FAO SPECIFICATIONS AND EVALUATIONS

FOR PLANT PROTECTION PRODUCTS

PARAQUAT DICHLORIDE

1,1'-dimethyl-4,4'-bipyridinium dichloride

2003

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PARAQUAT DICHLORIDE

Disclaimer[1]

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

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

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

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

INTRODUCTION

FAO establishes and publishes specifications* for technical material and related formulations of plant protection products 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.

Since 1999 the development of FAO specifications has followed the **New Procedure**, first described in the 5th edition of the "Manual on the development and use of FAO specifications for plant protection products" (FAO Plant Production and Protection Paper No. 149) and, subsequently, in the 1st edition of the "Manual for Development and Use of FAO and WHO Specifications for Pesticides" (FAO Plant Production and Protection Paper No. 173, 2002). 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).

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 1st edition of the "FAO/WHO Manual on Pesticide Specifications."

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

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

* Footnote: The publications are available on Internet under (http://www.fao.org/AG/AGP/AGPP/Pesticid/) or as hardcopy from the Plant Protection Information Officer.

PART ONE

SPECIFICATIONS

PARAQUAT DICHLORIDE

PARAQUAT DICHLORIDE INFORMATION
PARAQUAT DICHLORIDE TECHNICAL CONCENTRATES
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FAO SPECIFICATIONS AND EVALUATIONS FOR
PLANT PROTECTION PRODUCTS

PARAQUAT DICHLORIDE

INFORMATION

Common name (dication):

paraquat (E-ISO, (m)F-ISO, BSI, ANSI, WSSA, JMAF)

Synonyms:

methyl viologen

Chemical names:

dication -
IUPAC, 1,1'-dimethyl-4,4'-bipyridinium [1]
CA, 1,1'-dimethyl-4,4'-bipyridinium

dichloride -
IUPAC, 1,1'-dimethyl-4,4'-bipyridinium dichloride [1]
CA, 1,1'-dimethyl-4,4'-bipyridinium dichloride

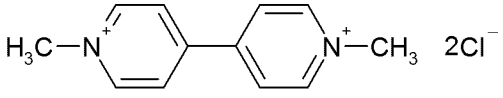
CAS No:

1910-42-5 (dichloride); 4685-14-7 (dication)

CIPAC No:

56 (dication); 56.302 (dichloride)

Structural formula (dichloride):



Molecular formula:

$C_{12}H_{14}Cl_2N_2$ (dichloride); $C_{12}H_{14}N_2$ (dication)

Relative molecular mass:

257.2 (dichloride); 186.3 (dication)

Identity tests (CIPAC G 56/SL/M-):

HPLC retention time; UV spectrum; addition of alkaline sodium dithionite to a dilute solution, where a blue colour indicates the presence of

[1] The IUPAC name for the bipyridinium moiety is alternatively expressed as “bipyridinediium” or “bipyridilium”.

paraquat. The presence of the dichloride salt is tested with silver nitrate solution.

PARAQUAT DICHLORIDE TECHNICAL CONCENTRATE (TK)

FAO Specification 56.302/TK (2003)

*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 reports (*56.302/2003*). It should be applicable to relevant products of 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 the products of other manufacturers. The evaluation reports (*56.302/2003*) as PART TWO forms an integral part of this publication.*

1 Description

The material shall consist of paraquat dichloride, together with related manufacturing impurities, in the form of an aqueous solution , free from visible extraneous matter, and must contain an effective emetic (Note 1). The material may also include colorants and olefactory alerting agents.

2 Active ingredient

2.1 **Identity tests** (56/SL/M/2, CIPAC G, p.128)

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 **Paraquat dichloride content** (56/SL/M/3, CIPAC E, p.167)

The paraquat dichloride content (Note 2) shall be declared (not less than 500 g/l at 20 ± 2ºC, Note 3) and, when determined, the average measured content shall not differ from that declared by more than ± 25 g/l.

3 Relevant impurities

3.1 **Free 4,4'-bipyridyl** (56/13/M/7.4, CIPAC 1A, p.1317)

Maximum: 1.0 g/kg (1000 ppm).

3.2 **Total terpyridines** (Note 4)

Maximum: 0.001 g/kg (1.0 ppm).

4 Physical properties

4.1 **pH range** (MT 75.3, CIPAC J, p. 131) (Note 5)

pH range: 2.0 to 6.0.

Note 1 An effective emetic, having the following characteristics, must be incorporated into the TK.

- It must be rapidly absorbed (more rapidly than paraquat) and be quick acting. Emesis must occur in about half an hour in at least 50% of cases.
- It must be an effective (strong) stimulant of the emetic centre of the brain, to produce effective emesis. The emetic effect should have a limited 'action period', of about two to three hours, to allow effective treatment of poisoning.
- It must act centrally on the emetic centre in the brain.
- It must not be a gastric irritant because, as paraquat is itself an irritant, this could potentiate the toxicity of paraquat.
- It must be toxicologically acceptable. It must have a short half-life in the body (to comply with the need for a limited action period).
- It must be compatible with, and stable in, the paraquat formulation and not affect the herbicidal efficacy or occupational use of the product.

To date, the only compound found to meet these requirements is 2-amino-4,5-dihydro-6-methyl-4-propyl-s-triazole-(1,5a)pyrimidin-5-one (PP796).

PP796 must be present in the TK at not less than 0.8 g/l.

The method for determination of PP796 content is given in Appendix 1 of the evaluation, which is Part Two of this publication.

Note 2 To obtain the paraquat dichloride content, multiply the paraquat ion content (as determined by CIPAC method 56/SL/M/3) by 1.38.

Note 3 The lower limit of 500 g/l corresponds nominally to 442 g/kg and thus the tolerance of ± 25 g/l corresponds to ± 5% on a g/kg basis. If, in a particular case, the declared concentration exceeds 566 g/l (>500 g/kg), the tolerance shall be ± 25 g/kg, not ± 25 g/l (± 22 g/kg). If the buyer requires specification of both g/l at 20°C and g/kg, then in case of dispute the analytical results shall be calculated as g/kg.

Note 4 The method for determination of total terpyridines content is given in Appendix 2 of the Evaluation, which is Part Two of this publication.

Note 5 The product must not be allowed to come into direct contact with metal. Containers may be manufactured from suitable polymeric materials or metal and must comply with pertinent national and international transport and safety regulations. If metal is used, containers must be lined with suitable polymeric material, or the internal surfaces treated to prevent corrosion of the container and/or deterioration of the contents.

PARAQUAT DICHLORIDE SOLUBLE CONCENTRATES (SL)

FAO Specification 56.302/SL (2003)

*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 reports (*56.302/2003*). It should be applicable to relevant products of 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 the products of other manufacturers. The evaluation reports (*56.302/2003*) as PART TWO forms an integral part of this publication.*

1 Description

The material shall consist of an aqueous solution of technical paraquat dichloride, complying with the requirements of FAO specification 56.302/TK(2003), together with necessary formulants and safening agents, which must include an effective emetic (Note 1) and appropriate colorants, and may include other safeners including olefactory alerting agents and thickeners. It shall contain not more than a trace of suspended matter, immiscible solvents and sediment.

2 Active ingredient

2.1 **Identity tests** (56/SL/M/2, CIPAC G, p.128)

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 **Paraquat dichloride content** (56/SL/M/3, CIPAC E, p.167, or 55+56/SL/M, CIPAC E, p.75, for mixed formulations containing diquat)

The paraquat dichloride content (Note 2) shall be declared (g/kg and/or g/l at 20 ± 2ºC, Note 3) and, when determined, the content obtained shall not differ from that declared by more than the following amounts.

Declared content, g/kg or g/l at 20 ± 2ºC	Permitted tolerance
25 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: the upper limit is included in each range.	

3 Relevant impurities

3.1 **Free 4,4'-bipyridyl** (56/13/M/7.4, CIPAC 1A, p.1317)

Maximum: 1.0 g/kg (1000ppm)[

3.2 **Total terpyridines** (Note 4)

Maximum: 0.001 g/kg (1.0ppm

4 Physical properties

4.1 **pH range** (CIPAC MT 75.3, CIPAC J, p. 131) (Note 5)

pH range of a 1% v/v dispersion: 6.0 to 8.0.

4.2 **Solution stability** (CIPAC MT 41, CIPAC F, p. 131)

The formulation, after the stability test at 54°C (see 5.2) and following dilution (Note 6) with CIPAC standard water D and standing at 30 ± 2°C for 18 h, shall give a clear or opalescent solution, free from more than a trace of sediment and visible solid particles. Any visible sediment or particles produced shall pass through a 45 μm test sieve (Note 7).

4.3 **Persistent foam** (CIPAC MT 47.2, CIPAC F, p. 152) (Note 8)

Maximum: 60 ml after one minute.

5 Storage stability

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

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

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

After storage at 54 ± 2ºC for 14 days, the determined average active ingredient content must not be lower than 97%, relative to the determined average content found before storage (Note 9), and the product shall continue to comply with the clauses for:

- pH range (4.1).

Note 1 An effective emetic, having the following characteristics, must be incorporated into the TK.

- It must be rapidly absorbed (more rapidly than paraquat) and be quick acting. Emesis must occur in about half an hour in at least 50% of cases.
- It must be an effective (strong) stimulant of the emetic centre of the brain, to produce effective emesis. The emetic effect should have a limited 'action period', of about two to three hours, to allow effective treatment of poisoning.
- It must act centrally on the emetic centre in the brain.
- It must not be a gastric irritant because, as paraquat is itself an irritant, this could potentiate the toxicity of paraquat.
- It must be toxicologically acceptable. It must have a short half-life in the body (to comply with the need for a limited action period).
- It must be compatible with, and stable in, the paraquat formulation and not affect the herbicidal efficacy or occupational use of the product.

To date, the only compound found to meet these requirements is 2-amino-4,5-dihydro-6-methyl-4-propyl-s-triazole-(1,5a)pyrimidin-5-one (PP796).

