DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Washington, DC 20204

April 24, 1998

Barry Hooberman, Ph.D.
Senior Science Advisor
Environ
4350 North Fairfax Drive, Suite 300
Arlington, VA 22203

Dear Mr. Hooberman;

This responds to your inquiry of February 3, 1998, and the enclosed copy of a revised description of a model food system and test protocol. You state that the test protocol is intended to demonstrate antimicrobial efficacy of a time release chlorine dioxide technology intended for use in food packaging materials. You state that you wish to receive comments from appropriate FDA review scientists regarding the proposed test protocol.

Our scientists have reviewed your revised efficacy test protocol and agree that you have addressed all of the comments and suggestions discussed at our meeting of November 24, 1997. Some of our reviewers thought that it would be important to remind you that your test protocol addresses only the efficacy requirements of a food additive petition. It does not provide data to establish the safety of the proposed use of chlorine dioxide.

Because chlorine dioxide is an oxidizing agent, food exposed to it should be assessed for the degree of oxidation. The Agency suggests that meat and poultry be evaluated by measuring the levels of fatty acids and (TBA) thiobarbituric acid values. In fruits and vegetables, it would be more appropriate to measure the levels of oxidation sensitive vitamins. The untreated food should be evaluated for comparison.

The reaction of chlorine dioxide with food may lead to the formation of chlorite. Chlorite is a suspected carcinogen, therefore its residues in food should be measured. Because chlorite is unstable at high temperatures, it would not be necessary to measure its residues on poultry, meat or other food that is expected to be cooked before consumption. If you have any further questions concerning this matter, please do not hesitate to contact us.

Sincerely,

Mark A. Hepp, Ph.D.
Division of Petition Control, HFS-215
Office of Premarket Approval
Center for Food Safety
and Applied Nutrition

APPENDICES

Volume 2 of 3

GENERALLY RECOGNIZED AS SAFE DETERMINATION FOR CHLORINE DIOXIDE GENERATED BY THE MICROSPHÈRE® SYSTEM IN LOW-DENSITY POLYETHYLENE FILM FOR FOOD-CONTACT PACKAGING MATERIAL

Prepared for

Bernàrd Technologies, Inc.
Chicago, Illinois

Prepared by

ENVIRON International Corporation
Arlington, Virginia

November 27, 2000

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APPENDIX H

NUTRITIONAL AND ORGANOLEPTIC QUALITY OF FOODS PACKAGED IN MS2500-CONTAINING FILM BAGS

CONTENTS OF APPENDIX H

1. Technical Report TR BTI # 00-11. Summary of Qualitative Studies of Chlorine Dioxide Generating Packaging Components
2. Technical Report TR BTI # 00-11 (A). A Qualitative Comparative Study Of Raw Poultry Products Stored In Polyethylene Bags Containing Either No Additives Or BTI Proprietary Technology.
    a. Whole Pieces of Raw Poultry Muscle Meat
    b. Cubed Pieces of Raw Poultry Muscle Meat

    Protocol Key-R-1-0200a and b

    Food evaluation characteristics (color, odor, TBA): Keystone Foods

    Chemical analyses (residual chlorite): Covance
3. Technical Report TR BTI # 00-11 (B). A Qualitative Comparative Study Of Partially Cooked Poultry Products Stored In Polyethylene Bags Containing Either No Additives Or BTI Proprietary Technology.

    Protocol Key-PCN-1-0200

    Food evaluation characteristics (color, odor, TBA): Keystone Foods

    Chemical analyses (residual chlorite): Covance
4. Technical Report TR BTI # 00-11 (C). A Qualitative Comparative Study Of Hamburger Buns Stored In Polyethylene Bags Containing Either No Additives Or BTI Proprietary Technology.

    Protocol EB-2-0200

    Food evaluation characteristics (visual assessment for mold, odor, texture, hardness, moisture, color): Merlin Development

    Chemical analyses (residual chlorite, thiamine, riboflavin): Covance
5. Technical Report TR BTI # 00-11 (D). A Qualitative Comparative Study Of Carrots Stored In Polyethylene Bags Containing Either No Additives Or BTI Proprietary Technology.

    Protocol TACS-2-0300

Food evaluation characteristics (visual assessment, odor, texture, color): Taniumra & Antle, Salinas, CA

Chemical analyses (residual chlorite, beta-carotene): Covance

6. Technical Report TR BTI # 00-11 (E). A Qualitative Comparative Study Of Primal Cuts of Beef Stored In Polyethylene Bags Containing Either No Additives Or BTI Proprietary Technology.

   IBP. 2000. Qualitative Comparative Study Of Primal Cut (Plate) Stored In Polyethylene Bags Containing Either No Additives Or BTI Proprietary Technology. Summary. May 17.

   Protocol IBP-RCB-1-0400

   Food evaluation characteristics (color, odor, TBA): IBP

   Chemical analyses (residual chlorite): Covance

7. Project Report. A Qualitative Comparative Study Of Shredded Carrots Stored In Polyethylene Bags Containing Either No Additives Or BTI Proprietary Technology.

   Protocol TACS-1-1199

   Food evaluation characteristics (visual assessment, odor, texture, color, total plate count, coliform bacteria): Taniumra & Antle, Salinas, CA

   Chemical analyses (residual chlorite, beta-carotene): Covance

8. Technical Report TR BTI # 00-11. Summary of Qualitative Studies of Chlorine Dioxide Generating Packaging Components. Methods

   a. Oxidative Rancidity Test (TBA) – Keystone

   b. Determination of Detection Limit for the Analysis of Chlorite by Iodometric Titration Chlorite – Southwest Research Institute

   c. Iodometric Titration for the Analysis of ClO_2 and Chlorite Anion Concentration – Bernard Technologies, Inc. SOP BTI # 99-01

9. Covance Laboratories. 2000. Analysis of Chlorite, Thiamine, Riboflavin, Beta-Carotene, and Vitamin A from Carotene in Various Foods. October 23.

BERNÀRD TECHNOLOGIES, INC.

A Health and Food Safety Technology Company

TECHNICAL REPORT

TR BTI # 00-11 (A through E)

Qualitative Studies
of
Chlorine Dioxide Generating Packaging Components

INTRODUCTION: The purposes of the studies described in this report were to determine whether there were any negative qualitative effects on a variety of foods exposed to BTI's system for the controlled generation of chlorine dioxide from precursor as Microsphère® CRT (Controlled Release Technology) incorporated in packaging material. Foods representing fresh produce, fresh muscle meat, fat, and baked goods were used in the studies. The studies address organoleptic characteristics, representative key nutrient stability, and residual chlorite. These studies complement the quantitative studies in the technical report entitled, Quantitative Studies – Chlorine Dioxide Generating Packaging Components, demonstrating positive efficacy of the CRT against bacteria, mold and yeast.

FOODS: Foods used in these studies represent the major food groups that may be packaged using CRT. These include fresh baked hamburger buns with propionate as a preservative, fresh baked hamburger buns without preservative, fresh carrots - shredded, whole pieces of fresh raw poultry muscle meat, cubed pieces of fresh raw poultry muscle meat, partially cooked whole pieces of poultry muscle meat, and fresh raw cuts of beef muscle meat.

CONCLUSION SUMMARY: Each food type was examined for the expression of negative effects on sensory characteristics and key nutrient(s), as well as whether there was residual chlorite anion in the food. There were no negative effects observed using the three levels of CRT tested for the baked goods, vegetable, and raw poultry. Abnormal effects were not observed for the fresh beef except with the highest level of CRT tested. Residual chlorite anion was not detected in any of the foods tested.

The CRT did not alter the amounts of thiamin and riboflavin in fresh buns stored for 9 days. Sensory characteristics such as off-odors, moistness, freshness, off flavor and overall acceptance were not affected when compared to control packaging. Chlorite residues were below the limits of detection. Results were similar for the buns containing or not containing propionate as a preservative.

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The CRT did not alter the concentrations of endogenous riboflavin or vitamin A from riboflavin in fresh shredded carrots stored for 24 days. There were no differences in sensory characteristics such as color, taste, and odor from control packaging. Chlorite residues were below the limit of detection.

The CRT did not alter the sensory characteristics of raw poultry muscle meat, whether whole pieces or cubes, including color and odor. In addition, the TBA values, a test for fat oxidation and rancidity, were comparable to the control packaged poultry. Similar findings were observed for the frozen partially cooked poultry pieces. The levels of TBA after 30 days of storage were higher than earlier periods; however, the increase appeared in the control as well as the CRT packaging and is typical for partially cooked frozen foods.

The CRT did not alter the sensory characteristics of raw beef muscle meat and associated fat, including color and odor for bags containing the low and medium levels of CRT. In addition, the TBA values, a test for fat oxidation and rancidity, were comparable to the control packaged red muscle meat. The meat packaged with the "high" bags showed a greenish tint after a few days, indicating oxidation of the endogenous myoglobin.

It is concluded that the Microsphère® CRT used as a packaging component does not negatively affect the qualitative attributes of fresh foods when concentrations of 40% or less are used as part of the packaging materials.

SUMMARY: The studies were conducted in conjunction with commercial food processors and suppliers according to a protocol for each type of food. All studies evaluated the variable parameters described in the protocols (see below) in conjunction with test packaging film in the form of heat sealable bags incorporating four levels of the controlled generation technology; zero or control, low, medium and high. Covance Laboratories, Madison, Wisconsin, conducted analyses for thiamin hydrochloride, riboflavin, beta-carotene, vitamin A from beta-carotene and residual chlorite anion. TBA analyses of fresh muscle meat were conducted by the food processor(s). Organoleptic evaluations were conducted by the food processors or a third party organization designated by a food processor. The results demonstrated that there were no significant or distinct differences in the foods between the control and test bags for nutrient stability and organoleptic characteristics. There were no detectable amounts of chlorite anion in any of the foods exposed in any of the test conditions. Specific results are reported in each subsection of this report.

TEST MATERIALS: Films used in this study were composed of butene based linear low density polyethylene that complies with FDA Regulation 21 CFR 177.1520 (c) 3.1 and may be used in articles that are intended to contact food except during cooking. They also contain low density polyethylene which complies with FDA Regulation 21 CFR 177.1530 (c) 2.1 and 2.2 and may be used in articles that are intended to contact non-alcoholic foods during cooking. The polyethylene food storage bags were approximately 12 inches by 15 inches and incorporated, via the normal manufacturing process, BTI's Microsphère® CRT system, at

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zero, low, medium or high amounts of the system (0%, 10%, 40%, and 50%, respectively). Once the bags were filled with the test foods, the bags were heat-sealed using typical commercial equipment and stored according to each protocol. The Microsphère® CRT contains Microsphère® 2500 for which an MSDS and report of oral safety (LD50>5000mg/kg) are in the Methods section.

METHODS: See each subsection for the following protocols:

TR BTI # 00- 11 A	Key-R-1-0200 a, b	Raw poultry muscle meat
TR BTI # 00- 11 B	Key-PCN-1-0200	Partially cooked poultry muscle meat
TR BTI # 00- 11 C	EB-2-0200	Hamburger buns
TR BTI # 00- 11 D	TACS-2-0300	Carrots, shredded
TR BTI # 00- 11 E	IBP-RCB-1-0400	Raw beef muscle meat & plate fat

Beta-carotene and Vitamin A from Beta-Carotene: Determined by HPLC using AOAC International, Official Methods of Analysis (modified), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, Virginia (1995). Quackenbush, F. W., Journal of Liquid Chromatography (modified), 10:643-653 (1987).

Chlorine Dioxide and Chlorite Anions: Standard Methods for Water Analysis Method 4500-ClO2 B. Iodometric Method. APHA, AWWA, and WPCF. Washington, D. C. (17th ed.) Pages 4-54 (modified as Southwest Research Institute BTI-SOP# 99-02. Aieta, E. M., P. V. Roberts, and M. Hernandez, J. Am. Water Works Assoc., 76(1), 64-70 (1984).

Riboflavin: AOAC International, 940.33 Riboflavin (Vitamin B2) in Vitamin Preparations (modified), Official Methods of Analysis, (ed.) Patricia Cuniff, Sixteenth Wd., Vol. 2, AOAC International: Gaithersburg, Maryland (1995).

