Food and Agriculture Organization
of the United Nations

FAO SPECIFICATIONS

FOR PLANT PROTECTION PRODUCTS

CARBOSULFAN (AGP:CP/315)

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

Rome, 1995

CONTENTS

DISCLAIMER [1]

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

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

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

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.

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

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

INTRODUCTION TO FAO SPECIFICATIONS DEVELOPED UNDER THE OLD PROCEDURE

Between 1975 and 2000, FAO published booklets of specifications for technical materials and related formulations of plant protection products. Revisions of, and additions to, already published specifications will be issued when necessary. However, all changes and revisions of FAO specifications are now subject to the new procedure described in the *Manual on the development and use of FAO and WHO Specifications for Plant Protection Products*, FAO Plant Production and Protection Paper No. 173, Rome 2002 (*Revised First Edition* available only on the FAO home page of the Internet at: http://www.fao.org/pest-and-pesticide-management/en/)

FAO specifications developed under the old procedure are based on the requirements defined in the Fourth Edition of the *Manual on the development and use of FAO specifications for plant protection products*, Plant Production and Protection Paper No. 128, Rome 1995.

This manual contained detailed definitions and other essential background information on basic procedures and technical principles adopted by the group on Pesticide Specifications of the FAO Panel of Experts on Pesticide Specifications, Registration Requirements, Application Standards and Prior Informed Consent, such as:

1. Categories of Specifications (Section 3.1 of the Manual)

   FAO Tentative Specifications (Code 'S/T', formerly 'TS') are those which have been recommended by FAO as preliminary specifications and which are based on minimum requirements. The methods of analysis cited are normally supplied by the manufacturer or may already have been published or be the subject of collaborative work.

   FAO Provisional Specifications [Code 'S/P', formerly ('S')] are those for which more evidence of the necessary parameters is available and where some collaborative study of the methods of analysis has been carried out.

   FAO (full) Specifications (Code 'S/F', formerly 'S').
   Specifications that have all necessary requirements together with CIPAC (full) methods, or other collaboratively studied (proven) methods.[2, 3]

   Wherever possible, standards for apparatus and common names for pesticides are those approved by the International Organization for Standardization (ISO).

2. Expression of active ingredient content (Section 4.2.5 of the Manual)

   - for solids, liquid technical materials, volatile liquids (of maximum boiling point 50°C) and viscous liquids (with minimum kinematic viscosity of 1×10^3 m^2/s at 20°C) the FAO Specification shall be based on expression of the content as g/kg;

- for all other liquids the active ingredient content of the product shall be declared in terms of g/kg *or* g/l at 20°C. If the customer requires both g/kg *and* g/l at 20°C, then in case of dispute the analytical results shall be calculated as g/kg.

3. Tolerance on content (Section 4.2.7 of the Manual)

A declared content of active ingredient must be included in all specifications, and one of the problems immediately arising is the level of tolerance acceptable about the nominal figure. The tolerance is influenced by (a) the reproducibility of the method of analysis, (b) the sampling error and (c) the manufacturing variance.

Allowable variations in analytical results (i.e. tolerances in content of active ingredient) with respect to specific pesticide consignments are intended to cover reasonable variations in the contents of active ingredients. For examples of such tolerances, see the table in Section 4.2.7 of the Manual.

4. Containers/packaging

FAO guidelines are in preparation.

Containers shall comply with pertinent national and international transport and safety regulations.

Technical materials, dustable powders and granules

Containers shall be suitable, clean, dry and as specified, and shall not adversely affect, or be affected by, the contents, but shall adequately protect them against external conditions.

Wettable powders

The product shall be packed in suitable, clean, dry containers as specified in the order. The container shall provide all necessary protection against compaction, atmospheric moisture, loss by vaporization and/or contamination to ensure that the product suffers no deterioration under normal transit and storage conditions.

The product shall be protected by an adequate moisture barrier. This may be a suitable bag of polyethylene or alternative means of giving equal or better protection.

Solutions and emulsifiable concentrates

Containers shall be lined, where necessary, with a suitable material, or the interior surfaces shall be treated to prevent corrosion and/or deterioration of the contents.

Additional information should be given in all specifications where particular pesticides present problems in packaging.

5. Biological information

Phytotoxicity

No test can be specified to cover the possible phytotoxicity of a formulation to all crops. When a crop is not mentioned in the instructions for use, purchasers should check with the supplier that the material is suitable, always provided that such a use is not restricted or legally forbidden.