PP796 must be present in the formulation at not less than 0.23% of the paraquat ion content[0.17% of the paraquat dichloride content].

The method for determination of PP796 content is given in Appendix 1 of the evaluation, which is Part Two of this publication.

Note 2 To obtain the paraquat dichloride content, multiply the paraquat ion content (as determined by CIPAC method 56/SL/M/3) by 1.38.

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

Note 4 The method for determination of total terpyridines content is given in Appendix 2 of the Evaluation, which is Part Two of this publication.

Note 5 The product must not be allowed to come into direct contact with metal. Containers may be manufactured from suitable polymeric materials or metal and must comply with pertinent national and international transport and safety regulations. If metal is used, containers must be lined with suitable polymeric material, or the internal surfaces treated to prevent corrosion of the container and/or deterioration of the contents.

Note 6 The concentration for the test should not be higher than the highest concentration recommended in the instructions for use.

Note 7 Some formulations containing additional wetter may show signs of layering and produce a trace of oily precipitate under the test conditions defined in MT41. This is acceptable, and does not affect biological efficacy or spray characteristics at normal spray dilution.

Note 8 The mass of sample used in the test should correspond to the highest concentration recommended in the instructions for use.

Note 9 Samples of the product taken before and after the storage stability test should be analyzed concurrently after the test to reduce the analytical error.

PARAQUAT DICHLORIDE WATER SOLUBLE GRANULES (SG)

FAO Specification 56.302/SG (2003)

*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 reports (*56.302/2003*). It should be applicable to relevant products of 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 the products of other manufacturers. The evaluation reports (*56.302/2003*) as PART TWO forms an integral part of this publication.*

1 Description

The material shall consist of granules containing technical paraquat dichloride complying with the requirements of the FAO specification 56/TK (2003), together with any necessary carriers, other formulants and safening agents, which must include an effective emetic (Note 1) and appropriate colorants, and may include other safeners. It shall be homogeneous, free from visible extraneous matter and/or hard lumps, free flowing, and nearly dust-free. Insoluble carriers and formulants shall not interfere with compliance with clause 4.2.

2 Active ingredient

2.1 **Identity tests** (56/SL/M/2, CIPAC G, p.128)

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 **Paraquat dichloride content** (*55+56/SG/M/-, CIPAC E, p.78, Note 2)

The paraquat dichloride content (Note 3) shall be declared (g/kg) and, when determined, the content measured shall not differ from that declared by more than the following amounts:

Declared content, g/kg or g/l at 20 ± 2ºC	Permitted tolerance
25 up to 100 Above 100 up to 250 Note: the upper limit is included in each range.	± 10% of the declared content ± 6% of the declared content

3 Relevant impurities

3.1 **Free 4,4'-bipyridyl** (56/13/M/7.4, CIPAC 1A, p.1317)

Maximum: 1.0 g/kg (1000ppm

3.2 **Total terpyridines** (Note 4)

Maximum: 0.001 g/kg (1.0ppm

4 Physical properties

4.1 **pH range** (CIPAC MT 75.3, CIPAC J, p. 131) (Note 5)

pH range of a 1% w/v dispersion: 6.0 to 8.0.

4.2 **Degree of dissolution and solution stability** (CIPAC MT 179, CIPAC H, p.307)

Residue of formulation retained on a 75um test sieve after dissolution in CIPAC Water D at 30 ± 2°C.

Maximum: 2% after 5 minutes.

Maximum: 2% after 18 hours.

4.3 **Persistent foam** (CIPAC MT 47.2, CIPAC F, p. 152) (Note 6)

Maximum: 30 ml after 1 minute.

4.4 **Dustiness** (CIPAC MT 171, CIPAC F, p. 425) (Note 7)

Nearly dust-free (maximum: 1 mg dust per 30 g sample, gravimetric method).

4.5 **Flowability** (CIPAC MT 172, CIPAC F, p. 430, and CIPAC MT 46.3, CIPAC J, p. 128)

At least 98% of the formulation shall pass through a 5 mm test sieve after 20 drops of the sieve.

4.6 **Attrition resistance** (CIPAC MT 178, CIPAC H, p. 304)

An attrition resistance of at least 99.5%

5 Storage stability

5.1 **Stability at elevated temperatures** (MT 46.3, CIPAC J, p. 128)

After storage at 54 ± 2°C for 14 days the determined average active ingredient content shall not be lower than 97%, relative to the determined average content found before storage (Note 8), and the formulation shall continue to comply with the clauses for:

- pH range (4.1);
- degree of dissolution and solution stability (4.2);
- dustiness (4.4);
- flowability (4.5);
- attrition resistance (4.6).

Note 1 An effective emetic, having the following characteristics, must be incorporated into the TK.

- It must be rapidly absorbed (more rapidly than paraquat) and be quick acting. Emesis must occur in about half an hour in at least 50% of cases.
- It must be an effective (strong) stimulant of the emetic centre of the brain, to produce effective emesis. The emetic effect should have a limited 'action period', of about two to three hours, to allow effective treatment of poisoning.
- It must act centrally on the emetic centre in the brain.
- It must not be a gastric irritant because, as paraquat is itself an irritant, this could potentiate the toxicity of paraquat.
- It must be toxicologically acceptable. It must have a short half-life in the body (to comply with the need for a limited action period).
- It must be compatible with, and stable in, the paraquat formulation and not affect the herbicidal efficacy or occupational use of the product.

To date, the only compound found to meet these requirements is 2-amino-4,5-dihydro-6-methyl-4-propyl-s-triazole-(1,5a)pyrimidin-5-one (PP796).

PP796 must be present in the formulation at not less than 0.23% of the paraquat ion content[0.17% of the paraquat dichloride content].

The method for determination of PP796 content is given in Appendix 1 of the evaluation, which is Part Two of this publication.

Note 2 For the analysis of SG formulations containing only paraquat, CIPAC method *55+56/SG/M/- should be used without the correction described for diquat.

Note 3 To obtain the paraquat dichloride content, multiply the paraquat ion content (as determined by CIPAC method 56/SL/M/3) by 1.38.

Note 4 The method for determination of total terpyridines content is given in Appendix 2 of the Evaluation, which is Part Two of this publication

Note 5 The product must not be allowed to come into direct contact with metal. Containers may be manufactured from suitable polymeric materials or metal and must comply with pertinent national and international transport and safety regulations. If metal is used, containers must be lined with suitable polymeric material, or the internal surfaces treated to prevent corrosion of the container and/or deterioration of the contents.

Note 6 The mass of sample to be used in the test should be specified at the highest recommended use rate.

Note 7 The optical method, MT 171, would not give reliable values at the levels of dust around the specified limit and should therefore not be used.

Note 8 Samples of the formulation taken before and after the storage stability test should be analyzed concurrently after the test in order to reduce the analytical error.

PART TWO

EVALUATION REPORTS

PARAQUAT

2003 FAO/WHO evaluation report based on submission of data from Syngenta, UK (TC, SL, SG).

FAO SPECIFICATIONS FOR PLANT PROTECTION PRODUCTS

FAO EVALUATION REPORT 56.302/2003

PARAQUAT

Explanation

The data for paraquat dichloride were evaluated in support of a review of existing FAO specifications (AGP:CP/344, Rome,1996).

Paraquat dichloride is not under patent.

Paraquat was reviewed by the World Health Organization (WHO) and the United Nations Environment Programme (UNEP) in 1983, resulting in the publication of Environmental Health Criteria 39 (WHO, 1984), and by the International Programme on Chemical Safety (IPCS, 1991), resulting in IPCS Health & Safety Guide No 51. Paraquat was reviewed by the FAO/WHO Joint Meeting on Pesticide Residues (JMPR) in 1986 and was scheduled for periodic re-evaluation in 2003. It has been evaluated by US EPA (USEPA, 1996) and is currently under evaluation by the European Commission.

The draft specification and the supporting data were provided by Syngenta Crop Protection AG, in 2002.

Uses

Paraquat dichloride is a non-selective contact herbicide, which is absorbed by foliage, with some translocation in the xylem. It is used in broad-spectrum control of broad-leaved weeds and grasses, in a wide range of agricultural applications, for general weed control on non-crop land and also for pasture restoration.

Identity

Common name (dication):

paraquat (E-ISO, (m)F-ISO, BSI, ANSI, WSSA, JMAF)

Synonyms:

methyl viologen

Chemical names:

dication -
IUPAC, 1,1'-dimethyl-4,4'-bipyridinium [1]
CA, 1,1'-dimethyl-4,4'-bipyridinium

dichloride -
IUPAC, 1,1'-dimethyl-4,4'-bipyridinium dichloride [1]

[1] The IUPAC name for the bipyridinium moiety is alternatively expressed as “bipyridinediium” or “bipyridilium”.

CA, 1,1'-dimethyl-4,4'-bipyridinium dichloride

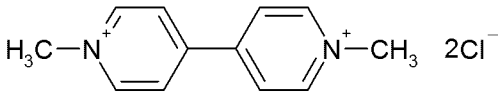
CAS No:

1910-42-5 (dichloride); 4685-14-7 (dication)

CIPAC No:

56 (dication); 56.302 (dichloride)

Structural formula (dichloride):



Molecular formula:

$C_{12}H_{14}Cl_2N_2$ (dichloride); $C_{12}H_{14}N_2$ (dication)

Relative molecular mass:

257.2 (dichloride); 186.3 (dication)

Identity tests (CIPAC G 56/SL/M-):

HPLC retention time; UV spectrum; addition of alkaline sodium dithionite to a dilute solution, where a blue colour indicates the presence of paraquat. The presence of the dichloride salt is tested with silver nitrate solution.

