

# The Goodyear Tire & Rubber Company

Akron, Ohio 44316 - 0001

June 19, 1996

(A)

## Certified Mail

OPPT Document Processing Center (TS-790)  
Attn: Section 8(e) Coordinator  
Office of Pollution Prevention and Toxics (OPPT)  
U. S. Environmental Protection Agency  
401 M Street, SW  
Washington, DC 20460

~~CONFIDENTIAL~~

**Contains No CBI**

96 JUN 25 11:11:30  
RECEIVED  
OPPT NCIC

Dear Ladies/Gentlemen:

Subject: TSCA Section 8(e) Notice



8EHQ-96-13675

This submittal does not contain Confidential Business Information.

The Goodyear Tire & Rubber Company submits this notice in accordance with Section 8(e) of the Toxic Substances Control Act (TSCA) and EPA's statement of Interpretation and Enforcement Policy, 43 Fed. Reg. 1110 (March 16, 1978).

Goodyear is advising EPA of the results of a battery of studies to examine the potential toxicity of a rubber antioxidant in the environment. The identity of the test material is as follows:

Chemical Abstract Name: Propanoic acid, 3-(dodecylthio)-, oxybis (2, 1-ethanediyl) ester

Chemical Abstract Number: 64253-30-1



88960000155

Submitted studies are as follows:

WINGSTAY SN-1: 48- Hour Flow-Through Acute Toxicity Test with the Cladoceran (Daphnia magna), Wildlife International Ltd. Project No: 414A-102, March 26, 1996.

WINGSTAY SN-1: A 72-Hour Toxicity Test with the Freshwater Alga (Selenastrum capricornutum), Wildlife International Ltd. Project Number: 414A-101, March 27, 1996.

WINGSTAY SN-1: Biodegradability (CO<sub>2</sub> Evolution Test), SLI Report No. 96-3-6432, Springborn Laboratories, Inc., May 30, 1996.

WINGSTAY SN-1: Determination of the n-Octanol/Water Partition Coefficient, SLI Report #95-4-5828, Springborn Laboratories, Inc., May 20, 1996.

96 JUN 22 AM 10:08  
RECEIVED  
OPPT NCIC

KS  
RECEIVED  
7/23/96

WINGSTAY SN-1: Determination of Water Solubility, SLI Report #95-5-5893, Springborn Laboratories, Inc. May 30, 1996.

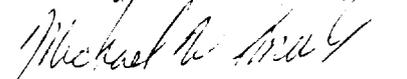
In this battery of tests, the results indicated that the test chemical had a 48-hour  $EC_{50}$  value for daphnid mortality of  $97\mu\text{g/L}$  and a 72-hour  $EC_{50}$  value for Selenastrum capricornutum of  $65\mu\text{g/L}$ . Further, the n-Octanol/Water Partition coefficient for the test chemical was concluded to be greater than  $10^5$ .

Goodyear is making this submission under 8(e) because the reported data indicate non-trivial effects and the possibility of bioaccumulation. However, it is unclear, given the additional data that the test chemical is extremely insoluble in water and is classified as readily biodegradable, as to whether or not such data require reporting under TSCA 8(e). Note that the two reported  $EC_{50}$  values are greater than the water solubility of the test compound, i.e.,  $47\mu\text{g/L}$  at  $20^\circ\text{C}$ . The fact that the test compound would not be soluble in the aquatic environment at the  $EC_{50}$  concentrations repudiates the relevancy of these experimental  $EC_{50}$  values and suggests that little to no actual toxicity would exist for these test organisms.

Goodyear would appreciate comments regarding whether or not this notice was required to be submitted. Please address comments and any questions to the following:

Michael W Smith  
The Goodyear Tire & Rubber Company  
Department 100D  
1144 East Market Street  
Akron, Ohio 44316-0001  
Telephone: (330) 796-2362

Sincerely,



Michael W. Smith  
Section Manager, Chemical Information  
Systems & Regulatory Affairs

MWS:jh  
s6m6a19

Attachments (5)

WINGSTAY SN-1:  
A 48-HOUR FLOW-THROUGH ACUTE TOXICITY TEST  
WITH THE CLADOCERAN (*Daphnia magna*)

FINAL REPORT

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 414A-102

OECD GUIDELINE 202

AUTHORS:

William C. Graves  
James P. Swigert, Ph.D.

STUDY INITIATION DATE: January 12, 1995

STUDY COMPLETION DATE: March 26, 1996

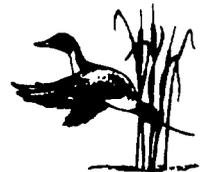
Submitted to

The Goodyear Tire & Rubber Company  
142 Goodyear Boulevard  
Akron, Ohio 44305



**WILDLIFE INTERNATIONAL LTD.**

8598 Commerce Drive  
Easton, Maryland 21601  
(410) 822-8600



Page 1 of 43

80 JUN -2 1996  
RECEIVED  
DEPT. NCIC

RECEIVED  
JUN 2 1996

**GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT**

SPONSOR: The Goodyear Tire & Rubber Company

TITLE: WINGSTAY SN-1: A 48-Hour Flow-Through Acute Toxicity Test with the Cladoceran (*Daphnia magna*)

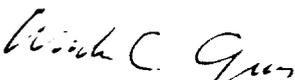
WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 414A-102

STUDY COMPLETION March 26, 1996

This study was conducted to conform with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 160, 17 August 1989; OECD, ISBN 92-84-12367-9, Paris 1982; and Japan MAFF (59 NohSan, Notification No. 3850, Agricultural Production Bureau), with the following exception:

The test substance characterization and stability under storage conditions at the testing site was not in compliance with Good Laboratory Practice Standards. The reference substance characterization was not in compliance with Good Laboratory Practice Standards.

STUDY DIRECTOR:

  
\_\_\_\_\_  
William C Graves  
Senior Aquatic Biologist

3-26-96  
\_\_\_\_\_  
DATE

SPONSOR APPROVAL:

\_\_\_\_\_  
Sponsor

\_\_\_\_\_  
DATE

\_\_\_\_\_  
Applicant/Submitter

\_\_\_\_\_  
DATE

- 3 -

## QUALITY ASSURANCE STATEMENT

This study was examined for conformance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 160, 17 August 1989; OECD, ISBN 92-84-12367-9, Paris 1982; and Japan MAFF (59 NohSan, Notification No. 3850, Agricultural Production Bureau). The dates of all inspections and audits and the dates that any findings were reported to the Study Director and Laboratory Management were as follows:

ACTIVITY	DATE CONDUCTED	DATE REPORTED TO:	
		STUDY DIRECTOR:	MANAGEMENT:
Test substance preparation	August 25, 1995	August 25, 1995	August 30, 1995
Test initiation and analytical sampling	August 29, 1995	August 29, 1995	September 1, 1995
Water chemistry measurements	August 30, 1995	August 30, 1995	September 1, 1995
Matrix fortification and sample preparation	August 30, 1995	August 31, 1995	September 5, 1995
Analytical instrument set-up	August 31, 1995	August 31, 1995	September 5, 1995
Biological Data and Draft Report	October 2-3, 1995	October 3, 1995	October 6, 1995
Analytical Data and Draft Report	October 3-4, 1995	October 5, 1995	October 13, 1995
Final Report	March 25, 1996	March 25, 1996	March 26, 1996

*Kimberly A. Hoxter*  
 \_\_\_\_\_  
 Kimberly A. Hoxter  
 Quality Assurance Representative

*3-26-96*  
 \_\_\_\_\_  
 DATE

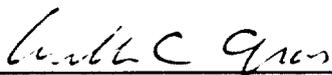
**REPORT APPROVAL**

SPONSOR: The Goodyear Tire & Rubber Company

TITLE: WINGSTAY SN-1: A 48-Hour Flow-Through Acute Toxicity Test with the Cladoceran (*Daphnia magna*)

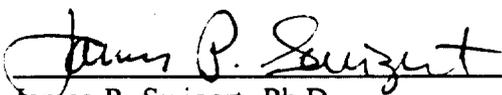
WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 414A-102

STUDY DIRECTOR:

  
\_\_\_\_\_  
William C. Graves  
Senior Aquatic Biologist

3-26-96  
\_\_\_\_\_  
DATE

MANAGEMENT:

  
\_\_\_\_\_  
James P. Swigert, Ph.D.  
Manager, Aquatic Toxicology

3/26/96  
\_\_\_\_\_  
DATE

**TABLE OF CONTENTS**

Title/Cover Page .....	1
Good Laboratory Practice Compliance Statement .....	2
Quality Assurance Statement .....	3
Report Approval .....	4
Table of Contents .....	5
Summary .....	7
Introduction .....	8
Objective .....	8
Experimental Design .....	8
Materials and Methods .....	9
Results and Discussion .....	14
Conclusion .....	15
References .....	16

**TABLES**

Table 1 - Summary of Analytical Chemistry Data .....	17
Table 2 - Temperature, Dissolved Oxygen and pH of Water in the Test Chambers .....	18
Table 3 - Cumulative Percent Mortality and Treatment-Related Effects .....	19
Table 4 - EC50 Values .....	20

**TABLE OF CONTENTS**

- Continued -

**APPENDICES**

Appendix I - Specific Conductance, Hardness, Alkalinity and pH of Well Water Measured During the 4-Week Period Immediately Preceding the Test . . .	21
Appendix II - Analyses of Pesticides, Organics, Metals and Other Inorganics Analyzed in Wildlife International Ltd. Well Water . . . . .	22
Appendix III - The Analysis of WINGSTAY SN-1 in Freshwater in Support of Wildlife International Ltd. Project No.: 414A-102 . . . . .	24
Appendix IV - Changes to Protocol . . . . .	42
Appendix V - Personnel Involved in the Study . . . . .	43

- 7 -

## SUMMARY

SPONSOR:	The Goodyear Tire & Rubber Company
CONTACT:	Mr. Richard Serva
LOCATION OF STUDY, RAW DATA AND FINAL REPORT:	Wildlife International Ltd. Easton, Maryland 21601

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER:	414A-102
TEST SUBSTANCE:	WINGSTAY SN-1 (diester of 3-(dodecylthio) propionic acid and tetraethylene glycol; CAS No. 64253-30-1)
STUDY:	WINGSTAY SN-1: A 48-Hour Flow-Through Acute Toxicity Test with the Cladoceran ( <i>Daphnia magna</i> )
MEAN MEASURED TEST CONCENTRATIONS:	Negative Control, Solvent Control, 0.035, 0.068, 0.10, 0.14 and 0.23 mg/L
TEST DATES:	Experimental Start - August 29, 1995 Biological Termination - August 31, 1995 Experimental Termination - August 31, 1995
LENGTH OF TEST:	48 Hours

TEST ORGANISM:	Neonate Cladocerans ( <i>Daphnia magna</i> )
SOURCE OF TEST ORGANISMS:	Wildlife International Ltd. Cultures Easton, Maryland 21601
AGE OF TEST ORGANISMS:	< 24 hours at test initiation

48-HOUR EC50:	0.097 mg/L
95% CONFIDENCE LIMITS:	0.082 and 0.11 mg/L
NO MORTALITY/IMMOBILITY CONCENTRATION:	<0.035 mg/L
NO-OBSERVED-EFFECT- CONCENTRATION:	<0.035 mg/L

- 8 -

## INTRODUCTION

This study was conducted by Wildlife International Ltd. for The Goodyear Tire & Rubber Company at the Wildlife International Ltd. aquatic toxicology facility in Easton, Maryland. The test was conducted from August 29, 1995 to August 31, 1995. Raw data generated by Wildlife International Ltd. and a copy of the final report are filed under Project Number 414A-102 in archives located on the Wildlife International Ltd. site.

## OBJECTIVE

The objective of this study was to evaluate the acute toxicity of WINGSTAY SN-1 to the cladoceran (*Daphnia magna*) during a 48-hour exposure period under flow-through test conditions.

## EXPERIMENTAL DESIGN

Daphnids were exposed to a geometric series of five test concentrations, a solvent control and a negative (well water) control. Two replicate test chambers were maintained in each treatment and control group, with 10 daphnids in each test chamber. Nominal test concentrations were selected in consultation with the Sponsor, and were based upon results of an exploratory range finding toxicity test. Nominal test concentrations selected were 0.052, 0.086, 0.14, 0.24 and 0.40 mg/L. Mean measured test concentrations were determined from samples of test water collected from alternate replicates of each treatment and control group at the beginning of the test, at 24 hours, and at test termination.

Delivery of the test substance was initiated approximately four days prior to the introduction of the daphnids to the test water in order to achieve equilibrium of the test substance in the test chambers. Daphnids were impartially assigned to exposure chambers at test initiation. Observations of mortality/immobility and other clinical signs were made approximately 6, 24 and

48 hours after test initiation. Cumulative percent mortality and immobility observed in the treatment groups were used to determine EC50 values at 24 and 48 hours. The no-observed-effect-concentration (NOEC) and no mortality/immobility concentration were estimated by examination of the mortality, immobility and clinical observation data.

### MATERIALS AND METHODS

The study was conducted according to the procedures outlined in the protocol, WINGSTAY SN-1: A 48-Hour Flow-Through Acute Toxicity Test with the Cladoceran (*Daphnia magna*). The protocol was based on procedures outlined in OECD Guideline for Testing of Chemicals, 202: *Daphnia* sp. *Acute Immobilization Test (24-Hour) and Reproduction Test* (1), and *ASTM Standard E729-88 Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians* (2).

#### Test Substance

The test substance was received from the Goodyear Tire & Rubber Company on December 13, 1994 and was assigned Wildlife International Ltd. identification number WIL #3080 upon receipt. The test substance was an off white waxy solid, identified on the label as Wingstay SN-1; Lot # 130893, Notebook # 10024-64-3. Information provided by the Sponsor indicated that the solubility limit of the test substance in water was ~ 46.7 µg/L at 20°C. The test substance was stored at ambient room temperature.

#### Preparation of Test Concentrations

One stock solution was prepared for each of the five concentrations tested. The first stock solution was prepared by dissolving Wingstay SN-1 in acetone (Burdick & Jackson Lot No. BK196) at a concentration of 4.0 mg Wingstay SN-1/mL. The stock solution was inverted several times to aid solubilization of Wingstay SN-1. Aliquots of the stock solution were diluted with acetone to prepare four additional stock solutions at concentrations of 2.4, 1.4, 0.86, and 0.52 mg/mL. Stock solutions were prepared one time during the test period. The five stocks

were injected into the diluter mixing chambers where they were mixed with well water to achieve the desired test concentrations. The resultant test concentrations were not adjusted for purity of the active ingredient in the test substance. The solvent concentration in the treatment and solvent control groups was 0.10 mL/L.

### Test Organism

The cladoceran, *Daphnia magna*, was selected as the test species for this study. Daphnids are representative of an important group of aquatic invertebrates and were selected for use in the test based upon past history of use and ease of culturing in the laboratory. Daphnid neonates used in the test were less than 24-hours old and were obtained from cultures maintained by Wildlife International Ltd., Easton, Maryland.

Adult daphnids were cultured at approximately the same temperature as used during the test in Wildlife International Ltd. well water supplemented with selenium. Daphnids in the cultures were held for 19 days prior to collection of the juveniles for testing. The adults showed no signs of disease or stress during the holding period. During the 14-day holding period preceding the test, water temperatures ranged from 21.0 to 21.9°C. The pH of the water ranged from 8.3 to 8.4, and dissolved oxygen ranged from 7.8 to 8.6 mg/L. Instrumentation used for water measurements are described in the *Environmental Conditions* section of this report.

Neonate daphnids were obtained for testing from individual adult daphnids. The progeny from eight adults were used in the test. At test initiation, the juvenile daphnids were collected from the cultures and placed in small containers. The daphnids were then transferred to the test chambers. Daphnids in the cultures were fed a mixture of yeast, Cerophyll®, and trout chow, as well as suspension of the freshwater green alga, *Selenastrum capricornutum*. The adults were fed prior to test initiation, but neonates were not fed during the test.

### Test Apparatus

A continuous-flow diluter was used to deliver each concentration of the test substance, a solvent control, and a negative (well water) control. Syringe pumps (Harvard Apparatus Model No. 22) were used to deliver the five test substance stock solutions and the solvent for the solvent control into mixing chambers assigned to each treatment and the solvent control. The stock solutions were diluted with well water in the mixing chambers in order to obtain the desired test concentrations. The flow of dilution water to the mixing chambers was controlled by rotameters. Rotameters were calibrated prior to test initiation. The flow of test water from each mixing chamber was split and allowed to flow into replicate test chambers. The proportion of test water that was split into each replicate was checked prior to the test to ensure that flow rates varied by no more than  $\pm 10\%$  of the mean for the two replicates.

The diluter was adjusted so that each test chamber received approximately 13 volume additions of test water every 24 hours. The delivery pumps were calibrated before the test, and the general operation of the diluter was checked visually at least two times per day during the test and at least once at the beginning and end of the test.

Test compartments were constructed from 300-mL glass beakers approximately 6.5 cm in diameter and 12 cm in height. Nylon screen was attached to an opening on each side of the test compartments to allow water to flow in and out of the test compartments. The beakers were suspended in Teflon®-lined, 8-L polyethylene test chambers filled with approximately 6.5 L of test water. The depth of the test water in each test compartment was approximately 6.9 cm, whereas the depth in each test chamber was approximately 18 cm. Test chambers were impartially positioned in a temperature-controlled water bath designed to maintain a temperature of  $20 \pm 1^\circ\text{C}$ . The water bath was enclosed in a plexiglass ventilation hood in order to minimize potential for cross-contamination. Test chambers were labelled with the project number, test concentration and replicate.

### Dilution Water

The water used for culturing and testing was freshwater obtained from a well 45 meters deep located on the Wildlife International Ltd. site. The well water is characterized as moderately-hard water. The specific conductance, hardness, alkalinity, and pH of the well water during the four-week period immediately preceding the test are presented in Appendix I.

The well water was passed through a sand filter to remove particles greater than approximately 25  $\mu\text{m}$ , and pumped into a 37,800-L storage tank where the water was aerated with spray nozzles. Prior to delivery to the diluter system, the water again was filtered to remove microorganisms and particles. The results of periodic analyses performed to measure the concentrations of selected contaminants in well water used by Wildlife International Ltd. are presented in Appendix II.

### Environmental Conditions

Lighting used to illuminate the cultures and test chambers during culturing and testing was provided by fluorescent tubes that emitted wavelengths similar to natural sunlight (Colortone® 50). A photoperiod of 16 hours of light and 8 hours of darkness was controlled with an automatic timer. A 30-minute transition period of low light intensity was provided when lights went on and off to avoid sudden changes in lighting. Light intensity prior to the test was approximately 368 lux at the surface of the water.

Temperature was measured in each test chamber at the beginning and end of the test using a hand-held thermometer. Temperature also was measured continuously in one negative control replicate using a Fulscope ER/C Recorder. The target test temperature during the study was  $20 \pm 1^\circ\text{C}$ . The pH and dissolved oxygen content of the water in alternate replicate test chambers of each treatment and control group were measured at 24-hour intervals. Hardness, alkalinity and specific conductance were measured in the dilution water at test initiation.

Measurements of pH were made using a Fisher Accumet Model 915 pH meter, and dissolved oxygen was measured using a Yellow Springs Instrument Model 51B dissolved oxygen meter. Specific conductance was measured using a Yellow Springs Instrument Model 33 Salinity-Conductivity-Temperature meter. Hardness and alkalinity measurements were made by titration in accordance with *Standard Methods for the Examination of Water and Wastewater* (3).

### Observations

Observations of mortality, immobilization and clinical signs of toxicity were made at approximately 6, 24, and 48 hours. Immobilization is defined as a lack of movement except for minor spontaneous random movement of the appendages.

### Statistical Analyses

The data were analyzed using the computer program of C. E. Stephan (4). The program was designed to estimate the EC50 value and the 95% confidence interval by probit analysis, the moving average method, or binomial probability with nonlinear interpolation (5, 6, 7). In this study, the 24-hour EC50 was estimated from the mortality/immobility data, while the 48-hour EC50 was determined using the probit method. The no-observed-effect-concentration and no mortality/immobility concentration were estimated by inspection of the mortality, immobility and clinical observation data.

### Analytical Chemistry

Samples were collected from one replicate test chamber in each treatment and control group at 0, 24, and 48 hours to measure concentrations of the test substance. Samples were also collected from the two lowest and the highest treatment groups prior to test initiation to verify diluter equilibration. The samples were collected in glass vials and analyzed without storage. Analytical procedures used in the analysis of the samples are provided in Appendix III.

## RESULTS AND DISCUSSION

### Measurement of Test Concentrations

Results of analyses to measure concentrations of Wingstay SN-1 in water samples collected during the test are presented in Table 1 and in the analytical chemistry report (Appendix III). Nominal concentrations selected for use in this study were 0.052, 0.086, 0.14, 0.24, and 0.40 mg/L. Although information provided by the Sponsor indicated that these levels were all greater than the solubility of Wingstay SN-1 in water (46.7 µg/L), all test solutions appeared clear and colorless with no visible signs of precipitate. Samples collected on Day 0 had measured values that ranged from 58 to 76% of nominal values. Measured values for samples taken at 24 and 48 hours ranged from 48 to 71 and 58 to 108% of nominal, respectively. When measured concentrations of samples collected at initiation, 24 hours, and at test termination were averaged, the mean measured concentrations for the study were 0.035, 0.068, 0.10, 0.14, and 0.23 mg/L. Mean measured concentrations were used in the calculations of EC50 values.

### Observations and Measurements

Measurements of temperature, dissolved oxygen, and pH are presented in Table 2. Water temperatures were within the  $20 \pm 1^\circ\text{C}$  range established for the test. Dissolved oxygen concentrations exceeded 93% of saturation throughout the test. Measurements of water pH ranged from 8.3 to 8.5.

Daily observations of mortality and other signs of toxicity observed during the test are shown in Table 3. Daphnids in the negative control group appeared healthy and normal throughout the test. One daphnid in the solvent control group and 0.035 mg/L treatment group died during the study and several of the remaining daphnids were observed floating. Once resubmerged, all the floating daphnids appeared normal. All daphnids in the 0.068 mg/L treatment group appeared normal within 24 hours of test initiation with only one daphnid observed to be floating. By test termination, two daphnids in the treatment group had died and one floating daphnid appeared lethargic. Eleven of the remaining seventeen daphnids were

- 15 -

observed to be floating, but once resubmerged, appeared normal. Six daphnids appeared normal at test termination. One daphnid in the 0.10 mg/L treatment group died and one daphnid was observed floating (and appeared normal) within 24 hours of test initiation. All remaining daphnids appeared normal. By test termination, twelve daphnids in the group had died and several daphnids were observed to be floating. Once resubmerged, all daphnids appeared normal. Mortality in the 0.14 mg/L treatment group was 10% within 24 hours of test initiation and two of the surviving daphnids appeared to be lethargic. By test termination, 16 (80%) of the daphnids had died and three floating daphnids appeared lethargic. One floating daphnid appeared normal. Mortality in the 0.23 mg/L treatment group, the highest concentration tested, was 45% within 24 hours of test initiation. By test termination, 19 (95%) of the daphnids in the treatment group had died and the surviving daphnid appeared to be lethargic. EC50 values and 95% confidence limits at 24 and 48 hours were calculated from the mortality/immobility data, and are shown in Table 4.

### CONCLUSION

The 48-hour EC50 value for daphnids exposed to Wingstay SN-1 was 0.097 mg/L. The 95% confidence limits were 0.082 and 0.11 mg/L, and the slope of the concentration-response curve was 4.7. The 48-hour no-observed-effect-concentration and no mortality/immobility concentration, determined by an examination of the mortality/immobility and observations data, was <0.035 mg/L, the lowest concentration tested.

## REFERENCES

- 1 Organization for Economic Cooperation and Development, 1984. *Daphnid sp. Acute Immobilization Test (24-Hour) and Reproduction Test.*, OECD Guideline for Testing of Chemicals. Guideline 202. Paris.
- 2 ASTM Standard E729-88. 1988. *Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians*, American Society for Testing and Materials.
- 3 APHA, AWWA, WPCF. 1985. *Standard Methods for the Examination of Water and Wastewater*. 16th Edition, American Public Health Association. American Water Works Association. Water Pollution Control Federation, New York.
- 4 Stephan, C.E. 1978. U.S. EPA, Environmental Research Laboratory, Duluth, Minnesota. Personal communication.
- 5 Thompson, W.R. 1947. *Bacteriological Reviews*, Vol. II, No. 2: 115-145.
- 6 Stephan, C.E. 1977. Methods for Calculating an LC50, Pages 65-84 in *Aquatic Toxicology and Hazard Evaluations*, American Society for Testing and Materials. Publication Number STP 634. Philadelphia, PA.
- 7 Finney, D.J. 1971. *Statistical Methods in Biological Assay*, second edition. Griffin Press, London.

- 17 -

Table 1

## Summary of Analytical Chemistry Data

Sponsor:	The Goodyear Tire & Rubber Company				
Test Substance:	WINGSTAY SN-1				
Test Organism:	Cladoceran, <i>Daphnia magna</i>				
Dilution Water:	Well Water				
Nominal Test Concentration (mg/L)	Replicate	Sampling Time (hrs)	Measured Concentration <sup>1</sup> (mg/L)	Mean Measured Concentration (mg/L)	Percent of Nominal
Negative Control	A	0	<LOQ <sup>2</sup>	--	--
	B	24	<LOQ		
	A	48	<LOQ		
Solvent Control	A	0	<LOQ	--	--
	B	24	<LOQ		
	A	48	<LOQ		
0.052	A	0	0.039 <sup>3</sup>	0.035	67
	B	24	0.025 <sup>3</sup>		
	A	48	0.042		
0.086	A	0	0.0512 <sup>3</sup>	0.068	79
	B	24	0.0610		
	A	48	0.0928		
0.14	A	0	0.106	0.10	71
	B	24	0.0993		
	A	48	0.104		
0.24	A	0	0.140	0.14	58
	B	24	0.145		
	A	48	0.138		
0.40	A	0	0.235	0.23	58
	B	24	0.216		
	A	48	0.232		

<sup>1</sup> The concentration of Wingstay SN-1 is calculated based upon the measurement of the bis ester of tetraethylene glycol and n-dodecanethiol which is present at 73.1% in Wingstay SN-1.

<sup>2</sup> The limit of quantitation (LOQ) was based upon the lowest standard analyzed concurrently with the samples from the test and the factor of two dilution of the test samples.

<sup>3</sup> Measured value extrapolated from a peak area below the lowest standard.

- 18 -

Table 2

## Temperature, Dissolved Oxygen and pH of Water in the Test Chambers

Sponsor:		The Goodyear Tire & Rubber Company							
Test Substance:		WINGSTAY SN-1							
Test Organism:		Cladoceran, <i>Daphnia magna</i>							
Dilution Water:		Well Water							
Mean Measured Test Concentration (mg/L)	Replicate	0 Hour <sup>1</sup>			24 Hours		48 Hours		
		Temp <sup>2</sup> (°C)	DO <sup>3</sup> (mg/L)	pH	DO (mg/L)	pH	Temp °C	DO (mg/L)	pH
Negative Control	A	20.0	8.7	8.4	--	--	20.0	8.5	8.3
	B	20.0	--	--	8.7	8.4	20.0	--	--
Solvent Control	A	20.0	8.7	8.4	--	--	20.0	8.6	8.3
	B	20.0	--	--	8.6	8.4	20.0	--	--
0.035	A	19.9	8.8	8.4	--	--	19.9	8.7	8.3
	B	19.9	--	--	8.6	8.4	19.9	--	--
0.068	A	19.9	8.6	8.4	--	--	19.9	8.7	8.3
	B	20.0	--	--	8.6	8.4	19.9	--	--
0.10	A	19.8	8.7	8.5	--	--	19.8	8.8	8.4
	B	19.8	--	--	8.6	8.4	19.8	--	--
0.14	A	19.7	8.8	8.5	--	--	19.7	8.7	8.4
	B	19.7	--	--	8.6	8.4	19.7	--	--
0.23	A	19.8	8.9	8.5	--	--	19.7	8.8	8.4
	B	19.7	--	--	8.7	8.4	19.7	--	--

<sup>1</sup> The 0-hour dilution water measurements for hardness, alkalinity and specific conductance were 128 mg/L as CaCO<sub>3</sub>, 176 mg/L as CaCO<sub>3</sub>, and 320 µmhos/cm, respectively.

<sup>2</sup> Temperature measured continuously during the test ranged from approximately 19.5 to 20.5°C.

<sup>3</sup> A dissolved oxygen concentration of 5.4 mg/L represents 60% saturation at 20°C in freshwater.

Table 3

Cumulative Percent Mortality and Treatment-Related Effects<sup>1</sup>

Mean Measured Test Concentration (mg/L.)	Replicate	Daphnia/ Replicate	6 Hours			24 Hours			48 Hours			Percent Immobile and Dead
			Cumulative Dead	Number Immobile	Effects	Cumulative Dead	Number Immobile	Effects	Cumulative Dead	Number Immobile	Effects	
Negative Control	A	10	0	0	10 AN	0	0	10 AN	0	0	10 AN	0%
	B	10	0	0	10 AN	0	0	10 AN	0	0	10 AN	
Solvent Control	A	10	0	0	6AN;4Q,AN	0	0	10Q,AN	1	0	9Q,AN	5%
	B	10	0	0	7AN;3Q,AN	0	0	2AN;8Q,AN	0	0	1AN;9Q,AN	
0.035	A	10	0	0	9AN;1Q,AN	0	0	4AN;6Q,AN	1	0	2AN;7Q,AN	5%
	B	10	0	0	6AN;4Q,AN	0	0	2AN;8Q,AN	0	0	2AN;8Q,AN	
0.068	A	10	0	0	8AN;2Q,AN	0	0	10 AN	0	0	6AN;4Q,AN	10%
	B	10	0	0	7AN;3Q,AN	0	0	9AN;1Q,AN	2	0	7Q,AN;1Q,C	
0.10	A	10	0	0	9AN;1Q,AN	1	0	9 AN	7	0	3Q,AN	60%
	B	10	0	0	8AN;2Q,AN	0	0	9AN;1Q,AN	5	0	1AN;4Q,AN	
0.14	A	10	0	0	10 AN	1	0	7AN;2C	8	0	1Q,C;1Q,AN	80%
	B	10	0	0	9AN;1Q,AN	1	0	9 AN	8	0	2Q,C	
0.23	A	10	0	0	10 AN	4	0	6 AN	10	0	..	95%
	B	10	0	0	10 AN	5	0	5 AN	9	0	1C	

<sup>1</sup> Observed Effects: AN = Appears Normal; C = Lethargy; Q,AN = daphnid floating, resubmerged appeared normal; Q,C = daphnid floating, resubmerged appeared lethargic.

- 20 -

Table 4

## EC50 Values

Sponsor:	The Goodyear Tire & Rubber Company			
Test Substance:	WINGSTAY SN-1			
Test Organism:	Cladoceran, <i>Daphnia magna</i>			
Dilution Water:	Well Water			
Time	EC50 (mg/L)	Lower 95% Confidence Limits	Upper 95% Confidence Limits	Statistical Method
24 Hours	> 0.23	-- <sup>1</sup>	--	NA
48 Hours	0.097	0.082	0.11	Probit
<sup>1</sup> Confidence limits could not be calculated with the mortality data obtained. NA - Statistical method not used for estimation made by visual interpretation of the mortality/immobility data.				

## APPENDIX I

Specific Conductance, Hardness, Alkalinity and pH of Well Water Measured  
During the 4-Week Period Immediately Preceding the Test

Sponsor:	The Goodyear Tire & Rubber Company	
Test Substance:	WINGSTAY SN-1	
Test Organism:	Cladoceran, <i>Daphnia magna</i>	
Dilution Water:	Well Water	
	Mean	Range
Specific Conductance ( $\mu\text{mhos/cm}$ )	(N = 4) 315	310 - 320
Hardness (mg/L as $\text{CaCO}_3$ )	(N = 4) 132	128 - 136
Alkalinity (mg/L as $\text{CaCO}_3$ )	(N = 4) 179	172 - 182
pH	(N = 4) 8.3	8.2 - 8.4

## APPENDIX II

Analyses of Pesticides, Organics, Metals and Other Inorganics  
Analyzed in Wildlife International Ltd. Well Water<sup>1</sup>


---

Sponsor: The Goodyear Tire & Rubber Company  
 Test Substance: WINGSTAY SN-1  
 Test Organism: Cladoceran, *Daphnia magna*  
 Dilution Water: Well Water

---

ANALYSIS	MEASURED CONCENTRATION	
<b>Organophosphorus &amp; Organonitrogen Pesticides</b>		
Azodrin (Monochrotophos)	<	2.50 µg/L
Bolstar	<	0.266 µg/L
Chlorpyrifos	<	0.267 µg/L
Coumaphos	<	0.500 µg/L
Demeton	<	0.265 µg/L
Diazinon	<	0.265 µg/L
Dichlorvos	<	0.260 µg/L
Dimethoate	<	0.250 µg/L
Disulfoton	<	0.255 µg/L
EPN	<	0.500 µg/L
Ethoprop	<	0.275 µg/L
Fenthion	<	0.252 µg/L
Fensulfothion	<	0.512 µg/L
Guthion (Methyl Azinphos)	<	0.500 µg/L
Malathion	<	0.270 µg/L
Merphos	<	0.246 µg/L
Mevinphos	<	0.255 µg/L
Naled	<	1.34 µg/L
Methylparathion	<	0.250 µg/L
Parathion	<	0.288 µg/L
Phorate	<	0.242 µg/L
Ronnel	<	0.257 µg/L
Stirofos	<	0.500 µg/L
Sulfotepp	<	0.260 µg/L
Tepp	<	1.04 µg/L
Tokuthion	<	0.276 µg/L
Trichloronate	<	0.265 µg/L
<b>Metals and Other Inorganics</b>		
Aluminum	<	50.0 µg/L
Arsenic	<	2.5 µg/L
Beryllium	<	5.0 µg/L
Cadmium	<	5.0 µg/L
Calcium		32800 µg/L
Chromium	<	10.0 µg/L
Copper	<	5.0 µg/L
Iron	<	45.0 µg/L
Lead	<	2.0 µg/L
Magnesium		13.1 mg/L
Manganese	<	5.0 µg/L
Nickel	<	15.0 µg/L
Potassium		6730 µg/L
Selenium	<	2.5 µg/L
Silver	<	5.0 µg/L
Sodium		21200 µg/L
Zinc	<	30.0 µg/L
Mercury	<	0.20 µg/L
Molybdenum	<	10.0 µg/L

---

<sup>1</sup> Analyses performed by Environmental Science & Engineering, Inc., Gainesville, Florida for samples collected on May 10, 1994.

---

APPENDIX II (Continued)  
Analyses of Pesticides, Organics, Metals and Other Inorganics  
Analyzed in Wildlife International Ltd. Well Water<sup>1</sup>

Sponsor:	The Goodyear Tire & Rubber Company
Test Substance:	WINGSTAY SN-1
Test Organism:	Cladoceran, <i>Daphnia magna</i>
Dilution Water:	Well Water

ANALYSIS	MEASURED CONCENTRATION	
<b>Miscellaneous Measurements</b>		
Total Dissolved Solids	248	mg/L
Ammonia Nitrogen	< 0.050	mg/L
Total Organic Carbon <sup>2</sup>	< 0.5	mg/L
Total Cyanide	< 0.003	mg/L
<b>Organochlorines and PCBs</b>		
Aldrin	< 0.005	µg/L
Alpha BHC	< 0.005	µg/L
Beta BHC	< 0.005	µg/L
Delta BHC	< 0.005	µg/L
Gamma BHC (Lindane)	< 0.005	µg/L
Chlordane	< 0.025	µg/L
DDD, pp'	< 0.005	µg/L
DDE, pp'	< 0.005	µg/L
DDT, pp'	< 0.005	µg/L
Dieldrin	< 0.005	µg/L
Endosulfan, A	< 0.005	µg/L
Endosulfan, B	< 0.005	µg/L
Endosulfan Sulfate	< 0.005	µg/L
Endrin	< 0.005	µg/L
Endrin Aldehyde	< 0.005	µg/L
Heptachlor	< 0.005	µg/L
Methoxychlor	< 0.005	µg/L
Heptachlor Epoxide	< 0.005	µg/L
Toxaphene	< 0.500	µg/L
PCB-1016	< 0.100	µg/L
PCB-1221	< 0.100	µg/L
PCB-1232	< 0.100	µg/L
PCB-1242	< 0.100	µg/L
PCB-1248	< 0.100	µg/L
PCB-1254	< 0.100	µg/L
PCB-1260	< 0.100	µg/L
<b>Chlorophenoxy Acid Herbicides</b>		
2,4-D, Total	< 0.020	µg/L
2,4-DB	< 0.020	µg/L
2,4,5-T Water	< 0.020	µg/L
2,4,5-TP/Silvex	< 0.020	µg/L
Dalapon	< 0.020	µg/L
Dicamba (Banvel)	< 0.020	µg/L
Dichloroprop	< 0.020	µg/L
Dinoseb	< 0.020	µg/L
MCPA	< 0.410	µg/L
MCPP	< 0.400	µg/L

<sup>1</sup> Analyses performed by Environmental Science Wingstay SN-1 Engineering, Inc., Gainesville, Florida for samples collected on May 10, 1994.

<sup>2</sup> Analyses performed by Wildlife International Ltd. for the sample collected on May 27, 1994.

- 24 -

APPENDIX III

THE ANALYSIS OF WINGSTAY SN-1 IN FRESHWATER  
IN SUPPORT OF  
WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 414A-102

## APPENDIX III

Introduction

Freshwater samples were collected from an acute aquatic toxicity study designed to determine the effects of Wingstay SN-1 to the cladoceran (*Daphnia magna*). The study was conducted by Wildlife International Ltd. and identified as WIL Project No.: 414A-102. The analyses of these water samples were performed at Wildlife International Ltd. by high performance liquid chromatography (HPLC) with UV detection. The analytical method was verified on August 22, 1995 at Wildlife International Ltd. Samples were received for analysis on August 28, 29, 30 and 31, 1995 and analyzed between August 28 and August 31, 1995.

Test Substance and Analytical Standard

The test substance, received from The Goodyear Tire and Rubber Company on December 13, 1994, was used to prepare matrix fortification samples. The test substance was an off-white waxy solid, identified on the label as: Wingstay SN-1; Lot # 130893; Notebook # 10024-64-3. The test substance did not have a reported purity or an expiration date. Upon receipt, the test substance was assigned Wildlife International Ltd. identification number WIL #3080 and stored under ambient conditions.

The analytical standard, received from The Goodyear Tire and Rubber Company on March 30, 1995, was used to prepare calibration standards. The analytical standard was a white solid, identified on the label as: bis component of Wingstay SN-1; Notebook Number 9988-5Z; Goodyear Notebook # 10024-65-5. The analytical standard had a reported purity of 98.06% and no expiration date was given. Upon receipt, the analytical standard was assigned Wildlife International Ltd. identification number WIL #3177 and stored under ambient conditions.

## APPENDIX III

Analytical Method

The method used for the analysis of the water samples was based upon methodology provided by The Goodyear Tire & Rubber Company and entitled: "Wingstay® SN-1 Determination of Water Solubility".

The analytical method consisted of diluting an aliquot of the aqueous sample with an equal volume of acetonitrile (Burdick & Jackson Lot No. BK329). These solutions were then placed in crimp top vials prior to analysis by HPLC with UV detection. Concentrations of Wingstay SN-1 in the samples were determined by high performance liquid chromatography (HPLC) using a Hewlett-Packard Model 1090 HPLC equipped with a diode array detector (DAD). HPLC separations were achieved using a C8 guard column and Betasil C8 column (25 cm x 3 mm I.D., 5 µm particle size). The instrument parameters are summarized in Table 1. A method flow chart is provided in Figure 1.

Calibration Curve, Limit of Detection and Limit of Quantitation

Calibration standards of the bis ester of tetraethylene glycol and n-dodecanethiol, ranging in concentration from 0.020 to 0.40 mg/L, were analyzed with each series of samples. Linear regression equations were generated using the peak area responses versus the respective concentrations of the calibration standards. An example of a calibration curve is presented in Figure 2. The concentration of test substance in the samples was determined by substituting the peak area responses of the analyte into the applicable linear regression equation and converting to test substance concentration. Representative chromatograms of low and high calibration standards are shown in Figures 3 and 4, respectively.

The instrument limit of detection (LOD) was set based upon the injection volume (200 µL) and the lowest standard concentration (0.020 mg/L). The LOD was set at 4 ng injected on-column. The method limit of quantitation (LOQ) for these analyses was set at 0.040 mg/L

## APPENDIX III

based upon the lowest standard analyzed concurrently with the samples from the test and dilution with an equal volume of acetonitrile.

Method Trials

The analytical method was verified for freshwater by analyzing a series of 15 fortification samples at five concentrations (0.05, 0.10, 0.20, 0.50 and 5.0 mg/L). The recoveries ranged from 82 to 118% of the nominal concentrations (Table 2). No interferences at or above the LOQ were observed for the six matrix blanks analyzed during the method trial.

Matrix Blank and Fortification Samples

Along with the actual sample analyses, three matrix blanks were analyzed to determine possible interference. No interferences were observed at or above the LOQ during the sample analyses (Table 3). A representative chromatogram of a matrix blank is presented in Figure 5.

Freshwater samples were fortified at 0.05, 0.10 and 0.50 mg/L and analyzed concurrently with the samples to determine the mean procedural recovery (Table 4). The mean procedural recovery was 88%. The samples were not corrected for the procedural recovery because the analytical method involved dilution and injection of the samples. A representative chromatogram of a matrix fortification is presented in Figure 6.

RESULTSSample Analysis

Prior to test initiation, water samples taken to evaluate diluter equilibration and test concentration verifications at the 0.052, 0.086 and 0.40 mg/L gave 73, 66 and 30% of nominal values, respectively (Table 5). These values were not used to calculate the mean measured concentration during the study. Freshwater samples were collected from the acute toxicity study

- 28 -

## APPENDIX III

with the cladoceran (*Daphnia magna*) at test initiation (Day 0), August 29, 1995, and at 24-hour intervals during the test through test termination (48 hours) on August 31, 1995. The concentrations of Wingstay SN-1 in the samples collected at initiation of exposure of the test organisms (Day 0) ranged from 58 to 76% of the nominal concentrations (Table 6). Samples collected at 24 hours and 48 hours (test termination) had measured concentration ranges of 48 to 71% and 58 to 108% of nominal values, respectively. A representative chromatogram of a sample is shown in Figure 7.

