxon	<0.06%	<0.06%	VAM 203-01	
EC, 500 g/L, batch 31I				Knold, 2003b
Malathion	473 g/kg	462 g/kg	VAM 001-01	
MeOOSPS-triester	0.98%	0.98%	VAM 203-01	
MeOSSPO-triester	<0.1%	<0.1%	AM 461	
MeOOOPS-triester	0.20%	0.21%	AM 461	
Isomalathion	0.18%	0.19%	VAM 203-01	
MeOOSPO-triester	<0.1%	<0.1%	AM 461	
Malaoxon	<0.06%	<0.06%	VAM 203-01	
EC, 830 g/L, batch 31E				Knold, 2003b
Malathion	711 g/kg	686 g/kg	VAM 001-01	
MeOOSPS-triester	0.99%	1.02%	VAM 203-01	
MeOSSPO-triester	<0.1%	<0.1%	AM 461	
MeOOOPS-triester	0.20%	0.20%	AM 461	
Isomalathion	0.27%	0.52%	VAM 203-01	
MeOOSPO-triester	<0.1%	<0.1%	AM 461	
Malaoxon	<0.06%	<0.06%	VAM 203-01	
EW, 440 g/L, batch 718-AMH-20A				Knold, 2003a
Malathion	404 g/kg	392 g/kg	CIPAC/12/EW/(M3)	
MeOOSPS-triester	1.01%	1.01%	AM 461	
MeOSSPO-triester	<0.1%	<0.1%	AM 461	
MeOOOPS-triester	0.20%	0.19%	AM 461	
Isomalathion	0.15%	0.27%	AM 461	
MeOOSPO-triester	<0.1%	0.10%	AM 461	
Malaoxon	<0.1%	0.12%	VAM 202-01	

Compound	Before storage	After storage	Method	Reference
Impurities as % of malathion content				
EW, 440 g/L, batch 718-AMH-20B				Knold, 2003a
Malathion	408 g/kg	397 g/kg	CIPAC/12/EW/(M3)	
MeOOSPS-triester	1.00%	1.00%	AM 461	
MeOSSPO-triester	<0.1%	<0.1%	AM 461	
MeOOOPS-triester	0.22%	0.20%	AM 461	
Isomalathion	<0.1%	0.17%	AM 461	
MeOOSPO-triester	<0.1%	0.10%	AM 461	
Malaoxon	<0.1%	<0.1%	VAM 202-01	
4DP, lot 49064				Knold, 2003c
Malathion	39.6 g/kg	37.2 g/kg	CIPAC/12/DP/(M3)	
MeOOSPS-triester	1.2%	0.73%	VAM 206-01	
MeOSSPO-triester	0.02%	0.08%	VAM 206-01	
MeOOOPS-triester	0.10%	<0.04%	VAM 206-01	
Isomalathion	0.44%	1.8%	VAM 005-03	
MeOOSPO-triester	0.09%	0.11%	VAM 201-01	
Malaoxon	<0.08%	<0.08%	VAM 208-01	
4DP, lot 48763				Knold, 2003c
Malathion	39.9 g/kg	37.6 g/kg	CIPAC/12/DP/(M3)	
MeOOSPS-triester	1.3%	0.63%	VAM 206-01	
MeOSSPO-triester	0.02%	0.07%	VAM 206-01	
MeOOOPS-triester	0.13%	<0.04%	VAM 206-01	
Isomalathion	0.66%	2.0%	VAM 005-03	
MeOOSPO-triester	0.10	0.11%	VAM 201-01	
Malaoxon	<0.08%	<0.08%	VAM 208-01	
TC, batch no. 20507-02				Knold, 2003d
Malathion	969.4 g/kg	962.6 g/kg	VAM 001-01	
MeOOSPS-triester	0.92%	0.93%	VAM 006-02	
MeOSSPO-triester	<0.01%	<0.01%	VAM 008-02	
MeOOOPS-triester	0.23%	0.23%	VAM 006-02	
Isomalathion	0.08%	0.29%	VAM 005-03	
MeOOSPO-triester	<0.05%	<0.05%	VAM 008-02	
Malaoxon	<0.04%	<0.04%	VAM 008-02	
TC, batch no. 20521-02				Knold, 2003d
Malathion	968.1 g/kg	961.4 g/kg	VAM 001-01	
MeOOSPS-triester	1.0%	1.0%	VAM 006-02	
MeOSSPO-triester	<0.01%	<0.01%	VAM 008-02	
MeOOOPS-triester	0.23%	0.23%	VAM 006-02	
Isomalathion	0.08%	0.30%	VAM 005-03	
MeOOSPO-triester	<0.05%	<0.05%	VAM 008-02	
Malaoxon	<0.04%	<0.04%	VAM 008-02	

The decline of malathion levels during the storage test depended on the formulation, with 0.7%, 2.6%, 2.8% and 5.9% declines in the TC, EC, EW and DP, respectively.

Isomalathion levels increased during storage. Average levels in the TC, EC, EW and DP after the storage test were 0.30%, 0.33%, 0.22% and 1.9%, respectively (all expressed as % of the malathion content). Only a small proportion (3-4%) of the malathion was converted to isomalathion in the EC and EW formulations but 23% and 31% was converted to isomalathion in the DP and TC, respectively.

Analysis of fresh and stored samples of malathion TC (Bjorholm, 2002)

Fresh samples of malathion TC were analyzed within a few days of production. Samples of five batches were then stored at 20°C for 24.9, 25.5, 26.2, 27.4 and 30.4 months, for re-analysis by methods similar to those used in the 5-batch analyses.

Malathion levels declined by 3-8 g/kg during storage (original levels 965-969 g/kg; water concentrations 0.4-0.5 g/kg). Storage did not produce malaoxon, MeOOSPO-triester or MeOSSPO-triester at levels above the LOQ (0.4 g/kg). Storage produced very small reductions in the levels of MeOOSPS-triester and water.

The major effect of the storage was to increase levels of isomalathion from 0.3-1.1 g/kg (mean 0.54 g/kg) in fresh samples to 2.5-3.5 g/kg (mean 3.0 g/kg) in stored samples. The generation of isomalathion during storage accounted for 26-83% (mean 44%) of the decline in malathion. The decline of malathion content observed in the TC during 14 days at 54°C (0.7%) was in good agreement with the observed decline in malathion in TC during 25-30 months at 20°C (0.3-0.8%, mean 0.64%). The observed generation of isomalathion in the TC during 14 days at 54°C (0.22%) was in good agreement with the observed generation of isomalathion in TC during 25-30 months at 20°C (0.21-0.26%, mean 0.24%).

Containers and packaging

No special requirements for containers and packaging were identified. However, due to potential corrosion and/or decomposition of the malathion, containers of iron, steel, tin plate and copper should not be used unless lined with suitable material.

Expression of the active ingredient

The active ingredient content is expressed as malathion, in g/kg or g/l (for liquid formulations at 20°).

Appraisal

Malathion is an organophosphorus insecticide that has been widely used for many years. Existing FAO specifications for the TC, DP, WP, OL and EC were developed under the old procedure in 1988. Existing WHO specifications for the TC, WP, EC and DP were developed in 1999 under the old procedure. For the present review, draft specifications for malathion TC, DP, UL, EC and EW, intended for use in agriculture and public health, were submitted together with supporting data. The data submitted were in accordance with the requirements of the WHO/FAO Manual (1st edition).

The main formulation types are DP, UL, EC and EW and the corresponding specifications proposed were similar for both agricultural and public health products.

The water solubility of malathion is 148 mg/l at 25°C. It is reasonably stable to hydrolysis at pH 5, but hydrolyses more readily as pH increases. It is generally stable to photolysis.

No special requirements for containers and packaging were identified. However because of possible corrosion, containers of iron, steel, tin plate and copper should not be used unless lined with suitable material.

The Meeting was provided with commercially confidential information on the manufacturing process and batch analysis data on all impurities present at or above 1 g/kg. Analyses of 5 batches of malathion, produced in 2001, accounted for 99.0-99.7% of the material. These data agreed with those submitted to the UK Pesticide Safety Directorate (PSD), except for differences in the maximum levels of MeOOSPS-triester and isomalathion and the presence of an impurity which was not listed in the UK specification (Pim, 2003).

Isomalathion, a potentiator of malathion-induced toxicity, and malaoxon are more toxic than malathion and their manufacturing limits exceeded or equalled the guideline level of 1 g/kg. Isomalathion levels were shown to increase during storage. The meeting agreed that isomalathion and malaoxon are relevant impurities and should be limited to 4 and 1 g/kg, respectively, in the TC, based on practical quality control limits in manufacture.

The toxicological and ecotoxicological data provided (Tables 3-6) were generally derived from malathion having impurity profiles similar to those described in Table 2 but there were exceptions: either where the malathion content was below 950 g/kg or where the malathion content and impurity profile were not known.

Ecotoxicological study summaries on fish, *Daphnia*, algae, birds, bees and earthworms were provided. Malathion appeared to be more hazardous to *Daphnia* than to other species.

Numerous studies are available on the toxicity of malathion and summaries were provided for this evaluation. Malathion was the subject of a full JMPR toxicological review in 1997. The ADI for malathion was set at 0-0.3 mg/kg bw. The IPCS hazard classification is Class III (slightly hazardous).