Physicochemical properties

Table 1. Physicochemical properties of pure paraquat dichloride

Parameter	Value(s) and conditions	Purity %	Method reference
Vapour pressure	<<1x10-8kPa at 25ºC (extrapolated)	99.5%	OECD 104
Melting point, boiling point and/or temperature of decomposition	Melting point: >400ºC Boiling point: not applicable Decomposition temperature: 340ºC	99.5%	OECD 102
Solubility in water	620g/l at 20 °C across pH range	99.5%	OECD 105 (flask method)
Octanol/water partition coefficient	log P_{ow} = -4.5 at 20ºC	99.5%	OECD 107 (flask method)
Hydrolysis characteristics	Paraquat dichloride is hydrolytically stable under acidic, neutral and alkaline conditions, no significant decrease in concentration having been recorded at pH 5, 7 and 9 after 30days at 25ºC and 40ºC.	Not stated	Analysis of sterile aqueous buffer solutions containing known amounts of paraquat dichloride before and after storage.
Photolysis characteristics	The environmental half-life of paraquat dichloride in water under mid-European conditions was calculated to be between 2 and 820 years, depending upon seasonal sunlight and depth of water.	99.7%	Measurement of molar extinction coefficients and quantum yield, then these data used in the Frank and Klöpffer model to obtain an estimate of half-life.

Parameter	Value(s) and conditions	Purity %	Method reference
Dissociation characteristics	In aqueous solution the paraquat dichloride is completely dissociated.	Not applicable	

Table 2. Chemical composition and properties of paraquat dichloride (TK).

Manufacturing process, maximum limits for impurities ≥ 1 g/kg, 5 batch analysis data	Confidential information supplied and held on file by FAO. Mass balances were 98.1-99.3% and percentages of unknowns were 1.9-0.7%.
Declared minimum paraquat dichloride content	500 g/l (442 g/kg).
Relevant impurities ≥ 1 g/kg and maximum limits for them	4,4 bipyridyl, 1 g/kg (1000 ppm).
Relevant impurities < 1 g/kg and maximum limits for them	Total terpyridines 0.001 g/kg (1.0 ppm)
Stabilisers or other additives and maximum limits for them	An effective emetic (reference to effective emetic criteria) – see below. PP796, 2-amino-4,5-dihydro-6-methyl-4-propyl-s-triazole-[1,5-a]pyrimidin-5-one is the only emetic known to meet these effective emetic criteria. If PP796 is the effective emetic employed, it must be present at a minimum level of 0.23% by weight of the paraquat ion content[0.17% on a paraquat dichloride basis]
Melting or boiling temperature range	340°C, at which decomposition occurs

Criteria for effective emesis.

- The emetic must be rapidly absorbed (more rapidly than paraquat) and be quick acting. Emesis must occur in about half an hour in at least 50% of cases.
- The emetic must be an effective (strong) stimulant of the emetic centre, to produce effective emesis. The emetic effect should have a limited "action period" of about two to three hours, to allow effective treatment of poisoning.
- The emetic must be act centrally on the emetic centre in the brain.
- The emetic must be not be a gastric irritant because, as paraquat is itself an irritant, this could potentiate the toxicity of paraquat.
- The emetic must be toxicologically acceptable. It must have a short half-life in the body (to comply with the need for a limited action period).
- The emetic must be compatible with, and stable in, the paraquat formulation and not affect the herbicidal efficacy or occupational use of the product.

Toxicological summaries

Notes.

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

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

Table 3. Toxicology profile of paraquat dichloride TK, based on acute toxicity, irritation and sensitization.

Species	Test	Duration and conditions or guideline adopted	Result (paraquat dichloride technical / paraquat cation).
Rat, Alpk:ApfSD,	oral	OECD 401, 14 day	MLD = 344 [246 – 457] mg paraquat dichloride technical / kg bw, equivalent

Species	Test	Duration and conditions or guideline adopted	Result (paraquat dichloride technical / paraquat cation).
male		observation	to 113.5 mg/kg bw expressed as paraquat cation.
Rat, Alpk:ApfSD, female	oral	OECD 401, 14 day observation	MLD = 283 [182 – 469] mg paraquat dichloride technical / kg bw, equivalent to 93.4 mg/kg bw expressed as paraquat cation.
Rat, Alpk:ApfSD, male and female	dermal	OECD 402, 24 hour, occluded, 14 day observation	MLD = >2000 mg paraquat dichloride technical / kg bw equivalent to >660 mg/kg bw expressed as paraquat cation.
Rat, Alpk:Ap, male and female	inhalation	OECD 403, 4 hour nose only*, 14 day observation	LC_{50} = 0.83 – 1.93 mg/m^3 expressed as paraquat cation.
Rabbit, New Zealand White, female	skin irritation	OECD 404, 4 hour, occluded, 34 day, observation	Slight but persistent skin irritant.
Rabbit, New Zealand White, female	eye irritation	OECD 405, 28 day observation	Persistent, moderate to severe irritant to the rabbit eye [Class 5 on a 1-8 scale].
Guinea pigs, Dunkin Hartley, female	skin sensitization	OECD 406, Magnusson and Kligman maximization test, 24 hour, occluded, 48 hour observation	Negative, not a skin sensitizer.

* Paraquat dichloride is non-volatile and formulations containing paraquat are not applied through equipment which will generate a significant proportion (>1% w/w) of spray droplets of diameter less than 50 µm. Therefore, respirable vapour or droplets of paraquat dichloride will not be produced in practice and these toxicity data are not relevant to assessment of human risks.

Table 4. Toxicology profile of paraquat TK, based on repeated administration (sub-acute to chronic).

Species	Test	Duration and conditions or guideline adopted	Result
Rabbit, New Zealand White, male and female	Short-term dermal toxicity	21-day dermal toxicity	NOEL = 1.57 mg paraquat dichloride/kg bw/day equivalent to 1.15 mg/kg bw/day, expressed as paraquat cation. LOEL = 3.61 mg paraquat dichloride /kg bw/day, equivalent to 2.6 mg/kg bw/day, expressed as paraquat ion.
Mouse, ICR-CRJ SPF, male and female	Short-term toxicity	13-week dietary	NOEL = 100 ppm, equivalent to approximately 12 and 14 mg/kg bw/day, expressed as paraquat ion in males and females, respectively. LOEL = 300 ppm, equivalent to approximately 36 and 42 mg/kg bw/day, expressed as paraquat ion in males and females, respectively.
Rat, Fischer CDF (F344), male and female	Short-term toxicity	13-week dietary	NOEL = 100 ppm, equivalent to approximately 6 and 7 mg/kg bw/day, expressed as paraquat ion in males and females, respectively. LOEL = 300 ppm, equivalent to approximately 20 and 21 mg/kg bw/day, expressed as paraquat ion

Species	Test	Duration and conditions or guideline adopted	Result
			in males and females, respectively.
Dog, Beagle, male and female	Short-term toxicity	13-week dietary	NOEL = 20 ppm, equivalent to approximately 0.6 and 0.7 mg/kg bw/day, expressed as paraquat ion in males and females, respectively. LOEL = 60 ppm, equivalent to approximately 2 mg/kg bw/day, expressed as paraquat ion in males and females.
Dog, Beagle, male and female	Short-term toxicity	1-year dietary	NOEL = 15 ppm, equivalent to approximately 0.45 and 0.48 mg/kg bw/day, expressed as paraquat ion in males and females, respectively. LOEL = 30 ppm, equivalent to approximately 0.9 and 1.0 mg/kg bw/day, expressed as paraquat ion in males and females, respectively.
Mouse, Alpk Swiss-derived, male and female	Carcinogenicity	99-week dietary	Not tumorigenic. NOAEL = 12.5 ppm, equivalent to approximately 1.5 mg/kg bw/day, expressed as paraquat ion in males. NOEL = 37.5 ppm, equivalent to approximately 4.3 mg/kg bw/day, expressed as paraquat ion in females.
Rat, Fischer 344, male and female	Chronic toxicity / carcinogenicity	113-117 weeks for males and 122-124 weeks for females	Not carcinogenic. NOEL = 25 ppm, equivalent to approximately 1.25 mg/kg bw/day, expressed as paraquat ion. LOEL = 75 ppm, equivalent to approximately 3.75 mg/kg bw/day, expressed as paraquat ion.
Rat, Alpk:APfSD, male and female	Reproductive toxicity	3-generation, 2 litters per generation	No effect on reproductive parameters. NOEL for toxicity = 25 ppm, equivalent to approximately 2.3 mg/kg bw/day, expressed as paraquat ion. NOEL for reproductive effects = >150 ppm, equivalent to approximately 13 mg/kg bw/day, expressed as paraquat ion.
Mice, Crl:CD1 (ICR) BR, female	Developmental toxicity	Gavage	NOEL for both maternal and developmental toxicity = 15 mg/kg bw/day expressed as paraquat ion.
Mice, Alpk SPF, female	Developmental toxicity	Gavage	Not teratogenic. No significant influence on embryonic or foetal development. NOEL for developmental toxicity = >10 mg/kg bw/day expressed as paraquat ion.
Rat, Alpk:SPF, female	Developmental toxicity	Gavage	Not teratogenic. NOEL for maternal and developmental toxicity > 1mg/kg bw/day expressed as paraquat ion.
Rat, Alpk:APfSD	Developmental toxicity	Gavage	Not teratogenic. NOAEL for maternal and developmental toxicity = 3 mg/kg bw/day expressed as paraquat ion.

Table 5. Mutagenicity profile of paraquat dichloride TK, based on *in vitro* and *in vivo* tests.

Species	Test	Conditions	Result
Mouse, lymphocytes (L5178Y)	OECD 476, L5178Y mouse lymphoma assay (*in vitro*)	Doses of 23 – 361 µg/ml	Negative
Human lymphocytes	OECD 473, Cytogenetic study (*in vitro*)	Dosed at 90, 903 and 1807 µg/ml	Positive
Chinese hamster lung fibroblasts	OECD 479, Sister chromatid exchange assay (*in vitro*)	Dosed at 0.9, 1.8, 9, 18, 90 and 177 µg/ml	Positive
Rat hepatocytes	OECD 482, DNA damage and repair/unscheduled DNA synthesis (*in vitro*)	Dosed at 0.19 ng/ml to 1.86 mg/ml	Negative
Rat somatic cells	Rat cytogenetic assay (*in vivo*)	Male and female Wistar rats given a single oral dose at 15, 75 and 150 mg/kg	Negative
Mouse somatic cells	OECD 474, Micronucleus test (*in vivo*)	Male and female C57BL/6J/Alpk mice given a single oral dose at 52 and 83 mg/kg	Negative
Rat somatic cells	UDS assay (*in vivo*)	Single oral dose at 42 to 120 mg/kg	Negative
Mouse germ cells	Dominant lethal (*in vivo*)	Male CD1 mice dosed orally at 0, 0.04, 0.4 and 4.0 mg/kg for 5 days.	Negative

Table 6. Ecotoxicology profile of paraquat dichloride TK.