Thiamin Hydrochloride: Official Methods of Analysis, 16th Edition, Method 942.23, Thiamine (Vitamin B1) in Foods, 953.17, Thiamine (Vitamin B1) in Grain Products, and 95.7.17, Thiamine (Vitamin B1) in Bread, modified, Chapter 45, AOAC, Gaithersburg, Maryland (1995).

TBA: Standard method conducted by Keystone Foods, L.L.C., Corporate Laboratory as TBA Test or Oxidative Rancidity Test (copy of method enclosed in the Methods section). A similar AOAC method was used by IBP, Inc.

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FILM RESULTS: The films containing the different levels of CRT were formed into food storage bags, approximately 12 inches by 15 inches. The average bag weighed 15.7 grams. The CRT system incorporated into the bags has 1.2% sodium chlorite (w/w) that is equivalent to 0.96% chlorite anion available for conversion to chlorine dioxide. The maximum amount of chlorine dioxide that potentially could be generated from the film

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containing the CRT, should the system be exhausted to completion, would be 15.07 mg for the 10% film, 60.29 mg for the 40% film and 75.40 mg for the 50% film. The profile for the generation of chlorine dioxide from these films in a sealed container having an atmosphere of 90% Relative Humidity as measured using an electrochemical detection cell (City Technologies, UK) is in Figure 1 and the data in Table 1.

FOOD RESULTS: See each subsection for results for each food type.

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TR BTI # 00-11 (A)

Protocol: Key-R-1-0200a and b

A QUALITATIVE COMPARATIVE STUDY OF RAW POULTRY PRODUCTS STORED IN POLYETHYLENE BAGS CONTAINING EITHER NO ADDITIVES OR BTI PROPRIETARY TECHNOLOGY

Whole Pieces of Raw Poultry Muscle Meat

1. Protocol
2. Table of Sensory Evaluations
3. Photographs of Test Samples
4. Table of TBA Values
5. Graphs of TBA Values
6. Results of TBA Analyses
7. Results of Chlorite Anion Analyses

Cubed Pieces of Raw Poultry Muscle Meat

1. Protocol
2. Table of Sensory Evaluations
3. Photographs of Test Samples
4. Table of TBA Values
5. Graphs of TBA Values
6. Results of TBA Analyses
7. Results of Chlorite Anion Analyses

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Protocol: Key-R-1-0200a

A QUALITATIVE COMPARATIVE STUDY OF RAW WHOLE POULTRY PIECES STORED IN POLYETHYLENE BAGS CONTAINING EITHER NO ADDITIVES OR BTI PROPRIETARY TECHNOLOGY

Objective: To determine any differences in selected characteristics of raw poultry products packaged in polyethylene (LDPE) bags or LDPE bags containing BTI proprietary technology.

Materials:

Raw poultry products prepared by Keystone Foods, Folcroft, PA.

1. Whole pieces of raw poultry muscle meat, boneless & skinless breast meat

Packaging film/bags

1. LDPE bags without active additives
2. LDPE opaque bags containing BTI additive at low %
3. LDPE opaque bags containing BTI additive at medium %
4. LDPE opaque bags containing BTI additive at high %

Packages:

1. Two pieces of whole raw poultry pieces per package
2. Four (4) replicates per sample type (2 for Keystone, 2 for Covance)
3. Total Packages: 160 (4 types of film)*(4 replicates/type)*(5 storage periods)*(2 products)
4. Total Packages per Test Lab: 80
5. Packages containing samples will be stored in a refrigerated cooler at 4° to 8°C

Analyses as listed in attached chart (Page 2):

1. Food evaluation characteristics to be conducted by Keystone Foods; Project Contact is Jade Lau; Test and Analytical Protocols to be provided to BTI by Keystone Foods.
2. Chemical analyses to be conducted by Covance, Madison, WI; Project Contact is Wayne Ellefson; Protocols to be provided by Covance.

Schedule:

- Day 0 — Prepare raw poultry products/obtain baseline data
- Day 1 — Package in test bags with labels and store as indicated
- Day 1 — Ship bags containing product to Covance test labs via FedEx

Samples for Covance may be frozen and all shipped at once on Day 10

- Day 1 — Test Labs conduct analyses as DAY 1 samples
- Day 2 — Test Labs conduct analyses as DAY 2 samples
- Day 5 — Test Labs conduct analyses as DAY 5 samples

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- Day 7 — Test Labs conduct analyses as DAY 7 samples
- Day 9 — Test Labs conduct analyses as DAY 9 samples
- Day -- — Preliminary Report of Results
- Day -- — Final Reports from Covance and Keystone Foods

Analyses:

Samples from each treatment will be analyzed for, color, odor, TBA and residual chlorite on each of the following days: 1, 2, 5, 7, 9.

Color and Odor by Keystone Labs:

If possible, color and odor comments will be obtained for each sample prior to the rinse. Color change may be documented using a digital camera.

TBA Analyses:

TBA analyses will be conducted by Keystone Labs.

Residual Chlorite:

A duplicate samples will be shipped to a COVANCE lab for residual chlorite analysis.

Chlorine Dioxide and Chlorite Anions: Standard Methods for Water Analysis Method 4500-ClO2 B. Iodometric Method. APHA, AWWA, and WPCF. Washington, D. C. (17th ed.) Pages 4-54 (modified as Southwest Research Institute BTI-SOP# 99-02. Aieta, E. M., P. V. Roberts, and M. Hernandez, J. Am. Water Works Assoc., 76(1), 64-70 (1984).

TBA: See Methods section.

Day	
0	Collect baseline data on test and control products
1 - 10	Store samples at 38°F
1	Sample: color, odor, TBA, residual chlorite
2	Sample: color, odor, TBA, residual chlorite
5	Sample: color, odor, TBA, residual chlorite
7	Sample: color, odor, TBA, residual chlorite
9	Sample: color, odor, TBA, residual chlorite

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RESULTS

Sensory: The CRT did not alter the sensory characteristics of raw poultry muscle meat as whole skinless pieces, including color and odor. At all storage times, the color, as seen the photographs, of the low, medium, and high CRT packaging was similar to the control.

TBA: The TBA values either as a function of film type or duration of storage were within the normal limits of the untreated muscle meat. The values for the poultry muscle meat stored in the bags containing the CRT technology were either below the control film bags or within the normal limits of sample-to-sample variability.

Chlorite: Residual chlorite anions were not detected. The cooking process would destroy any residual chlorite.

CONCLUSION

Packaging materials containing the Microsphère® CRT at levels of loading up to 50% may be used for the storage of fresh poultry without demonstrating any negative qualitative effects.

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[redacted]

COMPANY	Keystone Foods	

TEST ARTICLE	*Poultry-Raw Whole Pieces*

BTI SAMPLE FORM:	Microsphère® bags: zero, low, medium, high %

Temperature of Test:	30 to 38°C	

Day	Sample Evaluation					
	Color	Odor	Appearance		Chemical	
			(see photos)		TBA	Chlorite Residual
						ppm
Day 1						
Zero	Pink	Fresh	No discoloration		0.22	None Detected
Low	Pink	Fresh	No discoloration		0.08	None Detected
Medium	Pink	Fresh	No discoloration		0.17	None Detected
High	Pink	Fresh	No discoloration		0.13	None Detected
Day 2						
Zero	pale-yellow	low	No discoloration		0.18	None Detected
Low	pale yellow	low	No discoloration		0.10	None Detected
Medium	pale yellow	low	No discoloration		0.07	None Detected
High	pale yellow	low	No discoloration		0.10	None Detected
Day 5						
Zero	dk. pink	moderate	sticky film		0.19	None Detected
Low	dk pink	moderate	sticky film		0.14	None Detected
Medium	dk pink	moderate	sticky film		0.16	None Detected
High	dk. pink	moderate	sticky film		0.32	None Detected
Day 7	same as day 5					
Zero	dk. pink	High	slimy film		0.08	None Detected
Low	dk. pink	High	slimy film		0.13	None Detected
Medium	dk. pink	High	slimy film		0.11	None Detected
High	dk. pink	High	slimy film		0.08	None Detected
Day 9						
Zero	Very dk pink	Extremely	Heavy film		0.19	None Detected
Low	Very dk pink	Extremely	Heavy film		0.08	None Detected
Medium	Very dk pink	Extremely	Heavy film		0.11	None Detected
High	Very dk pink	Extremely	Heavy film		0.07	None Detected

Sensory Tests
Name of Person Conducting Tests: _____

Signature: _____ Date: 5/11/00

TBA Analyses
Name of Person Conducting Tests: _____

Signature: _____ Date: 5-15-00

Chlorite Analyses
Name of Person Conducting Tests: Conducted at Covance Labs - see Covance Report