Wetting of crops

The dilute spray should satisfactorily wet the leaves of the specified crops when used in accordance with the instructions. Test method MT 53.2, CIPAC F, p.162, may be useful.

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[1] *Should national pesticide specifications developed from these approved FAO specifications deviate from them, the National Authority responsible for making such changes is requested to inform the FAO Plant Protection Service of the nature of, and the reasons for, the modifications.*

[2] *Methods of analysis and miscellaneous techniques referred to in these specifications have been developed and adopted by CIPAC (Collaborative International Pesticides Analytical Council Ltd.). See CIPAC Handbooks 1 (1970), 1A (1980), 1B (1983), 1C (1985), D (1988), E (1993), F (1995), G (1995), CIPAC Proceedings 1980 and 1981, obtainable from Black Bear Press Limited, King's Hedges Road, Cambridge CB4 2PQ, England. The page numbers of specific methods are given in parentheses in the specifications. Copies of methods not yet published can be obtained from the FAO Plant Protection Service.*

[3] *Information on standard waters for laboratory evaluation of pesticidal formulations will be found in CIPAC Monograph 1*, Standard Waters and an FAO Survey on Naturally Occurring Waters *(1972), Black Bear Press Limited, King's Hedges Road, Cambridge CB4 2PQ, England.*

SUBMISSION OF DRAFT SPECIFICATIONS TO FAO

Any organization, commercial firm or interested individual is encouraged to submit relevant specifications, or proposals for revision of existing specifications, for pesticide products for consideration and possible adoption by FAO. Correspondence should be addressed to the Pesticide Management Group, Plant Production and Protection Division, FAO, Viale delle Terme di Caracalla, 00153 Rome, Italy.

General guidelines on preparing draft specifications are given in the *Manual on the development and use of FAO and WHO Specifications for Plant Protection Products*, FAO Plant Production and Protection Paper No. 173, Rome 2002 (Revised First Edition available only on the FAO home page of the Internet at: http://www.fao.org/pest-and-pesticide-management/en/).

Specifications which are considered suitable for further processing are assigned priorities and circulated to appropriate organizations and specialists to comment. Comments, together with other relevant information, are then reviewed in detail by the Group on Specifications of the FAO Panel of Experts on Pesticide Specifications, Registration Requirements, Application Standards and Prior Informed Consent. The drafts are converted into FAO Provisional Specifications, or full FAO Specifications.

AGP:CP/315, 1995

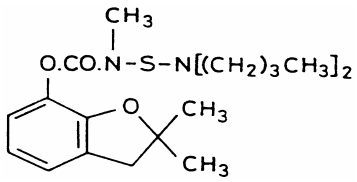
CARBOSULFAN

2,3-dihydro-2,2-dimethylbenzofuran-7-yl (dibutylaminothio)methylcarbamate

INFORMATION

COMMON NAME: carbosulfan

STRUCTURAL FORMULA:



EMPIRICAL FORMULA: $C_{20}H_{32}N_2O_3S$

RMM: 380.5

CAS REGISTRY NUMBER: 55285-14-8

CIPAC CODE NUMBER: 417

CHEMICAL NAMES:

2,3-dihydro-2,2-dimethylbenzofuran-7-yl (dibutylaminothio)methylcarbamate (IUPAC)

2,3-dihydro-2,2-dimethyl-7-benzofuranyl [(dibutylamino)thio]methylcarbamate (CA)

CARBOSULFAN TECHNICAL

FAO Specification 417/TC/S/F (1991)

1. DESCRIPTION

The material shall consist of carbosulfan together with related manufacturing impurities and shall be a brown viscous liquid free from visible extraneous matter and added modifying agents, other than stabilizers.

2. ACTIVE INGREDIENT

2.1 Identity tests (417/TC/M/2, CIPAC E, p.35)

An identity test is required if the identity of the active ingredient is in doubt.

2.2 Carbosulfan (417/TC/M/3, CIPAC E, p.36)

The carbosulfan content shall be declared (not less than 890 g/kg) and, when determined, the content obtained shall not differ from that declared by more than ± 25 g/kg.

3. IMPURITIES

3.1 Carbofuran*

Maximum: 20 g/kg.

3.2 Water (MT 30.1, CIPAC F, p.91)

Maximum: 2 g/kg.

4. PHYSICAL PROPERTIES

4.1 Alkalinity (MT 31, CIPAC F, p.96)

Maximum alkalinity: 0.2 g/kg calculated as NaOH.