In mammals, the safety of malathion has been attributed to the rapid hydrolytic degradation by carboxylesterases. Impurities that inhibit carboxylesterase activity thus have the ability to potentiate the toxicity of malathion.

In acute oral LD₅₀ tests on rats (Table 3 and summarized below), malathion of higher purity seems less toxic than malathion of lower purity.

<u>Test material</u>	<u>Year</u>	<u>Rat oral LD₅₀</u>
Malathion TC, purity not specified	1978	5000 mg/kg bw
Malathion TC, purity not specified, stored 1 year at 5°C	1979	4100 mg/kg bw
Malathion TC, purity not specified, stored 1 year at 20-25°C	1979	3450 mg/kg bw
Malathion TC, 96-98%, isomalathion <0.1%	1986	5550 mg/kg bw
Malathion TC, 96.8%	1991	5100 mg/kg bw
Malathion TC, 94.6%	1991	1650 mg/kg bw
Malathion TC, 96.2%, containing 0.44% isomalathion (spike)	2002	1850 mg/kg bw
Malathion TC, 96.2%, containing 0.2% isomalathion	2003	2400 mg/kg bw

Although insufficient details were available to support definite conclusions, the toxicity of malathion containing 0.44% isomalathion was higher than most of the other malathion samples

The manufacturing QC limit for isomalathion in TC is 4 g/kg and the proposed specifications were:

TC	4 g/kg	
DP	2.5% of malathion,	equivalent to 2.5 g/kg product (for a 100 g/kg product)
UL	0.4% of malathion,	equivalent to 4 g/kg product
EC	0.8% of malathion,	equivalent to 4 g/kg product (for a 500 g/kg product)
EW	0.6% of malathion,	equivalent to 3 g/kg product (for a 500 g/kg product)

The meeting noted that malathion containing 2 g/kg isomalathion was assessed in UK, compared with the proposed limit of 4 g/kg for TC in FAO/WHO specifications. Although data were provided to the Meeting in support of an LD₅₀ for malathion containing isomalathion at 4.4 g/kg (see Table 3), at the time of the initial review these data had not been assessed formally by a national registration authority or WHO/PCS and this was required to support the 4 g/kg limit proposed for this relevant impurity. Post-meeting, information became available (Pim J., 2004) indicating that data supporting the 4 mg/kg limit were under consideration by the EU. However, the manufacturer stated (Jensen, 2004a) that, for administrative (not toxicological) reasons, the company had subsequently reverted to a 2 g/kg limit within the EU. In a separate post-meeting review of the data (Aitio A., 2004) the WHO/PCS opinion was that the specification limit (4 g/kg) for isomalathion in malathion does not increase the hazards caused by exposure to malathion to an unreasonable extent, and is thus acceptable.

Upon storage, malathion levels decline and, in dustable powder, malathion is converted to isomalathion and consequently the proposed specification limit for isomalathion in the DP was proposed at 2.5% of the malathion concentration. Although this was not a reason for rejecting the proposed specification limit, WHO/PCS noted that the maximum allowed concentration of isomalathion approximately doubles the acute toxicity of the DP and that therefore the hazards associated with it are potentially greater than might be expected from the low concentrations of malathion associated the DP formulations (which contain a maximum of 100g active ingredient/kg product).

Six impurities could be considered as potentially relevant, because of their toxicological properties:

- isomalathion, CAS 2244-12-5;
- malaoxon, CAS 1634-78-2;
- MeOSSPO-triester, CAS 22608-53-3;
- MeOOSPO-triester, CAS 152-20-5;
- MeOOOPS-triester, CAS 152-18-1;
- MeOOSPS-triester, CAS 2953-29-9.

MeOSSPO-triester and MeOOSPO-triester are toxic but they were not detected in the technical malathion described in the current submission. Small amounts occurred in the DP formulation but the meeting agreed that they should not be considered as relevant impurities.

MeOOSPS-triester, a potentiator of malathion induced toxicity, and MeOOOPS-triester are more toxic than malathion and can occur at levels of up to 12 and 2 g/kg, respectively, in the technical malathion evaluated for the current submission. Levels of these impurities do not increase during storage.

The meeting agreed that MeOOSPS-triester is a relevant impurity, to be controlled with a maximum limit in the TC of 15 g/kg, based on the practical quality control limit in manufacture. The limit agreed for the UL (which is, in effect, a TC) was 1.6% of the malathion content. The slight difference between the 15 g/kg and 1.6% arose from the fact that malathion represents less than 100% of the TC. The meeting also agreed that MeOOOPS-triester is also a relevant impurity, to be controlled with a maximum limit in the TC of 5 g/kg, based on the practical quality control limit in manufacture.

When fresh samples of technical malathion were stored for 25-30 months at 20°C, malathion levels declined by 3-8 g/kg and isomalathion levels increased from starting levels of 0.3-1.1 g/kg to 2.5-3.5 g/kg. Levels of other impurities were either very low or were not influenced by the storage. The decline of malathion content and generation of isomalathion in TC during 14 days storage at 54°C agreed closely with the results from the 25-30 months storage at 20°C.

The decline in malathion content of formulations during 14 days of storage at 54°C depended on the formulation: EC 2.6%, EW 2.8% and DP 5.9% (all declines expressed as % of active ingredient). The generation of isomalathion during 14 days of storage at 54°C also depended on the formulation, with average final levels (expressed as % of active ingredient): EC 0.11%, EW 0.095% and DP 1.35%.

The meeting acknowledged that isomalathion tends to be generated during storage of DP, EC and EW formulations and, given that such products cannot be purified, therefore accepted that the specification limits (relative to malathion content) must be higher for the formulations than for the TC.

The analytical method for the determination active ingredient content, GC using a non-polar capillary column and FID, was collaboratively tested and shown to be suitable for TC, EC, EW and DP. The method was adopted by CIPAC in 2002. GC retention time and IR spectrum provide identity tests. UL formulations consist of technical malathion, so the meeting agreed that the analytical methods for active ingredient and impurities in TC are appropriate for the UL.

Relevant impurities in TC may be determined by HPLC or GC, but ³¹P-NMR spectroscopy is required for successful analysis of some formulations, down to the required levels. The ³¹P-NMR spectroscopy methods measure the ratio between the impurity and malathion contents and the impurity concentration is then calculated from the malathion content, as measured by GC. Methods for determination of impurities were subjected to within-laboratory validation and, for impurities in the TC and some formulations, were also subjected to independent laboratory validation

(see also Addenda 1 and 2). Analytical methods for the impurities are described in Appendices 1-6 and are applicable as follows:

	Appendix No.				
	TC	UL	EW	DP	EC
malaoxon	4	4	1	6	2
isomalathion	3	3	3	3	2
MeOOOPS	5	5	5	5	2
MeOOSPS	5	5	5	5	2

Recommendations

Subject to amendment of the draft specifications in accordance with the appraisal, above, the Meeting recommended:

- (i) withdrawal of existing (1988) FAO specifications for malathion TC, WP, EC, DP and OL;
- (ii) withdrawal of existing (1999) WHO specifications for malathion TC, WP, EC and DP;
- (iii) adoption by FAO of the proposed (2003) specifications for malathion TC, DP, EW, EC and UL.
- (iv) adoption by WHO of the proposed (2003) specifications for malathion TC, DP, EC and UL.
- (v) that specifications for very dilute (readu-to-use) EW should be considered when suitably validated methods of analysis and supporting data are provided by the manufacturer.

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ADDENDUM 1 TO THE EVALUATION (FEBRUARY, 2004)

MALATHION

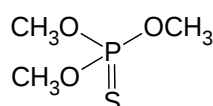
Explanation

The 2003 JMPS included MeOOOPS-triester as a relevant impurity in malathion. At that time, validated methods were available for determination of MeOOOPS-triester in TC and EW and DP formulations.

Method VAM 203-01, which relies on ^{31}P -NMR spectroscopy and which was previously used for the determination of malaoxon, isomalathion and MeOOSPS-triester impurities in malathion EC, has now been extended to the determination of MeOOOPS-triester in malathion EC formulations.

Methods of Analysis and Testing

MeOOOPS-triester, in EC



CAS 152-18-1

Method VAM 203-01 uses ^{31}P -NMR spectroscopy to measure the MeOOOPS-triester content of EC formulations. A portion of the test formulation is dissolved in deuterated chloroform and the molar ratio between MeOOOPS-triester and malathion is determined by ^{31}P -NMR spectroscopy. The MeOOOPS-triester content in the formulation is then calculated from the malathion content determined by GC (Hald, 2003ab).

Validation data (within-laboratory validation) for MeOOOPS-triester in malathion technical material and formulations are summarized in Table 1 (Hald, 2003c).

Table 1. Validation data for MeOOOPS-triester in malathion EC.