Species	Test	Duration and conditions	Result
Daphnia magna, (water flea)	Acute toxicity	EEC Method C2, Static system, 20-21°C, 48-hour observation	24 and 48 hour EC_{50} = 11.8 and 4.4 mg/l, expressed as paraquat ion, respectively. 48 hour NOEC = 2.2 mg/l expressed as paraquat ion.
Daphnia magna, (water flea)	Chronic toxicity	21-day exposure, based on OECD Guideline 202, modified by individually separating the Daphnia static system, growth and reproduction monitored	NOEC = 0.12 mg/l expressed as paraquat ion.
Oncorhynchus mykiss, (rainbow trout)	Acute toxicity	EEC Method C1, static system at 15°C	24, 48, 72 and 96 hour LC_{50} = 33, 22, 22 and 19 mg/l, expressed as paraquat ion, respectively. 96 hour NOEC = <0.3 mg/l, expressed as paraquat ion
Cyprinus carpio, (mirror carp)	Acute toxicity	EEC Method C1, static system at 22°C	24, 48, 72 and 96 hour LC_{50} = >112, >112, >112 and 98 mg/l expressed as paraquat ion, respectively.

Species	Test	Duration and conditions	Result
			96 hour NOEC = 60 mg/l expressed as paraquat ion.
Oncorhynchus mykiss, (rainbow trout)	Chronic toxicity	21-day fish juvenile growth test, based upon OECD Method 204, with the exposure period extended to 21 days. Broadly in agreement with the draft OECD guideline 'Fish, juvenile growth test - 28 days', except that the exposure was for 21 days. Flow through system at 15°C	NOEC = 8.5 mg/l expressed as paraquat ion.
Selenastrum capricornutum, (green alga)	Effect on growth	Based on OECD Guideline 201 but with an extension of the exposure period to 96 hours. Static system at 24°C, biomass and growth rate observed	EbC_{50} = 0.075 mg/l expressed as paraquat ion. ErC_{50} = 0.20 mg/l expressed as paraquat ion. NOEC = 0.016 mg/l expressed as paraquat ion.
Eisenia foetida, (earthworm)	Acute toxicity	Laboratory study in artificial soil	LC_{50} = >1000 mg/kg dry soil, expressed as paraquat ion
Apis mellifera (honey bee)	Acute oral toxicity	Based on UK data requirements for approval under the Control of Pesticides Regulations, Working Document D3 (revised 1979). Consistent with EPPO guideline 170. Controlled environment at 22°C	24, 48, 72, 96 and 120 hour LD_{50} = 154, 50.9, 26.3, 19.5 and 11.2 µg/bee, expressed as paraquat ion, respectively.
Apis mellifera (honey bee)	Acute contact toxicity	Based on UK data requirements for approval under the Control of Pesticides Regulations, Working Document D3 (revised 1979). Consistent with EPPO guideline 170. Controlled environment at 22°C	72, 96 and 120 hour LD_{50} = 108, 89.1 and 50.9 µg/bee, expressed as paraquat ion, respectively.
Colinus virginianus, (bobwhite quail)	Acute toxicity	Oral intubation in distilled water, 14 day observation	LD_{50} = 127 mg/kg bw expressed as paraquat ion. LLD = 115 mg/kg bw expressed as paraquat ion. NOEL = 72 mg/kg bw expressed as paraquat ion.
Anas platyrhynchos, (mallard duck)	Acute toxicity	Oral intubation in propylene glycol, 14 day observation	LD_{50} = 144 mg/kg bw expressed as paraquat ion.
Colinus virginianus, (bobwhite quail)	Short-term toxicity	5 days treatment, 3 days observation	LC_{50} = 711 mg/kg diet expressed as paraquat ion.
Anas platyrhynchos, (mallard duck)	Short-term toxicity	5 days treatment, 3 days observation	LC_{50} = 2932 mg/kg diet expressed as paraquat ion.
Coturnix japonica, (Japanese quail)	Short-term toxicity	5 days treatment, 3 days observation	LC_{50} = 703 mg/kg diet expressed as paraquat ion
Colinus virginianus,	Reproductive	18 week dietary treatment.	NOEC for toxicity and

Species	Test	Duration and conditions	Result
(bobwhite quail)	toxicity	Egglaying and collection started after 10 weeks on treated diet and lasted for 8 weeks.	reproduction = 100 mg/kg diet expressed as paraquat ion.
Anas platyrhynchos, (mallard duck)	Reproductive toxicity	18 week dietary treatment. Egglaying and collection started after 10 weeks on treated diet and lasted for 8 weeks.	NOEC for toxicity = 100 mg/kg diet expressed as paraquat ion. NOEC for reproduction = 30 mg/kg diet expressed as paraquat ion.

Paraquat dichloride was evaluated by WHO (WHO, 1984), by IPCS (IPCS, 1991) and by the FAO/WHO JMPR in 1986 (by which it is subject to a periodic re-evaluation in 2003). The IPCS (1991) review concluded that residue levels of paraquat in food and drinking-water, resulting from its normal use, are unlikely to pose a health hazard for the general population.

The WHO/PCS hazard classification (WHO 2002) of paraquat dichloride is: moderately hazardous, class II.

The US EPA concluded, from acute toxicity studies on laboratory animals, that paraquat is highly toxic by the inhalation route and was placed in Toxicity Category I (the highest of four levels) for acute inhalation effects. However, the EPA established that the large droplets arising in agricultural practice (400 to 800 µm) are well beyond the respirable range and therefore inhalation toxicity is not a toxicological endpoint of concern. Paraquat is moderately toxic (Category II) by the oral route and slightly toxic (Category III) by the dermal route. Paraquat will cause moderate to severe eye irritation and minimal dermal irritation and has been placed in Toxicity Categories II and IV for these effects (USEPA, 1997). Paraquat was classified as a "Group E" chemical, i.e. one showing evidence of non-carcinogenicity to humans. The no observed effect levels (NOEL) for maternal toxicity are equal to, or more conservative (protective) than, the NOEL based on developmental toxicity. There is no evidence that paraquat is associated with reproductive effects. Paraquat also shows no evidence of causing mutagenicity. The US EPA has determined that there is a reasonable certainty that no harm will result to infants and children or to the general population from aggregate exposure to paraquat dichloride residues. The EPA does not believe that the effects produced by paraquat would be cumulative with those of other, structurally related, compounds.

Formulations

The main formulation types available are SL and SG.

The SL formulations are registered and sold in many countries throughout the world. SG formulations are registered in Europe and sold mainly in the UK.

Methods of analysis and testing

Analytical methods for the active ingredient (including identity tests) were published in CIPAC Handbook E, pp. 75 and 167, and utilise a colorimetric procedure based on the

blue free-radical ion produced by paraquat. The method(s) for determination of impurities are based on GC-FID, GC-MS and CE.

Relevant impurity, 4,4'-bipyridyl, is determined by GC-FID (CIPAC 56/13) the group of relevant impurities, the terpyridines, are determined by GC-MS.

The method for terpyridines is currently being peer reviewed, which is expected to should be completed by the end of 2003. A method for the emetic, PP796 is also undergoing peer review against the same timescale.

Test methods for determination of physico-chemical properties of the technical active ingredient were essentially OECD methods, with CIPAC procedures being used for formulation assessment (as indicated in the specifications).

Physical properties

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

Containers and packaging

Detailed requirements for containers are given in the specifications, as a note, but it is important to prevent paraquat dichloride from coming into contact with metals.

Expression of the active ingredient

The active ingredient is expressed as paraquat dichloride.

Appraisal

Data submitted were in accordance with the FAO/WHO Manual (2002, 1st edition) and supported the proposed specifications.

Paraquat dichloride specifications were previously developed under the old FAO procedure in 1994 (TK and SL) and published by FAO. Revised FAO specifications (TK and SL) and an additional specification (SG) for paraquat dichloride were proposed under the new procedure by Syngenta Crop Protection AG.

Paraquat dichloride is no longer under patent.

Paraquat dichloride is a non-selective contact herbicide, highly soluble and stable in water (pH 5-9), only very slowly subject to photolysis and essentially non-volatile. It very readily, and essentially irreversibly, binds to soils and sediments.

The proposer provided the meeting with commercially confidential information on the two manufacturing processes (a third manufacturing process was no longer in use) for paraquat dichloride and concomitant impurities. Data for five batches from each of the two manufacturing processes were provided for the TK. Addition of water and an emetic (after reactions are complete) complete the TK manufacturing process. Other safening additives, such as warning colorants, stenching agents and thickeners (for liquid formulations) are also incorporated. Mass balances were good: 99.0-99.3%

characterized one manufacturing process, while 98.1-99.0% characterized the second process.

The proposer identified two relevant impurities of manufacturing (4,4'-bipyridyl and total terpyridines), both of which are normally below 0.5 g/kg. Minimum levels were specified for the emetic additive, and maximum levels for the two proposed relevant impurities, in the draft specifications for paraquat dichloride TK, SL and SG. Data submitted to FAO for TK purity, impurities and emetic content were similar to those submitted for registration of paraquat dichloride in the UK. A difference between the two sets of data was that terpyridines were not included in the UK data, because the concentrations are well below 1 g/kg. Both the terpyridines and 4, 4' bipyridyl were below 1 g/kg in batch analysis data submitted to FAO, regardless of which of the two current manufacturing processes was employed. The proposer noted that terpyridines are highly toxic, whilst, in some respects, 4,4'-bipyridyl is rather more toxic than paraquat dichloride. WHO/PCS opinion was to accept these views. The proposed new limit of 1 g/kg for 4,4'-bipyridyl is below the level of the previous FAO Specification (56/TK/S/F-1994). The Meeting agreed that the two impurities should be considered as relevant.