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* The analytical method for determination of the relevant impurity is available from the Pesticide Management Group of the FAO Plant Protection Service, or can be downloaded here

CARBOSULFAN EMULSIFIABLE CONCENTRATES

FAO Specification 417/EC/S/F (1991)

1. DESCRIPTION

The material shall consist of technical carbosulfan, complying with the requirements of FAO specification 417/TC/S/F (1991), dissolved in suitable solvents together with any other necessary formulants. It shall be in the form of a stable liquid, free from visible suspended matter and sediment.

2. ACTIVE INGREDIENT

2.1 Identity tests (417/EC/M/2, CIPAC E, p.40)

An identity test is required if the identity of the active ingredient is in doubt.

2.2 Carbosulfan (417/EC/M/3, CIPAC E, p.40)

The carbosulfan content shall be declared (g/kg or g/l at 20°C)1 and, when determined, the content obtained shall not differ from that declared by more than the following amounts.

Declared content	Permitted tolerance
Up to 500 g/kg or g/l	± 5% of the declared content

3. IMPURITIES

3.1 Carbofuran*

Maximum: 2% of the carbosulfan content found under 2.2.

3.2 Water (MT 30.1, CIPAC F, p.91)

Maximum: 2 g/kg.

4. PHYSICAL PROPERTIES

4.1 Alkalinity (MT 31, CIPAC F, p.96)

Maximum alkalinity: 1 g/kg calculated as NaOH.

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* The analytical method for determination of the relevant impurity is available from the Pesticide Management Group of the FAO Plant Protection Service, or can be downloaded here

4.2 Emulsion stability and re-emulsification (MT 173, CIPAC F, p.431. Note 2)

The product, when diluted at 30°C (Notes 3 and 4) with CIPAC Standard Waters A and C, shall comply with the following:

Time after dilution	Limits of stability
0 h	Initial emulsification complete
0.5 h	Minimum: 95%
2.0 h	Minimum: 90%
24 h (Note 5)	Re-emulsification complete
24.5 h (Note 5)	Minimum: 95%

Alternatively, if the buyer requires other CIPAC Standard Waters to be used, then this shall be specified when ordering.

4.3 Flash point (MT 12, CIPAC F, p.31. Note 6)

If required, the flash point of the product shall not be lower than the minimum declared flash point. A closed cup method shall be used and the method stated.

5. STORAGE STABILITY

5.1 Stability at 0°C (MT 39.1, CIPAC F, p.128)

After storage at 0 ± 1°C for 7 days, the volume of solid and/or liquid which separates shall not be more than 0.3 ml.

5.2 Stability at 54°C (MT 46.1.3, CIPAC F, p.150)

After storage at 54 ± 2°C for 14 days, the determined average active ingredient content must not be lower than 97% relative to the determined average content found before storage (Note 7) and the product shall continue to comply with 4.1 and 4.2.

NOTES

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

2. This test will normally be carried out only after the heat stability test 5.2.

3. Unless another temperature is specified.

4. The product should be tested at the highest and lowest rates of use recommended by the supplier.

5. These tests need be carried out only in case of doubt as to the result of the 2-hour emulsion stability test.

6. Attention is drawn to the appropriate national and/or international regulations on the handling and transport of flammable materials.

7. Samples of the product taken before and after the storage stability test should be analysed together after the test to reduce the analytical error.

CARBOSULFAN GRANULES

(For application by mechanical equipment)

FAO Specification 417/GR/S/F (1991)

1. DESCRIPTION

The material shall consist of granules containing technical carbosulfan complying with the requirements of FAO specification 417/TC/S/F (1991) together with suitable carriers and any other necessary formulants. It shall be dry, free from visible extraneous matter and hard lumps, free-flowing, essentially non-dusty and intended for application by machine.

2. ACTIVE INGREDIENT

2.1 Identity tests (417/GR/M/2, CIPAC E, p.41)

An identity test is required if the identity of the active ingredient is in doubt.

2.2 Carbosulfan (417/GR/M/3, CIPAC E, p.41)

The carbosulfan content shall be declared (g/kg) and, when determined, the content obtained shall not differ from that declared by more than the following amount.

Declared content	Permitted tolerance
Up to 100 g/kg	± 10% of the declared content

3. IMPURITIES

3.1 Carbofuran*

Maximum: 2% of the carbosulfan content found under 2.2.

4. PHYSICAL PROPERTIES

4.1 Acidity (MT 31, CIPAC F, p.96)

Maximum acidity: 0.5 g/kg calculated as H_2SO_4.