Signature: _____ Date: _____

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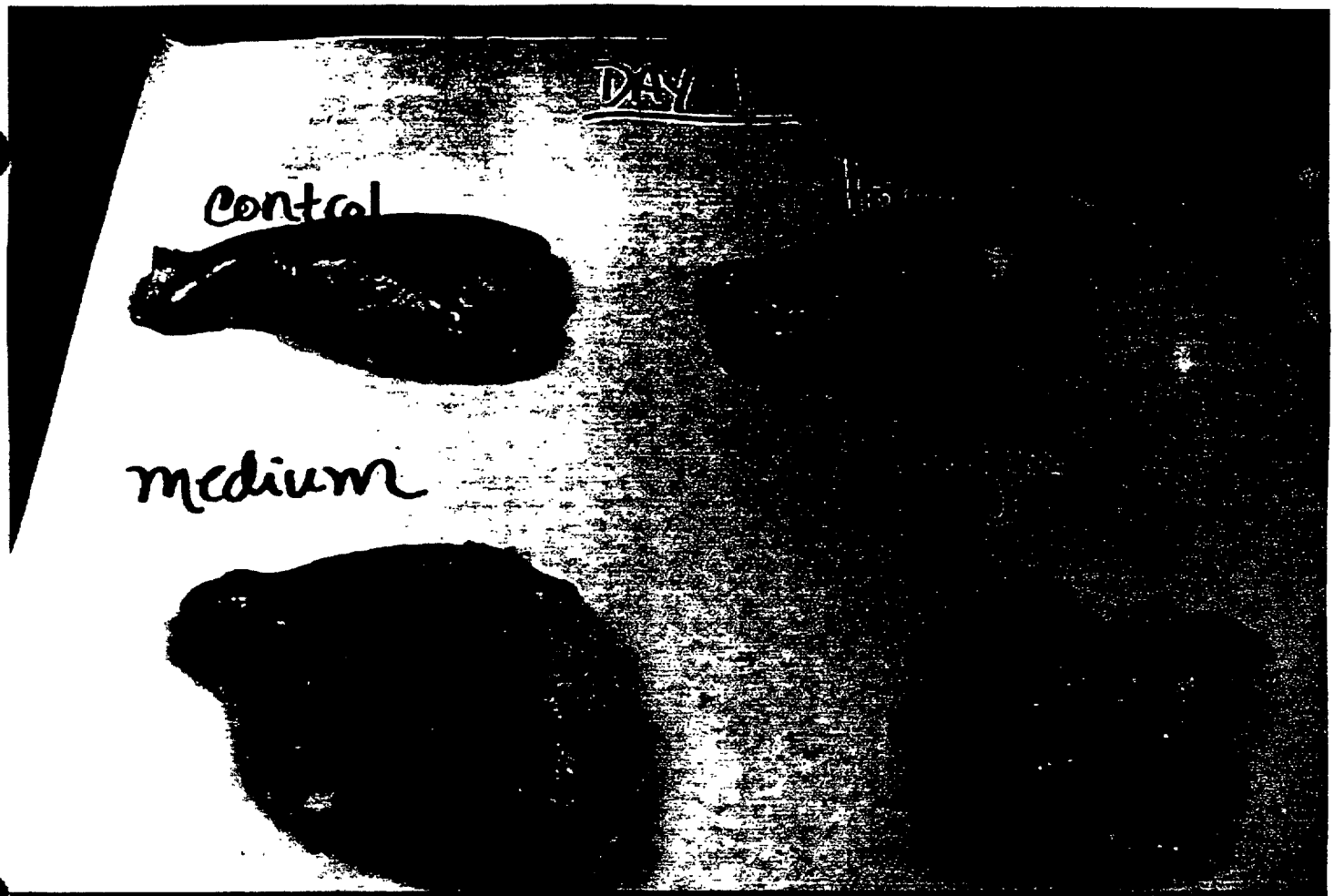
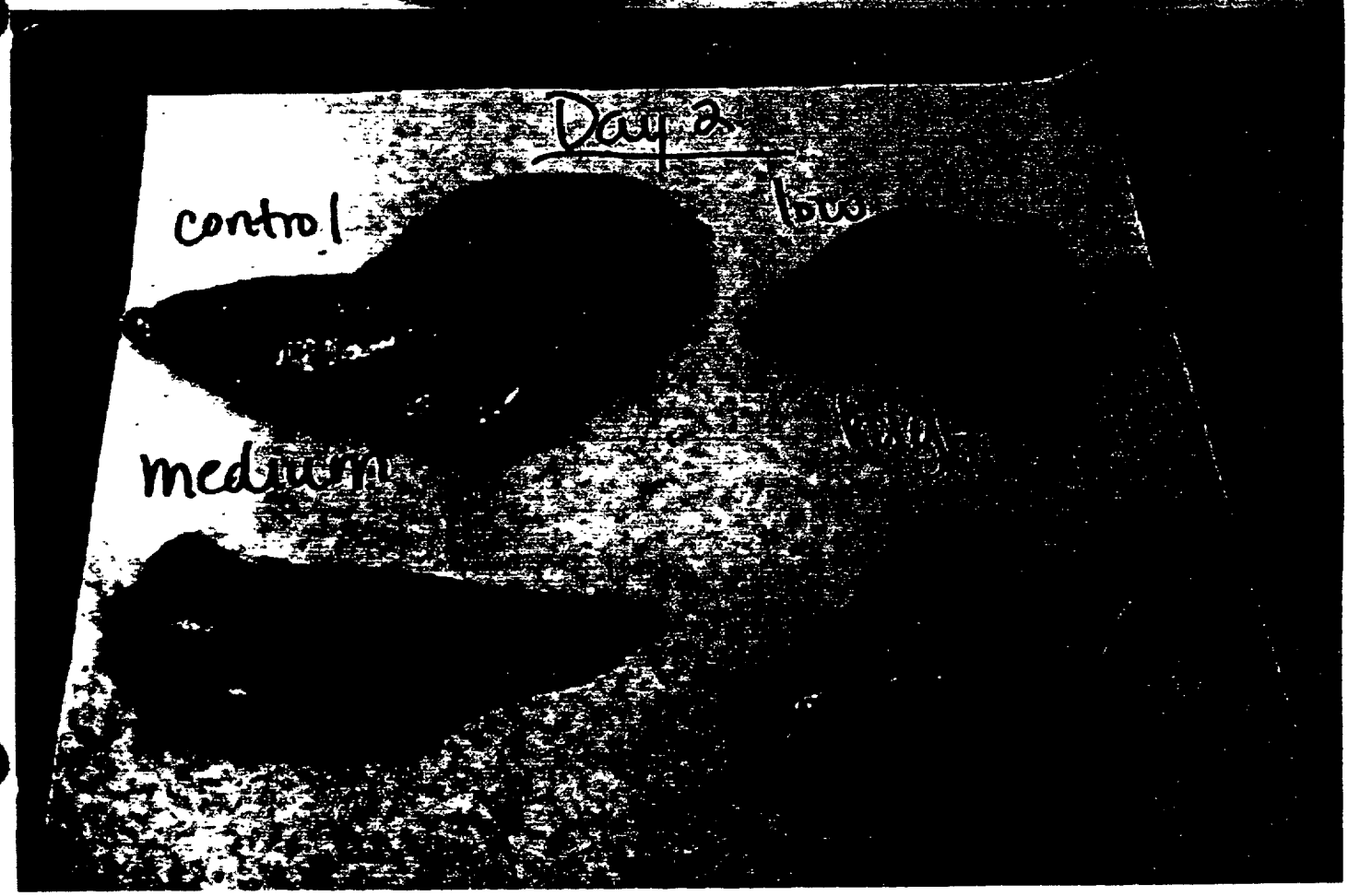

DAY
Control
medium

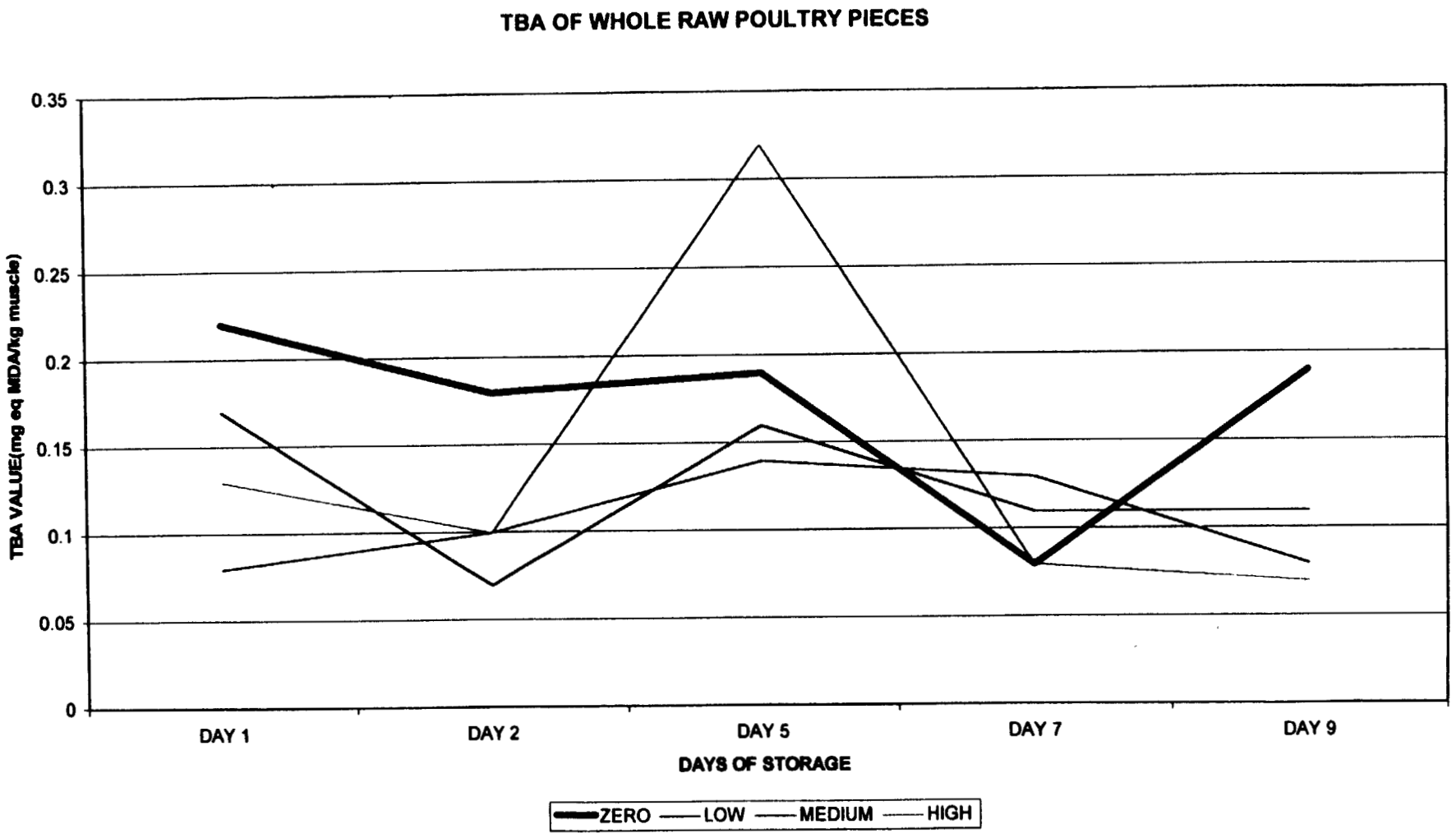

Day 2
control
medium

medium length

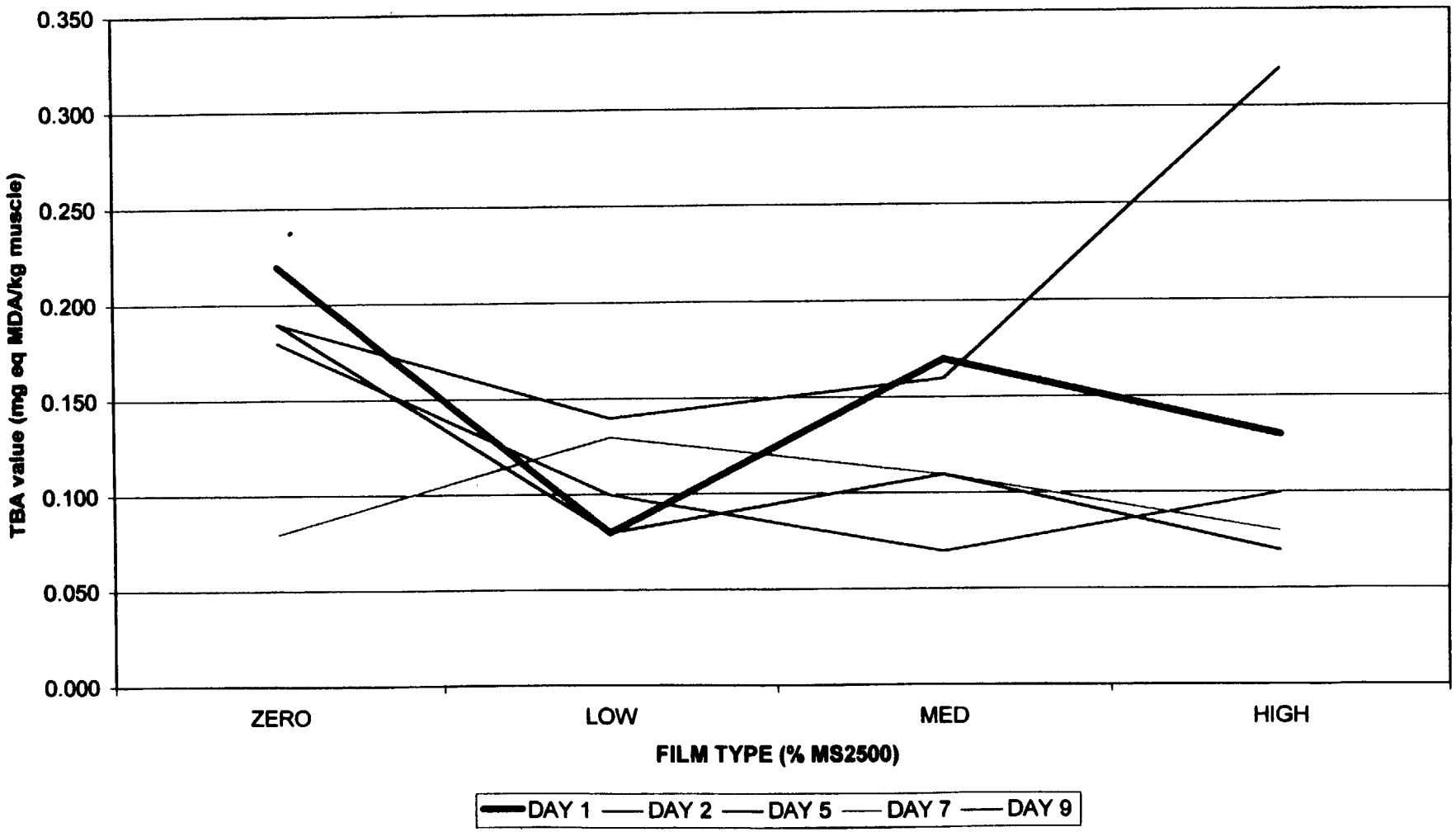
TBA VALUE
RAW POULTRY
WHOLE PIECES AND CUBED PIECES
(Conducted by Keystone Foods)