- 29 -

## APPENDIX III

Table 1

## Typical HPLC Operational Parameters

---

---

INSTRUMENT:	Hewlett-Packard Model 1090 High Performance Liquid Chromatograph with Diode Array Detector (DAD)
GUARD COLUMN:	Javelin Betasil C8 (3.0 mm x 20 mm)
ANALYTICAL COLUMN:	Keystone Scientific, Inc. Betasil C8 (3.0 mm x 250 mm, 5 $\mu$ m particle size)
STOP TIME:	15 minutes
POST TIME:	0.0 minute
FLOW RATE:	0.60 mL/min.
OVEN TEMPERATURE:	40°C
SOLVENT A:	100% Acetonitrile (Fisher Optima Lot No. 953205 and Burdick & Jackson Lot No. BK329)
INJECTION VOLUME:	200 $\mu$ L
WINGSTAY SN-1 PEAK RETENTION TIME:	Approximately 8.5 minutes
PRIMARY ANALYTICAL WAVELENGTH:	210 nm (4nm bandwidth)
SECONDARY ANALYTICAL WAVELENGTH:	450 (80 nm bandwidth)

---

---

## APPENDIX III

Table 2

## Method Trial Recoveries for Wingstay SN-1 in Freshwater

Number (414A-102-)	Sample Type	Concentration of Wingstav SN-1 (mg/L) <sup>1</sup>		Percent Recovery
		Fortified	Measured <sup>2</sup>	
VMAB-1	Matrix Blank	0.0	<0.04	--
VMAB-2	Matrix Blank	0.0	<0.04	--
VMAB-3	Matrix Blank	0.0	<0.04	--
VMAB-4	Matrix Blank	0.0	<0.04	--
VMAB-5	Matrix Blank	0.0	<0.04	--
VMAB-6	Matrix Blank	0.0	<0.04	--
VMAS-10	Matrix Fortification	0.050	0.0517	103
VMAS-11	Matrix Fortification	0.050	0.0451	90
VMAS-12	Matrix Fortification	0.050	0.0591	118
VMAS-13	Matrix Fortification	0.10	0.0815	82
VMAS-14	Matrix Fortification	0.10	0.0854	85
VMAS-15	Matrix Fortification	0.10	0.0837	84
VMAS-16	Matrix Fortification	0.20	0.183	92
VMAS-17	Matrix Fortification	0.20	0.203	102
VMAS-18	Matrix Fortification	0.20	0.190	95
VMAS-4	Matrix Fortification	0.50	0.530	106
VMAS-5	Matrix Fortification	0.50	0.485	97
VMAS-6	Matrix Fortification	0.50	0.516	103
VMAS-7	Matrix Fortification	5.0	4.69	94
VMAS-8	Matrix Fortification	5.0	5.17	103
VMAS-9	Matrix Fortification	5.0	4.95	99

Mean Recovery = 97%  
Standard Deviation = ±9.6%  
N = 15

<sup>1</sup> The concentration of Wingstay SN-1 is calculated based upon the measurement of the bis ester of tetraethylene glycol and n-dodecanethiol which is present at 73.1% in Wingstay SN-1.

<sup>2</sup> The limit of quantitation (LOQ) was based upon the lowest standard analyzed concurrently with the samples from the test and the factor of two dilution of the test samples.

## APPENDIX III

Table 3

## Matrix Blanks Analyzed Concurrently During Sample Analysis

Sample		Measured Concentration of Wingstay SN-1 (mg/L) <sup>1,2</sup>
Number (414A-102-)	Type	
MAB-1	Matrix Blank	<0.04
MAB-2	Matrix Blank	<0.04
MAB-3	Matrix Blank	<0.04

<sup>1</sup> The concentration of Wingstay SN-1 is calculated based upon the measurement of the bis ester of tetraethylene glycol and n-dodecanethiol which is present at 73.1% in Wingstay SN-1.

<sup>2</sup> The limit of quantitation (LOQ) was based upon the lowest standard analyzed concurrently with the samples from the test and the factor of two dilution of the test samples.

- 32 -

## APPENDIX III

Table 4

## Matrix Fortifications Analyzed Concurrently During Sample Analysis

Sample Number (414A-102-)	Concentrations of Wingstay SN-1 (mg/L) <sup>1</sup>		Percent Recovered
	Fortified	Measured	
MAS-1	0.050	(0.036) <sup>2</sup>	(72) <sup>3</sup>
MAS-4	0.050	(0.013) <sup>2</sup>	(26) <sup>3</sup>
MAS-7	0.050	0.042	(84) <sup>3</sup>
MAS-2	0.10	0.0785	79
MAS-5	0.10	0.0908	91
MAS-8	0.10	0.111	111
MAS-3	0.50	0.255	51
MAS-6	0.50	0.487	97
MAS-9	0.50	0.494	99
			Mean = 88%
			Standard Deviation = ±21%
			N = 6

<sup>1</sup> The concentration of Wingstay SN-1 is calculated based upon the measurement of the bis ester of tetraethylene glycol and n-dodecanethiol which is present at 73.1% in Wingstay SN-1.

<sup>2</sup> Measured value extrapolated from a peak area below the peak area of the lowest standard analyzed with the samples.

<sup>3</sup> Recovery value not used to calculate the mean recovery.

## APPENDIX III

Table 5  
Measured Concentrations of Wingstay SN-1 in Pre-Test Diluter Verification Samples<sup>1</sup>

Nominal Concentration (mg/L)	Sample Number (414A-102-)	Sampling Time Prior to Test (Day)	Wingstay SN-1 Measured <sup>2</sup> Concentration (mg/L)	Mean	Mean Percent of Nominal
0.052	PT-1	-1	(0.0396) <sup>3</sup>	0.038	73
	PT-2	-1	(0.0354) <sup>3</sup>		
0.086	PT-3	-1	0.0610	0.057	66
	PT-4	-1	0.0536		
0.40	PT-5	-1	0.0971	0.121	30
	PT-6	-1	0.145		

<sup>1</sup> For the quality control samples, the matrix blank had no detectable peak and the recovery of the matrix fortifications at 0.050, 0.100 and 0.50 mg/L were 63%, 71% and 119%, respectively.

<sup>2</sup> The concentration of Wingstay SN-1 is calculated based upon the measurement of the bis ester of tetraethylene glycol and n-dodecanethiol which is present at 73.1% in Wingstay SN-1.

<sup>3</sup> Measured value extrapolated from a peak area below the peak area of the lowest standard.

## APPENDIX III

Table 6

## Measured Concentrations of Wingstay SN-1 in Freshwater Samples from a Daphnia Acute Toxicity Test

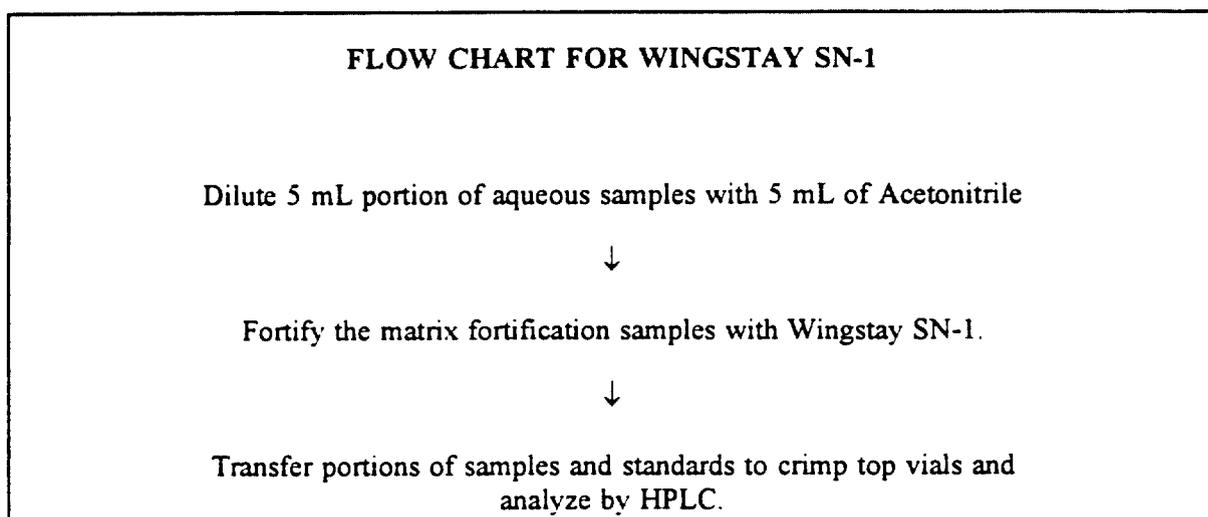
Nominal Concentration (mg/L)	Sample Number (414A-102-)	Sampling Time (Hours)	Wingstay SN-1 Measured Concentration <sup>1</sup> (mg/L)	Mean	Mean Percent of Nominal
0.0 (Negative Control)	1	0	<0.04 <sup>2</sup>		
	8	24	<0.04		
	15	48	<0.04		
0.0 (Solvent Control)	2	0	<0.04		
	9	24	<0.04		
	16	48	<0.04		
0.052	3	0	(0.039) <sup>3</sup>	0.035	67
	10	24	(0.025) <sup>3</sup>		
	17	48	0.042		
0.086	4	0	(0.0512) <sup>3</sup>	0.068	79
	11	24	0.0610		
	18	48	0.0928		
0.14	5	0	0.106	0.10	71
	12	24	0.0993		
	19	48	0.104		
0.24	6	0	0.140	0.14	58
	13	24	0.145		
	20	48	0.138		
0.40	7	0	0.235	0.23	58
	14	24	0.216		
	21	48	0.232		

<sup>1</sup> The concentration of Wingstay SN-1 is calculated based upon the measurement of the bis ester of tetraethylene glycol and n-dodecanethiol which is present at 73.1% in Wingstay SN-1.

<sup>2</sup> The limit of quantitation (LOQ) was based upon the lowest standard level (0.020 mg/L) analyzed concurrently with the test samples and the factor of two dilution of the samples.

<sup>3</sup> Mean value extrapolated from a peak area below the peak area of the lowest standard.

APPENDIX III



**Figure 1.** Analytical method flow chart for the analysis of Wingstay SN-1 in freshwater.

- 36 -

## APPENDIX III

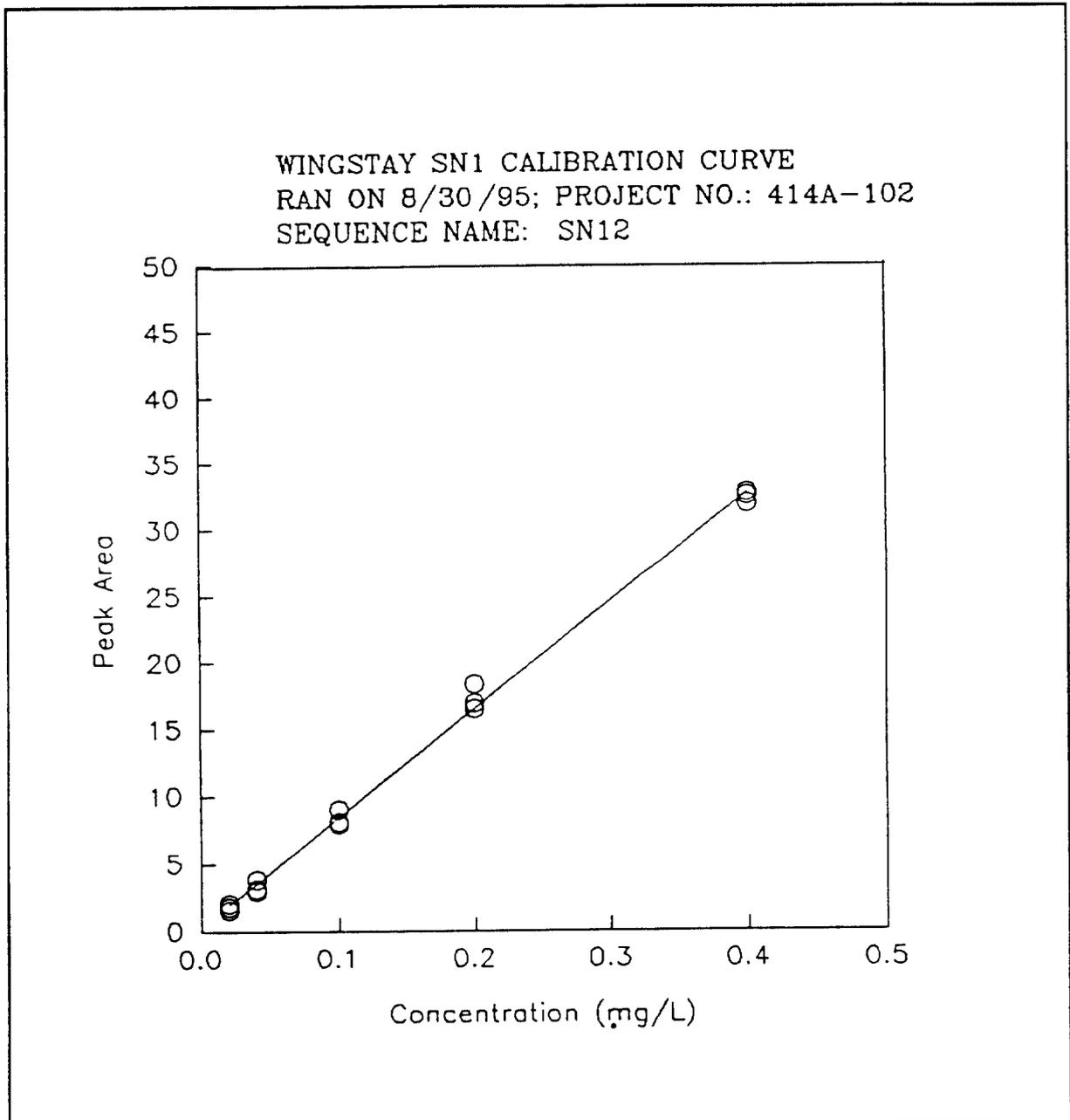
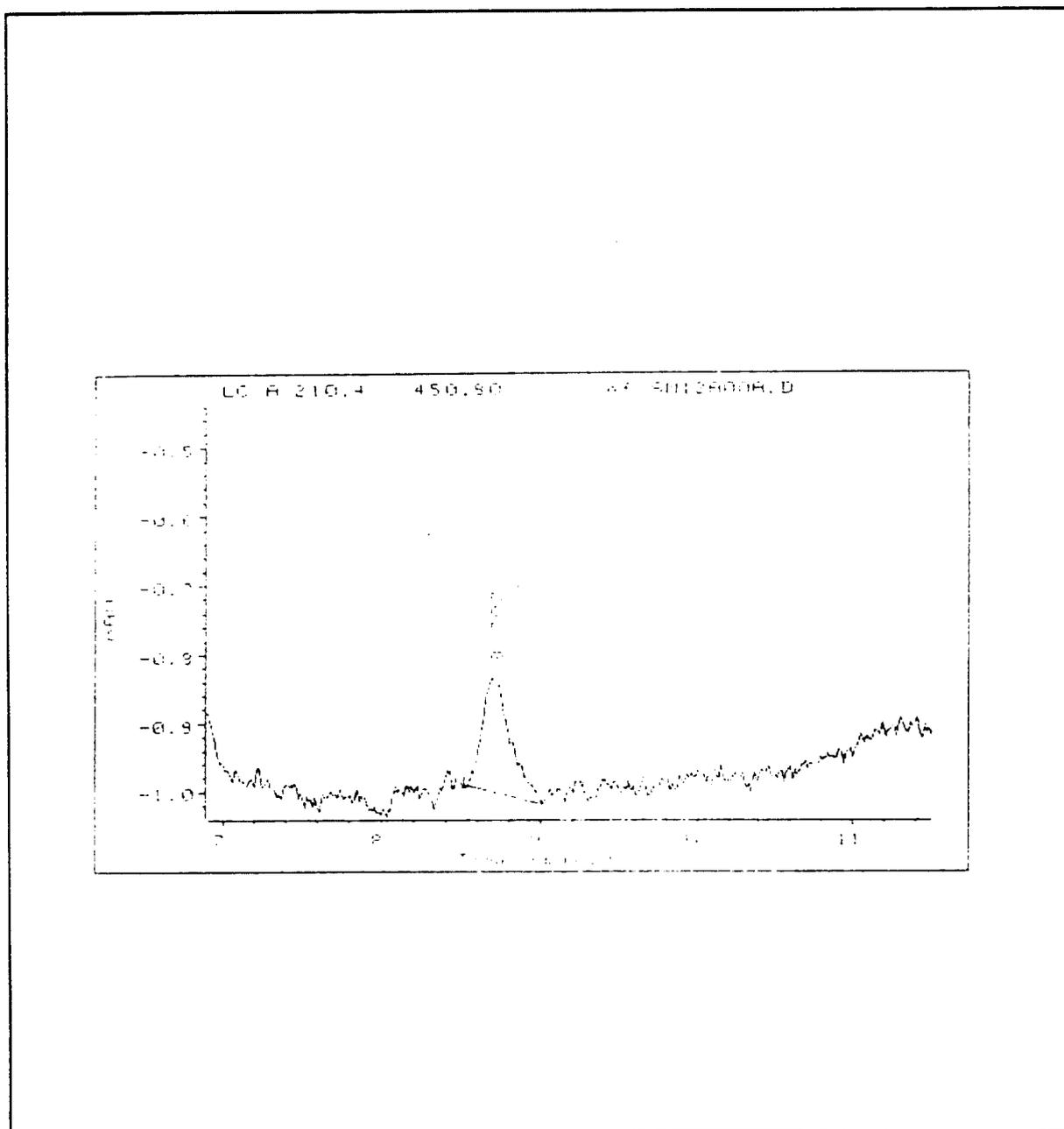


Figure 2. Representative calibration curve for Wingstay SN-1. Slope = 80.9549; Intercept = 0.2872.

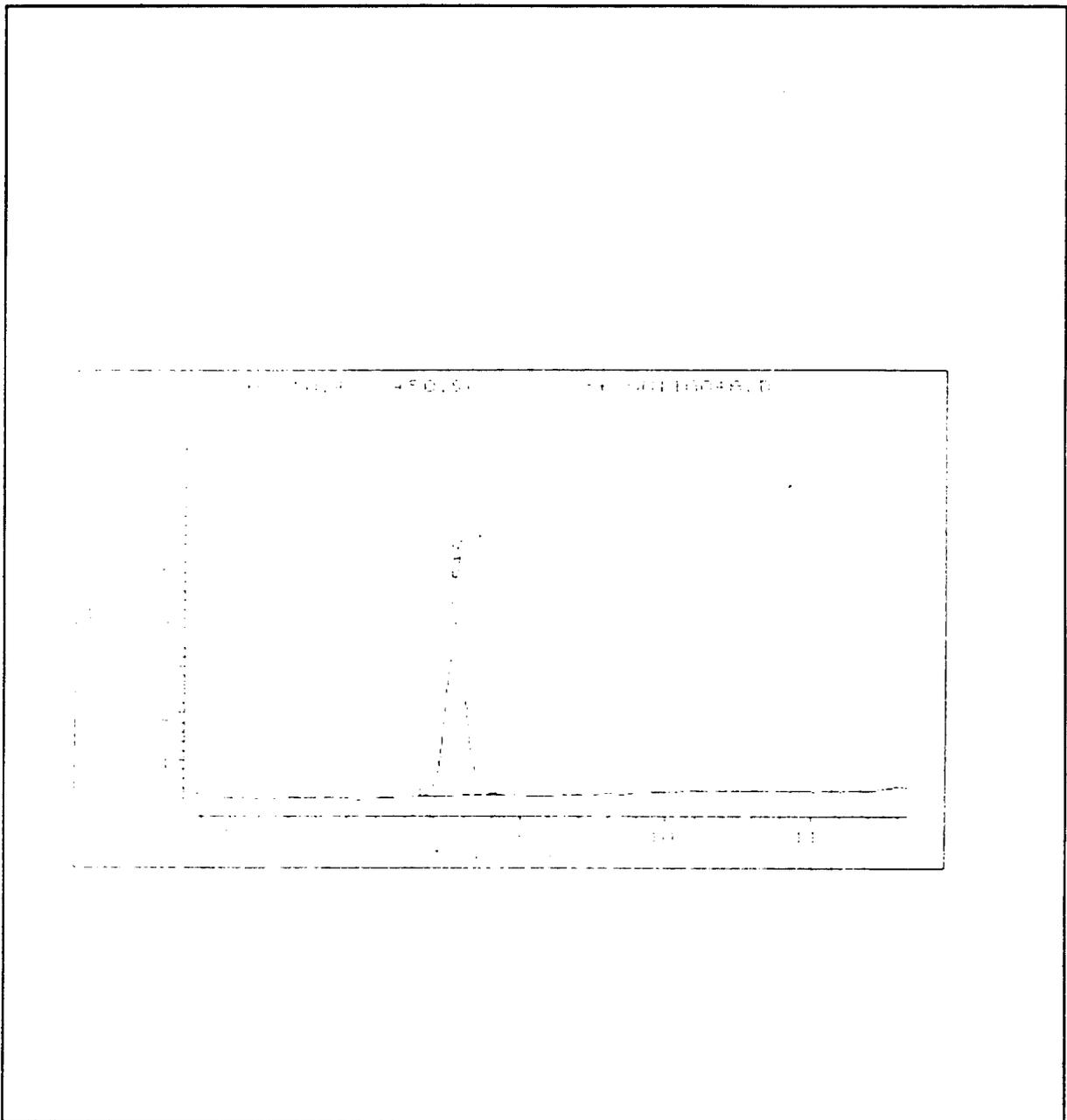
- 37 -

## APPENDIX III



**Figure 3.** A representative chromatogram of a 0.020 mg/L standard (4 ng on-column) of the bis ester of tetraethylene glycol and n-dodecanethiol.

APPENDIX III



**Figure 4.** A representative chromatogram of a 0.40 mg/L standard (80 ng on-column) of the bis ester of tetraethylene glycol and n-dodecanethiol.

APPENDIX III

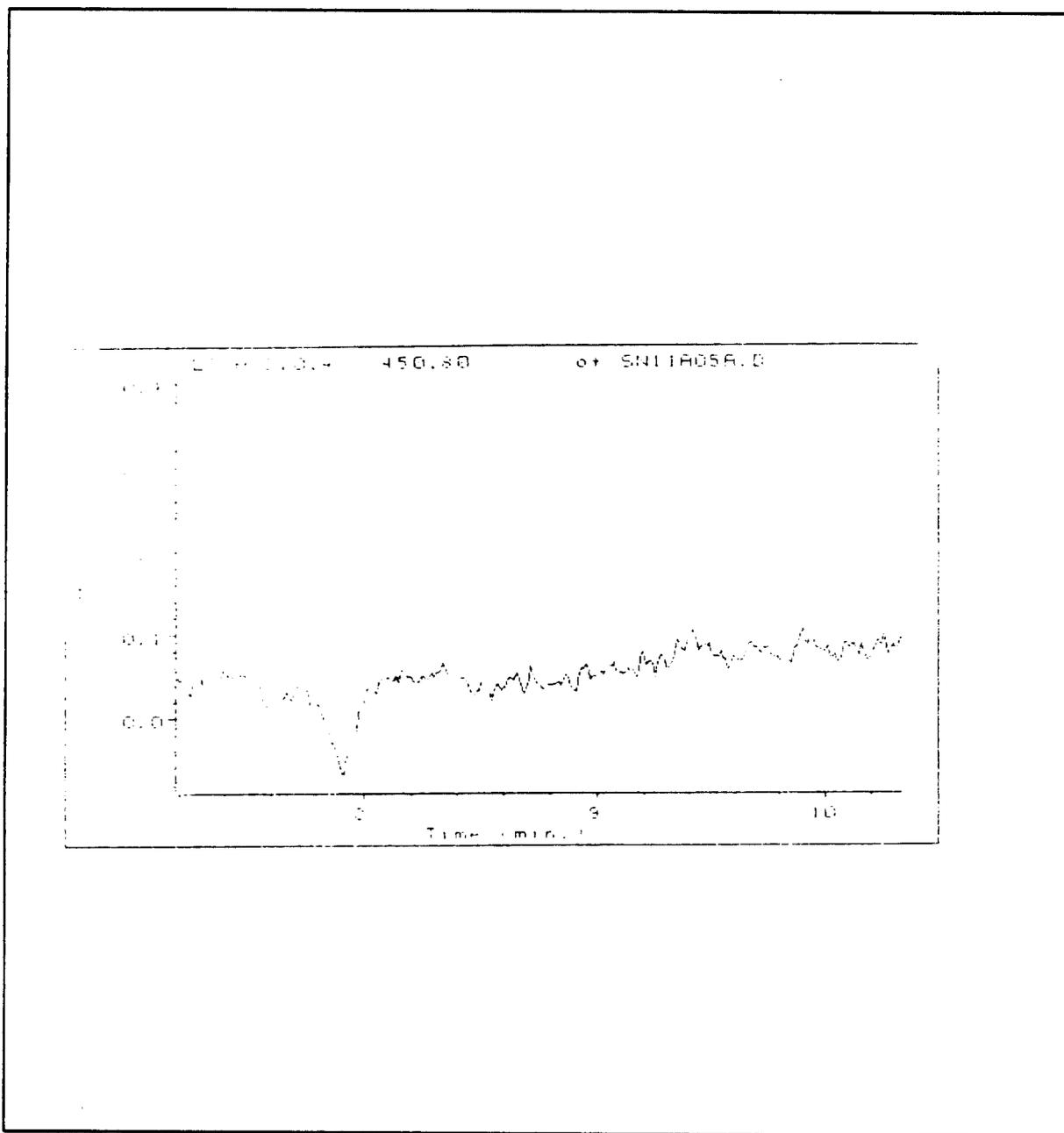
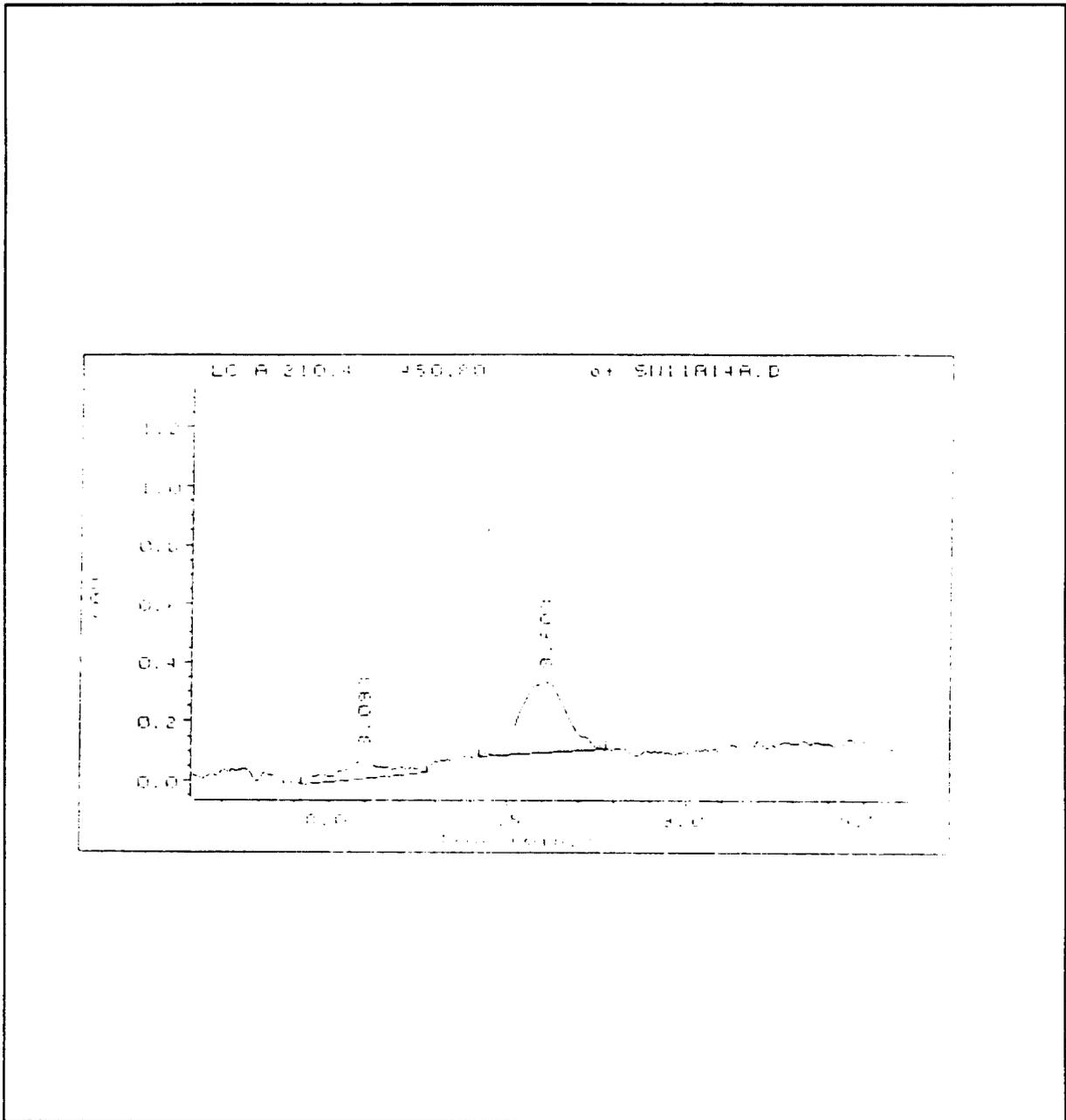


Figure 5. A representative chromatogram of a matrix blank, 414A-102-MAB-1.

APPENDIX III



**Figure 6.** A representative chromatogram of a matrix fortification, 414A-102-MAS-2 (0.10 mg/L).

APPENDIX III

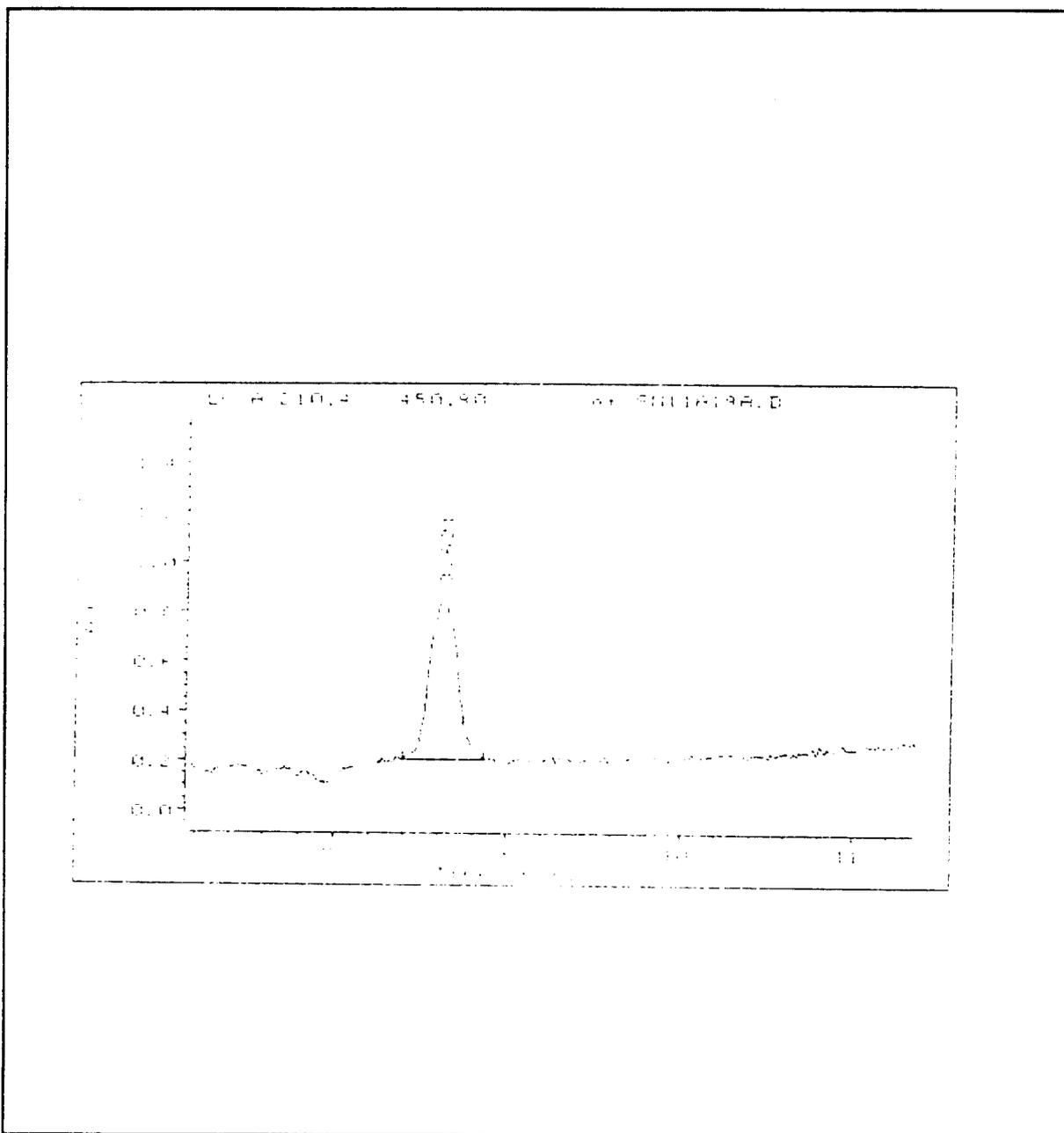


Figure 7. A representative chromatogram of a sample on Day 0, 414A-102-7 (0.40 mg/L nominal concentration).

## APPENDIX IV

## Changes to Protocol

This study was conducted in accordance with the approved Protocol with the following changes:

1. The protocol was amended to add the proposed experimental start and termination dates, study room and test concentrations.
2. The protocol was amended to clarify the frequency of water and feed analyses.
3. The protocol was amended to clarify the number of pretest samples to be collected and analyzed.
4. The analytical method verification scheme was added by amendment.
5. The syringes used to deliver the test substance stock solutions were constructed of plastic.
6. The analytical method used in the study was more closely related to an additional method provided by the Sponsor than the method indicated in Appendix II.

In the opinion of the Study Director, the above changes in the approved Protocol did not adversely affect the results of this study.

APPENDIX V

Personnel Involved in the Study

The following key personnel were involved in the conduct or management of this study:

1. James P. Swigert, Ph.D., Manager, Aquatic Toxicology
2. Ray L. Hanson, Ph.D., Supervisor, Aquatic Chemistry
3. William C. Graves, Senior Aquatic Biologist
4. Cynthia A. Roberts, Senior Aquatic Biologist
5. Mark A. Mank, Aquatic Biologist
6. Victor V. Shigaev, Ph.D., Aquatic Biologist

**Contains No Cd**

WINGSTAY SN-1:  
A 72-HOUR TOXICITY TEST WITH THE  
FRESHWATER ALGA (*Selenastrum capricornutum*)

FINAL REPORT

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 414A-101

OECD GUIDELINE 201

AUTHORS:

Cynthia A. Roberts  
James P. Swigert, Ph.D.

STUDY INITIATION DATE: January 16, 1995

STUDY COMPLETION DATE: March 27, 1996

Submitted to

The Goodyear Tire & Rubber Company  
142 Goodyear Boulevard  
Akron, Ohio 44305



**WILDLIFE INTERNATIONAL LTD.**

8598 Commerce Drive  
Easton, Maryland 21601  
(410) 822-8600



Page 1 of 54

80:0111W 2-70F 96

RECEIVED  
OFFICE

96 JUN 25 11:31

RECEIVED  
OFFICE

**GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT**

SPONSOR: The Goodyear Tire & Rubber Company

TITLE: Wingstay SN-1: A 72-Hour Toxicity Test with the Freshwater Alga  
(*Selenastrum capricornutum*)

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 414A-101

STUDY COMPLETION: March 27, 1996

This study was conducted to conform with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 160, 17 August 1989; OECD, ISBN 92-84-12367-9, Paris 1982; and Japan MAFF (59 NohSan, Notification No. 3850, Agricultural Production Bureau), with the following exceptions

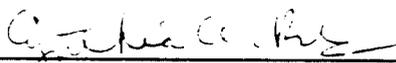
Test substance characterization was not performed in accordance with Good Laboratory Practice Standards.

The stability of the test substance under storage conditions at the testing facility was not known for this study.

The reference substance characterization was not performed in compliance with Good Laboratory Practice Standards.

Due to the limitations of the analytical methodology, the 0.72, 1.8, 4.5, 11 and 28 µg Wingstay SN-1/L test concentrations were not verified.

STUDY DIRECTOR:

  
\_\_\_\_\_  
Cynthia A. Roberts  
Senior Aquatic Biologist

3/27/96  
\_\_\_\_\_  
DATE

SPONSOR APPROVAL:

\_\_\_\_\_  
Sponsor

\_\_\_\_\_  
DATE

\_\_\_\_\_  
Applicant/Submitter

\_\_\_\_\_  
DATE

- 3 -

## QUALITY ASSURANCE STATEMENT

This study was examined for conformance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 160, 17 August 1989; OECD, ISBN 92-84-12367-9, Paris 1982; and Japan MAFF (59 NohSan, Notification No. 3850, Agricultural Production Bureau). The dates of all inspections and audits and the dates that any findings were reported to the Study Director and Laboratory Management were as follows:

ACTIVITY	DATE CONDUCTED:	DATE REPORT TO	
		STUDY DIRECTOR:	MANAGEMENT:
Test substance preparation	September 29, 1995	October 2, 1995	October 30, 1995
Standards preparation	September 29, 1995	October 2, 1995	October 4, 1995
Biological sampling	October 2, 1995	October 2, 1995	October 30, 1995
Analytical sampling	October 2, 1995	October 2, 1995	October 30, 1995
Analytical Data and Draft Report	October 24 and 25, 1995	October 25, 1995	November 2, 1995
Biological Data and Draft Report	October 30 and 31, 1995	October 31, 1995	November 2, 1995
Final Report	March 27, 1996	March 27, 1996	March 27, 1996

  
 \_\_\_\_\_  
 Marshall T. Hynson  
 Quality Assurance Program Supervisor

3/27/96  
 \_\_\_\_\_  
 DATE

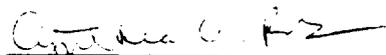
**REPORT APPROVAL**

SPONSOR: The Goodyear Tire & Rubber Company

TITLE: Wingstay SN-1: A 72-Hour Toxicity Test with the Freshwater Alga  
(*Selenastrum capricornutum*)

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 414A-101

STUDY DIRECTOR

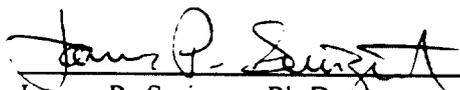


Cynthia A. Roberts  
Senior Aquatic Biologist

3/27/96

DATE

MANAGEMENT:



James P. Swigert, Ph.D.  
Manager, Aquatic Toxicology

3/27/96

DATE

**TABLE OF CONTENTS**

Title/Cover Page .....	1
Good Laboratory Practice Compliance Statement .....	2
Quality Assurance Statement .....	3
Report Approval .....	4
Table of Contents .....	5
Summary .....	7
Introduction .....	8
Objective .....	8
Experimental Design .....	8
Materials and Methods .....	9
Results and Discussion .....	14
Conclusion .....	15
References .....	16

**TABLES**

Table 1 - Summary of Analytical Chemistry Data .....	17
Table 2 - Temperature Measurements .....	18
Table 3 - pH Measurements .....	19
Table 4 - Mean Area Under the Growth Curve and Percent Inhibition .....	20
Table 5 - EC50 Values Over the 72-Hour Exposure Period .....	21

## TABLE OF CONTENTS

-Continued-

## FIGURE

Figure 1 - Dose-Response Curve for <i>Selenastrum capricornutum</i> Exposed to Wingstay SN-1 for 72 Hours .....	22
---	----

## APPENDICES

Appendix I - Freshwater Algal Medium .....	23
Appendix II - Analyses of Pesticides, Organics, Metals and Other Inorganics Analyzed in Wildlife International Ltd. Well Water .....	24
Appendix III - The Analysis of Wingstay SN-1 in Freshwater Algal Medium in Support of Wildlife International Ltd. Project No.: 414A-101 .....	26
Appendix IV - Cell Densities for Each Replicate Over the 72-Hour Exposure Period .....	51
Appendix V - Area Under the Growth Curve for Each Replicate Over the 72-Hour Exposure Period .....	52
Appendix VI - Changes to Protocol .....	53
Appendix VII - Personnel Involved in the Study .....	54

- 7 -

## SUMMARY

SPONSOR:	The Goodyear Tire & Rubber Company
CONTACT:	Mr. Richard Serva
LOCATION OF STUDY, RAW DATA AND FINAL REPORT:	Wildlife International Ltd. Easton, Maryland 21601

WILDLIFE INTERNATIONAL LTD PROJECT NUMBER:	414A-101
TEST SUBSTANCE:	Wingstay SN-1 (diester of 3-(dodecylthio) propionic acid and tetraethylene glycol), CAS No.: 64253-30-1
STUDY:	Wingstay SN-1: A 72-Hour Toxicity Test with the Freshwater Alga ( <i>Selenastrum capricornutum</i> )
NOMINAL TEST CONCENTRATIONS:	Negative Control; Solvent Control; 0.72, 1.8, 4.5, 11, 28 and 70 µg Wingstay SN-1/L
TEST DATES	Experimental Start - September 29, 1995 Exposure Termination - October 2, 1995 Experimental Termination - November 6, 1995
LENGTH OF TEST:	72 Hours

TEST ORGANISM:	Freshwater Alga ( <i>Selenastrum capricornutum</i> )
SOURCE OF TEST ORGANISMS:	Wildlife International Ltd. Easton, Maryland 21601

72-HOUR EC50:	65 µg Wingstay SN-1/L
95% CONFIDENCE LIMITS:	Undetermined
72-HOUR NO-OBSERVED- EFFECT-CONCENTRATION:	4.5 µg Wingstay SN-1/L

## INTRODUCTION

This study was conducted by Wildlife International Ltd. for The Goodyear Tire & Rubber Company at the Wildlife International Ltd. aquatic toxicology facility in Easton, Maryland. The test was conducted from September 29, 1995 to October 2, 1995. Raw data generated by Wildlife International Ltd. and a copy of the final report are filed under Project Number 414A-101 in archives located on the Wildlife International Ltd. site.

