# FAO SPECIFICATIONS AND EVALUATIONS

# FOR AGRICULTURAL PESTICIDES

## MALATHION

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


FOOD AND AGRICULTURE ORGANIZATION *of* THE UNITED NATIONS

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

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

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

## INTRODUCTION

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

From 2002, the development of FAO specifications follows the **New Procedure**, described in the Manual on Development and Use of FAO and WHO Specifications for Pesticides, which is available only on the internet through the FAO and WHO web sites. This **New Procedure** follows a formal and transparent evaluation process. It describes the minimum data package, the procedure and evaluation applied by FAO and the Experts of the FAO/WHO Joint Meeting on Pesticide Specifications (JMPS). [Note: prior to 2002, the Experts were of the FAO Panel of Experts on Pesticide Specifications, Registration Requirements, Application Standards and Prior Informed Consent, which now forms part of the JMPS, rather than the JMPS.]

FAO Specifications now only apply to products for which the technical materials have been evaluated. Consequently from the year 2000 onwards the publication of FAO specifications under the **New Procedure** has changed. Every specification consists now of two parts namely the specifications and the evaluation report(s):

**Part One**: **The Specification** of the technical material and the related formulations of the pesticide in accordance with chapters 4 to 9 of the "Manual on development and use of FAO and WHO specifications for pesticides".

**Part Two**: **The Evaluation Report(s)** of the plant protection product reflecting the evaluation of the data package carried out by FAO and the JMPS. The data are to be provided by the manufacturer(s) according to the requirements of Appendix A, annex 1 or 2 of the "Manual on the development and use of FAO specifications for plant protection products" and supported by other information sources. The Evaluation Report includes the name(s) of the manufacturer(s) whose technical material has been evaluated. Evaluation reports on specifications developed subsequently to the original set of specifications are added in a chronological order to this report.

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

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

* NOTE: PUBLICATIONS ARE AVAILABLE ON THE INTERNET AT (http://www.fao.org/agriculture/crops/core-themes/theme/pests/pm/jmps/en/)

# PART ONE

# SPECIFICATIONS

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## MALATHION

## MALATHION

INFORMATION

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

*Synonyms*: maldison, malathon, mercaptothion, mercaptotion, carbofos

*Chemical name*:

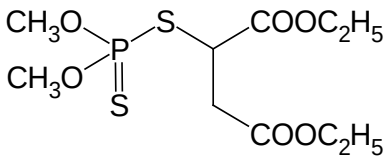
*IUPAC*: *S*-1,2-bis(ethoxycarbonyl)ethyl *O,O*-dimethyl phosphorodithioate

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

*CAS No*: 121-75-5

*CIPAC No*: 12

*Structural formula*:

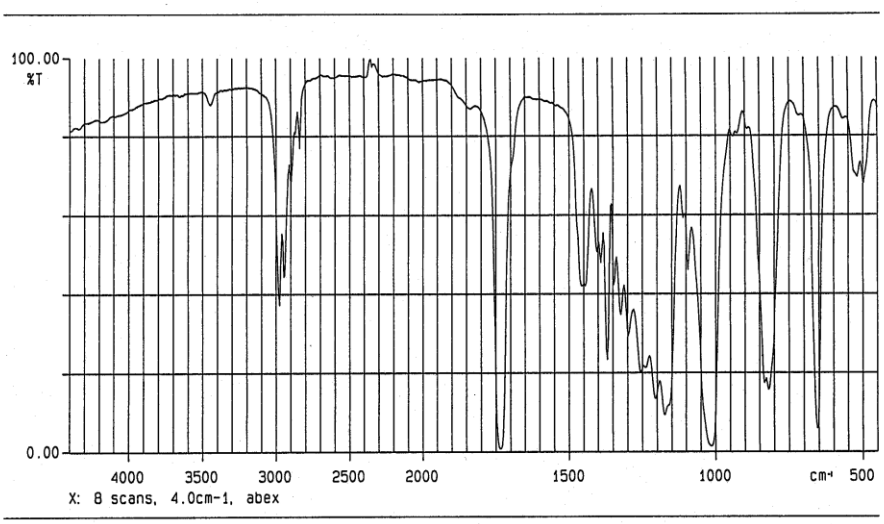



*Molecular formula*: $C_{10}H_{19}O_6PS_2$

*Relative molecular mass*:

330.36

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



# MALATHION TECHNICAL MATERIAL

FAO specification 12/TC (March 2013*)

*This specification, which is PART ONE of this publication, is based on an evaluation of data submitted by the manufacturer whose name is listed in the evaluation report (*12/2003*). It should be applicable to TC produced by this manufacturer but it is not an endorsement of those products, nor a guarantee that they comply with the specifications. The specification may not be appropriate for TC produced by other manufacturers. The evaluation report (*12/2003*) 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** (CIPAC 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** (CIPAC 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: http://www.fao.org/agriculture/crops/core-themes/theme/pests/pm/jmps/en/.

