

# FAO SPECIFICATIONS AND EVALUATIONS FOR AGRICULTURAL PESTICIDES

## GLYPHOSATE

N-(phosphonomethyl)glycine



FOOD AND AGRICULTURE ORGANIZATION *of* THE UNITED NATIONS

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## DISCLAIMER<sup>1</sup>

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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.

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

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

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<sup>1</sup> This disclaimer applies to all specifications published by FAO.

## INTRODUCTION

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

From 2002, the development of WHO specifications follows the **New Procedure**, described in the 1st edition of “Manual for Development and Use of FAO and WHO Specifications for Pesticides” (2002) and amended with the supplement of this manual (2006), 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 plant protection product in accordance with chapter 4, 5 and 6 of the 5<sup>th</sup> edition of the “Manual on the development and use of FAO specifications for plant protection products”.

**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 (November and year) of publication of the current version. Dates of publication of the earlier versions, if any, are identified in a footnote. 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/ag/agp/agpp/pesticid/>) OR IN HARDCOPY FROM THE PLANT PROTECTION INFORMATION OFFICER.

## **PART ONE**

### **SPECIFICATIONS**

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## GLYPHOSATE

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### INFORMATION

*ISO common name for glyphosate (acid) and its variants*<sup>2</sup>

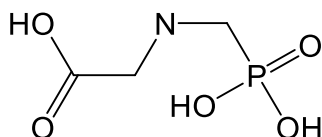
Glyphosate (acid, ISO 1750 published)

*Chemical names*

IUPAC N-phosphonomethylglycin

CA N-phosphonomethylglycin

*Structural formula*



*Molecular formula*

C<sub>7</sub>H<sub>14</sub>N<sub>4</sub>O<sub>3</sub>

*Relative molecular mass*

202.21 g/mole

*CAS Registry number*<sup>1</sup>

165252-70-0

*CIPAC number*

284

*Identity tests*

IR, retention time in reversed-phase HPLC

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<sup>2</sup> When this substance is used as a salt, its identity should be stated, for example (CAS Registry numbers in brackets) glyphosate-diammonium [69254-40-6], glyphosate-dimethylammonium [34494-04-7], glyphosate-isopropylammonium [38641-94-0], glyphosate-monoammonium [40465-66-5], glyphosate-potassium [70901-20-1], glyphosate-sesquisodium [70393-85-0], glyphosate-trimesium [81591-81-3].

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## GLYPHOSATE ACID TECHNICAL MATERIAL

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### FAO Specification 284 / TC (November 2014\*)

*This specification, which is PART ONE of this publication, is based on an evaluation of data submitted by the manufacturers whose names are listed in the evaluation reports (284/2000, 284/2001, 284/2012.1 & 284/2012.2). It should be applicable to technical materials of these manufacturers but it is not an endorsement of those products, nor a guarantee that they comply with the specifications. The specification may not be appropriate for the technical materials of other manufacturers. The evaluation reports (284/2000, 284/2001, 284/2012.1 & 284/2012.2) as PART TWO form an integral part of this publication.*

#### 1 Description

The material shall consist of glyphosate, together with related manufacturing impurities. It shall be a white dry powder, free from visible extraneous matter and added modifying agents.

#### 2 Active Ingredient

##### 2.1 Identity tests (284/TC/(M)/2, CIPAC Handbook 1C, 1985, p. 2132)

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 Glyphosate acid (284/TC/(M)/3, CIPAC Handbook 1C, 1985, p. 2132)

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

#### 3 Relevant Impurities

##### 3.1 Formaldehyde (Note 1)

Maximum 1.3 g/kg of the glyphosate acid content found under 2.2.

##### 3.2 N-Nitrosoglyphosate (Note 2)

Maximum 1 mg/kg

Note 1 The analytical method for determination of Formaldehyde is provided in Appendix 1.

Note 2 The analytical method for determination of N-Nitrosoglyphosate is provided in Appendix 2.

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\* 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/jmps/ps-new/en/>



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## GLYPHOSATE ACID TECHNICAL CONCENTRATE

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FAO Specification 284 / TK (November 2014<sup>\*</sup>)

*This specification, which is PART ONE of this publication, is based on an evaluation of data submitted by the manufacturers whose names are listed in the evaluation reports (284/2000, 284/2001, 284/2012.1 and 284/2012.2). It should be applicable to technical concentrates of these manufacturers but it is not an endorsement of those products, nor a guarantee that they comply with the specifications. The specification may not be appropriate for the products of other manufacturers. The evaluation reports (284/2000, 284/2001, 284/2012.1 and 284/2012.2) as PART TWO form an integral part of this publication.*

### .1 Description

The material shall consist of glyphosate acid together with related manufacturing impurities. It shall be a white to greyish wet cake, free from visible extraneous matter and added modifying agents.

### 2 Active Ingredient

#### 2.1 Identity tests (284/TC/(M)/2, CIPAC 1C, , p.2132, 1985),

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 Glyphosate acid (284/TC/(M)/3, CIPAC 1C, p.2132; AOAC 983.10, 1990)

The glyphosate acid content shall be declared (not less than 950 g/kg on a dry weight basis) and, when determined, and, when determined, the average measured content shall not be lower than the declared minimum content.

### 3 Relevant impurities

#### 3.1 Formaldehyde (Note 1)

Maximum 1.3 g/kg of the glyphosate acid content found under 2.2.

#### 3.2 N-Nitrosoglyphosate (Note 2)

Maximum 1 mg/kg

#### 3.3 Loss on drying (MT 17.3, CIPAC Handbook F, p. 57, 1995)

Sample weight: 10 g; temperature: 105°C, time: 3 hours.).

The loss on drying shall be declared and, when measured the average loss shall be not more than 200 g/kg.

Note 1 The analytical method for determination of Formaldehyde is provided in Appendix 1.

Note 2 The analytical method for determination of N-Nitrosoglyphosate is provided in Appendix 2.

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<sup>\*</sup> 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/jmps/ps-new/en/>

## GLYPHOSATE ISOPROPYLAMINE SALT TECHNICAL CONCENTRATES

FAO Specification 284.105 / TK (November 2014<sup>\*</sup>)

*This specification, which is PART ONE of this publication, is based on an evaluation of data submitted by the manufacturers whose names are listed in the evaluation reports (284/2000, 284/2001 and 284/2012.1). It should be applicable to technical concentrates of these manufacturers but it is not an endorsement of those products, nor a guarantee that they comply with the specifications. The specification may not be appropriate for the products of other manufacturers. The evaluation reports (284/2000, 284/2001 and 284/2012.1) as PART TWO form an integral part of this publication.*

### 1 Description

The material shall consist of technical glyphosate, complying with the requirements of FAO specification 284/TC (November 2014), together with related manufacturing impurities in the form of the isopropylamine salt, and shall be a solution in water, free from visible extraneous matter and added modifying agents except for the diluent.

### .2 Active ingredient

#### 2.1 Identity tests (284/TC/(M)/2, CIPAC 1C, p.2132)

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 Glyphosate acid (284/TC/(M)/3, CIPAC 1C, p.2132; AOAC 983.10, 1990)

The glyphosate acid content shall be declared, (g/l or g/kg at 20 ± 2°C) and, when determined, the average measured content shall not differ from that declared by more than the appropriate tolerance, given in the table of tolerances:

Declared content in g/kg or g/l at 20±2°C	Tolerance
above 250 up to 500	± 5 % of the declared content
above 500	± 25 g/kg or g/l

### 3 Relevant impurities

#### 3.1 Formaldehyde (Note 1)

Maximum 1.3 g/kg of the glyphosate acid content found under 2.2.

#### 3.2 N-Nitrosoglyphosate (Note 2)

Maximum 1 mg/kg

<sup>\*</sup> 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/jmps/ps-new/en/>

- 3.3 **Loss on drying** (MT 17.3, CIPAC Handbook F, p. 57, 1995)  
(Sample weight: 10 g; temperature: 105°C, time: 3 hours.).  
The loss on drying shall be declared and, when measured the average loss shall be not more than 200 g/kg.

.4 **Physical properties**

- 4.1 **pH range** (MT 75.3, CIPAC Handbook J, p. 131, 2000)  
pH 4.5 to pH 6.8

Note 1      The analytical method for determination of Formaldehyde is provided in Appendix 1.

Note 2      The analytical method for determination of N-Nitrosoglyphosate is provided in Appendix 2.

## GLYPHOSATE SOLUBLE CONCENTRATES

FAO Specification 284 / SL (November 2014\*)

*This specification, which is PART ONE of this publication, is based on an evaluation of data submitted by the manufacturers whose names are listed in the evaluation reports (284/2000, 284/2001, 284/2012.1 and 284/2012.2). It should be applicable to technical concentrates of these manufacturers but it is not an endorsement of those products, nor a guarantee that they comply with the specifications. The specification may not be appropriate for the products of other manufacturers. The evaluation reports (284/2000, 284/2001 and 284/2012.1) as PART TWO form an integral part of this publication.*

### 1 Description

The material shall consist of a solution of technical glyphosate, complying with the requirements of FAO specification 284 / TC (November 2014) in the form of a soluble salt (Note 1), dissolved in water, together with any necessary formulants. It shall be in the form of a clear or opalescent liquid, free from suspended matter and sediment, to be applied as a true solution of the glyphosate salt in water.

### 2 Active ingredient

#### 2.1 Identity tests (284/TC/(M)/2, CIPAC 1C, p.2132, 1985)

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 Glyphosate content (284/SL/(M)/3, CIPAC 1C, p.2134, 1985; AOAC 983.10, 1990)

The glyphosate acid content shall be declared for each specific soluble concentrate (g/kg or g/l at  $20 \pm 2$  °C, Note 2) and, when determined, the content measured shall not differ from that declared by more than the following amounts:

Declared content in g/kg or g/l	Tolerance
up to 25	15 % of the declared content
25 to 100	10 % of the declared content
100 to 250	6 % of the declared content
250 to 500	5 % of the declared content
above 500	25 g/kg or g/l
in each range the upper limit is included	

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

### 3 Relevant impurities

#### 3.1 Formaldehyde (Note 3)

Maximum 1.3 g/kg of the glyphosate acid content found under 2.2.

#### 3.2 N-Nitrosoglyphosate (Note 4)

Maximum 1 mg/kg of the glyphosate acid content found under 2.2.

### .4 Physical properties (Note 5)

#### 4.1 Solution stability (MT 41, CIPAC Handbook F, p. 131, 1995)

After the stability test at 54°C (5.2), the product, after dilution with CIPAC Standard Water D and standing for 18 h at 30 ± 2°C (Note 5), shall give a clear or opalescent solution, free from more than a trace of sediment or, particles produced shall pass through a 45 µm test sieve.

#### 4.2 Persistent foam (MT 47.3, Note 6)

Maximum 60 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 be not 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 average determined glyphosate content must not be lower than 95 % relative to the determined content found before storage and the formulation shall continue to comply with the clause for:

- solution stability (4.2)

Note 1 See footnote 1 in the information section for a list of glyphosate salts.

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

Note 3 The analytical method for determination of formaldehyde is provided in Appendix 1.

Note 4 The analytical method for determination of N-Nitrosoglyphosate is provided in Appendix 2.

Note 5 In the case of isopropylamine salt containing formulations and depending on the climatical conditions the pH of the formulation has to be taken into account because the equilibrium glyphosate acid-glyphosate monoisopropylamine salt-diisopropylamine salt and properties of the formulants added will determine the stability towards crystallisation of glyphosate acid

## GLYPHOSATE WATER SOLUBLE GRANULES

FAO Specification 284 / SG (November 2014<sup>\*</sup>)

*This specification, which is PART ONE of this publication, is based on an evaluation of data submitted by the manufacturers whose names are listed in the evaluation reports (284/2000 & 284/2001 & 284/2012.1). It should be applicable to technical concentrates of these manufacturers but it is not an endorsement of those products, nor a guarantee that they comply with the specifications. The specification may not be appropriate for the products of other manufacturers. The evaluation reports (284/2000, 284/2001 & 284/2012.1) as PART TWO form an integral part of this publication.*

### 1 Description

The material shall consist of granules containing technical glyphosate, complying with the requirements of FAO specification 284 / TC (November 2014), in the form a suitable salt together with suitable carriers and formulants. It shall be homogeneous, free from visible extraneous matter and/or hard lumps, free flowing, and essentially non-dusty. The glyphosate salt shall be soluble in water (Note 1). Insoluble carriers and formulants shall not interfere with compliance with clause 4.2.

### .2 Active Ingredient

#### 2.1 Identity tests (284/SG/(M)/2, CIPAC Handbook H, p.182, 1998), (Note 2)

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

#### 2.2 Glyphosate content (284/SG/(M)/3, CIPAC Handbook H, p.182, 1998)

The glyphosate acid or salt content shall be declared (g/kg) and, when determined, the content obtained shall not differ by more than the appropriate tolerance, given in the table of tolerances:

Declared content in g/kg	Tolerance
100 to 250	6 % of the declared content
250 to 500	5 % of the declared content
above 500	25 g/kg
in each range the upper limit is included	

<sup>\*</sup> 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/jmps/ps-new/en/>

### 3 Relevant impurities

#### 3.1 Formaldehyde (Note 2)

Maximum 1.3 g/kg of the glyphosate acid content found under 2.2.