Product	Test (note 1)	Result (note 2)			
Method VAM 203-01 (³¹ P NMR). Validation: Hald, 2003c.					
EC	Linearity	Acceptable linearity over test range 0.1 – 1.4% of the ai (tests on 3 formulations). The line of best fit passed close to the origin.			
	Specificity	No overlap of the peak of the MeOOOPS triester and the peaks of the other impurities or the malathion peak.			
	Precision	S _r = 0.019-0.031 at MeOOOPS-triester levels of 0.12-0.22% of ai and 0.0029-0.020 at levels of 0.6-1.1% of the ai (n=5).			
		Test sample (note 3)	MeOOOPS added	MeOOOPS conc as % of ai	S _r n=5
		450 g/l EC (purified malathion)	0.1%	0.22%	0.031
		450 g/l EC (purified malathion)	0.5%	1.1%	0.020
		450 g/l EC (malathion TC)		0.20%	0.021
		500 g/l EC (purified malathion)	0.1%	0.20%	0.019
		500 g/l EC (purified malathion)	0.5%	1.0%	0.0029
		500 g/l EC (malathion TC)		0.20%	0.028
		830 g/l EC (purified malathion)	0.1%	0.13%	0.025
		830 g/l EC (purified malathion)	0.5%	0.64%	0.010
		830 g/l EC (malathion TC)		0.20%	0.023

Product	Test (note 1)	Result (note 2)
	LOQ	0.12% of the ai.
	Recovery	Mean (5 replicate analyses) recovery (3 samples × 2 fortification levels) 94-104% at MeOOOPS-triester levels of 0.13 -1.1% of the ai.

Note 1: LOQ: lowest concentration tested at which an acceptable mean recovery and relative standard deviation were obtained.

Note 2: S_r is relative standard deviation.

Note 3: ECs were prepared either from normal TC or a purified sample of malathion containing no detectable MeOOOPS.

Appraisal

The proposed specification for MeOOOPS-triester content of malathion EC formulations is: "maximum 0.5% of the malathion content found under specification 2.2".

The ^{31}P -NMR spectroscopic method, VAM 203-01, was shown to produce acceptable accuracy (average recovery) and precision (S_r) for the determination of MeOOOPS-triester, in the concentration range 0.13 -1.1% of the malathion content and is thus suitable for testing for compliance with the proposed specification of 0.5%. The method was subjected to within-laboratory validation.

Recommendation

The Meeting recommended that method VAM 203-01 be accepted as extended to malathion EC formulations, for the determination of MeOOOPS-triester impurity.

References

- Hald M., 2003a Analytical method VAM 203-01 (version xx). Determination of isomalathion (CAS No. 3344-12-5), MeOOSPS-triester (CAS No. 2953-29-9), MeOOOPS triester (CAS No. 152-18-1) and malaoxon (CAS No. 1634-78-2) in malathion EC formulations. Cheminova study NVAL 203-01. Unpublished.
- Hald M., 2003b Analytical method VAM 203-01 (version 2). Determination of isomalathion (CAS No. 3344-12-5), MeOOSPS-triester (CAS No. 2953-29-9), MeOOOPS triester (CAS No. 152-18-1) and malaoxon (CAS No. 1634-78-2) in malathion EC formulations. Cheminova study NVAL 203-01, Amendment 1. Unpublished.
- Hald M., 2003c Extension of analytical method VAM 203-01 for determination of isomalathion (CAS No. 3344-12-5), MeOOSPS-triester (CAS No. 2953-29-9) and malaoxon (CAS No. 1634-78-2) in malathion EC formulations in order to include the determination of MeOOOPS triester (CAS No. 152-18-1) in the method. Cheminova study NVAL 203-01. Unpublished.

ADDENDUM 2 TO THE EVALUATION (MARCH, 2004)

MALATHION

Explanation

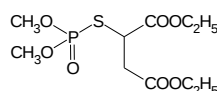
The 2003 JMPS included malaoxon, isomalathion, MeOOSPS-triester and MeOOOPS-triester as relevant impurities in malathion.

Methods VAM 203-01 and VAM 202-01, rely on ^{31}P -NMR spectroscopy for the determination of malaoxon, isomalathion, MeOOSPS-triester and MeOOOPS-triester impurities in malathion EC and EW formulations.

Information on independent laboratory validation of the methods became available after the 2003 JMPS and was provided for evaluation.

Methods of Analysis and Testing

Malaoxon, in EC, EW
CAS 1634-78-2



Method VAM 202-01 uses ^{31}P -NMR spectroscopy to measure the malaoxon content of EW formulations. A portion of the test formulation is dissolved in 10% deuterated acetone in acetone and the molar ratio between malaoxon and malathion is determined by ^{31}P -NMR spectroscopy. The malaoxon content in the formulation is then calculated from the malathion content determined by GC (Hald, 2002a). Method VAM 203-01 is a similar procedure, but with the sample dissolved in deuterated chloroform, which is applied to EC formulations (Hald, 2002c).

Independent laboratory validation data for malaoxon in malathion EC and EW formulations are summarized in Table 1.

Table 1. Validation data for determination of malaoxon in malathion EC and EW formulations.

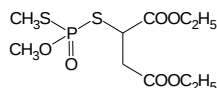
Substrate	Test (note 1)	Result (note 2)		
Method VAM 202-01 (³¹ P NMR). Validation: Wollborn, 2004a.				
EW	Linearity	Acceptable linearity over test range 0.26-2.4% of the ai (tests on 1 formulation). The line of best fit passed close to the origin.		
	Specificity	No overlap in responses from malaoxon and from other impurities.		
	Precision	S _r = 0.063 at a malaoxon level of 0.46% of ai (n = 5).		
	LOQ	0.28% of the ai.		
	Recovery	mean (n = 5)	range	conc., % of a.i.
		95%	81-100%	0.28 %
		101%	99-104%	1.5%
Method VAM 203-01 (³¹ P NMR). Validation: Wollborn 2004b.				
EC	Linearity	Acceptable linearity over test range 0.29-1.3% of the ai (tests on 1 formulation). The line of best fit passed close to the origin.		
	Specificity	No overlap in responses from malaoxon and from other impurities.		
	Precision (of recovery tests)	S _r = 0.125 and 0.047 at malaoxon levels of 0.086% and 0.80% of the a.i. respectively (n = 5).		

LOQ	0.09%% of the a.i.		
Recovery	mean (n = 5)	range	conc, as % a.i.
	83%	67-92%	0.09%
	95%	91-102%	0.80%

Note 1. LOQ: was the lowest concentration tested at which acceptable mean recovery and relative standard deviation were obtained.

Note 2. S_r is the relative standard deviation.

Isomalathion, in EC
CAS 3344-12-5



Method VAM 203-01 uses ^{31}P -NMR spectroscopy to measure the isomalathion content of EC formulations. A portion of the test formulation is dissolved in deuterated chloroform and the molar ratio between isomalathion and malathion is determined by ^{31}P -NMR spectroscopy. The isomalathion content in the formulation is then calculated from the malathion content determined by GC (Hald, 2002c).

Independent laboratory validation data for the determination of isomalathion in malathion EC formulations are summarized in Table 2.

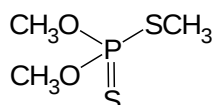
Table 2. Validation data for the determination of isomalathion in malathion EC formulations.

Formulations:				
Substrate	Test (note 1)	Result (note 2)		
Method VAM 203-01 (³¹ P NMR). Validation: Wollborn, 2004b.				
EC	Linearity	Acceptable linearity over test range 0.27-2.6% of the ai (tests on 1 formulation). The line of best fit passed close to the origin.		
	Specificity	No overlap in responses from isomalathion and from other impurities.		
	Precision	S _r = 0.119 at an isomalathion level of 0.11% of a.i. (n = 5).		
	LOQ	0.24% of the a.i.		
	Recovery	mean (n = 5)	range	conc., % a.i.
		99%	92-107%	0.24%
		96%	94-100%	1.5%

Note 1. LOQ is lowest concentration tested at which an acceptable mean recovery and relative standard deviation were obtained.

Note 2. S_r is the relative standard deviation.

MeOOSPS-triester, in EC
CAS 2953-29-9



Method VAM 203-01 uses ^{31}P -NMR spectroscopy to measure the MeOOSPS-triester content of EC formulations. A portion of the test formulation is dissolved in deuterated chloroform and the molar ratio between MeOOSPS-triester and malathion is determined by ^{31}P -NMR spectroscopy. The MeOOSPS-triester content in the formulation is then calculated from the malathion content determined by GC (Hald, 2003ab).

Independent laboratory validation data for MeOOSPS-triester in malathion EC formulations are summarized in Table 3.

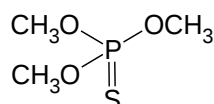
Table 3. Validation data for the determination of MeOOSPS-triester in malathion EC formulations.

EC Formulations:				
Substrate	Test (note 1)	Result (note 2)		
Method VAM 203-01 (³¹ P NMR). Validation: Wollborn, 2004b.				
EC	Linearity	Acceptable linearity over the test range, 0.30-4.35% of the a.i. (tests on 1 formulation). The line of best fit passed close to the origin.		
	Specificity	No overlap of the peak of the MeOOSPS-triester and the peaks of the other impurities or the malathion peak.		
	Precision	S _r = 0.0094 at a MeOOSPS-triester level of 0.87% of ai (n = 5).		
	LOQ	0.28% of the a.i.		
	Recovery	mean (n = 5)	range	conc., % a.i.
		97%	92-100%	0.28%
		97%	96-100%	4.1%

Note 1. LOQ is the lowest concentration tested at which an acceptable mean recovery and relative standard deviation were obtained.