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** (CIPAC MT 31)

Maximum: 2 g/kg, calculated as $H_2SO_4$.

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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 006-02 for MeOOSPS-triester and MeOOOPS-triester.

# MALATHION DUSTABLE POWDER

FAO specification 12/DP (March 2013*)

*This specification, which is PART ONE of this publication, is based on an evaluation of data submitted by the manufacturer whose name is listed in the evaluation report (*12/2003*). 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*) as PART TWO forms an integral part of this publication.*

## 1 Description

The material shall consist of an homogeneous mixture of technical malathion, complying with the requirements of FAO specification 12/TC (March 2013), 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** (CIPAC 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** (CIPAC 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<br>Note: the upper limit is included in the range | -10% to +25% of the declared content |

## 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: http://www.fao.org/agriculture/crops/core-themes/theme/pests/pm/jmps/en/.

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 31, CIPAC Handbook F, p.96, 1995)

Maximum: 1 g/kg, calculated as $H_2SO_4$.

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

After storage at 54 ± 2°C for 14 days, the determined average active ingredient content must not be lower than 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).

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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 should be analyzed together after the test in order to reduce the analytical error.

# MALATHION ULTRA LOW VOLUME LIQUID

FAO specification 12/UL (March 2013*)

*This specification, which is PART ONE of this publication, is based on an evaluation of data submitted by the manufacturer whose name is listed in the evaluation report (*12/2003*). 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*) as PART TWO forms an integral part of this publication.*

## 1 Description

The material shall consist of technical malathion, complying with the requirements of FAO specification 12/TC (March 2013) together with any necessary formulants. 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** (CIPAC 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** (CIPAC 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.

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.

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

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, CIPAC Handbook F, p.96, 1995)

Maximum: 2 g/kg, calculated as $H_2SO_4$.

## 5 Storage stability

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

After storage at 0 ± 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 46.3, CIPAC Handbook J, p.128, 2000)

After storage at 54 ± 2°C for 14 days, the determined average active ingredient content must not be lower than 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).

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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 006-02 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 2013-01 (Appendix 2) should be used.

Note 2 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.

# MALATHION EMULSIFIABLE CONCENTRATE

FAO specification 12/EC (March 2013*)

*This specification, which is PART ONE of this publication, is based on an evaluation of data submitted by the manufacturer whose name is listed in the evaluation report (*12/2003*). 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*) as PART TWO forms an integral part of this publication.*

## 1 Description

The material shall consist of technical malathion complying with the requirements of FAO specification 12/TC (March 2013), 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 (CIPAC 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 (CIPAC 12/EC/(M3)/3, CIPAC Handbook K, p.91, 2003)

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<br>above 500<br><br>Note in each range the upper limit is included. | ± 5% of the declared content<br>± 25 g/kg or g/l |

## 3 Relevant impurities (Note 3)

### 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: http://www.fao.org/agriculture/crops/core-themes/theme/pests/pm/jmps/en/.

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 31, CIPAC Handbook F, p.96, 1995)

Maximum: 2 g/kg calculated as $H_2SO_4$.

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

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<br>"Free oil", maximum: 0.5 ml |
| 24 h | Re-emulsification complete |
| 24.5 h | "Cream", maximum: 4 ml<br>"Free oil", maximum: 0.5 ml |
| Note: tests after 24 h are required only where results at 2 h are in doubt. | |

4.3 **Persistent foam** (MT 47.3) (Notes 5 and 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 46.3, CIPAC Handbook J, p.128, 2000)

After storage at 54 ± 2°C for 14 days, the determined average active ingredient content must not be lower than:

(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).

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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 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 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 CIPAC method MT 47.3 for the determination of persistent foam created when formulations are added to water before use (CIPAC/4835) was accepted as a provisional CIPAC method in 2012. Prior to its publication in a Handbook, copies of the method may be obtained through the CIPAC website, http://www.cipac.org/cipacpub.htm.