## OBJECTIVE

The objective of this study was to evaluate the acute toxicity of Wingstay SN-1 to the freshwater alga, *Selenastrum capricornutum*, during a 72-hour exposure period under static test conditions.

## EXPERIMENTAL DESIGN

The freshwater alga, *Selenastrum capricornutum*, was exposed to a geometric series of six test concentrations, a solvent control, and a negative (culture medium) control under static conditions for 72 hours. Three replicate test chambers were maintained for each treatment and control group. Nominal test concentrations were selected in consultation with the Sponsor and were based upon toxicity data provided by the Sponsor and the results of an exploratory range finding test. Nominal test concentrations selected were 0.72, 1.8, 4.5, 11, 28 and 70  $\mu\text{g}$  Wingstay SN-1/L. Measured concentrations were determined from samples of test medium collected from each treatment and control group at the beginning and end of the test.

At test initiation an inoculum of the algal cells was prepared from the stock culture at a concentration of approximately  $1.0 \times 10^6$  cells/mL. The concentration of algal cells was verified and 1.0 mL was added to each test chamber to achieve a nominal concentration of approximately

10,000 cells/mL. Samples were collected from each replicate test chamber at approximately 24-hour intervals during the exposure to determine cell densities. Cell densities were used to calculate area under the growth curve values, which were subsequently used to calculate percent inhibition values relative to the controls over the 72-hour exposure period. EC50 values (i.e., the theoretical toxicant concentrations that would produce a 50% reduction in area under the growth curve) were estimated when possible, for each 24-hour interval of the exposure period. The no-observed-effect-concentration (NOEC) was determined through evaluation of the 72-hour statistical results and the dose-response pattern.

## MATERIALS AND METHODS

Test methods were based on procedures outlined in the protocol, Wingstay SN-1: A 72-Hour Toxicity Test with the Freshwater Alga (*Selenastrum capricornutum*). The protocol was based on procedures outlined in OECD Guideline for Testing of Chemicals 201: *Alga, Growth Inhibition Test* (1).

### Test Substance

The test substance was received from The Goodyear Tire & Rubber Company on December 13, 1994 and was assigned Wildlife International Ltd. identification number WIL #3080 upon receipt. The test substance was an off white waxy solid, identified on the label as: Wingstay SN-1; Lot # 130893; Notebook # 10024-64-3. The test substance was stored at ambient room temperature.

### Preparation of Test Concentrations

A primary test substance stock was prepared by dissolving Wingstay SN-1 in dimethylformamide (DMF) (Burdick & Jackson, Lot No BGI66). The concentration of the stock was 0.001 g Wingstay SN-1/mL. Five secondary stock solutions were prepared by diluting 4.0 mL of the appropriate working stock to 10 mL in DMF. Thirty-five  $\mu$ L aliquots of the primary stock

- 10 -

and the five secondary stocks were diluted to 500 mL with culture medium to prepare the 0.72, 1.8, 4.5, 11, 28 and 70  $\mu\text{g}$  Wingstay SN-1/L nominal test concentrations. Stock concentrations and the resultant test concentrations were prepared on a total product basis (i.e., the concentrations were not corrected for test substance purity). A solvent control was prepared by diluting 35  $\mu\text{L}$  of DMF to 500 mL with culture medium to yield a solvent concentration equivalent to that in the treatment groups.

#### Test Organism

The freshwater alga, *Selenastrum capricornutum*, was selected as the test species for this study. The species is representative of an important group of freshwater algae and was selected for use in the test based upon a past history of use and ease of culturing in the laboratory. Original algal cultures were obtained from UTEX - The Culture Collection of Algae at the University of Texas at Austin and have been maintained in culture medium at Wildlife International Ltd, Easton, Maryland. Algal cells used in this test were obtained from Wildlife International Ltd cultures that had been actively growing in culture medium for at least two weeks prior to test initiation. The negative control organisms were expected to exhibit exponential growth over the 72-hour exposure period. Exponential growth phase, defined as the period of growth where the algal cells are dividing at a constant rate, is indicated by the linear section of the growth curve (Figure 1)

#### Culture Medium

The algal cells were cultured and tested in freshwater algal medium (2). Stock nutrient solutions were prepared by adding reagent-grade chemicals to Wildlife International Ltd. well water purified by reverse osmosis. The test medium was prepared by adding the appropriate volumes of stock nutrient solutions to purified well water (Appendix I). The pH was adjusted to  $7.5 \pm 0.1$  using 0.1 N NaOH and/or 10% HCl, and the medium was sterilized by filtration (0.22  $\mu\text{m}$ ) prior to use. Analyses were performed at least once annually to determine the concentrations of selected organic and inorganic constituents in the well water. The results of

analyses performed to measure the concentrations of selected contaminants in well water used by Wildlife International Ltd. are presented in Appendix II.

#### Test Apparatus

Test chambers were sterile 250-mL Erlenmeyer flasks plugged with gauze-wrapped cotton stoppers, and containing 100 mL of test or control medium. The test flasks were labelled with the project number, test concentration and replicate and were indiscriminately positioned on a mechanical shaker in an environmental chamber designed to maintain the desired test temperature throughout the test. The test flasks were shaken continuously at 100 rpm.

#### Environmental Conditions

Test flasks were held in an environmental chamber at a temperature of  $24 \pm 2^\circ\text{C}$ . The temperature of a container of water adjacent to the test chambers in the environmental chamber was recorded twice daily during the test using a hand-held mercury thermometer.

The algae were held under continuous cool-white fluorescent lighting throughout the test. The target light intensity was  $8000 \pm 1600$  lux. Light intensity was measured at five locations surrounding the test flasks at test initiation.

The pH of the medium in each treatment and control group was measured at test initiation and termination using a Fisher Accumet Model 915 pH meter. Samples for pH measurement at test initiation were collected from individual batches of test solution prepared for each treatment and control group. At test termination, samples of test medium were collected from each replicate test chamber and were pooled by treatment group for pH measurement.

Algal Growth Measurements

Test medium samples were collected from each replicate of the treatment and control groups for the determination of algal cell densities. Samples were collected at approximately 24-hour intervals during the 72-hour exposure and were held under refrigerated conditions until cell counts could be performed. Cell counts were conducted using an electronic particle counter (Model 2M; Coulter Electronics, Inc. Hialeah, Florida). Each sample was diluted using an electrolyte solution (Isoton®), as needed, and three, 0.5-mL volumes of the sample were counted.

Statistical Analyses

Cell densities, area under the growth curve values and percent inhibition values were calculated using "Lotus 1-2-3, Release 3.1" (3), while statistical analyses were conducted using "ICPIN Version 2.0" (4) and "TOXSTAT Release 3.2" (5). Area under the growth curve was calculated for the control and treatment group using the following formula:

$$A = ((N_1 - N_0)/2)(t_1) + ((N_1 + N_2 - 2N_0)/2)(t_2 - t_1) + ((N_{n-1} + N_n - 2N_0)/2)(t_n - t_{n-1})$$

where:

- A = Area
- $N_0$  = Nominal number of cells/mL at  $t_0$
- $N_1$  = Mean measured number of cells/mL at  $t_1$
- $N_2$  = Mean measured number of cells/mL at  $t_2$
- $N_n$  = Mean measured number of cells/mL at  $t_n$
- $t_1$  = time of first measurement after beginning of test (hours)
- $t_2$  = time of second measurement after beginning of test (hours)
- $t_n$  = time of  $n^{\text{th}}$  measurement after beginning of test (hours)

Percent inhibition values were calculated for each treatment group as the percent reduction in area under the growth curve relative to the control replicates. The following formula was used:

$$\text{Percent Inhibition} = \frac{\text{Mean Area}_{\text{Pooled Control}} - \text{Mean Area}_{\text{Treatment}}}{\text{Mean Area}_{\text{Pooled Control}}} \times 100$$

Area under the growth curve values were analyzed statistically using the computer program of ICPIN (4) to estimate the EC50 values (i.e., the theoretical test concentrations that would produce a 50% reduction in area under the growth curve) and 95% confidence limits for each 24-hour interval over the 72-hour exposure period. This program was designed to calculate the EC50 values and 95% confidence limits by linear interpolation. The negative and solvent control groups were compared using a t-test, and based upon the results, all statistical evaluations were made relative to the pooled control replicates. The 72-hour cell densities and area under the growth curve values were evaluated for normality and homogeneity of variances using the Chi-Square test (5) and Bartlett's test (5), respectively and were analyzed statistically using Bonferroni T-test (5). The 72-hour no-observed-effect-concentration (NOEC) was determined through evaluation of the statistical results and the percent inhibition values.

#### Analytical Chemistry

Samples of test medium were collected from the treatment and control groups at 0 and 72 hours to measure concentrations of the test substance. A sample of the primary stock solution used to dose the test solutions was also collected on Day 0 of the test. Samples collected at 0 hours were taken from individual batches of test medium prepared at test initiation. Samples collected at 72 hours consisted of composited test medium from each of the three replicates in each respective treatment and control group. The samples were collected in glass bottles with Teflon®-lined caps and were analyzed immediately. The samples were analyzed according to an analytical method that was provided by the Sponsor, and previously verified by Wildlife International Ltd. The analytical methodology was validated in culture medium concurrently with the analysis of definitive samples. Analytical procedures used in the analysis of the samples are provided in Appendix III.

## RESULTS AND DISCUSSION

### Measurement of Test Concentrations

Analytical measurements were performed to verify exposure concentrations of Wingstay SN-1 in the test medium. Results of those analyses are presented in Table 1 and in the analytical chemistry report (Appendix III). No method was available for analyzing lower level test concentrations, therefore, all measurements are reported as nominal concentrations. Nominal concentrations selected for use in this study were 0.72, 1.8, 4.5, 11, 28 and 70 µg Wingstay SN-1/L. Samples collected at 0 hours showed measured values of less the limit of quantitation (LOQ) except for the 70 µg Wingstay SN-1/L concentration which showed a measured value of 53.2 µg Wingstay SN-1/L, representing 76% of nominal. The stock solution sample measured at 0 hours showed a measured value of 874,000 µg Wingstay SN-1/L representing 87% of nominal. The measured concentrations of samples collected at 72 hours were all below the LOQ.

### Observations and Measurements

Measurements of temperature are presented in Table 2, while measurements of pH are presented in Table 3. The temperatures ranged from 24.1 to 25.8°C and were within the 24±2°C range established for the test. Measurements of pH ranged from 7.3 to 7.4 at 0 hours and from 7.5 to 8.9 for the treatment and control groups at 72 hours. The increase of pH relative to algal population was typical for tests conducted with *Selenastrum capricornutum*. The light intensity ranged from 6450 to 7830 lux, which was within the desired range of 8000 ± 1600 lux.

The toxicity of Wingstay SN-1 to *Selenastrum capricornutum* was determined by measuring differences in area under the growth curve at the end of the 72-hour exposure period. Mean cell densities at 72 hours were used to calculate area under the growth curve values, which were subsequently used to calculate percent inhibition values. Mean area under the growth curve values are presented in Table 4, along with the corresponding percent inhibition values. EC50 values and 95% confidence limits calculated based upon area under the growth curve are given

in Table 5. Cell densities are presented graphically in Figure 1 and are listed in Appendix IV. Area under the growth curve values for the individual replicates are presented in Appendix V.

Changes in mean cell density indicated that exponential growth occurred in the negative and solvent control replicates (Figure 1). There were no statistically significant ( $p>0.05$ ) differences in area under the growth curve between the 0.72, 1.8, 4.5, 11 and 28  $\mu\text{g}$  Wingstay SN-1/L treatment groups and the pooled control replicates. The 72-hour percent inhibition values calculated relative to area under the growth curve for those treatments were 9.4, -7.3, 6.0, 21 and 15, respectively. Although not statistically significant ( $p>0.05$ ) compared to the pooled control group, the percent inhibition values for the 11 and 28  $\mu\text{g}$  Wingstay SN-1/L treatments could not be precluded as a treatment-related effect. Statistically significant differences ( $p<0.05$ ) were observed in area under the growth curve between the pooled controls and 70  $\mu\text{g}$  Wingstay SN-1/L treatment group. The growth inhibition value for this treatment group was 55% at 72 hours.

### CONCLUSION

The 72-hour EC50 value for *Selenastrum capricornutum* exposed to Wingstay SN-1 was determined to be 65  $\mu\text{g}$  Wingstay SN-1/L. The 72-hour no-observed-effect-concentration was determined to be 4.5  $\mu\text{g}$  Wingstay SN-1/L based on percent inhibition values.

**REFERENCES**

- 1 OECD. 1984. Guideline for Testing of Chemicals, 201: *Alga, Growth Inhibition Test*.
- 2 ASTM Standard Guide 1218-90E. *Standard Guide for Conducting Static 96-Hour Toxicity Tests with Microalgae*. August 1990.
- 3 Lotus Development Corporation, "Lotus 1-2-3 Release 3.1." Copyright 1990.
- 4 Norberg-King, T.J. *A Linear Interpolation Method for Sublethal Toxicity: The Inhibition Concentration (ICp) Approach (Version 2.0)*. U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, Minnesota. June 1993.
- 5 Gulley, D.D. "TOXSTAT Release 3.2," The University of Wyoming, July 1990.

- 17 -

Table 1

## Summary of Analytical Chemistry Data

Sponsor:	The Goodyear Tire & Rubber Company		
Test Substance:	Wingstay SN-1		
Test Organism:	Freshwater Alga, <i>Selenastrum capricornutum</i>		
Dilution Water:	Freshwater Algal Medium		
Nominal Test Concentration ( $\mu\text{g}$ Wingstay SN-1/L)	Sampling Time <sup>1,2</sup> (Days)	Concentration <sup>3,4</sup> ( $\mu\text{g}$ Wingstay SN-1/L)	Percent of Nominal
Negative Control	0	< 40	--
	3	< 80	
Solvent Control	0	< 40	--
	3	< 80	
0.72	0	< 40	--
	3	< 80	
1.8	0	< 40	--
	3	< 80	
4.5	0	< 40	--
	3	< 80	
11	0	< 40	--
	3	< 80	
28	0	< 40	--
	3	< 80	
70	0	53.2	76
	3	< 80	

<sup>1</sup> 0-hour Samples were collected from individual batches of test solution prepared for the treatment and control groups at test initiation.

<sup>2</sup> 72-hour samples were composites of test solution collected from each of the three replicates per treatment and control group.

<sup>3</sup> The limit of quantitation (LOQ) was based upon the lowest standard used in the calibration curve and the factor of two dilution of the test samples.

<sup>4</sup> The concentration of Wingstay SN-1 is calculated based upon the measurement of the bis ester of tetraethylene glycol and n-dodecanethiol which is present at 73.1% in Wingstay SN-1.

- 18 -

Table 2  
Temperature Measurements

Sponsor:	The Goodyear Tire & Rubber Company	
Test Substance:	Wingstay SN-1	
Test Organism:	Freshwater Alga, <i>Selenastrum capricornutum</i>	
Dilution Water:	Freshwater Algal Medium	
	Temperature (°C)	
Time (Hours)	Measurement 1	Measurement 2 <sup>1</sup>
0	24.1	24.2
24	24.2	24.4
48	24.2	25.8
72	24.4	24.4
<sup>1</sup> Temperature Measurement 2 was taken at least 4 hours after Measurement 1, with the exception of 72 hours.		

- 19 -

Table 3  
pH Measurements

Sponsor:	The Goodyear Tire & Rubber Company	
Test Substance:	Wingstay SN-1	
Test Organism:	Freshwater Alga. <i>Selenastrum capricornutum</i>	
Dilution Water:	Freshwater Algal Medium	
Nominal Test Concentration ( $\mu\text{g}$ Wingstay SN-1/L)	pH Measurements	
	0 Hours <sup>1</sup>	72 Hours <sup>2</sup>
Negative Control	7.3	7.5
Solvent Control	7.4	8.9
0.72	7.4	8.6
1.8	7.4	8.7
4.5	7.4	8.6
11	7.4	8.2
28	7.4	8.5
70	7.4	7.8
<sup>1</sup> 0-hour samples were collected from individual batches of test solution prepared for the treatment and control groups at test initiation.		
<sup>2</sup> 72-hour samples were composites of test solution collected from each of the three replicates per treatment and control group.		

- 20 -

Table 4

## Mean Area Under the Growth Curve and Percent Inhibition

Sponsor:		The Goodyear Tire & Rubber Company				
Test Substance:		Wingstay SN-1				
Test Organism:		Freshwater Alga, <i>Selenastrum capricornutum</i>				
Dilution Water:		Freshwater Algal Medium				
Nominal Test Concentration ( $\mu\text{g}$ Wingstay SN-1/L)	0 - 24 Hours		0 - 48 Hours		0 - 72 Hours	
	Mean Area	Percent Inhibition <sup>1</sup>	Mean Area	Percent Inhibition <sup>1</sup>	Mean Area	Percent Inhibition <sup>1</sup>
Negative Control	472,320	--	5,601,640	--	33,683,680	--
Solvent Control	539,480	--	6,352,280	--	35,921,040	--
Pooled Control	505,900	--	5,976,960	--	34,802,360	--
0.72	516,520	-2.1	5,239,360	12	31,524,640	9.4
1.8	489,840	3.2	5,541,720	7.3	37,330,240	-7.3
4.5	457,240	9.6	5,549,120	7.2	32,722,240	6.0
11	302,320	40	3,765,760	37	27,528,560	21
28	365,400	28	4,237,120	29	29,583,760	15
70	385,680	24	2,563,880	57	15,764,320	55 <sup>2</sup>

<sup>1</sup> Percent inhibition values were calculated relative to the pooled controls.

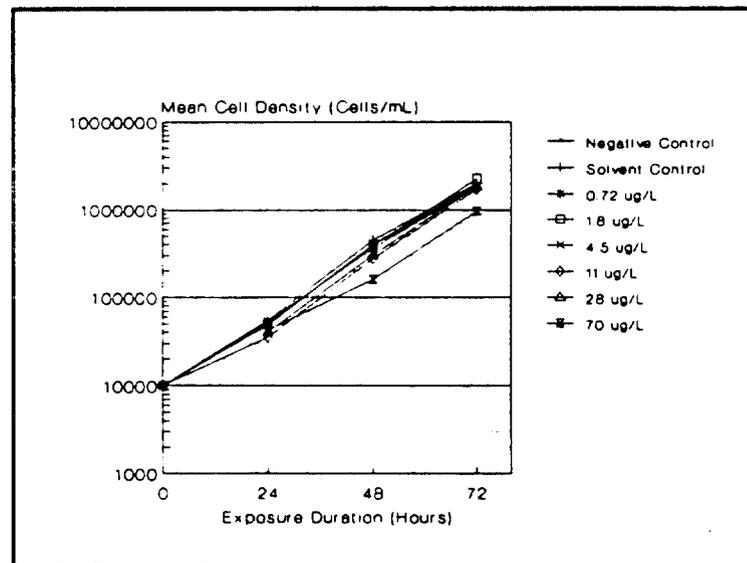
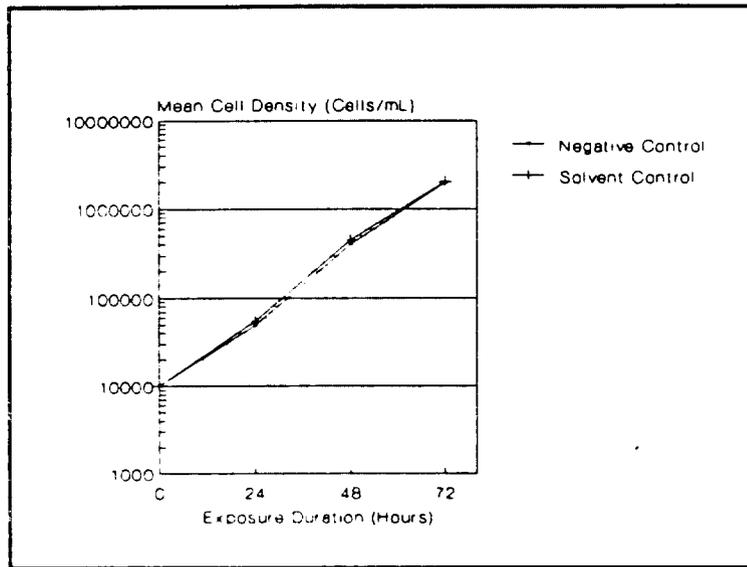
<sup>2</sup> Statistically significant ( $p < 0.05$ ) compared to the negative control.

- 21 -

Table 5  
EC50 Values Over the 72-Hour Exposure Period

Sponsor:	The Goodyear Tire & Rubber Company	
Test Substance:	Wingstay SN-1	
Test Organism:	Freshwater Alga, <i>Selenastrum capricornutum</i>	
Dilution Water:	Freshwater Algal Medium	
Time	EC50 <sup>1</sup> (µg Wingstay SN-1/L)	95% Confidence Limits <sup>3</sup> (µg Wingstay SN-1/L)
24 Hours	-- <sup>2</sup>	--
48 Hours	59	--
72 Hours	65	--
<sup>1</sup> EC50 values were calculated using linear interpolation method. <sup>2</sup> The growth rate pattern did not allow for the calculation of an EC50 value. <sup>3</sup> The pattern of inhibition did not allow for the calculation of 95% confidence limits.		

Figure 1. Dose-Response Curve for *Selenastrum capricornutum* Exposed to Wingstay SN-1 for 72 Hours.



## APPENDIX I

Freshwater Algal Medium<sup>1</sup>

Sponsor:	The Goodyear Tire & Rubber Company
Test Substance:	Wingstay SN-1
Test Organism:	Freshwater Alga. <i>Selenastrum capricornutum</i>
Dilution Water:	Freshwater Algal Medium

Compound <sup>2</sup>	Nominal Concentration
MgCl <sub>2</sub> •6H <sub>2</sub> O	12.16 mg/L
CaCl <sub>2</sub> •2H <sub>2</sub> O	4.40 mg/L
H <sub>3</sub> BO <sub>3</sub>	0.1856 mg/L
MnCl <sub>2</sub> •4H <sub>2</sub> O	0.416 mg/L
ZnCl <sub>2</sub>	3.28 µg/L
FeCl <sub>3</sub> •6H <sub>2</sub> O	0.1598 mg/L
CoCl <sub>2</sub> •6H <sub>2</sub> O	1.428 µg/L
Na <sub>2</sub> MoO <sub>4</sub> •2H <sub>2</sub> O	7.26 µg/L
CuCl <sub>2</sub> •2H <sub>2</sub> O	0.012 µg/L
Na <sub>2</sub> EDTA•2H <sub>2</sub> O	0.300 mg/L
NaNO <sub>3</sub>	25.50 mg/L
MgSO <sub>4</sub> •7H <sub>2</sub> O	14.70 mg/L
K <sub>2</sub> HPO <sub>4</sub>	1.044 mg/L
NaHCO <sub>3</sub>	15.0 mg/L

<sup>1</sup> The pH was adjusted, as necessary, to 7.5 ± 0.1 using 0.1 N NaOH and/or 10% HCl

<sup>2</sup> Manufacturers and lot numbers for individual chemical constituents of algal medium are maintained as facility records

- 24 -

## APPENDIX II

Analyses of Pesticides, Organics, Metals and Other Inorganics  
in Wildlife International Ltd. Well Water<sup>1</sup>

Sponsor:	The Goodyear Tire & Rubber Company
Test Substance:	Wingstay SN-1
Test Organism:	Freshwater Alga, <i>Selenastrum capricornutum</i>
Dilution Water:	Freshwater Algal Medium

ANALYSIS	MEASURED CONCENTRATION	
<b>Organophosphorus &amp; Organonitrogen Pesticides</b>		
Azodrin (Monochrotophos)	<	2.50 µg/L
Bolstar	<	0.266 µg/L
Chlorpyrifos	<	0.267 µg/L
Coumaphos	<	0.500 µg/L
Demeton	<	0.265 µg/L
Diazinon	<	0.265 µg/L
Dichlorvos	<	0.260 µg/L
Dimethoate	<	0.250 µg/L
Disulfoton	<	0.255 µg/L
EPN	<	0.500 µg/L
Ethoprop	<	0.275 µg/L
Fenthion	<	0.252 µg/L
Fensulfothion	<	0.512 µg/L
Guthion (Methyl Azinphos)	<	0.500 µg/L
Malathion	<	0.270 µg/L
Merphos	<	0.246 µg/L
Mevinphos	<	0.255 µg/L
Naled	<	1.34 µg/L
Methylparathion	<	0.250 µg/L
Parathion	<	0.288 µg/L
Phorate	<	0.242 µg/L
Ronnel	<	0.257 µg/L
Surofos	<	0.500 µg/L
Sulfotepp	<	0.260 µg/L
Tepp	<	1.04 µg/L
Tokuthion	<	0.276 µg/L
Trichloronate	<	0.265 µg/L
<b>Metals and Other Inorganics</b>		
Aluminum	<	50.0 µg/L
Arsenic	<	2.5 µg/L
Beryllium	<	5.0 µg/L
Cadmium	<	5.0 µg/L
Calcium	<	32800 µg/L
Chromium	<	10.0 µg/L
Copper	<	5.0 µg/L
Iron	<	45.0 µg/L
Lead	<	2.0 µg/L
Magnesium	<	13.1 mg/L
Manganese	<	5.0 µg/L
Nickel	<	15.0 µg/L
Potassium	<	6730 µg/L
Selenium	<	2.5 µg/L
Silver	<	5.0 µg/L
Sodium	<	21200 µg/L
Zinc	<	30.0 µg/L
Mercury	<	0.20 µg/L
Molybdenum	<	10.0 µg/L

<sup>1</sup> Analyses performed by Environmental Science & Engineering, Inc., Gainesville, Florida for samples collected on May 10, 1994.

APPENDIX II (Continued)  
Analyses of Pesticides, Organics, Metals and Other Inorganics  
in Wildlife International Ltd. Well Water<sup>1</sup>

Sponsor:	The Goodyear Tire & Rubber Company
Test Substance:	Wingstay SN-1
Test Organism:	Freshwater Alga, <i>Selenastrum capricornutum</i>
Dilution Water:	Freshwater Algal Medium

ANALYSIS	MEASURED CONCENTRATION	
<b>Miscellaneous Measurements</b>		
Total Dissolved Solids	248	mg/L
Ammonia Nitrogen	< 0.050	mg/L
Total Organic Carbon <sup>2</sup>	< 0.5	mg/L
Total Cyanide	< 0.003	mg/L
<b>Organochlorines and PCBs</b>		
Aldrin	< 0.005	µg/L
Alpha BHC	< 0.005	µg/L
Beta BHC	< 0.005	µg/L
Delta BHC	< 0.005	µg/L
Gamma BHC (Lindane)	< 0.005	µg/L
Chlordane	< 0.025	µg/L
DDD, pp'	< 0.005	µg/L
DDE, pp'	< 0.005	µg/L
DDT, pp'	< 0.005	µg/L
Dieldrin	< 0.005	µg/L
Endosulfan, A	< 0.005	µg/L
Endosulfan, B	< 0.005	µg/L
Endosulfan Sulfate	< 0.005	µg/L
Endrin	< 0.005	µg/L
Endrin Aldehyde	< 0.005	µg/L
Heptachlor	< 0.005	µg/L
Methoxychlor	< 0.005	µg/L
Heptachlor Epoxide	< 0.005	µg/L
Toxaphene	< 0.500	µg/L
PCB-1016	< 0.100	µg/L
PCB-1221	< 0.100	µg/L
PCB-1232	< 0.100	µg/L
PCB-1242	< 0.100	µg/L
PCB-1248	< 0.100	µg/L
PCB-1254	< 0.100	µg/L
PCB-1260	< 0.100	µg/L
<b>Chlorophenoxy Acid Herbicides</b>		
2,4-D, Total	< 0.020	µg/L
2,4-DB	< 0.020	µg/L
2,4,5-T Water	< 0.020	µg/L
2,4,5-TP/Silvex	< 0.020	µg/L
Dalapon	< 0.020	µg/L
Dicamba (Banvel)	< 0.020	µg/L
Dichloroprop	< 0.020	µg/L
Dinoseb	< 0.020	µg/L
MCPA	< 0.410	µg/L
MCPP	< 0.400	µg/L

<sup>1</sup> Analyses performed by Environmental Science & Engineering, Inc., Gainesville, Florida for samples collected on May 10, 1994.

<sup>2</sup> Analyses performed by Wildlife International Ltd. for the sample collected on May 27, 1994.

- 26 -

APPENDIX III

THE ANALYSIS OF WINGSTAY SN-1 IN FRESHWATER ALGAL MEDIA  
IN SUPPORT OF  
WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 414A-101

## APPENDIX III

Introduction

Freshwater alga media samples were collected from an acute aquatic toxicity study designed to determine the effects of WINGSTAY SN-1 to the freshwater alga (*Selenastrum capricornutum*). This study was conducted by Wildlife International Ltd. and identified as WIL Project No. 414A-101. The analyses of these algal media samples were performed at Wildlife International Ltd. by high performance liquid chromatography (HPLC) with UV detection. The analytical method was verified on August 22, 1995 at Wildlife International Ltd and reported under WIL Project No. 414A-102. Samples were received for analysis on September 29 and October 2, 1995 and analyzed between September 29 and October 2, 1995 and reanalyzed on November 5 and 6, 1995.

Test Substance and Analytical Standard

The test substance, received from The Goodyear Tire and Rubber Company on December 13, 1994, was used to prepare matrix fortification samples. The test substance was an off-white waxy solid, identified on the label as: Wingstay SN-1; Lot # 130893; Notebook # 10024-64-3. The test substance did not have a reported purity or an expiration date. Upon receipt, the test substance was assigned Wildlife International Ltd. identification number WIL #3080 and stored under ambient conditions.

The analytical standard, received from The Goodyear Tire and Rubber and Company on March 30, 1995, was used to prepare calibration standards. The analytical standard was a white solid, identified on the label as: bis component of Wingstay SN-1; Notebook Number 9988-5Z; Goodyear Notebook # 10024-65-5. The analytical standard had a reported purity of 98.06% and no expiration date was given. Upon receipt, the analytical standard was assigned Wildlife International Ltd. identification number WIL #3177 and stored under ambient conditions.

## APPENDIX III

Analytical Method

The method used for the analysis of the water samples was based upon methodology provided by The Goodyear Tire and Rubber Company and entitled: "Wingstay® SN-1 Determination of Water Solubility".

The analytical method consisted of diluting an aliquot of the aqueous sample with an equal volume of acetonitrile (Burdick & Jackson, Lot No. BK932; Fisher 54019). These solutions were then placed in crimp or snap top vials prior to analysis by HPLC with UV detection. Concentrations of Wingstay SN-1 in the samples were determined by high performance liquid chromatography (HPLC) using a Hewlett-Packard Model 1090 HPLC equipped with a diode array detector (DAD). HPLC separations were achieved using a C8 guard column and Betasil C8 column (3.0 mm I.D. x 250 mm, 5 µm particle size). The instrument parameters are summarized in Table 1. A method flow chart is provided in Figure 1.

Because of an interference peak in the samples from the end of the test, all the samples were reanalyzed. The HPLC separations were achieved using a Zorbax RX-C8 guard column and Zorbax RX-C8 column (4.6 mm I.D. x 150 mm, 5 µm particle size). The instrument parameters are summarized in Table 2.

Calibration Curve, Limit of Detection and Limit of Quantitation

Calibration standards of the bis ester of tetraethylene glycol and n-dodecanethiol, ranging in concentration from 20 to 1000 µg/L, were analyzed with each series of samples. Linear regression equations were generated using the peak area responses versus the respective concentrations of the calibration standards. Examples of calibration curves are presented in Figures 2 and 3. The concentration of test substance in the samples was determined by substituting the area responses of the analyte into the applicable linear regression equation and

## APPENDIX III

converting to test substance concentration. Representative chromatograms of low and high calibration standards are shown in Figures 4 and 5, and Figures 6 and 7 from reanalysis.

The instrument limit of detection (LOD) was set based upon the injection volume (200  $\mu\text{L}$  or 250  $\mu\text{L}$ ) and the lowest standard concentration (20  $\mu\text{g/L}$ ). The LOD was set at 4 ng or 5 ng injected on-column. The method limit of quantitation (LOQ) for these analyses was set at 40  $\mu\text{g/L}$  or 80  $\mu\text{g/L}$  based upon the lowest standard used in the calibration curves and dilution of samples with an equal volume of acetonitrile.

Matrix Blank and Fortification Samples

Along with the actual sample analyses, three matrix blanks were analyzed to determine possible interference. No interferences were observed near the retention time of the analyte at or above the LOQ during the sample analyses (Table 3). Representative chromatograms of matrix blanks are presented in Figures 8 and 9.

Freshwater algal media samples were fortified at 0.5, 10, 40, 50, 100, 500 and 1000  $\mu\text{g/L}$  and analyzed concurrently with the samples to determine the mean procedural recovery (Table 4). The mean procedural recovery was 106% for fortifications at 50, 100, 500 and 1000  $\mu\text{g/L}$ . The samples were not corrected for the procedural recovery because the analytical method involved dilution and direct injection of the samples. Representative chromatograms of matrix fortifications are presented in Figure 10 and 11.

RESULTS

During method development, an interference was found (Figure 12). Due to the interference observed, it was not possible to develop a method capable of quantitating test concentration below 40  $\mu\text{g/L}$ . The interference peak found in the Day 3 samples with algae

## APPENDIX III

present eluted prior to the analyte (Figure 13). Better resolution of the interference was achieved upon reanalysis of the study samples.

Sample Analysis

Freshwater algal media samples were collected from the acute toxicity test with the freshwater alga (*Selenastrum capricornutum*) at test initiation (Day 0), September 29, 1995, and at test termination (Day 3) on October 2, 1995. The concentration of Wingstay SN-1 in the 70 µg/L test sample collected at initiation of exposure of the test organisms (Day 0) was 76% of the nominal concentration and 86% upon reanalysis of the sample (Table 5). Chromatograms of Day 0 sample 70 µg/L are shown in Figures 14 and 15. The stock solution used to prepare exposure solutions was analyzed and found to be 87% of the nominal concentration and 74% upon reanalysis

- 31 -

## APPENDIX III

Table 1

## Typical HPLC Operational Parameters

---

---

INSTRUMENT:	Hewlett-Packard Model 1090 High Performance Liquid Chromatograph with a Photo Diode Array Detector (DAD)
GUARD COLUMN	Javelin Betasil C8 (3.0 mm x 20 mm)
ANALYTICAL COLUMN	Keystone Scientific, Inc. Betasil C8 (3.0 mm x 250 mm, 5 $\mu$ m particle size)
STOP TIME	15 minutes
POST TIME	0.0 minute
FLOW RATE	0.60 mL/minute
OVEN TEMPERATURE	40°C
SOLVENT A	100% Acetonitrile (Burdick & Jackson, Lot No. BK329)
INJECTION VOLUME	200 $\mu$ L
BIS ESTER COMPONENT PEAK RETENTION TIME:	Approximately 8.4 minutes
PRIMARY ANALYTICAL WAVELENGTH	210 nm (4 nm bandwidth)
SECONDARY ANALYTICAL WAVELENGTH:	450 (80 nm bandwidth)

---

---

- 32 -

## APPENDIX III

Table 2

## Reanalysis HPLC Operational Parameters

INSTRUMENT	Waters 616 Pump with 6005 controller, 717 Plus Autosampler, 486 Tunable Absorbance Detector with Millenium 2.10 Software and a Keystone Scientific Column Oven
GUARD COLUMN:	Zorbax RX-C8 (4.6 mm I.D. x 1.25 mm, 5 $\mu$ m particle size))
ANALYTICAL COLUMN	Zorbax RX-C8 (4.6 mm I.D. x 150 mm, 5 $\mu$ m particle size)
STOP TIME	20 minutes
POST TIME	0.0 minute
FLOW RATE:	1.0 mL/minute
OVEN TEMPERATURE:	40°C
SOLVENT A	95% Acetonitrile (Burdick & Jackson, Lot No. BK932)
SOLVENT B	5% Water
INJECTION VOLUME:	250 $\mu$ L
BIS ESTER COMPONENT PEAK RETENTION TIME:	Approximately 9.4 minutes
PRIMARY ANALYTICAL WAVELENGTH:	210 nm

## APPENDIX III

Table 3

## Matrix Blanks Analyzed Concurrently During Sample Analysis

Sample		Measured Concentration of Wingstay SN-1 ( $\mu\text{g/L}$ ) <sup>1,2</sup>
Number (414A-101-)	Type	
MAB-1	Matrix Blank	< 40
MAB-2	Matrix Blank	< 80
MAB-3 <sup>3</sup>	Matrix Blank	< 40

<sup>1</sup> The concentration of Wingstay SN-1 is calculated based upon the measurement of the bis ester of tetraethylene glycol and n-dodecanethiol which is present at 73.1% in Wingstay SN-1.

<sup>2</sup> The limit of quantitation (LOQ) was based upon the lowest standard used in the calibration curve and the factor of two dilution of the test samples.

<sup>3</sup> Result from reanalysis.

- 34 -

## APPENDIX III

Table 4

## Matrix Fortifications Analyzed Concurrently During Sample Analysis

Sample Number (414A-101-)	Concentrations of Wingstay SN-1 ( $\mu\text{g/L}$ )		Percent Recovered
	Fortified	Measured <sup>1,2</sup>	
MAS-4	0.50	< 40	--
MAS-5	10	< 80	--
MAS-8 <sup>4</sup>	40	< 40	--
MAS-1	50	59.3	119
MAS-6	50	(30.2) <sup>3</sup>	60
MAS-9 <sup>4</sup>	100	108	108
MAS-2	500	510	102
MAS-3	1000	1000	100
MAS-7	1000	1020	102
			Mean = 106%
			Standard Deviation = 7.8%
			N = 5

<sup>1</sup> The concentration of Wingstay SN-1 is calculated based upon the measurement of the bis ester of tetraethylene glycol and n-dodecanethiol which is present at 73.1% in Wingstay SN-1.

<sup>2</sup> The limit of quantitation (LOQ) was based upon the lowest standard used in the calibration curve and the factor of two dilution of the test samples.

<sup>3</sup> Extrapolated value since peak area is less than the peak area of the lowest standard (40.16  $\mu\text{g/L}$ ) in the calibration curve. Not used to calculate mean recovery.

<sup>4</sup> Results from reanalysis.

## APPENDIX III

Table 5

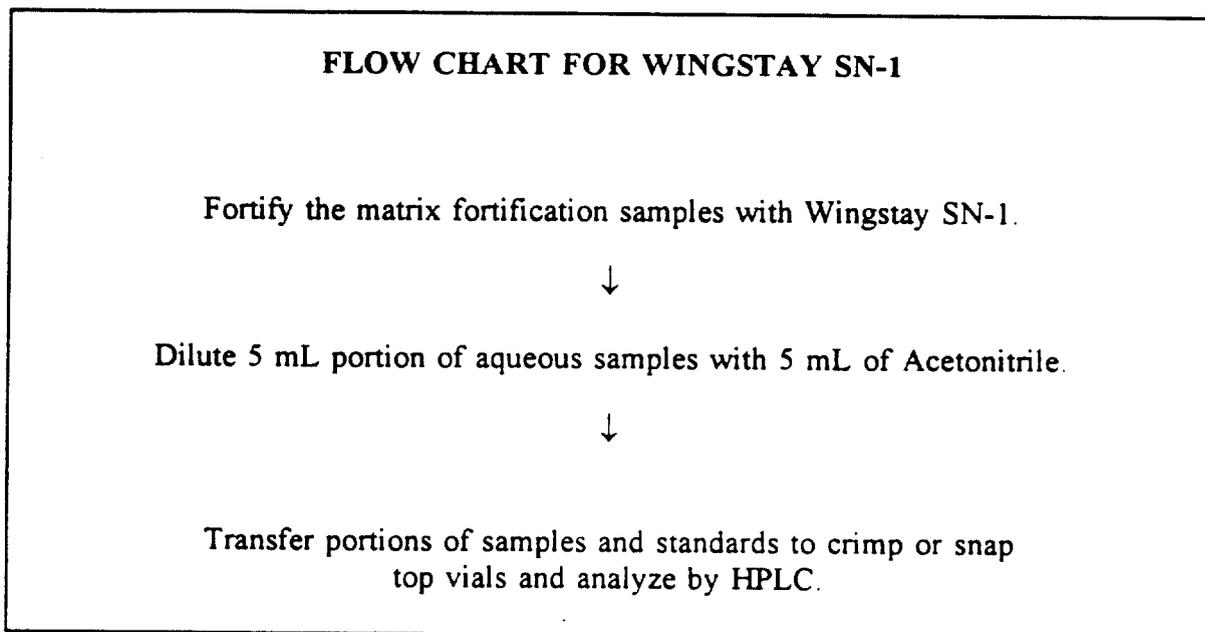
Measured Concentrations of Wingstay SN-1 in Freshwater Algal Medium Samples  
from an Acute Toxicity Test with the Freshwater Alga (*Selenastrum capricornutum*)

Nominal Concentration (µg/L)	Sample Number (414A-101-)	Sampling Time (Days)	Wingstay SN-1 Concentration <sup>1,2</sup> (µg/L)	Percent of Nominal	Reanalysis Wingstay SN-1 Concentration <sup>1,2</sup> (µg/L)	Percent of Nominal
0.0 (Negative Control)	1	0	< 40	--	< 40	--
	10	3	< 80	--	< 40	--
0.0 (Solvent Control)	2	0	< 40	--	< 40	--
	11	3	< 80	--	< 40	--
0.72	3	0	< 40	--	< 40	--
	12	3	< 80	--	< 40	--
1.8	4	0	< 40	--	< 40	--
	13	3	< 80	--	< 40	--
4.5	5	0	< 40	--	< 40	--
	14	3	< 80	--	< 40	--
11	6	0	< 40	--	< 40	--
	15	3	< 80	--	< 40	--
28	7	0	< 40	--	< 40	--
	16	3	< 80	--	< 40	--
70	8	0	53.2	76	60.1	86
	17	3	< 80	--	< 40	--
1000000	9	0	874000	87	743000	74

<sup>1</sup> The concentration of Wingstay SN-1 is calculated based upon the measurement of the bis ester of tetraethylene glycol and n-dodecanethiol which is present at 73.1% in Wingstay SN-1.