	WHOLE PIECES	CUBED PIECES
	ZERO	ZERO
DAY 1	0.22	0.17
DAY 2	0.18	0.19
DAY 5	0.19	0.18
DAY 7	0.08	0.14
DAY 9	0.19	0.26
	LOW	LOW
DAY 1	0.08	0.24
DAY 2	0.1	0.14
DAY 5	0.14	0.2
DAY 7	0.13	0.14
DAY 9	0.08	0.12
	MEDIUM	MEDIUM
DAY 1	0.17	0.19
DAY 2	0.07	0.14
DAY 5	0.16	0.39
DAY 7	0.11	0.18
DAY 9	0.11	0.12
	HIGH	HIGH
DAY 1	0.13	0.11
DAY 2	0.1	0.17
DAY 5	0.32	0.19
DAY 7	0.08	0.2
DAY 9	0.07	0.12

Pieces were packaged in polyethylene film film containing zero, low, medium and high % of BTI's proprietary chlorine dioxide generating product


TBA OF WHOLE RAW POULTRY PIECES
TBA VALUE(mg eq MDA/kg muscle)
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
DAY 1
DAY 2
DAY 5
DAY 7
DAY 9
DAYS OF STORAGE
ZERO
LOW
MEDIUM
HIGH

TBA of WHOLE RAW POULTRY PIECES
as a
FUNCTION of FILM for ANY GIVEN DAY of STORAGE

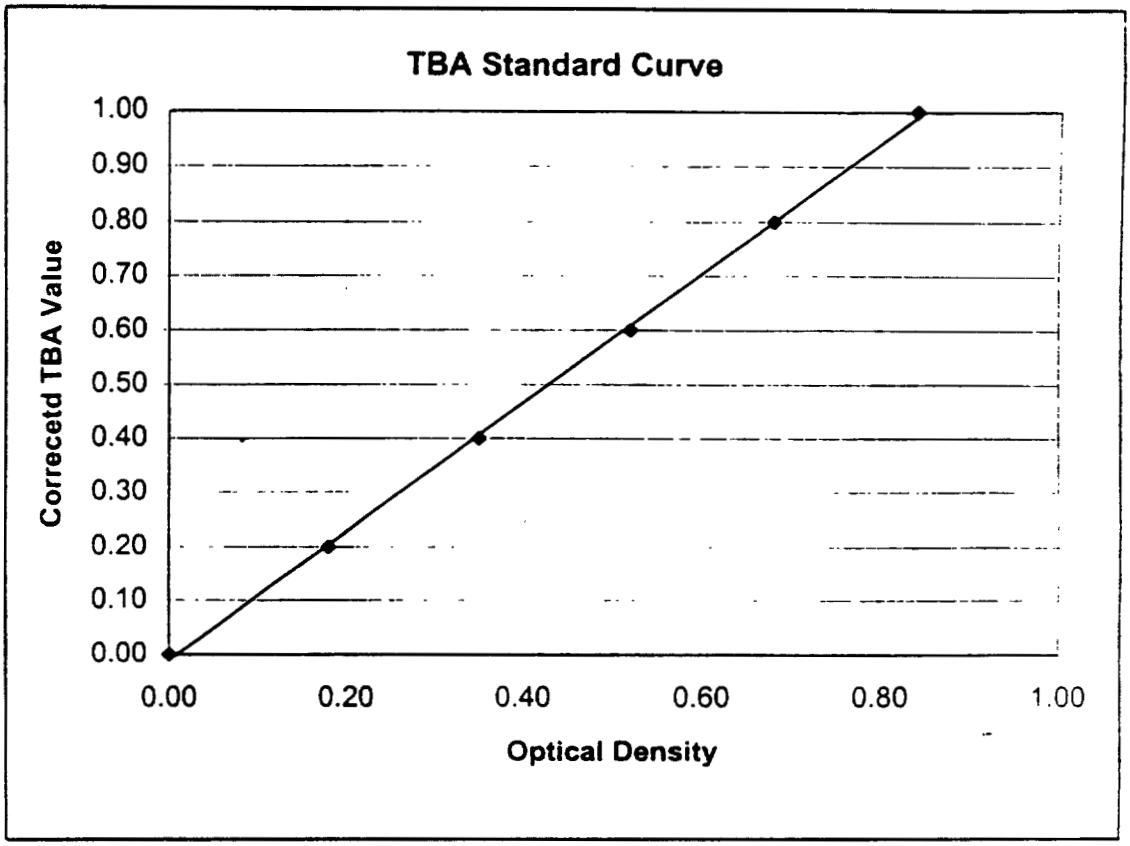


TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



Sample # : KEY-R-Z-219 -Day 1 whole raw

Observed Optical Density: 0.19

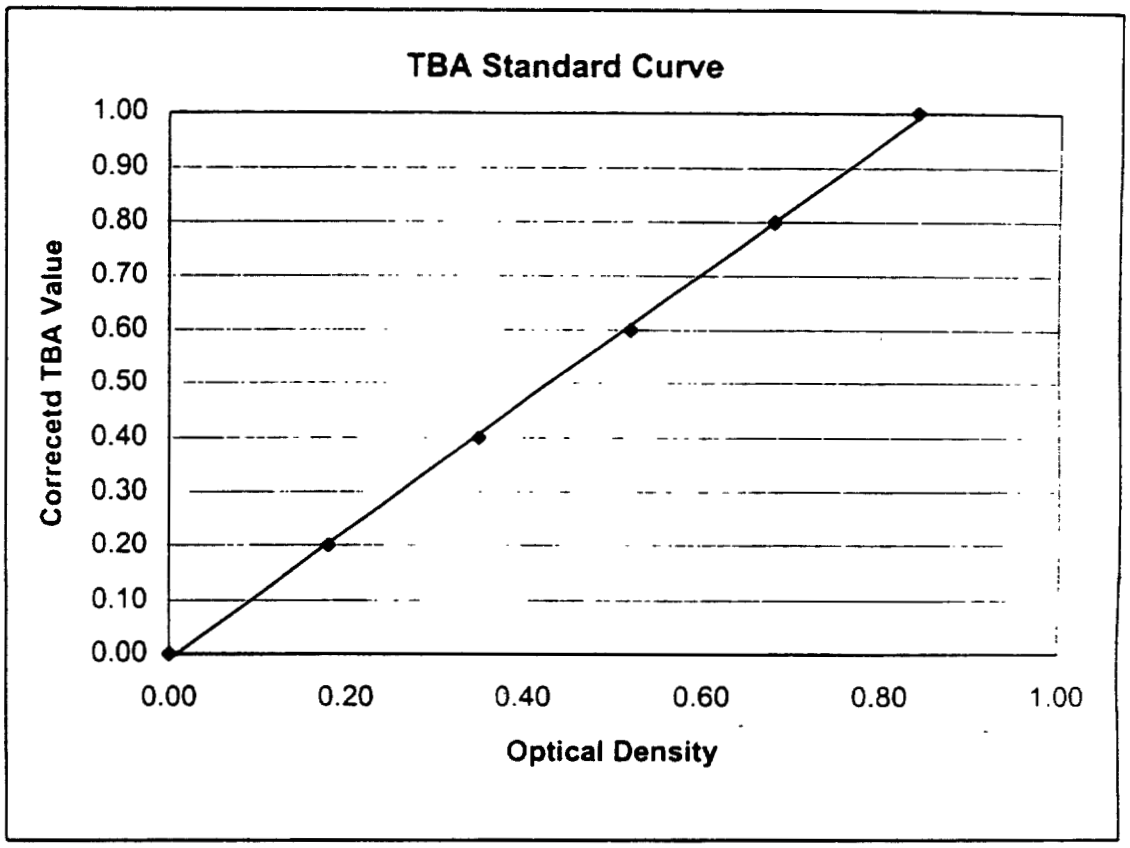
Calculated TBA Value: 0.22

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



Sample # : KEY-R-LO-107 - Day 1 whole raw

Observed Optical Density: 0.08

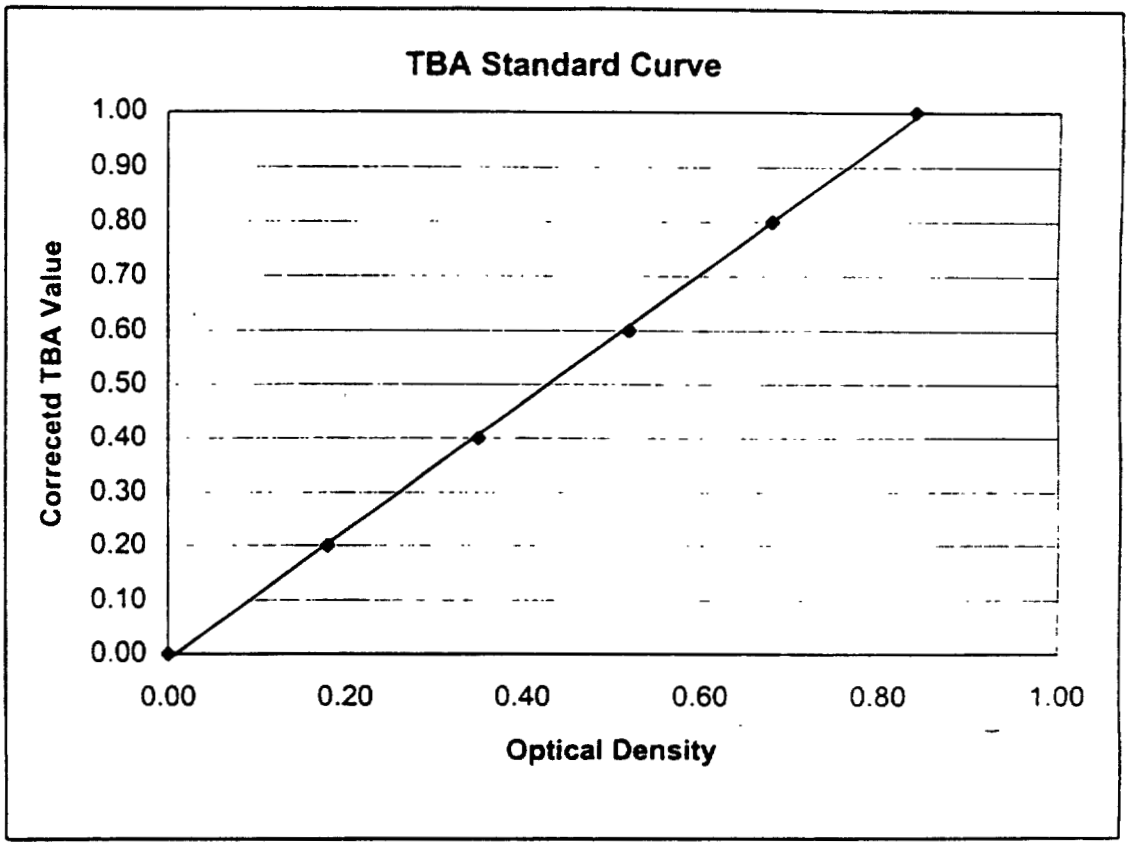
Calculated TBA Value: 0.08

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



Sample # :	KEY-R-MED-139
Observed Optical Density:	0.15
Calculated TBA Value:	0.17