Note 2. S_r is the relative standard deviation.

MeOOOPS-triester, in EC
CAS 152-18-1



Method VAM 203-01 uses ³¹P-NMR spectroscopy to measure the MeOOOPS-triester content of malathion EC formulations. A portion of the test formulation is dissolved in deuterated chloroform and the molar ratio between MeOOOPS-triester and malathion is determined by ³¹P-NMR spectroscopy. The MeOOOPS-triester content of the formulation is then calculated from the malathion content determined by GC (Hald, 2003ab).

Independent laboratory validation data for MeOOOPS-triester in malathion EC formulations are summarized in Table 4 (Wollborn, 2004b).

Table 4. Validation data for the determination of MeOOOPS-triester in malathion EC formulations.

EC formulations:				
Substrate		Test (note 1)		Result (note 2)
Method VAM 203-01 (³¹ P NMR). Validation: Wollborn, 2004b.				
EC	linearity	acceptable linearity over test range 0.30-1.35% of the ai (tests on 1 formulation). The line of best fit passes close to the origin.		
	specificity	no overlap of the peak of the MeOOOPS-triester and the peaks of the other impurities or the malathion peak		
	precision	S _r = 0.014 at a MeOOOPS-triester level of 0.20% of ai (n=5).		
	LOQ	0.26% of the ai		
	recovery	mean (n=5)	range	conc, as %ai
		90%	87-92%	0.26%
		96%	94-98%	1.4%

Note 1. LOQ is the lowest concentration tested at which an acceptable mean recovery and relative standard deviation were obtained.

Note 2. S_r is the relative standard deviation.

Appraisal

Methods VAM 203-01 and VAM 202-01 rely on ³¹P-NMR spectroscopy for the analysis of malaoxon, isomalathion, MeOOSPS-triester and MeOOOPS-triester impurities in malathion EC and EW formulations. Information on independent laboratory validation of the methods became available after the 2003 JMPS and was provided for evaluation.

Methods for impurities in malathion EC and EW formulations were subjected to independent-laboratory validation, as summarized below.

Analyte	Formulation	Method code	Method	LOQ, % a.i.	Validation
Isomalathion	EC	VAM 203-01	³¹ P-NMR spectroscopy	0.24	independent lab.
Malaoxon	EW	VAM 202-01	³¹ P-NMR spectroscopy	0.28	independent lab.
Malaoxon	EC	VAM 203-01	³¹ P-NMR spectroscopy	0.09	independent lab.
MeOOSPS-triester	EC	VAM 203-01	³¹ P-NMR spectroscopy	0.28	independent lab.
MeOOOPS-triester	EC	VAM 203-01	³¹ P-NMR spectroscopy	0.26	independent lab.

The proposed maximum malaoxon content of EC formulations is 0.1% of the malathion content. The within-laboratory validation LOQ was 0.06% (of malathion content) and the independent laboratory LOQ was 0.09% of malathion content. The method was accepted as validated for the purpose.

The ³¹P-NMR spectroscopy methods VAM 203-01 and VAM 202-01 have been shown in independent laboratory validations to produce acceptable recoveries and precision for the impurities in concentration ranges suitable for testing the proposed specifications for EC and EW formulations.

Recommendation

The Meeting recommended that methods VAM 203-01 and VAM 202-01 be accepted as extended to malathion EC and EW formulations for determination of the impurities malaoxon, isomalathion, MeOOSPS-triester and MeOOOPS-triester.

References

From previous

- Hald, 2002a Analytical method VAM 202-01: determination of malaoxon (CAS. No. 1634-78-2) in malathion EW formulations. Cheminova A/S. Study: VAM 202-01. Unpublished.
- Hald, 2002c Analytical method VAM 203-01: determination of isomalathion (CAS No. 3344-12-5), MeOOSPS-triester (CAS No. 2953-29-9) and malaoxon (CAS No. 1634-78-2) in malathion EC formulations. Cheminova A/S. Study: VAM 203-01. Unpublished.
- Hald, 2003a Analytical method VAM 203-01 (version xx). Determination of isomalathion (CAS No. 3344-12-5), MeOOSPS-triester (CAS No. 2953-29-9), MeOOOPS-triester (CAS No. 152-18-1) and malaoxon (CAS No. 1634-78-2) in malathion EC formulations. Cheminova study NVAL 203-01. Unpublished.
- Hald, 2003b Analytical method VAM 203-01 (version 2). Determination of isomalathion (CAS No. 3344-12-5), MeOOSPS-triester (CAS No. 2953-29-9), MeOOOPS-triester (CAS No. 152-18-1) and malaoxon (CAS No. 1634-78-2) in malathion EC formulations. Cheminova study NVAL 203-01, Amendment 1. Unpublished.
- Hald, 2003c Extension of analytical method VAM 203-01 for determination of isomalathion (CAS No. 3344-12-5), MeOOSPS-triester (CAS No. 2953-29-9) and malaoxon (CAS No. 1634-78-2) in malathion EC formulations in order to include the determination of MeOOOPS-triester (CAS No. 152-18-1) in the method. Cheminova study NVAL 203-01. Unpublished.

Addendum 2 references

- Wollborn, 2004a Validation of the analytical method VAM 202-01 (Cheminova A/S): "Determination of malaoxon (CAS No. 1634-78-2) in malathion EW formulations. Bayer Industry Services. Unpublished.

Wollborn, 2004b Validation of the analytical method VAM 203-01 (Cheminova A/S):
"Determination of isomalathion (CAS No. 3344-12-5), MeOOSPS-triester (CAS
No. 2953-29-9), MeOOOPS-triester (CAS No. 152-18-1) and malaoxon (CAS No.
1634-78-2) in malathion EC formulations. Bayer Industry Services.
Unpublished.

Appendix 1

Determination of malaoxon in malathion EW

(Adapted from Cheminova analytical method VAM 202-01)

Principle

The malaoxon:malathion molar ratio is determined by quantitative ^{31}P -NMR spectroscopy and the malaoxon content (% of malathion) is calculated directly, using the ratio of molecular weights.

Apparatus

NMR spectrometer, Bruker Avance DPX 250 spectrometer (250 MHz) or equivalent, equipped with a 5 mm QNP liquid probe (HFCP) or equivalent.

Ultrasonic bath; centrifuge; mixer; 4 ml sample bottles.

Typical operating parameters

Pulse programme	Standard one X-pulse experiment with inverse gated decoupling: relaxation delay - 90° -X-pulse - acquisition (with proton decoupling).
Nucleus	^{31}P .
Relaxation delay	60s.
Pulse width	The 90° pulse width is chosen from the most recent calibration of the ^{31}P pulse in a polar solvent.
Nucleus for decoupler	^1H .
Decoupler mode	Inverse gated.
Decoupler modulation mode	Broadband Waltz decoupling.
Transmitter offset	Midway between malaoxon and malathion signals.
Number of points i FID	256k.
Number of scans	64
Spectral width	200ppm
Temperature	25°C.

Preparation of test solutions

Shake the EW formulation thoroughly to ensure homogeneity and make duplicate test solutions (A and B), as follows. Transfer 0.5ml of EW formulation to a 4 ml sample bottle. Add 1 ml of 10% d_6 -acetone in acetone and mix well, using a mixer appropriate for the bottles. Place the bottle in an ultrasonic water bath for 10 minutes. Centrifuge the sample for 10 min. and transfer 0.6 ml of the upper liquid layer into a 5mm NMR-tube.

Spectroscopic procedure

Set the operating parameters according to the values listed above or to equivalents that are appropriate for the particular instrument used. This parameter set is used for all samples.

Place the test solutions in the autosampler, record all necessary all information about them and start the acquisition process.

When the data-acquisition is complete, process the FID's obtained in the following way. A Lorentzian line-broadening of 1 Hz is applied to the FID and Fourier transformation (256k points) is executed. The spectrum obtained is phased

manually. Careful phasing of the spectrum is of the utmost importance. Baseline correction is applied.

The malathion signal, which is easily identified as the most intense in the spectrum, is set to 95.88 ppm. Integral regions are then set manually. The malathion signal is integrated (I mal) using a region of ± 1 ppm, while the malaoxon signal (at approximately 29.0 ppm*) is integrated (I oxon) using a region of ± 0.1 ppm around the signal. Normalize the integral of malathion to a value of 1000. See Figure 1.

* The chemical shift is approximately 29.1 ppm in test solutions of 92 g/l malathion EW and approximately 28.7 ppm in test solutions of 440 g/l malathion EW. For formulations with malathion concentrations between these concentration values, an estimate of the shift of malaoxon can be obtained by interpolating between these two values, assuming a linear relationship between malathion concentration and malaoxon chemical shift. In cases of doubt about the identity of the malaoxon peak, the position should be confirmed by spiking the sample with a malaoxon standard.