<sup>2</sup> The limit of quantitation (LOQ) was based upon the lowest standard used in the calibration curve and the factor of two dilution of the test samples.

APPENDIX III



**Figure 1.** Analytical method flow chart for the analysis of Wingstay SN-1 in freshwater algal medium.

APPENDIX III

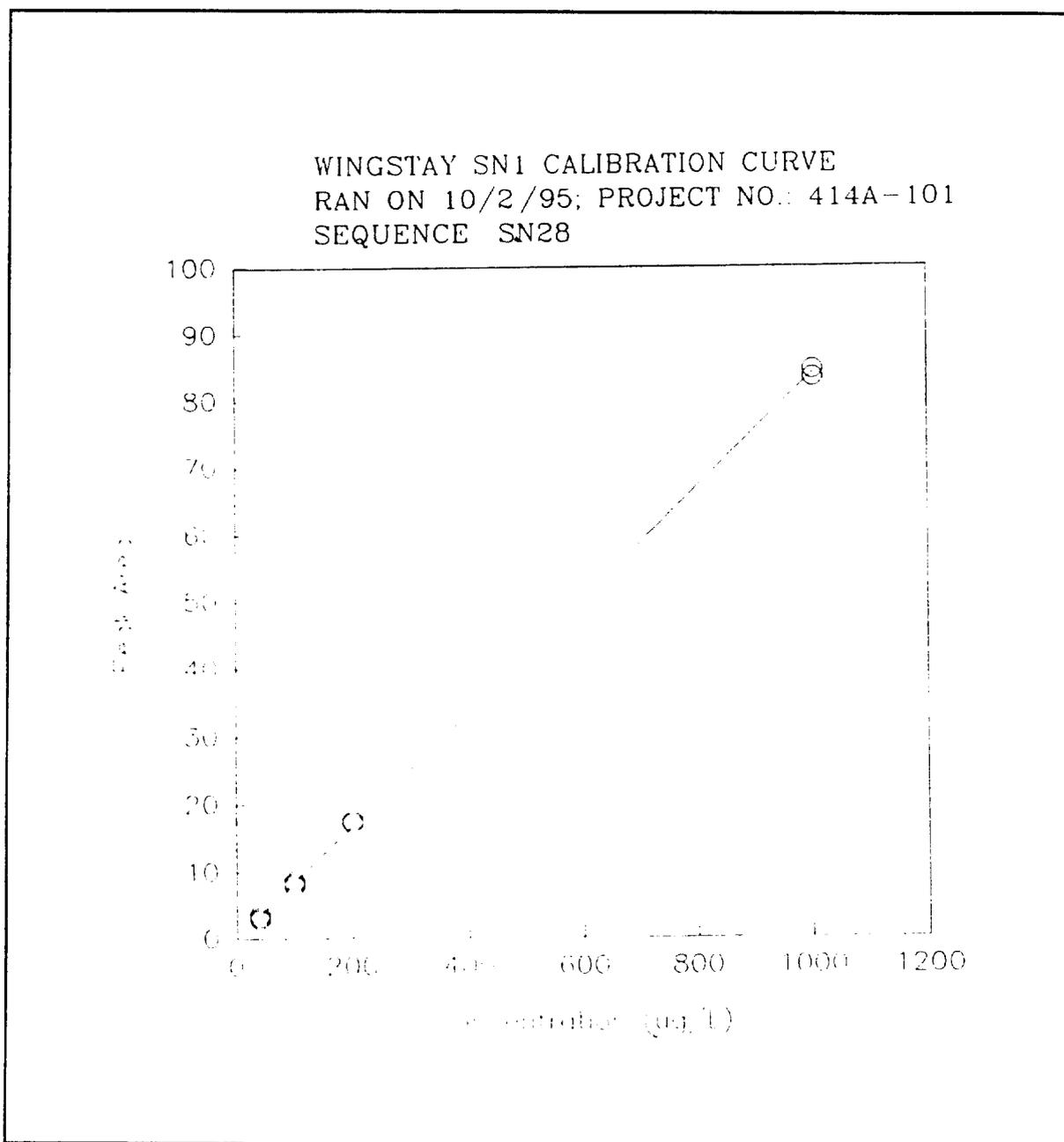
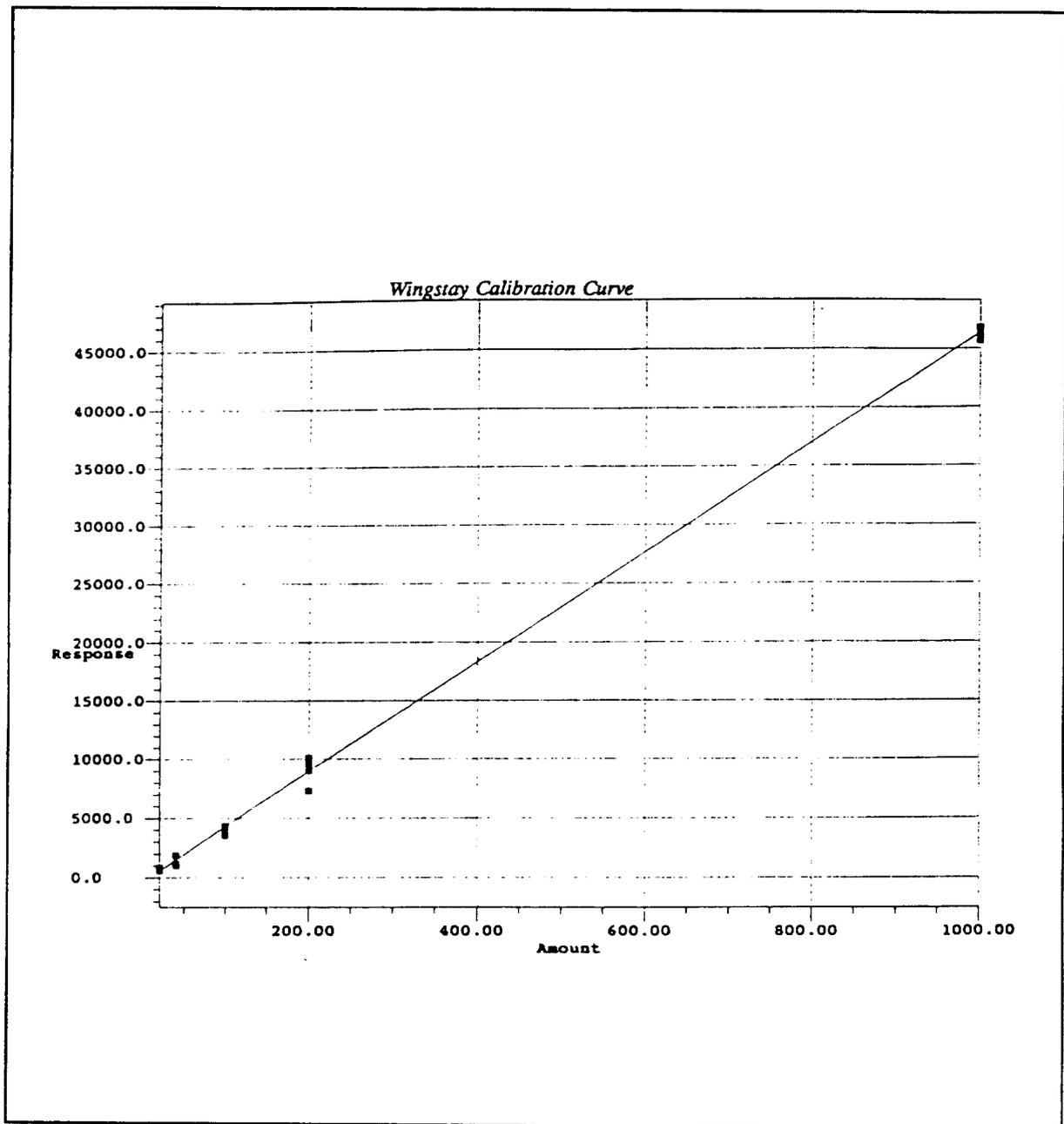


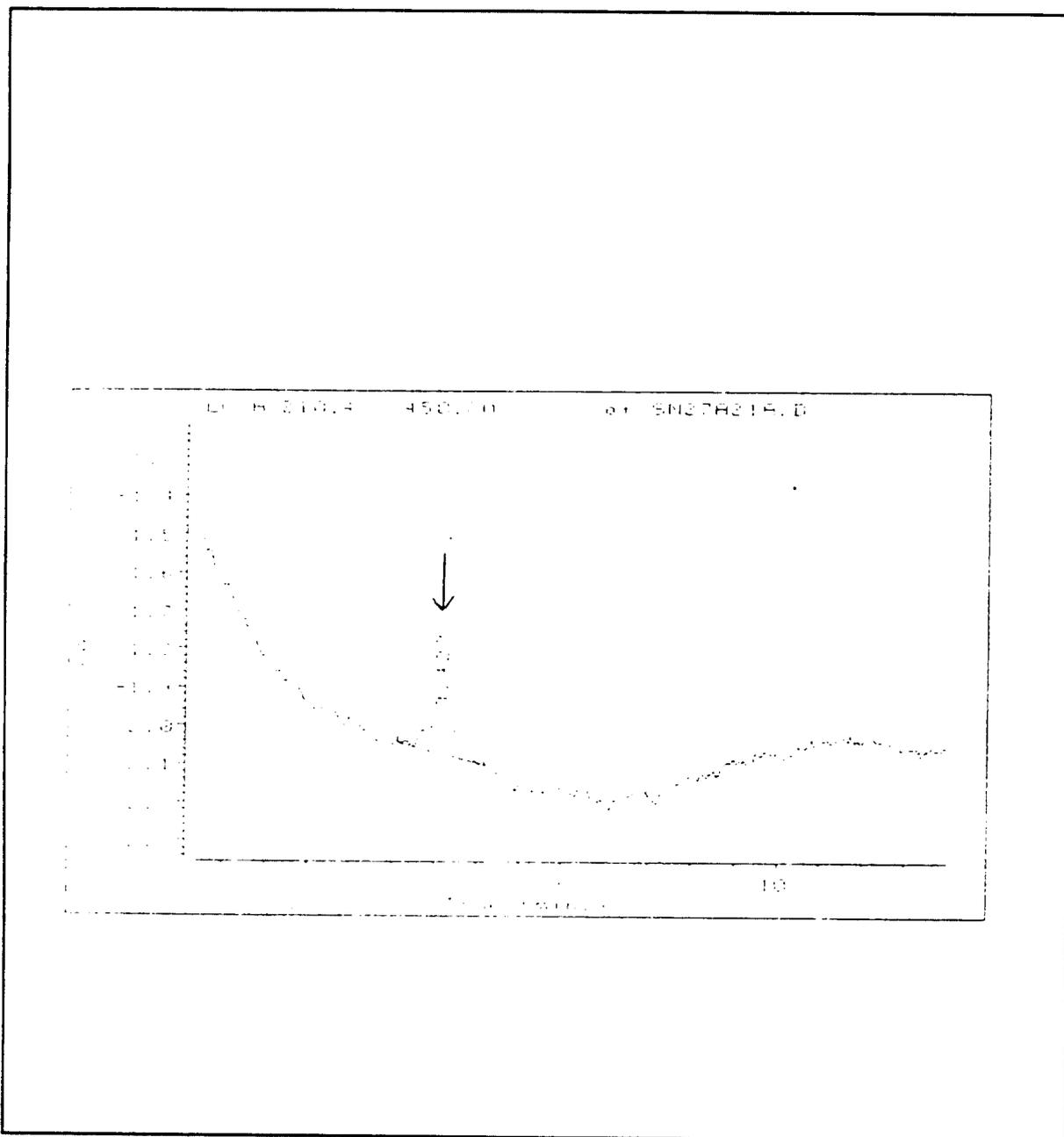
Figure 2. A representative calibration curve for Wingstay SN-1. Slope = 0.0836; Intercept = 0.0891.

APPENDIX III



**Figure 3.** A representative calibration curve for Wingstay SN-1. Slope = 46.778960; Intercept = -408.718365.

APPENDIX III



**Figure 4.** A representative chromatogram of a 20 µg/L Wingstay SN-1 standard (4 ng on-column).

APPENDIX III

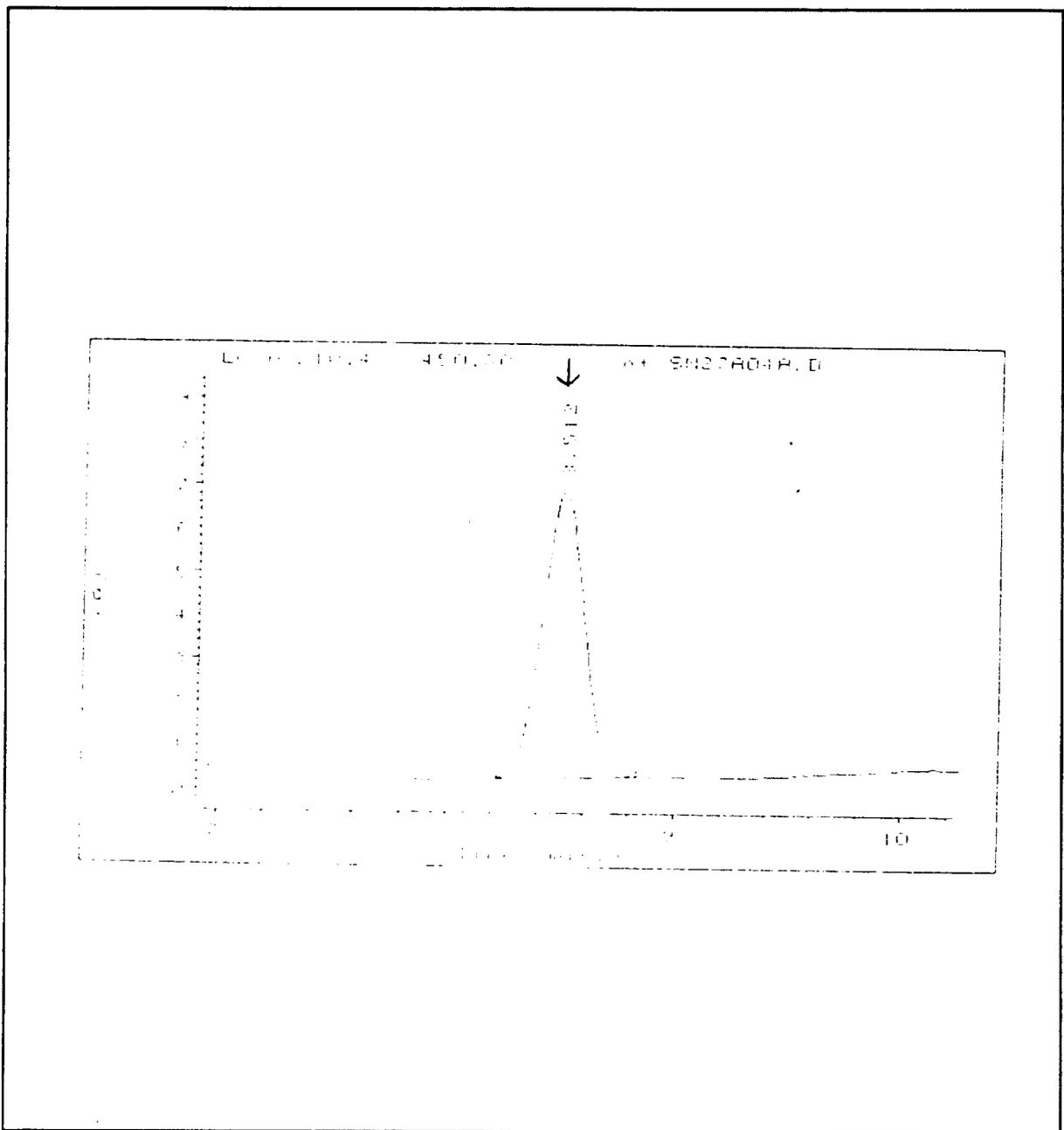


Figure 5. A representative chromatogram of a 1000 µg/L Wingstay SN-1 standard (200 ng on-column).

APPENDIX III

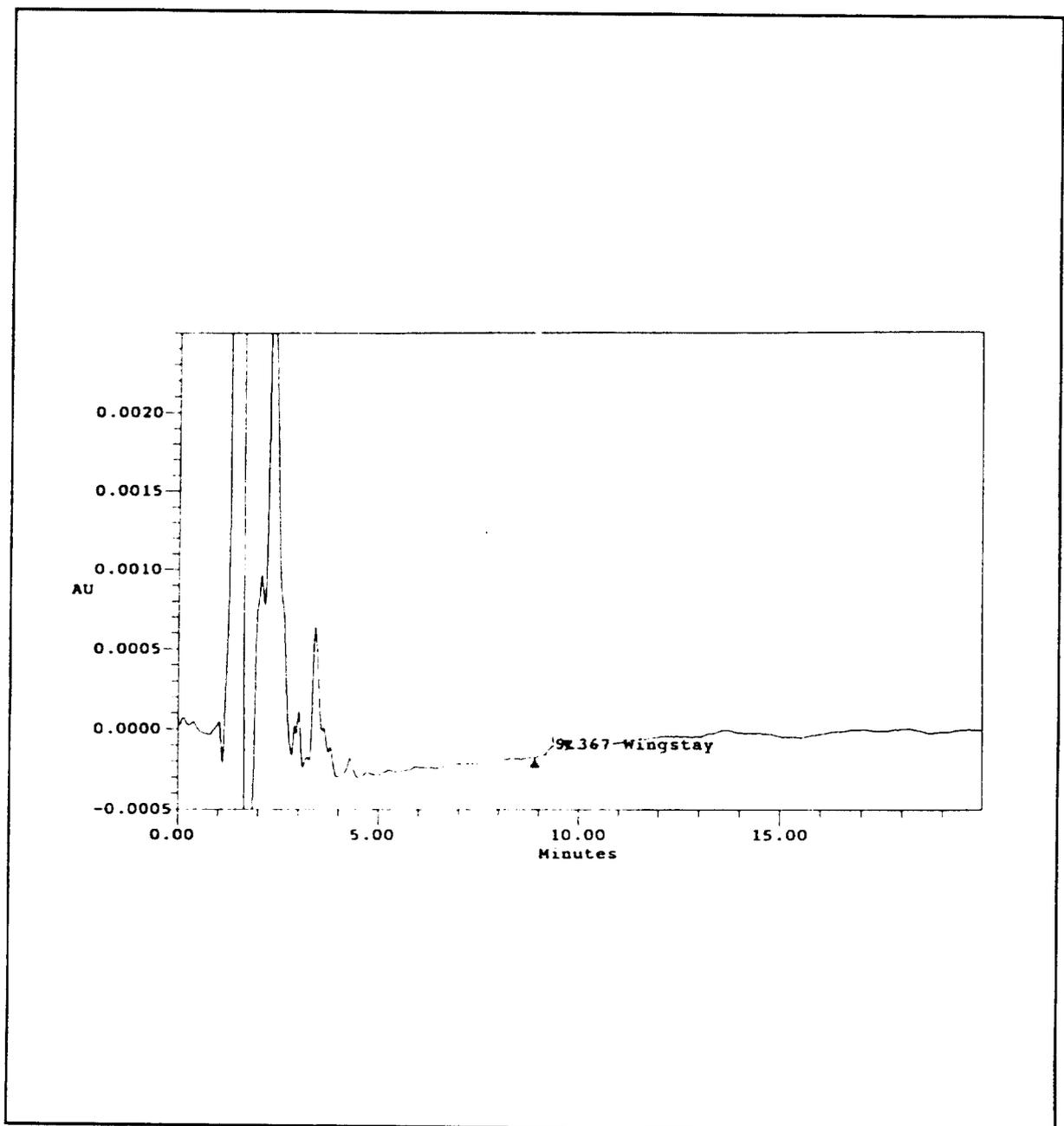


Figure 6. A representative chromatogram of a 20 µg/L Wingstay SN-1 standard (5 ng on-column).

APPENDIX III

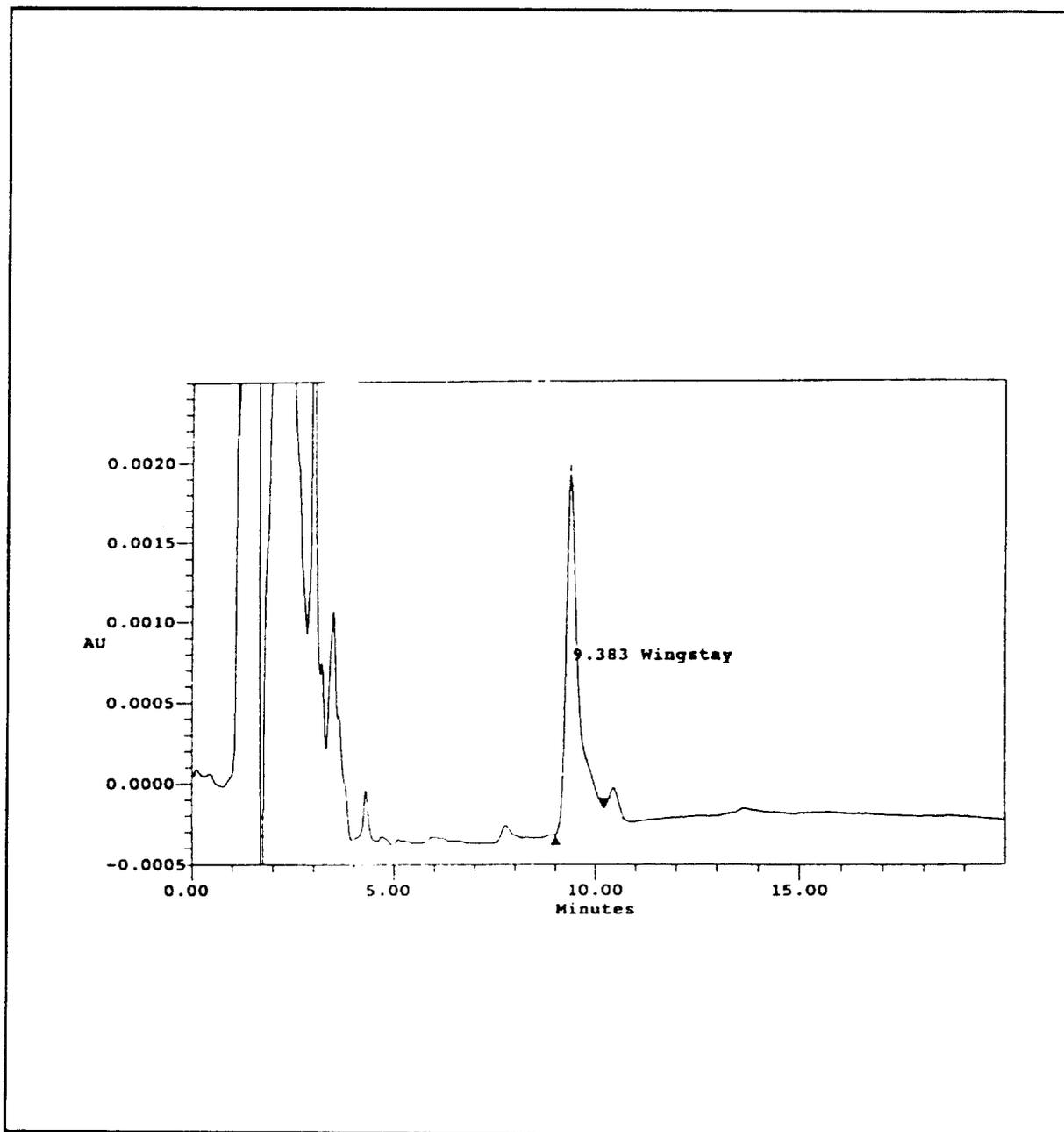
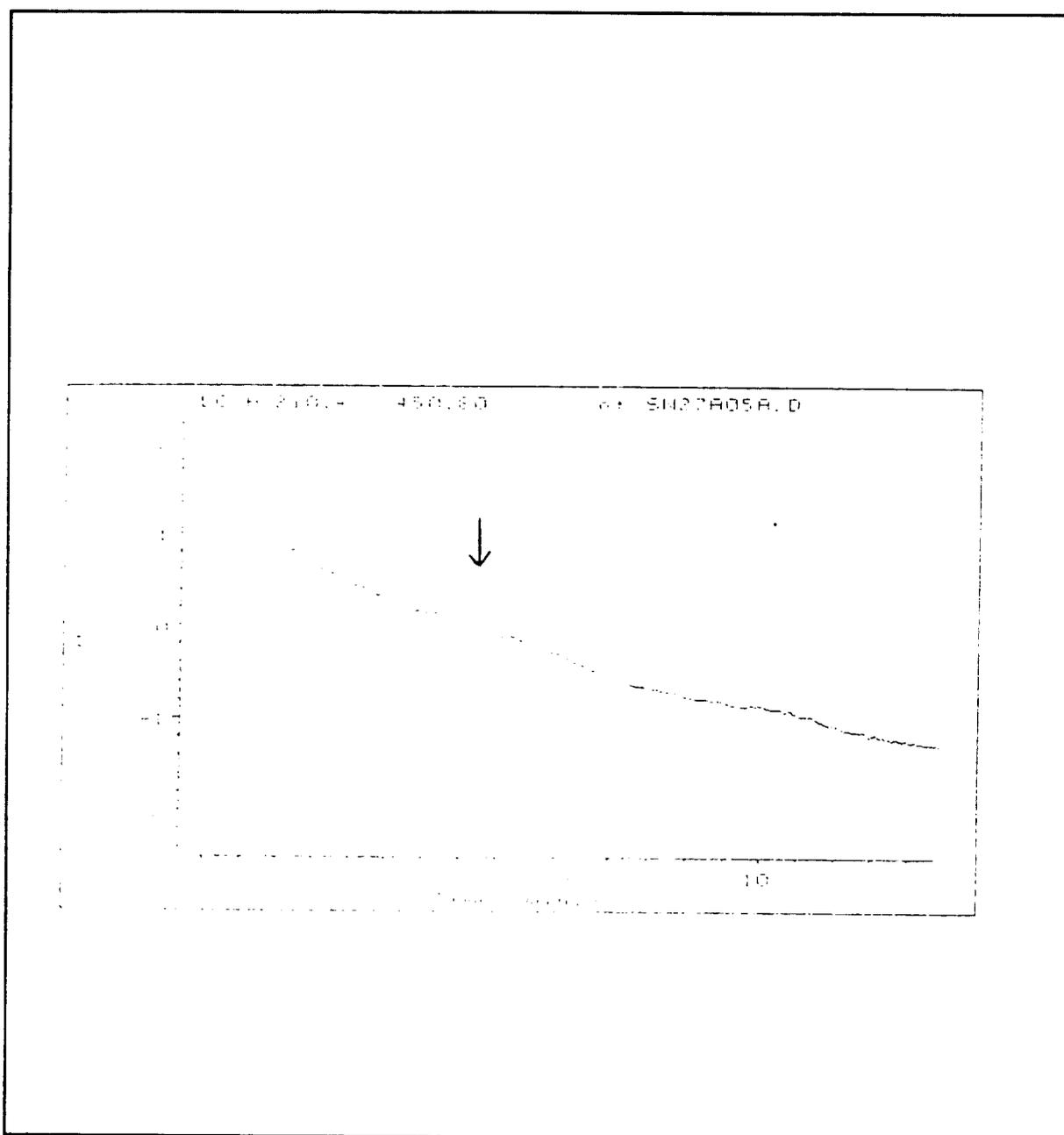


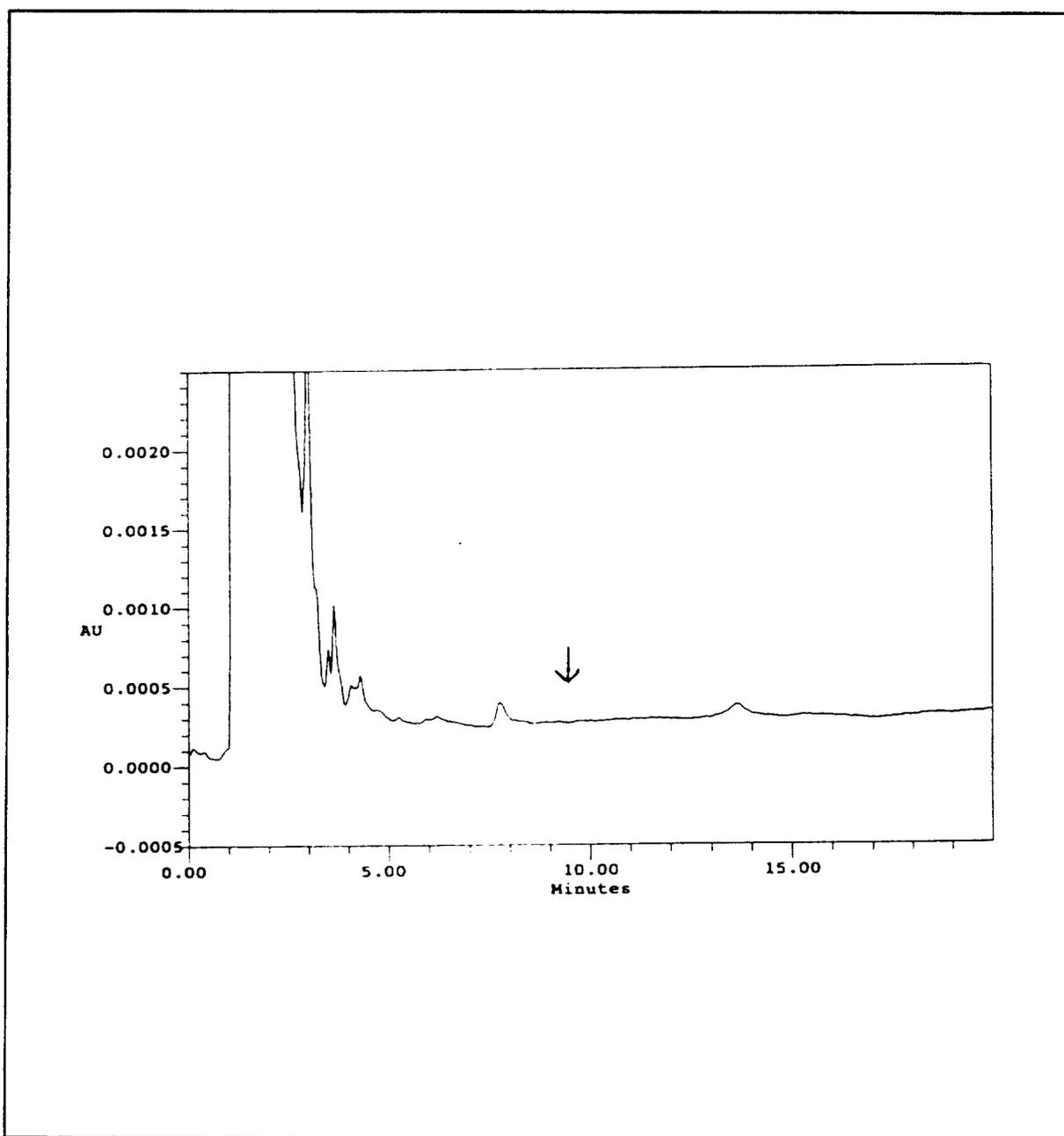
Figure 7. A representative chromatogram of a 1000 µg/L Wingstay SN-1 standard (250 ng on-column).

APPENDIX III



**Figure 8.** A representative chromatogram of a matrix blank, 414A-101-MAB-1. Arrow indicates the retention time of Wingstay SN-1.

APPENDIX III



**Figure 9.** A representative chromatogram of a matrix blank, 414A-101-MAB-3. Arrow indicates the retention time of Wingstay SN-1.

APPENDIX III

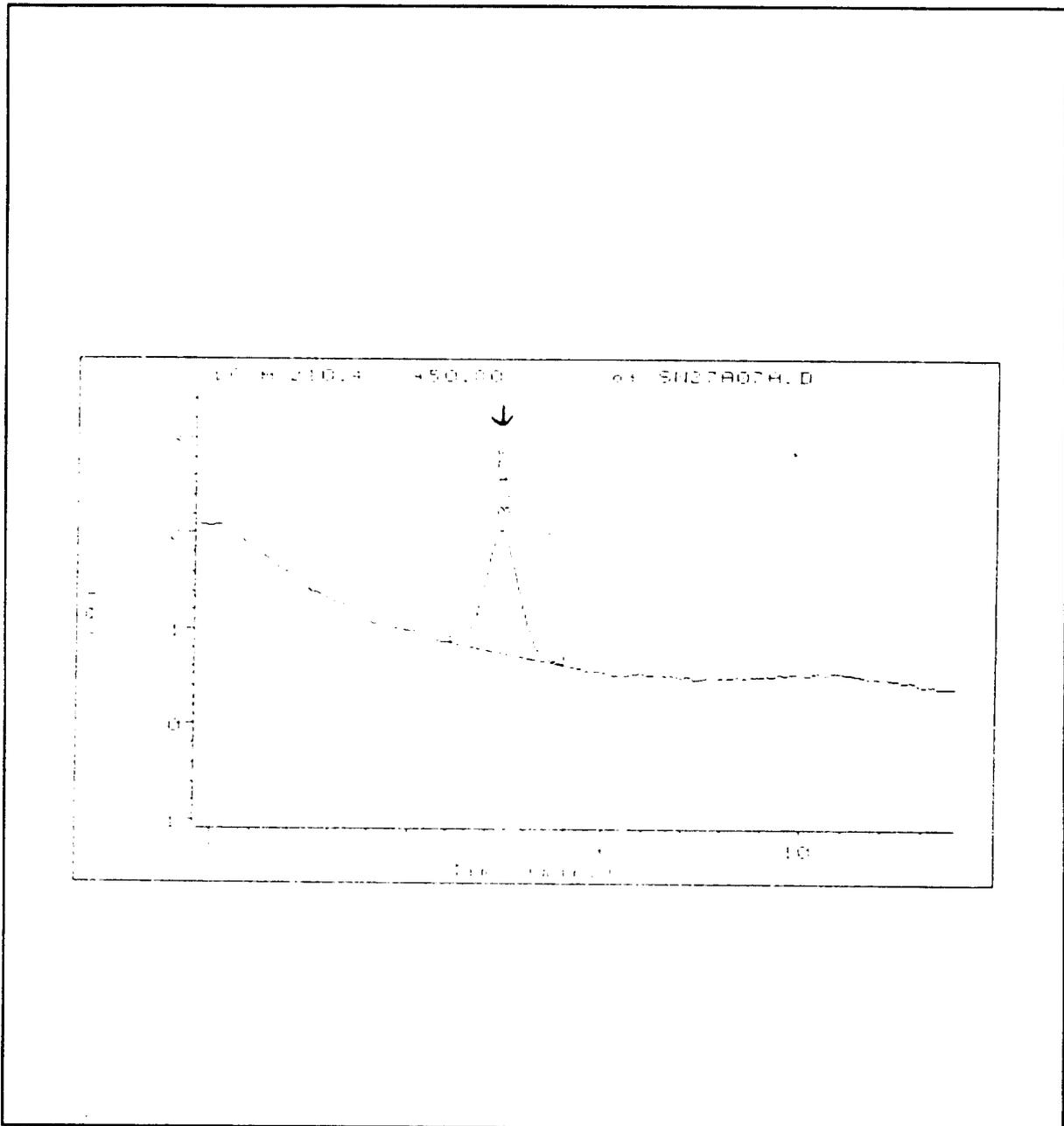
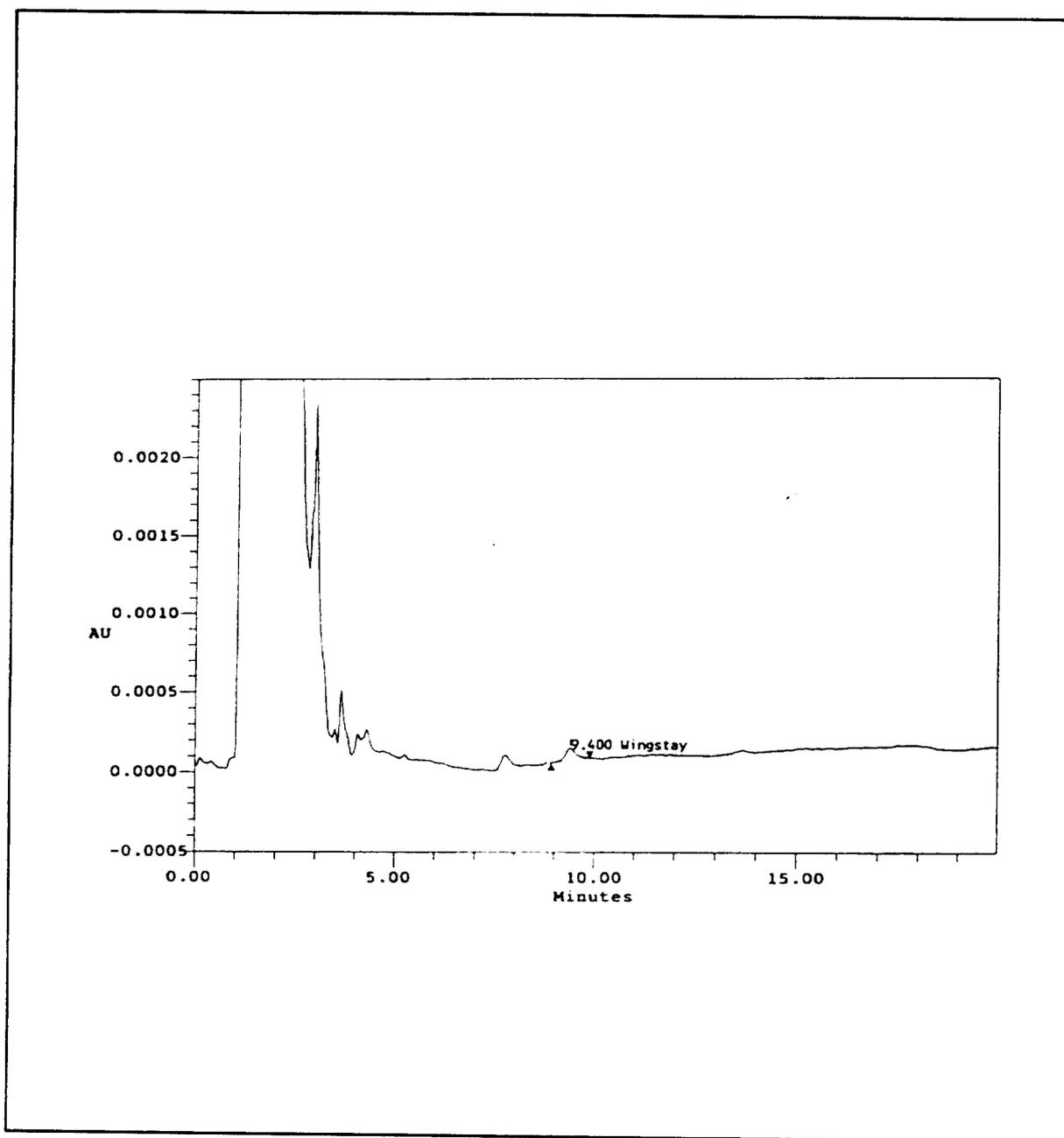


Figure 10. A representative chromatogram of a matrix fortification, 414A-101-MAS-2, (500 µg/L Wingstay SN-1).

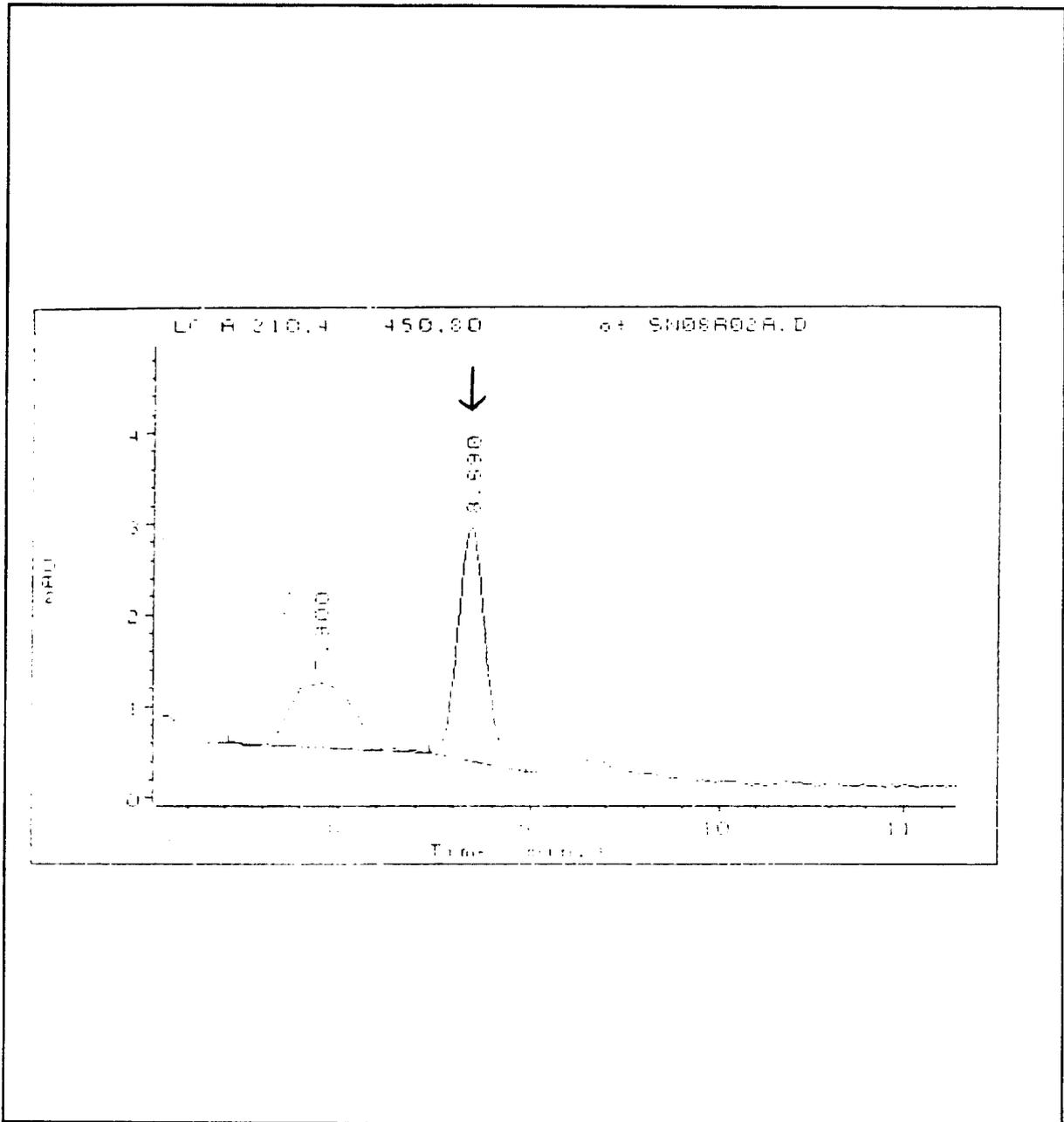
- 46 -

## APPENDIX III



**Figure 11.** A representative chromatogram of a matrix fortification, 414A-101-MAS-9, (100  $\mu\text{g/L}$  Wingstay SN-1).