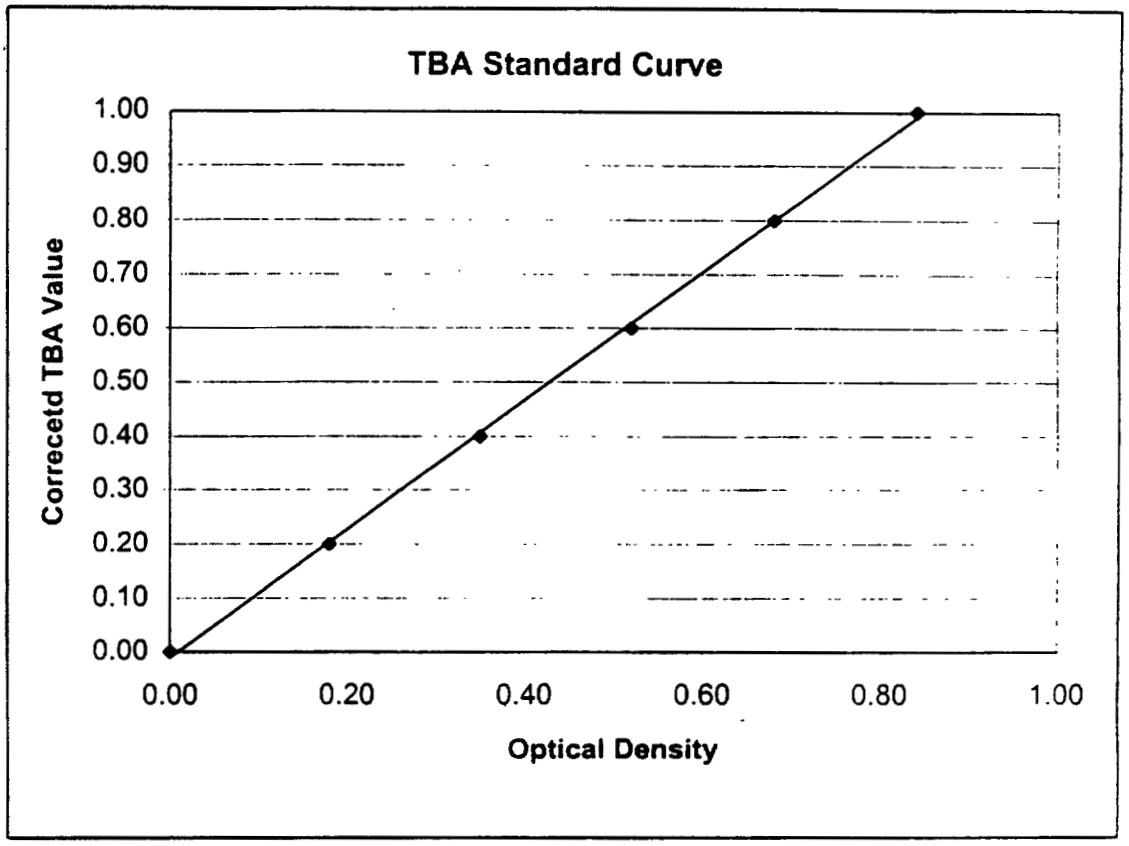
-Day 1 whole raw

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



Sample # : KEY-R-HI-274 – Day 1 whole raw

Observed Optical Density: 0.12

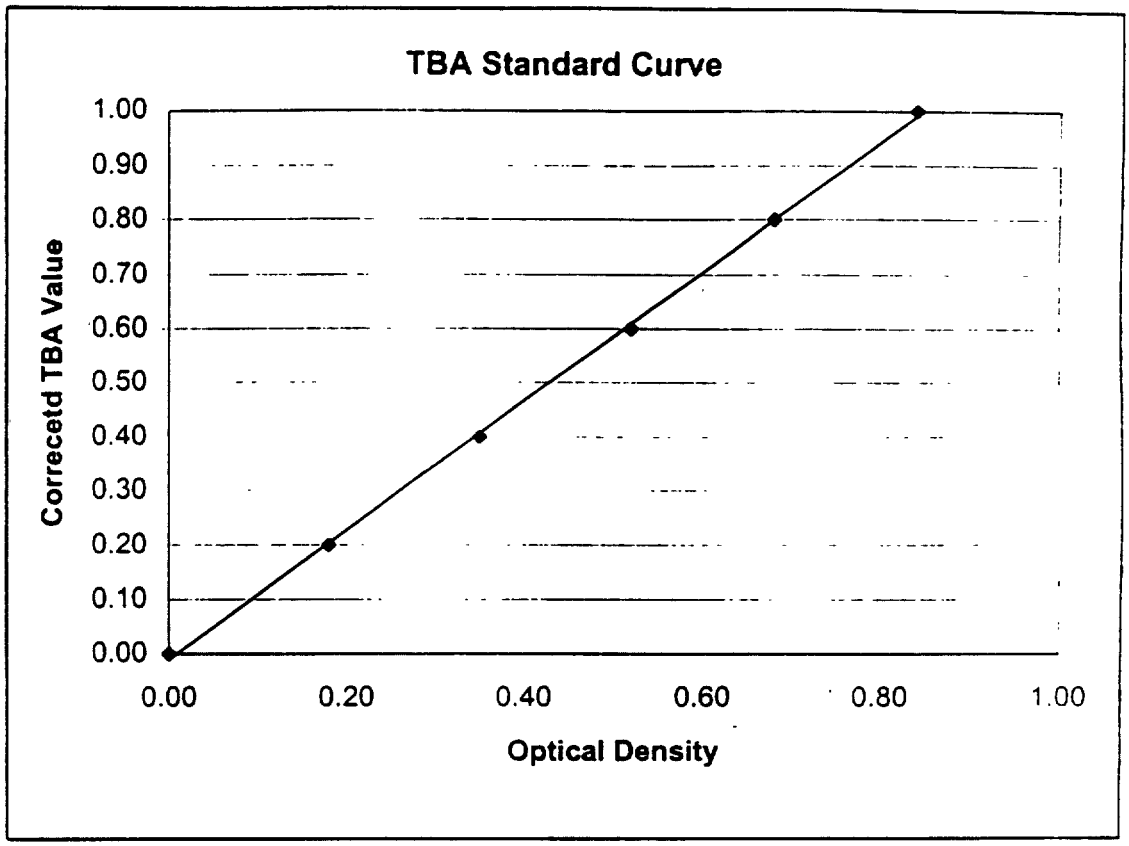
Calculated TBA Value: 0.13

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



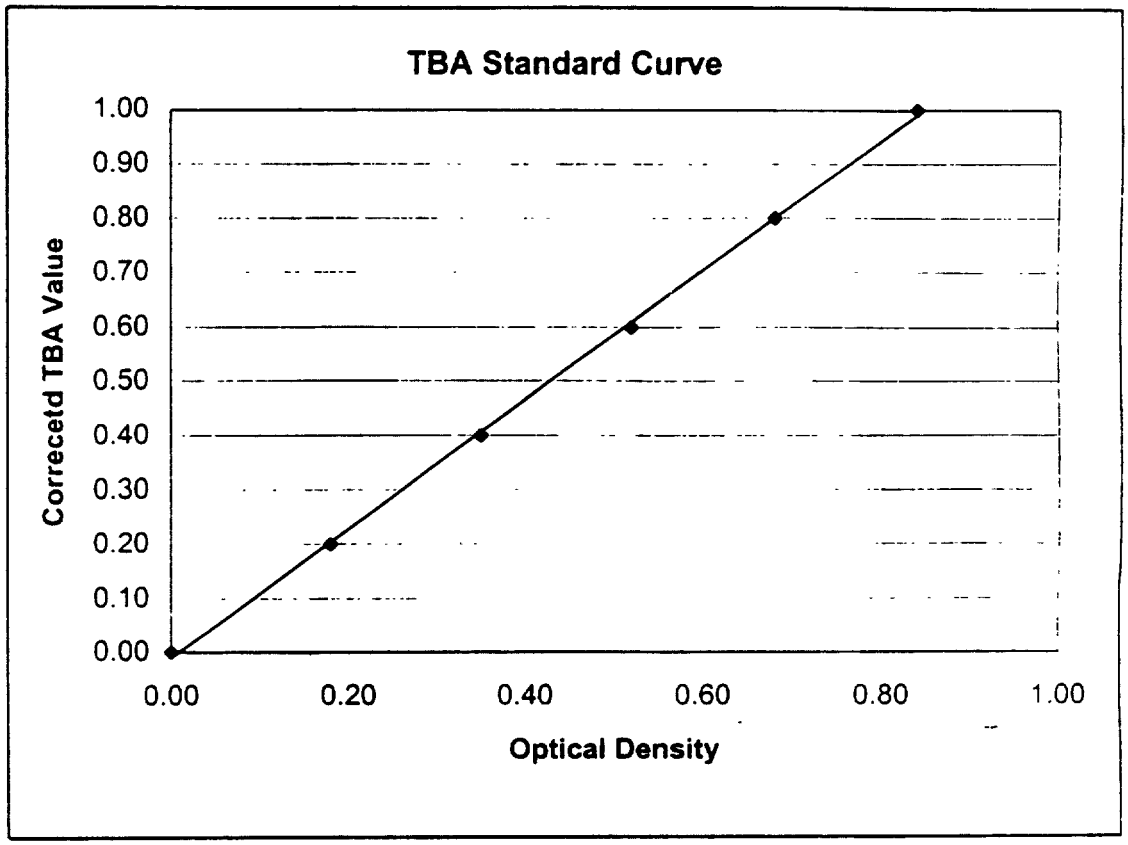
Sample # :	KEY-R-Z-218 WHOLE PIECES – Day 2
Observed Optical Density:	0.16
Calculated TBA Value:	0.18

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



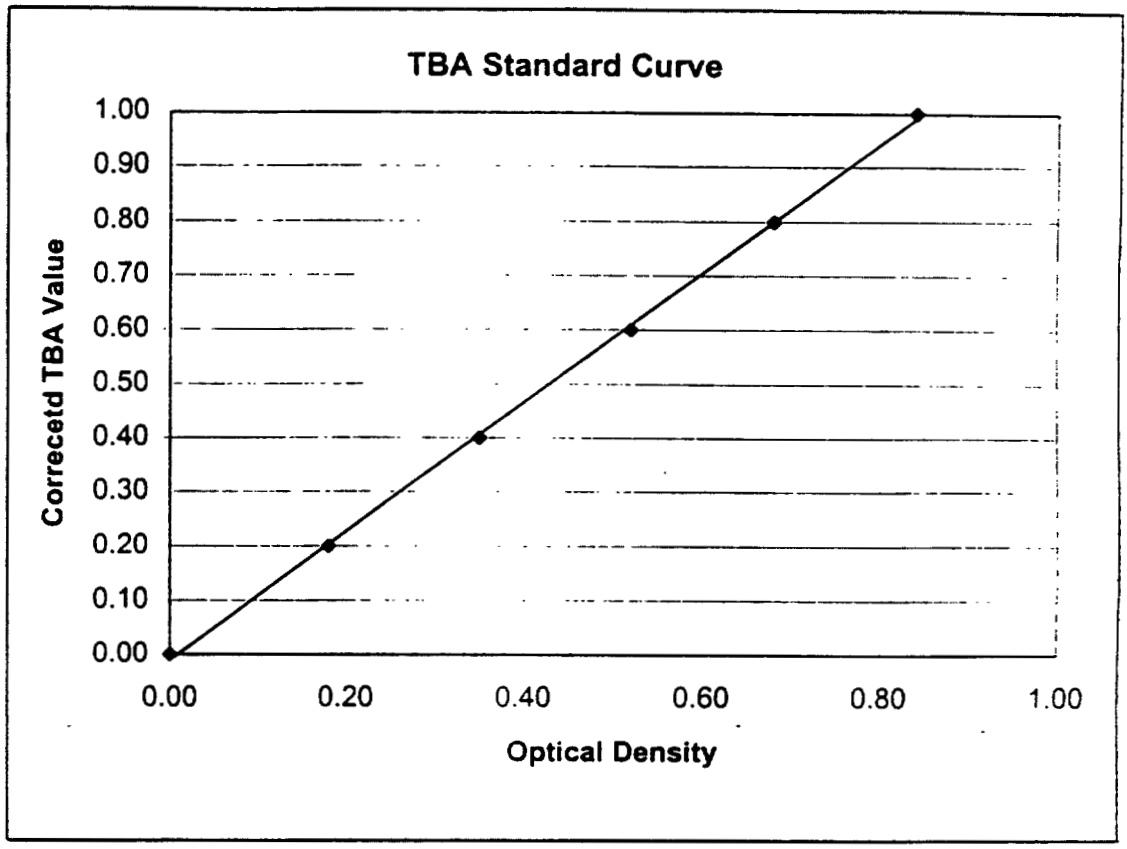
Sample # :	KEY-R-LO-082 WHOLE PIECES - Day 2
Observed Optical Density:	0.09
Calculated TBA Value:	0.10