Specifications for relevant impurities in the SL and SG are given on a formulation basis, not an active ingredient basis. The unusual basis is due to limitations in the available analytical methods, which are unable to determine lower levels in the formulations, with acceptable accuracy. The Meeting agreed that consistent maximum levels should be adopted for these impurities, based on the analytical performance that can currently be achieved routinely and therefore the same limit is applied to SL and SG, irrespective of paraquat content.

The method of analysis for paraquat dichloride is based on a colorimetric procedure, in which the blue paraquat radical, formed upon addition of alkaline sodium dithionite, is measured (CIPAC Handbook E, pages 75-78 and 167-168). The presence of paraquat as the dichloride salt may be identified by a check for chloride, using silver nitrate solution. Paraquat SL and SG may be formulated with or without diquat. CIPAC methods for the determination of paraquat are available for both mixed and single formulations, although the method for SG was validated by CIPAC only for mixed formulations. For this reason, the SL specification references both methods, with an explanatory note. The SG specification references the method intended for mixed formulations, with a note indicating that the absorbance correction for diquat is not necessary for formulations containing only paraquat.

Methods for impurities are based on GC-FID (4,4' bipyridyl, CIPAC Handbook E, p.168 and CIPAC Handbook 1A, p. 1245) or GC-MS (terpyridines). Determination of the content of emetic, PP796, is based on capillary GC. The Meeting noted that the CIPAC method for bipyridyl is based on packed column GC, which is no longer available in many laboratories. This is a problem associated with many older CIPAC methods and it was expected that capillary GC would form an appropriate and preferable alternative in this case. The methods for the emetic and terpyridines have under gone satisfactory peer validation for the TK but further validation is underway for analysis of the formulations.

The proposer stated that physiochemical properties of paraquat dichloride were essentially determined using OECD methods, with CIPAC procedures used for assessment of formulation characteristics, as indicated in the specifications.

In the description clause of the SL specification, the phrase "...free from visible suspended matter and sediment..." was replaced by "...shall contain not more than a trace of suspended matter, immiscible solvents and sediment...". The proposer explained that certain batches of wetters used in current formulations result in the presence of fine oily droplets but that these do not affect the sprayability or other characteristics of the product and have not resulted in complaints from customers. The Meeting accepted the modified wording.

The limits for dustiness in the SG specification are more stringent than usual, to minimize the potential emission of dust containing paraquat and the consequent risks to operators. SG specifications do not normally include a clause for attrition resistance but, with risks from dust inhalation after handling, transport and storage in mind, the Meeting agreed that a clause and stringent limit should be included. The proposer confirmed that the CIPAC method for attrition resistance (MT 178), developed for GR formulations, is appropriate for testing paraquat SG.

Paraquat dichloride was evaluated by WHO IPCS (1983 and 1991) with a classification of moderately hazardous assigned. The acceptable daily intake estimated by the FAO/WHO JMPR is 0-0.004 mg/kg. The US EPA has assigned a Category II acute toxicity to paraquat dichloride, which indicates it is moderately toxic. However, once paraquat is ingested and absorbed in sufficient amount, poisoning is essentially irreversible, with death as the probable end-point. Thus, all paraquat products must contain an effective emetic, to reduce the risk of accidental or deliberate ingestion and absorption. Paraquat is of low dermal toxicity but the US EPA classified paraquat dichloride in its highest toxicity class, Category I, for inhalation hazard. Nonetheless, the agency noted that, because the spray droplets produced in normal agricultural uses are too large to be respirable, the inhalation risk is actually very low. Paraquat dichloride is moderately toxic to aquatic invertebrates, slightly toxic to fish, moderately toxic to avian species and relatively non-toxic to bees.

As a result of evaluation of paraquat under Directive 91/414/EEC, the European Commission is proposing to make a colorant, an effective emetic and a stenching (or other olfactory alerting) agent, mandatory requirements for paraquat formulations. The proposer recommended the revised specifications be amended to reflect these same standards. The Meeting accepted the requirements for a stenching agent and emetic in paraquat product descriptions. The Meeting also agreed that a note to the specifications should identify the only emetic currently known to be satisfactory and provide both a minimum concentration an a suitable analytical method for it. The Meeting agreed that the note on emetic content should allow for a possible alternative compound, by describing the characteristics required for an effective emetic.

Paraquat dichloride is not mutagenic and EPA placed it in Group E for chemicals showing evidence of being non-carcinogenic to humans. Further, the evidence

available indicates that paraquat dichloride has no effect on reproduction parameters and is non-teratogenic.

Paraquat will corrode most metals, over a wide pH range. The effect is exacerbated at low pH (as, for example, with the TK) but it is essential that paraquat does not come into contact with metals and the packaging must be designed to avoid loss of container integrity. Details are contained in each specification, as a note.

Certain amendments were made to the draft specifications, as agreed between the Meeting and the proposer. Apart from the exceptional requirements identified in the appraisal, the specifications were in accordance with the normal requirements of the FAO/WHO Manual.

Recommendations

The meeting recommended that the specification for paraquat dichloride TK, as amended, should be adopted by FAO. The Meeting recommended that the specifications for SL and SG, as amended should be adopted by FAO, subject to satisfactory completion of peer validation of the analytical methods for terpyridines and the emetic.

References

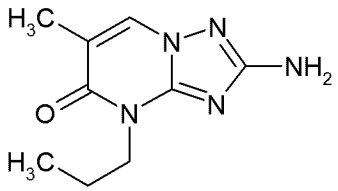
WHO, 1984	Environmental Health Criteria 39: Paraquat and diquat. World Health Organization, Geneva, 1984.
IPCS, 1991	Health and Safety Guide No. 51. Paraquat Health and Safety Guide. World Health Organization, Geneva. 1991.
WHO, 2002	The WHO Recommended Classification of Pesticides by Hazard and Guidelines to Classification 2000-2002 (WHO/PCS/01.5). World Health Organisation, Geneva, 2002.
USEPA, 1997	R.E.D. Facts. Paraquat dichloride (EPA-738-F-96-018). United States Environmental Protection Agency, 1997.
US EPA, 1996	Reregistration Eligibility Decision (RED), Paraquat dichloride. List A Case 0262. United States Environmental Protection Agency, 1996.

Appendix 1

Determination of PP796 (emetic) in paraquat dichloride technical concentrates (TK)

Information

IUPAC name: 2-amino-4,5-dihydro-6-methyl-4-propyl-*s*-triazole-[1,5-a]pyrimidin-5-one
CA name: 2-amino-6-methyl-4-propyl-(1,2,4)triazolo[1,5-a]pyrimidine-5-(4*H*)-one (9CI)
CAS Registry No: *[27277-00-5]*
Molecular structure:



Molecular formula: $C_9H_{13}N_5O$
Relative molecular mass: 207.2

Scope of method

This capillary gas chromatography (GC) method is for the determination of PP796 emetic, as % w/w, in paraquat dichloride technical concentrates.

Summary of method

A portion of the TK (aqueous solution) is made basic with NaOH and partitioned into dichloromethane, containing octadecane as an internal standard. The extract is analyzed by capillary GC-FID, measuring peak areas.

Safety information

Paraquat dichloride salts are toxic, particularly by inhalation of particulates or ingestion, and, because there is no antidote or treatment for the progressive symptoms which can develop, exposure must be avoided. Paraquat dichloride solutions are irritant if splashed in the eyes and harmful if allowed to contact the skin or open cuts. Wear eye protection and protective gloves when handling paraquat dichloride analytical standards, the TK or formulated materials. Solid paraquat materials must only be handled in a fume cupboard.

If in any doubt about the nature and hazards of the chemicals used in this method, consult the Material Safety Data Sheet (MSDS) or an appropriate safety manual such as:

"Hazards in the Chemical Laboratory", Luxon, Ed., Royal Society of Chemistry, 5th Edn, 1992, London, ISBN 0-85186-220-2.

"The Sigma-Aldrich Library of Chemical Safety Data", Lenga, Ed., 2nd Edn, 1999, Milwaukee, WI, ISBN 0-941633-16-0.

Chemicals

Dichloromethane, HPLC grade.

Octadecane, laboratory reagent grade. Weigh approximately 50 mg into a 100 ml volumetric flask and add about 80 ml dichloromethane. Shake to dissolve, make to the mark with dichloromethane and mix well, to produce an internal standard solution of approximately 0.5 mg/ml.

Sodium hydroxide solution, 1M.

PP796 emetic, analytical standard grade (obtainable from Syngenta). Weigh accurately about 10 mg into two separate 25 ml volumetric flasks and add 5.0 ml of internal standard solution. Shake to dissolve, make to the mark with dichloromethane and mix well, to produce two solutions (Solutions A_1 and A_2) containing PP796 at 0.4 mg/ml.

Laboratory detergent, non-ionic, e.g. Decon Neutracon.

Dimethyldichlorosilane (DMCS), laboratory reagent grade.

Hexane, laboratory reagent grade.

Methanol, water-free.

Acetone, laboratory reagent grade

Apparatus

Gas chromatograph, equipped with split/splitless injection system and flame ionisation detection, operated in split mode, with automatic injector and electronic data capture and handling system. All gases should be purified through molecular sieves. The carrier gas should be further purified through an oxygen trap.

Injection Liner, straight silica liner (4 mm ID) packed with silanized fused silica wool plug (e.g. Restek cat No. 20790). Contaminated split injection liners should be treated as follows.

Immerse in detergent (10% solution) for about 1 hour, then wash in purified water and dry in an oven at 120°C. Take the liner, while still warm, and immerse in a 5% solution of dimethylchlorosilane in hexane for 5 min. Remove the liner and immerse in fresh dry methanol for 1 hour. Wash the liner with acetone and dry thoroughly. The fused silica liner is ready for packing with silanized fused silica wool.