Calculation

Using the integrals obtained for malathion and malaoxon the content of malaoxon, as a percentage of the malathion content of the formulation, can be calculated using the following equation:

$$\text{malaoxon (\% of malathion)} = \frac{(I \text{ malaoxon}) \times (\text{MW malaoxon}) \times 100}{(I \text{ malathion}) \times (\text{MW malathion})}$$

where:

malathion g/kg = concentration (g/kg) of malathion in the sample, as determined by GC;

(I malathion) = integral of the malathion signal in the NMR spectrum;

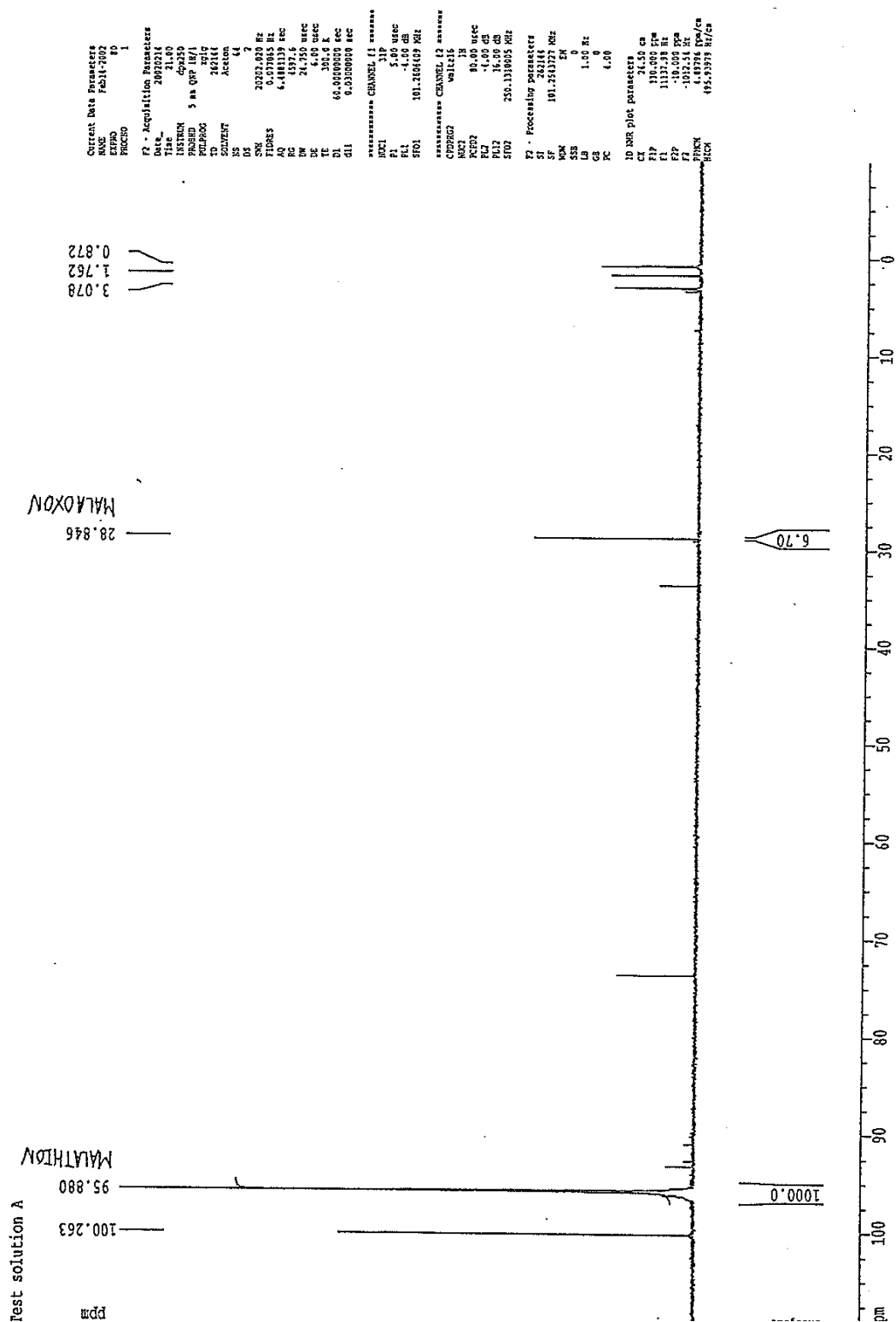
(I malaoxon) = integral of the malaoxon signal in the NMR spectrum;

(MW malathion) = molecular weight of malathion, 330.36 g/mol.

(MW malaoxon) = molecular weight of malaoxon (314.30 g/mol).

Calculate the average of the values obtained from test solutions A and B.

Figure 1. Typical NMR spectrum of a test solution



Appendix 2

Determination of isomalathion, MeOOSPS-triester, MeOOOPS-triester and malaoxon in malathion EC

(Adapted from Cheminova analytical method VAM 203-01)

Principle

The molar ratios between the four impurities (isomalathion, MeOOSPS triester, MeOOOPS triester and malaoxon) and malathion is determined by quantitative ^{31}P -NMR spectroscopy and the impurity content (g/kg) of the formulation is calculated using the ratio and the malathion content (g/kg) of the formulation, determined by GC.

Apparatus

NMR spectrometer, Bruker Avance DPX 250 spectrometer (250 MHz) or equivalent, equipped with a 5 mm QNP liquid probe (HFCP) or equivalent.

Ultrasonic bath; centrifuge; mixer; 4 ml sample bottles.

Typical operating parameters

Pulse programme	Standard one X-pulse experiment with inverse gated decoupling: relaxation delay - 90° -X-pulse - acquisition (with proton decoupling).
Nucleus	^{31}P .
Relaxation delay	60s.
Pulse width	The 90° pulse width is chosen from the most recent calibration of the ^{31}P pulse in a polar solvent.
Nucleus for decoupler	^1H .
Decoupler mode	Inverse gated.
Decoupler modulation mode	Broadband Waltz decoupling.
Transmitter offset	80.0ppm (with malathion set to 95.88ppm).
Number of points i FID	256k.
Number of scans	64
Spectral width	200 ppm
Temperature	25°C.

Preparation of test solutions

Shake the EW formulation thoroughly to ensure homogeneity and make duplicate test solutions (A and B), as follows. Transfer a volume of EC formulation, corresponding to approximately 0.25 g malathion, to a 4 ml sample bottle. Add 0.5 ml of deuterated chloroform (CDCl_3) and mix well, using a mixer appropriate for the bottles. Transfer 0.6 ml of the solution into a 5 mm NMR-tube.

Spectroscopic procedure

Set the operating parameters according to the values listed above or to equivalents that are appropriate for the particular instrument used. This parameter set is used for all samples.

Place the test solutions in the autosampler, record all necessary all information about them and start the acquisition process.

When the data-acquisition is complete, process the FID's obtained in the following way. A Lorentzian line-broadening of 1 Hz is applied to the FID and Fourier

transformation (256k points) is executed. The spectrum obtained is phased manually. Careful phasing of the spectrum is of the utmost importance. Baseline correction is applied.

The malathion signal, which is easily identified as the most intense in the spectrum, is set to 95.88 ppm. Integral regions are then set manually. MeOOSPS-triester yields one signal at approximately 100.3 ppm. MeOOOPS-triester yields one signal at approximately 73.5 ppm. Isomalathion yields two signals (due to the presence of two diastereoisomers) at approximately 56.6 ppm and 58.0 ppm. Malaoxon yields one signal at approximately 28.0 ppm. In cases of doubt about the identity of the impurity peaks, the position(s) should be confirmed by spiking the sample with a suitable standard.

Integral regions are set manually. Careful integration of the signals is of the utmost importance. Normalize the integral of malathion to a value of 1000. Plot the spectrum and print an integral list. See Figure 1.

Calculation

Using the integrals obtained for isomalathion, MeOOSPS triester, MeOOOPS triester, malaoxon and malathion, together with the concentration of malathion in the sample (obtained from GC measurements as malathion in g/kg), the concentrations of the four impurities (as % of malathion) can be calculated using the following equations:

$$\text{MeOOSPS-triester (\% of malathion)} = \frac{(I \text{ MeOOSPS-triester}) \times (\text{MW MeOOSPS-triester}) \times 100}{(I \text{ malathion}) \times (\text{MW malathion})}$$

$$\text{MeOOOPS-triester (\% of malathion)} = \frac{(I \text{ MeOOOPS-triester}) \times (\text{MW MeOOOPS-triester}) \times 100}{(I \text{ malathion}) \times (\text{MW malathion})}$$

$$\text{isomalathion (\% of malathion)} = \frac{(I \text{ iso-1} + I \text{ iso-2}) \times (\text{MW isomalathion}) \times 100}{(I \text{ malathion}) \times (\text{MW malathion})}$$

$$\text{malaoxon (\% of malathion)} = \frac{(I \text{ malaoxon}) \times (\text{MW malaoxon}) \times 100}{(I \text{ malathion}) \times (\text{MW malathion})}$$

where:

(I malathion) = integral of the malathion signal in the NMR spectrum;

(I MeOOSPS) = integral of the MeOOSPS-triester signal in the NMR spectrum;

(I MeOOOPS) = integral of the MeOOOPS-triester signal in the NMR spectrum;

(I iso-1 + iso-2) = sum of integrals of the two isomalathion signals in the NMR spectrum;

(I malaoxon) = integral of the malaoxon signal in the NMR spectrum;

(MW malathion) = molecular weight of malathion, 330.36 g/mol;