APPENDIX III



**Figure 12.** A chromatogram of algal medium. Two hundred mL of algal medium passed through an Empore C-8 disk, eluted with acetonitrile, and concentrated to a final volume of 2 mL before analysis by HPLC.

APPENDIX III

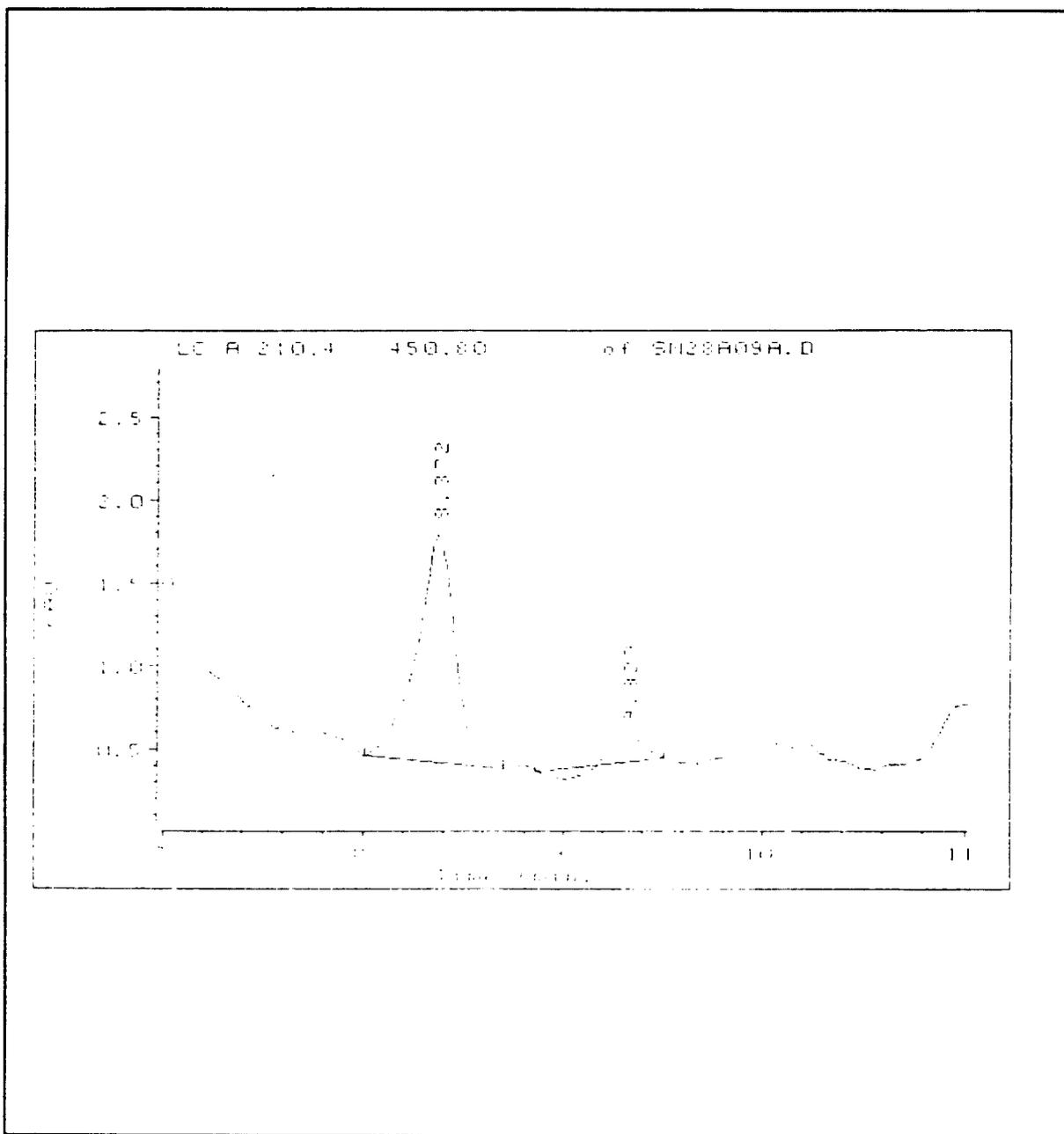
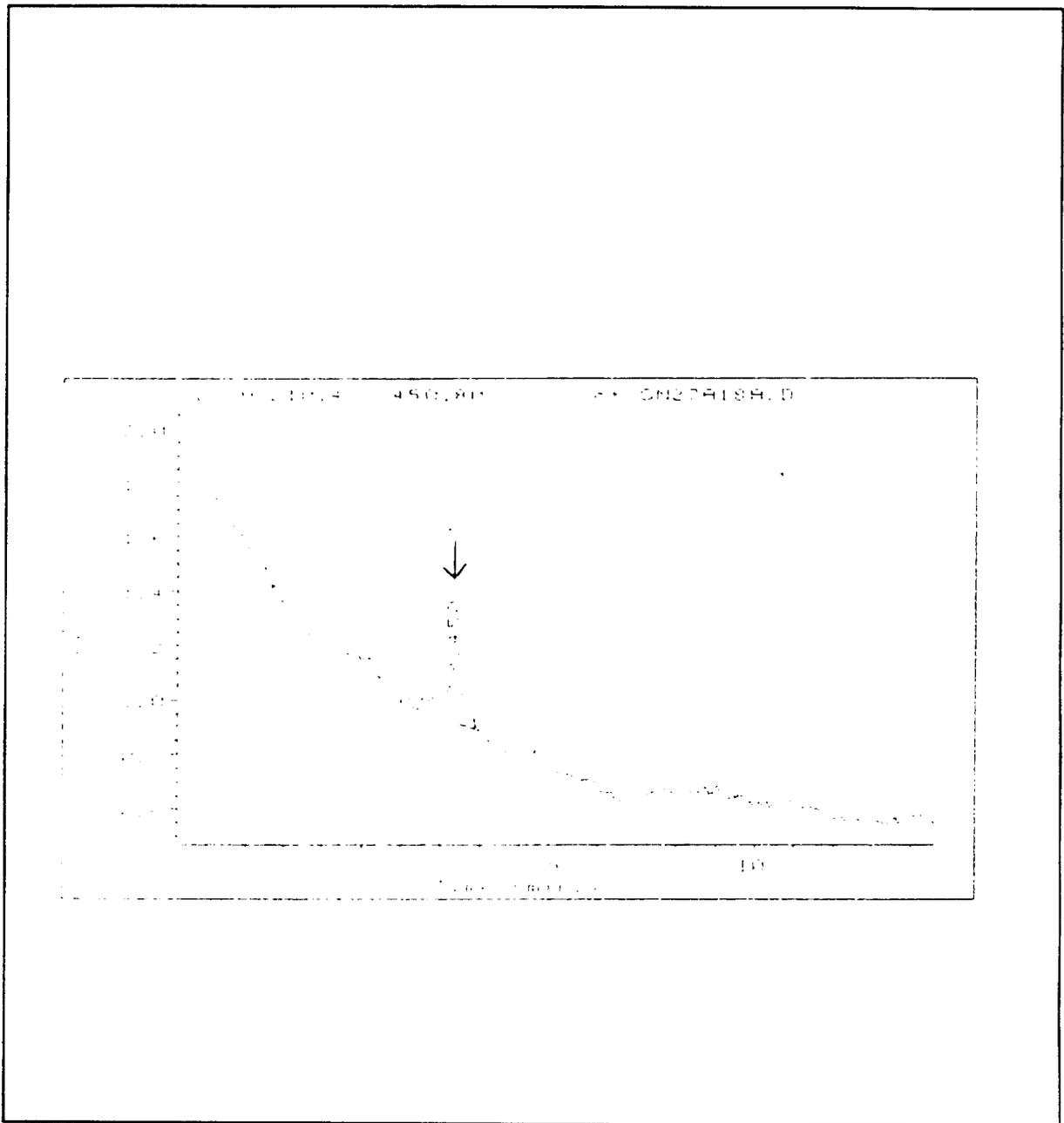


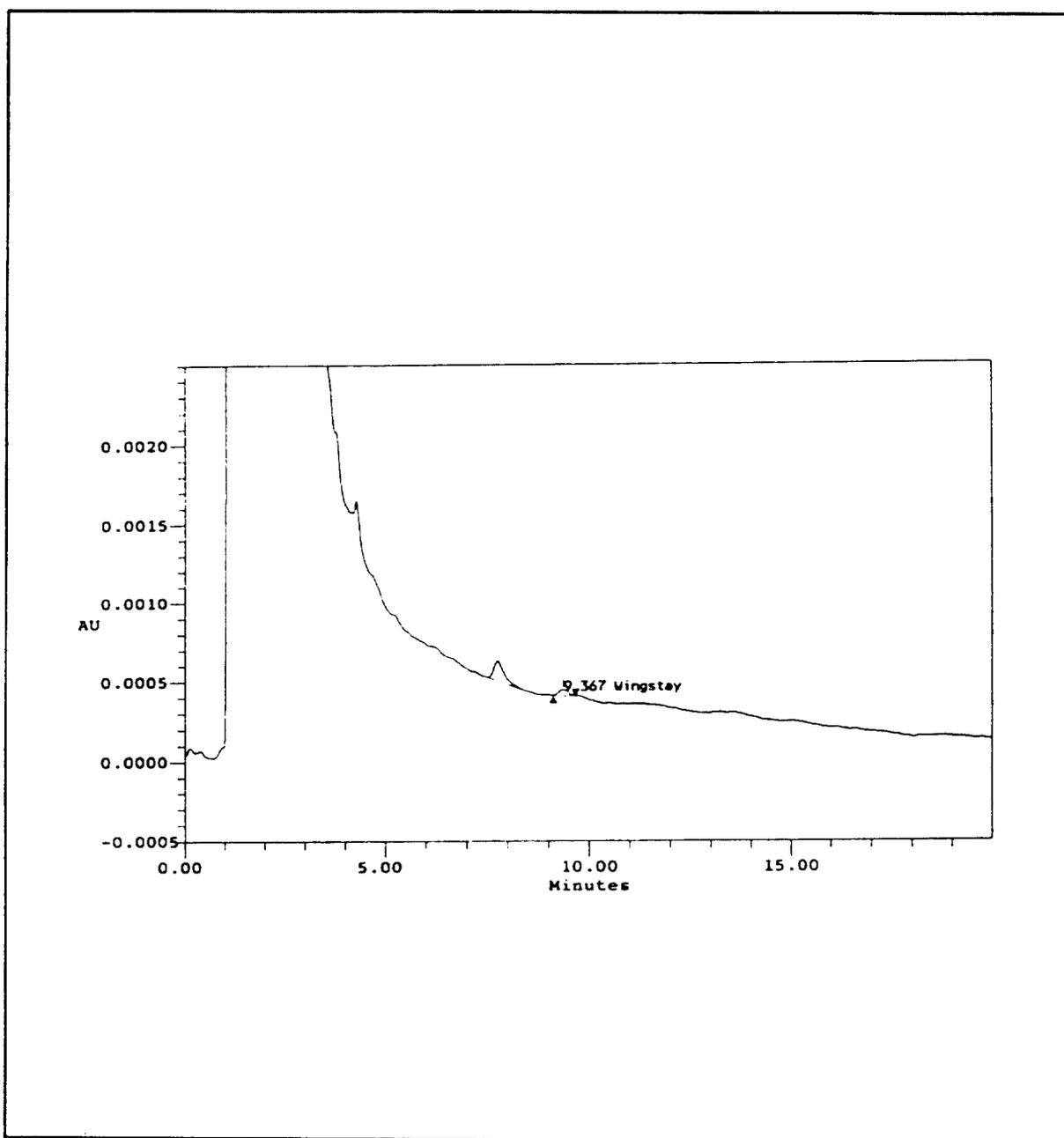
Figure 13. A chromatogram of a negative control sample, 414A-101-10 from the end of the test.

APPENDIX III



**Figure 14.** A chromatogram of a Day 0 sample, 414A-101-8 (70 µg/L nominal concentration).

## APPENDIX III



**Figure 15.** A chromatogram of a Day 0 sample, 414A-101-8 (70  $\mu\text{g/L}$  nominal concentration).

- 51 -

## APPENDIX IV

Cell Densities for Each Replicate Over the  
72-Hour Exposure Period

Sponsor:		The Goodyear Tire & Rubber Company			
Test Substance:		Wingstay SN-1			
Test Organism:		Freshwater Alga, <i>Selenastrum capricornutum</i>			
Dilution Water:		Freshwater Algal Medium			
Nominal Test Concentration ( $\mu\text{g}$ Wingstay SN-1/L)	Replicate	Cell Densities (Cells/mL) <sup>1</sup>			72-Hour Mean
		24 Hours	48 Hours	72 Hours	
Negative Control	A	54,150	300,250	1,454,980	1,962,087
	B	56,810	579,400	2,256,760	
	C	37,120	314,600	2,174,520	
Solvent Control	A	55,730	331,770	1,921,060	2,034,620
	B	53,200	551,520	2,696,100	
	C	55,940	465,040	1,486,700	
0.72	A	57,760	285,490	1,387,640	1,849,913
	B	50,170	502,610	1,912,200	
	C	51,200	293,480	2,249,900	
1.8	A	52,240	387,780	2,359,820	2,278,873
	B	49,710	421,980	2,112,860	
	C	50,510	360,750	2,363,940	
4.5	A	49,980	353,230	1,497,280	1,888,207
	B	52,560	564,510	2,320,500	
	C	41,770	270,920	1,846,840	
11	A	39,560	285,760	1,301,960	1,726,807
	B	33,920	267,960	1,789,300	
	C	32,100	266,560	2,089,160	
28	A	30,940	261,950	1,350,780	1,830,027
	B	40,730	282,540	1,821,100	
	C	49,680	362,090	2,318,200	
70	A	44,450	120,410	810,620	960,660 <sup>2</sup>
	B	41,670	175,840	980,620	
	C	40,300	181,880	1,090,740	

<sup>1</sup> The initial cell density of the stock culture was determined and an inoculum volume was administered to each test chamber to yield a cell density of approximately 10,000 cells/mL at test initiation (0 hours).

<sup>2</sup> Statistically significant ( $p < 0.05$ ) compared to the negative control.

- 52 -

## APPENDIX V

Area Under the Growth Curve for Each Replicate Over the  
72-Hour Exposure Period

Sponsor: The Goodyear Tire & Rubber Company				
Test Substance: Wingstay SN-1				
Test Organism: Freshwater Alga, <i>Selenastrum capricornutum</i>				
Dilution Water: Freshwater Algal Medium				
Nominal Test Concentration ( $\mu\text{g}$ Wingstay SN-1/L)	Replicate	Cumulative Area Under the Growth Curve		
		0 - 24 Hours	0 - 48 Hours	0 - 72 Hours
Negative Control	A	529,800	4,542,600	25,365,360
	B	561,720	7,956,240	41,750,160
	C	325,440	4,306,080	33,935,520
Solvent Control	A	548,760	4,958,760	31,752,720
	B	518,400	7,535,040	46,266,480
	C	551,280	6,563,040	29,743,920
0.72	A	573,120	4,452,120	24,289,680
	B	482,040	6,875,400	35,613,120
	C	494,400	4,390,560	34,671,120
1.8	A	506,880	5,547,120	38,278,320
	B	476,520	5,896,800	36,074,880
	C	486,120	5,181,240	37,637,520
4.5	A	479,760	5,078,280	27,044,400
	B	510,720	7,675,560	42,055,680
	C	381,240	3,893,520	29,066,640
11	A	354,720	4,018,560	22,831,200
	B	287,040	3,669,600	28,116,720
	C	265,200	3,609,120	31,637,760
28	A	251,280	3,525,960	22,638,720
	B	368,760	4,008,000	29,011,680
	C	476,160	5,177,400	37,100,880
70	A	413,400	2,151,720	13,084,080
	B	380,040	2,750,160	16,387,680
	C	363,600	2,789,760	17,821,200

## APPENDIX VI

## Changes to Protocol

This study was conducted in accordance with the approved Protocol with the following changes:

1. The protocol was amended to add the proposed experimental start and termination dates, test concentrations, study room, test substance number and receipt date.
2. Cell densities were determined using an electronic particle counter instead of a hemacytometer and microscope.
3. Statistical analyses were based on measurements of the area under the growth curve but not on cell density.
4. The protocol was amended to add the analysis of the primary stock at test initiation.
5. The analytical method used in the study was more closely related to an additional method provided by the Sponsor than that provided in Appendix II.
6. The reference substance used to prepare the analytical standard was not amended to the protocol.

APPENDIX VII

Personnel Involved in the Study

The following key personnel were involved in the conduct or management of this study:

1. James P. Swigert, Ph.D., Manager, Aquatic Toxicology
2. Ray L. Hanson, Ph.D., Supervisor, Aquatic Chemistry
3. Cynthia A. Roberts, Senior Aquatic Biologist
4. Cary A. Sutherland, Aquatic Biologist

**Contains No CBI**

Page 1 of 48

**WINGSTAY® SN-1 - BIODEGRADABILITY  
(CO<sub>2</sub> EVOLUTION TEST)**

**Guideline Reference Number: 301B**

**Submitted to:**

**The Goodyear Tire & Rubber Company  
142 Goodyear Boulevard  
Akron, Ohio 44305**

**SLI Report No. 96-3-6432**

**SLI Study No. 13537.1195.6129.745**

**Study Director: Alex Armitage**

**Springborn Laboratories, Inc.  
Health and Environmental Sciences  
790 Main Street  
Wareham, Massachusetts 02571-1075**

**30 May 1996**

**FINAL REPORT**

96 JUN 26 11:31

RECEIVED  
MILLS

96 JUL -2 AM 10:08

RECEIVED  
OPPT NCIC

---

**GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT**

The data and report presented for "**Wingstay<sup>®</sup> SN-1- Biodegradability (CO<sub>2</sub> Evolution Test)**" were produced and compiled in accordance with all pertinent OECD Good Laboratory Practice regulations, with the following exceptions: routine water contaminant screening analyses for pesticides, PCBs, and metals were conducted using standard U.S. EPA procedures by Lancaster Laboratories, Lancaster, Pennsylvania and total organic carbon analysis of the test substance was conducted by Galbraith Laboratories, Inc., Knoxville, Tennessee. These analyses were not conducted in accordance with Good Laboratory Practice procedures (i.e., no distinct protocol, Study Director, etc.). Stability, characterization, verification of the test substance identity, and maintenance of records on the test substance are the responsibility of the Study Sponsor at test termination.

SPRINGBORN LABORATORIES, INC.

*Alek Armitage* 5/30/96  
Alek Armitage Date  
Study Director

## TABLE OF CONTENTS

	Page
GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT .....	2
LIST OF TABLES .....	5
LIST OF FIGURES .....	6
SUMMARY .....	7
1.0 INTRODUCTION .....	8
2.0 MATERIALS AND METHODS .....	8
2.1 Protocol .....	8
2.2 Test Substance, Reference Substance and Standard Reagents .....	8
2.2.1 Test Substance .....	8
2.2.2 Reference Substance .....	9
2.2.3 Standard Reagents .....	9
2.3 Stock Solution Preparation .....	10
2.4 Microbial Inoculum (Activated Sludge) .....	10
2.5 Test System .....	10
2.6 Test Initiation .....	11
2.7 Test Maintenance and Sampling .....	12
3.0 ANALYSIS .....	12
3.1 Titration of Evolved CO <sub>2</sub> .....	12
3.2 Dissolved Organic Carbon (DOC) Analysis .....	13
4.0 RESULTS .....	14
4.1 Test Conditions (Temperature and pH) .....	14
4.2 Observations .....	14
4.3 Titration Analysis .....	14
4.4 DOC Analysis .....	15
5.0 CONCLUSION .....	15
PROTOCOL DEVIATIONS .....	16
QUALITY ASSURANCE UNIT STATEMENT .....	17
REFERENCES .....	18

---

<b>TABLES</b> .....	19
<b>FIGURES</b> .....	25
<b>SIGNATURES AND APPROVAL</b> .....	30
<b>6.0 APPENDIX I - STUDY PROTOCOL</b> .....	31
<b>7.0 APPENDIX II - CERTIFICATES OF ANALYSIS</b> .....	40
<b>8.0 APPENDIX III - ORGANIC CARBON ANALYSIS OF THE TEST SUBSTANCE</b> .....	44
<b>9.0 APPENDIX IV - WATER ANALYSIS</b> .....	46

## LIST OF TABLES

	Page
Table 1. <b>Composition of stock solutions for mineral salts medium</b> .....	20
Table 2. <b>pH measurements taken at initiation and termination of the 33-day biodegradation study with Wingstay<sup>®</sup> SN-1</b> .....	21
Table 3. <b>Cumulative CO<sub>2</sub> evolved from test vessels containing Wingstay<sup>®</sup> SN-1 or sodium benzoate, as measured by titration</b> .....	22
Table 4. <b>Cumulative CO<sub>2</sub> evolved from sterile, toxicity, and blank control replicates, as measured by titration</b> .....	23
Table 5. <b>Net dissolved organic carbon (DOC) measured in test vessels containing Wingstay<sup>®</sup> SN-1 or sodium benzoate</b> .....	24

## LIST OF FIGURES

	Page
Figure 1. Diagram of the chemical structure of the components of Wingstay <sup>®</sup> SN-1 .....	26
Figure 2. Reaction unit for testing biodegradation in water and series of effluent traps .....	27
Figure 3. Cumulative percent CO <sub>2</sub> evolved from flasks containing Wingstay <sup>®</sup> SN-1 and sodium benzoate during the 33-day study .....	28
Figure 4. Percent net dissolved organic carbon (DOC) in flasks containing Wingstay <sup>®</sup> SN-1 or sodium benzoate .....	29

**SUMMARY****Wingstay® SN-1 - Biodegradability (CO<sub>2</sub> Evolution Test)**

**SPONSOR:** The Goodyear Tire & Rubber Company

**PROTOCOL TITLE:** "Protocol for Determining the Biodegradability of a Test Substance Based on OECD Method 301B (CO<sub>2</sub> Evolution Test)," Springborn Laboratories Protocol #: 102095/13537/OECD 301B.

**REPORT NUMBER:** 96-3-6432

**STUDY NUMBER:** 13537.1195.6129.745

**TEST SUBSTANCE:** Wingstay® SN-1, Batch No. 130893, CAS Registry No. 64253-30-1, a white, waxy crystal with calculations based on a purity of 100%, was received from Goodyear Research on 8 November 1995

**EXPERIMENTAL TEST DATES:** 12 January to 14 February 1996

**RESULTS:** The mean cumulative carbon dioxide (CO<sub>2</sub>) evolved from aqueous medium fortified with Wingstay® SN-1, at 20 mg carbon per liter, was 70.8% of the theoretical concentration, according to titration measurements. The mean net dissolved organic carbon (DOC) was determined to be 1.96 mg/L on day 0 and 0.783 mg/L on day 33 indicating the insolubility of Wingstay® SN-1 in the test system.

In the procedural control, the mean cumulative CO<sub>2</sub> evolved from aqueous medium fortified with the reference substance, sodium benzoate, at 10 mg carbon per liter, was 82.3% of the theoretical concentration, according to titration measurements. The mean net DOC decreased from 8.77 mg/L on day 0 to 0 mg/L on day 33, indicating that approximately 100% of the carbon added as sodium benzoate was released as CO<sub>2</sub>.

**CONCLUSION:** Based on the results of this study, Wingstay® SN-1 was classified as readily biodegradable by the OECD definition since more than 60% biodegradation was observed over a 28 day period.

## 1.0 INTRODUCTION

This study was performed to determine the potential for biodegradation of Wingstay® SN-1 in water by the carbon dioxide evolution method following OECD Guideline 301B. The amount of carbon dioxide (CO<sub>2</sub>) released upon biodegradation of the test substance and a reference substance, sodium benzoate, was measured. Two blank controls, a sterile control containing test substance, and a toxicity control containing reference and test substances were also established to account for background, abiotic degradation, and toxicity, respectively. Test flasks were incubated aerobically in the dark for a period of 33 days. The study was initiated on 6 December 1995, the date the Study Director signed the protocol, and was completed on the day the Study Director signed the final report. The experimental phase of the definitive test was conducted from 12 January to 14 February 1996 at Springborn Laboratories, Inc. (SLI), *Health and Environmental Sciences*, in Wareham, Massachusetts. All original raw data and the final report produced during this study are stored in Springborn's archives at the above location.

## 2.0 MATERIALS AND METHODS

### 2.1 Protocol

Procedures used in this study followed those described in the Springborn protocol entitled "Protocol for Determining the Biodegradability of a Test Substance Based on OECD Method 301B (CO<sub>2</sub> Evolution Test)," Springborn Laboratories Protocol #: 102095/13537/OECD 301B (Appendix I).

### 2.2 Test Substance, Reference Substance and Standard Reagents

**2.2.1 Test Substance.** The test substance, Wingstay® SN-1, was received from Goodyear Research, Akron, Ohio on 8 November 1995. Upon receipt at Springborn, the test substance was stored at room temperature (approximately 20 °C) in a dark, ventilated cabinet. Structures of the test chemical's components are presented in Figure 1. The following information describes the test substance received:

Chemical Name: diester of 3-(dodecylthio) propionic acid and tetraethylene glycol

Physical Appearance:	white, waxy crystal
Batch No.:	130893
CAS Registry No.:	64253-30-1
Purity:	used as 100% (Certificate of Analysis, GPC analysis of Wingstay® SN-1, Batch No. 130893, Appendix II)
Molecular Weight:	706.04 g/mol
Density:	0.990 ± 0.009 g/mL at 25 °C (determined at Springborn, SLI Report # 95-8-6026)
Water Solubility:	46.7 µg/L at approximately 20 °C (determined at Springborn, SLI Report # 95-5-5893)
Vapor Pressure:	below the detection limit of $1.33 \times 10^{-5}$ Pascal ( $1.00 \times 10^{-7}$ mm Hg) at 20.5 °C (determined at Springborn, SLI Report # 95-6-5928)

A subsample of the test substance (5 g) was sent to Galbraith Laboratories, Inc., Knoxville, Tennessee, for determination of total organic carbon (TOC) content. Total organic carbon was determined to comprise 64.2% of Wingstay® SN-1, by weight (Appendix III).

**2.2.2 Reference Substance.** Sodium benzoate, Lot No. F33711 (Baker), CAS No. 532-32-1, was used as the reference substance. At experimental initiation, the supplier did not provide a purity for this lot of the reference substance. A purity of 100% was assumed for calculations.

**2.2.3 Standard Reagents.** All solutions were prepared using reagent water (meeting ASTM Type IIA requirements) obtained with a Sybron/Barnstead NANOpure® II system. The source water for this system was well water which has been shown to be free of contaminants which could affect study results (Appendix IV). The filter-sterilized water typically has greater than 16.7 Mohm-cm resistivity and total organic carbon below 1 mg/L (the detection limit) in routine monthly analyses. The reagent water was heat-sterilized by autoclaving at 121 °C, 15 psi for 30 minutes and cooled before use in preparing the mineral salts medium and its component solutions (Table 1). The dissolved organic carbon (DOC) content of the sterilized water was measured prior to use, with a result of 0.562 mg/L. All chemicals were at least reagent grade and were obtained from commercial sources.

### 2.3 Stock Solution Preparation

A sodium benzoate stock solution was prepared by dissolving and diluting 428.5 mg of sodium benzoate to 25 mL with reagent water to produce a stock solution with a concentration of 17.1 mg/mL sodium benzoate (10 mg carbon/L).

### 2.4 Microbial Inoculum (Activated Sludge)

The activated sludge used for this study was obtained from the Wareham Wastewater Treatment Plant, Wareham, Massachusetts. This treatment facility receives primarily domestic sewage. Activated sludge was collected on 11 January 1996 and transported to Springborn. The sludge was used immediately.

The solids content of the activated sludge was determined using an automated Moisture Analyzer (Sartorius), and the sludge was adjusted by dilution with mineral salts medium to produce an inoculum containing 3000 mg suspended solids/L.

### 2.5 Test System

Each test system consisted of a 4-L glass bottle (Kimax) with a No. 8 rubber stopper into which one stainless steel tube with a Luer-Lok connection and two pieces of glass tubing were inserted (Figure 2). Prior to test initiation, the test vessels were acid-washed, rinsed repeatedly with reagent water and sterilized by autoclave at 121 °C, 18 psi for 30 minutes. The stainless steel tube served as a sampling port for solution samples. Sampling was accomplished using a piece of Teflon<sup>®</sup> tubing extended below the stopper into the test solution. A rubber cap was used to cover the top of the sample port. The glass tubing provided the inlet and outlet ports for air exchange. Compressed, CO<sub>2</sub>-free synthetic air (79% N<sub>2</sub>, 21% O<sub>2</sub>, Liquid Carbonics, Inc.) was provided to the system under positive pressure at a rate of 1 to 2 bubbles per second (approximately 50 to 100 mL/min). A rehydration flask, containing reagent water preceded each test vessel to moisten the dry compressed air and prevent excessive evaporation from the test vessels. The outlet port of each system was fitted with three CO<sub>2</sub> traps, each consisting of a 200-mL bottle containing 100 mL of 0.025 N Ba(OH)<sub>2</sub>. The Ba(OH)<sub>2</sub> solution was filtered and analyzed before use. A schematic diagram of the test system appears in Figure 2. The test

vessels were identified with the project number, replicate number and treatment type. The test systems were kept in the dark, in an environmental chamber designed to maintain a temperature of  $22 \pm 2$  °C.

## 2.6 Test Initiation

Eight test systems were established: two Wingstay® SN-1 non-sterile systems, one Wingstay® SN-1 sterile control, one toxicity control (test substance and reference substance combined), two sodium benzoate procedural controls and two blank controls.

On day-1, 2400 mL of mineral salts medium were added to each test vessel except the sterile control. The sterile control vessel received 3000 mL of mineral salts medium without inoculum. For each test substance, toxicity control, reference substance and blank control vessel, a 30-mL aliquot of the solids-adjusted sludge was dispensed into a graduated cylinder, then diluted to 600 mL with additional mineral salts medium. This volume was then added to the test vessel, producing a final volume of 3 L that contained 30 mg/L suspended solids. The sterile control was treated with 1 mL of a 30 mg/mL mercuric chloride solution to ensure sterility. The vessels were placed on magnetic stir plates and attached to the aeration apparatus. The systems were purged with CO<sub>2</sub>-free air for approximately 24 hours with continuous mixing.

On day 0, aeration was suspended in each vessel for dosing. Each designated test substance vessel was fortified with Wingstay® SN-1 at a level of approximately 20 mg carbon per liter. Test substance was dispensed onto a glass microfiber filter (Whatman) that had previously been cleaned and sterilized by repeated boiling in reagent water. The sterile control vessel was fortified with 93.3 mg of Wingstay® SN-1, as described above. The toxicity control vessel was fortified with 94.0 mg of Wingstay® SN-1, as described above, and with 3.00 mL of the 17.1 mg/mL sodium benzoate stock solution, for a sodium benzoate level of 10 mg carbon per liter and a total fortification level of 30 mg carbon per liter. The designated sodium benzoate reference substance vessels each were fortified with 3.00 mL of the sodium benzoate stock solution, for a final concentration of 10 mg carbon per liter. The blank control and reference substance test vessels each received a non-treated sterile glass filter.

After treatment, aeration was resumed at a rate of 1 to 2 bubbles per second (approximately 50 to 100 mL/min). After approximately 30 minutes of continuous aeration and mixing, samples were taken from each test vessel for analysis of pH and dissolved organic carbon (DOC) content.

## 2.7 Test Maintenance and Sampling

CO<sub>2</sub>-free air was used to provide oxygen for aerobic bacteria and to capture evolved carbon dioxide in all test systems. Aeration was continuous for 33 days under positive pressure. A minimum-maximum thermometer (Fisher Scientific) was maintained with the test systems to provide continuous monitoring of temperature.

On days 0, 28, and 33, samples were taken from each test vessel, for analysis of pH and DOC content. Samples were removed using a sterile disposable syringe through the sampling port. The sample (30-mL) was dispensed through a 0.5- $\mu$ m filter disc into an acid-washed, baked, amber glass sample bottle and preserved by the addition of sulfuric acid for DOC analysis. An additional 10 mL of the unfiltered sample was dispensed into a vial for pH analysis. Measurements of pH were taken using a Jenco Model 611 pH meter.

On days 2, 4, 6, 9, 13, 18, 23, 28, and 33 the first Ba(OH)<sub>2</sub> carbon dioxide trap from each test system was removed for analysis of CO<sub>2</sub> concentration. The second trap was moved up to position one, the third trap was moved up to position two, and a new CO<sub>2</sub> trap containing 100 mL of freshly prepared 0.025 N Ba(OH)<sub>2</sub> was placed on the test system in position three. On day 33, after samples were taken for pH and DOC analysis, 1 mL of concentrated HCl was added to each test vessel to terminate biological activity. Aeration was continued overnight to maximize the capture of evolved CO<sub>2</sub> and all traps were subsequently removed from each test system for CO<sub>2</sub> analysis.

## 3.0 ANALYSIS

### 3.1 Titration of Evolved CO<sub>2</sub>

For the determination of evolved CO<sub>2</sub>, the amount of unreacted Ba(OH)<sub>2</sub> in the traps at selected intervals was quantified by titrating 25-mL aliquots of trapping solution with standardized

0.05 N hydrochloric acid to the phenolphthalein endpoint using a digital buret (Brinkman). The trapping solution from each test system was allowed to settle to remove precipitated  $\text{Ba}(\text{CO}_3)_2$ ; and a 25-mL aliquot was removed for  $\text{CO}_2$  analysis by titration. Titration of the contents of the first  $\text{CO}_2$  trap from each test system was performed on days 2, 4, 6, 9, 13, 18, 23, 28, and 34. At test termination, all three traps from each test system were analyzed by titration. Prior to each sampling and titration, all glassware used was acid-washed and rinsed repeatedly with reagent water.

The amount of  $\text{CO}_2$  evolved from each test system was calculated using the following formula:

$$\text{mg CO}_2 = \frac{\text{mL HCl blank} - \text{mL treatment}}{2} \times 0.05 \text{ mmoles/mL} \times 44 \text{ mg/mmole}$$

The volume of HCl used in the treatment is corrected by subtracting the mean volume of HCl used in titration of the blank controls. Results for each treatment are expressed as cumulative mg  $\text{CO}_2$  evolved and as cumulative percent biodegradation (or percent of theoretical  $\text{CO}_2$  production). The percent biodegradability for each test system was calculated using the formula:

$$\% \text{ CO}_2 \text{ evolved} = \frac{\text{mg CO}_2 \text{ produced}}{\text{mg TOC added in test} \times 3.67} \times 100\%$$

where 3.67 is the molecular weight conversion factor for carbon to carbon dioxide.

### 3.2 Dissolved Organic Carbon (DOC) Analysis

DOC is the fraction of organic carbon (OC) that passes through a 0.5- $\mu\text{m}$  filter. Quantitation of DOC was performed on days 0, 28, and 33 using a Dohrmann DC-80 Carbon Analyzer. Duplicate analysis of each filtered acid-preserved 20-mL sample was performed, using a 1000- $\mu\text{L}$  injection volume. The mean of duplicate analysis was reported. The mean DOC value for the blank control (i.e., inoculum DOC) was subtracted from the DOC values for the inoculum-containing test systems to obtain net DOC values.

## 4.0 RESULTS

### 4.1 Test Conditions (Temperature and pH)

The temperature recorded daily during the study ranged from 20 to 22 °C. The pH measurement on day 0 was 7.6 in all vessels. Measurements of pH on day 33 ranged from 7.2 to 7.5 in all vessels. Measurements of pH recorded for individual test vessels are presented in Table 2.

### 4.2 Observations

In the aqueous medium fortified with Wingstay<sup>®</sup> SN-1, both test substance replicates, procedure controls, and the toxicity control (containing test and reference substances), the glass filter was observed to have dissociated in each test vessel and organic matter was visible. In the blank controls, the glass filter had dissociated in one replicate only, (Replicate 2) with organic matter visible in both replicates. In the sterile control, the glass filter had dissociated but no organic matter was visible.

### 4.3 Titration Analysis

At test termination (day 33) the mean cumulative CO<sub>2</sub> evolved from the aqueous medium fortified with Wingstay<sup>®</sup> SN-1 was 64.8% and 70.8% (day 28 and 34, respectively) of the theoretical amount, based on the titration measurements. The cumulative CO<sub>2</sub> evolved from the sterile control over the same time period was 0%, indicating no significant abiotic degradation. The mean cumulative CO<sub>2</sub> evolved from the sodium benzoate procedural controls (aqueous medium containing sodium benzoate) was 82.3% of the theoretical amount at test termination, with 67.6% degradation occurring by day 9. This rapid degradation of the reference substance confirmed the presence of microbial community adequate for biodegradation testing. The cumulative CO<sub>2</sub> evolved from the toxicity control (containing test and reference substances) was 43.3% of the theoretical concentration on day 13, which meets the OECD guidelines of at least 25% degradation within the first 14 days. This indicates that the degradation of sodium benzoate was not inhibited by the presence of Wingstay<sup>®</sup> SN-1. The mean cumulative CO<sub>2</sub> evolved from the blank was 30.2 mg/L. This value is consistent with the OECD (1992) requirement that the CO<sub>2</sub> evolved from the blanks not exceed 40 mg/L. Cumulative CO<sub>2</sub> evolution for each sampling interval is reported

in Table 3 for the test substance and procedural control replicates and in Table 4 for the sterile, toxicity, and blank control for each sampling interval and is depicted in Figure 3 (as percent of theoretical).

#### 4.4 DOC Analysis

In the test substance systems, net DOC values were 1.96 mg/L on day 0 and 0.783 mg/L on day 33. The DOC in the sterile control was 5.63 mg/L on day 0 and 8.40 on day 33. Net DOC values for each treatment are presented in Table 5 and depicted in Figure 4 (as percent of theoretical).

The mean net DOC measured in the sodium benzoate systems (i.e., procedural control replicates 1 and 2) decreased from 8.77 mg/L to 0 mg/L over the course of the study, confirming the expected biodegradation of the reference substance.

## 5.0 CONCLUSION

Based on the results of this study, Wingstay<sup>®</sup> SN-1 was classified as readily biodegradable by the OECD definition since more than 60% biodegradation was observed.

---

**PROTOCOL DEVIATIONS**

There were no protocol deviations during the course of this study.

SPRINGBORN LABORATORIES, INC.

*Alex Armitage* 5/30/96  
Alex Armitage Date  
Study Director



## REFERENCES

- OECD (1981). Good Laboratory Practices as acknowledged in the EEC Council Directive 87/320/EEC of 9 June 1988.
- OECD, Paris 1992. Test Guideline 301B, adopted by the Council on 17 July 1992.
- Official Journal of the European Communities. 1992. C.4. Biodegradation: Determination of "ready" biodegradability. C.4-C: Carbon dioxide (CO<sub>2</sub>) evolution. L383 A, Volume 35, 29 December 1992.

**TABLES**

**Table 1. Composition of stock solutions for mineral salts medium.<sup>a</sup>**

Solution <sup>b</sup>	Chemical Formula of Components	Amount (g) per L of Stock	mL of Stock per L of Medium <sup>a</sup>
A. Phosphate buffer	KH <sub>2</sub> PO <sub>4</sub>	8.50	10
	K <sub>2</sub> HPO <sub>4</sub>	22.0	
	Na <sub>2</sub> HPO <sub>4</sub>	17.7	
	NH <sub>4</sub> Cl	0.500	
B. Calcium chloride	CaCl <sub>2</sub>	27.2	1
C. Magnesium sulfate	MgSO <sub>4</sub> •7H <sub>2</sub> O	22.5	1
D. Ferric chloride	FeCl <sub>3</sub> •6H <sub>2</sub> O	0.25	1

<sup>a</sup> Based on OECD Document 301B (1992).

<sup>b</sup> Solutions were prepared using sterile reagent water (see Section 2.2.3). The prepared mineral salts medium solutions A through D were prepared separately and were kept refrigerated (approximately 4 °C) until use on day<sup>-1</sup>.