Column, 25 m x 0.25 mm ID fused silica capillary column with 0.25 μm film of BPX-5 (ex SGE) or Chrompack CP-Sil 8CB, or equivalent. Maximum programmed operating temperature 350ºC.

Typical operating conditions

Oven temperature programme:	initial temperature 50ºC for 2 min. programme 1, rate 20ºC min^{-1} to 100ºC, held for 2 min. programme 2, rate 20ºC min^{-1} to 280ºC, held for 10 min. total run time, 25.5 min.
Injector temperature:	300ºC
Detector temperature:	325ºC
Gas flow rates:	hydrogen carrier gas, 50 cm/sec (e.g. 10 psi head pressure) nitrogen make-up gas, 30 ml/min. hydrogen flame gas, 30 ml/min. air, 450 ml/min. Split flow , 50 ml/min.
Injection volume:	1 μl (by autosampler)
Typical retention times:	octadecane, 12-14 min; PP796, 13-15 min.

Sample extraction

Weigh accurately, in duplicate, about 2 g of paraquat dichloride technical concentrate into two 100 ml separating funnels. In each case, add 0.5 ml 1M sodium hydroxide solution and swirl the separating funnel taking care not to foul the stopper. Add 2.0 ml octadecane internal standard solution and swirl the separating funnel, taking care not to foul the stopper. Carefully release the gas pressure, shake well and again carefully release the gas pressure. Leave standing until two clear layers are obtained.

Collect the lower (dichloromethane) layer into a 14 ml glass screw-capped (trident) vial and retain the aqueous layer in the separating funnel. Add 2 ml dichloromethane to the separating funnel and shake well. Leave standing until two clear layers are obtained. Combine the lower (dichloromethane) layer with the initial extract in the glass vial and retain the aqueous layer in the separating funnel. Repeat the extraction with a further 2 ml dichloromethane, combining all three extracts in the glass vial and add 5 ml dichloromethane to the glass vial. Identify the duplicate extracts as Solutions B_1 and B_2.

Determination

Make replicate injections of Solution A_1 and/or A_2 at about 2 min. intervals, to equilibrate the GC system. Wait 19 min. then inject Solution A_1 or A_2 again and check that the retention times of the octadecane (12-14 min.) and PP796 (13-15 min.) are within the expected time windows. If not, the column head pressure may be adjusted ± 1 psi, or column temperature programme 1 final temperature may be adjusted ± 10°C. If column performance deteriorates substantially during use, check the condition of the split injection liner and replace if necessary.

Perform replicate injections of calibration and sample solutions in an appropriate sequence, such as: A_1, B_1, A_1, B_1, A_1, B_1, A_2, B_2, A_2, B_2, A_2, B_2. Measure peak areas.

Confirm the identity of PP796 in Solution B by GC-MS or, alternatively, by spiking an aliquot of Solution B with an aliquot of Solution A and check for exact co-elution.

Calculations

Calculate the relative response factor, RF, for each injection of the standard Solution A as follows.

$$RF = \frac{A \times VI \times 100}{P \times I \times WR}$$

Where: WR = weight of PP796 standard (mg);
VI = volume of internal standard added in ml;
A = peak area of PP796 peak;
I = peak area of octadecane peak;
P = % w/w purity of PP796 standard.

Calculate the percentage PP796 content (w/w) of each sample Solution B as follows.

$$\%\ w/w = \frac{A' \times VI \times 100}{I' \times WS \times RF}$$

Where: WS = weight of sample (mg);
VI = volume of internal standard added in ml;
A' = peak area of PP796 peak;
I' = peak area of octadecane peak;
RF = relative response factor for PP796 obtained from the preceding standard Solution A.

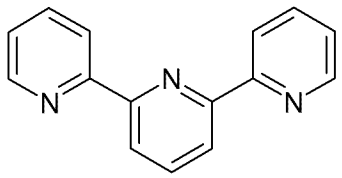
Calculate the average PP796 content from Solutions B_1 and B_2.

Appendix 2

Determination of total terpyridines in paraquat dichloride technical concentrates (TK)

Information

A number of terpyridine isomers can exist and the following name, CAS No. and structure are for one of them. The molecular formula and relative molecular mass are applicable to all.
IUPAC name: 2,2':6',2"-terpyridine
CA name: 2,2':6',2"-terpyridine
CAS Registry No: *[1148-79-4]*
Molecular structure:



Molecular formula: $C_{15}H_{11}N_3$
Relative molecular mass: 233.3

Scope

This capillary gas chromatography/mass spectrometry (GC-MS) method is for screening and determination of terpyridine isomers, in mg/kg, in paraquat dichloride technical concentrates.

Summary of method

A portion of the TK (aqueous solution) is made basic with NaOH, partitioned into ethyl acetate and the extract is analyzed by capillary GC-MS. Screening for the presence of terpyridines is done using full-scan MS conditions (*m/z* 35-290). For quantification of terpyridines, an internal standard (diphenyl-2-pyridylmethane) is added and the MS performed in SIM mode, monitoring ions at *m/z* 167 and 244 ($[M-H]^+$) for the internal standard and *m/z* 205 and 233 ($[M]^+$) for the terpyridines, measuring the sums of ion peak areas. The 2,2':6',2"-terpyridine isomer standard response is calibrated relative to the internal standard. It is assumed that the spectra (and relative responses) of other terpyridine isomers are quantitatively similar to that of 2,2':6',2"-terpyridine. Examples of typical total ion chromatograms are given in Figures 1 and 2 and examples of full scan spectra are given in Figures 3 and 4.

Safety information

Terpyridines are highly toxic by ingestion, inhalation and in particular dermal/eye absorption. Wear gloves, protective clothing and full face visor and handle in a fume cupboard at all times. Samples and waste materials should be disposed of in such a way as to prevent exposure to people and contamination of the environment.

Paraquat dichloride salts are toxic, particularly by inhalation of particulates or ingestion, and, because there is no antidote or treatment for the progressive symptoms which can develop, exposure must be avoided. Paraquat dichloride solutions are irritant if splashed in the eyes and harmful if allowed to contact the skin or open cuts. Wear eye protection and protective gloves when handling paraquat dichloride analytical standards, the TK or formulated materials. Solid paraquat materials must only be handled in a fume cupboard.

If in any doubt about the nature and hazards of the chemicals used in this method, consult the Material Safety Data Sheet (MSDS) or an appropriate safety manual such as:

"Hazards in the Chemical Laboratory", Luxon, Ed., Royal Society of Chemistry, 5th Edn, 1992, London, ISBN 0-85186-220-2.

"The Sigma-Aldrich Library of Chemical Safety Data", Lenga, Ed., 2nd Edn, 1999, Milwaukee, WI, ISBN 0-941633-16-0.

Chemicals

Ethyl acetate, HPLC, or higher, grade.

Sodium hydroxide solution, 1M.

Diphenyl-2-pyridylmethane, 99+% (e.g. from Acros Organics). Weigh 30 mg diphenyl-2-pyridylmethane into a 100 ml volumetric flask, add 50 ml ethyl acetate and swirl to dissolve. Make to the mark with ethyl acetate to the mark and mix well. This IS solution contains 0.3 mg/ml diphenyl-2-pyridylmethane.

2,2':6',2"-Terpyridine, analytical standard, (HIGHLY TOXIC, obtainable from Syngenta). Accurately weigh, in duplicate, approximately 10 mg of 2,2':6',2"-terpyridine into 25 ml volumetric flasks. Add 15 ml of ethyl acetate and swirl to dissolve. If necessary, to aid dissolution, place in an ultrasonic bath and sonicate until complete dissolution is achieved. Add ethyl acetate to the mark and mix well. Using accurately calibrated pipettes to dispense the internal standard and terpyridine solutions, these two solutions, $\mathbf{A_1}$ and $\mathbf{A_2}$, are used to prepare a series of diluted solutions, as follows.

- Solution $\mathbf{B_1}$. To a 25ml volumetric flask containing approximately 20 ml of ethyl acetate, transfer 250 µl of solution A_2. Make up to the mark with ethyl acetate and mix well.
- Solution $\mathbf{B_2}$. To a 25ml volumetric flask containing approximately 20 ml of ethyl acetate, transfer 125 µl of solution A_2. Make up to the mark with ethyl acetate and mix well.
- Solution $\mathbf{C_1}$ (20 mg/kg terpyridine equivalent), nominal concentration 4×10^{-3} mg/ml. To a 30 ml powder jar (with a tightly fitting lid), add 20 ml ethyl acetate and then 5.0 ml of IS solution. Transfer 250 µl of Solution A_1 to the powder jar and mix well.
- Solution $\mathbf{C_2}$ (0.8 mg/kg terpyridine equivalent), nominal concentration 1.5×10^{-4} mg/ml. To a 30 ml powder jar (with a tightly fitting lid), add 20 ml ethyl acetate and then 5.0 ml of IS solution. Transfer 1.0 ml of Solution B_1 to the powder jar and mix well.
- Solution $\mathbf{C_3}$ (8 mg/kg terpyridine equivalent), nominal concentration 1.6×10^{-3} mg/ml. To a 30 ml powder jar (with a tightly fitting lid), add 20 ml ethyl acetate and then 5.0 ml of IS solution. Transfer 100 µl of Solution A_2 to the powder jar and mix well.
- Solution $\mathbf{C_4}$ (2 mg/kg terpyridine equivalent), nominal concentration 4×10^{-4} mg/ml. To a 30 ml powder jar (with a tightly fitting lid), add 15 ml ethyl acetate and then 5.0 ml of IS solution. Transfer 5.0 ml of Solution B_2 to the powder jar and mix well.
- Solution $\mathbf{C_5}$ (0.4 mg/kg terpyridine equivalent), nominal concentration 8×10^{-5} mg/ml. To a 30 ml powder jar (with a tightly fitting lid), add 20 ml ethyl acetate and then 5.0 ml of IS solution. Transfer 1.0 ml of Solution B_2 to the powder jar and mix well.