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 should be analyzed together after the test in order to reduce the analytical error.

# MALATHION EMULSION, OIL IN WATER

FAO specification 12/EW (March 2013*)

*This specification, which is PART ONE of this publication, is based on an evaluation of data submitted by the manufacturer whose name is listed in the evaluation report (*12/2003, 12/2012*). 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, 12/2012*) 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 FAO specification 12/TC (March 2013), in an aqueous phase together with suitable formulants (Note 1). After gentle agitation, the formulation shall be homogeneous and suitable for dilution in water (Note 2).

## 2 Active ingredient

### 2.1 **Identity tests** (CIPAC 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** (CIPAC 12/EW/(M)/3, CIPAC Handbook K, p.92, 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 50 up to 100 | ± 10% of the declared content |
| above 100 up to 250 | ± 6% of the declared content |
| above 250 up to 500 | ± 5% of the declared content |
| Note in each range the upper limit is included. | |

## 3. Relevant impurities (Note 4)

### 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.

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

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.

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 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<br>“Free oil”, none |
| 24 h | Re-emulsification complete |
| 24.5 h | “Cream”, maximum: 2 ml<br>“Free oil”, none |
| Note: tests after 24 h are required only where results at 2 h are in doubt. | |

4.4 **Persistent foam** (MT 47.3) (Notes 6 and 7)

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 ± 2°C for 7 days, no separation of particulate or oily matter shall be visible after gentle agitation.

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

After storage at 54 ± 2°C for 14 days, the determined average active ingredient content must not be lower than:

(i) 90% relative to the determined average content found before storage (Note 8), for products with a declared content of malathion above 25 up to 500 g/l;

(ii) 80% relative to the determined average content found before storage (Note 8), for products with a declared content of malathion up to 25 g/l;

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).

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

Note 2 Ready-for-use EW formulations are not inteded for further dilution with water.

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

Note 4 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 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 CIPAC method MT 47.3 for the determination of persistent foam created when formulations are added to water before use (CIPAC/4835) was accepted as a provisional CIPAC method in 2012. Prior to its publication in a Handbook, copies of the method may be obtained through the CIPAC website, http://www.cipac.org/cipacpub.htm.

Note 7 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 8 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

**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 receveid 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.

# 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 UK[1] 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

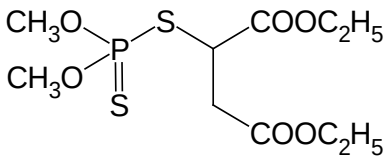
CA: butanedioic acid, [(dimethoxyphosphinothioyl)thio]-, diethyl ester

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 | 25ºC: $4.5 \pm 1.1 \times 10^{-4}$ Pa, $3.4 \pm 0.82 \times 10^{-6}$ mm Hg.<br>35ºC: $3.1 \pm 0.84 \times 10^{-3}$ Pa, $2.3 \pm 0.63 \times 10^{-5}$ mm Hg.<br>45ºC: $1.9 \pm 0.47 \times 10^{-2}$ Pa, $1.4 \pm 0.35 \times 10^{-4}$ mm Hg. | 98.9 | US EPA D63-9 | Teeter and Blasberg, 1988 |
| Boiling point | No value could be determined due to decomposition above approx. 174ºC. | 99.1 | EEC A2 | Cuthbert and Mullee, 2001 |
| Melting point | Melting point: below -20ºC. | 99.1 | EEC A1 | Cuthbert and Mullee, 2001 |
| Temperature of decomposition | Onset of decomposition at 174ºC at 100 kPa. | 99.1 | EEC A2 | Cuthbert and Mullee, 2001 |
| Solubility in water | 148 mg/l at 25 ± 1ºC | [$^{14}$C]-malathion radiochemical purity: 98.4 | US EPA D63-8 | Kabler, 1989 |
| Octanol/water partition coefficient | log $K_{ow}$ = 2.7 at 25ºC | [$^{14}$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).<br>pH 5: Half-life 107 days; Rate constant 0.0065 day$^{-1}$<br>pH 7: Half-life 149 hours; Rate constant 0.00463 hours$^{-1}$<br>pH 9: Half-life 11.8 hours; Rate constant 0.0587 hours$^{-1}$ | [$^{14}$C]-malathion radiochemical purity >98 | US-EPA N161-1 | Teeter, 1988 |
| 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).<br>Sensitised (acetone): 70.4% remaining after 30 days; Rate constant 0.00972 day$^{-1}$; Half-life 71 days<br>Non-sensitised: 78.7% remaining after 30 days; Rate constant 0.00707 day$^{-1}$; Half-life 98 days<br>Dark controls: ~90% | [$^{14}$C]-malathion (radiochemical purity 94.9; unlabelled malathion purity 98.4 | US-EPA N161-2 | Carpenter, 1990 |

| 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:

| | Product as % applied dose | | |
|---|---|---|---|
| Hydrolysis product | 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:

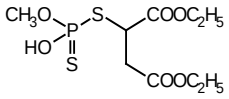



Table 2. Chemical composition and properties of malathion technical material (TC).

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

| | |
|---|---|
| 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<br>CAS No: 1634-78-2<br>CAS name: butanedioic acid, (dimethoxyphosphinothioyl)-, diethyl ester | 1 g/kg |
| Isomalathion<br>CAS No: 3344-12-5<br>CAS name: succinic acid, mercaptodiethylester, *S*-ester with *O,S*-dimethyl phosphorodithioate | 4 g/kg |
| MeOOSPS-triester<br>CAS No: 2953-29-9<br>CAS name: phosphorodithioic acid, *O,O,S*-trimethyl ester | 15 g/kg |
| MeOOOPS-triester<br>CAS No: 152-18-1<br>CAS name: phosphorothioic acid, *O*,*O*,*O*-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<br>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<br>Females: $LD_{50}$ = 4061 mg/kg bw | Malathion (Fyfanon) technical, purity 96.8% | Fischer, 1991b<br>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 $\pm$ 385 mg/kg bw | Malathion (Fyfanon) technical purity not specified. | Terrell, 1978<br>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<br>Female: $LD_{50}$ = 4400 mg/kg bw | Malathion technical (Fyfanon) purity not specified Stored for 1 year at 5ºC 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<br>Females: $LD_{50}$ = 3700 mg/kg bw | Malathion technical (Fyfanon) purity not specified Stored for 1 year at 20-25ºC 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<br>Females: $LD_{50}$ = 5700 mg/kg bw | Malathion technical (Fyfanon) purity 96-98%. Isomalathion content <0.1% | Kynoch, 1986a<br>JMPR, 1997 |
| Rabbit M/F | Acute dermal $LD_{50}$ on New Zealand albino rabbits | No guideline | Combined males and females group $LD_{50}$ = 8790 $\pm$ 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 hyper-sensitivity 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 neuro-toxicity 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 *et al.* 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 conjuctival 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 hyper-sensitivity | 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 *et al*., 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% | Schellen-berger 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 *et al*., 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 *et al.*, 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 *et al.*, 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 | | | | | |
| *in vitro* *Salmonella typhimurium* | 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 |
| *in vitro* 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 |
| *in vitro* 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 |
| *In vitro* 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 |
| *In vivo* 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 |
| *In vivo* rat hepatocytes | *In vivo* 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 | | | | | |
| *Cyprinodon variegatus* (Sheepshead minnow) | Acute toxicity, flow-through system: US EPA, EPA-660/3-75-009, April 1979, minor deviations | 96 hours at 21 to 23ºC | 24 h $LC_{50}$ = 0.043 mg/l<br>48 h $LC_{50}$ = 0.043 mg/l<br>96 h $LC_{50}$ = 0.040 mg/l<br>NOEC = 0.018 mg/l | Malathion (Cythion), technical, purity 94% | Bowman, 1989 |
| *Lepomis macrochirus* (Bluegill sunfish) | Acute toxicity, flow-through system: OECD 203 | 96 hours at 23 ± 1ºC | 24 h $LC_{50}$ = 0.12 mg/l<br>48 h $LC_{50}$ = 0.12 mg/l<br>72 h $LC_{50}$ = 0.076 mg/l<br>96 h $LC_{50}$ = 0.054 mg/l<br>NOEC = 0.032 mg/l | Malathion (Fyfanon) technical, purity 96.9% | Gries and Purghart, 2001a |
| *Oncorhynchus mykiss* (Rainbow trout) | Acute toxicity, flow-through system: OECD 203 | 96 hours at 16 ± 1ºC | 24-h $LC_{50}$ = 0.41 mg/l<br>48 h $LC_{50}$ = 0.37 mg/l<br>72-h $LC_{50}$ = 0.27 mg/l<br>96-h $LC_{50}$ = 0.18 mg/l<br>NOEC = 0.091 mg/l | Malathion technical (Fyfanon), purity 96.9% | Gries and Purghart, 2001b |
| *Cyprinus carpio* (Common carp) | Acute toxicity, flow-through system: OECD 203 | 96 hours at 23 ± 1ºC | 96 h $LC_{50}$ = >10 mg/l<br>NOEC = 1 mg/l | Malathion technical (Fyfanon), purity 96.9% | Gries and van der Kolk, 2002 |
| *Gasterosteus aculeatus* (Three-spined stickleback) | Acute toxicity, flow-through system: OECD 203 | 96 hours at 10 ± 2ºC | 96 h $LC_{50}$ = 0.022 mg/l<br>NOEC = 0.005 mg/l | Malathion technical (Fyfanon), purity 96.9% | Gries *et al.*, 2002b |
| *Pimephales promelas* (Fathead minnow) | Acute toxicity, flow-through system: OECD 203 | 96 hours at 23 ± 1ºC | 96 h $LC_{50}$ = >8.0 mg/l<br>NOEC = 0.98 mg/l | Malathion technical (Fyfanon), purity 96.9% | Gries *et al.*, 2002a |
| *Oncorhynchus mykiss* (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 13.6ºC | NOEC = 0.021 mg/l | Malathion technical (Cythion), purity 94% | Cohle, 1989 |
| Daphnia | | | | | |
| *Daphnia magna* (Daphnids, water flea) <24 hours old | Chronic toxicity, flow-through system: OECD 202-I | 48 hours at 20 ± 1ºC | 48 h $EC_{50}$ = 0.72 μg/l.<br>NOEC = 0.21 μg/l | Malathion technical (Fyfanon), purity 96.9% | Gries and Purghart, 2001c |
| *Daphnia magna* (Daphnids, water flea) <24 hours old | Chronic toxicity, flow-through system: OECD 202, May 1981 | 21 days at 21 to 22ºC | NOEC = 0.06 μg/l | Malathion technical (Cythion), purity 94% | Blakemore and Burgess, 1990 |