#### 3.2 N-Nitrosoglyphosate (Note 3)

Maximum 1 mg/kg

### 4 Physical Properties

#### 4.1 Degree of dissolution and solution stability (MT 179, CIPAC Handbook H, p. 307, 1998)

Residue of formulation retained on a 75 µm test sieve after dissolution in CIPAC Water D at 30 ± 2°C. Maximum: 2 % after 5 minutes. Maximum: 0.05 % after 18 hours.

#### 4.2 Persistent foam (MT 47.3, Note 4) Maximum: 40 ml after 1 minute.

#### 4.3 Dustiness (MT 171, CIPAC Handbook F, p. 425, 1995).(Note 5). Essentially non-dusty.

#### 4.4 Flowability (MT 172, CIPAC Handbook F, p. 430, 1995)

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

### 5 Storage Stability

#### 5.1 Stability at elevated temperatures (MT 46.3, CIPAC Handbook K, p. 137, 2003)

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

- degree of dissolution and solution stability (4.1)
- dustiness (4.3)
- flowability (4.4)

Note 1 Glyphosate acid as the sodium- or ammonium salt.

Note 2 The analytical method for determination of formaldehyde is provided in Appendix 1.

Note 3 The analytical method for determination of N-Nitrosoglyphosate is provided in Appendix 2.

Note 4 The revised MT method 47.3 was adopted as provisional CIPAC method in 2012 and became a full method in 2013. Prior to its publication in the next Handbook, copies of the method can be obtained through [www.cipac.org/prepub.htm](http://www.cipac.org/prepub.htm)

Note 5 The optical method, MT 171, usually shows good correlation with the gravimetric method and can, therefore, be used as an alternative where the equipment is available. Where the correlation is in doubt, it must be checked with the formulation to be tested. In case of dispute the gravimetric method shall be used.

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

## PART TWO

### EVALUATION REPORTS

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## GLYPHOSATE

### FAO/WHO EVALUATION REPORT 284/2012.2

#### Recommendations

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The Meeting recommended that

- i) the glyphosate TC proposed by Helm AG (Helm) to be accepted as equivalent to the glyphosate reference profile (based on Tier-2).
- (ii) to extend the existing TC specification to the technical material produced by Helm AG, after the adoption of the Monsanto revised reference profile.

#### Appraisal

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The data on glyphosate TC were provided by Helm in support of an equivalence determination with the reference profile that supports the existing glyphosate FAO specifications 284/TC (2012 A). The data submitted were broadly in accordance with the requirements of the [FAO/WHO Manual, 2010]. The technical material of Helm is produced by three different manufacturing processes (routes 1 to 3) in three different plants.

Glyphosate of Helm is currently registered in Argentina. As the registration authorities of Argentina confirmed, the confidential data for evaluation of equivalence of glyphosate of the three manufacturing routes are identical for one of them, while for the rest two are similar to those submitted to FAO.

The Meeting was provided with Helm's commercially confidential information on the three different manufacturing processes and respective 5-batch analysis data on impurities present at or above 1g/kg. The Helm maximum limits for the two impurities formaldehyde and N-nitroso-N-phosphonomethyl-glycine that are considered as relevant in the existing FAO specification of glyphosate TC comply with the limits specified. Mass balances ranged from 99.19 to 100.26% (for route 1), 99.13 to 99.52% (for route 2) and 99.1 to 99.5% (for route 3) in the 5-batch data, respectively.

The declared minimum active ingredient content (950 g/kg) agrees with that of the FAO specification, although it is not statistically justified in all cases (based on the Helm's five batches). A new impurity was identified, an existing impurity was increased above the acceptable range (according to the equivalence criteria as laid down in the FAO/WHO Manual) and its synthetic pathways are clearly different from that of Monsanto, therefore the equivalence cannot be decided based on Tier-1. In addition to the *in-vitro* mutagenicity studies required in Tier-1, toxicology studies were submitted for equivalence determination as required. The presence of that new impurity and the increased limits for the existing one in Helm's technical material is considered to have no toxicological significance based on a battery of toxicological tests conducted with technical materials

representing the three different routes. Toxicity studies were conducted by using one batch of the 5-batches in each case, giving the following results:

- Rat acute oral: LD<sub>50</sub> >2000 mg/kg bw
- Rat acute dermal: LD<sub>50</sub> >2000 mg/kg bw
- Rat acute inhalation: LC<sub>50</sub> >5.02 mg/kg bw
- Rabbit eye irritation: non-irritant
- Rabbit skin irritation: non-irritant
- Guinea pig skin sensitisation: non-sensitiser
- Mutagenicity test (Ames test with *S. typhimurium*, strains TA98, TA100, TA 1535, TA1537, TA102): negative.

Considering the results of the toxicity studies, the Meeting concluded that the glyphosate technical materials produced by Helm was not more hazardous and hence equivalent to the toxicology profile of the reference TC based on Tier-2 evaluations.

The analytical methods used for the determination of the active ingredient content of glyphosate in each of the three different manufacturing processes were different between each other and different from that of CIPAC method [Glyphosate content (284/TC/(M)/2, CIPAC 1C, p.2132)]. It should be mentioned that in all cases RP-HPLC with UV detection was used, but with different eluents and chromatographic columns.

Impurities were also determined by HPLC-UV or LC-MS. Validation data were provided for glyphosate and the impurities. Methods for the impurities were validated to limits of quantitation of 0.004 -10 mg/kg in the TC.

SUPPORTING INFORMATION  
FOR  
EVALUATION REPORT 284/2012.2

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## INFORMATION

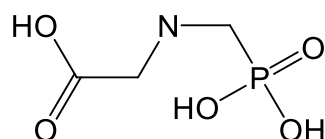
### *ISO common name for glyphosate (acid) and its variants*<sup>3</sup>

Glyphosate (acid, ISO 1750 published)

#### *Chemical names*

IUPAC	N-phosphonomethylglycin
CA	N-phosphonomethylglycin

#### *Structural formula*



#### *Molecular formula*

C<sub>7</sub>H<sub>14</sub>N<sub>4</sub>O<sub>3</sub>

#### *Relative molecular mass*

202.21 g/mole

#### *CAS Registry number*<sup>1</sup>

165252-70-0

#### *CIPAC number*

284

#### *Identity tests*

IR, retention time in anion-exchange HPLC.

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<sup>3</sup> When this substance is used as a salt, its identity should be stated, for example (CAS Registry numbers in brackets) glyphosate-diammonium [69254-40-6], glyphosate-dimethylammonium [34494-04-7], glyphosate-isopropylammonium [38641-94-0], glyphosate-monoammonium [40465-66-5], glyphosate-potassium [70901-20-1], glyphosate-sesquisodium [70393-85-0], glyphosate-trimesium [81591-81-3].

Table 1a. Chemical composition and properties of glyphosate technical material (TC) (manufacturing process 1)

Manufacturing process, maximum limits for impurities $\geq 1$ g/kg, 5 batch analysis data		Confidential information supplied and held on file by FAO. Mass balances were 99.19 – 100.26 % and percentages of unknowns were in the range 0 - 0.81%		
Declared minimum [a.i.] content		950 g/kg		
Relevant impurities $\geq 1$ g/kg and maximum limits for them		Formaldehyde 1.3 g/kg		
Relevant impurities $< 1$ g/kg and maximum limits for them:		N-nitrosoglyphosate 1.0 mg/kg		
Stabilisers or other additives and maximum limits for them:		None.		
Parameter	Value and conditions	Purity %	Method reference	Study number
Melting temperature range of the TC and/or TK	Not available			
Solubility in organic solvents	Not available			

Table 1b. Chemical composition and properties of glyphosate technical material (TC) (manufacturing process 2)

Manufacturing process, maximum limits for impurities $\geq 1$ g/kg, 5 batch analysis data		Confidential information supplied and held on file by FAO. Mass balances were 99.13 - 99.52%.		
Declared minimum [a.i.] content		950 g/kg		
Relevant impurities $\geq 1$ g/kg and maximum limits for them		Formaldehyde 1.3 g/kg.		
Relevant impurities $< 1$ g/kg and maximum limits for them:		N-nitrosoglyphosate 1.0 mg/kg		
Stabilisers or other additives and maximum limits for them:		None.		
Parameter	Value and conditions	Purity %	Method reference	Study number
Melting temperature range of the TC and/or TK	Not available			
Solubility in organic solvents	Not available			

Table 1c. Chemical composition and properties of glyphosate technical material (TC) (manufacturing process 3)

Manufacturing process, maximum limits for impurities $\geq$ 1 g/kg, 5 batch analysis data		Confidential information supplied and held on file by FAO. Mass balances were 99.1-99.5%.		
Declared minimum [a.i.] content		950 g/kg		
Relevant impurities $\geq$ 1 g/kg and maximum limits for them		Formaldehyde 1.3 g/kg		
Relevant impurities $<$ 1 g/kg and maximum limits for them:		N-nitrosoglyphosate 1.0 mg/kg		
Stabilisers or other additives and maximum limits for them:		None		
Parameter	Value and conditions	Purity %	Method reference	Study number
Melting temperature range of the TC and/or TK	Not available			
Solubility in organic solvents	Not available			

#### **METHODS OF ANALYSIS AND TESTING: MANUFACTURING PROCESS 1**

The analytical method for the active ingredient (including identity tests) is a modification of the CIPAC Method No. 284/TC/M/3. The Glyphosate is determined by HPLC with UV detection at 195 nm.

The method(s) for determination of relevant impurities are based on HPLC, following derivatisation and UV detection at 240 nm with internal standardisation (for formaldehyde) and ion chromatography with UV detection at 244 nm (for N-Nitrosoglyphosate).

#### **METHODS OF ANALYSIS AND TESTING: MANUFACTURING PROCESS 2**

The analytical method for the active ingredient (including identity tests) is another modification of the CIPAC Method No. 284/TC/M/3. The Glyphosate is determined by HPLC with UV detection at 195 nm.

The method(s) for the determination of the relevant impurities are based on pre-column derivatisation with Hantzsch solution and HPLC with detection at 412 nm (for formaldehyde), reverse phase chromatography and UV detection at 245 nm (for N-nitrosoglyphosate).

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

### **METHODS OF ANALYSIS AND TESTING: MANUFACTURING PROCESS 3**

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The analytical method for the active ingredient (including identity tests) is a validated method and the identity was confirmed by FTIR. The Glyphosate is determined by reverse phase HPLC using UV detection at 196 nm and external standardisation.

The method(s) for the determination of the relevant impurities are based on derivatisation and reverse phase chromatography with UV detection for both formaldehyde and N-Nitroso glyphosate.

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

### **CONTAINERS AND PACKAGING**

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No special requirements for containers and packaging have been identified.

### **EXPRESSION OF THE ACTIVE INGREDIENT**

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The active ingredient content is expressed as glyphosate acid in g/l (liquid formulations) and g/kg (dry formulations).

## **ANNEX 1**

### **HAZARD SUMMARY PROVIDED BY THE PROPOSER**

Notes.

- (i) The proposer confirmed that the toxicological and ecotoxicological data included in the summary below were derived from glyphosate 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 1. Mutagenicity profile of the glyphosate technical material based on *in vitro* and *in vivo* tests (manufacturing process 1)

Species	Test	Purity % Note <sup>4</sup>	Guideline	Dosage	Result	Study number
Manufacturing process 1						
<i>S. typhimuri-um</i>	Ames Test – <i>in vitro</i>	95.8	OECD 471	31.6, 100,316, 1'000,2'500 and 5'000 µg/plate -S9/+S9	Negative	101268
Manufacturing process 2						
<i>Salmonella typhimurium</i>	Ames Test – <i>in vitro</i>	96.4	OECD 471	31.6, 100,316, 1'000,and 3'160 µg/plate -S9/+S9	Negative	24880
Manufacturing process 3						
<i>Salmonella typhimurium</i>	Ames Test – <i>in vitro</i>	98.8	OECD 471	31.6, 100,316, 1'000,and 3'160 µg/plate -S9/+S9	Negative	23916
Rat – Bone Marrow Cells	Micronucleus Test – <i>in vivo</i>	98.8	OECD 474	500, 1'000, 2'000 mg/kg bw	Negative	23917

<sup>4</sup> Note: Purity is the content of pure active ingredient in the technical material, expressed as a percentage.