Solutions to inject into the GC-MS are C_1, C_2, C_3, C_4 and C_5.

Laboratory detergent, non-ionic, e.g. Decon Neutracon.

Dimethyldichlorosilane (DMCS), laboratory reagent grade.

Hexane, laboratory reagent grade.

Methanol, water-free.

Acetone, laboratory reagent grade

Apparatus

Coupled gas chromatograph and mass spectrometer system, for example, Finnigan Voyager quadrupole mass spectrometer linked to an Agilent HP6890 gas chromatograph, equipped with split/splitless injection system, operated in split mode, with automatic injector. The mass spectrometer must be tuned and mass calibrated according to the manufacturer's instructions before starting analyses. The carrier gas should be purified through molecular sieves and an oxygen trap. Analyte carryover and contamination should be minimized by adequate syringe washing cycles, between injections, with ethyl acetate. If the syringe is suspected of contaminating the analytical solutions in autosampler vials, replace the syringe.

Injection liner, straight silica liner (4 mm ID) packed with base-deactivated fused silica wool plug (e.g. Restek Cat No. 20999). Contaminated injection liners should be treated as follows.

Immerse in detergent (10% solution) for about 1 hour, then wash in purified water and dry in an oven at 120°C. Take the liner, while still warm, and immerse in a 5% solution of dimethylchlorosilane in hexane for 5 min. Remove the liner and immerse in fresh dry methanol for 1 hour. Wash the liner with acetone and dry thoroughly. The fused silica liner is ready for packing with silanized fused silica wool.

Column, 30 m x 0.25 mm ID fused silica capillary column, coated with 0.25 μm Chrompack CP-Sil 8CB for amines (or equivalent giving similar performance). Maximum operating temperature (programmable) 350ºC.

Note. The column used should be dedicated to this analysis. If column performance deteriorates substantially during use, check the condition of the injection liner and replace if necessary. If a-poor peak shape is obtained, or no peak is detected, from 2,2':6',2"-terpyridine in the calibration solutions but a good peak shape is obtained from the internal standard, replace the injection liner, deactivated as described above, if required. If the poor peak shape persists, a new capillary column may be required, as the columns are known to deteriorate with use for this purpose.

Centrifuge, bench-top, suitable for use with the tubes described in the section on procedure.

Typical operating conditions, general

Oven temperature programme:	initial temperature 150ºC for 1 min. programme 1, rate 40ºC min^{-1} to 260ºC, immediately progressing to programme 2, rate 2ºC min^{-1} to 270ºC, immediately progressing to programme 3, rate 40ºC min^{-1} to 320ºC, held for 2 min. total run time, 12 min.
Injector temperature:	300ºC
MS interface temperature:	300ºC
MS (EI) source temperature:	250ºC
Gas flow rates:	helium carrier gas, 42 cm/sec at 150ºC (constant pressure mode, 16 psi, vacuum corrected)
Typical retention times:	diphenyl-2-pyridylmethane (IS), 2.5-6.2 min; terpyridines, 6.2-10 min.

Typical operating conditions, first screening analysis (to determine whether detectable levels of terpyridines are present in the sample)

MS analyzer mode	full scan (FS), 35-290 amu
Split ratio	27:1
Injection volume	2 μl
Ionisation mode and energy	EI+, 70 eV
Solvent delay	2.5 min.
Electron multiplier voltage	500 V

Typical operating conditions, second screening analysis (required only if the first screening analysis produces doubtful results)

MS analyzer mode	full scan (FS), 35-290 amu
Split ratio	1:1
Injection volume	1 μl
Ionisation mode and energy	EI+, 70 eV
Solvent delay	2.5 min.
Electron multiplier voltage	500 V

Typical operating conditions for quantification (required only if screening analyses show the presence of terpyridines)

MS analyzer mode	Selected ion monitoring (SIM), *m/z* 167 and 244 for diphenyl-2-pyridylmethane, *m/z* 205 and 233 for terpyridine isomers. SIM acquisition windows adjusted according to actual retention times.
Split ratio	27:1
Injection volume	1 μl
Ionisation mode and energy	EI+, 70 eV
Dwell time:	0.2 sec.
Mass span	0.20 amu
Solvent delay	2.5 min.
Electron multiplier voltage	350 V for diphenyl-2-pyridylmethane, 550 V for terpyridines; the voltage being adjusted to avoid detector saturation.

Procedure for screening (full scan MS) analysis (internal standard not required)

Preparation of sample solution

Place approximately 2g (approximately 1.8 ml) of paraquat dichloride TK into a 14 ml glass, screw-cap (trident) vial. Add 2.0 ml of 1M sodium hydroxide and gently swirl the solution, taking care not to foul the vial cap. Add 6 ml of ethyl acetate and shake well. Place the 14 ml glass vial inside a larger solvent-resistant leak-proof capped plastic tube and centrifuge the sample for about 2 minutes, at a speed sufficient to provide adequate separation of the two layers. *Note: samples must be double-contained, to prevent possible contamination of the centrifuge with highly toxic material.* Remove the top layer (sample solution **A**) with a pasteur pipette, taking care not to transfer any of the dark coloured, aqueous bottom layer.

System suitability check, equilibration and adjustment of retention time

The GC-MS system should be sufficiently sensitive in full scan mode to detect terpyridines in a sample equivalent to 0.4 mg/kg. Make replicate injections of calibration solution $\mathbf{C_5}$, to equilibrate the system and ensure that the terpyridine peak is detected. The retention time for the terpyridine standard should be 6.2-8.0 min and, if not, the carrier gas head pressure may be adjusted ± 2 psi.

Determination

Make replicate injections of sample solution **A** and calibration solution $\mathbf{C_5}$, using the conditions described above for (full scan) first screening analysis. Display reconstructed ion-chromatograms for m/z 205 and 233 and check the background-subtracted mass spectrum of any peak in which these ions are coincident. The background selected for subtraction should be chosen for its minimal, or constant, contributions of these ions. Positive identification of terpyridine isomers is achieved when the relative abundance of the *m/z* 205 ion is approximately 15-40% of the *m/z* 233 ion.

If there is uncertainty as to whether or not terpyridine isomers have been detected (usually at low levels), a further full scan screen should be carried out, following the conditions outlined for the second screening analysis. If the first or second full scan screening analyses do not detect the presence of terpyridine isomers, it may be assumed that the sample complies with specification limit of 1 mg/kg (0.001 g/kg) for terpyridines.

Procedure for quantitative (SIM) analysis

Preparation of sample solution

Weigh accurately, in duplicate, approximately 2g (approximately 1.8 ml) of paraquat dichloride TK into a 14 ml glass, screw-cap (trident) vial. Add 2.0 ml of 1M sodium hydroxide and gently swirl the solution, taking care not to foul the vial cap. Add 2.0 ml IS solution and 4.0 ml ethyl acetate and shake well.

Place the 14 ml glass vial inside a larger solvent-resistant leak-proof capped plastic tube and centrifuge the sample for about 2 minutes, at a speed sufficient to provide adequate separation of the two layers. *Note: samples must be double-contained, to prevent possible contamination of the centrifuge with highly toxic material.* Transfer as much of the top layer as possible using a pasteur pipette into a clean 14 ml glass, screw-cap (trident) vial, taking care not to transfer any of the dark coloured, aqueous bottom layer. Add 2 ml ethyl acetate to the remaining dark coloured, aqueous material, shake well, centrifuge as before and again remove as much of the top layer as possible (taking care not to transfer any of the dark coloured, aqueous bottom layer) and combine it with the first ethyl acetate extract. Repeat the extraction with another 2 ml of ethyl acetate, as described above and combine and mix the three ethyl acetate extracts (Solution **E**, approximately 10 ml). If Solution **E** appears to be cloudy, it may be contaminated with a small amount of the aqueous bottom layer. If so, centrifuge Solution **E,** to aid the separation of two layers and take an aliquot of the top layer for analysis. Two solutions, $\mathbf{E_1}$ and $\mathbf{E_2}$, should be prepared from each sample.

System suitability check, equilibration and adjustment of retention time

Perform replicate injections of calibration solution $\mathbf{C_4}$ until consistent peak areas are obtained for 2,2':6',2"-terpyridine and the internal standard. If the 2,2':6',2"-terpyridine peak tails excessively, carry out the actions recommended under **Apparatus**, *Column*. Check the retention times for the internal standard (2.5-6.2 min) and 2,2':6',2"-terpyridine (6.2-8.0 min) and, if they are not within the expected time windows, the carrier gas head pressure may be adjusted ± 2 psi.

Determination

Make replicate injections of calibration solutions $\mathbf{C_1}$, $\mathbf{C_2}$, $\mathbf{C_3}$, $\mathbf{C_4}$ and $\mathbf{C_5}$ and sample solutions $\mathbf{E_1}$, $\mathbf{E_2}$, under the conditions described above for SIM analysis, in a sequence such as: $\mathbf{C_1}$, $\mathbf{E_1}$, $\mathbf{E_1}$, $\mathbf{C_2}$, $\mathbf{E_1}$, $\mathbf{E_2}$, $\mathbf{C_3}$, $\mathbf{E_2}$, $\mathbf{C_4}$, $\mathbf{E_2}$, $\mathbf{C_5}$. Detector responses to injections of the range of calibration solutions must encompass those of the sample solutions. Sums of peak areas for the ions at *m/z* 167 and 244 for the IS and *m/z* 205 and 233 for the terpyridines, obtained from calibration solutions C1, C2, C3, C4 and C5, are used to calculate response factors, from which a calibration curve and equation are derived. In the SIM chromatograms obtained from sample solutions $\mathbf{E_1}$ and $\mathbf{E_2}$, peaks corresponding to the ions at *m/z* 205 and 233 are measured only where they have been identified as terpyridine isomers by the full scan screening analysis.