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

| Earthworms | | | | | |
|---|---|---|---|---|---|
| *Eisenia foetida foetida* (Earthworm) | Acute toxicity: OECD 207, April 1984 | 14 days exposure, 18.5 to 22ºC, pH 7.5 to 7.8 | 14 day $LC_{50}$ = 613 mg/kg soil<br>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)
- 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

Isomalathion (rat oral $LD_{50}$ 113 mg/kg bw, and a potentiator of malathion-induced toxicity) and malaoxon (rat oral $LD_{50}$ 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 $LD_{50}$ 26 mg/kg bw) and MeOOSPO-triester (reported $LD_{50}$ 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 $LD_{50}$ 562 mg/kg bw) and MeOOSPS-triester (reported rat oral $LD_{50}$ 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.

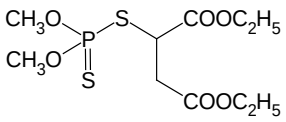
## Methods of analysis and testing

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*



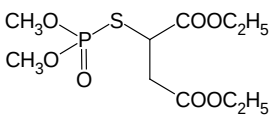

*CAS 121-75-5*

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*




*CAS 1634-78-2*

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.

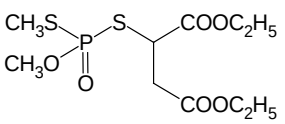
| Substrate | Test (note 1) | Result (note 2) |
|---|---|---|
| Method VAM 008-02 (HPLC). Validation: Hinz, 2001b. | | |
| TC | Linearity | Acceptable linearity over test range, 7-53 mg/kg in injection solution. |
| | Specificity | Interference did not occur from the following compounds, with retention 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<br>0.12 / 0.06%<br>0.058 / 0.10%<br>0.10 / 0.11%<br>0.020 / 0.34%<br>0.015 / 0.58%<br>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*




CAS 3344-12-5

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 $^{31}$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).

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

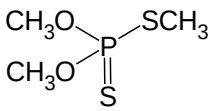
Table 9. Validation data for isomalathion in malathion TC and formulations.

| 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 × 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.

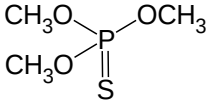
Table 10. Validation data for MeOOSPS-triester in malathion TC and formulations.

| 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 × 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 (1$^{st}$ 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 ±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 ± 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 54ºC (±2ºC), 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 54ºC 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%).