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



Sample # : KEY-R-MED-142 WHOLE PIECES - Day 2

Observed Optical Density: 0.07

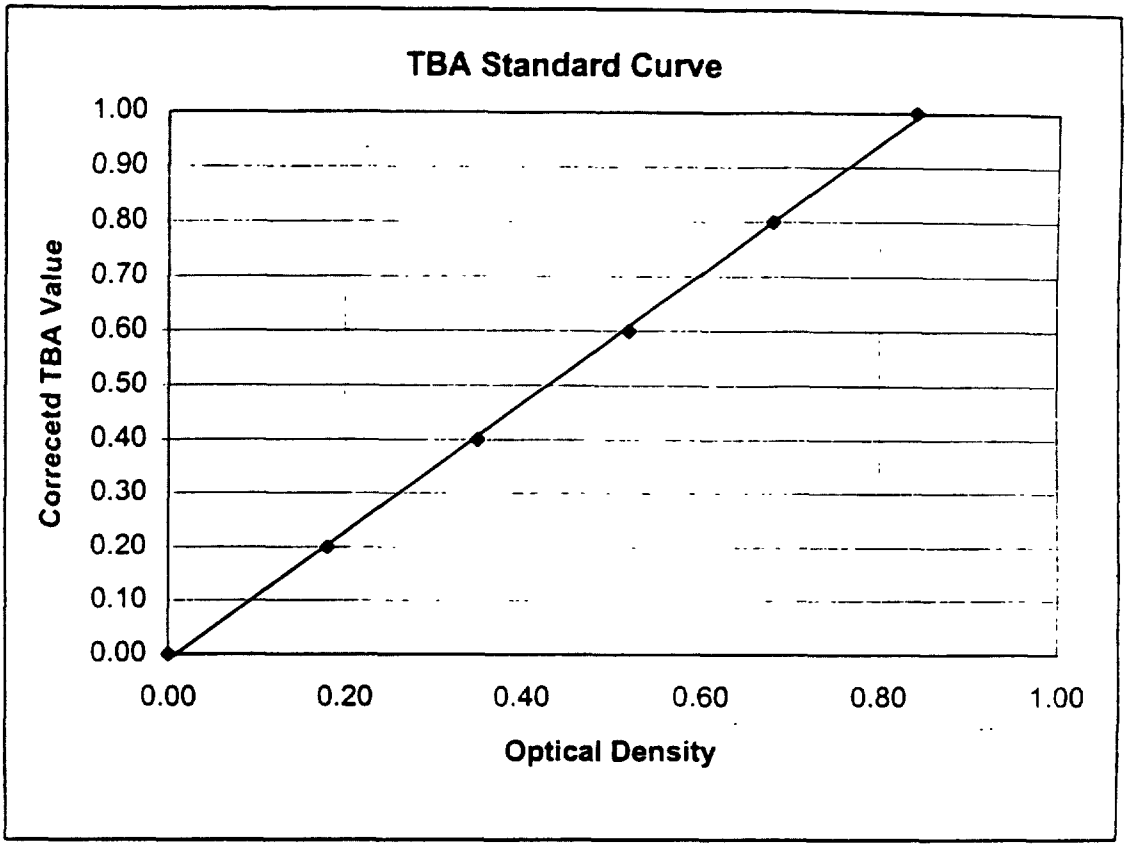
Calculated TBA Value: 0.07

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



Sample # : KEY-R-HI-269 WHOLE PIECES – Day 2

Observed Optical Density: 0.09

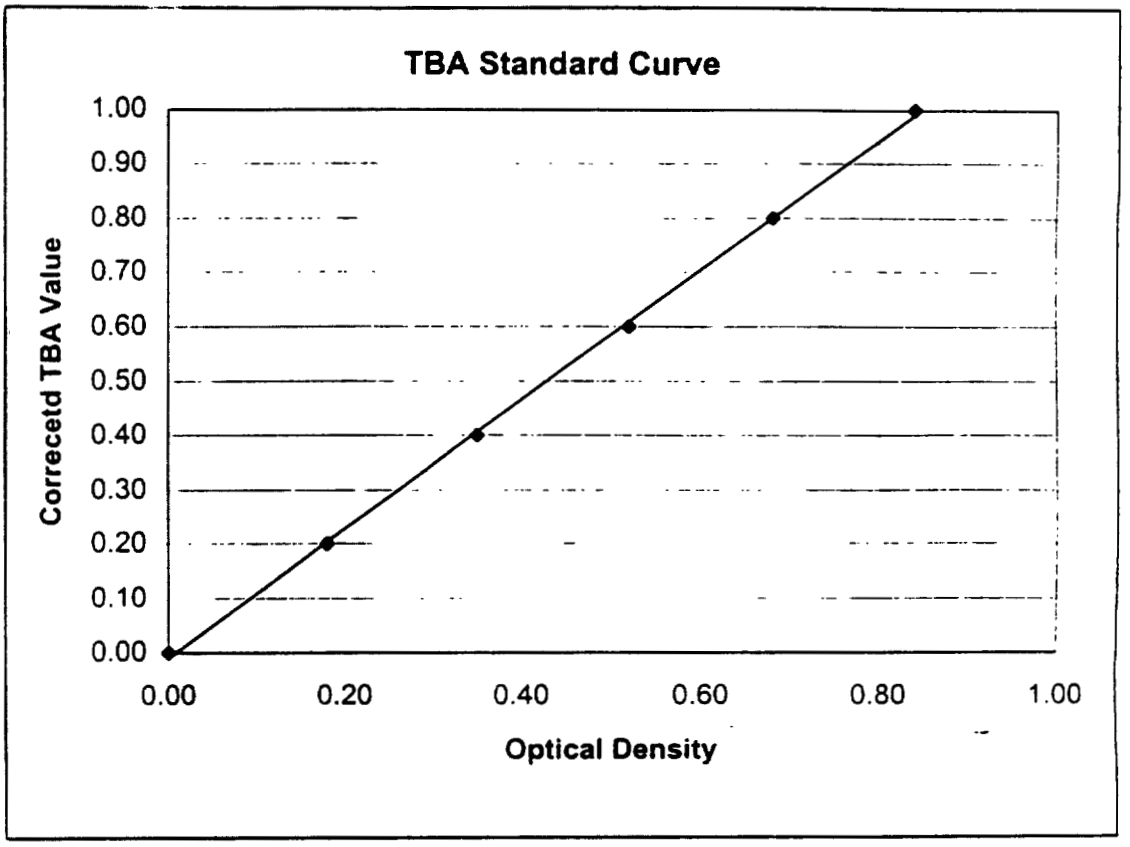
Calculated TBA Value: 0.10

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



Sample # : KEY-R-Z-215 RAW WHOLE PIECES - Day 5

Observed Optical Density: 0.17

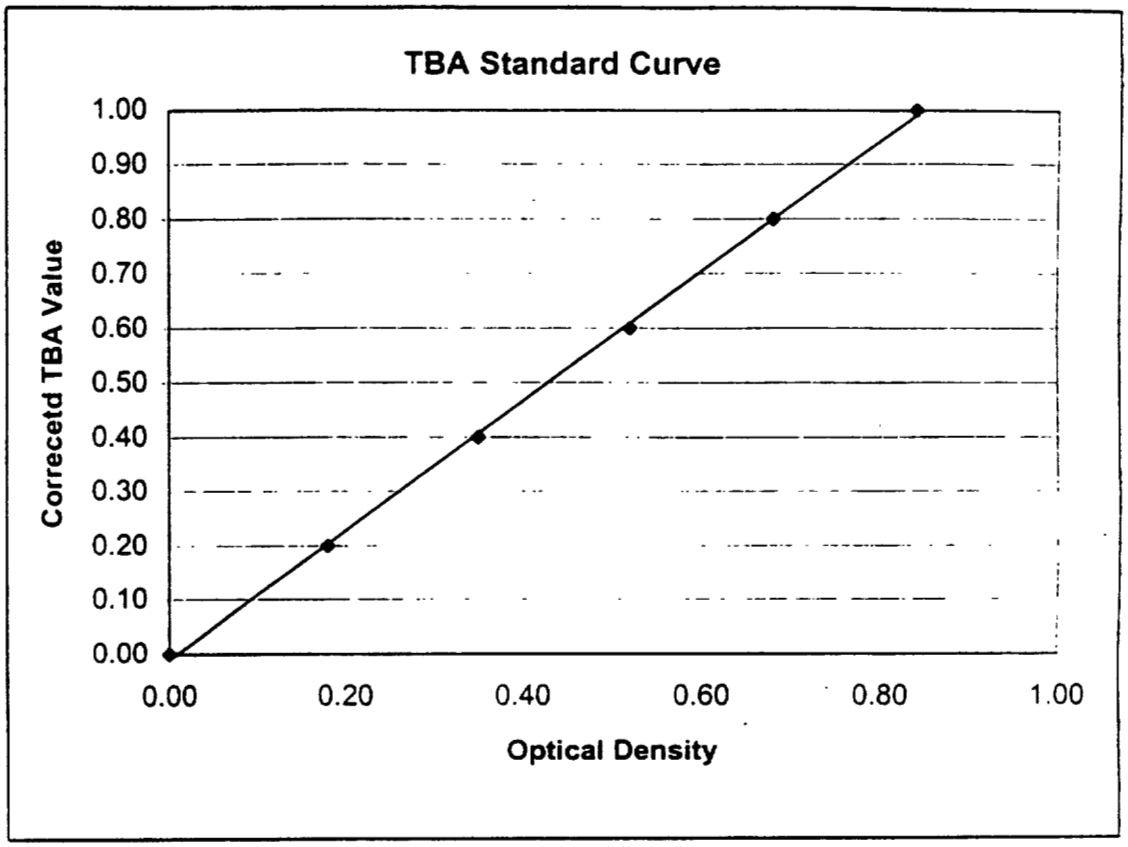
Calculated TBA Value: 0.19

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



Sample # : KEY-R-LO-097 RAW WHOLE PIECES - Day 5

Observed Optical Density: 0.13

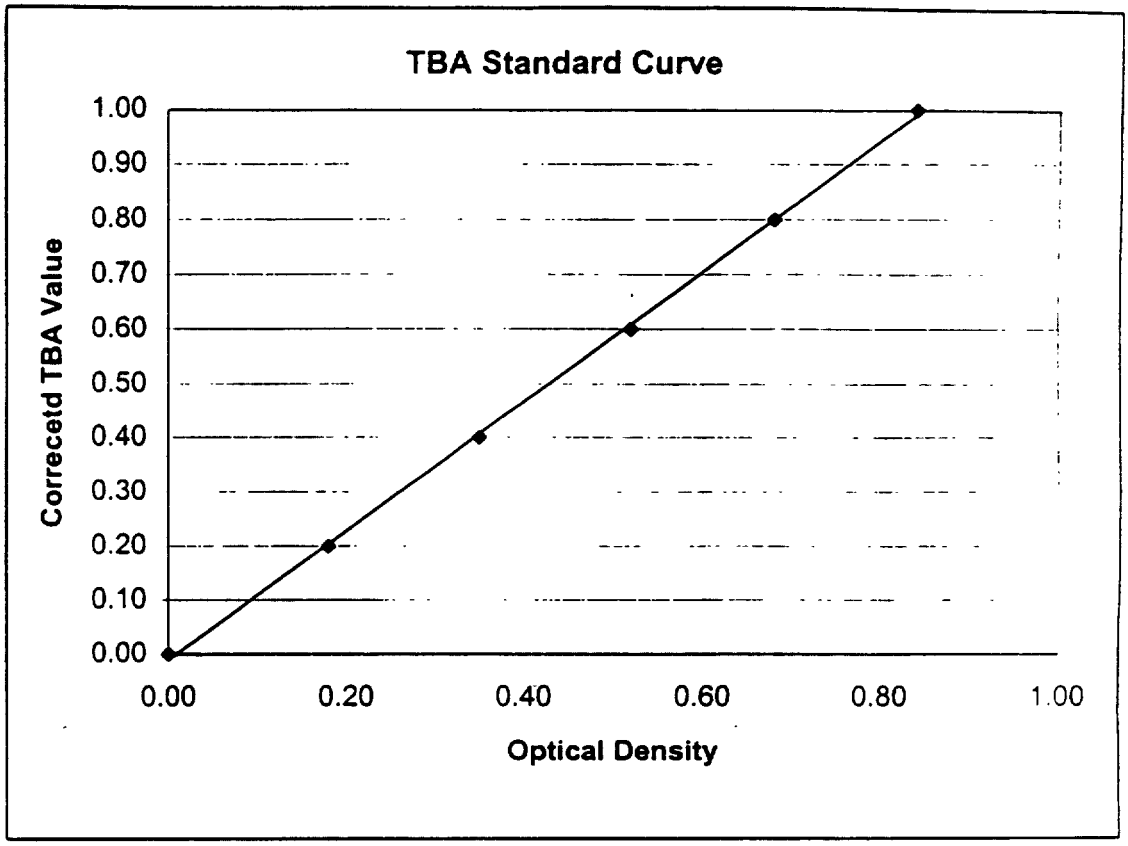