Calculations

Calculate the *peak area ratio* (PAR) for the terpyridine peak in each of the calibration solutions $\mathbf{C_1}$, $\mathbf{C_2}$, $\mathbf{C_3}$, $\mathbf{C_4}$ and $\mathbf{C_5}$ as follows:

$$\text{PAR } \mathbf{C_1} = \frac{\text{A } \mathbf{C_1}}{\text{I } \mathbf{C_1}}$$

$$\text{PAR } \mathbf{C_2} = \frac{\text{A } \mathbf{C_2}}{\text{I } \mathbf{C_2}}$$

$$\text{PAR } \mathbf{C_3} = \frac{\text{A } \mathbf{C_3}}{\text{I } \mathbf{C_3}}$$

$$\text{PAR } \mathbf{C_4} = \frac{\text{A } \mathbf{C_4}}{\text{I } \mathbf{C_4}}$$

$$\text{PAR } \mathbf{C_5} = \frac{\text{A } \mathbf{C_5}}{\text{I } \mathbf{C_5}}$$

where: A $\mathbf{C_1}$ = sum of ion peak areas of terpyridine in calibration solution $\mathbf{C_1}$;
A $\mathbf{C_2}$ = sum of ion peak areas of terpyridine in calibration solution $\mathbf{C_2}$;
A $\mathbf{C_3}$ = sum of ion peak areas of terpyridine in calibration solution $\mathbf{C_3}$;
A $\mathbf{C_4}$ = sum of ion peak areas of terpyridine in calibration solution $\mathbf{C_4}$;
A $\mathbf{C_5}$ = sum of ion peak areas of terpyridine in calibration solution $\mathbf{C_5}$;
I $\mathbf{C_1}$ = sum of ion peak areas of internal standard in calibration solution $\mathbf{C_1}$;

I $\mathbf{C_2}$ = sum of ion peak areas of internal standard in calibration solution $\mathbf{C_2}$;
I $\mathbf{C_3}$ = sum of ion peak areas of internal standard in calibration solution $\mathbf{C_3}$;
I $\mathbf{C_4}$ = sum of ion peak areas of internal standard in calibration solution $\mathbf{C_4}$;
I $\mathbf{C_5}$ = sum of ion peak areas of internal standard in calibration solution $\mathbf{C_5}$.

Calculate the *amount*, AM (mg), of the terpyridine in calibration solutions $\mathbf{C_1}$, $\mathbf{C_2}$, $\mathbf{C_3}$, $\mathbf{C_4}$ and $\mathbf{C_5}$, as follows:

$$\text{AM } \mathbf{B_1} = \frac{(\text{WR } \mathbf{A_1} \times P) \times 0.25}{25 \times 100}$$

$$\text{AM } \mathbf{B_2} = \frac{(\text{WR } \mathbf{A_2} \times P) \times 0.125}{25 \times 100}$$

$$\text{AM } \mathbf{C_1} = \frac{(\text{WR } \mathbf{A_1} \times P) \times 0.25}{25 \times 100}$$

$$\text{AM } \mathbf{C_2} = \frac{\text{AM } \mathbf{B_1} \times 1}{25}$$

$$\text{AM } \mathbf{C_3} = \frac{(\text{WR } \mathbf{A_2} \times P) \times 0.1}{25 \times 100}$$

$$\text{AM } \mathbf{C_4} = \frac{\text{AM } \mathbf{B_2} \times 5}{25}$$

$$\text{AM } \mathbf{C_5} = \frac{\text{AM } \mathbf{B_2} \times 1}{25}$$

where: WR $\mathbf{A_1}$ = weight of standard taken to make solution $\mathbf{A_1}$ (mg);
WR $\mathbf{A_2}$ = weight of standard taken to make solution $\mathbf{A_2}$ (mg);
P = purity, % w/w, of the 2,2':6',2"-terpyridine standard.

Calculate the *peak area ratio*, PAR S, for each terpyridine isomer in the sample solution E as follows:

$$\text{PAR S} = \frac{A'}{I'}$$

where: A' = sum of ion peak areas of terpyridine isomer in sample;
I' = sum of ion peak areas of internal standard in sample.

Calculate the *amount*, AM' (mg), of each terpyridine isomer in the sample solution E, using a data system, Microsoft Excel®, or similar package. For example:

1. Produce an Excel Workbook containing the raw peak area data from the terpyridine and internal standard peaks in the calibration and sample solutions.
2. Calculate the peak area ratios: PAR $\mathbf{C_5}$, PAR $\mathbf{C_2}$, PAR $\mathbf{C_4}$, PAR $\mathbf{C_3}$ and PAR $\mathbf{C_1}$. Plot a graph of the calibration amounts (AM $\mathbf{C_5}$, AM $\mathbf{C_2}$, AM $\mathbf{C_4}$, AM $\mathbf{C_3}$ and AM $\mathbf{C_1}$) against the PAR values, ensuring that the equation of the line is shown after the 2nd-order polynomial trendline has been selected.
3. The equation will have the form $y = ax^2 + bx + c$ and the numerical values for the coefficients a, b and c should be formatted to give 15 decimal places.
4. Calculate the amount AM' of each terpyridine isomer in each injection as follows:

$$\text{AM'} = \frac{-b + \sqrt{(b^2 - 4a(c - PAR\ S))}}{2a}$$

where a, b and c are the numerical coefficients from the equation of the line.

For sample solution E, the *terpyridine content in mg/kg* is calculated as follows.

$$\text{terpyridine, mg/kg} = \frac{AM' \times 10^6 \times IS\ S}{WS \times IS\ C}$$

where: WS = weight of sample (mg);
IS S = volume of IS solution added to samples (ml);
IS C = volume of IS solution added to calibration solutions (ml).

The total terpyridine content is the sum of the mean content of the individual terpyridine isomers.

Figure 1. SIM chromatogram of 2,2':6',2''-terpyridine calibration solution **C_4**.

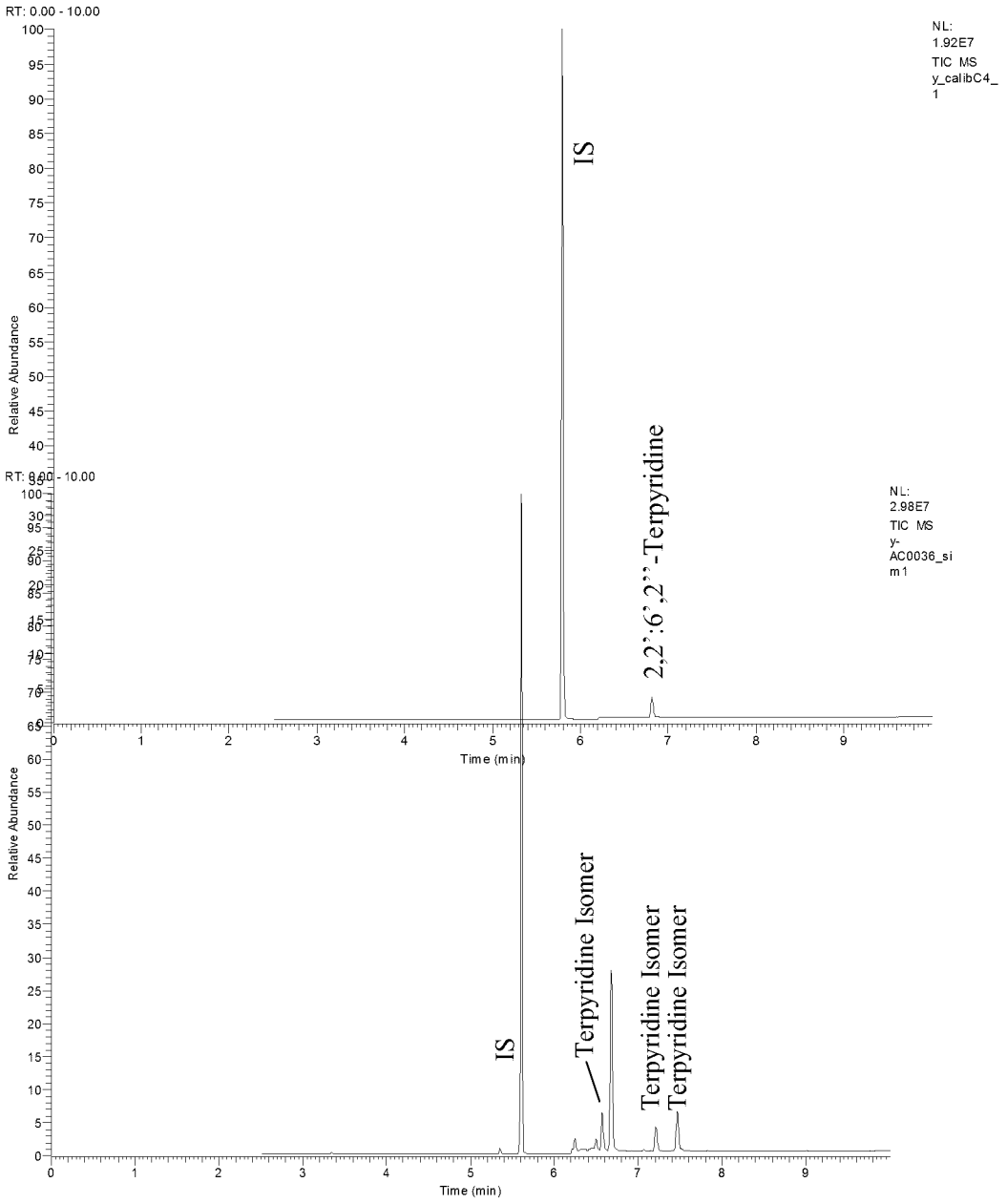


Figure 2. SIM chromatogram of a typical sample solution E, extracted from paraquat dichloride technical concentrate.

Figure 3. EI mass spectrum of 2,2':6',2''-terpyridine standard at chromatographic peak apex.

Figure 4. EI mass spectrum of a terpyridine isomer peak obtained from a paraquat dichloride technical concentrate extract.