* The analytical method for determination of the relevant impurity is available from the Pesticide Management Group of the FAO Plant Protection Service, or can be downloaded here

4.2 Apparent density after compaction (MT 159, CIPAC F, p.390)

The apparent density of the product after compaction shall be declared, where required, and shall be not less than 1.5 g/ml.

4.3 Nominal size range (MT 58.2, CIPAC F, p.173)

The nominal size range of the product shall be declared. The ratio of the lower to the upper limit shall not normally exceed 1:4 (Note 1). Not less than 90% of the product shall be within the declared nominal size range.

4.4 Material retained on a 125 µm test sieve (MT 58.2, CIPAC F, p.173. Note 2)

Minimum: 995 g/kg retained on a 125 µm test sieve.

The carbosulfan content of the material retained on the sieve shall not be less than 95% of that found under 2.2 (Note 3).

5. STORAGE STABILITY

5.1 Stability at 54°C (MT 46.1.1, CIPAC F, p.149)

After storage at 54 ± 2°C for 14 days, the determined average active ingredient content must not be lower than 97% relative to the determined average content found before storage (Note 4) and the product shall continue to comply with 4.1, 4.3 and 4.4.

NOTES

1. Higher ratios may increase the risk of segregation which may adversely affect the flow rate. This should be checked with the machine to be used. The purchaser should check that the nominal size range is suitable for his requirements, since the size range may affect the biological activity.

2. This requirement is only valid for granules having a lower limit of the size range of at least 300 µm. For microgranules having a lower limit of less than 300 µm, the mass percent of material passing a 63 µm test sieve shall be declared.

3. For example, if the carbosulfan content of the product determined under 2.2 is 100 g/kg, the proportion of the product retained on the 125 µm test sieve is used in the test, then the amount of active ingredient should be not less than 95% of (0.995 x 10) g, i.e. 9.45 g.

4. Samples of the product taken before and after the storage stability test should be analysed together after the test to reduce the analytical error.

CARBOSULFAN ULTRA LOW VOLUME LIQUIDS

FAO Provisional Specification 417/UL/S/P (1993)

1. DESCRIPTION

The material shall consist of technical carbosulfan, complying with the requirements of FAO specification 417/TC/S/F (1991), together with any necessary formulants. It shall be in the form of a stable liquid, free from visible suspended matter and sediment.

2. ACTIVE INGREDIENT

2.1 Identity tests (417/TC/M/2, CIPAC E, p.35)

An identity test is required if the identity of the active ingredient is in doubt.

2.2 Carbosulfan (417/EC/M/3, CIPAC E, p.40. Note 1)

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

Declared content (g/kg or g/l)	Permitted tolerance
Up to 25	± 15% of the declared content
Above 25 up to 100	± 10% of the declared content
Above 100 up to 250	± 6% of the declared content
Above 250 up to 500	± 5% of the declared content

3. IMPURITIES

3.1 Carbofuran*

Maximum: 2% of the carbosulfan content found under 2.2.

3.2 Water (MT 30.1, CIPAC F, p.91)

Maximum: 2 g/kg.

4. PHYSICAL PROPERTIES

4.1 Alkalinity (MT 31, CIPAC F, p.96)

Maximum alkalinity: 1 g/kg calculated as NaOH.

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* The analytical method for determination of the relevant impurity is available from the Pesticide Management Group of the FAO Plant Protection Service, or can be downloaded here

4.2 Flash point (MT 12, CIPAC F, p.31. Note 3)

The flash point of the product shall not be lower than 22.8°C. A closed cup method shall be used and the method stated.

4.3 Kinematic viscosity range (MT 22, CIPAC F, p.75)

The kinematic viscosity determined at 30°C shall be in the range 1 to 10 mm^2/s.

4.4 Volatility (MT 56.2, CIPAC F, p.170)

The measured volatility of the product shall not exceed 100 g/kg.

5. STORAGE STABILITY

5.1 Stability at 0°C (MT 39.1, CIPAC F, p.128)

After storage at 0 ± 1°C for 7 days, the volume of solid and/or liquid which separates shall not be more than 0.3 ml.

5.2 Stability at 54°C (MT 46.1.3, CIPAC F, p.150. Note 4)

After storage at 54 ± 2°C for 14 days, the determined average active ingredient content must not be lower than 97% relative to the determined average content found before storage (Note 5) and the product shall continue to comply with 4.1 and 4.3.