**Table 2a.** *Toxicology profile of the glyphosate technical material, based on acute toxicity, irritation and sensitization (via manufacturing process 1)*

Species	Test	Purity % Note <sup>5</sup>	Guideline, duration, doses and conditions	Result	Study number
Rat, ♀	oral	97.3	OECD 423	LD <sub>50</sub> > 2'000 mg/kg bw	24602
Rat, ♀♂	dermal	97.3	OECD 402	LD <sub>50</sub> > 2'000 mg/kg bw	24604
Rat, ♀♂	inhalation	97.3	OECD 403	LC <sub>50</sub> > 5.18 mg/L	24603
Rabbit, ♂	skin irritation	97.3	OECD 404	Non-irritant	24605
Rabbit, ♂	eye irritation	97.3	OECD 405	Non-irritant	24606
Guinea Pig, ♂	skin sensitization	97.3	OECD 406	No sensitiser (Magnusson and Kligman Test)	24607

**Table 2b.** *Toxicology profile of the glyphosate technical material, based on acute toxicity, irritation and sensitization (via manufacturing process 2)*

Species	Test	Purity % Note <sup>6</sup>	Guideline, duration, doses and conditions	Result	Study number
Rat, ♀	oral	96.4	OECD 423	LD <sub>50</sub> > 2'000 mg/kg bw	24874
Rat, ♀♂	dermal	96.4	OECD 402	LD <sub>50</sub> > 2'000 mg/kg bw	24876
Rat, ♀♂	inhalation	96.4	OECD 403	LC <sub>50</sub> > 5.02 mg/L	24875
Rabbit, ♂	skin irritation	96.4	OECD 404	No-irritant	24877
Rabbit, ♂	eye irritation	96.4	OECD 405	Non-irritant	24878
Guinea Pig, ♀	skin sensitization	96.4	OECD 406	No sensitiser (Magnusson and Kligman Test)	24879

<sup>5</sup> Note: Purity is the content of pure active ingredient in the technical material, expressed as a percentage.

<sup>6</sup> Note: Purity is the content of pure active ingredient in the technical material, expressed as a percentage.

*Table 2c. Toxicology profile of the glyphosate technical material, based on acute toxicity, irritation and sensitization (via manufacturing process 3)*

Species	Test	Purity % Note <sup>7</sup>	Guideline, duration, doses and conditions	Result	Study number <small>Fehler! Textmarke nicht definiert.</small>
Rat, ♀	Oral	98.8	OECD 423	LD <sub>50</sub> > 2'000 mg/kg bw	23910
Rat, ♀♂	Dermal	98.8	OECD 402	LD <sub>50</sub> > 2'000 mg/kg bw	23912
Rat, ♀♂	Inhalation	98.8	OECD 403	LC <sub>50</sub> > 5.12 mg/L	23911
Rabbit, ♂	Skin irritation	98.8	OECD 404	Non-irritant	23913
Rabbit, ♂	Eye irritation	98.8	OECD 405	Non-irritant	23914
Guinea Pig, ♂	Skin sensitisation	98.8	OECD 406	No sensitiser (Magnusson and Kligman Test)	23915

<sup>7</sup> Note: Purity is the content of pure active ingredient in the technical material, expressed as a percentage.

## Annex 2

### References

#### Manufacturing pathway 1

Study number	Author(s)	year	Study title. Study identification number. Report identification number. GLP [if GLP]. Company conducting the study.
	FAO/WHO	2006	Manual on development and use of FAO and WHO specifications for pesticides. February 2006 Revision of First Edition. FAO Plant Production and Protection Paper. Revised. <a href="http://www.fao.org/ag/AGP/AGPP/Pesticid/Default.htm">www.fao.org/ag/AGP/AGPP/Pesticid/Default.htm</a> and <a href="http://whqlibdoc.who.int/publications/2006/9251048576_eng_update2.pdf">http://whqlibdoc.who.int/publications/2006/9251048576_eng_update2.pdf</a>
24602		2010	Acute Oral Toxicity Study of Glyphosate TC in Rats. Report 24602. GLP. Germany. Unpublished Confidential Report of Helm AG.
24604		2010	Acute Dermal Toxicity Study of Glyphosate TC in CD Rats. Report 24604. GLP.. Unpublished Confidential Report of Helm AG.
24603		2010	Acute Inhalation Toxicity Study of Glyphosate TC in Rats. Report 24603. GLP. Germany. Unpublished Confidential Report of Helm AG.
24605		2010	Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC in Rabbits. Report 24605. GLP. Unpublished Confidential Report of Helm AG.
24606		2010	Acute Eye Irritation/Corrosion Test of Glyphosate TC in Rabbits. Report 24606. GLP. Unpublished Confidential Report of Helm AG.
24607	Haferkorn J	2010	Examination of Glyphosate TC in the Skin Sensitisation Test in Guinea Pigs according to Magnusson and Kligman (Maximisation Test). Report 24607. GLP. Unpublished Confidential Report of Helm AG.
101268	Wallner B	2010	Reverse Mutation Assay using Bacteria (Salmonella typhimurium) with Glyphosate TC. Report 101268. GLP. Unpublished Confidential Report of Helm AG.

#### Manufacturing pathway 2

Study number	Author(s)	year	Study title. Study identification number. Report identification number. GLP [if GLP]. Company conducting the study.
	FAO/WHO	2006	Manual on development and use of FAO and WHO specifications for pesticides. February 2006 Revision of First Edition. FAO Plant Production and Protection Paper. Revised. <a href="http://www.fao.org/ag/AGP/AGPP/Pesticid/Default.htm">www.fao.org/ag/AGP/AGPP/Pesticid/Default.htm</a> and <a href="http://whqlibdoc.who.int/publications/2006/9251048576_eng_update2.pdf">http://whqlibdoc.who.int/publications/2006/9251048576_eng_update2.pdf</a>
24874		2010	Acute Oral Toxicity Study of Glyphosate TC in Rats. Report 24874. GLP. Unpublished Confidential Report of Helm AG.
24876		2010	Acute Dermal Toxicity Study of Glyphosate TC in CD Rats. Report 24876. GLP. Unpublished Confidential Report of Helm AG.
24875		2010	Acute Inhalation Toxicity Study of Glyphosate TC in Rats. Report 24875. GLP. Unpublished Confidential Report of Helm AG.

<b>Study number</b>	<b>Author(s)</b>	<b>year</b>	<b>Study title. Study identification number. Report identification number. GLP [if GLP]. Company conducting the study.</b>
24877		2009	Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC in Rabbits. Report 24877. GLP. Unpublished Confidential Report of Helm AG.
24878		2009	Acute Eye Irritation/Corrosion Test of Glyphosate TC in Rabbits. Report 24878. GLP. Unpublished Confidential Report of Helm AG.
24879		2010	Examination of Glyphosate TC in the Skin Sensitisation Test in Guinea Pigs according to Magnusson and Kligman (Maximisation Test). Report 24879. GLP. Unpublished Confidential Report of Helm AG.
24880		2010	Mutagenicity Study of Glyphosate TC in the Salmonella typhimurium Reverse Mutation Assay (in vitro). Report 24880. GLP. Unpublished Confidential Report of Helm AG.

### **Manufacturing pathway 3**

<b>Study number</b>	<b>Author(s)</b>	<b>year</b>	<b>Study title. Study identification number. Report identification number. GLP [if GLP]. Company conducting the study.</b>
	FAO/WHO	2006	Manual on development and use of FAO and WHO specifications for pesticides. February 2006 Revision of First Edition. FAO Plant Production and Protection Paper. Revised. <a href="http://www.fao.org/ag/AGP/AGPP/Pesticid/Default.htm">www.fao.org/ag/AGP/AGPP/Pesticid/Default.htm</a> and <a href="http://whqlibdoc.who.int/publications/2006/9251048576_eng_update2.pdf">http://whqlibdoc.who.int/publications/2006/9251048576_eng_update2.pdf</a>
23910		2009	Acute Oral Toxicity Study of Glyphosate TC in Rats. Report 23910. GLP. Unpublished Confidential Report of Helm AG.
23912		2009	Acute Dermal Toxicity Study of Glyphosate TC in CD Rats. Report 23912. GLP. Unpublished Confidential Report of Helm AG.
23911		2009	Acute Inhalation Toxicity Study of Glyphosate TC in Rats. Report 23911. GLP. Unpublished Confidential Report of Helm AG.
23913		2009	Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC in Rabbits. Report 23913. GLP. Unpublished.
23914		2009	Acute Eye Irritation/Corrosion Test of Glyphosate TC in Rabbits. Report 23914. GLP. Unpublished Confidential Report of Helm AG.
23915		2009	Examination of Glyphosate TC in the Skin Sensitisation Test in Guinea Pigs according to Magnusson and Kligman (Maximisation Test). Report 23915. GLP. Unpublished Confidential Report of Helm AG.
23916		2009	Mutagenicity Study of Glyphosate TC in the Salmonella typhimurium Reverse Mutation Assay (in vitro). Report 23916. GLP. Unpublished Confidential Report of Helm AG.
23917		2009	Micronucleus Test of Glyphosate TC in Bone Marrow Cells of the CD Rat by oral administration. Report 23917. GLP. Unpublished Confidential Report of Helm AG.

## GLYPHOSATE

### FAO/WHO EVALUATION REPORT 284/2012.1

#### Recommendations

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The Meeting recommended that the revised specification for glyphosate TC proposed by Monsanto Company, as amended, should be adopted by FAO after the some clarifications.

#### Appraisal

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A data package for glyphosate was submitted by Monsanto Company in support of a revision of the published FAO specifications for TC (2000/2001). The revision was conducted at the request of Monsanto Company, because a slight change in the manufacturing process had resulted in the formation of a new impurity which was not present in the previous FAO submission and a higher concentration is specified for a previously existing impurity. In addition, the limits of four impurities were decreased and one impurity was removed from the specifications, compared with the Monsanto's technical specifications evaluated for the Annex I inclusion of glyphosate (DAR Addendum 2, 2002). The data submitted were in accordance with the FAO Manual (FAO/WHO Manual 2010) and the proposed FAO specifications for glyphosate were almost the same to the existing ones. However, a maximum of 4g/kg is currently specified for insolubles (without reference to the analytical method used), while in the published FAO specifications a maximum 0.1 g/kg is specified for insolubles in 1M NaOH (MT 71). The company explained, that a transcription error in the initial data submission had occurred and that the intention already in the first submission had been to propose 0.1 g/kg. The Meeting concluded, that this lower limit of 0.1 g/kg renders the insolubles irrelevant and agreed to remove the clause.

The minimum purity of glyphosate acid technical remained at 950 g/kg. The limits for the two relevant impurities, formaldehyde (1.3 g/kg) and the N-nitrosoglyphosate (1 mg/kg) comply with the existing FAO specifications. The increased specification of the previously existing impurity is acceptable (the increase comply with the respective FAO criteria). Although, a new impurity is present which indicates that the equivalence cannot be decided upon Tier-1 assessment, this impurity is not toxicologically significant as one of its use is as a food additive.

Additionally, Monsanto submitted further information on the physical-chemical properties (data on glyphosate solubility in the organic solvents) and toxicological data that were not previously submitted.

Furthermore, five batch analysis of glyphosate isopropylamine salt and glyphosate potassium salt from Monsanto's plants were provided, indicating a minimum purity 439 g/kg and 456 g/kg respectively. However, in the case of TK the declared content, with the respective tolerances, should be provided. It should be mentioned that, the existing FAO specifications refer only to glyphosate isopropylamine salt and not to glyphosate potassium salt.

The Meeting was provided with commercially confidential information on the manufacturing process for glyphosate and 5-batch analysis data on the purity and impurities  $\geq 1$ g/kg. Mass balances were from 98.92 to 100.32 % in the 5-batch data. Confidential data were similar to those submitted for registration in EU.

Analytical methods for the determination of glyphosate in TC and all formulations type available are full CIPAC methods, in which glyphosate is determined by high performance liquid chromatography using UV detection at 195nm, an anion exchange column and external standardization (CIPAC Handbooks C and H). The retention time of HPLC-UV method provides a mean for identifying glyphosate.

The analytical method for the determination of formaldehyde, the relevant impurity identified in glyphosate technical material, is available. The method is based on the reaction of the Hantzsch reagent with formaldehyde. The resulting derivative, diacetyldihydrolutidine (DDL) is determined by reversed phase high performance liquid chromatography with UV detection. The link in the footnote refers to this method.

The analytical method for the determination of N-Nitrosoglyphosate, the relevant impurity identified in glyphosate technical material, is also available. The N-Nitrosoglyphosate is identified and quantified with the use of HPLC with a strong ion exchange column. The effluent from HPLC system is then sent into a post column reactor where the N-Nitrosoglyphosate is converted to purple azo dye which is detected at 550nm. The link in the footnote refers to this method.

In particular, the several glyphosate specifications from 2001 were revised as follows:

TC and TK for glyphosate acid

The insolubles in 1 M NaOH were removed. The reason for this is a transcription error – the previous limit of 4 g was wrong, reduced to 0.1 g per kg and the Meeting agreed that this amount could be considered as non-relevant.

Glyphosate isopropylammonium TK

A correct amount for the declared value of the active ingredient (459 g/kg) with the corresponding tolerances is now specified.