## Container 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 $LD_{50}$ tests on rats (Table 3 and summarized below), malathion of higher purity seems less toxic than malathion of lower purity.

| Test material | Year | Rat oral $LD_{50}$ |
|---|---|---|
| Malathion TC, purity not specified | 1978 | 5000 mg/kg bw |
| Malathion TC, purity not specified, stored 1 year at 5ºC | 1979 | 4100 mg/kg bw |
| Malathion TC, purity not specified, stored 1 year at 20-25ºC | 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 $LD_{50}$ 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 apporpriate for the UL.

Relevant impurities in TC may be determined by HPLC or GC, but $^{31}$P-NMR spectroscopy is required for successful analysis of some formulations, down to the required levels. The $^{31}$P-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.

## References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ADDENDUM 1 TO THE EVALUATION (FEBRUARY, 2004)

## MALATHION

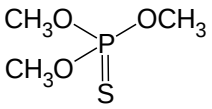
### Explanation

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) | | | |
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| 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.

## Recommendations

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

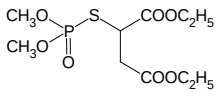
The 2003 JMPS included malaoxon, isomalathion, MeOOSPS-triester and MeOOOPS-triester as relevant impurities in malathion.

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.

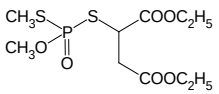
Table 1. Validation data for determination of malaoxon in malathion EC and EW formulations.

| 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).

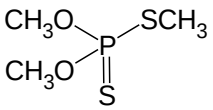
Independent laboratory validation data for the determination of isomalathion in malathion EC formulations are summarized in Table 2.

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

| 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).

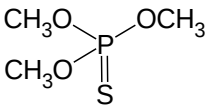
Independent laboratory validation data for MeOOSPS-triester in malathion EC formulations are summarized in Table 3.

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

| 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 $^{31}$P-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 $^{31}$P-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.

| 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 $^{31}$P-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 $^{31}$P-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.

## Recommendations

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 - 90º-X-pulse - acquisition (with proton decoupling). |
| Nucleus | $^{31}$P. |
| Relaxation delay | 60s. |
| Pulse width | The 90º pulse width is chosen from the most recent calibration of the $^{31}$P pulse in a polar solvent. |
| Nucleus for decoupler | $^{1}$H. |
| 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 | 25ºC. |

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 outmost 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:

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

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;

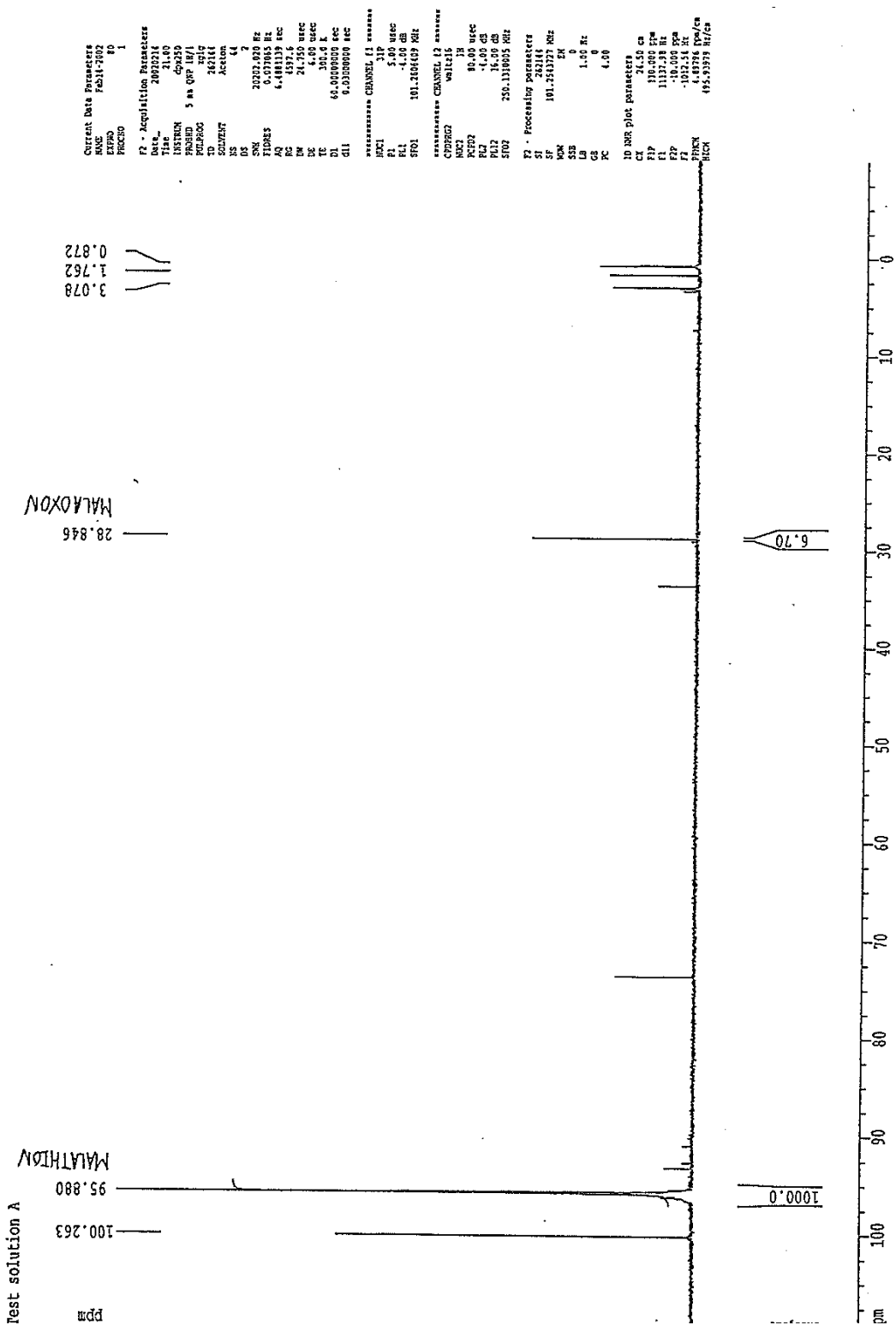
(I malaoxon) = integral of the malaoxon signal in the NMR spectrum;