Calculated TBA Value: 0.14

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



Sample # : KEY-R-MED-159 RAW WHOLE PIECES -Day 5

Observed Optical Density: 0.14

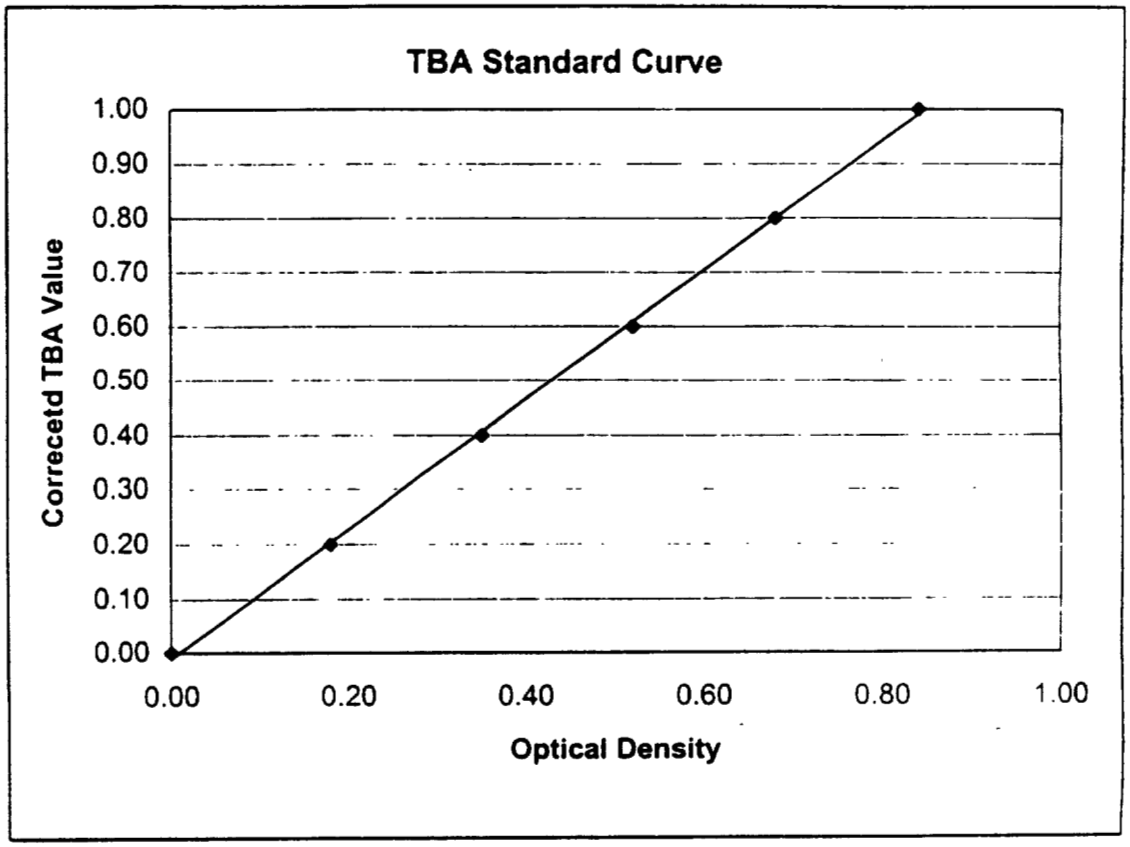
Calculated TBA Value: 0.16

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



Sample # : KEY-HI-278 RAW WHOLE PIECES - Day 5

Observed Optical Density: 0.28

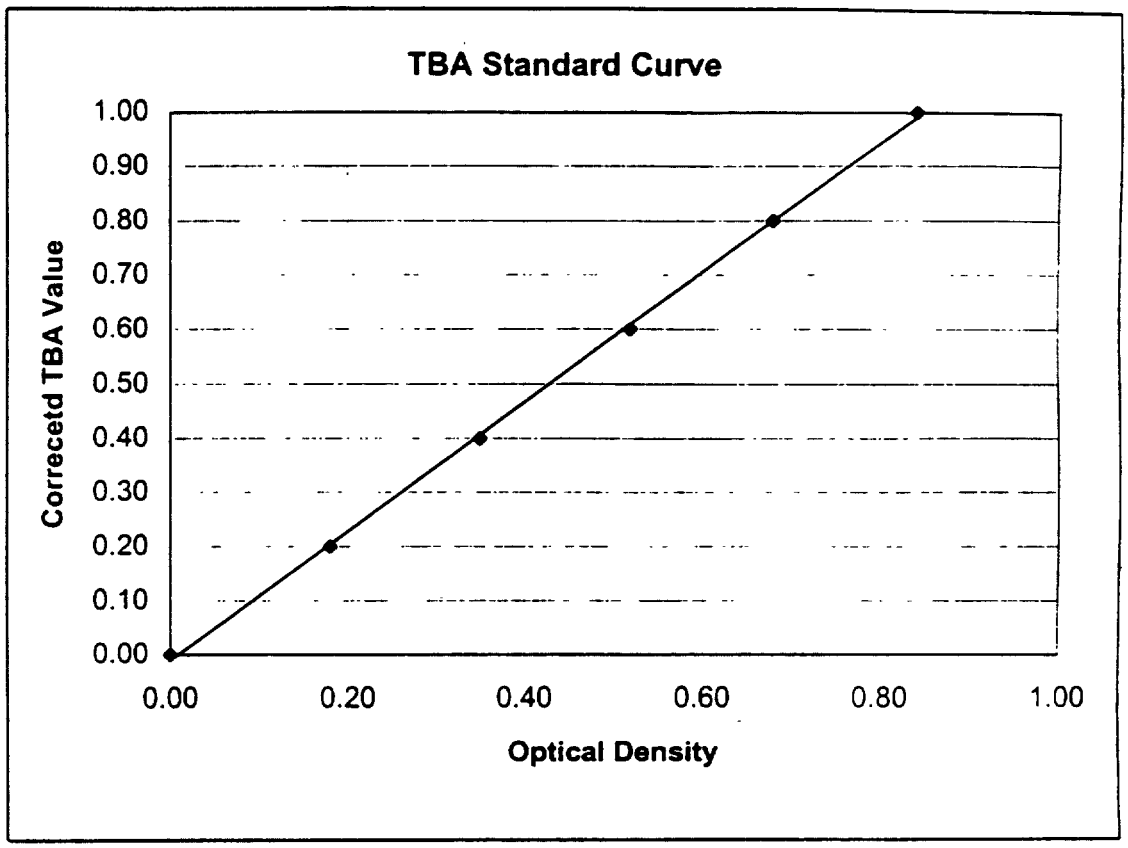
Calculated TBA Value: 0.32

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



Sample # : KEY-R-Z-200 RAW WHOLE PIECES - DAY 7

Observed Optical Density: 0.08

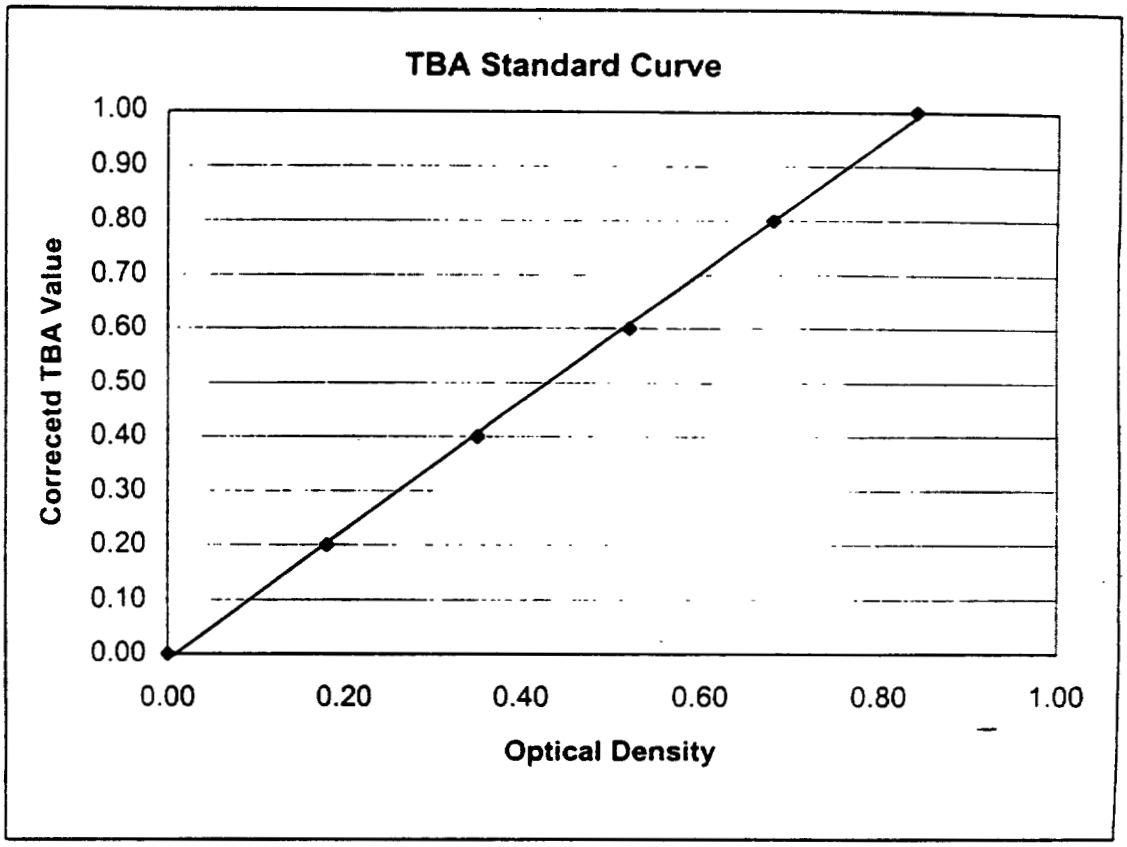
Calculated TBA Value: 0.08

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



Sample # : KEY-R-LO-091 RAW WHOLE PIECES - DAY 7

Observed Optical Density: 0.12

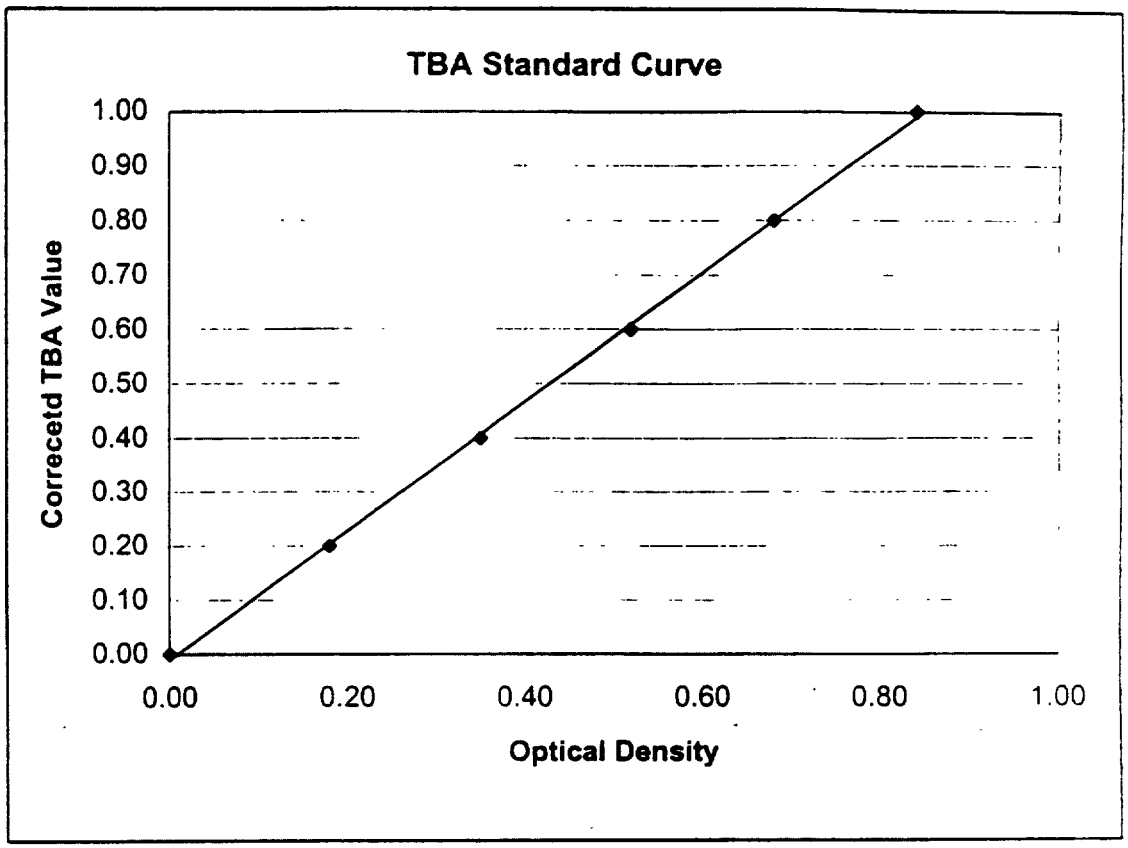