#### Specifications for SL and GR

In the 2001 version, clauses for testing after the high temperature storage stability included the analyses for the relevant impurities formaldehyde and *N*-nitrosoglyphosate. As formaldehyde and *N*-Nitrosoglyphosate cannot be formed on storage, these clauses were removed based on the rules of the FAO/WHO Manual, November 2010 - second revision of the First Edition.

#### Hazard data

The IPCS hazard classification of Glyphosate is: slightly hazardous, class III.



SUPPORTING INFORMATION  
FOR  
EVALUATION REPORT 284/2012.1

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## USES

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Glyphosate is a systemic non-selective foliar applied herbicide belonging to the group of the glycines, which is used for the control of a wide range of monocot and dicot weeds in a range of situations. Glyphosate is classified by HRAC in Group G.

Glyphosate is taken up by green tissue of the leaves and stems of treated plants. It is transported systemically (via apoplastic and symplastic pathways) throughout the plant including the roots, rhizomes and stolons but especially to areas of metabolic activity in the plant (sinks), where it inhibits the shikimic acid pathway. Glyphosate binds to and blocks the activity of its target enzyme EPSPS (5-enolpyruvylshikimate-3-phosphate synthase), an enzyme of the aromatic amino acid biosynthetic pathway. The inhibition of the enzyme prevents the plant from synthesizing the essential aromatic amino acids needed for protein biosynthesis.

### Identity of the active ingredient

#### *ISO common name*

Glyphosate (ISO-accepted)

#### *Chemical name(s)*

IUPAC

N-(phosphonomethyl)-glycine

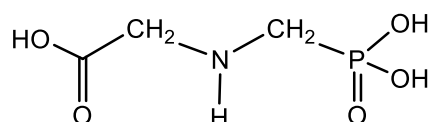
CA

glycine, N-(phosphonomethyl)-

#### *Synonyms*

none

#### *Structural formula*



#### *Molecular formula*

C<sub>3</sub>H<sub>8</sub>NO<sub>5</sub>P

#### *Relative molecular mass*

169.1 g/mol

#### *CAS Registry number*

1071-83-6  
CIPAC number  
284

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

Parameter	Value(s) and conditions	Purity % <sup>8</sup>	Method reference (and technique if the reference gives more than one)	Study number
Vapour pressure	1.31 x 10 <sup>-5</sup> Pa at 25 °C	98.6	OECD 104, by extrapolation	676/2-AR
Melting point.	189.5 °C	99.9	OECD 102	NA 89 9641/I
Temperature of decomposition	199 °C	99.9	OECD 102	NA 89 9641/I
Solubility in water	10.5 g/l at 20 °C in distilled water pH 2 ‡	99.5	OECD 105	257207
Octanol/water partition coefficient	log P <sub>OW</sub> = < -3.2 at 25 °C pH 5,7 and 9	99.9	OECD 107	MSL-7241
Hydrolysis characteristics	Half-life <sup>9</sup> = >>30 days at 25°C °C at pH 5,7 and 9.	96.6	US EPA 161-1	238500
Photolysis characteristics	<10% photodegradation of 14C-glyphosate was observed at pH 5, 7, or 9.	100	US EPA 161-2	MSL-10575
Dissociation characteristics	pKa = 2.74 5.63 and 10.2 at 25°C	98.6	OECD 112, titration method	11704.04 92.6121-885
Solubility in organic solvents	0.078 g/l acetone at 20 °C 0.233 g/l dichloromethane at 20 °C 0.012 g/l ethyl acetate at 20 °C 0.026 g/l hexane at 20 °C 0.231 g/l methanol at 20 °C 0.020 g/l propane-2-ol at 20 °C 0.036 g/l toluene at 20 °C	98.6	OECD 105	6759-676/5

<sup>8</sup> Note: Purity is the content of pure active ingredient in the technical material, expressed as a percentage

**Table 2. Chemical composition and properties of glyphosate technical materials (TC, TK and its variants)**

<b>Glyphosate acid (TC)</b>				
Manufacturing process, maximum limits for impurities $\geq 1$ g/kg, 5 batch analysis data		Confidential information supplied and held on file by FAO. Mass balances were 98.92 –100.32 % (dry weight) and percentages of unknowns were 0 –1.08 %.		
Declared minimum [a.i.] content		950 g/kg		
Relevant impurities $\geq 1$ g/kg and maximum limits for them		None		
Relevant impurities $< 1$ g/kg and maximum limits for them:		Formaldehyde (maximum 1 g/kg) N-Nitrosoglyphosate (maximum 1 mg/kg)		
Stabilisers or other additives and maximum limits for them:		None		
Parameter	Value and conditions	Purity %	Method reference	Study number
Melting temperature range of the TC and/or TK	189.5 °C decomposition occurs at 199°C	99.9*	OECD 102	NA 89 9641/I
Solubility in organic solvents	0.078 g/l acetone at 20 °C 0.233 g/l dichloromethane at 20 °C 0.012 g/l ethyl acetate at 20 °C 0.026 g/l hexane at 20 °C 0.231 g/l methanol at 20 °C 0.020 g/l propane-2-ol at 20 °C 0.036 g/l toluene at 20 °C	98.6*	OECD 105	6759-676/5

<b>Glyphosate isopropylamine salt (TK)</b>	
Manufacturing process, maximum limits for impurities $\geq 1$ g/kg, 5 batch analysis data	Confidential information supplied and held on file by FAO. Mass balances were 47.42-49.85 % and percentages of unknowns were proportional to those in the parent glyphosate acid TK
Declared minimum [a.i.] content	439 g/kg
Relevant impurities $\geq 1$ g/kg and maximum limits for them	None
Relevant impurities $< 1$ g/kg and maximum limits for them:	Formaldehyde (maximum 1.3 g/kg of the glyphosate acid content) N-Nitrosoglyphosate (maximum 1 mg/kg)
Stabilisers or other additives and maximum limits for them:	None

<b>Glyphosate potassium salt (TK)</b>	
Manufacturing process, maximum limits for impurities $\geq 1$ g/kg, 5 batch analysis data	Confidential information supplied and held on file by FAO. Mass balances were 48.64 -50.03% and percentages of unknowns were proportional to those in the parent glyphosate acid TK
Declared minimum [a.i.] content	456 g/kg
Relevant impurities $\geq 1$ g/kg and maximum limits for them	None
Relevant impurities $< 1$ g/kg and maximum limits for them:	Formaldehyde (maximum 1.3 g/kg of the glyphosate acid content) N-Nitrosoglyphosate (maximum 1 mg/kg)
Stabilisers or other additives and maximum limits for them:	None

## **ANNEX 1**

### **HAZARD SUMMARY PROVIDED BY THE PROPOSER**

Notes.

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

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

No change in end points since the previous evaluation (unless indicated)

Species	Test	Purity % Note <sup>10</sup>	Guideline, duration, doses and conditions	Result [(iso-mer/form)]	Study number
Rat	oral	98.6	OECD 401, dose rate 5000 mg/kg bw	LD <sub>50</sub> > 5000 mg/kg bw	IRI 5883
Rabbit	dermal	97.76	US EPA 40 CFR part 160	LD <sub>50</sub> > 5000 mg/kg bw	FD-88-29
Rat*	inhalation	98.6	Dust aerosol, 4-hour exposure, snout only, 4.98 mg/l	LC <sub>50</sub> = 5 g/m <sup>3</sup>	IRI 5993
Rabbit	skin irritation	98.6	OECD 404, 0.5 g moistened with water; intact skin	Essentially non-irritating	IRI 5885
Rabbit	eye irritation	98.6	OECD 405, 100 mg pure	Moderate/severe irritation	IRI 5886
Guinea pig	skin sensitisation	98.6	OECD 406 induction, 1% in water; challenge 25% in water	Not a dermal sensitizer	IRI 5887

\*Study not previously submitted.

**Table 4.** *Toxicology profile of the technical material based on repeated administration (subacute to chronic)*

Species	Test	Purity % Note <sup>11</sup>	Guideline, duration, doses and conditions	Result	Study number
Rat	oral	95.21	OECD 408, 90 days Dose rates 0, 1000, 5000, 20000 ppm	NOAEL =1267 mg/kg bw/d (males) NOAEL =1623 mg/kg bw/d (females)	ML-86-351
Mouse	oral	98.7	OECD 408, 90 days Dose rates 0, 5000, 10000, 50000 ppm	NOAEL =1870 mg/kg bw/d (males) NOAEL =2740 mg/kg bw/d (females)	BDN 77-419
Dog*	oral	62.49% isopropyl-amine salt	OECD 452, 6 No- vembers Dose rates 0, 10, 60, 300 mg/kg bw/d	NOAEL 300 mg/kg bw/d . Calculated as acid.	ML-81-368

<sup>10</sup> Note: Purity is the content of pure active ingredient in the technical material, expressed as a percentage.

<sup>11</sup> Note: Purity is the content of pure active ingredient in the technical material, expressed as a percentage.

Species	Test	Purity % Note <sup>1</sup>	Guideline, duration, doses and conditions	Result	Study number
Rat	carcinogenicity	98.7	2 years Dose rates 0,3,10,31 mg/kg bw/d in male rats Dose rates 0, 3.4, 11,34 mg/kg bw/d in females	NOAEL was > 31 mg/kg/day (highest-dose tested). No treatment-related effects observed.	BDN-77-416
Rat	carcinogenicity	96.5	2 years	NOAEL was 362 and 457 mg/kg/day for males and females, respectively. Effects at high-dose: decreased weight gain in females and increased incidence of cataracts in males. Not carcinogenic.	MSL 10495
Mouse	carcinogenicity	99.7	OECD 451, 2 years. Dose rates 4000, 5000, 30000 mg/kg bw/d	NOAEL was 814 and 955 mg/kg/day for males and females, respectively. Effects at high-dose: decreased body weight gain, hepatocyte hypertrophy or necrosis and urinary bladder epithelial hyperplasia. Not carcinogenic.	BDN 77-420
Rat	Three generation reproduction	97.7	Dose rates 0, 3, 10, 30 mg/kg bw/d	NOAEL > 30 mg/kg bw/d No effects observed at any dose level.	BDN-77-417
Rat	Two generation reproduction	97.7	Dose rates 0, 2000, 10000, 30000mg/kg bw/d	NOEL > 722 mg/kg bw/d (males) NOEL >757 mg/kg bw/d (females)	MSL-10387
Rat	teratogenicity , maternal toxicity and developmental toxicity	98.7	Dose rates 0,300,1000,3500 mg/kg bw/d	Maternal and developmental NOAEL was 1000 mg/kg/day. No birth defects observed. Effects at 3500 mg/kg/day: (dams) diarrhea, body weight loss, inactivity, death; (offspring) decreased body weights, increased post-implantation loss.	IRI-79-016
Rabbit	teratogenicity , maternal toxicity and developmental toxicity	98.7	Dose rates 0, 75, 175, 350 mg/kg bw/d	Maternal NOAEL was 175 mg/kg/day. Developmental NOAEL was ≥ 175 mg/kg/day. Effects at 350 mg/kg/day: (dams) diarrhea, nasal discharge, death; (offspring) although too few litters to were available to fully assess developmental toxicity, no birth defects or developmental toxicity was observed at any dose level.	IR-79-018

\*Replaces study submitted in 1999.

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

Species	Test	Purity % Note <sup>12</sup>	Guideline, duration, doses and conditions	Result	Study number
Salmonella typhimurium TA98, TA100, TA1535 TA 1537; <i>E. coli</i> WP2 hcr strain	Bacterial mutation assay with and without metabolic activation	98.4	Dose rate 10 – 5000 µg/plate	negative	ET 78-241
Chinese Hamster ovary	Mammalian cell gene mutation assay with and without metabolic activation	98.7	Dose range: -S9 : 5 – 22.5 mg/ml; +S9: 5 – 25 mg/ml	negative	ML-83-155
Human Lymphocytes (chromosomal aberrations)	mammalian cell cytogenetic assay	96	Dose range: -S9 mix: 33 – 333 µg/ml; +S9 mix: 237 – 562 µg/ml (both experiments taken together)	negative	141918
Rat hepatocytes UDS assay	Rat hepatocyte culture unscheduled DNA synthesis assay	98.7	Assay to assess the potential of the test material to produce DNA damage in mammalian cells which possess endogenous metabolic capability.	negative	AH-83-181
Mouse bone marrow micronucleus test	Mouse bone marrow	98.6	0 – 5000 mg/kg bw. Sampling after 24h 48 and 72 hours	negative	12324
Mouse lymphoma test	In vitro mammalian cell gene mutation test	98.6	OECD 476 -S9/ 0.61 – 5.0 mg/ml. +S9/ 0.52 – 4.2 mg/ml	negative	12325

**Table 6. Ecotoxicology profile of the technical material**

Species	Test	Purity %	Guideline, duration, doses and conditions	Result	Study number
Bobwhite quail	Acute toxicity	83	Single dose	LD <sub>50</sub> > 3851 mg/kg bw	WL-78-27
Bobwhite quail	Acute and short-term toxicity	>98	5 day dietary exposure, plus 3 days observation	LC <sub>50</sub> > 4640 mg/kg feed LDD <sub>50</sub> > 1127 mg/kg bw/d	HL-73-76
Mallard duck	Acute and short-term toxicity	>98	5 day dietary exposure, plus 3 days observation	LC <sub>50</sub> > 4640 mg/kg feed LDD <sub>50</sub> > 1242 mg/kg bw/d	HL-73-15

<sup>12</sup> Note: Purity is the content of pure active ingredient in the technical material, expressed as a percentage.