(MW MeOOSPS) = molecular weight of MeOOSPS-triester, 172.2 g/mol;

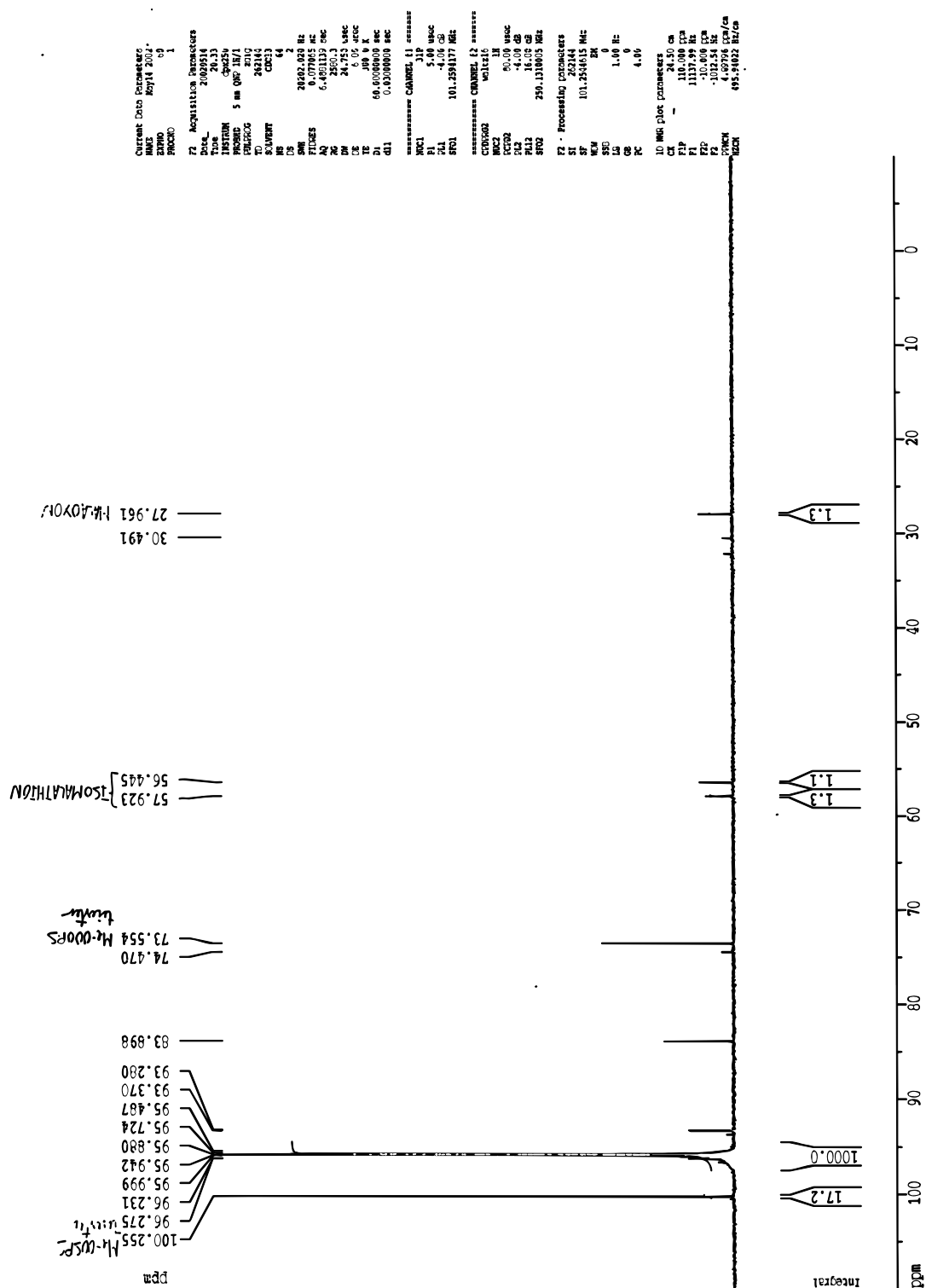
(MW MeOOOPS) = molecular weight of MeOOOPS-triester, 156.1 g/mol;

(MW isomalathion) = molecular weight of isomalathion, 330.36 g/mol;

(MW malaoxon) = molecular weight of malaoxon (314.30 g/mol).

Calculate the average of the values obtained from test solutions A and B.

Figure 1. NMR spectrum of a test solution



Appendix 3

Determination of isomalathion in malathion TC, UL, DP and EW

(Adapted from Cheminova analytical method VAM 005-03)

Principle

Isomalathion is separated by reversed-phase HPLC and determined by UV-absorption, with external standardization.

Chemicals

Acetonitrile, Lichrosolv®, Merck Art.14291, or equivalent (solvent B).

Water, HPLC grade (solvent A).

Isomalathion, reference standard, as pure as practicable.

Prepare an approximately 1% solution of the reference material by weighing accurately about 0.1 g (a g) into a tared 12 ml sample bottle with screw cap. Add 10 ml of 75% v/v acetonitrile/water, weigh again (b g) and mix well (stock solution).

Weigh accurately 180 µl of the stock solution (c g) into a tared sample bottle with screw cap. Add 10 ml of 75% v/v acetonitrile/water, weigh again (d g) and mix well (Solution 1).

Weigh an aliquot of 2 ml of Solution 1 (e g.) into a tared sample bottle. Add 6 ml of 75% v/v acetonitrile/water, weigh again (f g) and mix well (Solution 2).

Weigh an aliquot of 1 ml of Solution 2 into a tared sample bottle. Add 2 ml of 75% v/v acetonitrile/water, weigh again and mix well (Solution 3).

Weigh an aliquot of 2 ml of Solution 3 into a tared sample bottle. Add 2 ml of 75% v/v acetonitrile/water, weigh again and mix well (Solution 4).

Solutions 1, 2, 3 and 4 are injected into the liquid chromatograph.

Apparatus

HPLC system, equipped with binary eluent delivery system, autosampler, photodiodearray detector and data handling system.

Analytical column, Phenomenex Sphereclone ODS2, 5 µm, 120 mm x 4.6 mm, or equivalent.

Guard column, Phenomenex Sphereclone ODS2, 5 µm, 50 mm x 4.6 mm, or equivalent.

Typical operating parameters

Gradient and flow programme	time (min.)	% B	flow (ml/min.)
	0.0	40	1.0
	8.0	40	1.0
	9.0	95	2.0
	12.0	95	2.0
	12.5	40	1.0
Stop time:	14 min.		
Post time:	1 min.		
DAD stop time:	8 min.		
Column temperature:	50°C.		

Signals	sample, 200 nm (8 nm bandwidth) interference check, 225 nm (8 nm bandwidth) reference (360 nm (100 nm bandwidth)
Slit width	4 nm
Spectrum	190-400 nm in 2 nm steps
Injection volume	25 µl
Integration	peak area
Typical retention time	6.3 min (isomalathion)

System suitability checks

Repeatability

Inject Solution 2 at least three times or until the peak area obtained from isomalathion does not differ by more than 5.0% between two successive measurements.

Linearity

Inject Solutions 1, 2, 3 and 4 and measure the peak areas of the isomalathion. Having calculated the concentrations of the solutions from the weights measured, calculate the linear regression coefficient (r^2) of the calibration curve, which should be >0.998 .

Carry over

Inject a blank solution after Solution 2 and measure the peak area obtained for isomalathion. The "carry over" from the previous injection is acceptable if $\leq 2.0\%$ of solution 2.

Interference

Ensure that there is clear baseline separation between the isomalathion and malathion in a test solution.

Preparation of test solutions

Technical material

Weigh accurately 0.3 g malathion TC (g g) into a tared 12 ml sample bottle with a screw cap. Add 10 ml of 75% v/v acetonitrile/water, weigh again (h g) and mix well. This solution is injected into the HPLC.

EW formulations

Weigh accurately sufficient EW to contain about 0.06 g malathion (g g) into a tared 12 ml sample bottle. Add 10 ml of 75% v/v acetonitrile/water, weigh again (h g) and mix well. Sonicate the solution in an ultrasonic bath for 10 minutes. Approximately half-way through the period, handshake the mixture vigorously. Centrifuge the solution if it appears cloudy and transfer 1-2 ml of the upper liquid to an autosampler vial.

DP formulations

Weigh accurately sufficient DP to contain about 0.3 g of malathion (g g) into a tared 12 ml sample bottle. Add 10 ml of 75% v/v acetonitrile/water, weigh again (h g) and mix well. Sonicate the solution in an ultrasonic bath for 10 minutes. Approximately half-way through the period, handshake the mixture vigorously. Centrifuge the solution and transfer 1-2 ml of the upper liquid to an autosampler vial.

All test solutions

If the area of the isomalathion peak observed exceeds that obtained from the most concentrated calibration solution, dilute the solution accordingly.

HPLC analysis

Inject the test and standard solutions in the following sequence:

Solution 2, T₁, T₂, T₃, T₄, T₅, T₆, T₇, T₈, Solution 2, T₉, ..., T₁₆ Solution 2, T₁₇... etc.
Where T₁...T_n are test solutions 1 to n and only one injection is made from each vial. Recalculate the response factor after each Solution 2 measurement and end the sequence with an injection of Solution 2.