NOTES

1. The CIPAC method 417/EC/M/3 for EC formulations may be used for the UL formulation.

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

3. Attention is drawn to the appropriate national and/or international regulations on the handling and transport of flammable materials.

4. The samples should be stored in sealed glass ampoules.

5. Samples of the product taken before and after the storage stability test should be analysed together after the test to reduce the analytical error.

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Issued: December 7, 2001

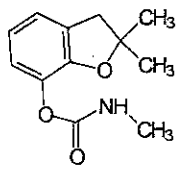
TEST METHOD APG NO. 61A

61A HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF CARBOFURAN AND 7-HYDROXY IMPURITIES IN CARBOSULFAN TECHNICAL

61A.1 INTRODUCTION

This test method describes a high performance liquid chromatographic (HPLC) separation of carbofuran and 7-hydroxy impurities in technical carbosulfan. This method employs external standard calibration techniques utilizing peak area measurements for quantitative determinations. The reversed phase isocratic HPLC mode of fractionation with a Zorbax® Rx-C8 column is utilized.

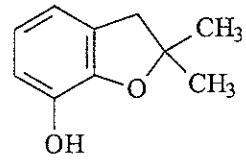
The chemical designation and structure for carbofuran follow:



CA Name: 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate
(CAS No. 1563-66-2)

IUPAC Name: 2,3-dihydro-2,2-dimethylbenzofuran-7-yl-methylcarbamate

The chemical designation and structure for 7-hydroxy follow:



CA Name: 2,3-dihydro-2,2-dimethyl-7-benzofuranol
(CAS No. 1563-38-8)

61A.2 SAFETY CONSIDERATIONS

This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and to determine the applicability of regulatory limitations prior to use. Standard safety practices should be applied at all times during the implementation of this procedure. Exposure to all reagents, samples and standards should be reduced to the lowest possible levels by whatever means are available. Safety practices should be applied when handling all glassware and operating analytical instrumentation. Gloves and disposable lab coats are recommended anytime the standards or samples are transferred between containers. All laboratory personnel involved in this procedure should be familiar with safety data available on all chemicals used.

61A.3 PRECISION AND ACCURACY

In order to determine the variance of this method, we analyzed two weights of a carbosulfan technical sample injected four times each. The relative standard deviation for the analysis of carbofuran in carbosulfan was found to be less than 0.7% in samples containing approximately 1.2% carbofuran. The relative standard deviation for the analysis of 7-hydroxy in carbosulfan was found to be less than 3.0% in samples containing approximately 0.04% 7-hydroxy.

A standard curve over a concentration range of 200 µg carbofuran to 2000 µg carbofuran per mL acetonitrile produced a line with a correlation coefficient of greater than 0.999 (Note 1). A standard curve over a concentration range of 40 µg 7-hydroxy to 160 µg 7-hydroxy per mL acetonitrile produced a line with a correlation coefficient of greater than 0.99 (Note 2).

61A.4 REAGENTS

Carbofuran, Analytical Standard, available from Agricultural Products Group, FMC Corporation, Princeton, NJ

7-hydroxy, Analytical Standard, available from Agricultural Products Group, FMC Corporation, Princeton, NJ

Acetonitrile, J.T. Baker® HPLC Grade, or equivalent

Methanol, J.T. Baker® HPLC Grade, or equivalent

Water, Millipore Organex® Q, or equivalent HPLC Grade water

61A.5 INSTRUMENTATION AND SPECIFICATIONS

Instrument:	Hewlett-Packard (HP) Model 1050 HPLC system, or equivalent
Detector:	HP Model 1050 Diode Array Detector, or equivalent, set at a wavelength of 280 nm
Column:	Zorbax® Rx-C8, 5 µm, 4.6 mm i.d. x 250 mm length
Column Heater:	Perkin-Elmer Model 101 LC column oven, or equivalent, set at 40° C
Injection Volume:	10 µL
Date Reduction:	HP ChemStation®, or equivalent
Semi-micro analytical Balance:	Mettler Model AT250, or equivalent, capable of ± 0.01 mg readability
Mobile Phase:	35% Water / 65% Methanol (A/B)

Analysis Mode: Isocratic (with gradient cleanup)

Time (min)	% A Water	% B Methanol	Flow Rate (mL/min)
0	35	65	1.0
10	35	65	1.0
11	0	100	1.0
26	0	100	1.0
27	35	65	1.0
37	35	65	1.0

Analysis Time: Approximately 37 minutes, including cleanup

Peak Profile: Typical elution times of approximately 4.8 minutes for carbofuran and 5.0 minutes for 7-hydroxy.