Species	Test	Purity %	Guideline, duration, doses and conditions	Result	Study number
Bobwhite quail	One generation reproduction	>96	Exposure via feed for a period of 20 weeks]	NOEC > 2250 mg/kg diet	123-186
Mallard duck	One generation reproduction	>96	Exposure via feed for a period of 21 weeks]	NOEC > 2250 mg/kg diet	123-187
Bluegill sunfish	Acute toxicity	83	96 hours, static	LC <sub>50</sub> = 120 mg/L	AB-78-123
Rainbow trout	Acute toxicity	83	96 hours, static	LC <sub>50</sub> = 86 mg/L	AB-78-165
Rainbow trout	Acute toxicity	>97	21-day flow through	NOEC = 50 mg/L for behaviour and mortality	AB-89-36
<i>Daphnia magna</i> (waterflea)	Acute toxicity	83	48 hours, static	EC <sub>50</sub> = 780 mg/L	AB-78-201
<i>Skeletonema costatum</i> (marine diatom)	Acute toxicity	>95	120 hours	EC <sub>50</sub> = 12 mg/L	BL5684/B
<i>Eisenia fetida</i> (earthworm)	Acute toxicity	>98	14 days	LC <sub>50</sub> > 1000 mg/ kg soil dry weight	250784
<i>Apis mellifera</i> (honeybee)	Acute oral toxicity	tech	48 hours	LD <sub>50</sub> = 100 µg/bee	HU85X094
<i>Apis mellifera</i> (honeybee)	Acute dermal toxicity	tech	48 hours	LD <sub>50</sub> > 100 µg/bee	HU85X094

## METHODS OF ANALYSIS AND TESTING

The following analytical methods for active ingredient (including identity tests) are available:

- AOC-CIPAC method 284/TC/(M)/3, CIPAC 1C, p.2132, and AOAC 983.10, 1990.
- AOAC-CIPAC method 284/SG/(M)/3, CIPAC H, p. 182, and AOAC Official Method 996.12, 1997. The principle is HPLC using anion exchange column on a strong anion exchange column and UV detection at 195 nm and quantification by external standardisation.
- Spectrophotometric method. Reaction of glyphosate with sodium nitrite under acidic conditions to form *N*-nitroso-glyphosate. UV determination at 243 nm.

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

## FORMULATIONS

The main formulation types available are soluble liquid (SL) and soluble granule (SG). Glyphosate may be co-formulated with MCPA, dicamba and 2,4-D (as examples).

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

#### **CONTAINERS AND PACKAGING**

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No special requirements for containers and packaging have been identified.

#### **EXPRESSION OF THE ACTIVE INGREDIENT**

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The active ingredient content is expressed as glyphosate acid in g/l (liquid formulations) and g/kg (dry formulations).

## Annex 2

### References

Study number	Author(s)	year	Study title. Study identification number. Report identification number. GLP [if GLP]. Company conducting the study.
676/2-AR		1991	Glyphosate: Determination of vapour pressure. project no: 676/2-AR. GLP, not published
NA 89 9641/I		1989	Determination of the melting point of the test sample glyphosate acid 99,9% acc. To OECD-Guideline 102. Report No.: NA 89 9641/I GLP, not published
257207		1990	Solubility determination of glyphosate (PMG) in water. report no 257207. GLP, not published
MSL-7241		1987	Octanol/water partition coefficient of Glyphosate and MON 7200.Monsanto Company report no MSL-7241 (amended). GLP, not published
238500		1990	Hydrolysis determination of <sup>14</sup> C-glyphosate (PMG) at different pH values. report no 238500. GLP, not published
MSL-10575	S	1990	Photodegradation of [14C]Glyphosate in a buffered aqueous solution at pH 5, 7 and 9 by natural sunlight. Report no. MSL-10575/PTRL 233-W-1. GLP, not published
11704.049 2.6121-885		1995	Glyphosate – Product chemistry studies: Dissociation constant and pH. report no. 11704.0492.6121-885. GLP, not published
6759-676/5		1991	Glyphosate: Determination of solubility in organic solvents. Report no 6759-676/5. GLP, not published
IRI 5883		1989	Glyphosate technical: Acute oral toxicity (limit) test in rats; report no.5883 not published.
FD-88-29		1988	Acute Dermal Toxicity Study of Glyphosate batch /lot/NBR no. XLI-55 in New Zealand White Rabbits. GLP, not published.
IRI 5993		1989	Glyphosate technical: Acute inhalation toxicity study in rats (Limit test); report no.5993 not published.
IRI 5885		1989	Glyphosate technical: Primary skin irritation study in rabbits. report no.5885 not published.
IRI 5886		1989	Glyphosate technical: Primary eye irritation study in rabbits. Inveresk Research International report no.5886 not published.
IRI 5887		1989	Glyphosate technical: Magnusson-Kligman maximisation test in guinea pigs. report no.5887 not published.
ML-86-351		1987	90 day study of glyphosate administered in feed to Sprague/Dawley rats. Monsanto ML-86-351 not published.
BDN 77-419		1979	A three November feeding study of glyphosate (Roundup technical) in mice. Project no. 77-2111 not published.
ML-81-368		1983	Six November study of MON 0139 administered by gelatine capsule to beagle dogs. Monsanto ML-81-368 not published.
BDN-77-416		1981	A Lifetime Feeding Study of Glyphosate (Roundup Technical) in Rats. Report no.BDN-77-416 not published..
MSL 10495		1990	Chronic study of Glyphosate administered in feed to albino rats (6 volumes).Generated by : Monsanto Agricultural Company Submitted by : Monsanto Company Report No. : MSL 10495/ML-87-148not published
BDN 77-420		1983	A chronic feeding study of glyphosate(Roundup technical) in mice. report no. BDN-77-420 not published.
BDN-77-417		1981	A three generation reproduction study in rats with glyphosate. report no. BDN-77-417 not published.

MSL-10387	1990 Two generation reproduction feeding study with glyphosate on Sprague-Dawley rats. Monsanto report no.MSL-10387not published.
IR-79-016	1980 Teratology study in rats.. IR-79-016 not published.
IR-79-018	1980 Teratology study in rabbits. no. IR-79-018 not published.
ET 78-241	1978 Microbial mutagenicity testing o, CP 67573 (glyphosate) Study no. ET 78.241 published by Li and Long (1988).
ML-83-155	1983 CHO/HGPRT gene mutation assay with glyphosate. Monsanto ML-83-155 Published Li and Long (1988)
141918	1995 Evaluation of the ability of glyphosate to induce chromosome aberrations in cultured peripheral human lymphocytes (with independent repeat. The Netherlands report no. 141918 GLP not published.
AH-83-181	1983 The hepatocyte primary culture/DNA repair assay on compound JJN-1020 (glyphosate) using rat hepatocytes in culture., report no.AH-83-181 not published.
12324	1991 Mutagenicity test: Micronucleus test with glyphosate. report no.12324 GLP not published.
12325	1991 Mutagenicity test: In vitro mammalian cell gene mutation test with glyphosate. report no.12325 GLP not published.
WL-78-27	1978 Acute oral LD50 – bobwhite quail. Technical glyphosate. report no.WL-78-27 not published.
HL-73-76	1973 Eight day dietary LC50-bobwhite quail technical CP67573. report no. HL-73-76.GLP not published.
HL-73-15	1973 Eight day dietary LC50-mallard duck technical CP67573. Hazelton Laboratories, report no. HL-73-15.GLP not published
	1978 One-generation reproduction study – bobwhite quail – glyphosate technical. Report no.WL-78-52 GLP not published
WL-78-53	1978 One-generation reproduction study – mallard duck – glyphosate technical. WL-78-53, GLP not published.
AB-78-123	1978 Static acute bioassay report. Acute toxicity of technical glyphosate to bluegill sunfish ( <i>Lepomis macrochirus</i> ). Report no. : AB-78-123, not published.
AB-78-165	1978 Acute Toxicity of Technical Glyphosate to Rainbow Trout ( <i>Salmo gairdneri</i> ). report no.AB-78-165 not published.
AB-89-36	1989 Flow-through of glyphosate to rainbow Trout ( <i>Salmo gairdneri</i> ) for 21-day duration period Report no. AB-89-36.
BP-78-4-031	1978 Toxicity of seven test materials to the marine alga, <u>Skeletonema costatum</u> . EG&G, no. : BP-78-4-031 not published
250784	1990 Acute toxicity (LC50) of glyphosate to earthworms. Report no. 250784 not published.
HU85X094	1972 The acute contact and oral toxicities of CP67573 and MON 2139 to worker honey bees. Report No. : HU85X094 not published.

## FAO SPECIFICATIONS AND EVALUATIONS FOR PLANT PROTECTION PRODUCTS

### GLYPHOSATE

#### EVALUATION REPORT 284/2000

#### EXPLANATION

Glyphosate was scheduled as an existing FAO specification to be reviewed in 1999 under the procedure introduced by FAO in 1998 (FAO Panel, 1998).

The current FAO specifications for glyphosate acid technical concentrates (FAO Specification 284/TK/S, 1991) and glyphosate soluble concentrates (FAO Specification 284/SL/S, 1991) were published in 1992 (AGP:CP/301) with a correction in 1994 (AGP:CP/311).

Glyphosate was evaluated for the first time by JMPR for toxicology and residues in 1986, for residues again in 1988 and 1994, and for toxicology and residues in 1997.

The new draft specifications were submitted 1999 by Monsanto and Cheminova jointly. Data were provided by both companies.

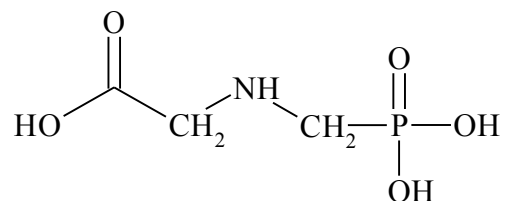
#### USES

Glyphosate is a non-selective contact herbicide with a broad spectrum of applications in agriculture, horticulture viticulture, forestry orchards, plantation crops, amenities, home gardening and greenhouses for the control of annual and perennial grasses and broad-leaved weeds. Furthermore it is used for weed control on aquatic areas, industrial areas, railroad tracks and on other non-cultivated areas. Besides the weed control it is used for root sucker control, for reseeding of grassland and to facilitate harvest. In addition there are uses in transgenic crops which are tolerant to glyphosate (rape, maize, soybeans, in sugar and fodder beets, cotton).

## IDENTITY

ISO common name : Glyphosate  
Chemical name  
IUPAC: *N*-(phosphonomethyl)glycine  
CA: *N*-(phosphonomethyl)glycine  
CAS No: 1071-83-6  
EINECS No: 213-997-4  
CIPAC No: 284  
Synonyms: MON 0573  
CP 67573

Structural formula:



Molecular formula: C<sub>3</sub>H<sub>8</sub>NO<sub>5</sub>P

Molecular weight: 169

Identity test: HPLC method (284/TC/(M)/3, CIPAC 1C, p.2132), retention time.

Spectrophotometric method:

Reaction of glyphosate under acidic conditions to form *N*-nitroso-glyphosate. UV determination at 243 nm.

## PHYSICAL AND CHEMICAL PROPERTIES OF PURE ACTIVE INGREDIENT

Vapour pressure: 1.3 × 10<sup>-5</sup> Pa at 25°C  
Method: EEC A4  
Substance purity: 986 g/kg

Melting point: 189.5°C ± 0.5°C  
Method: OECD 102  
Substance purity: 999 g/kg

Temperature of decomposition: 199°C ± 1°C  
Method: OECD 102  
Substance purity: 999 g/kg

Solubility in water: 10.5 g/l at 20°C  
Method OECD 105  
Substance purity: 995 g/kg

Octanol/water partition coefficient: log K<sub>ow</sub> = < -3.2 at 25°C  
equivalent K<sub>ow</sub> = < 6 × 10<sup>-4</sup>  
(same K<sub>ow</sub> was found at pH 5, 7 and 9)  
Method OECD 107  
Substance purity: 974 g/kg

Hydrolysis:	glyphosate can be considered hydrolytically stable at pH 3, 6 and 9 at 5 or 35°C (half-life >> 30 days). <sup>14</sup> C-glyphosate can be considered hydrolytically stable at pH 5, 7 and 9 at 25°C (half-life >> 30 days). Method US EPA similar to OECD 111. Substance purity: 974 g/kg
Photolysis	No change noted after 24 hours exposure to sunlight. Method: US EPA FIFRA subdivision D- no 63-13.