Sequential injections of Solution 2 should produce peak areas within 5% of each other. Examine spectra or wavelength ratios obtained across the isomalathion peaks detected, to ensure that there is no significant interference from other components.

Calculation

Measure the peak areas of isomalathion both from the reference solution and the test solution.

Determine the isomalathion content of the test sample, in g/kg, as follows:

$$\text{isomalathion (g/kg)} = \frac{\text{peak area of isomalathion in test solution} \times h \times r_f}{g}$$

where: g and h are the weights (g) measured in the preparation of the test solutions, described above;
r_f is the response factor, determined from Solution 2 as follows:

$$r_f = \frac{\text{purity (g/kg) of isomalathion (ref. material)} \times a \times c \times e}{\text{peak area isomalathion in Solution 2} \times b \times d \times f}$$

where: a, b, c, d, e and f are the weights (g) measured in the preparation of Solution 2, described above.

Determine the isomalathion content of formulation samples, in % w/w of the malathion content, as follows:

$$\text{isomalathion (\% w/w of malathion)} = \frac{\text{isomalathion (g/kg)} \times 100}{\text{malathion content (g/kg)}}$$

Appendix 4

Determination of malaoxon in malathion TC and UL

(Adapted from Cheminova analytical method VAM 008-02)

Principle

Malaoxon is separated by reversed-phase HPLC, detected by UV absorption and determined by and external standardization.

Chemicals

Acetonitrile, Lichrosolv®, Merck Art.14291, or equivalent (solvent B).

Water, HPLC grade (solvent A).

Malaoxon, reference standard, as pure as practicable.

Prepare an approximately 1% solution of the reference material by weighing accurately about 0.1 g (a g) into a tared 12 ml sample bottle with screw cap. Add 10 ml acetonitrile, weigh again (b g) and mix well (stock solution).

Weigh accurately 50 µl of the stock solution (c g) into a tared sample bottle with screw cap. Add 10 ml of 75% v/v acetonitrile/water, weigh again (d g) and mix well (Solution 1).

Weigh an aliquot of 5 ml of Solution 1 (e g.) into a tared sample bottle. Add 5 ml of 75% v/v acetonitrile/water, weigh again (f g) and mix well (Solution 2).

Weigh an aliquot of 2 ml of Solution 2 (g g) into a tared sample bottle. Add 2 ml of 75% v/v acetonitrile/water, weigh again (h g) and mix well (Solution 3).

Weigh an aliquot of 2 ml of Solution 3 (i g) into a tared sample bottle. Add 2 ml of 75% v/v acetonitrile/water, weigh again (j g) and mix well (Solution 4).

Solutions 1, 2, 3 and 4 are injected into the liquid chromatograph.

Apparatus

HPLC system, equipped with binary eluent delivery system, autosampler, photodiodearray detector and data handling system.

Analytical column, Phenomenex Prodigy ODS2, 5 µm, 150 mm x 4.6 mm, or equivalent. Two columns are connected in series to form a column of 300 mm length.

Guard column, Phenomenex Prodigy ODS2, 5 µm, 30 mm x 4.6 mm, or equivalent.

Typical operating parameters

Gradient programme	time (min.)	% B
	0.0	35
	10.0	35
	10.5	95
	15.5	95
	16.0	35
Flow rate	1.5 ml/min.	
Stop time:	20 min.	
Post time:	10 min.	
DAD stop time:	15 min.	
Column temperature:	50°C.	

Signals	sample, 215 nm (8 nm bandwidth) interference check, 230 nm (8 nm bandwidth) reference (400 nm (100 nm bandwidth)
Slit width	8 nm
Spectrum	190-400 nm in 2 nm steps
Injection volume	50 µl
Integration	peak area
Typical retention times	10.9 min (malaoxon) 14.1 (malathion)

System suitability checks

Lamp test

Check the lamp intensity and the wavelength calibration of the detector (holmium oxide check) as described in the operating manual for the liquid chromatograph and make sure they meet the defined criteria.

Repeatability

Inject Solution 2 at least three times or until the peak area obtained from malaoxon does not differ by more than 10% between two successive measurements.

Linearity

Inject Solutions 1, 2, 3 and 4 and measure the peak areas of the malaoxon. Having calculated the concentrations of the solutions from the weights measured, calculate the linear regression coefficient (r^2) of the calibration curve, which should be >0.998 .

Carry over

Inject a blank solution after Solution 2 and measure the peak area obtained for malaoxon. The "carry over" from the previous injection is acceptable if $\leq 2.0\%$ of solution 2.

Interference

Ensure that there is clear baseline separation between the isomalathion and malathion in a test solution.

Preparation of test solutions

Technical material

Weigh accurately 0.3 g malathion TC (k g) into a tared 12 ml sample bottle with a screw cap. Add 10 ml of 75% v/v acetonitrile/water, weigh again (l g) and mix well. Prepare duplicate test solutions for each test sample. These solutions are injected into the HPLC.

If the area of the malaoxon peak observed exceeds that obtained from the most concentrated calibration solution, dilute the solutions accordingly, using 75% v/v acetonitrile/water.

HPLC analysis

Inject the test and standard solutions in the following sequence:

Solution 2, T₁, T₂, T₃, T₄, T₅, T₆, T₇, T₈, Solution 2, T₉, T₁₆ Solution 2, T₁₇... etc.

Where $T_1 \dots T_n$ are test solutions 1 to n and only one injection is made from each vial. Recalculate the response factor after each Solution 2 measurement and end the sequence with an injection of Solution 2.

Sequential injections of Solution 2 should produce peak areas within 5% of each other. Examine spectra or wavelength ratios obtained across the malaoxon peaks detected, to ensure that there is no significant interference from other components.

Calculation

Measure the peak areas of malaoxon both from the reference solution and the test solution.

Determine the malaoxon content of the test sample, in g/kg, as follows:

$$\text{malaoxon (g/kg)} = \frac{\text{peak area of malaoxon in test solution} \times l \times r_f}{k}$$

where: k and l are the weights (g) measured in the preparation of the test solutions, described above;

r_f is the response factor, determined from Solution 2 as follows:

$$r_f = \frac{\text{purity (g/kg) of malaoxon (ref. material)} \times a \times c \times e}{\text{peak area of malaoxon in Solution 2} \times b \times t \times f}$$

where: $t = d + c$, which are the weights (g) measured in the preparation of Solution 1, described above.

a, b and c are the weights (g) measured in the preparation of Solution 1, described above.

Appendix 5

Determination of MeOOOPS-triester and MeOOSPS-triester in malathion TC, UL, EW and DP

(Adapted from Cheminova analytical method VAM 206-01)

Principle

MeOOOPS-triester and MeOOSPS-triester are separated by capillary GC, using a non-polar column, FID and external standardization.

Chemicals

Acetonitrile, Lichrosolv®, Merck Art.14291, or equivalent.

MeOOOPS-triester, reference standard, as pure as practicable.

MeOOSPS-triester, reference standard, as pure as practicable.

Prepare an approximately 1% solution of each reference material by weighing accurately about 0.1 g (a_1 , a_2 g) into a tared 12 ml sample bottle with screw cap. Add 10 ml acetonitrile, weigh again (b_1 , b_2 g) and mix well (stock solutions).

Weigh accurately a 100 μ l aliquot of the MeOOOPS stock solution (c_1 g) and a 300 μ l aliquot of MeOOSPS stock solution (c_2 g), into a tared sample bottle with screw cap. Add acetonitrile to 10 ml, weigh again (d g) and mix well (Solution 1).

Weigh an aliquot of 2 ml of Solution 1 (e g.) into a tared sample bottle. Add 2 ml acetonitrile, weigh again (f g) and mix well (Solution 2).

Weigh an aliquot of 120 μ l of Solution 2 into a tared sample bottle. Add 2 ml acetonitrile, weigh again and mix well (Solution 3).

Solutions 1, 2 and 3 are injected into the gas chromatograph.

Apparatus

GC system, equipped with split/splitless injection system, autosampler, flame ionization detector and data handling system.

GC column, Agilent HP-1, 10 m x 0.53 mm, 2.65 μ m film thickness, or equivalent.

Injection liner, Agilent part No. 19251-60540, or equivalent.

Injection, splitless.

Typical operating parameters

Injector temperature	150°C
Detector temperature	300°C
Column temperature programme	initial 2 min at 60°C; 10°C/min to 210°C, hold 0 min; 25°C/min to 250°C, hold 2 min; 35°C/min to 280°C, hold 5 min.
Run time	26.5 min.
Carrier gas:	helium
Total flow rate:	80 ml/min.
Head pressure:	1.9 psi.
Injector purge on time	0.5 min.

Injection volume	2 µl
Injection syringe wash solvents	acetonitrile
Injection parameters:	
Sample washes	6
Sample pumps	6
Injection volume	1.0 µl
Syringe size	5.0 µl
Post Inj solvent A washes	6
PostInj solvent B washes	6
Viscosity delay	0 sec.
Plunger speed	fast
PreInjection dwell	0.00 min.
PostInjection dwell	0.00 min.
Integration	peak area
Typical retention times	MeOOOPS-triester, 6.0 min. MeOOSPS-triester, 9.3 min.