**Table 2. pH measurements taken at initiation and termination of the 33-day biodegradation study with Wingstay<sup>®</sup> SN-1.**

	Replicate	Measured pH		
		Day 0	Day 28	Day 33
<b>Test Substance</b>	Rep 1	7.6	7.3	7.2
	Rep 2	7.6	7.3	7.2
<b>Sterile (Abiotic) Control</b>	Rep 1	7.6	7.5	7.5
<b>Toxicity Control</b>	Rep 1	7.6	7.4	7.3
<b>Procedural Control (Sodium Benzoate)</b>	Rep 1	7.6	7.4	7.4
	Rep 2	7.6	7.4	7.4
<b>Blank Control</b>	Rep 1	7.6	7.4	7.3
	Rep 2	7.6	7.4	7.3

**Table 3. Cumulative CO<sub>2</sub> evolved from test vessels containing Wingstay<sup>®</sup> SN-1 or sodium benzoate, as measured by titration.<sup>a</sup>**

Test Day	Cumulative mg CO <sub>2</sub>			Cumulative Percent CO <sub>2</sub>		
	Rep 1	Rep 2	Mean	Rep 1	Rep 2	Mean
<b>Wingstay<sup>®</sup> SN-1</b>						
2	0.29	0.07	0.18	0.13	0.03	0.08
4	10.5	8.71	9.59	4.76	3.96	4.36
6	33.4	22.7	28.0	15.2	10.3	12.7
9	63.3	40.7	52.0	28.8	18.5	23.6
13	98.9	60.1	79.5	45.0	27.3	36.1
18	133	82.8	108	60.6	37.7	49.1
23	156	106	131	70.7	48.0	59.3
28	162	124	143	73.5	56.2	64.8
34 (trap 1)	163	144	154	74.2	65.5	69.9
34 (trap 2)	163	148	156	74.2	67.4	70.8
34 (trap 3)	163	148	156	74.2	67.4	70.1
<b>Sodium Benzoate</b>						
2	23.2	23.9	23.6	21.1	21.7	21.4
4	48.1	47.3	47.7	43.7	43.0	43.4
6	65.1	61.7	63.4	59.2	56.1	57.6
9	75.8	72.9	74.3	68.9	66.2	67.6
13	81.1	80.0	80.5	73.7	72.7	73.2
18	84.3	84.2	84.3	76.7	76.6	76.6
23	88.3	86.3	87.3	80.3	78.4	79.4
28	91.5	86.3	88.9	83.2	78.4	80.8
34 (trap 1)	93.9	86.3	90.1	85.4	78.4	81.9
34 (trap 2)	94.5	86.3	90.4	85.9	78.4	82.2
34 (trap 3)	94.5	86.3	90.4	86.2	78.4	82.3

<sup>a</sup> The rounded values presented in this table were calculated based on unrounded experimental results. All values are corrected for mean blank CO<sub>2</sub> production.

**Table 4. Cumulative CO<sub>2</sub> evolved from sterile, toxicity, and blank control replicates, as measured by titration.<sup>a</sup>**

Test Day	Sterile Control <sup>b</sup>		Toxicity Control <sup>c</sup>		Blank Control	
	(mg)	(%)	(mg)	(%)	(mg)	(%)
2	0.00	0.00	17.5	5.29	14.9	NA <sup>d</sup>
4	0.00	0.00	45.3	13.7	32.7	NA
6	0.00	0.00	77.1	23.4	48.2	NA
9	0.00	0.00	109	33.1	62.8	NA
13	0.00	0.00	143	43.3	74.1	NA
18	0.00	0.00	180	54.5	83.1	NA
23	0.00	0.00	208	62.9	85.0	NA
28	0.00	0.00	222	67.4	85.0	NA
34 (trap 1)	0.00	0.00	230	69.7	86.1	NA
34 (trap 2)	0.00	0.00	232	70.2	86.1	NA
34 (trap 3)	0.00	0.00	232	70.2	86.8	NA

<sup>a</sup> The rounded values presented in this table were calculated based on unrounded experimental results. All values are corrected for mean blank CO<sub>2</sub> production.

<sup>b</sup> Sterile control contained Wingstay<sup>®</sup> SN-1

<sup>c</sup> Toxicity control contained Wingstay<sup>®</sup> SN-1 and sodium benzoate.

<sup>d</sup> NA = not applicable

**Table 5. Net dissolved organic carbon (DOC) measured in test vessels containing Wingstay® SN-1 or sodium benzoate.<sup>a</sup>**

	Replicate	Theoretical DOC (mg C/L)	Net DOC (mg C/L) <sup>b</sup>			
			Day 0	Percent Theoretical	Day 33	Percent Theoretical
<b>Test Substance</b>	Rep 1	20	2.24	11.2	0.673	3.37
	Rep 2	20	1.67	8.35	0.892	4.46
	Mean	20	1.96	9.78	0.783	3.92
<b>Sterile (Abiotic) Control<sup>c</sup></b>	Rep 1	20	5.63	28.2	8.40	42.0
<b>Toxicity Control</b>	Rep 1	30	11.1	37.0	0.607	2.02
<b>Procedural Control</b>	Rep 1	10	8.86	88.6	0.00	0.00
	Rep 2	10	8.68	86.8	0.00	0.00
	Mean	10	8.77	87.7	0.00	0.00

	Replicate	DOC (mg/L)	
		Day 0	Day 33
<b>Blank Control</b>	Rep 1	1.02	0.97
	Rep 2	0.886	0.74
	Mean	0.954	0.85

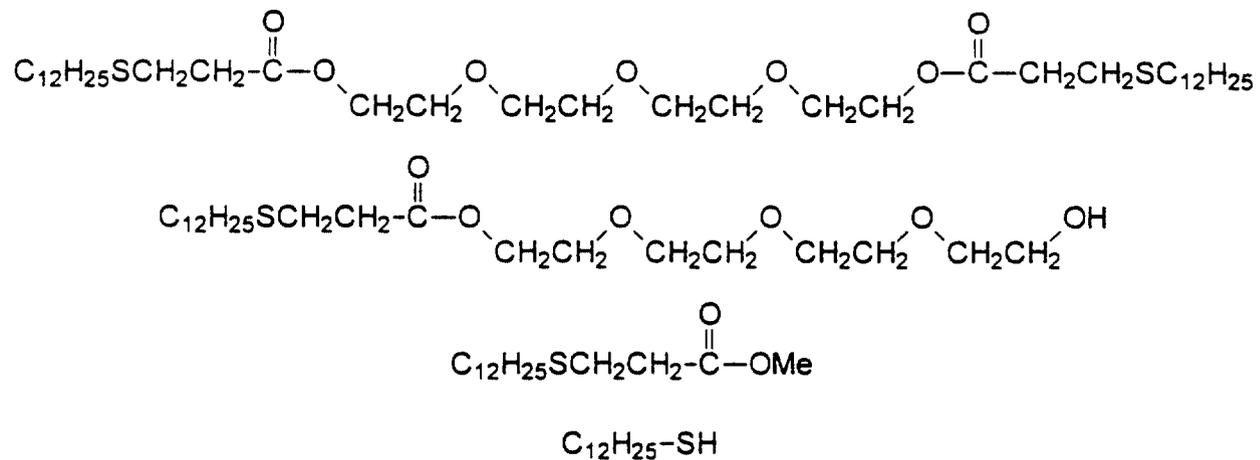
<sup>a</sup> The rounded values presented in this table were calculated based on unrounded experimental results; each DOC value presented represents the mean of two measurements.

<sup>b</sup> For each test day, mean DOC values in the control blanks were subtracted from the DOC in each inoculum-containing treatment replicate (i.e., the test substance replicates, toxicity control replicate and procedural control replicates) in order to account for the inoculum contribution to DOC.

<sup>c</sup> Sterile control did not contain inoculum, therefore was not corrected for DOC of the blanks.

**FIGURES**

Figure 1. Diagram of the chemical structure of the components of Wingstay® SN-1.



**Figure 2. Reaction unit for testing biodegradation in water and series of effluent traps.**

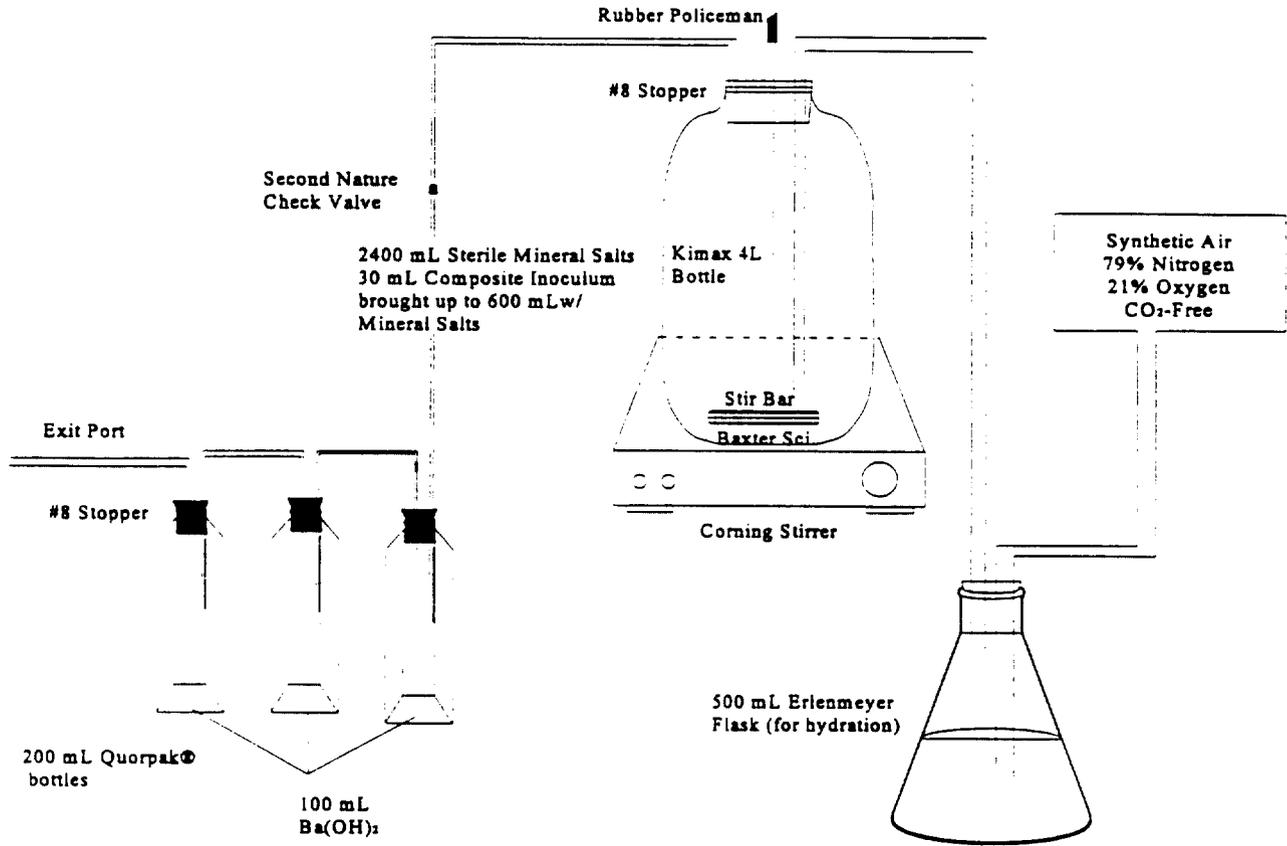
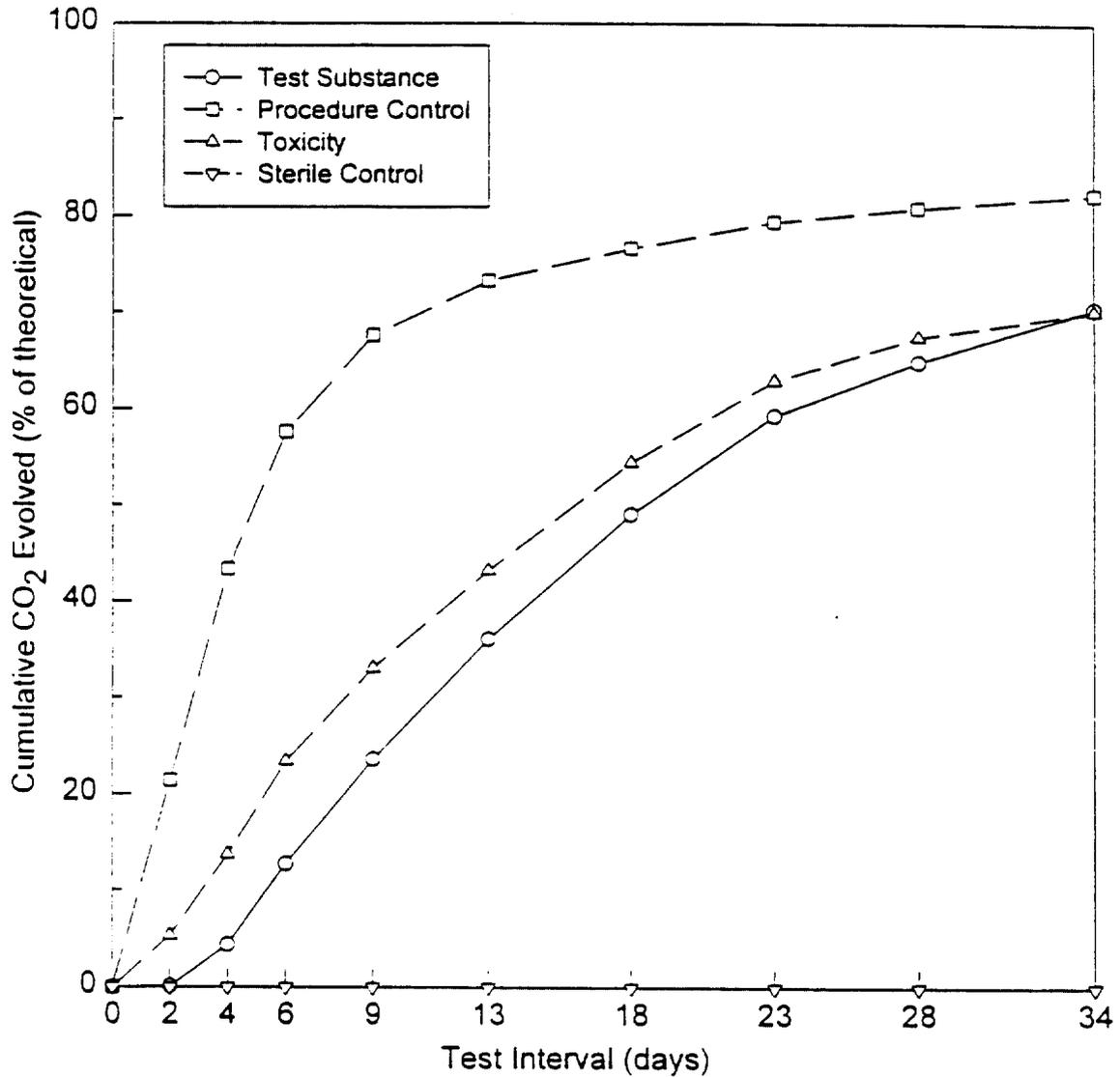
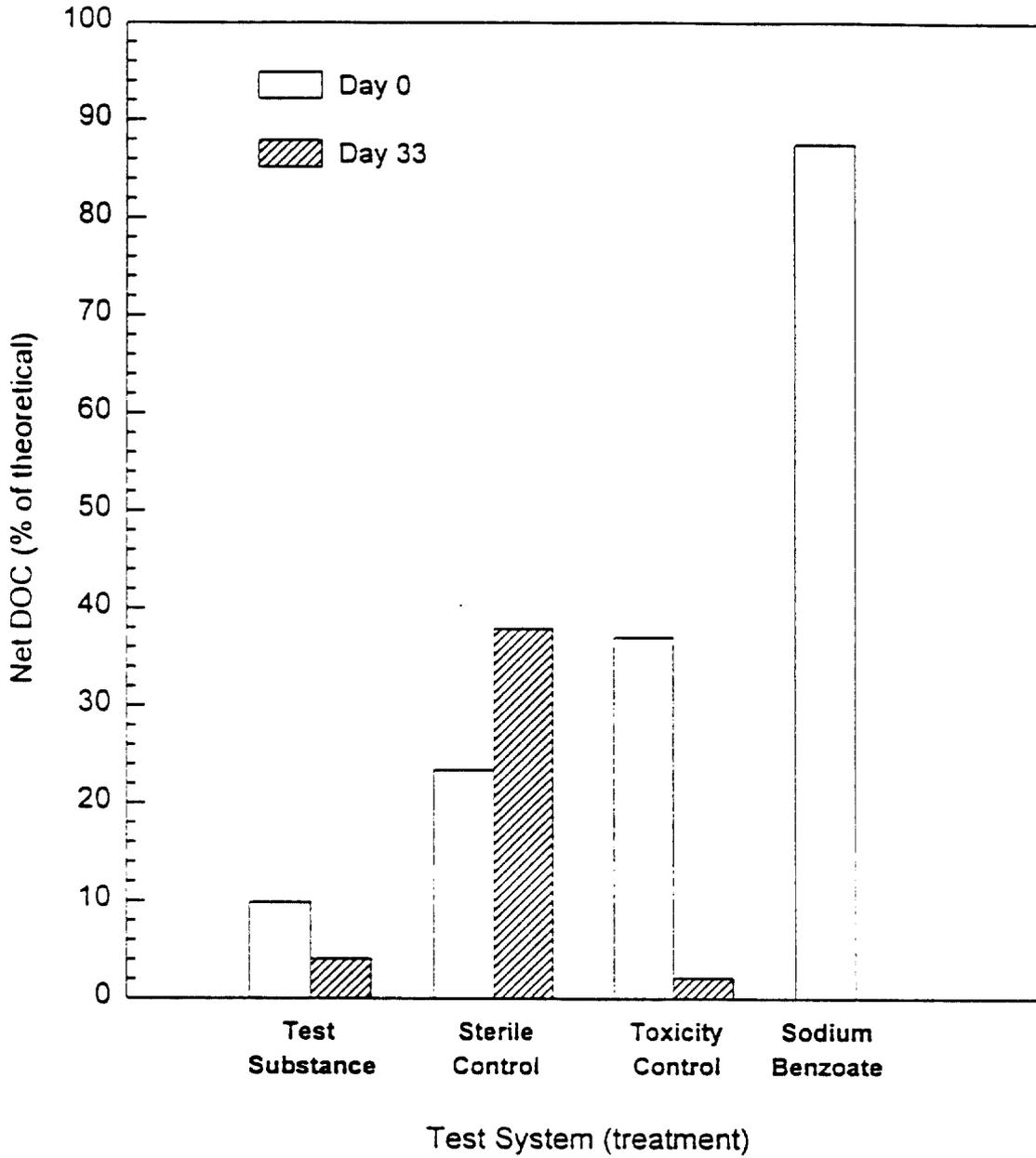


Figure 3. Cumulative percent CO<sub>2</sub> evolved from flasks containing Wingstay® SN-1 and sodium benzoate during the 33-day study.<sup>a,b</sup>



- <sup>a</sup> A two day lag time was observed for the degradation of test substance Wingstay® SN-1. the slope of a linear regression of the linear segment of the curve (day 0 - day 34) is 2.32.
- <sup>b</sup> No lag time was observed for sodium benzoate. The slope of a linear regression of the linear segment of the curve (day 0 to 6) is 9.74.

Figure 4. Percent net dissolved organic carbon (DOC) in flasks containing Wingstay® SN-1 or sodium benzoate.



**SIGNATURES AND APPROVAL**

**SUBMITTED BY:**

Springborn Laboratories Inc.  
Health and Environmental Sciences  
790 Main Street  
Wareham, Massachusetts 02571-1075

**PREPARED BY:**

*Alex Armitage* 5/30/96  
Alex Armitage Date  
Study Director

*Kurt R. Andrews* 30 May 96  
Kurt R. Andrews Date  
Principal Investigator

**APPROVED BY:**

*Paul H. Fackler* 30 May 96  
Paul H. Fackler Date  
Director,  
Technical Operations

*Doreen S. Newhouse* 30 May 96  
Doreen S. Newhouse Date  
Manager,  
Quality Assurance Unit

This final report has been signed in accordance with SLI SOP No. 4.3.07(1).

**6.0 APPENDIX I - STUDY PROTOCOL**

Springborn Laboratories, Inc.  
 Environmental Sciences Division  
 790 Main Street • Wareham, Massachusetts 02571-1075 • (508) 295-2550 • Telex 4436041 • Facsimile (508) 295-8107

TEST PROTOCOL

PROTOCOL TITLE: Protocol for Determining the Biodegradability of a Test Substance Based on OECD Method 301B (CO<sub>2</sub> Evolution Test).

TO BE COMPLETED BY THE STUDY SPONSOR:	
Study Sponsor: Goodyear Tire & Rubber Company	
Address: 142 Goodyear Boulevard	
Akron, Ohio 44305	Phone: (216) 796-2963
Sponsor Protocol/Project No.:	
Test Substance Name(s): Wingstay SN-1 Lot # 132893 note made no. 10024-65-3	
Additional Comments and Modifications:	
Sponsor Approval <i>RJ Sm</i>	Date 10-31-95

TO BE COMPLETED BY SPRINGBORN LABORATORIES BEFORE TEST INITIATION:

Testing Facility: Springborn Laboratories, 790 Main St., Wareham, MA 02571-1075

Study Director: *Alex Amitage* Study No.: 13537-1195-6129-745

Test Concentration: *30mg C / liter* *2/2/96*

Dosing Solvent Used: *NA* CAS# or Lot # *NA*

Proposed Experimental Dates: (Start) *01/12/96* (Termination) *02/13/96*

Study Director Signature: *Alex Amitage* 01/16/95 Study Initiation Date

Springborn Laboratories' Protocol No.: 102095/13537/OECD 301B

Page No. 1 of 8  


LETTERS AND REPORTS. Springborn Laboratories, Inc. letters and reports are issued for the exclusive use of the clients to whom they are addressed. No disclosure from reports or use of the Springborn Laboratories, Inc. name is permitted except as expressly authorized in writing. Letters and reports apply only to the specific materials, products or processes tested, examined or surveyed and are not necessarily indicative of the quality of separately ordered or other materials, products or processes. The liability of Springborn Laboratories, Inc. with respect to services rendered shall be limited to the amount of the compensation paid for such services and not include any consequential damages.

PROTOCOL TITLE: Protocol for Determining the Biodegradability of a Test Substance  
Based on OECD Method 301B (CO<sub>2</sub> Evolution Test).

### 1.0 SUMMARY

The test substance is added to a chemically defined liquid medium at a concentration between 10-20 mg DOC or TOC per liter. The test is conducted in the dark, in glass bottles. The solution is inoculated with a small population of microorganisms, and aerated at 22±2°C. At regular sampling intervals the degradation of the test substance is monitored by measurement of CO<sub>2</sub> production in the test vessels. Control vessels are tested concurrently to measure endogenous CO<sub>2</sub> evolved in the absence of the test substance. In addition, a reference material, such as sodium benzoate, is tested concurrently to monitor the suitability of the microbial population. The test procedures follow "Ready Biodegradability: 301B CO<sub>2</sub> Evolution Test" pertaining to the OECD 1992 "Guidelines For Testing of Chemicals" and those published in the Official Journal, C.4-C, 1992. A modified dosing method is used for testing a substance that is not soluble in water.

### 2.0 METHODS AND MATERIALS

- 2.1 **Inoculum** - Activated sludge from the aeration basin of the Wareham Wastewater Treatment Plant is used as the microbial inoculum (other sources may be used to derive the inoculum, such as surface waters and soils). The Wareham Wastewater Treatment Plant receives predominantly domestic sewage. After collection, the activated sludge is passed through a fine sieve, if needed, to remove large particles. The sludge is then settled and centrifuged. The supernatant is discarded. The sludge may be washed in mineral medium. The concentrated sludge is suspended in mineral medium to yield a concentration of 3-5 grams suspended solids per liter. The moisture content of a 4.0- or 5.0-mL sample of well-mixed settled sludge is determined using a Sartorius moisture balance. From that measurement, the solids content of the sludge is determined, and the sludge is diluted accordingly. Thereafter, the sludge is aerated until used. The sludge inoculum is used on the day it is prepared. Therefore, no preconditioning or pre-adaptation to the test substance will take place.
- 2.2 **Nutrient Solution**- The aqueous medium for testing provides the essential nutrients necessary to sustain the inoculum throughout the testing period. The ingredients of the testing medium are detailed the OECD Method 301A (paragraph 5 and 6).
- 2.3 **Water**- High purity reagent grade water, free from inhibitory concentrations of toxic substances (e.g. Cu<sup>2+</sup> ions), is used for the preparation of the nutrient solution and all dosing stock solutions. Only one batch of water, previously checked by DOC analysis is used. The water must contain no more than 10% of the organic carbon content introduced by the test substance.
- 2.4 **CO<sub>2</sub> Trapping Apparatus** - Barium hydroxide (0.0125 M) is used to collect and contain CO<sub>2</sub> in the scrubbing apparatus. Exactly 4.0 g of Ba(OH)<sub>2</sub> · 8 H<sub>2</sub>O are dissolved per liter of water. The solution is filtered to remove any precipitates and is stored in a sealed bottle under a

nitrogen atmosphere to prevent absorption of CO<sub>2</sub> from air. The strength of this solution will be determined immediately before use by titration with standard acid.

The air source is CO<sub>2</sub>-free air. Alternatively, air containing CO<sub>2</sub> from a pressurized source is sparged at a constant rate through a series of scrubbing solutions connected in series using inert tubing to the test vessels. Scrubbing solutions for the system consist of four 1-L vessels containing 700 mL of 10 N (10 M) NaOH; one 1-L vessel containing 700 mL of the barium hydroxide solution (see above) and one empty 1-L vessel to prevent liquid carry-over. The strength of the barium hydroxide will be determined by titration with a standard acid, and the solution will be stored under nitrogen gas.

- 2.5 **Glassware Cleaning and Sterilization-** All test vessels and handling equipment are cleaned using a commercial detergent, followed by a 10% solution of nitric acid (HNO<sub>3</sub>) rinse and repetitive rinses with deionized water. The test vessels and equipment are then autoclaved for sterilization.
- 2.6 **Test Conditions-** A 1 percent inoculum (30 mg suspended solids/L) is used in the CO<sub>2</sub> Evolution Test. This is achieved by adding 2,400 mL of mineral medium to each 4-L glass test vessel. An appropriate volume of the prepared activated sludge is added to give a concentration of suspended solids of not more than 30 mg/L in the final 3 liters of inoculated mixture. All test vessels are agitated at approximately the same speed using stir plates and magnetic stir bars. The speed is sufficient to suspend the solids in the test solution, but not so high as to draw a vortex. Before dosing, this mixture is aerated at 22±2 °C with CO<sub>2</sub>-free air for 24 hours to purge the system of carbon dioxide. These solutions will be maintained at a temperature of 22±2 °C throughout the duration of the test:
- 2.7 **Test Dosing Method-** Test substance is added to the appropriate flasks, as listed in the following section of this protocol, to begin the test period. If the test substance is not readily soluble in water, it is added to a filter which is dropped into the test vessels. The amount added to the treated test systems is enough to add 10-20 mg carbon per liter in the test flask, if it all were dissolved, in excess of the blank organic carbon concentration. Calculations of the percent biodegradation are based on the amount of carbon added to the system.

The pH of the test solution is adjusted, if it falls outside the range of 6 to 8, by addition of 1.0 N HCl or NaOH under vigorous mixing.

- 2.8 **Test Vessels** - Test vessels consist of 4-L glass bottles. A total of seven test vessels will be used for the study as follows:
- Flasks 1 & 2: containing test substance (on a filter, if needed), mineral medium and inoculum (**test suspension**);
  - Flasks 3 & 4: containing only inoculum, mineral medium and, if needed, the same size fiber filter without test substance (**inoculum blank**);
  - Flask 5: containing reference compound, e.g. sodium benzoate, mineral medium, inoculum and the same size clean filter (**procedure control**);
  - Flask 6: containing test substance on a filter and sterilizing agent, e.g. HgCl<sub>2</sub>,

(abiotic sterile control);  
Flask 7: containing test substance on a filter, reference compound and inoculum (toxicity control).

If more than one compound is evaluated at once, additional procedural controls may be added. The final solution volume in each test vessel is 3 L. The CO<sub>2</sub> evolved from each test vessel is collected in three 100-mL barium hydroxide absorber bottles connected in series with flexible plastic tubing. The bottles are covered with aluminum foil to prevent light infiltration.

The test is started by bubbling CO<sub>2</sub>-free air through the solution at a rate of 50 to 100 mL per minute per test vessel (one to two bubbles per second). Samples for DOC measurement are taken from all test flasks at test initiation. This measurement will provide supporting evidence of degradation of the reference substance and may provide evidence for degradation of the test substance if significant solubilization occurs. The DOC samples are split, and one subsample is used to determine total carbon content. This will allow determination of inorganic carbon content.

- 2.9 **Sampling and Analysis-** CO<sub>2</sub> titrations are performed on the barium hydroxide trap nearest to the test vessel at each sampling interval (2, 4, 6, 9, 13, 18, 23 and 29 days), and the remaining two absorbers are moved one place closer to the test vessel. A new absorber containing 100 mL of fresh 0.025 N Ba(OH)<sub>2</sub> is placed to the far end of the series. Titrations may be made more frequently if precipitate is noted in the second CO<sub>2</sub> trap. If precipitate is noted, this absorber trap is titrated also, and subsequently replaced with a new absorber.

CO<sub>2</sub> produced on the test vessels reacts with the barium hydroxide and is precipitated as barium carbonate. The amount of CO<sub>2</sub> produced is determined by titrating a portion of the trapping solution in the trap closest to the test vessel with 0.05 N standardized HCl to the phenolphthalein endpoint. Solution handling is kept to a minimum, and no effort is made to remove precipitate prior to centrifuging, in order to decrease potential CO<sub>2</sub> absorption from air contact.

The pH of the test vessel solution is measured again on the 28th day, or on the second to last day of the test if the test duration is extended beyond 28 days. Samples are taken for DOC analysis, and then 1 mL of concentrated HCl is added to each of the test vessels to drive off inorganic carbonate. The test vessels are aerated overnight, and samples are removed from each test vessel for DOC analysis. The final titration is made on day 29, unless the CO<sub>2</sub> evolution pattern requires a prolonged testing period.

Upon study termination, a vessel inspection of the stored test substance is made and recorded to ensure stability during the experimental period.

- 2.10 **Test Duration** - The standard test duration is 28 days, unless the CO<sub>2</sub> evolution pattern indicates the test should continue.

### 3.0 CALCULATIONS

The biodegradation is defined as the CO<sub>2</sub> produced by the test substance, as a percentage of the theoretical CO<sub>2</sub> it should have produced ("ThCO<sub>2</sub>"), calculated from the organic carbon content of the substance. The organic carbon content is determined empirically for compounds of known molecular structure, and experimentally by dichromate oxidation and titration for mixtures and compounds of unknown structure. The ThCO<sub>2</sub> is based on the amount of test substance added.

The amount of CO<sub>2</sub> produced by a test substance is calculated by the difference (in mL of titrant) between the experimental and blank barium hydroxide traps. This difference is then multiplied by the factor 1.1 which is obtained by the following equation:

$$\text{mg CO}_2 = (((0.05 \text{ mmoles/mL}) \times (\text{mL HCl titrated for blank} - \text{mL titrated for treatment})) / 2) \times 44 \text{ mg/mmmole}$$

or

$$\text{mg CO}_2 = 1.1 \times \text{net mL of titrant}$$

The percent biodegradability is calculated at each sampling interval according to the following equation:

$$\% \text{ Biodegradability} = \frac{\text{mg CO}_2 \text{ produced}}{\text{mg TOC added in test} \times 3.67} \times 100\%$$

Where 3.67 is the conversion factor (44/12) for carbon to carbon dioxide.

The course of degradation will be displayed graphically and a 10-day window, lag phase, and slope will be indicated, if appropriate. The percent removal achieved at the plateau, at the end of the test and/or at the end of the 10-day window is calculated and reported where appropriate. Test substances yielding greater than 60 percent biodegradation within 28 days inside a 10-day window are regarded as readily biodegradable.

For each DOC sampling interval, percent DOC removal is calculated using net DOC values calculated for blanks as follows:

$$D_t = \left[ 1 - \frac{C_t - C_{\text{blank}(t)}}{C_0 - C_{\text{blank}(0)}} \right] \times 100$$

where

- D<sub>t</sub> = % removal at time t
- C<sub>0</sub> = starting concentration of DOC in the inoculated medium containing test substance (mg/L)
- C<sub>t</sub> = concentration of DOC in the inoculated medium containing test substance at time t (mg/L)

$C_{DO}(0)$  = mean starting concentration of DOC in blank inoculated medium at time 0 (mg/L)

$C_{DO}(t)$  = mean concentration of DOC in blank inoculated medium at time t (mg/L)

- 3.1 **Acceptance Criteria** - The test is considered valid if the reference material exhibits greater than 60 percent biodegradation within 14 days. Failure to exhibit this level of degradation indicates an inadequate inoculum, and the test is repeated using an inoculum from a different source.

The difference between replicates at the plateau or at the end of the test (10-day window should not exceed 20%). The inorganic carbon content of the test substance suspension at the beginning of the test should be <5% of the total carbon content.

The CO<sub>2</sub> evolution in blank controls should not exceed 70 mg per liter of medium at the end of the test. If the toxicity control does not show at least 25% degradation within 14 days, the test substance may be considered toxic to the inoculum. In that case, future testing should include consideration of reduced concentration and alternate detection methods.

#### 4.0 RECORDS TO BE MAINTAINED

Records to be maintained include, but are not limited to, correspondence and other documents relating to the interpretation and evaluation of data, as well as all raw data and documentation generated as a result of the study.

#### 5.0 REPORTING

The raw data and draft report are reviewed by the Quality Assurance Unit and Study Director. All values are reported to different levels of significance depending on the accuracy of the measuring devices employed during any one process. A single copy of the draft report is initially submitted to the Study Sponsor for review. Upon acceptance by the Sponsor, three copies of the final report are submitted. All reports include, but are not limited to, the following information:

- The report and project numbers from Springborn Laboratories, Inc. and Sponsor Study number (if any).
- Laboratory and site, dates of testing and personnel involved in the study, i.e., Quality Assurance Unit, Program Coordinator (if applicable), Study Director and Principal Investigator.
- All information pertaining to the test substance which appears on the sample bottle, e.g., its empirical formula, molecular structure, source, percent active ingredient, physical properties, Sponsor's test substance I.D., and sample number if available. In addition, a statement of the visible stability of the compound in the original container throughout the study will be included.

- A full description of the experimental design and procedures followed and a description of the test equipment used.
- Methods of preparation of the test substance and reference substance, including procedures for enhancing their dispersion into the test medium, if used.
- Temperature range recorded during the test period.
- The principal mathematical equations and statistics packages, if used in generating and analyzing the data as well as calculations using these equations.
- Tabular and graphical representations of the cumulative mg CO<sub>2</sub> and percent of theoretical CO<sub>2</sub> accumulated over time and at the plateau for every flask (except the blank flask, for which only the cumulative mg CO<sub>2</sub> produced will be reported). Tabular data will be reported for each replicate and the mean unless only one replicate was used or unless one replicate must be excluded on scientific grounds, in which case an explanation for the exclusion will be provided. The graphical presentation will show the lag phase, degradation phase, slope and time windows (time window means a 10-day period, starting from the time that the observed level of degradation first exceeded 10%), if appropriate.
- Ingredients of the nutrient solution.
- Source of the inoculum as well as date of collection and storage, and handling procedures.
- Description of any difficulties experienced and their resolution.
- Statement of Objectives
- Dry weight of settled sludge expressed as mg solids per L.
- The carbon content of the test and reference substances and the methods used to measure each.
- The duration of the test.
- Any observed abiotic degradation (from the sterile control), and a discussion of how these results might have impacted the study.
- Any observed inhibition phenomena (from the toxicity control), and a discussion of how these results might have impacted the study.
- Percent DOC removal and discussion thereof for each time period at which DOC was measured, along with the method of calculation.
- Discussion of results
- Deviations from the protocol not addressed in protocol amendments will be listed, together

- with an explanation for the change, as well as a discussion of the impact on the study.
- Good Laboratory Practice (GLP) compliance statement signed by the Study Director.
  - Date(s) of Quality Assurance audit(s) and certification of report approval.
  - Location of raw data and report.

#### 6.0 PROTOCOL CHANGES

All amendments to the approved protocol must be documented in writing and signed by both the Study Director and the Sponsor's contact or representative. Protocol amendments and deviations must include the reasons for the change and the predicted impact of the change on the results of the study, if any. If necessary, amendments initially may be verbally authorized, followed by Springborn Laboratories' written documentation. In such cases, the effective date of the amendment will be the date of verbal authorization.

#### 7.0 SPECIAL PROVISIONS

**GOOD LABORATORY PRACTICES (GLP):** All test procedures, documentation, records, and reports will comply with the Organization for Economic Co-operation and Development (OECD) Good Laboratory Practices as set forth in the OECD Guidelines for Testing Chemicals.

**TEST SUBSTANCE DISPOSAL:** Sixty days after issuing the final report, the test substance will be returned to the Sponsor's project officer, at Sponsor expense. A surcharge will be levied for all test substances refused to cover the expenses of waste disposal.

**ARCHIVAL:** All raw data will be archived by Springborn Laboratories until different arrangements are made with the Sponsor for final deposition. A copy of the final report will be archived at Springborn Laboratories, Inc.

#### 8.0 REFERENCES

OECD. Pans 1992. Test Guideline 301B. Adopted by the Council on 17<sup>th</sup> July 1992.

Official Journal of the European Communities. 1992. C.4. Biodegradation: determination of the "ready" biodegradability. C.4-C. Carbon dioxide (CO<sub>2</sub>) evolution. L383 A, Volume 35, 29 December 1992.

**7.0 APPENDIX II - CERTIFICATES OF ANALYSIS**

THE GOODYEAR TIRE & RUBBER COMPANY  
 BAYPORT CHEMICAL PLANT  
 P. O. BOX 669  
 LA PORTE, TEXAS 77572  
 (713) 474-0027

CERTIFICATE OF ANALYSIS  
 WINGSTAY-SNL

LOT NO.	(SPEC. LIMITS)	METHOD	
			130893
BATCH NUMBER			7
CRYSTAL POINT C	>27.0	E-867	27.0
COLOR GARDNER SCALE	3 MAX.	Visual	1
SPECIFIC GRAVITY @ 40 C	0.970-0.980	E-201	0.978
BROOKFIELD VIS. CPS 40 C	20-40cps	E-138	32.20
BIS ESTER CONTENT, %	65 - 80	E-920	66.61
MCNO ESTER CONTENT, %	20 MAX.	E-920	17.57
STAGE 1, %		E-920	11.63
TEG, %	2 MAX.	E-920	0.56
STG. 1 RESIDUAL MERCAPTAN, %	1 MAX.	E-920	0.11
APPARENT CHARGE RATIO	1.85-2.10		1.97
TOTAL DRUMS SHIPPED @ 400#			unavail.
SHIPPED TO: GOODYEAR			
PO #			
CODE NO.			

I the undersigned, certify that all test were performed in accordance with approved test methods and that the results are correct.

\_\_\_\_\_  
 Greg Prill, Manager  
 Quality Assurance

Prepared by: M. Lee  
 Date: January 12, 1994

95079RDS1062

RESEARCH & DEVELOPMENT ANALYTICAL SERVICES  
LABORATORY WORK REPORT 95-0350

Date: March 20, 1995

TO: R J Serva

FROM: E J Lauck

Subject: GPC Analysis of Wingstay SN-1

## SUMMARY:

A sample of Wingstay SN-1 and a sample of its relatively pure bis ester, were submitted by the Health, Safety, Environmental, Toxicology and Regulatory Compliance department for GPC analysis to determine their relative purities. This information is needed to support current toxicological testing.

## EXPERIMENTAL:

The samples were prepared for analysis by dissolving 0.15g sample in 10 ml of THF followed by filtration through a 0.45um filter prior to analysis. The samples were examined by GPC using refractive index detection. The results are summarized in the following table.

9988-52 Bis Std	
RT(min)	Area%
-----	
34.86	.03
36.51	98.06
38.84	.64
43.73	.01
51.41	.27
53.06	.37
57.01	.59
60.41	.03

Wingstay SN-1 Lot# 130893		
	RT(min)	Area%
-----		
Heavy	33.08	0.2
	34.25	1.9
	35.26	1.7
Bis	36.52	73.1
other	38.17	1.3
Mono	38.73	9.9
other	40.69	1.6
1st stage	41.74	9.0
Tetraethylene glycol	44.05	1.3

Attachments: YES\_\_\_NO\_\_X\_\_\_      Signature: E J Lauck  
Date Received: 03/15/95      Date Completed: 03/20/95  
cc: R T Prudence  
A Krishen  
Disoss

**8.0 APPENDIX III - ORGANIC CARBON ANALYSIS OF THE TEST SUBSTANCE**



13537 1195 6123 745  
**GALBRAITH LABORATORIES, INC.**  
*Accuracy with speed - since 1950*

13537 1195 6129 745

LABORATORY REPORT

Kurt R Andrews  
 Springborn Laboratories  
 790 Main Street  
 Wareham MA 02571-1075

Sample Received: 01/03/96  
 Report Date: 01/09/96  
 Purchase Order #: 29647

SAMPLE ID	LAB ID	ANALYSIS	RESULTS	
K-52-49A	N-9964	Total Carbon	77.86	%
		Carbonate calculated as Carbon	<0.02	%
		Total Organic Carbon (by difference)	77.84	%
SN-1-52-50A	N-9965	Total Carbon	64.26	%
		Carbonate calculated as Carbon	<0.02	%
		Total Organic Carbon (by difference)	64.24	%

Authorized Release of Data

*Shannon G. Augé*  
 Shannon G. Augé, Technical Manager

SGA:le

## 9.0 APPENDIX IV - WATER ANALYSIS

Well <sup>1</sup> Water Sample*		
Date Submitted: 7/28/95 Date Reported: 9/11/95		
OC & OP Pesticides in Water	Result As Received	Limit of Quantitation
Alpha BHC	< 0.01 µg/l	0.01 µg/l
Beta BHC	< 0.01 µg/l	0.01 µg/l
Gamma BHC - Lindane	< 0.01 µg/l	0.01 µg/l
Delta BHC	< 0.01 µg/l	0.01 µg/l
Heptachlor	< 0.01 µg/l	0.01 µg/l
Aldrin	< 0.01 µg/l	0.01 µg/l
Heptachlor Epoxide	< 0.01 µg/l	0.01 µg/l
p,p-DDE	< 0.01 µg/l	0.01 µg/l
p,p-DDD	< 0.01 µg/l	0.01 µg/l
p,p-DDT	< 0.01 µg/l	0.01 µg/l
o,p-DDE	< 0.01 µg/l	0.01 µg/l
o,p-DDD	< 0.01 µg/l	0.01 µg/l
o,p-DDT	< 0.01 µg/l	0.01 µg/l
HCB	< 0.01 µg/l	0.01 µg/l
Mirex	< 0.01 µg/l	0.01 µg/l
Methoxychlor	< 0.05 µg/l	0.05 µg/l
Dieldrin	< 0.01 µg/l	0.01 µg/l
Endrin	< 0.01 µg/l	0.01 µg/l
Teodrin	< 0.01 µg/l	0.01 µg/l
Chlordane	< 0.3 µg/l	0.3 µg/l
Toxaphene	< 4 µg/l	4 µg/l
Endosulfan Sulfate	< 0.03 µg/l	0.03 µg/l
Ronnel	< 0.01 µg/l	0.01 µg/l
Ethion	< 0.02 µg/l	0.02 µg/l
Trithion	< 0.05 µg/l	0.05 µg/l
Diazinon	< 0.1 µg/l	0.1 µg/l
Methyl Parathion	< 0.02 µg/l	0.02 µg/l
Ethyl Parathion	< 0.02 µg/l	0.02 µg/l
Malathion	< 0.05 µg/l	0.05 µg/l
Endosulfan I	< 0.01 µg/l	0.01 µg/l
Endosulfan II	< 0.01 µg/l	0.01 µg/l
PCB-1016	< 1 µg/l	1 µg/l
PCB-1221	< 1 µg/l	1 µg/l
PCB-1232	< 1 µg/l	1 µg/l
PCB-1242	< 1 µg/l	1 µg/l
PCB-1248	< 1 µg/l	1 µg/l
PCB-1254	< 1 µg/l	1 µg/l
PCB-1260	< 1 µg/l	1 µg/l

<sup>1</sup> Well water supplemented by Town of Wareham water

\* Analyzed by Lancaster Laboratories, Inc.