## **CHEMICAL COMPOSITION AND PROPERTIES OF THE TECHNICAL MATERIAL (TC and TK)**

All necessary information on the manufacturing process and the impurity profile including batch analysis was presented by both of the data submitters in the proposal.

### **Methods of manufacture –**

A summary of the commercially confidential manufacturing process was provided to the Meeting from both of the companies. The Meeting was also provided with information on the nature of the impurities at or exceeding 1 g/kg and their maximum limits in technical material.

Purity (content of active ingredient): glyphosate content in technical material, not less than 950 g/kg.

The impurity profile submitted by Monsanto was different from that provided to the German authorities before with regard to the maximum limits of the specified impurities, but no new impurities were specified. The impurity profile of Cheminova was in line with the information submitted to the German authorities. The impurity profiles have been compared by the German authorities and were regarded to be equivalent with regard to toxicological and ecotoxicological properties.

The Meeting was provided with commercially confidential information on the manufacturing process and batch analysis data on impurities present at or above 1 g/kg, from both companies. The mean mass balances of the batches were 994.5 (Monsanto) and 1045 g/kg (Cheminova).

## HAZARD SUMMARY

Evaluations referred to: JPMR 1986/97  
ICPS Environmental Health Criteria 159  
Agriculture Canada, Discussion Document 1991

Hazard classification. WHO: Unlikely to present acute hazard in normal use

**Table 1. Acute toxicity of glyphosate acid technical material**

Species	Test	Test result
Rat	Oral LD <sub>50</sub>	> 5000 mg/kg
Rat	Dermal LD <sub>50</sub>	> 5000 mg/kg
Rabbit	Skin irritancy	essentially non-irritating
Rabbit	Eye irritancy	moderate/severe irritation
Guinea Pig	Skin sensitization	not a dermal sensitizer

**Table 2. Summary of NOAELs for studies on short term toxicity, long term toxicity and carcinogenicity (EHC 159, 1994\*)**

Species	Test compound	Dose levels mg kg <sup>-1</sup> diet unless otherwise stated	Effects, dose level (mg/kg diet)	NOAEL [mg/kg diet] mg kg <sup>-1</sup> b.w. d <sup>-1</sup>
<b>Short-term studies</b>				
Mouse	Technical glyphosate	5000, 10000, 50000	decreased growth and increased weights in brain, heart, kidneys (50000)	[10000] 1890 m, 2730 f
Mouse	Technical glyphosate	3125, 6250, 12500, 25000, 50000	reduced weight gain (50 000), lesions of salivary glands (≥ 6250)	[3125] 507
Rat	Technical glyphosate	1000, 5000, 20000	no adverse effects	[20000]** 1267**m 1623**f
Rat	Technical glyphosate	200 to 12500	no adverse effects	[12500] NG**
Rat	Technical glyphosate	3125, 6250, 12500, 25000, 50000	increased AP and ALAT (≥6250), increased haematocrit and red cell parameters (≥12 500), increased bile acids, decreased sperm counts (≥25 000), histological alterations in salivary glands (≥3 125), reduced weight gain (≥25 000)	[< 3125] < 205 m < 213 f
Dogs	Technical glyphosate	20, 100, 500 mg kg <sup>-1</sup> bw	no adverse effects	500**



Cattle	Roundup	400, 500, 630, 790 mg kg <sup>-1</sup> bw	decreased feed intake ( $\geq 630$ mg kg <sup>-1</sup> bw d <sup>-1</sup> ), diarrhoea ( $\geq 500$ ), increased blood parameters (790)	400
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Long-term studies				
Mouse	technical glyphosate	1000, 5000, 30000	decreased growth (30 000), increased incidence of hepatocyte hypertrophy and necrosis (30 000), increased incidence of urinary bladder epithelial hyperplasia (30 000)	[5000] 814
Rat	technical glyphosate	2000, 8000, 20000	decreased growth (20 000), increased liver weights (20 000), increased incidences of degenerative lens changes (20 000) and of gastric inflammation (8000 and 20 000)	[8000] 410
Rat	technical glyphosate	60, 200, 600	slightly decreased growth (600)	a

\* note taken of corrigenda on the IPCS web site; m = males; f = females;

\*\* Highest dose tested; NG, not given;

<sup>a</sup> The slight effect at 600 mg/kg diet (32 mg/kg bw) is considered marginal in the light of the absence of an effect on growth at higher dose levels (2000 and 8000 mg/kg diet) in a more recent 2-year study in rats.

**Table 3. Summary of teratogenicity and reproduction studies on glyphosate (EHC 159)**

Species	Test compound	Dose levels	Effects, dose level	NOAEL <sup>a</sup> mg kg <sup>-1</sup> b.w. d <sup>-1</sup>
Rat	technical glyphosate	300, 1000, 3500 mg kg <sup>-1</sup> diet d <sup>-1</sup> gestation days 6-19	mortality, clinical signs and decreased growth in dams, early resorptions, decreased numbers of implantations and visible fetuses, decreased ossification of fetal sternebrae (all at 3500 only); no fetal malformations	1000
Rabbit	technical glyphosate	75, 175, 350 mg/kg body weight, gestation days 6-27	diarrhoea and soft stools (350, slight at 175), nasal discharge (350)	175
Rat	technical glyphosate	3, 10, 30 mg/kg body	increased incidence of renal tubular dilation in	< 30 <sup>b</sup>

		weight given in diet, 3 generations	F <sub>3b</sub> male pups (30)	
Rat	technical glyphosate	2000, 10 000, 30 000 mg/kg diet, 2 generations	soft stools of parents (30 000), decreased litter size (30 000), decreased body weights of parents and pups (30 000 and 10 000)	100 <sup>b</sup> [2000 mg/kg diet]

<sup>a</sup> Based on all observed effects (both in dams and offspring)

<sup>b</sup> There is some discrepancy in the results, and in the NOAELs, of the two reproduction studies carried out with technical glyphosate; the renal effects in the 3-generation study were not reproduced in the more recent 2-generation study with higher dose levels.

**Table 4. Genotoxicity testing, in Vitro Mutagenicity studies (Monsanto)**

Test system	Target cells	Results
Bacterial mutation assay with and without metabolic activation	Salmonella typhimurium TA98, TA100, TA1535 TA 1538; <i>B. subtilis</i> ; <i>E. coli</i>	negative
Mammalian cell gene mutation assay with and without metabolic activation	Chinese Hamster ovary	negative
Mammalian cell cytogenetic Assay	Human Lymphocytes (chromosomal aberrations)	negative
Rat hepatocyte culture unscheduled DNA synthesis assay	Rat hepatocytes UDS	negative

**Table 5. In vivo Mutagenicity studies (Monsanto)**

Test system	Target cells	Results
Mouse bone marrow Micronucleus assay	Mouse bone marrow	negative

## **Acute toxicity**

Glyphosate acid and its salts exhibited a low acute toxicity in laboratory animals by the oral and dermal route with LD<sub>50</sub> values greater than 5000 mg/kg bw

Regarding primary irritation, glyphosate acid and the salts were found to be non-irritant, at least to intact skin. In contrast, undiluted glyphosate acid was found to be strongly irritant to rabbit eyes. There was markedly less eye irritation observed with the salts.

Sensitization was not observed with either glyphosate acid or the salts.

## **Short-term toxicity**

Subacute and subchronic oral toxicity studies also show a low toxicity of glyphosate. Repeated dermal exposure of rabbits and rats to glyphosate did not result in any systemic effects. Dermal irritation was not observed.

## **Mutagenicity / carcinogenicity**

Glyphosate was examined for mutagenicity in a wide range of test systems covering all relevant endpoints in vitro as well as in vivo.

From this large database, it can be concluded that the active ingredient does not exhibit a mutagenic risk to humans. It should be also taken into consideration that there is no evidence of carcinogenic effects in humans, although glyphosate products have been in world-wide use for many years.

## **Reproduction toxicity**

Multigeneration studies in rats did not indicate a specific hazard of glyphosate for reproduction.

Glyphosate is not teratogenic. The NOEL for developmental effects was 1000 mg/kg bw/day in rats and 175 mg/kg bw/day in rabbits.

## **Metabolites**

The metabolite AMPA was investigated for acute and subchronic effects, mutagenicity and teratogenicity. These studies have shown that AMPA has a lower toxicity than the parent compound and is devoid of a mutagenic or teratogenic potential.

## Ecotoxicology

**Table 6. Acute and chronic toxicity of Glyphosate to aquatic organisms**

Species	Test duration/type	EC <sub>50</sub> /LC <sub>50</sub>	Assessment
<i>Daphnia magna</i> (with aeration)	48-hr EC <sub>50</sub>	37 mg/L	Slightly toxic
<i>Daphnia magna</i> (Without aeration)	48-hr EC <sub>50</sub>	24 mg/L	Slightly toxic
<i>Daphnia magna</i>	48-hr EC <sub>50</sub>	13 mg/L	Slightly toxic
<i>Gammarus pseudolimnaeus</i> (Flow-through water)	48-hr EC <sub>50</sub>	42 mg/L	Slightly toxic
Carp	96-hr EC <sub>50</sub>	19.mg/L	Slightly toxic
Bluegill Sunfish (Static water)	96-hr LC <sub>50</sub>	34.0 mg/L	Slightly toxic
Bluegill Sunfish (Flow-through water)	96-hr LC <sub>50</sub>	5.8 mg/L	Moderately toxic
Rainbow trout (Static water)	96-hr LC <sub>50</sub>	15-26 mg/L	Slightly toxic
Rainbow trout (Flow-through water)	96-hr LC <sub>50</sub>	8.2 mg/L	Moderately toxic
Channel Catfish	96-hr LC <sub>50</sub>	39 mg/L	Slightly toxic
Fathead minnow	96-hr LC <sub>50</sub>	23 mg/L	Moderately toxic
Coho Salmon	96-hr LC <sub>50</sub>	22mg/L	Slightly toxic
Chinook Salmon	96-hr LC <sub>50</sub>	20 mg/L	Slightly toxic
Pink Salmon	96-hr LC <sub>50</sub>	14-33mg/L	Slightly toxic

**Table 7. Acute and chronic toxicity of Glyphosate to birds**

Bird Species	Toxicity (mg a.i./kg)
Bobwhite quail acute and short term	8-day LC <sub>50</sub> > 4640 mg/kg Non-toxic 14-day LD <sub>50</sub> > 3851 mg/kg Non toxic
Bobwhite quail Reproduction	NOEC >1000 mg/kg diet
Mallard duck acute and short term	LC <sub>50</sub> > 4640 mg/kg Non toxic
Mallard duck Reproduction	NOEC >1000 mg/kg diet
Chicken	LD <sub>50</sub> >2500 mg/kg Non-toxic

**Table 8. Toxicity\* to bees**

Exposure Route	Toxicity Response
Oral LD <sub>50</sub>	> 100 µg/bee (Non-toxic)
Dermal LD <sub>50</sub>	> 100 µg/bee (Non-toxic)

\* determined with formulated product

On the basis of toxicity data and application rates for the active substance glyphosate, the risks for birds, mammals, aquatic organisms, bees, earthworms and micro-organisms in soil in observance of corresponding risk management measures are regarded as slight.

## FORMULATIONS

Glyphosate liquid formulations (GIFAP code SL) and glyphosate water soluble granules (GIFAP code SG).

Registered and sold in most countries of the world.

## METHODS OF ANALYSIS AND TESTING

- **Chemical analytical methods for active ingredient (including identity tests):**

AOAC-CIPAC method 284/TC/(M)/3, CIPAC 1C, p.2132, and AOAC 983.10, 1990.

AOAC-CIPAC method 284/SG/(M)/3, CIPAC H, p. 182, and AOAC Official Method 996.12, 1997.

The principle is HPLC using an anion exchange column, UV detection at 195 nm and quantification by external standardisation.

### Identity Tests

- AOAC-CIPAC method 284/TC/(M)/2, CIPAC 1C, p.2132, retention time.
- AOAC-CIPAC method 284/SG/(M)/2, CIPAC H, p.182 for SG's, retention time.
- Record the UV scan of the main peak of the chromatogram and compare with an UV scan of the calibration solution.
- Spectrophotometric method. Reaction of glyphosate with sodium nitrite under acidic conditions to form *N*-nitroso-glyphosate. UV determination at 243 nm.
- 

- **Method(s) for determination of relevant impurities in the technical material**

Formaldehyde is determined by a reversed phase HPLC column, off-line derivatization with Hatzsch reagent and UV-VIS detection at 412 nm. This method has been validated from 10 - 300 ppm. (Monsanto Method No AQC 678-86).

*N*-Nitroso-*N*-phosphonomethylglycine (NNG) is determined by strong anion exchange HPLC with UV-visible detection. Samples are dissolved in water and reacted with hydrobromic acid to form a nitrosyl cation; the nitrosyl cation reacts with *N*-(1-naphthyl)ethylenediamine and sulfanilamide to form a purple azo dye that is detected at 550 nm. Because nitrite ion will react with glyphosate to form NNG, all glassware and equipment must be rinsed with sulfamic acid. This method has been validated to 200 ppb in glyphosate technical and 100 ppb in formulated products (Monsanto method no AQC 684-86).