61A.6 PREPARATION OF ANALYTICAL STANDARD SOLUTIONS

A stock analytical standard solution containing a mixture of carbofuran and 7-hydroxy is prepared in acetonitrile, and working standards are prepared by appropriately diluting the stock solution with acetonitrile. The working standard solutions should be prepared to cover the concentration range of 20 ug to 2000 ug impurity per mL acetonitrile.

For example:

A primary stock solution of carbofuran is prepared by weighing approximately 100 mg of carbofuran analytical standard, to the nearest 0.01 mg. Acetonitrile is added to achieve a final concentration of approximately 4.0 mg/mL.

A primary stock solution of 7-hydroxy is prepared by weighing approximately 50 mg of 7-hydroxy analytical standard, to the nearest 0.01 mg. Acetonitrile is added to achieve a final concentration of approximately 1.0 mg/mL.

Both stock solutions should be shaken thoroughly until dissolution is complete, and stored under refrigeration at approximately 4°C until needed.

Working standards containing a mixture of carbofuran and 7-hydroxy are prepared by further dilution of the stock solutions. Place the following volumes of stock solution into individual 10 mL volumetric flasks:

Volume of Carbofuran Stock Solution (mL)	Volume of 7-Hydroxy Stock Solution (mL)	Final Dilution Volume (mL)	Final Concentration Carbofuran Standards (ug/mL)	Final Concentration 7-Hydroxy Standards (ug/mL)
0.50	0.20	10	200	20
1.00	0.40	10	400	40
2.00	0.70	10	800	70
4.00	1.00	10	1600	100
5.00	1.50	10	2000	150

Dilute the samples to volume with acetonitrile, and mix thoroughly until dissolution is complete.

61A.7 SAMPLE PREPARATION AND ANALYSIS

The samples are prepared in acetonitrile to give an expected impurity concentration within the ranges set by the working standard solutions.

For example:

Weigh approximately 60 mg of carbosulfan technical sample, to the nearest 0.01 mg. Dilute the sample volumetrically with approximately 10 mL of acetonitrile, and mix thoroughly until dissolution is complete. The resulting sample concentration is approximately 60.0 mg/mL.

Prepare at least two replicate weights of each sample. All sample solutions should be stored under refrigeration at approximately 4°C until needed.

Fill the autosampler vials with the appropriate working standard and sample solutions and load the autosampler tray. Fill the sample loop and inject the working analytical standard or sample solution onto the HPLC column. Generate a minimum of two chromatograms with area percent reports for each analytical standard or sample solution (Notes 3 and 4).

61A.8 CALCULATIONS

Determine the response factor (RF) of each individual impurity from the standard injections:

$$RF = C_{STD} / A_{STD}$$

Where, C_{STD} = Concentration of the working standard (mg/mL), adjusted for purity

A_{STD} = Area counts for individual impurity peak in the working standard solution

An average response factor (RF_{AVG}) is calculated for all working standard solutions run with the samples.

The weight percent of the carbofuran impurity is calculated for each sample solution injection as follows:

$$Wt\% = [(A_{SPL} \times RF_{AVG}) / C_{SPL}] \times 100$$

C_{SPL} = Concentration of sample solution in mg/mL

A_{SPL} = Area counts for carbofuran peak in the sample solution

RF_{AVG} = Average standard RF for all carbofuran working standard solutions

Similarly, the weight percent of the 7-hydroxy impurity is calculated for each sample solution injection as follows:

$$Wt\% = [(A_{SPL} \times RF_{AVG}) / C_{SPL}] \times 100$$

C_{SPL} = Concentration of sample solution in mg/mL

A_{SPL} = Area counts for 7-hydroxy peak in the sample solution

RF_{AVG} = Average standard RF for all 7-hydroxy working standard solutions

61A.9 NOTES

1. Figure 1 shows the linear regression curve for carbofuran analytical standard solutions.

2. Figure 2 shows the linear regression curve for 7-hydroxy analytical standard solutions.

3. Figure 3 shows a typical HPLC chromatogram for an analytical standard solution containing carbofuran and y-hydroxy.

4. Figure 4 shows a typical HPLC chromatogram for a carbosulfan technical sample solution containing carbofuran and 7-hydroxy impurities.