Well <sup>1</sup> Water Sample*		
Date Submitted: 7/28/95 Date Reported: 9/11/95		
Analysis	Result As Received	Limit of Quantitation
Arsenic	< 0.10 mg/l	0.10 mg/l
Selenium	< 0.20 mg/l	0.20 mg/l
Boron	< 0.050 mg/l	0.050 mg/l
Thallium	< 0.50 mg/l	0.50 mg/l
Aluminum	< 0.20 mg/l	0.20 mg/l
Antimony	< 0.20 mg/l	0.20 mg/l
Barium	< 0.10 mg/l	0.10 mg/l
Beryllium	< 0.010 mg/l	0.010 mg/l
Cadmium	< 0.010 mg/l	0.010 mg/l
Calcium	10.4 mg/l	0.20 mg/l
Chromium	< 0.030 mg/l	0.030 mg/l
Cobalt	< 0.050 mg/l	0.050 mg/l
Copper	< 0.025 mg/l	0.025 mg/l
Iron	< 0.10 mg/l	0.10 mg/l
Lead	< 0.10 mg/l	0.10 mg/l
Lithium	< 0.020 mg/l	0.020 mg/l
Magnesium	2.66 mg/l	0.10 mg/l
Manganese	< 0.010 mg/l	0.010 mg/l
Molybdenum	< 0.050 mg/l	0.050 mg/l
Nickel	< 0.050 mg/l	0.050 mg/l
Potassium	1.49 mg/l	0.50 mg/l
Silver	< 0.020 mg/l	0.020 mg/l
Sodium	17.3 mg/l	0.40 mg/l
Strontium	0.057 mg/l	0.010 mg/l
Tin	< 0.30 mg/l	0.30 mg/l
Titanium	< 0.020 mg/l	0.020 mg/l
Vanadium	< 0.015 mg/l	0.015 mg/l
Mercury	< 0.00020 mg/l	0.00020 mg/l
Zinc	0.027 mg/l	0.025 mg/l
Total Organic Carbon***	< 1. mg/l	1. mg/l
Total Suspended Solids	< 9. mg/l	9. mg/l
Nitrate Nitrogen	< 0.5 mg/l	0.50 mg/l
Chlorine Residual	< 0.1 mg/l	0.10 mg/l

<sup>1</sup> Well water supplemented by Town of Wareham water

\* Analyzed by Lancaster Laboratories, Inc.

\*\*\* Represents "non-purgeable TOC"

**Container No. 991**

**WINGSTAY® SN-1 - DETERMINATION  
OF THE n-OCTANOL/WATER PARTITION COEFFICIENT**

**Official Journal of the European Communities  
L383A, Part A, Section A.8.**

**Submitted to:**

**The Goodyear Tire & Rubber Company  
142 Goodyear Boulevard  
Akron, Ohio 44305**

**SLI Report # 95-4-5828**

**SLI Study #13537.1294.6108.705**

**Study Director: Deborah A. Hartley**

**Springborn Laboratories, Inc.  
Health and Environmental Sciences  
790 Main Street  
Wareham, Massachusetts 02571-1075**

**30 May 1996**

**FINAL REPORT**

96 JUL -2 AM 10: 09

RECEIVED  
OPT MGRS

96 JUL 26 11:31

RECEIVED  
MGRS



## TABLE OF CONTENTS

	Page
GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT .....	2
LIST OF TABLES .....	5
LIST OF FIGURES .....	6
SUMMARY .....	7
1.0 INTRODUCTION .....	8
2.0 METHODS AND MATERIALS .....	8
2.1 Protocol .....	8
2.2 Test Substance and Reagents .....	8
2.2.1 Test Substance .....	8
2.2.2 Reagents .....	9
2.3 Test Solution Preparation .....	9
2.3.1 Preliminary Testing .....	9
2.3.2 Partitioning Testing .....	9
2.4 n-Octanol/Water Saturation .....	9
2.5 Test System .....	10
2.6 Test Procedure .....	10
2.6.1 Preliminary Test .....	10
2.6.2 Partitioning Test .....	11
2.6.3 Chemical Analysis .....	11
3.0 CALCULATIONS .....	12
4.0 RESULTS .....	13
4.1 Preliminary Test Results .....	13
4.2 Partitioning Test Results .....	14
5.0 CONCLUSIONS .....	14
PROTOCOL DEVIATION .....	15
QUALITY ASSURANCE UNIT STATEMENT .....	16
REFERENCES .....	17
TABLES .....	18
FIGURE .....	23
SIGNATURES AND APPROVAL .....	25

---

6.0 APPENDIX I - STUDY PROTOCOL .....	26
7.0 APPENDIX II - CERTIFICATES OF ANALYSIS .....	33

## LIST OF TABLES

	Page
Table 1. Analytical results of test solution concentrations determined by HPLC during the preliminary and partitioning test. ....	19
Table 2. Preliminary test results for the partitioning of Wingstay® SN-1 between n-octanol and water .....	20
Table 3. Partitioning test results for the partitioning of Wingstay® SN-1 between n-octanol and water .....	21
Table 4. Analytical results for the quality control samples analyzed with the test samples .....	22

---

LIST OF FIGURES

	<b>Page</b>
<b>Figure 1. Diagram of Wingstay® SN-1 .....</b>	<b>24</b>

---

**SUMMARY****Wingstay® SN-1 - Determination of the  
n-Octanol/Water Partition Coefficient**

**SPONSOR:** The Goodyear Tire & Rubber Company

**PROTOCOL TITLE:** Test Substance: Determining the Partitioning Coefficient (n-Octanol/Water by Flask-shaking Method) of a Test Substance Following the Official Journal of the European Communities, L383 A - Part A.8. Springborn Laboratories Protocol #: 112394/EC-A.8 (Appendix I)

**REPORT NUMBER:** 95-4-5828

**STUDY NUMBER:** 13537.1294.6108.705

**TEST SUBSTANCE:** Wingstay® SN-1, Batch No. 130893, CAS Registry No. 64253-30-1, a white solid with calculations based on a purity of 100%, was received from Goodyear Research on 13 December 1994.

**EXPERIMENTAL TEST DATES:** 2 to 8 February 1995

**RESULTS:** The  $P_{ow}$  of Wingstay® SN-1 is concluded to be greater than  $10^4$ .

**CONCLUSION:** Chemicals with n-octanol/water partition coefficients of less than 10 are not expected to significantly bioconcentrate in living organisms or sorb to organic particles; chemicals with n-octanol/water partition coefficients of  $10^4$  or greater may bioconcentrate or sorb significantly (Veith et al., 1985). Wingstay® SN-1 with a log  $P_{ow}$  greater than 4, may significantly accumulate in lipid tissue and sorb onto organic particles.

## 1.0 INTRODUCTION

The objective of this study was to determine the n-octanol/water partition coefficient for Wingstay<sup>®</sup> SN-1 by the shake-flask method. This study was initiated on 16 December 1994, the day the Study Director signed the protocol, and was completed on the day the Study Director signed the final report. The experimental phase of this study was conducted from 2 to 8 February 1995 at Springborn Laboratories, Inc. (SLI), *Health and Environmental Sciences*, located in Wareham, Massachusetts. All original raw data generated and the final report produced during this study are stored in Springborn's archives at the above location.

## 2.0 METHODS AND MATERIALS

### 2.1 Protocol

This study was conducted according to the Springborn Laboratories, Inc. (SLI) standard operating procedures and the protocol entitled, "Test Material: Determining of the Partitioning Coefficient (n-Octanol/Water by Flask-shaking Method) of a Test Material Following the Official Journal of the European Communities, L383 A - Part A.8." Springborn Laboratories Protocol #: 112394/EC-A.8 (Appendix I).

### 2.2 Test Substance and Reagents

**2.2.1 Test Substance.** The test substance, Wingstay<sup>®</sup> SN-1, was received from Goodyear Research, Akron, Ohio on 13 December 1994. Upon receipt at Springborn, the test substance was stored at room temperature (approximately 20 °C) in a dark, ventilated cabinet. A diagram of the chemical structure is presented in Figure 1. The following information describes the test substance received:

Chemical Name:	diester of 3-(dodecylthio) propionic acid and tetraethylene glycol
Physical Appearance:	white solid
Batch No.:	130893
CAS Registry No.:	64253-30-1
Purity:	used as 100% (GPC Analysis, Certificate of Analysis, Appendix II)
Molecular Weight:	706.04 g/mol

Density:	0.990 ± 0.009 g/mL at 25 °C (determined at Springborn, SLI Report # 95-8-6026)
Water Solubility:	46.7 µg/L at approximately 20 °C (determined at Springborn, SLI Report # 95-5-5893)
Vapor Pressure:	below the detection limit of $1.33 \times 10^{-5}$ Pascal ( $1.00 \times 10^{-7}$ mm Hg) at 20.5 °C (determined at Springborn, SLI Report # 95-6-5928)

**2.2.2 Reagents.** All aqueous solutions were prepared using reagent water (meeting ASTM Type IIA requirements) obtained with a Sybron/Barnstead NANOpure® II system. The filter-sterilized water typically shows greater than 16.7 Mohm-cm resistivity and less than 1 mg/L total organic carbon, which is the detectable limit. All chemicals were at least reagent grade from commercial sources. The n-octanol (Lot No. 04536AG, 99+% pure) was obtained from Aldrich Chemical Company, Milwaukee, Wisconsin.

## 2.3 Test Solution Preparation

**2.3.1 Preliminary Testing.** A Wingstay® SN-1 test solution was prepared for preliminary testing by dissolving 176.5 mg of test substance to a final volume of 25 mL with water saturated n-octanol. This produced a test solution with a nominal concentration of  $1.00 \times 10^{-2}$  M. This solution was analyzed in triplicate by high performance liquid chromatography (HPLC). The results of the HPLC analysis are presented in Table 1.

**2.3.2 Partitioning Testing.** A Wingstay® SN-1 test solution was prepared for partitioning testing by dissolving 1412.1 mg test substance to a final volume of 200 mL with water saturated n-octanol. This produced a test solution with a nominal concentration of  $1.00 \times 10^{-2}$  M. This solution was analyzed in triplicate by HPLC. The results of the HPLC analysis are presented in Table 1.

## 2.4 n-Octanol/Water Saturation

For the preliminary and partitioning test, 1000 mL of reagent water and 500 mL of n-octanol were combined in an Erlenmeyer flask, placed on a shaker table (Lab-Line Model 3520), and shaken at approximately 150 revolutions per minute (rpm) for 24 hours to saturate both phases.

The aqueous and n-octanol phases were separated using a separatory funnel after being allowed to stand until a distinct phase boundary formed.

## 2.5 Test System

The test system used in this study consisted of 50-mL glass centrifuge tubes with Teflon<sup>®</sup>-lined screw caps. The centrifuge tubes were hand shaken in an environmental chamber and designed to maintain temperature at  $25 \pm 1$  °C.

## 2.6 Test Procedure

**2.6.1 Preliminary Test.** A preliminary test was performed in triplicate in order to provide an estimate of the partition coefficient for Wingstay<sup>®</sup> SN-1. This was determined at one nominal concentration of the test substance,  $1.0 \times 10^{-2}$  M, in water saturated n-octanol solution prior to initiation of the partitioning study.

Samples were prepared in three separate 50-mL centrifuge tubes by equilibrations with 40.0- mL of the n-octanol saturated water and 5.00 mL of the test solution, dissolved in water saturated n-octanol. Each tube was tightly sealed with a Teflon<sup>®</sup>-lined screw cap. All tubes were hand rotated through 180 degrees about their transverse axis so that any trapped air would rise through the phases. One hundred rotations in five minutes was performed in the environmental chamber.

After shaking, the tubes were centrifuged for 20 minutes at approximately 1000 rpm to completely separate the two layers and break up any emulsions. The samples were then placed back in the environmental chamber for one hour to re-equilibrate at the test temperature. Approximately 4.0 mL of the n-octanol phase (upper layer) from each tube was transferred to an HPLC vial using a disposable pipet. The remainder of the n-octanol was removed and discarded. The aqueous phase was sampled using a glass syringe with a removable needle. The syringe, partially filled with air, was slowly expelled as the needle was inserted into the water phase. Approximately 4.0 mL of the aqueous phase (lower layer) was removed and transferred to an HPLC vial. Each transfer vessel was wiped down with a tissue and pre-rinsed with a portion of the

equilibrium phase prior to transfer. In addition, samples were collected from the unused n-octanol saturated water, water-saturated n-octanol and from the test solution. These were designated as blank water, blank n-octanol and test solutions, respectively. Aqueous and n-octanol samples were analyzed by high performance liquid chromatography with ultraviolet detection (HPLC-UV). Quality Control (QC) samples were analyzed concurrently with the test samples.

**2.6.2 Partitioning Test.** A test solution for the partitioning test was prepared at a nominal concentration of  $1.0 \times 10^{-2}$  M Wingstay<sup>®</sup> SN-1, prepared in water saturated n-octanol. Three tests were conducted in duplicate by equilibrating either 20, 40, or 10 mL of the test solution with 40 mL of the n-octanol-saturated water. The centrifuge tubes designated as replicates 1 and 2 of sets A, B, and C respectively, were sealed with Teflon<sup>®</sup>-lined screw caps. The tubes were hand rotated through 180° about their traverse axis. A total of 100 rotations within 5 minutes was performed in the environmental chamber.

The tubes were centrifuged for 20 minutes at 1000 rpm, then placed back in the environmental chamber to re-equilibrate for one hour. The tubes were sampled in the same manner as the preliminary testing.

**2.6.3 Chemical Analysis.** Test samples were subjected to compound-specific analysis by HPLC-UV analysis of Wingstay<sup>®</sup> SN-1 by methodology developed at Springborn. Aqueous and n-octanol phase test samples were processed and analyzed with QC samples. Dilutions of the n-octanol test samples were prepared in n-octanol, when necessary.

For analysis of the aqueous and n-octanol samples, instrumentation consisted of a Hewlett-Packard Model 1050 or a Waters Model M6000A solvent pump, a Hewlett-Packard Model 1050 or a Waters Model 710B autosampler, an Applied Biosystems Model 759A or a Hewlett-Packard Model 1050 VW (variable wavelength) detector and a Hewlett-Packard Model 3393A or Model 3396A integrator or similar equipment.

The HPLC analysis was conducted utilizing the following (or similar) instrumental conditions:

Column: Metachem Technologies Inertsil Zorbax R<sub>x</sub> (C-8), 250 mm (length) x 4.6 mm (I.D.)  
Mobile Phase: 95/5 acetonitrile (Lot# BJ166)/reagent water  
Flow Rate: 2.0 mL/minute  
Wavelength: 210 nm  
Injection Volume: 10.0, 100, or 200 µL

A 1.00 mg/mL primary stock solution was prepared by dissolving 50.0 mg of Wingstay<sup>®</sup> SN-1 to a final volume of 50.0 mL high purity acetonitrile (Lot# BJ166). This stock, and dilutions of this stock were used to prepare QC and analytical calibration standards.

Calibration standards were prepared by diluting aliquots of the Wingstay<sup>®</sup> SN-1 stock solution in reagent water and n-octanol for the aqueous and n-octanol analysis, respectively. Introduction of samples and standards into the chromatographic system was performed by programmed injection.

During each phase of testing, a standard curve was constructed for each matrix (aqueous or n-octanol) by plotting the peak responses of the Wingstay<sup>®</sup> SN-1 analytical calibration standards against their prepared concentrations (mg/L). A linear regression analysis was performed for each set of calibration standards, and the coefficient of determination, slope, y-intercept and the limit of quantitation was calculated.

The Wingstay<sup>®</sup> SN-1 concentration of test samples was determined by comparing the peak response of the test samples with the linear regression analysis obtained for the analytical calibration standards in the corresponding matrix.

### 3.0 CALCULATIONS

The following equations were used to calculate measured concentrations of Wingstay<sup>®</sup> SN-1 and analytical results:

$$DC = \frac{(\text{signal} - b)}{m}$$

$$A = DC \times DF$$

where:

signal	=	peak signal (height) from chromatogram
b	=	y-intercept from regression analysis
m	=	slope from regression analysis
DC	=	detected concentration (mg/L) in the diluted or extracted sample by HPLC
DF	=	dilution factor (final volume of the diluted or extracted sample divided by the original volume, if appropriate)
A	=	analytical result (mg/L), concentration in the original aqueous sample

The partition coefficient ( $P_{ow}$ ) for Wingstay<sup>®</sup> SN-1 was calculated by dividing the molar concentration of Wingstay<sup>®</sup> SN-1 in n-octanol by the molar concentration of Wingstay<sup>®</sup> SN-1 in water. The  $P_{ow}$  was calculated using the following equation:

$$P_{ow} = \frac{C\text{-octanol}}{C\text{-water}}$$

where:

C-octanol	=	the molar concentration in the n-octanol phase
C-water	=	the molar concentration in the aqueous phase

## 4.0 RESULTS

### 4.1 Preliminary Test Results

The temperature range in the environmental chamber during preliminary testing was 24.3 to 24.4 °C. Linear regression analyses of the calibration standards analyzed with the preliminary test samples yielded a coefficient of determination ( $r^2$ ) of 0.995 for the standards in n-octanol, indicating the appropriateness of the analytical methods for the quantification of Wingstay<sup>®</sup> SN-1 in the corresponding test samples. Test samples in the aqueous phase were less than 0.5 mg/L, the analytical detection limit. An approximate partition coefficient ( $P_{ow}$ ) was estimated during preliminary testing. Data from the preliminary test are presented in Table 2.

#### 4.2 Partitioning Test Results

Partitioning testing was conducted to further define the partitioning of Wingstay® SN-1 between n-octanol and water. The partitioning test provided measurements of the equilibrium concentrations of Wingstay® SN-1 in the n-octanol and aqueous phases, from which the corresponding partition coefficients ( $P_{ow}$ ) could be calculated. These coefficients and the concentration of Wingstay® SN-1 measured in each replicate n-octanol or aqueous phase is presented in Table 3.

A linear regression analysis of the calibration standards analyzed with the partitioning test samples yielded a coefficients of determination ( $r^2$ ) of 0.996 for the standards in n-octanol indicating the appropriateness of the analytical methods for the quantification of Wingstay® SN-1 in the corresponding test samples. Test sample results in the aqueous phase were less than 0.0312 mg/L, the analytical detection limit. This detection limit was used to calculate the log partition coefficient  $\geq 5.35$  for Wingstay® SN-1. During partitioning testing, the temperature in the environmental chamber was 24.6 to 24.7 °C. Recoveries for the QC samples analyzed for both the preliminary and partitioning test are presented in Table 4 and confirmed the maintenance of sample integrity during the study.

#### 5.0 CONCLUSIONS

The log  $P_{ow}$  for Wingstay® SN-1 is concluded to be greater than 4. Chemicals with n-octanol/water partition coefficients of less than 10 are not expected to significantly bioconcentrate in living organisms or sorb to organic particles; chemicals with partition coefficients of  $10^4$  or greater may bioconcentrate or sorb significantly. Wingstay® SN-1 can be expected to bioconcentrate or sorb to organic material in the environment.

---

PROTOCOL DEVIATION

There were no protocol deviations during the course of this study.

SPRINGBORN LABORATORIES, INC.

Deborah A. Hartley 30 May 1996  
Deborah A. Hartley Date  
Study Director

### QUALITY ASSURANCE UNIT STATEMENT

The raw data and report for "Wingstay® SN-1 - Determination of the n-Octanol/Water Partition Coefficient" were inspected by the Quality Assurance Unit (QAU) at Springborn Laboratories, Inc., *Health and Environmental Sciences*, to determine adherence with the study protocol and laboratory standard operating procedures. In addition, inspection of certain phases of the study conduct was performed. Dates of study inspections, dates reported to Study Director and to Management are listed below.

<u>Inspection Date</u>	<u>Inspection Type</u>	<u>Reported to Study Director</u>	<u>Reported to Management</u>
2/9/95	Phase inspection	2/13/95	2/24/95
3/23/95	Data audit	3/23/95	3/24/95
6/15/95	Data audit	6/19/95	6/30/95
6/21/95	Draft report audit	6/21/95	6/30/95
6/22/95	Draft report audit	6/23/95	6/30/95
4/18/96	Revised draft report audit	4/18/96	4/19/96
5/28-30/96	Final report audit	5/30/96	5/30/96

SPRINGBORN LABORATORIES, INC.

Doreen S Newhouse 30 May 96  
 Doreen S. Newhouse Date  
 Manager,  
 Quality Assurance Unit

---

**REFERENCES**

- EC (Official Journal of the European Communities), L383. December 29, 1992. Part A. Methods for the Determination of Physico-Chemical Properties, Section A.8.
- OECD Good Laboratory Practices (OECD, 1981) as acknowledged in the EEC Council Directive 88/320/EEC of 9 June 1988.
- OECD (1993) OECD Guideline for Testing of Chemicals. Partition Coefficient (n-octanol/water)(Flask-Shaking Method). Guideline #107. Adopted 12 May 1981.
- Veith, G.D., DeFoe, D. and M. Knuth. 1985. Structure-activity relationships for screening organic chemicals for potential ecotoxicity effects. Drug Metab. Rev. 15:1295-1303

**TABLES**



**Table 2. Preliminary test results for the partitioning of Wingstay<sup>®</sup> SN-1 between n-octanol and water.**

	<u>Aqueous Phase</u>			<u>n-Octanol Phase</u>			$P_{ow}$	Log $P_{ow}$	Total Recovered (mg)	% Recovery <sup>a</sup>
	mg/L	mg	Molarity	mg/L	mg	Molarity				
R1	<0.500	<2.00 x 10 <sup>-2</sup>	7.08x10 <sup>-7</sup>	6360	31.8	9.01x10 <sup>-3</sup>	>1.27 x 10 <sup>4</sup>	>4.10	31.8	101.8
R2	<0.500	<2.00 x 10 <sup>-2</sup>	7.08x10 <sup>-7</sup>	6400	32.0	9.07x10 <sup>-3</sup>	>1.28 x 10 <sup>4</sup>	>4.11	32.0	102.5
R3	<0.500	<2.00 x 10 <sup>-2</sup>	7.08x10 <sup>-7</sup>	6260	31.3	8.86x10 <sup>-3</sup>	>1.25 x 10 <sup>4</sup>	>4.10	31.3	100.1
Mean (n = 3)							>1.27 x 10 <sup>4</sup>	>4.10 <sup>b</sup>		101.5
Standard Deviation (n = 3)							>1.52 x 10 <sup>2</sup>			1.2

<sup>a</sup> To calculate percent recovery, the following data can be used R1, R2, and R3 prepared using 5-mL at 6.25 mg/mL = 31.3 mg Wingstay<sup>®</sup> L-HLS was added to each tube.

<sup>b</sup> This value is the log of the mean  $P_{ow}$  determined.

**Table 3. Partitioning test results for the partitioning of Wingstay® SN-1 between n-octanol and water.**

	Aqueous Phase			n-Octanol Phase			$P_{ow}$	Log $P_{ow}$	Total Recovered (mg)	% Recovery <sup>a</sup>
	mg/L	mg	Molarity	mg/L	mg	Molarity				
R1A	<0.0312	$<3.12 \times 10^{-4}$	$<4.42 \times 10^{-8}$	7620	153	$1.08 \times 10^{-2}$	$>2.44 \times 10^5$	$>5.39$	153	102.5
R2A	<0.0312	$<3.12 \times 10^{-4}$	$<4.42 \times 10^{-8}$	7590	152	$1.08 \times 10^{-2}$	$>2.43 \times 10^5$	$>5.39$	152	102.1
Average							$>2.44 \times 10^5$	$>5.39$		
R1B	<0.0312	$<3.12 \times 10^{-4}$	$<4.42 \times 10^{-8}$	7530	301	$1.07 \times 10^{-2}$	$>2.41 \times 10^5$	$>5.38$	301	101.2
R2B	<0.0312	$<3.12 \times 10^{-4}$	$<4.42 \times 10^{-8}$	7380	295	$1.05 \times 10^{-2}$	$>2.37 \times 10^5$	$>5.37$	295	99.3
Average							$>2.39 \times 10^5$	$>5.38^b$		
R1C	0.0365	$3.65 \times 10^{-4}$	$5.18 \times 10^{-8}$	7310	73.1	$1.04 \times 10^{-2}$	$2.00 \times 10^5$	5.30	73.1	98.3
R2C	0.0415	$4.15 \times 10^{-4}$	$5.87 \times 10^{-8}$	7310	73.1	$1.04 \times 10^{-2}$	$1.76 \times 10^5$	5.25	73.1	98.3
Average							$1.88 \times 10^5$	5.27		
Mean (n = 6)							$>2.24 \times 10^5$	$>5.35^c$		100.3
Standard Deviation (n = 6)							$2.87 \times 10^4$			1.9

<sup>a</sup> To Calculate percent recovery, the following data can be used:  
R1A and R2A were prepared using 20 mL of the test solution. 20 mL at 7.435 mg/mL = 148.7 mg of Wingstay® SN-1 was added to each tube.

R1B and R2B were prepared using 40 mL of the test solution. 40 mL at 7.435 mg/mL = 297.4 mg of Wingstay® SN-1 was added to each tube.

R1C and R2C were prepared with 10 mL of the test solution. 10 mL at 7.435 mg/mL = 74.35 mg of Wingstay® SN-1 was added to each tube.

<sup>b</sup> This value is the log of the average  $P_{ow}$  determined.

<sup>c</sup> This value is the log of the mean  $P_{ow}$  determined.

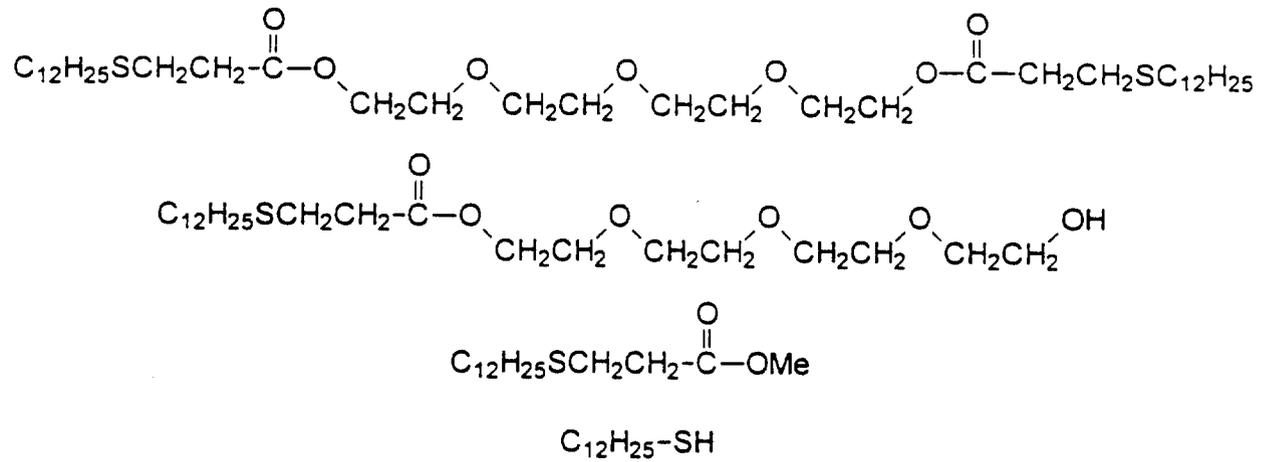
**Table 4. Analytical results for the quality control samples analyzed with the test samples.<sup>a</sup>**

Replicate	Phase	Concentration (mg/L)		Recovery (%)
		Fortified	Measured	
<b><u>Preliminary Test Samples</u></b>				
QA1	aqueous	1.00	0.921	92.1
QA1	n-octanol	10.0	15.1	115
QA2		50.0	56.3	112
QA3		100	111	111
<b><u>Partitioning Test Samples</u></b>				
QA1	aqueous	0.100	0.101	101
QA2		0.500	0.474	94.8
QA3		1.00	0.929	92.9
QA1	n-octanol	10.0	11.0	110
QA2		50.0	51.3	103
QA3		100	104	104

<sup>a</sup> Calculations were performed using actual unrounded analytical data, not the rounded values presented in this table. Minor discrepancies may be attributed to rounding.

**FIGURE**

Figure 1. Diagram of Wingstay® SN-1.



**SIGNATURES AND APPROVAL**

**SUBMITTED BY:**

Springborn Laboratories Inc.  
Health and Environmental Sciences  
790 Main Street  
Wareham, Massachusetts 02571

**PREPARED BY:**

Deborah A. Hartley 30 May 1996  
Deborah A. Hartley Date  
Study Director

Nigel D. Dix 30/5/96  
Nigel D. Dix Date  
Analytical Chemist

Scott M. Barrows 5/30/96  
Scott M. Barrows Date  
Principal Investigator

**APPROVED BY:**

Paul H. Fackler 30 May 96  
Paul H. Fackler Date  
Director,  
Technical Operations

Doreen S. Newhouse 30 May 96  
Doreen S. Newhouse Date  
Manager,  
Quality Assurance Unit

This final report has been signed in accordance with SLI SOP No. 4.3.07(1).

**6.0 APPENDIX I - STUDY PROTOCOL**

**Springborn Laboratories, Inc.**

Environmental Sciences Division

790 Main Street • Wareham, Massachusetts 02571-1075 • (508) 295-2550 • Telex 4436041 • Facsimile (508) 295-8107

**TEST PROTOCOL**

**PROTOCOL TITLE: Test Material: Determining the Partitioning Coefficient (n-Octanol/Water by Flask-shaking Method) of a Test Material Following the Official Journal of the European Communities, L383 A - Part A.8**

<b>TO BE COMPLETED BY THE STUDY SPONSOR:</b>	
Study Sponsor: Goodyear Tire & Rubber Company	
Address: 142 Goodyear Boulevard	
Akron, OH 44305	Phone: 216-796-2963
Sponsor Protocol/Project No.:	
Test Material Name(s): Wingstay SN1	
Purity:	CAS# or Lot # <i>CAS# 14253-30-1 Lot# 112093</i>
Additional Comments and Modifications: <i>Goodyear Sample # 10024-124-3</i>	
Sponsor Approval <i>RJ Sme</i>	Date <i>12-6-94</i>

**TO BE COMPLETED BY SPRINGBORN LABORATORIES BEFORE TEST INITIATION:**

Testing Facility: Springborn Laboratories, 790 Main St., Wareham, MA 02571

Study Director: *Deborah A. Hartley*

Study No.: *13537-1214-6108-705*

Test Concentration: 100% Test Material

Proposed Experimental Dates:

(Start *January, 1995* Termination *January, 1995*)

*Deborah A. Hartley*

*16 Dec 1994*

Study Director Signature *D*

Study Initiation Date

TEST MATERIAL: DETERMINING THE PARTITIONING COEFFICIENT  
(N-OCTANOL/WATER) BY THE FLASK-SHAKING METHOD FOLLOWING THE OFFICIAL  
JOURNAL OF THE EUROPEAN COMMUNITIES, L383 A - PART A.8

### 1.0 INTRODUCTION

The n-octanol/water partition coefficient ( $P_{ow}$ ) is the equilibrium ratio of the molar concentration of the test material in n-octanol and water in a dilute solution. The  $P_{ow}$  is determined by distributing the test material between n-octanol and water in a closed vessel at 20 to 25 + 1 °C and measuring the concentration in each phase after equilibration by specific chromatographic methods. The  $P_{ow}$  is determined from the following equation:

$$P_{ow} = \frac{C_{\text{octanol}}}{C_{\text{water}}}$$

where C is the molar concentration of the test material in n-octanol and water at equilibrium. The method includes preliminary experiments to determine the equilibration time. The test procedures follow OECD Method 107 (1981) and those published in the Official Journal of the European Communities (EC, 1992). This procedure is designed to determine the n-octanol/water partition coefficient in the range 0.01 - 10<sup>4</sup>. If the partition coefficient is outside this range, it will be characterized as less than 0.01 or greater than 10<sup>4</sup>. The procedure is not applicable to ionisable compounds without modifications. The use of buffer solutions and tests at multiple pH should be considered for such compounds.

### 2.0 METHODS AND MATERIALS

#### 2.1 Test Material:

Upon arrival at Springborn Laboratories, Inc., all test articles, materials, reference substances and standards are received by the Test Material Center. Records are maintained by GLP Standards, and a Chain-of-Custody is established. The condition of the external packaging of the test material is recorded and any damage is noted. The packaging is removed, the primary storage container is inspected for leakage or damage, and the condition is recorded. Any damage is reported to the Sponsor and/or manufacturer.

Each test material sample is given a unique sample ID number and is stored under the conditions specified by the Sponsor or manufacturer. Approximately 10 to 50 g of test material is required for these tests. Detection limits for measuring the test material concentration and other parameters will determine the exact amount needed. The following information is required from the Study Sponsor: test material lot or batch number, test material purity, water solubility (pH and temperature of solubility determination) vapor pressure, storage stability, methods of analysis of the test material in water, MSDS and safe handling procedures, and a verified expiration or reanalysis date, if applicable.

2.2 **n-Octanol and Water-** High purity distilled water and analytical grade n-octanol are used for testing. Necessary volumes of n-octanol saturated water and water saturated n-octanol are prepared prior to partitioning tests. Pure n-octanol and sufficient distilled water for saturation are added to a large vessel and then shaken for 24 hours on a mechanical shaker. The mixture is allowed to stand for phase separation long enough to allow the phases to separate and to achieve a saturation state. The same procedure is performed for n-octanol saturation of distilled water.

2.3 **Preliminary Estimate of  $P_{ow}$** - The partition coefficient is estimated based on the solubilities of the test material in the pure solvents, using the following equation:

$$P_{ow} = \frac{\text{Saturation } c_{n\text{-octanol}}}{\text{Saturation } c_{\text{water}}}$$

2.4. **Preliminary Test-** If the solubility of the test substance in either water or n-octanol is unknown, a simple preliminary experiment may be performed to estimate the approximate partition coefficient. The test material is dissolved in an appropriate volume of n-octanol, placed into a 50 mL glass centrifuge tube and the appropriate volume of water is then added. Generally, 5 mL of n-octanol and 40 mL water are used. A series of centrifuge tubes are then gently shaken, to minimize emulsion formation, at  $20 \pm 1$  °C. Both phases are analyzed after centrifugation to determine the approximate partition coefficient.

2.5 **Partitioning Test-** To minimize loss due to volatilization, the test vessel is nearly filled with the two solvents. The test vessel usually consist of a 50-mL glass centrifuge tube, although larger volume centrifuge tubes may be used. For hydrophobic test materials, a larger volume of water may be required, and, if necessary, preliminary and definitive testing will be performed in large ground-glass stoppered flasks. The volume ratio and quantities of the test substance are fixed by (1) the approximate partition coefficient determined in the preliminary test; (2) the minimum quantity of test material required for the analytical procedure; and (3) the limitation of a maximum concentration in either phase of 0.01 mol/L.

Three tests are conducted, each in duplicate:

1. The first test uses the approximate volume ratio determined from the preliminary test.
2. In the second test, twice the volume of n-octanol is added.
3. In the third test, half the volume of n-octanol is added.

A stock solution of the test material with a mass concentration between 1 and 100 mg/mL is prepared in n-octanol. The precise concentration of the test material in this stock solution is determined prior to use. The stock solution is stored under stable conditions. To initiate the tests, measured aliquots of the two solvents together with the

necessary quantity of the stock solution are placed in glass test vessels and tightly sealed. The test vessels are rotated by hand through 180° about its transverse axis so that trapped air rises through the two phases. About 100 rotations in 5 minutes usually establish partition equilibrium. After equilibration, centrifuge tubes are centrifuged to separate the n-octanol and water and to break-up any emulsions formed. The centrifuge tubes are reequilibrated at 20 to 25 ± 1 °C for one hour before analysis.

2.6 **Sampling/Analysis-** Sampling of the n-octanol and water phases after centrifugation for preliminary and definitive tests proceeds as follows:

- a) A known volume of the n-octanol phase is withdrawn by pipet and transferred to an analysis or dilution vessel. The outside of the pipet is wiped with a paper tissue prior to transfer.
- b) The remainder of the n-octanol phase and the interfacial layer is removed and discarded. Alternatively, for small aqueous volumes, the aqueous phase can be sampled to minimize the risk of including traces of the n-octanol as follows. A syringe with a removable needle is partly filled with air. Air is gently expelled while inserting the needle through the n-octanol phase. An adequate volume of aqueous phase is withdrawn into the syringe, the syringe is quickly removed and the needle detached. The contents of the syringe is then used as the aqueous sample.
- c) Another pipet is used to transfer a known volume of the water phase to an analysis or dilution vessel. The outside of the pipet is wiped with a paper tissue prior to transfer.

All transfer vessels are prewashed with a portion of the equilibrium phase prior to transfer for analysis.

Phase separation for large volumes is performed by transferring the two phase mixture to centrifuge tubes prewashed with the aqueous phase, and centrifuging as above. Aliquots of the phases separated are combined prior to analysis.

2.7 **Calculations-** The  $P_{ow}$  is calculated from the following equation:

$$P_{ow} = \frac{C_{\text{octanol}}}{C_{\text{water}}}$$

where C is the molar concentration in n-octanol and water at equilibrium.

### 3.0 RECORDS TO BE MAINTAINED

Records to be maintained include but are not limited to, correspondence and other documents relating to the interpretation and evaluation of data as well as all raw data and documentation generated as a result of the study.

### 4.0 REPORTING

The report will be a typed document, submitted in triplicate, describing the results of the study, and will be signed by the Study Director and the Quality Assurance Unit. It will include, but not be limited to, the following:

- Dates on which the study began and ended.
- Name and address of the testing laboratory.
- Location where the test was performed.
- Name(s) of principal investigator(s).
- Signatures of the senior scientific personnel responsible for the study.
- A full description of the experimental design and procedures followed and a description of the test equipment used.
- Identification of the test substance including chemical name, common name, CAS Registry number and percentage of active ingredient, molecular structure, and qualitative and quantitative descriptions of the chemical composition (Sponsor supplied).
- Manufacturer and lot and sample numbers of the test substance.
- Properties of the test substance including physical state, pH, and stability (Sponsor supplied).
- The principal mathematical equations used in generating and analyzing the data as well as calculations using these equations. Tabular representations of the data.
- Results of the preliminary equilibration study, including analytical method description and precision and accuracy data.
- Results of the measured concentrations of the test material in the n-octanol and water phases of the definitive test.
- Calculated  $P_{ow}$  for each concentration and pH tested including individual and mean (SD) data.

- Data evaluation and conclusions.
- Location of raw data and report.
- A complete description of any protocol deviations and the impact expected.
- Dates of Quality Assurance reviews to the Study Director and management, signed by the QA unit.

#### 5.0 PROTOCOL CHANGES

All amendments to the approved protocol must be documented in writing and signed by both the Study Director and the Sponsor's contact or representative. Protocol amendments and deviations must include the reasons for the change and the impact of the change on results of the study, if any. If necessary, amendments initially may be in the form of verbal authorization, followed by Springborn's written documentation of the amendment. In such cases, the effective date of the amendment will be the date of verbal authorization.

#### 6.0 SPECIAL PROVISIONS

**GOOD LABORATORY PRACTICES (GLP):** All test procedures, documentation, records, and reports will comply with the Community principles of Good Laboratory Practices as promulgated under the Council Directive 88/320/EEC of 9 June 1988.

**TEST MATERIAL DISPOSAL:** After 60 days of the issuance of the final report, the test material will be returned to the Sponsor's project officer, at Sponsor expense, unless different arrangements are made.

**ARCHIVAL:** All raw data will be archived by the Study Sponsor unless different arrangements are made. A copy of the final report will be archived at Springborn Laboratories, Inc.

#### 7.0 REFERENCES

OECD, Paris 1981. Test Guideline For Testing of Chemicals. Decision of the Council C(81) "Partition Coefficient ( $n_{\text{octanol/water}}$ ) (Flask-shaking Method). May 12, 1981.

EC (Official Journal of the European Communities), L383. December 29, 1992. Part A. Methods for the Determination of Physico-Chemical Properties, Section A.8

**7.0 APPENDIX II - CERTIFICATES OF ANALYSIS**

01/20/95 15:19 216 796 3304

CDYR RES RM 256

002

THE GOODYEAR TIRE & RUBBER COMPANY  
 BAYPORT CHEMICAL PLANT  
 P. O. BOX 669  
 LA PORTE, TEXAS 77572  
 (713) 474-0027

CERTIFICATE OF ANALYSIS  
 WINGSTAY-SM1

LOT NO.	(SPEC. LIMITS)	METHOD	
			130893
BATCH NUMBER			7
CRYSTAL POINT C	>27.0	E-867	27.0
COLOR GARDNER SCALE	3 MAX.	Visual	1
SPECIFIC GRAVITY @ 40 C	0.970-0.980	E-201	0.978
BROOKFIELD VIS. CPS 40 C	20-40cps	E-338	32.20
BIS ESTER CONTENT, %	65 - 80	E-920	66.61
MCNO ESTER CONTENT, %	20 MAX.	E-920	17.57
STAGE 1, %		E-920	11.63
TEG, %	2 MAX.	E-920	0.56
STG. 1 RESIDUAL MERCAPTAN, %	1 MAX.	E-920	0.11
APPARENT CHARGE RATIO	1.85-2.10		1.97
TOTAL DRUMS SHIPPED @ 400#			unavail.
SHIPPED TO: GOODYEAR			
PO #			
CODE NO.			