(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

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 - 90º-X-pulse - acquisition (with proton decoupling). |
| Nucleus | $^{31}$P. |
| Relaxation delay | 60s. |
| Pulse width | The 90º pulse width is chosen from the most recent calibration of the $^{31}$P pulse in a polar solvent. |
| Nucleus for decoupler | $^{1}$H. |
| 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 | 25ºC. |

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 outmost 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 outmost 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:

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

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

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

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

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;

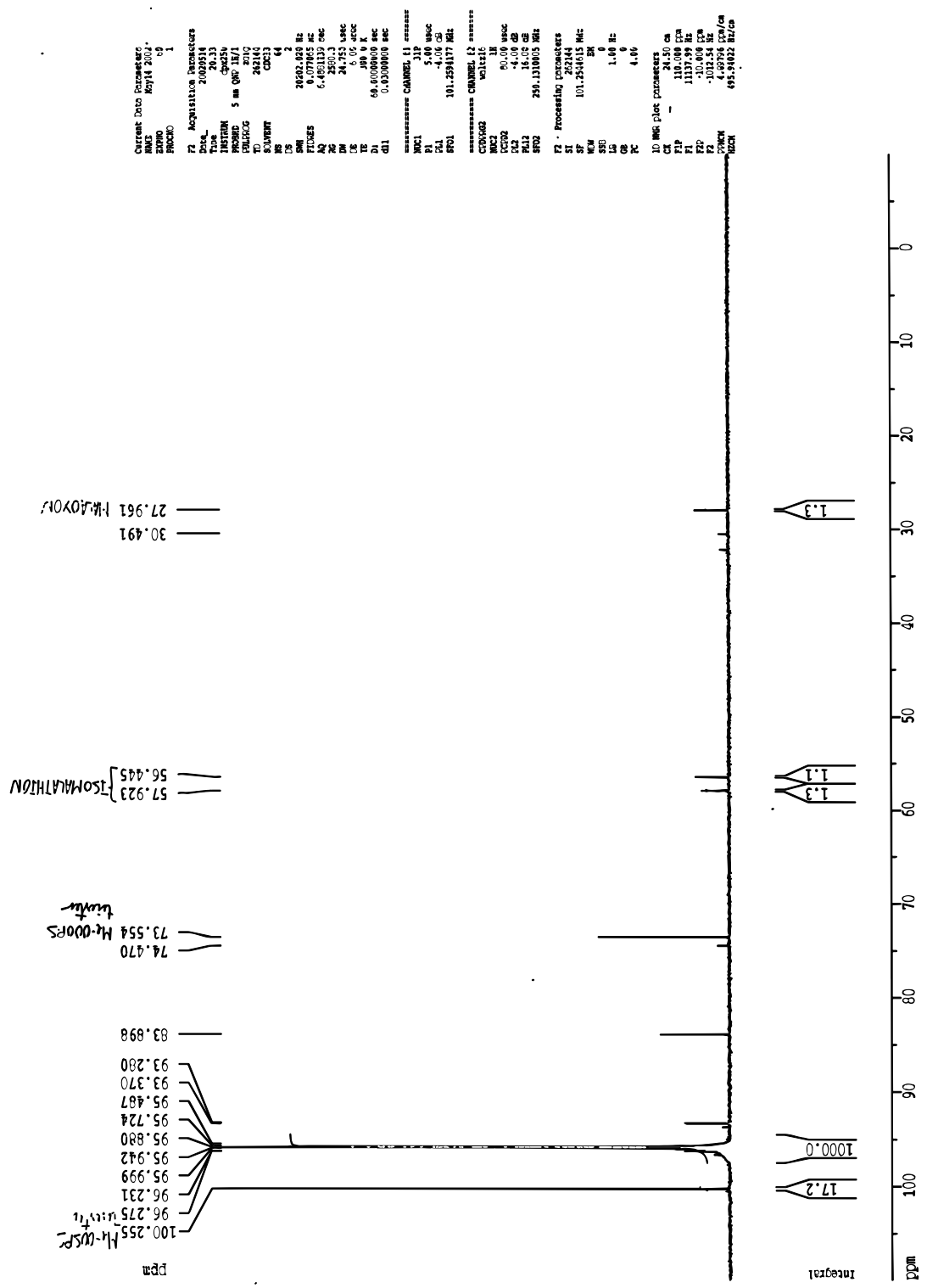
(MW MeOOOPS) = molecular weight of MeOOOPS-triester, 156.1 g/mol;