61A.10 REFERENCES

Reference: Springborn Laboratories, Inc., Environmental Sciences Division; Carbosulfan Product Chemistry Study Number 282-0194-6116-890

Method Prepared By:	Barbara L. Renze Barbara L. Renze	12-7-01 Date
Reviewed By:	Edward J. Kikta, Jr., Ph.D	12/7/01 Date
Approved By:	Jun H. Chang, Ph.D.	12/10/01 Date

FIGURE 1

Linear Regression Curve for Carbofuran
Analytical Working Standard Solutions

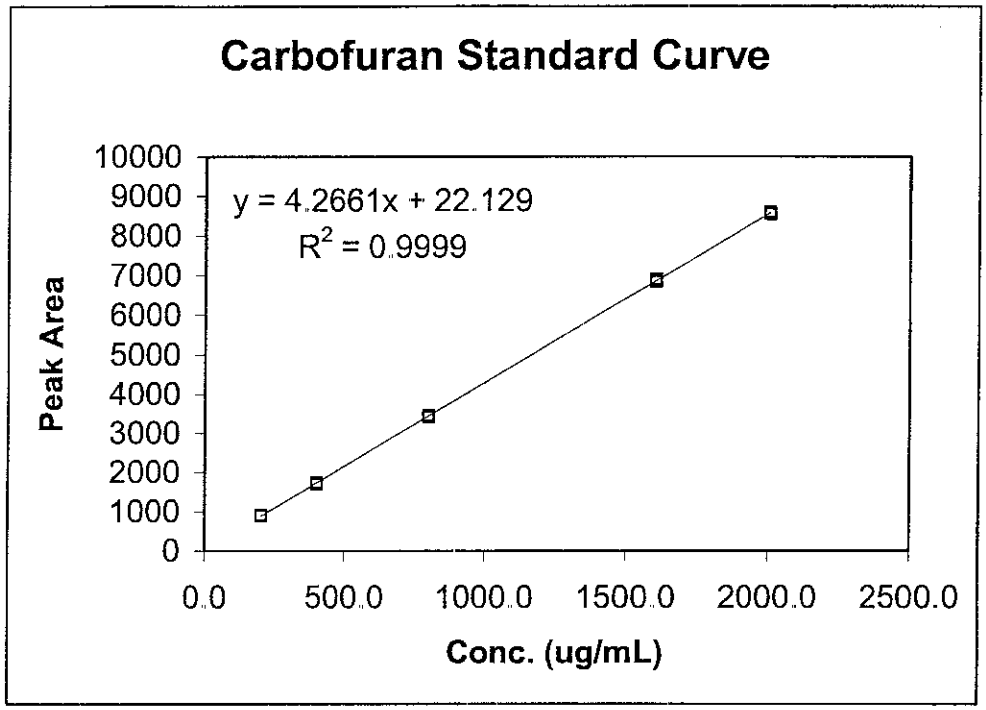


FIGURE 2

Linear Regression Curve for 7-Hydroxy
Analytical Working Standard Solutions

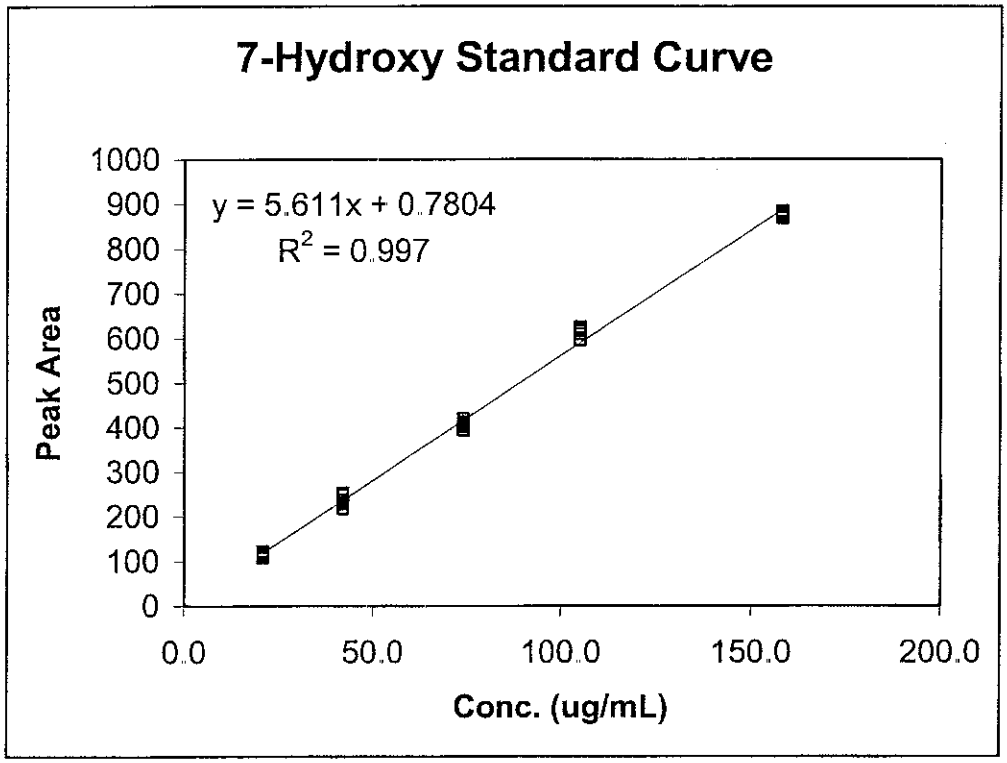


FIGURE 3

Typical HPLC Chromatogram for an Analytical Standard Solution Containing Carbofuran and 7-Hydroxy

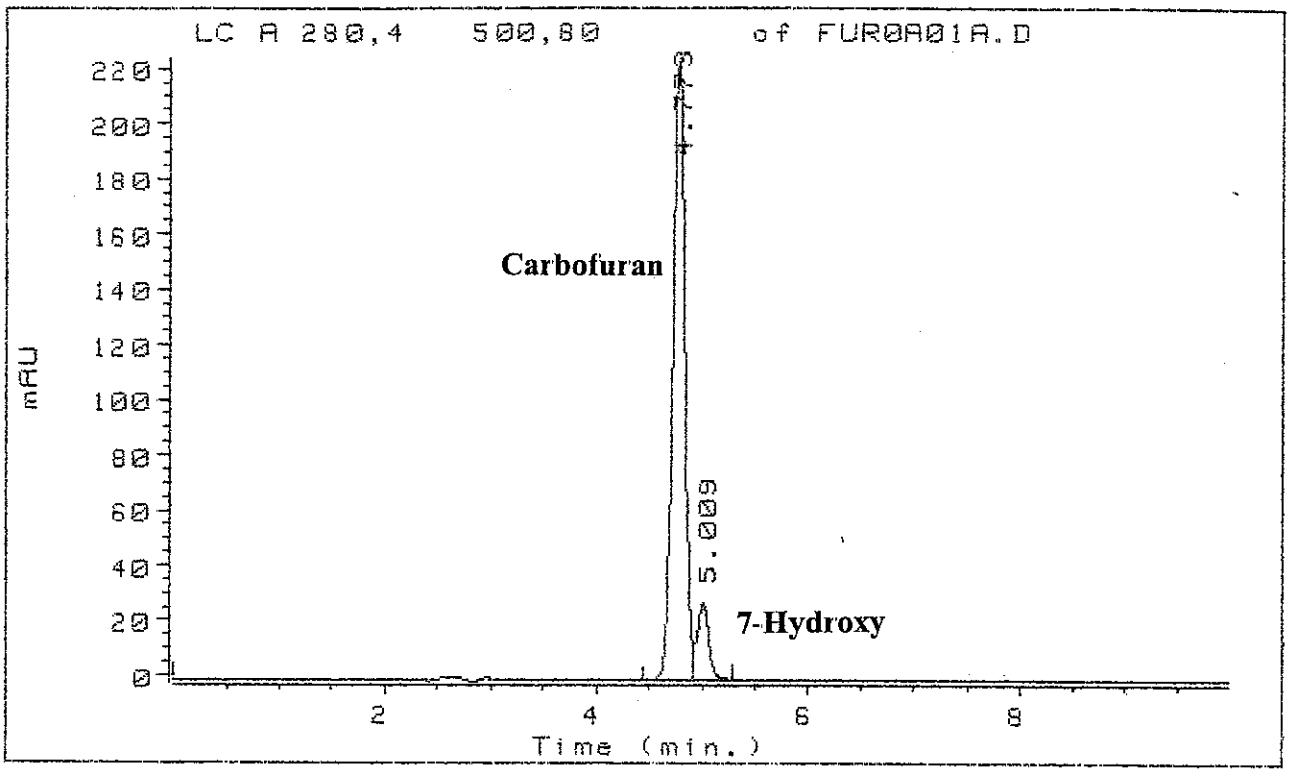


FIGURE 4

Typical HPLC Chromatogram for a Carbosulfan Technical Sample Solution