- Physical testing methods: See the specifications.

## - PHYSICAL PROPERTIES

The proposers declared that glyphosate produced and commercialised by Monsanto and Cheminova complies with the FAO specifications (2000).

The clause for specifying the pH range in the case of glyphosate isopropylamine salt concentrates (284.105/TK) and glyphosate soluble concentrates (284/SL) was introduced because, depending on the climatic conditions, the equilibrium glyphosate acid - glyphosate monoisopropylamine salt - diisopropylamine salt will determine the potential crystallisation of glyphosate acid, which has lower water solubility than its salts.

The clause specifying the flowability of soluble granules was changed from 100% to 98% because it was too stringent. Such granules sometimes have the tendency to form loose aggregates, which may remain on the sieve but readily disappear during dissolution in water.

## CONTAINERS AND PACKAGING

No special requirements have been reported for containers and packaging but metal containers should not be used unless lined with suitable material to resist the products if they are acidic.

## EXPRESSION OF ACTIVE INGREDIENT (Sections 4.2.5 and 4.2.7 of the Manual)

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

## APPRAISAL

The current FAO specifications for glyphosate acid technical concentrates (FAO Specification 284/TK/S, 1991) and glyphosate soluble concentrates (FAO Specification 284/SL/S, 1991) were based on data submitted from Monsanto and were published 1992 (AGP:CP/301) with a correction 1994 (AGP:CP/311). The proposers for the revised specification are Monsanto Agricultural Company and Cheminova Agro A/S.

Glyphosate acid is a colourless crystalline solid without odour. It melts at 189.5 °C. The acid is of medium water solubility (10 g/l), the salts are highly soluble in water. It is formulated as water soluble concentrates and water soluble granules, in both of which it is used as a salt (isopropylamine salt, ammonium salt or sodium salt). Glyphosate is stable to hydrolysis in the range of pH 5 to pH 9 and relatively stable to photodegradation.

The Meeting was provided with commercially confidential information on the manufacturing process and batch analysis data on impurities present at or above 1 g/kg, from both of the companies.

Two impurities were identified (formaldehyde and *N*-nitroso-*N*-phosphonomethylglycine, NNG) as relevant and maximum limits are specified.

The same absolute limit of 1 mg/kg for NNG has been set for the TC, TK's and the formulations because this impurity may be formed during the synthesis of glyphosate acid, as well as during the subsequent steps of acid neutralisation (formation of the salt) and during the final steps of formulation.



During the synthesis, the presence of nitrites in the process water, or the presence of  $[\text{NO}]_x$  in the air or oxygen, used in the oxidation process, are the main causes of the formation of *N*-nitrosoglyphosate (NNG).

During the step of acid to salt conversion, the presence of free nitrites in the water being used, might increase the level of *N*-nitrosoglyphosate.

Finally, the formulation or granulation steps, again might cause an increase in the NNG level due to the presence of free nitrites in the water used. Here also again, the  $[\text{NO}]_x$  present in the air, e.g. hot air being used to dry the granules, might cause increase of NNG.

For formaldehyde the limit was set to 1.3 g/kg on a glyphosate acid basis, according to the rules of FAO as published in the Manual. This limit corresponds closely to the limit in the US OSHA regulations which was set on "as is" basis and not on an acid basis.

The differences in the impurity profiles of the two sources had been assessed by the German authorities and were regarded to be of no relevance with regard to toxicological or ecotoxicological properties. This assessment included all toxicological and ecotoxicological studies available to the German authorities. Taking the more detailed Monsanto impurity profile as the reference profile the Cheminova profile is equivalent to the Monsanto impurity profile according to the criteria given in the Manual.

Glyphosate is of low acute toxicity and shows no adverse effects with regard to carcinogenicity, mutagenicity, teratogenicity or reproduction toxicity.

The proposal for an ADI of 0.3 mg/kg bw for glyphosate based on long term studies in rats is in line with the value published by WHO based on the JMPR evaluation of 1986.

Glyphosate is of low risk to birds, mammals, aquatic organisms, bees, earthworms and micro-organisms in soil.

The proposers declared that glyphosate produced and commercialized by Monsanto and Cheminova comply with the FAO specifications (1999)

## RECOMMENDATIONS

The draft specifications for glyphosate acid technical, glyphosate acid technical concentrates, glyphosate isopropylamine salt technical concentrates, glyphosate soluble concentrates and glyphosate water soluble granules, proposed jointly by Monsanto and Cheminova were regarded as acceptable by the Meeting. As the Cheminova impurity profile is covered by the Monsanto impurity profile the Meeting recommended that the Monsanto profile should be the reference profile.

## REFERENCES

- Manual on Development and Use of FAO Specifications for Plant Protection Products, January 1999, Rome.

- FAO Panel of Experts on Pesticide Specifications, Registration Requirements, Application Standards and Prior Informed Consent. Group of Experts on Pesticide Specifications, 3rd Session. 5 - 8 October 1998, Rome.
- IPCS Environmental Health Criteria 159, WHO 1994, Geneva.
- IPCS, The WHO Recommended Classification of Pesticides by Hazard and Guidelines to Classification 1998-1999, WHO 1999, Geneva.
- CIPAC Handbook 1C, 1985
- CIPAC Handbook F, 1995
- CIPAC Handbook H, 1998

FAO SPECIFICATIONS AND EVALUATIONS FOR  
PLANT PROTECTION PRODUCTS

**GLYPHOSATE**

EVALUATION REPORT 284/2001

**Explanation**

The data for glyphosate were evaluated in support of existing FAO specifications 284/TC, 284/TK, 284/SL, 284/SG (2000). The supporting data were provided by Syngenta to extend the scope of the existing specification to their product.

**Uses**

See Evaluation Report for glyphosate (2000).

**Identity**

ISO common name: Glyphosate

Chemical name:

IUPAC: *N*-(phosphonomethyl)-glycine

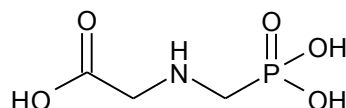
CA: *N*-(phosphonomethyl)-glycine

CAS No: 1071-83-6

CIPAC No: 284

Synonyms: none

Structural formula:



Molecular formula: C<sub>3</sub>H<sub>8</sub>NO<sub>5</sub>P

Relative molecular mass: 169.1

Identity tests: see FAO Specification 284/TC (2000)

## **Physico-chemical properties of pure glyphosate**

See FAO Specification 284/TC (2000)

## **Chemical composition and properties of glyphosate technical materials**

See FAO Specification 284/TC (2000) and confidential information to this report.

## **Hazard summary**

See Evaluation Report for glyphosate (2000).

It was recognised that the acute dermal toxicity given ( < 2000 mg/kg bw) by Syngenta was higher than stated in the Evaluation Report for glyphosate (2000) (< 5000 mg/kg bw).

### Justification submitted by Syngenta:

The guideline used in the acute dermal study [CTL/P/4464] was OECD 402 as specified in 91/414/EEC. In accordance with this guideline the limit dose of 2000 mg/kg was applied following a range finding test to set the dose. A limit dose at this level is, from a technical perspective, appropriate as this is approaching the maximum quantity that can be applied with reasonable confidence that the totality of the dose applied will remain in contact with the rat skin for the duration of the exposure. Applications of amounts greater than 2000 mg/kg are less likely to result in the total dose achieving and/or maintaining contact with the rat skin during the exposure period.

Hence a dermal topical application of 5000 mg/kg leading to an acute dermal MLD50 value of >5000 mg/kg does not signify a lower intrinsic acute dermal toxicity than an MLD50 of >2000 mg/kg resulting from a study using a limit dose of only 2000 mg/kg. The difference in endpoints being simply a reflection of limit dose set used in the individual studies.

It is therefore reasonable to consider that acute dermal MLD50 values in the rat of >2000 and >5000 mg glyphosate acid/kg, where the variance is only a reflection of the differing limit doses of the individual studies, indicate an equivalent profile of the acute dermal toxicity.

This justification was accepted by WHO.

## **Formulations**

Not submitted by Syngenta

## **Methods of analysis and testing**

Analytical method for the active ingredient (including identity tests): see FAO Specification 284/TC (2000).

Fully validated analytical methods for the impurities were provided by Syngenta.

### **Physical properties**

See FAO Specification 284/TC (2000)

### **Containers and packaging**

See FAO Specification 284/TC (2000)

### **Expression of the active ingredient**

See FAO Specification 284/TC (2000)

### **Appraisal**

The data submitted by Syngenta were in accordance with the requirements of the FAO Manual (5th edition) and supported the draft specification. The deviations from reference data set were justified by the proposer and regarded as acceptable by the evaluator.

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.

The manufacturing process and the impurity profile of Syngenta were different from those submitted with the reference specification. The deviations from reference data set were >50% or 3 g/kg in the case of R025029 and R290510 impurities. However, these differences do not lead to differences in toxicological assessment, as evidenced by the data submitted by the proposer for acute oral, dermal, inhalation, skin and eye irritation and sensitization. The Syngenta product is therefore considered to be equivalent to the products upon which the reference profile is based.

### **Recommendations**

The draft specification for technical glyphosate proposed by Syngenta was accepted by the Meeting. The proposer had requested a specification for this material as a TC but the Syngenta product is considered to be equivalent to the existing TK specification. The difference between TC acid and TK acid is the water content only and therefore the extension of the TK specification is recommended. From the production Syngenta isolates the TK acid as a wet paste with a minimum content of 760 g/kg glyphosate. This is within the reference specification for the TK.

### **References**

See Evaluation Report for glyphosate (2000).

## **APPENDIX 1**

### **ANALYSIS OF FORMALDEHYDE IN GLYPHOSATE WETCAKE, GLYPHOSATE ISOPROPYLAMINE SALT AND ROUNDUP<sup>®</sup> SAMPLES**

#### **1. Principle of Method**

This method describes a liquid chromatographic procedure for the selective determination of formaldehyde in glyphosate wetcake, glyphosate isopropylamine salt, and Roundup<sup>®</sup> samples

The Hantzsch reagent is used to react with formaldehyde present in aqueous glyphosate solutions. The resulting derivative, diacetyldihydrolutidine or DDL, is determined by reversed phase HPLC with UV detection.

Quantitation is based on the area of the DDL peak. This response is compared to the response of external standards prepared in the same manner as the samples.

#### **2. Safety**

Several of the solvents and reagents for this method are hazardous chemicals and should be used only with proper ventilation.

#### **3. Range and Sensitivity**

##### **Range**

This method has been validated for the range of 0.3 – 14 ug/mL formaldehyde in diluted samples of glyphosate wetcake, glyphosate isopropylamine salt, and Roundup<sup>®</sup>.

##### **Sensitivity**

Analytical response was found to be linear over the range of 0.3 – 14 ug/mL. The detection limit of the method is 1 ppm under these conditions.

#### **4. Interferences**

Formaldehyde generating substances such as N-isopropylhexahydrotriazine will interfere with this method.

Any compound with the same retention time as DDL, that responds at 412 nm will interfere with this method.

The presence of formaldehyde in reagents will cause a high background level, visible in reagent blanks.

Formaldehyde contamination from Bakelite caps must be avoided.

#### **5. Precision and Accuracy**

##### **Precision**

The pooled coefficient of variation of the analytical method in the range of 0.3-14 ug/mL is 0.222.

## **Accuracy**

Average spiked recoveries for formaldehyde standard spikes in the range of 0.3-14 ug/mL in simulated glyphosate wetcake samples were 85.9 -106.2%. Simulated wetcake was prepared from pure glyphosate and water. Average recoveries for spikes in the range of 26 – 521 ug/g in glyphosate isopropylamine salt and 5.0 -371 ug/g in Roundup<sup>®</sup> samples (equivalent in each case to concentrations of 0.3-14 ug/g in solution) were 88.5- 106.7% and 97.9- 102.0%.

## **6. Advantages and disadvantages**

### **Advantages**

The method is sensitive and selective and is unaffected by the glyphosate matrix.

### **Disadvantage**

Reaction time is at least 2 hours. Samples and standards should be prepared and analyzed on the same day due to the instability of DDL.

## **7. Apparatus**

HPLC pump – Perkin Elmer series 3B

Injector – Perkin Elmer 420B autosampler

Column oven – Perkin Elmer LC – 100

Detector – Perkin Elmer LC-75 spectrophotometric detector

Recorder – Monsanto chromatography data system and strip chart recorder

Analytical column –Dupont Zorbax ODS 4.6mm i.d. X 15 cm

Assorted glasswares

## **8. Reagents**

Formaldehyde – 37% solution Fisher F-79

Acetyl acetone – Fisher A-25

Ammonium acetate – Fisher A-637

Acetic acid – Glacial, Fisher A-38

Sodium hydroxide – Fisher S-318

HPLC water – Burdick and Jackson 365

Acetonitrile – Burdick and Jackson 015

## **9. Calibration and Standardization**

A series of aqueous formaldehyde standards in the range of interest are derivatized and analyzed using the same HPLC conditions and on the same day as the unknown or spiked samples. Formaldehyde is quantitated by comparison with calibration data generated. Standards are prepared by appropriate dilution of a 37% w/w (40% w/w) formaldehyde solution to the working range of 0-14 ppm. These aqueous formaldehyde standards should be prepared fresh for every analysis.