I the undersigned, certify that all test were performed in accordance with approved test methods and that the results are correct.

Greg Frill, Manager  
 Quality Assurance

Prepared by: M. Lee  
 Date: January 12, 1994

95079RDS1062

RESEARCH & DEVELOPMENT ANALYTICAL SERVICES  
LABORATORY WORK REPORT 95-0350

Date: March 20, 1995

TO: R J Serva

FROM: E J Lauck

Subject: GPC Analysis of Wingstay SN-1

## SUMMARY:

A sample of Wingstay SN-1 and a sample of its relatively pure bis ester, were submitted by the Health, Safety, Environmental, Toxicology and Regulatory Compliance department for GPC analysis to determine their relative purities. This information is needed to support current toxicological testing.

## EXPERIMENTAL:

The samples were prepared for analysis by dissolving 0.15g sample in 10 ml of THF followed by filtration through a 0.45um filter prior to analysis. The samples were examined by GPC using refractive index detection. The results are summarized in the following table.

9988-52 Bis Std	
RT(min)	Area%
-----	
34.86	.03
36.51	98.06
38.84	.64
43.73	.01
51.41	.27
53.06	.37
57.01	.59
60.41	.03

Wingstay SN-1 Lot# 130893		
	RT(min)	Area%
	-----	
Heavy	33.08	0.2
	34.25	1.9
	35.26	1.7
Bis	36.52	73.1
other	38.17	1.3
Mono	38.73	9.9
other	40.69	1.6
1st stage	41.74	9.0
Tetraethylene glycol	44.05	1.3

Attachments: YES\_\_\_NO\_\_X\_\_      Signature: E J Lauck  
Date Received: 03/15/95      Date Completed: 03/20/95  
cc: R T Prudence  
A Krishen  
Disoss

**Contains No CBI**

**WINGSTAY® SN-1 - DETERMINATION  
OF WATER SOLUBILITY**

**Official Journal of the European Communities,  
L383A, Part A, Section 6**

**Submitted to:**

**The Goodyear Tire & Rubber Company  
142 Goodyear Boulevard  
Akron, OH 44305**

**SLI Report #95-5-5893**

**SLI Study # 13537.1294.6107.700**

**Study Director: John Mao, Ph.D.**

**Springborn Laboratories, Inc.  
Health and Environmental Sciences  
790 Main Street  
Wareham, Massachusetts 02571-1075**

**30 May 1996**

**FINAL REPORT**

60 JUN 2 1996

RECEIVED  
OFFICE

RECEIVED  
96 JUN 2 11:31

**GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT**

The data and report presented for "**Wingstay<sup>®</sup> SN-1 - Determination of Water Solubility**" were produced and compiled in accordance with all pertinent OECD Principles of Good Laboratory Practice. Stability, characterization, verification of the test substance identity and maintenance of records on the test substance are the responsibility of the Study Sponsor. At the termination of the testing program, all remaining test substance will be sent to the Study Sponsor. Maintenance of a sample of the test substance is the responsibility of the Study Sponsor.

SPRINGBORN LABORATORIES, INC.

John Mao                      5/30/96  
John Mao, Ph.D.                      Date  
Study Director

## TABLE OF CONTENTS

	<b>Page</b>
GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT .....	2
LIST OF TABLES .....	4
LIST OF FIGURES .....	5
SUMMARY .....	6
1.0 INTRODUCTION .....	7
2.0 MATERIALS AND METHODS .....	7
2.1 Protocol .....	7
2.2 Test Substance and Standard Reagents .....	8
2.2.1 Test Substance .....	8
2.2.2 Standard Reagents .....	8
2.3 Stock Solution .....	8
2.4 Test System .....	9
2.5 Test Procedure .....	9
2.6 Analysis .....	9
3.0 CALCULATIONS .....	10
4.0 RESULTS AND CONCLUSIONS .....	11
QUALITY ASSURANCE UNIT STATEMENT .....	12
REFERENCES .....	13
TABLE .....	14
FIGURES .....	17
SIGNATURES AND APPROVAL .....	22
5.0 APPENDIX I - STUDY PROTOCOL .....	23
6.0 APPENDIX II - CERTIFICATES OF ANALYSIS .....	30

---

LIST OF TABLES

	<b>Page</b>
<b>Table 1.</b> Solubility of Wingstay <sup>®</sup> SN-1 in reagent water .....	15
<b>Table 2.</b> Quality control samples prepared in 50/50 CH <sub>3</sub> CN/reagent water .....	16

---

**LIST OF FIGURES**

	<b>Page</b>
<b>Figure 1.</b> Diagram of the chemical structure of Wingstay <sup>®</sup> SN-1 .....	18
<b>Figure 2.</b> HPLC chromatogram of Wingstay <sup>®</sup> SN-1 in a 0.05 mg/L standard solution .....	19
<b>Figure 3.</b> HPLC chromatogram of Wingstay <sup>®</sup> SN-1 sample .....	20
<b>Figure 4.</b> HPLC chromatogram of control sample .....	21

**SUMMARY**

**Wingstay® SN-1 - Determination of Water Solubility**

**SPONSOR:** The Goodyear Tire & Rubber Company

**PROTOCOL TITLE:** "Test Material: Determining the Water Solubility of a Substance Following The Official Journal of the European Communities, L 383 A - Part A.6.", Springborn Laboratories, Inc. Protocol #: 112294/EC A.6.

**REPORT NUMBER:** 95-5-5893

**STUDY NUMBER:** 13537.1294.6107.700

**TEST SUBSTANCE:** Wingstay® SN-1, Batch No. 130893, CAS Registry No. 64253-30-1, a white solid with calculations based on a purity of 100%, was received from Goodyear Research on 13 December 1994.

**EXPERIMENTAL TEST DATES:** 2 to 4 March 1995

**RESULTS:** Wingstay® SN-1 was shown to be extremely insoluble in water. The water solubility of Wingstay® SN-1 was determined by the column elution method to be 46.7 µg/L at approximately 20 °C.

## 1.0 INTRODUCTION

Information on physical and chemical characteristics is required to support registration of products in OECD and EC countries. This report describes the determination of the water solubility of Wingstay<sup>®</sup> SN-1. The test substance was analyzed by high performance liquid chromatography with ultraviolet detection (HPLC-UV).

Water solubility is an important property governing the mobility and distribution of a test substance within the environment. Generally, highly water soluble substances are more likely to be transported and distributed by the hydrologic cycle than relatively water-insoluble substances. The degree of water solubility influences the extent to which a substance may sorb to particulate matter or cross a lipid/water interface. The water solubility of a substance is specified by the saturation mass concentration of the substance in water and is a function of temperature. Knowledge of water solubility is also used to determine appropriate experimental design in ecological effects and fate tests.

The study was initiated on 14 December 1994, the date the Study Director signed the protocol, and terminated on the day the Study Director signed the final report. The experimental phase of this study was conducted from 2 to 4 March 1995 at Springborn Laboratories, Inc. (SLI), *Health and Environmental Sciences*, located in Wareham, Massachusetts. All original raw data generated and the final report produced during this study are stored in Springborn's archives at the above location.

## 2.0 MATERIALS AND METHODS

### 2.1 Protocol

This study was conducted according to the Springborn protocol entitled "Test Material: Determining the Water Solubility of a Substance Following The Official Journal of the European Communities, L 383 A - Part A.6.", Springborn Laboratories, Inc. Protocol #: 112294/EC A.6.

## 2.2 Test Substance and Standard Reagents

**2.2.1 Test Substance.** The test substance, Wingstay<sup>®</sup> SN-1, was received from Goodyear Research, Akron, Ohio on 13 December 1994. Upon receipt at Springborn, the test substance was stored at room temperature (approximately 20 °C) in a dark, ventilated cabinet. A diagram of the chemical structure is presented in Figure 1. The following information describes the test substance received:

Chemical Name:	diester of 3-(dodecylthio) propionic acid and tetraethylene glycol
Physical Appearance:	white solid
Batch No.:	130893
CAS Registry No.:	64253-30-1
Purity:	used as 100% (Certificate of Analysis, GPC analysis of Wingstay <sup>®</sup> SN-1, Batch No. 130893, Appendix II)
Molecular Weight:	706.04 g/mol
Density:	0.990 ± 0.009 g/mL at 25 °C (determined at Springborn, SLI Report # 95-8-6026)
Vapor Pressure:	below the detection limit of 1.33 x 10 <sup>-5</sup> Pascal (1.00 x 10 <sup>-7</sup> mm Hg) at 20.5 °C (determined at Springborn, SLI Report # 95-6-5928)

**2.2.2 Standard Reagents.** All aqueous solutions were prepared using reagent water (meeting ASTM Type IIA requirements) obtained with a Sybron/Barnstead NANOpure<sup>®</sup> II system. The filter-sterilized water typically shows greater than 16.7 Mohm-cm resistivity and less than 1 mg/L total organic carbon, which is the detectable limit. All chemicals were at least reagent grade from commercial sources.

## 2.3 Stock Solution

A 1.00 mg/mL primary stock solution was prepared by dissolving and diluting 0.0502 g of Wingstay<sup>®</sup> SN-1 to 50.0 mL with acetonitrile (lot # BJ484). A secondary dilution of this Wingstay<sup>®</sup> SN-1 primary stock solution was used to prepare HPLC-UV calibration standards and Quality Control (QC) samples.

A 10.0 mg/mL stock solution was prepared for study initiation by dissolving and diluting 0.251 g of Wingstay® SN-1 to 25.0 mL with acetone (lot #BJ534). Five milliliters of this stock solution were used to load the support material for the column elution definitive water solubility test.

#### 2.4 Test System

The column elution method was chosen for the definitive test. The test system consisted of duplicate double-walled columns. The support material consisted of silica gel (EM Science, CAS# 63231-67-4 ) and was packed in the columns. Both columns were placed in an environmental chamber maintained at approximately 20 °C throughout the test.

#### 2.5 Test Procedure

Five milliliters of the 10 mg/L stock solution was added to approximately 0.600 g of silica gel material in roundbottom flasks. The acetone solvent was allowed to evaporate using rotary evaporation and the treated silica gel was further dried under a stream of nitrogen gas. The treated silica gel was soaked for 2 hours in 5 mL of reagent water at room temperature. The duplicate columns were packed with treated silica gel coated in test substance. Reagent water was applied to the columns with HPLC-grade solvent pumps, one set at a flow rate of 0.20 mL/min and the other set at 0.40 mL/min. The test systems were allowed to equilibrate in the environmental chamber. Samples were collected on Day 2 for HPLC-UV analysis, and were also measured for pH using an Orion Model 420A meter and glass pH electrode. A total of five samples were collected at approximately 20-min intervals for each column.

#### 2.6 Analysis

Wingstay® SN-1 was measured using a reverse-phase HPLC-UV system. Samples were applied to the system by direct aqueous programmed injection. The system consisted of an Hewlett-Packard Series 1050 solvent pump equipped with an Hewlett-Packard 1050 autosampler, an Hewlett-Packard 1050 VW UV detector, an Hewlett-Packard 3396A integrator, an Eppendorf CH30 column heater and an Eppendorf TC55 column controller. The analyses were conducted under the following conditions:

Column:	Zorbax C8, (5 µm), 250 mm (length) x 4.6 mm I.D.
Mobile Phase:	95/5 acetonitrile/reagent water
Flow Rate:	2.0 mL/minute
Column Temperature:	40 °C
Injection Volume:	500 µL
Wavelength:	210 nm

HPLC calibration standards (0.0500, 0.100 and 0.200 mg/L) were prepared by fortifying 50/50 CH<sub>3</sub>CN/reagent water with the a 0.100 mg/mL Wingstay<sup>®</sup> SN-1 secondary stock solution. Two sets of standards were analyzed by HPLC-UV with the sample set. A standard curve was constructed by plotting the UV detection peak height of each analytical standard against the concentration (mg/L) of the standard injected. A linear regression of the data was performed, and the coefficient of determination (r<sup>2</sup>), slope, y-intercept and limit of detection were calculated. The sample concentration was determined by comparing the HPLC-UV peak height obtained for the sample with the regression of the calibration standards.

### 3.0 CALCULATIONS

The following equations were used to calculate measured concentrations and analytical results:

$$DC = \frac{(\text{signal} - b)}{m}$$

- where:
- DC = detected concentration (mg/L) in the sample on HPLC-UV
  - signal = peak signal (height) from chromatogram
  - b = y-intercept from regression analysis
  - m = slope from regression analysis
  - DF = analytical result (mg/L), concentration in the original aqueous sample

---

#### 4.0 RESULTS AND CONCLUSIONS

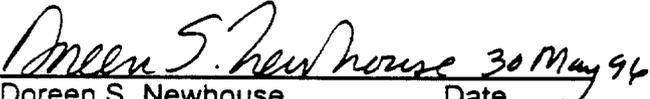
Duplicate columns were prepared at a high (0.40 mL/min) and a low flow rate (0.20 mL/min). Five sample fractions were chosen after column equilibration. For the lower flow rate the water solubility of Wingstay<sup>®</sup> SN-1 was  $46.4 \pm 3.5$  µg/L. For the higher flow rate, the water solubility of Wingstay<sup>®</sup> SN-1 was  $47.1 \pm 1.0$  µg/L. The average water solubility of Wingstay<sup>®</sup> SN-1 was determined to be 46.7 µg/L at approximately 20 °C. The pH ranged from 6.16 to 6.70 for all test samples. Measured concentrations of Wingstay<sup>®</sup> SN-1 and pH of test samples are presented in Table 1. The percent recovery for the QC samples are presented in Table 2 and verified the test sample integrity over the study duration.

### QUALITY ASSURANCE UNIT STATEMENT

The raw data and report for "**Wingstay<sup>®</sup> SN-1 - Determination of Water Solubility**" were inspected by the Quality Assurance Unit at Springborn Laboratories Inc., *Health and Environmental Sciences* to determine adherence with the study protocol and laboratory standard operating procedures. In addition, inspection of certain phases of the conduct for the study was performed. Dates of study inspections, dates reported to the Study Director and to Management are listed below.

<u>Inspection Date</u>	<u>Inspection Type</u>	<u>Reported to Study Director</u>	<u>Reported to Management</u>
3/2/95	Phase inspection	3/3/95	3/10/95
6/6-7/95	Data audit	6/7/95	6/16/95
6/6-7/95	Draft report audit	6/7/95	6/16/95
6/8/95	Draft report audit	6/8/95	6/16/95
4/18/96	Revised draft report audit	4/18/96	4/19/96
5/30/96	Final report audit	5/30/96	5/30/96

SPRINGBORN LABORATORIES, INC.

  
 Doreen S. Newhouse                      Date  
 Manager,  
 Quality Assurance Unit

### REFERENCES

OECD (1981). Good Laboratory Practices as acknowledged in the EEC Council Directive 88/320/EEC of 9 June 1988.

Official Journal of the European Communities (1992). L383A. Part A: Methods for the Determination of Physico-Chemical Properties. Section A.6. Water Solubility. 29 December 1992.

**TABLE**

**Table 1. Solubility of Wingstay® SN-1 in reagent water.<sup>a</sup>**

Replicate	<u>0.4 mL/min</u>	pH	<u>0.2 mL/min</u>	pH
	Concentration µg/L		Concentration µg/L	
1	45.9		50.8	
2	48.2		48.8	
3	48.1		44.5	
4	46.5		41.8	
5	46.7	6.70	45.9	6.16
	Mean	47.1	Mean	46.4
	Std. Dev.	±1.0	Std. Dev.	±3.5

<sup>a</sup> Calculations were performed using the actual unrounded analytical data and not the rounded values presented in this table.

**Table 2. Quality control samples prepared in 50/50 CH<sub>3</sub>CN/reagent water.**

---

	<b>Nominal Concentration (µg/L)</b>	<b>Measured Concentration (µg/L)</b>	<b>Percent Recovery (%)</b>
QC 1	50.0	50.6	101
QC 2	100	97.2	97.2
QC 3	200	199	99.3

---

**FIGURES**

Figure 1. Diagram of the chemical structure of Wingstay® SN-1.

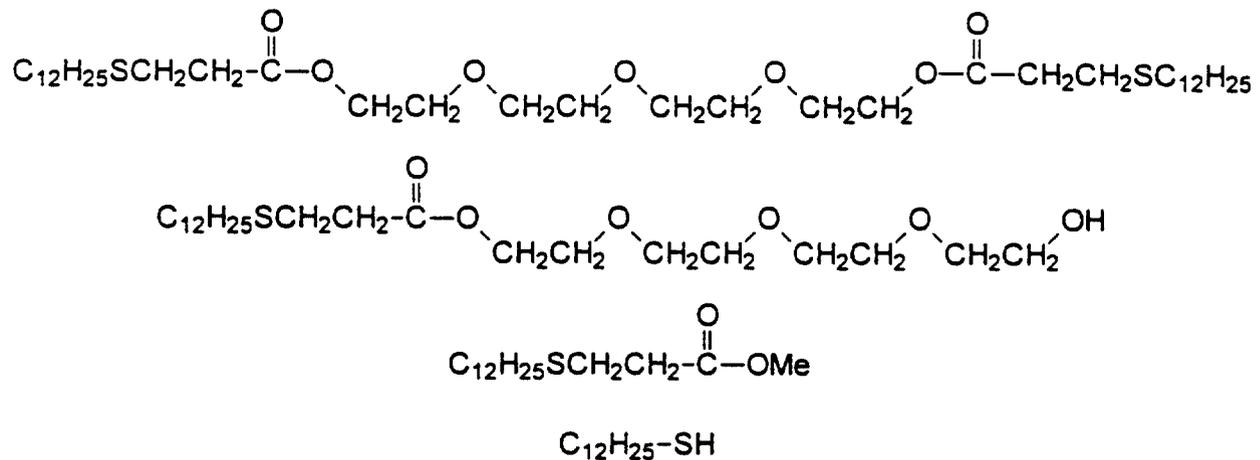


Figure 2. HPLC chromatogram of Wingstay® SN-1 in a 0.05 mg/L standard solution.

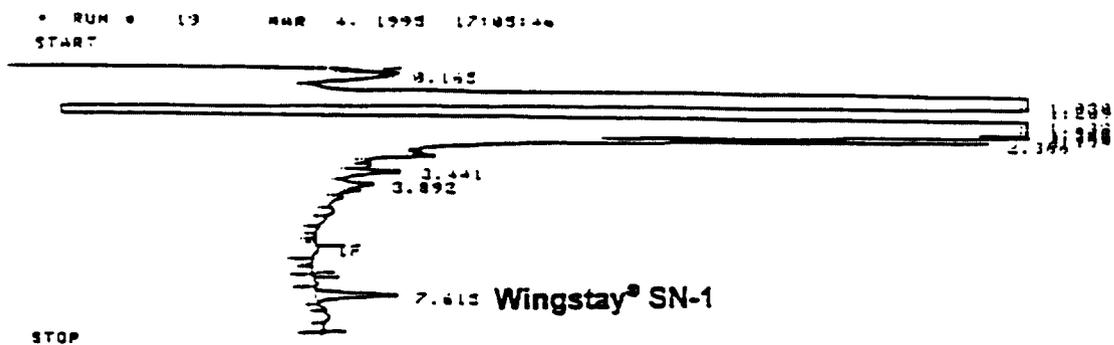


Figure 3. HPLC chromatogram of Wingstay® SN-1 sample.

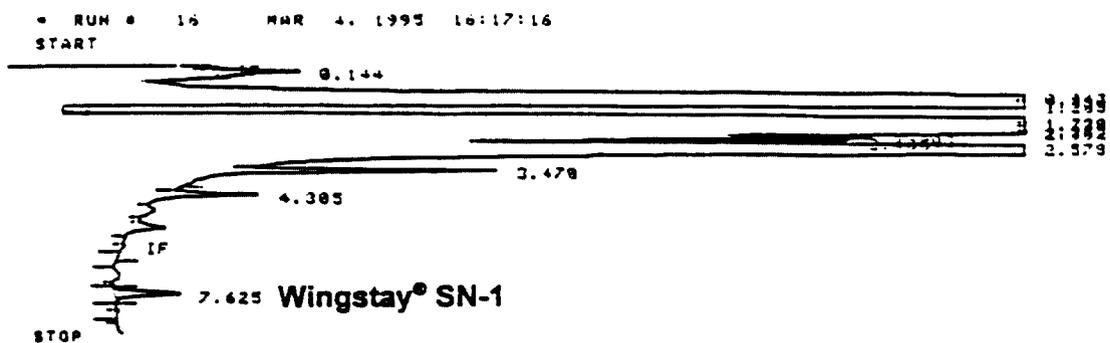
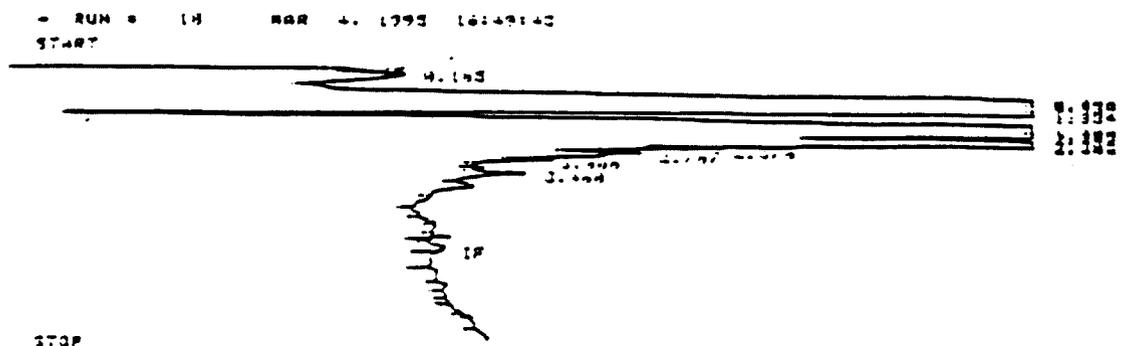


Figure 4. HPLC chromatogram of control sample.<sup>1</sup>



1

No peak corresponds to the retention time for Wingstay<sup>®</sup> SN-1 (Wingstay<sup>®</sup> SN-1 is detected at a retention time of approximately 7.6 minutes).

**SIGNATURES AND APPROVAL**

**SUBMITTED BY:**

Springborn Laboratories, Inc.  
Health and Environmental Sciences  
790 Main Street  
Wareham, Massachusetts 02571-1075

**PREPARED BY:**

John Mao 5/30/96  
John Mao, Ph.D. Date  
Study Director

5/30/96  
John Mao for Debra Marelli  
Debra Marelli Date  
Principal Investigator

**APPROVED BY:**

Paul H. Fackler 30 May 96  
Paul H. Fackler Date  
Director,  
Technical Operations

Doreen S. Newhouse 30 May 96  
Doreen S. Newhouse Date  
Manager,  
Quality Assurance Unit

This final report has been signed in accordance with SLI SOP No. 4.3.07(1).

**5.0 APPENDIX I - STUDY PROTOCOL**

**Springborn Laboratories, Inc.**

Environmental Sciences Division

790 Main Street • Wareham, Massachusetts 02571-1075 • (508) 295-2550 • Telex 4436041 • Facsimile (508) 295-8107

**TEST PROTOCOL**

**PROTOCOL TITLE: Test Material: Determining the Water Solubility of a Substance  
Following The Official Journal of the European Communities, L383 A - Part A.6.**

<b>TO BE COMPLETED BY THE STUDY SPONSOR:</b>	
Study Sponsor: Goodyear Tire & Rubber Company	
Address: 142 Goodyear Boulevard	
Akron, OH 44305	Phone: 216-796-2963
Sponsor Protocol/Project No.:	
Test Material Name(s): Wingstay SN1	
Purity:	CAS# or Lot # CAS # 64253-30-1 Lot # 13089-3
Additional Comments and Modifications: Goodyear Sample # 10024-64-3 Transferred 2/12/94	
Sponsor Approval <i>A. Philip Johnson</i>	Date 12-13-94

**TO BE COMPLETED BY SPRINGBORN LABORATORIES BEFORE TEST INITIATION:**

Testing Facility: Springborn Laboratories, 790 Main St., Wareham, MA 02571

Study Director: *J. Mac Ph.D.* Study No.: 13537-1394-6107-700

Test Concentration: 100% Test Material

Proposed Experimental Dates: (Start) 3/95 (Termination) 3/95  
*J. Mac* 12/14/94

Study Director Signature

Study Initiation Date

+LE  
5/30/96  
*J. Mac*

Springborn Laboratories Protocol #: 112294/EC A.6.

Page 1 of 6  
● Springborn

TEST MATERIAL: DETERMINING THE WATER SOLUBILITY OF A SUBSTANCE  
FOLLOWING THE OFFICIAL JOURNAL OF THE EUROPEAN COMMUNITIES,  
L383 A - PART A.6

## 1.0 INTRODUCTION

The mobility of a substance in the environment is largely determined by its water solubility. The water solubility of a substance is specified by the saturation mass concentration of the substance in water and is a function of temperature. The procedures described in this protocol illustrate two methods for the determination of the water solubility of a chemical substance. The "column elution method" is applicable to substances with low solubilities ( $< 10^{-2}$  g/L) and the "flask method" applies to relatively soluble substances ( $> 10^{-2}$  g/L).

Preliminary testing is performed in order to determine the approximate amount of sample and the time necessary to achieve the saturation mass concentration. Using the "column elution method" the elution of the test chemical in water, from a micro-column charged with an inert support material is monitored. The water solubility is determined when the mass concentration of the eluate is constant. Using the "flask method" the test substance is dissolved in water at a temperature slightly higher than the test temperature, the solution is then cooled to that of the test temperature and mixed to a state of equilibrium. The mass concentration of the substance (in the absence of any undissolved particulate matter) is determined using a suitable analytical method. Procedures described in this protocol are designed to conform with the Official Journal of the European Communities (EC, 1992).

## 2.0 MATERIALS AND METHODS

### 2.1 Test Materials:

Upon arrival at Springborn Laboratories, Inc., all test articles, materials, reference substances and standards are received by the Test Material Center. Records are maintained by GLP Standards, and a Chain-of-Custody is established. The condition of the external packaging of the test material is recorded and any damage is noted. The packaging is removed, the primary storage container is inspected for leakage or damage, and the condition is recorded. Any damage is reported to the Sponsor and/or manufacturer.

Each test material sample is given a unique sample ID number and is stored under the conditions specified by the Sponsor or manufacturer. Approximately 10 to 50 g of test material is required for these tests. Detection limits for measuring the test material concentration and other parameters will determine the exact amount needed. The following information is required from the Study Sponsor: test material lot or batch number, test material purity, water solubility (pH and temperature of solubility determination), vapor pressure, storage stability, methods of analysis of the test material in water, MSDS and safe handling procedures, and a verified expiration or reanalysis date, if applicable.

- 2.2. **Preliminary Testing.** Approximately 0.1 grams of test substance (solid substances must be pulverized) is added to a glass stoppered graduated cylinder. Increasing amounts of room temperature distilled water are added (refer to Table I). After each addition of the indicated amount of water is made, the mixture is shaken vigorously for 10 minutes and is visually checked for any undissolved particulate. If, after the addition of 10 mL of water, the sample or parts of it remains undissolved, the contents are transferred to a 100 mL graduated cylinder. At low water solubilities a period up to 24 hours may be required to completely dissolve a substance. The approximate solubility is listed in Table I under the amount of water required for complete dissolution of the sample. If the substance is still apparently insoluble, further dilution should be undertaken to ascertain whether the column elution or flask solubility method should be used.

Table I

mL of water	0.1	0.5	1	2	10	100	>100
- Solub. g/L	> 1000	1000 to 200	200 to 100	100 to 50	50 to 10	10 to 1	< 1

### 2.3 Definitive Testing

#### 2.3.1 Column Elution Method -

Approximately 600 mg of support material (ie., glass beads, silica) is weighed and transferred to a 50 mL roundbottom flask. An appropriate amount of test substance dissolved in a suitable solvent and added to the roundbottom flask, and the solvent is completely evaporated using rotary evaporation. The loaded material is allowed to soak for approximately 2 hours in approximately 5 mL of water, and the suspension is then added to the microcolumn. The test material is eluted from the support material with distilled water and is monitored by either of the two following methods:

- 2.3.1.1 **Leveling Vessel** - A connection to the leveling vessel is made by using a ground glass joint connected by PTFE tubing. At an approximately flow rate of 25 mL/hr successive eluate fractions are collected and analyzed by a suitable analytical method.

Fractions from the middle eluate range where the concentration are constant ( $\pm 30\%$ ) in at least five consecutive fractions are used to determine the solubility in water. The procedure is repeated at half the flow rate of the first. If results of the two runs are in agreement, the test is considered satisfactory. If this is not the case, then halving the flow rate must be continued until two successive runs yield the same water solubility. The pH of each sample should be recorded.

**2.3.1.2 Circulating Pump** - In order to use a circulating pump the microcolumn must be altered with a two-way stopcock. The circulating pump can be a peristaltic pump or a membrane pump. The flow through the column is started at an approximate flow rate of 25 mL/hr. (approximately 10 bed volumes per hour). The first five bed volumes are discarded to remove water soluble impurities. The recycling pump is connected and the apparatus allowed to run until equilibration is established, defined by five successive samples whose concentrations do not differ by more than  $\pm 30$  percent in a random fashion. Samples should be separated from each other by time intervals corresponding to the passage of at least ten bed volumes of the eluent. The amount of test material present in the eluent is measured using an appropriate analytical method.

In both cases (circulating pump and the leveling vessel) the fractions sampled should be checked for the Tyndall effect (light scattering). Presence of such particles invalidates the results, and the test should be repeated with improvements in the filtering action of the column. The pH of each sample should be recorded and a second run should be performed at the same temperature.

### 2.3.2 Flask Method

The quantity of material necessary to saturate the desired volume of water is estimated from the preliminary test. The volume of water required depends on the analytical method and the solubility range. About five times the quantity of material determined as described above is weighed into each of three glass vessels fitted with glass stoppers (i.e., centrifuge tubes, flasks). The chosen volume of water is added to each vessel and the vessels are agitated at 30 °C, using a shaking or stirring device capable of operating at a constant temperature (i.e., magnetic stirrer in a thermostatically controlled water bath).

After 24 hours one of the vessels is removed and re-equilibrated for 24 hours at the test temperature ( $20 \pm 0.5^\circ\text{C}$ ) with occasional shaking. The contents of the vessel are then centrifuged and the concentration of compound in the clear aqueous phase is determined by a suitable analytical method. The other two flasks are treated similarly after initial equilibration at 30 °C for two and three days, respectively. If the analytical results from at least the last two vessels agree with the required reproducibility (at least 15%), the test is considered to be satisfactory. The whole test should be repeated using longer equilibration times if the result from vessels 1, 2 and 3 show a tendency to increasing values.

#### 2.4. Analytical Procedure

A compound specific analytical method is required for these determinations since a small amount of soluble impurity could cause large errors in the measured water solubility. Examples of such methods are: gas or liquid chromatography, titrations, photometry or polarography. The method selected will be validated at Springborn Laboratories, Inc.

#### 3.0 RECORDS TO BE MAINTAINED

Records to be maintained include, but are not limited to, correspondence and other documents relating to the interpretation and evaluation of data as well as all raw data and documentation generated as a result of the study.

#### 4.0 REPORTING

The report will be a typed document, submitted in triplicate, describing the results of the study, and will be signed by the Study Director and Quality Assurance Unit. It will include, but not be limited to, the following:

- Dates on which the study began and ended.
- Name and address of laboratory conducting the test, name(s) of person(s) responsible for carrying out the test.
- A full description of the experimental design and procedures followed and a description of the test equipment used.
- Identification of the test substance including chemical name (CAS number) and percentage of active ingredient, empirical formula, molecular structure, qualitative and quantitative descriptions of the chemical composition (Sponsor supplied).
- The principal mathematical equations used in generating and analyzing the data as well as calculations using these equations. Tabular and graphical representations of the data (if applicable).
- Column elution method: individual concentrations, flow rates, pH of each sample, temperature of each run, method of analysis employed, nature of the carrier material, loading of carrier material, the mean water solubility reported as grams per mL water, and standard deviation from at least five samples on the saturation plateau, and the average of the two successive, acceptable runs.
- Flask Method: the test temperature, the analytical method employed, individual analytical determinations and the average where more than one value was determined

for one flask and the average of the value for the different flasks which were in agreement, reported as grams per mL of water and the pH of each sample.

- Location of raw data and report.
- A complete description of any protocol deviations and the impact expected.
- Dates of Quality Assurance audits reviews to the Study Director and management, signed by the QA unit.

#### 5.0 PROTOCOL CHANGES

All amendments to the approved protocol must be documented in writing signed by both the Study Director and the Sponsor's contact or representative. Protocol amendments and deviations must include the reasons for the change and the impact of the change on results of the study, if any. If necessary, amendments initially may be in the form of verbal authorization, followed by Springborn's written documentation of the amendment. In such a case, the effective date of the amendment will be the date of verbal authorization.

#### 6.0 SPECIAL PROVISIONS

**GOOD LABORATORY PRACTICES (GLP):** All test procedures, documentation, records, and reports will comply with the Community principles of Good Laboratory Practices as promulgated under the Council Directive 88/320/EEC of 9 June 1988.

**TEST MATERIAL DISPOSAL:** After 60 days of the issuance of the final report, the test material will be returned to the Sponsor's project officer, at Sponsor expense, unless different arrangements are made.

**ARCHIVAL:** All raw data will be archived by the Study Sponsor unless different arrangements are made. A copy of the final report will be archived at Springborn Laboratories, Inc.

#### 7.0 REFERENCE

EC (Official Journal of the European Communities), L383, December 29, 1992, Part A: Methods for the Determination of Physico-Chemical Properties, Section A.6.

**6.0 APPENDIX II - CERTIFICATES OF ANALYSIS**

01/20/95 15:19 216 796 3304

COYR RES RM 256

Q002

THE GOODYEAR TIRE & RUBBER COMPANY  
 BAYPORT CHEMICAL PLANT  
 P. O. BOX 669  
 LA PORTE, TEXAS 77572  
 (713) 474-0027

CERTIFICATE OF ANALYSIS  
 WINGSTAY-SNL

LOT NO.	(SPEC. LIMITS)	METHOD	
			130893
BATCH NUMBER			7
CRYSTAL POINT C	>27.0	E-867	27.0
COLOR GARDNER SCALE	3 MAX.	Visual	1
SPECIFIC GRAVITY @ 40 C	0.970-0.980	E-201	0.978
BROOKFIELD VIS. CPS 40 C	20-40cps	E-338	32.20
BIS ESTER CONTENT, %	65 - 80	E-920	66.61
MCNO ESTER CONTENT, %	20 MAX.	E-920	17.57
SEAGE 1, %		E-920	11.63
TEG, %	2 MAX.	E-920	0.56
STG. 1 RESIDUAL MERCAPTAN, %	1 MAX.	E-920	0.11
APPARENT CHARGE RATIO	1.85-2.10		1.97
TOTAL DRUMS SHIPPED @ 400#			unavail.
SHIPPED TO: GOODYEAR			
PO #			
CODE NO.			

I the undersigned, certify that all test were performed in accordance with approved test methods and that the results are correct.

Greg Frill, Manager  
 Quality Assurance

Prepared by: M. Lee  
 Date: January 12, 1994

95079RDS1062

RESEARCH & DEVELOPMENT ANALYTICAL SERVICES  
LABORATORY WORK REPORT 95-0150

Date: March 20, 1995

TO: R J Serva

FROM: E J Lauck

Subject: GPC Analysis of Wingstay SN-1

## SUMMARY:

A sample of Wingstay SN-1 and a sample of its relatively pure bis ester, were submitted by the Health, Safety, Environmental, Toxicology and Regulatory Compliance department for GPC analysis to determine their relative purities. This information is needed to support current toxicological testing.

## EXPERIMENTAL:

The samples were prepared for analysis by dissolving 0.15g sample in 10 ml of THF followed by filtration through a 0.45um filter prior to analysis. The samples were examined by GPC using refractive index detection. The results are summarized in the following table.

9988-52 Bis Std	
RT(min)	Area%
-----	
34.86	.03
36.51	98.06
38.84	.64
43.73	.01
51.41	.27
53.06	.37
57.01	.59
60.41	.03

Wingstay SN-1 Lot# 130893		
	RT(min)	Area%
-----		
Heavy	33.08	0.2
	34.25	1.9
	35.25	1.7
Bis	36.52	73.1
other	38.17	1.3
Mono	38.73	9.9
other	40.69	1.6
1st stage	41.74	9.0
Tetraethylene glycol	44.05	1.3

### Triage of 8(e) Submissions

Date sent to triage: 10/25/96

NON-CAP

CAP

Submission number: 13675 A

TSCA Inventory:

Y

N

D

Study type (circle appropriate):

Group 1 - Gordon Cash (1 copy total)

ECO

AQUATO

Group 2 - Ernie Falke (1 copy total)

ATOX

SBTOX

SEN

w/NEUR

Group 3 -HERD (1 copy each)

STOX

CTOX

EPI

RTOX

GTOX

STOX/ONCO

CTOX/ONCO

IMMUNO

CYTO

NEUR

Other (FATE, EXPO, MET, etc.): \_\_\_\_\_

Notes:

- This is the **original 8(e)** submission; refile after triage evaluation.
- This **original** submission has been **split**; rejoin after triage evaluation.
- Other:

#### Photocopies Needed for Triage Evaluation

entire document: 0    1    2    3

front section and CECATS: 0    1    2    3

Initials: \_\_\_\_\_

Date: \_\_\_\_\_

CECATS TRIAGE TRACKING DBASE ENTRY FORM

CECATS DATA: Submission # BEHQ-0696-13675 SEQ. A

TYPE: (N) SUPP FLWP

SUBMITTER NAME: Goodyear Tire + Rubber Company

SUB. DATE: 06/19/96 OTS DATE: 06/26/96 CSRAD DATE: 07/23/96

CHEMICAL NAME: PROPANOIC acid, 3-(podecy1+thio)-, oxybis (2,1-ethanedij) ester  
Wingstay SN-1  
 CAS# 64253-30-1

- VOLUNTARY ACTIONS:**  
 (N) NO ACTION REPORTED  
 0402 STUDIES PLANNED/IN PROGRESS  
 0403 NOTIFICATION OF WORK IN PROGRESS  
 0404 LABEL/MSDS CHANGES  
 0405 PROCESS/HANDLING CHANGES  
 0406 APP/USE DISCONTINUED  
 0407 PRODUCTION DISCONTINUED  
 0408 CONFIDENTIAL

- INFORMATION REQUESTED - FLWP DATE:**  
 0501 NO INFO REQUESTED  
 0502 INFO REQUESTED (TECH)  
 0503 INFO REQUESTED (VOL. ACTIONS)  
 0504 INFO REQUESTED (REPORTING RATIONALE)  
**DISPOSITION:**  
 (N) REFER TO CHEMICAL SCREENING  
 0678 CAP NOTICE

INFORMATION TYPE:	P F C	INFORMATION TYPE:	P F C	INFORMATION TYPE:	P F C
0201 ONCO (HUMAN)	01 02 04	0216 EPICLIN	01 02 04	0241 IMMUNO (ANIMAL)	01 02 04
0202 ONCO (ANIMAL)	01 02 04	0217 HUMAN EXPOS (PROD CONTAM)	01 02 04	0242 IMMUNO (HUMAN)	01 02 04
0203 CELL TRANS (IN VITRO)	01 02 04	0218 HUMAN EXPOS (ACCIDENTAL)	01 02 04	0243 CHEMPHYS PROP	01 02 04
0204 MUTA (IN VITRO)	01 02 04	0219 HUMAN EXPOS (MONITORING)	01 02 04	0244 CLASTO (IN VITRO)	01 02 04
0205 MUTA (IN VIVO)	01 02 04	0220 ECO/AQUA TOX	01 02 04	0245 CLASTO (ANIMAL)	01 02 04
0206 REPRO/TERATO (HUMAN)	01 02 04	0221 ENV. OCCUR/REL/FATE	01 02 04	0246 CLASTO (HUMAN)	01 02 04
0207 REPRO/TERATO (ANIMAL)	01 02 04	0222 EMER INCI OF ENV CONTAM	01 02 04	0247 DNA DAM/REPAIR	01 02 04
0208 NEURO (HUMAN)	01 02 04	0223 RESPONSE REQEST DELAY	01 02 04	0248 PROD/USE/PROC	01 02 04
0209 NEURO (ANIMAL)	01 02 04	0224 PROD/COMP/ID	01 02 04	MSDS	01 02 04
0210 ACUTE TOX. (HUMAN)	01 02 04	0225 REPORTING RATIONALE	01 02 04	OTHER	01 02 04
0211 CHR. TOX. (HUMAN)	01 02 04	0226 CONFIDENTIAL	01 02 04		
0212 ACUTE TOX. (ANIMAL)	01 02 04	0227 ALLERG (HUMAN)	01 02 04		
0213 SUB ACUTE TOX (ANIMAL)	01 02 04	0228 ALLERG (ANIMAL)	01 02 04		
0214 SUB CHRONIC TOX (ANIMAL)	01 02 04	0229 METAB/PHARMACO (ANIMAL)	01 02 04		
0215 CHRONIC TOX (ANIMAL)	01 02 04	0240 METAB/PHARMACO (HUMAN)	01 02 04		

TRIAGE DATA	NON-CBI INVENTORY	ONGOING REVIEW	SPECIES	TOXICOLOGICAL CONCERN:	USE:	PRODUCTION:
CAS SR	YES	YES (DROP/REFER)	Daphnia	LOW		
	NO	NO (CONTINUE)	Alga	MED		
		REFER		HIGH		

11/11/96

# Tox Concern

ID  
13675A

AQUATO

H

## COMMENT

AQUATIC TOXICITY TO DAPHNIA MAGNA IS OF HIGH CONCERN WITH A 48 HOUR EC50 OF 97.0 UG/L. NOEC<35UG/L. MEASURED CONCENTRATIONS AND A FLOW THROUGH TEST WERE USED.

AQUATIC TOXICITY TO THE ALGA, S. CAPRICORNUTUM IS OF HIGH CONCERN WITH A 72 HOUR EC50 OF 65 UG/L. NOEC=4.5 UG/L. NOMINAL CONCENTRATIONS WERE USED. THE CHEMICAL HAD A SOLUBILITY OF 46.7 UG/L.