(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.

### Apparatus and chemicals

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: | 50ºC. |
| Signals | sample, 200 nm (8 nm bandwidth)<br>interference check, 225 nm (8 nm bandwidth)<br>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 ≤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 , $T_1$, $T_2$, $T_3$, $T_4$, $T_5$, $T_6$, $T_7$, $T_8$, Solution 2, $T_9$, ….$T_{16}$ Solution 2, $T_{17}$… etc.
Where $T_1$….$T_n$ 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.

### Apparatus and chemicals

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: | 50ºC. |
| Signals | sample, 215 nm (8 nm bandwidth)<br>interference check, 230 nm (8 nm bandwidth)<br>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)<br>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 ≤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 , $T_1$, $T_2$, $T_3$, $T_4$, $T_5$, $T_6$, $T_7$, $T_8$, Solution 2, $T_9$, ….$T_{16}$ Solution 2, $T_{17}$… etc.

Where $T_1 \ldots T_n$ 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:

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

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:

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

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.

### Apparatus and chemicals

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 ul aliquot of the MeOOOPS stock solution ($c_1$ g) and a 300 ul 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 µl 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 µm flim thickness, or equivalent.

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

*Injection*, splitless.

Typical operating parameters

| | |
|---|---|
| Injector temperature | 150ºC |
| Detector temperature | 300ºC |
| Column temperature programme | initial 2 min at 60ºC;<br>10ºC/min to 210ºC, hold 0 min;<br>25ºC/min to 250ºC, hold 2 min;<br>35ºC/min to 280ºC, 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.<br>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 ≤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 , $T_1$, $T_2$, $T_3$, $T_4$, $T_5$, $T_6$, $T_7$, $T_8$, Solution 2, $T_9$, ….$T_{16}$ Solution 2, $T_{17}$… etc.

Where $T_1$….$T_n$ 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: $a_{1 \text{ or } 2}$, $b_{1 \text{ or } 2}$, $c_{1 \text{ or } 2}$, 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.

### Apparatus and chemicals

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: | 50ºC. |
| Signals | sample, 215 nm (8 nm bandwidth)<br>interference check, 230 nm (8 nm bandwidth)<br>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)<br>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 ≤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 , $T_1$, $T_2$, $T_3$, $T_4$, $T_5$, $T_6$, $T_7$, $T_8$, Solution 3, $T_9$, ….$T_{16}$ Solution 3, $T_{17}$… etc.
Where $T_1$….$T_n$ 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.