## **10. Procedure**



## **Cleaning of Equipment**

All glassware used for this method should be washed with liquid detergent and rinsed thoroughly with deionized water.

## **Collection and Shipping of Samples**

Samples should be placed in a tightly sealed container with minimal headspace to avoid drying.

## **Sample Preparation**

The HPLC mobile phase is prepared by adding 800 mL of HPLC water to 200 mL of acetonitrile followed by mixing and degassing with helium.

The Hantzsch reagent is prepared by placing 150 g ammonium acetate, 3 mL acetic acid, and 2 mL acetyl acetone in a 1 L volumetric flask and diluting to volume with HPLC water.

The 11% NaOH solution is prepared by diluting 110 g NaOH to 1000 mL with HPLC water.

Prepare an aqueous solution of the glyphosate wetcake to be analyzed in the range of 1% - 20% w/v depending upon the anticipated formaldehyde level. A concentration of 1 ppm formaldehyde can be quantitated in a 20% wetcake. Add 3 mL 11% NaOH per gram of wetcake. Shake until all solids dissolve and dilute to volume with deionized water.

Samples of glyphosate salt and Roundup<sup>®</sup> should also be diluted such that the concentration of formaldehyde in the diluted samples falls within the range of 0.3 -14 ug/g. In all cases, at least slight dilution of these matrices is recommended to reduce potential viscosity issues associated with sample injection via an autosampler.

In a small vial, combine equal volumes of sample or standard solution, containing less than 14 ppm formaldehyde, and Hantzsch reagent. Shake well and allow to stand at ambient temperature for at least 2 hours.

## **Analysis of Prepared Sample**

The derivatized samples and standards are injected onto the HPLC system alternately and the peak area of DDL is recorded. HPLC conditions are: flow = 1.0 mL/min, wavelength = 412 nm, column temperature = 60°C, average retention time of DDL is 6.1 min under these conditions, injection volume = 50 ul.

The amount of formaldehyde in the samples is determined from the established calibration curve in the range of interest using linear regression

## **Special Comments**

If too much formaldehyde is present in the original sample solution, DDL will eventually precipitate out of solution. The yellow color of DDL will fade with time, especially in sunlight.

## 11. Calculations

Quantitation is based upon comparison of the peak areas of the samples and standards. The concentration of formaldehyde in the original solution is determined from the established calibration curve in ppm. From this, the total weight of formaldehyde in the original sample is calculated and is then divided by the original sample weight to give ppm formaldehyde in the sample matrix

$$\text{ppm formaldehyde} = \frac{\text{formaldehyde found(ug)}}{\text{original sample weight(g)}}$$

## 12. Discussion

Due to inconsistent distribution of water content in glyphosate wetcake, there is some difficulty in weighing out representative samples. For validation purposes, glyphosate wetcake was simulated by weighing out dry recrystallized glyphosate and then adding 15% deionized water during spiking. This resulted in consistent samples containing 15% water.

A small background response was observed in the dry recrystallized glyphosate used to simulate wetcake and in the reagent blanks. This response was subtracted from the spike responses when calculating found concentrations. When quantitating low levels of formaldehyde, a reagent blank should always be run.

## 13. References

T. Nash, Biochem J. (London), 55:416-421 (1953)

R. LaMonica, HPLC Assay for Formaldehyde in CMA, Unpublished Results, MAPC (1983)

## **APPENDIX 2**

### **METHOD FOR THE ANALYSIS OF N-NITROSOGLYPHOSATE , (NNG) (IMPURITY- GLYPHOSATE)**

#### **1. Principle of Method**

A one millilitre injection of sample is made into the HPLC system. The NNG is separated from other compounds on a strong anion exchange (SAX) column. The effluent from the HPLC system is then sent into a Griess post column reactor where NNG reacts with HBr to form nitrosyl cation. Nitrosyl cation then reacts with N-( 1-Naphthyl)ethylenediamine and sulphanilamide to form a purple azo dye which is detected at 550 nm.

#### **2. Safety**

Hydrobromic acid and hydrochloric acids are very corrosive. Sulfamic acid, sodium hydroxide, and hydrogen peroxide are also corrosive. Avoid any contact with the skin.

All solutions should be made in a fume hood. Proper gloves are recommended when handling these chemicals.

#### **3. Range and Sensitivity**

##### **Range**

This method has been validated in the range of 200 - 400 ppb NNG in glyphosate wetcake. The standard curve range is 10 – 200 ppb NNG.

##### **Sensitivity**

The sensitivity of the method is 1 mV/ppb NNG.

#### **4. Interferences**

Other N- nitroso compounds and nitrate ion give response with the Griess post column reactor. These interferences should be removed by the analytical method.

There are no known interferences for this method, however nitrate ion will react with glyphosate to form NNG. All glassware and equipment must be rinsed with sulfamic acid to remove any nitrite ions. A solution of sodium hydroxide/hydrogen peroxide is added to the samples and standards to help prevent the formation of NNG.

#### **5. Precision and accuracy**

##### **Precision**

The pooled coefficient of variation for glyphosate wetcake is 0.014 and the correlation coefficient is 0.9991.

##### **Accuracy**

The average recovery for glyphosate wetcake is 93%.

## **6. Advantages and disadvantages**

### **Advantages**

This method, using HPLC/post-column reactor methodology, gives a procedure that is sensitive to NNG at the parts per billion level. The large injection volume gives the needed sensitivity without the need for any concentration steps.

### **Disadvantages**

The use of the post column reactor adds to the complexity of the method and the analysis time.

## **7. Apparatus**

### **Equipment**

Dupont 8800 Pump Module.

Sample injector – Waters Intelligent Sample Processor (WISP) 710B.

Technicon Proportioning Pump III.

Technicon single Channel Colorimeter Equipped with 2.0 X 50 mm Flow Cell and 550 nm filters

Technicon Oil Bath Cartridge Kit, Type A.

Electronic Filter- Spectrum 1021 Filter and Amplifier.

Strip Chart Recorder, 0 – 100 mV span.

Millipore Solvent Filtering Apparatus \_ Type GS, 0.22 micron Filters.

Monsanto Chromatography Data System – A Computer Data handling System.

Technicon Mixing Coils and Tees

HPLC column, Whatman Partisil 10 SAX, 25 cm X 4.6 mm I. D.

Pump Tubing

Orange – Orange Silicon, 0.42 mL/min, Fisher Catalog Number 116-0497-090.

Orange – White, PVC, 1.00 mL/min, Fisher Catalog Number 14-190-75.

Gray – Gray, PVC, 1.00 mL/min, Fisher Catalog number 14-190-80

Standard Laboratory Glassware.

System Auto-Zero (optional), P.J. Cobert Catalog Number AZ\_1436.

### **HPLC Operating Conditions**

HPLC Pump Flow Rate: 1.5 mL/min

Sample Injecton Size: 0.100 mL.

Run Time(WISP): 5 min.

Run Time(MCDS): 20 min.

Detection Wavelength: 550 nm.

Detector settings:

DAMP-NORMAL

Std. Cal. – 6.00

Output – Telemetry Plug (5 volts Full Scale)

Spectrum Filter Settings:

Cutoff Frequency – 0.01  
Attenuation – 1.0  
Post-Column Reactor Oil Bath – 94 deg. C.

## **8. Reagents**

### **Chemicals**

Sulfamic Acid(Fisher A-295)  
Sodium Hydroxide, 2.5 N (Fisher, SO-414)  
Hydrogen Peroxide, 30% (Fisher, H-325)  
Ammonium Phosphate Monobasic (Fisher, A-684)  
Methanol, HPLC Grade (Fisher, A-452)  
N-(1-Naphthyl)ethylenediamine Dihydrochloride (NED). (Eastman,4835)  
Hydrobromic Acid, 48% (Fisher,A-140)  
Sulfanilamide (Fisher, 0-4525)  
Hydrochloric acid, 12 N (Fisher, A-144)  
Phosphoric acid, 85% (Fisher, A-242)  
Brij 35, 30% (Fisher, Cs-285-2)  
High Temperature Bath Oil (Fisher, 0-2)

### **HPLC Mobile Phase**

Mix 20 g ammonium phosphate monobasic into 2.0 liter deionized water. Add 400 mL methanol and bring total volume to 4.0 liters with deionized water. Adjust pH to 2.1 with 85% phosphoric acid, filter and degas mobile phase through a 0.22 micron filter.

### **NED/HBr Solution**

Dissolve 4.35 g N-(1-naphthyl)ethylenediamine dihydrochloride in 400 mL deionized water. Add 500 mL 48% HBr and bring volume to 1.0 L with deionized water.

### **Sulfanilamide Solution**

To 2.0 liters deionized water, add 400 mL concentrated HCl. Add 40.0 g sulphanilamide and 135 mL 30% Brij 35. Bring volume to 4.0 liters with deionized water.

### **Sulfamic Acid Solution**

Dissolve 20 g sulfamic acid in 1.0 L deionized water.

## **9. Calibration and Standardization**

### **NOTE**

**All glassware must be rinsed with the sulfamic acid solution and then with copious amounts of deionized water prior to use. For each volume of sulfamic acid solution used, use an equal volume of deionized water for each rinse. Nalgene sample bottles must also be washed and rinsed before using.**

### **Preparation of Standards**

Standards and samples are prepared and diluted on a weight per weight basis. Measure and record weight to the proper significant figure.

### **Stock Solutions**

A 1000 ppm NNG stock solution is prepared by weighing  $0.10000 \pm 0.00001$  g of analytical grade NNG into a 100 mL volumetric flask and diluting to  $100.00 \pm 1.00$  deionized water.

A 5.000 ppm NNG working stock solution is made by weighing  $0.5000 \pm 0.0010$  g of the 1000 ppm nitrite stock solution into a 100 mL volumetric flask and diluting to  $100.00 \pm 1.00$  g. This working solution should be made fresh weekly along with all standard solutions.

### **Standards**

Standards in the range of 10 – 200 ppb NNG are prepared by the appropriate dilutions of the 5.000 ppm nitrite standard into  $50.00 \pm 1.00$  g deionized water.

Weight 5.000 ppm NNG Stock Solution	NNG concentration
0.1000 g	10 ppb
0.2000 g	20 ppb
0.5000 g	50 ppb
1.0000 g	100 ppb
2.0000 g	200 ppb

### **Calibration**

A series of external standards in the range of 0 – 200 ppb NNG are prepared and analyzed. The height of the NNG peak is measured and a calibration curve is prepared.

## **10. Procedure**

### **Cleaning of Equipment**

All glassware and nalgene bottles must be rinsed with the sulfamic acid solution and then with copious amounts of deionized water. This should remove any trace amounts of nitrite and any other contaminants.

### **Collection and Shipping of Samples**

Samples should be collected in brown nalgene bottles that have been washed with sulfamic acid and rinsed with deionized water. Failure to use sulfamic acid and deionized water washed bottles will compromise sample integrity.

### **Sample Preparation**

Samples and standards are prepared and diluted on a weight per weight basis. Measure and record weights to the proper significant figure.

## **Wetcake**

Weigh out  $0.4000 \pm 0.100$  g glyphosate wetcake into a clean sample bottle. Add 0.85 mL 2.5 N NaOH/0.3% hydrogen peroxide and dilute to  $10.00 \pm$  g with deionized water.

## **Analysis of prepared Samples**

Start all reagents and mobile phase flowing. Once a good baseline is obtained, start injections, alternating samples and standards throughout the analysis. Measure the height of the NNG peak for standards and samples. Prepare a calibration curve. For wetcake samples find the amount of NNG in the sample from the calibration curve and the equation in section 11.

## **11. Calculations**

The concentration of NNG in the sample is calculated using the weight of the sample (sample weight), the total weight of the prepared sample (total weight), and the amount of NNG injected which is found using the calibration curve (ppb NNG injection). The equation is :

$$\text{ppb NNG} = \frac{(\text{total weight})(\text{ppb NNG injection})}{(\text{sample weight})}$$

## **12. Discussion**

The retention of NNG onto the column is reduced over a period of time. The amount of salt in the mobile phase can be reduced to maintain the same retention time. A precolumn filter and/or guard column may also help to increase column lifetime.

Analysis of other types of samples for NNG may also be possible. Spike recoveries will help to determine if quantitation is correct. It is also recommended that about 10% of the samples analyzed be spiked with NNG so that good quality control can be insured.

## **13. References**

C. A. Pelezo, Fayetteville Standard Analytical Method Number F-62-84. Revision 1, July, 1984.

D.J. Augustin and F.Y. Triebe. Monsanto Analytical Method, ANMET Number 80, March, 1984.