System suitability checks

Repeatability

Inject Solution 2 at least three times or until the peak areas obtained from MeOOOPS or MeOOSPS do not differ by more than 5.0% between two successive measurements.

Linearity

Inject a solvent blank and Solutions 1, 2 and 3 and measure the peak areas of MeOOOPS and MeOOSPS. Having calculated the concentrations of the solutions from the weights measured, calculate the linear regression coefficient (r^2) of the calibration curve, which should be >0.98 .

Carry over

Inject a blank solution after Solution 1 and measure the peak areas obtained for MeOOOPS and MeOOSPS. The "carry over" from the previous injection is acceptable if $\leq 1.0\%$ of solution 1.

Interference

Ensure that there is clear baseline separation between MeOOOPS and MeOOSPS.

Preparation of test solutions

Technical material

Weigh accurately 0.1 g malathion TC (i g) into a tared 12 ml sample bottle with a screw cap. Add 10 ml acetonitrile, weigh again (j g) and mix well. This solution is injected into the GC.

EW formulations

Weigh accurately sufficient EW to contain about 0.05 g malathion (g g) into a tared 12 ml sample bottle. Add 5 ml acetonitrile, weigh again (h g) and mix well. Sonicate the solution in an ultrasonic bath for 10 minutes. Approximately half-way through the period, handshake the mixture vigorously. Centrifuge the solution if it appears cloudy and transfer 1-2 ml of the upper liquid to an autosampler vial.

DP formulations

Weigh accurately sufficient DP to contain about 0.05 g of malathion (g g) into a tared 12 ml sample bottle. Add 5 ml of acetonitrile, weigh again (h g) and mix well. Sonicate the solution in an ultrasonic bath for 10 minutes. Approximately half-way through the period, handshake the mixture vigorously. Centrifuge the solution and transfer 1-2 ml of the upper liquid to an autosampler vial.

All test solutions

If the areas of the MeOOOPS and MeOOSPS peaks observed exceed those obtained from the most concentrated calibration solution, dilute the test solution accordingly with acetonitrile.

GC analysis

Inject the test and standard solutions in the following sequence:

Solution 2, T₁, T₂, T₃, T₄, T₅, T₆, T₇, T₈, Solution 2, T₉, ..., T₁₆ Solution 2, T₁₇... etc.
Where T₁...T_n are test solutions 1 to n and only one injection is made from each vial. Recalculate the response factor after each Solution 2 measurement and end the sequence with an injection of Solution 2.

Sequential injections of Solution 2 should produce peak areas within 5% of each other. Examine the peaks detected, to ensure that there is no significant interference from other components.

Calculation

Measure the peak areas of MeOOOPS and MeOOSPS peaks obtained from the reference and test solutions.

Determine the MeOOOPS and MeOOSPS content of the test sample, in g/kg, as follows:

$$\text{MeOOOPS or MeOOSPS (g/kg)} = \frac{\text{peak area of impurity in test solution} \times h \times r_f}{g}$$

where: g and h are the weights (g) measured in the preparation of the test solutions, described above;

r_f is the response factor for the impurity, determined from Solution 2 as follows:

$$r_f = \frac{\text{purity (g/kg) of impurity (ref. material)} \times a_{1 \text{ or } 2} \times c_{1 \text{ or } 2} \times e}{\text{peak area of impurity in Solution 1} \times b_{1 \text{ or } 2} \times d \times f}$$

where: a_{1 or 2}, b_{1 or 2}, c_{1 or 2}, d, e and f are the weights (in g) measured in the preparation of Solution 2, described above.

Appendix 6

Determination of malaoxon in malathion DP

(Adapted from Cheminova analytical method VAM 208-01)

Principle

Malaoxon is separated by reversed-phase HPLC, detected by UV absorption and determined by and external standardization.

Chemicals

Acetonitrile, Lichrosolv®, Merck Art.14291, or equivalent. (solvent B)

Water, HPLC grade (solvent A).

Malaoxon, reference standard, as pure as practicable.

Prepare an approximately 1% solution of the reference material by weighing accurately about 0.1 g (a g) into a tared 12 ml sample bottle with screw cap. Add 10 ml acetonitrile, weigh again (b g) and mix well (stock solution).

Weigh accurately 50 µl of the stock solution (c g) into a tared sample bottle with screw cap. Add 10 ml of 75% v/v acetonitrile/water, weigh again (d g) and mix well (Solution 1).

Weigh an aliquot of 5 ml of Solution 1 (e g.) into a tared sample bottle. Add 5 ml of 75% v/v acetonitrile/water, weigh again (f g) and mix well (Solution 2).

Weigh an aliquot of 5 ml of Solution 2 (g g) into a tared sample bottle. Add 5 ml of 75% v/v acetonitrile/water, weigh again (h g) and mix well (Solution 3).

Weigh an aliquot of 2 ml of Solution 3 (i g) into a tared sample bottle. Add 3 ml of 75% v/v acetonitrile/water, weigh again (j g) and mix well (Solution 4).

Weigh an aliquot of 1 ml of Solution 4 (k g) into a tared sample bottle. Add 1.5 ml of 75% v/v acetonitrile/water, weigh again (l g) and mix well (Solution 5).

Solutions 1, 2, 3, 4 and 5 are injected into the liquid chromatograph.

Apparatus

HPLC system, equipped with binary eluent delivery system, autosampler, photodiodearray detector and data handling system.

Analytical column, Phenomenex Prodigy ODS2, 5 µm, 150 mm x 4.6 mm, or equivalent. Two columns are connected in series to form a column of 300 mm length.

Guard column, Phenomenex Prodigy ODS2, 5 µm, 30 mm x 4.6 mm, or equivalent.

Typical operating parameters

Gradient programme	time (min.)	% B
	0.0	35
	10.0	35
	10.5	95
	15.5	95
	16.0	35
Flow rate	1.5 ml/min.	
Stop time:	20 min.	

Post time:	10 min.
DAD stop time:	15 min.
Column temperature:	50°C.
Signals	sample, 215 nm (8 nm bandwidth) interference check, 230 nm (8 nm bandwidth) reference (400 nm (100 nm bandwidth)
Slit width	8 nm
Spectrum	190-400 nm in 2 nm steps
Injection volume	50 µl
Integration	peak area
Typical retention times	11.1 min (malaoxon) 14.1 (malathion)

System suitability checks

Lamp test

Check the lamp intensity and the wavelength calibration of the detector (holmium oxide check) as described in the operating manual for the liquid chromatograph and make sure they meet the defined criteria.

Repeatability

Inject Solution 2 at least three times or until the peak area obtained from malaoxon does not differ by more than 10% between two successive measurements.

Linearity

Inject Solutions 1, 2, 3, 4 and 5 and measure the peak areas of the malaoxon. Having calculated the concentrations of the solutions from the weights measured, calculate the linear regression coefficient (r^2) of the calibration curve, which should be >0.98 .

Carry over

Inject a blank solution after Solution 2 and measure the peak area obtained for malaoxon. The "carry over" from the previous injection is acceptable if $\leq 2.0\%$ of solution 2.

Interference

Ensure that there is clear baseline separation between the isomalathion and malathion in a test solution.

Preparation of test solutions

DP formulations

Weigh accurately 2 g malathion DP (m g) into a tared 12 ml sample bottle with a screw cap. Tare the bottle and add 4 ml of 75% v/v acetonitrile/water, weigh again (n g) and sonicate the sample for 10 min. Centrifuge the solution for 5 min. and weigh accurately an aliquot of 0.5 ml of the clear liquid (o g) into a tared 12 ml sample glass. Add 1.5 ml of 65% v/v acetonitrile/water, weigh again (p g) and mix well.

Prepare duplicate test solutions for each test sample. These solutions are injected into the HPLC.

If the area of the malaoxon peak observed exceeds that obtained from the most concentrated calibration solution, dilute the solutions accordingly, using 75% v/v acetonitrile/water.

HPLC analysis

Inject the test and standard solutions in the following sequence:

Solution 3, T₁, T₂, T₃, T₄, T₅, T₆, T₇, T₈, Solution 3, T₉, T₁₆ Solution 3, T₁₇... etc.
Where T₁...T_n are test solutions 1 to n and only one injection is made from each vial.
Recalculate the response factor after each Solution 3 measurement and end the sequence with an injection of Solution 3.

Sequential injections of Solution 3 should produce peak areas within 5% of each other. Examine spectra or wavelength ratios obtained across the malaoxon peaks detected, to ensure that there is no significant interference from other components.

Calculation

Measure the peak areas of malaoxon both from the reference solution and the test solution.

Determine the malaoxon content of the test sample, in g/kg, as follows:

$$\text{malaoxon (g/kg)} = \frac{\text{peak area of malaoxon in test solution} \times n \times p \times r_f}{m \times o}$$

where: m, n, o and p are the weights (g) measured in the preparation of the test solutions, described above;

r_f is the response factor, determined from Solution 3 as follows:

$$r_f = \frac{\text{purity (g/kg) of malaoxon (ref. material)} \times a \times c \times e \times g}{\text{peak area of malaoxon in Solution 3} \times b \times d \times f \times h}$$

where: a, b, c, d, e, f, g and h are the weights (g) measured in the preparation of Solution 3, described above.