Calculated TBA Value: 0.13

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



Sample # : KEY-R-MED-153 RAW WHOLE PIECES - DAY 7

Observed Optical Density: 0.10

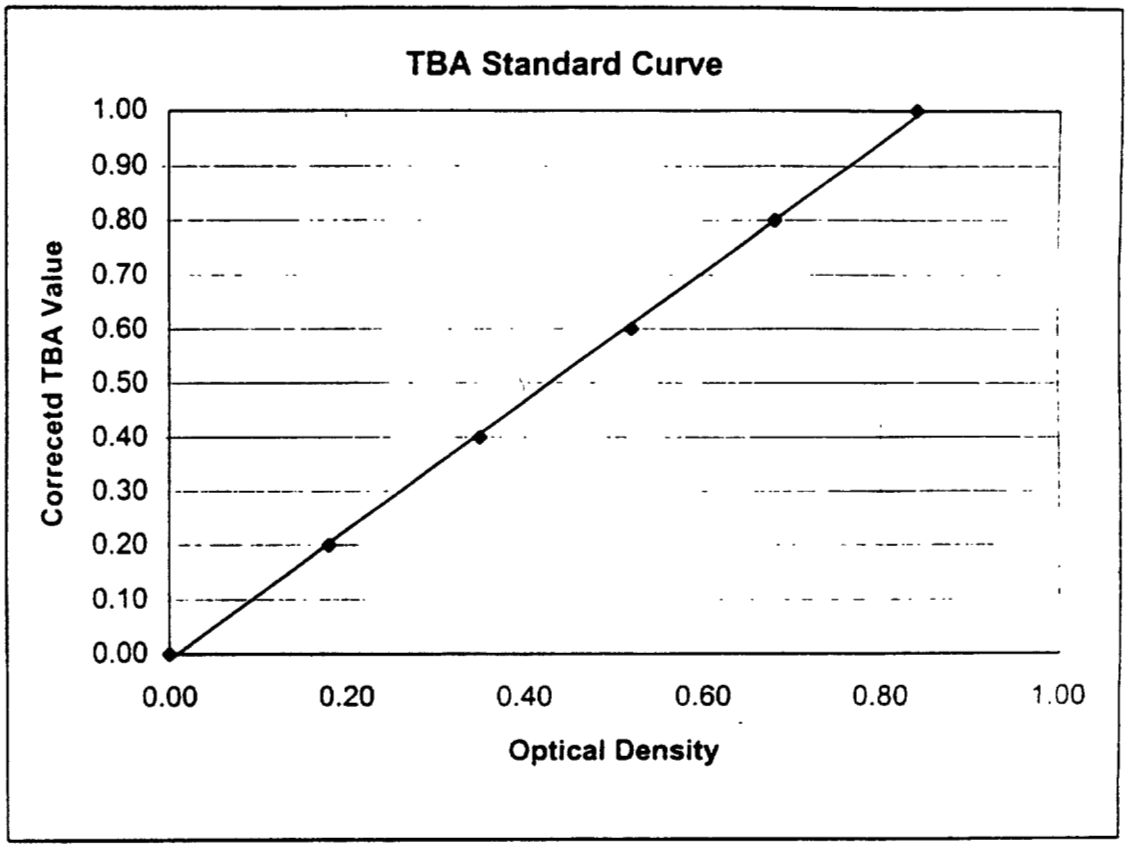
Calculated TBA Value: 0.11

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



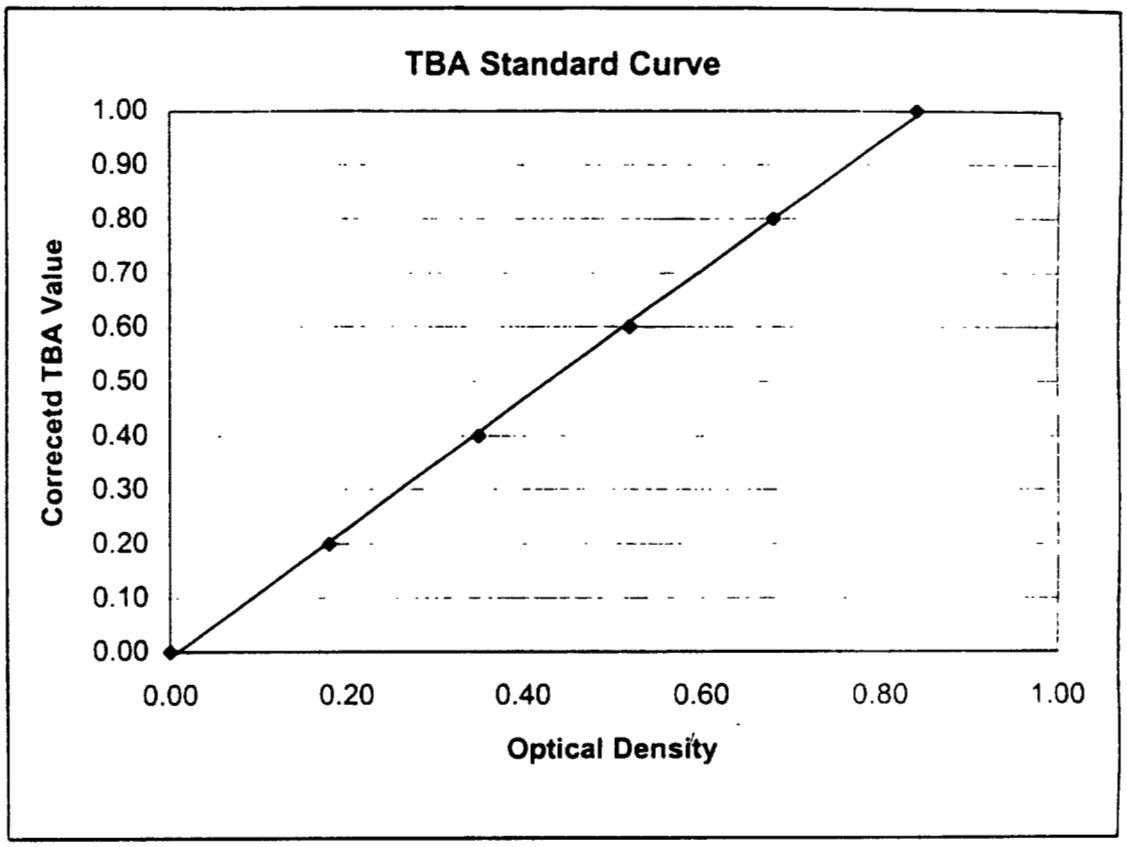
Sample # :	KEY-R-HI-287 RAW WHOLE PIECES - DAY 7
Observed Optical Density:	0.08
Calculated TBA Value:	0.08

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



Sample # : KEY-R-Z-193 RAW WHOLE PIECES – Day 1

Observed Optical Density: 0.17

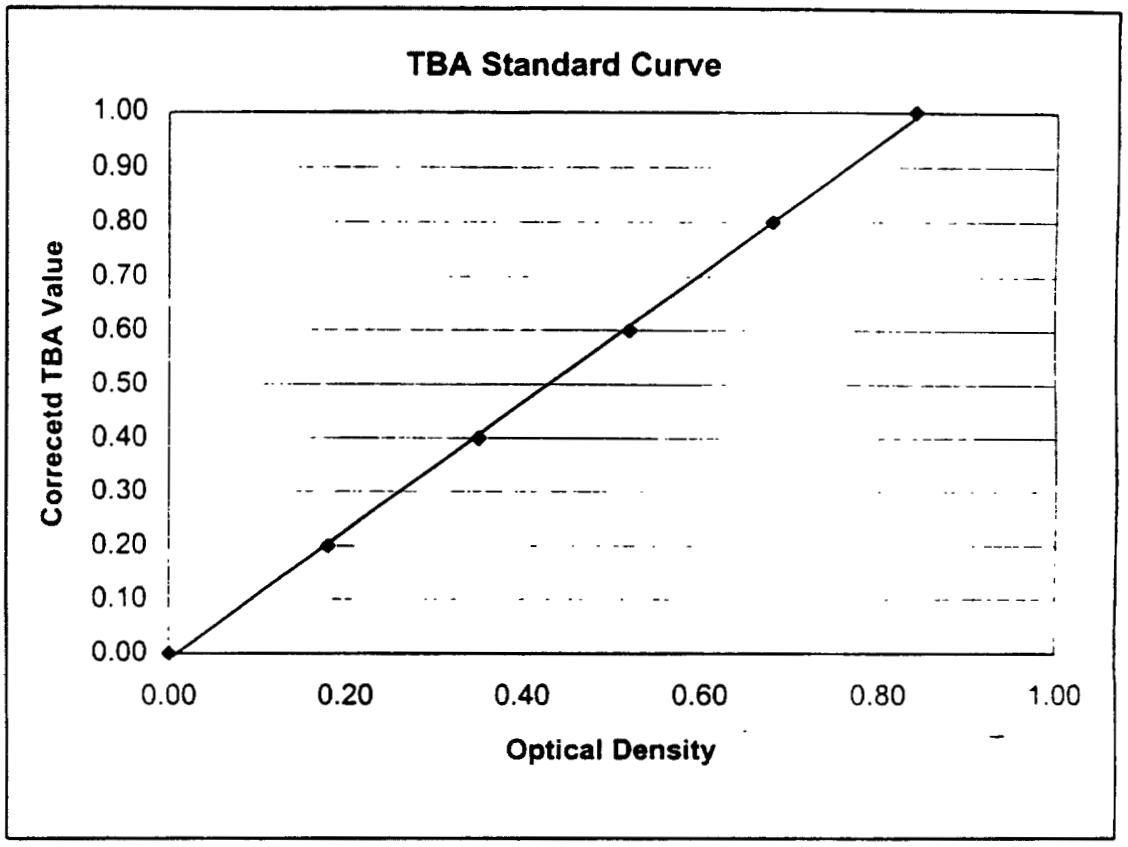
Calculated TBA Value: 0.19

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



Sample # : KEY-R-LO-087 RAW WHOLE PIECES - Day 4

Observed Optical Density: 0.08

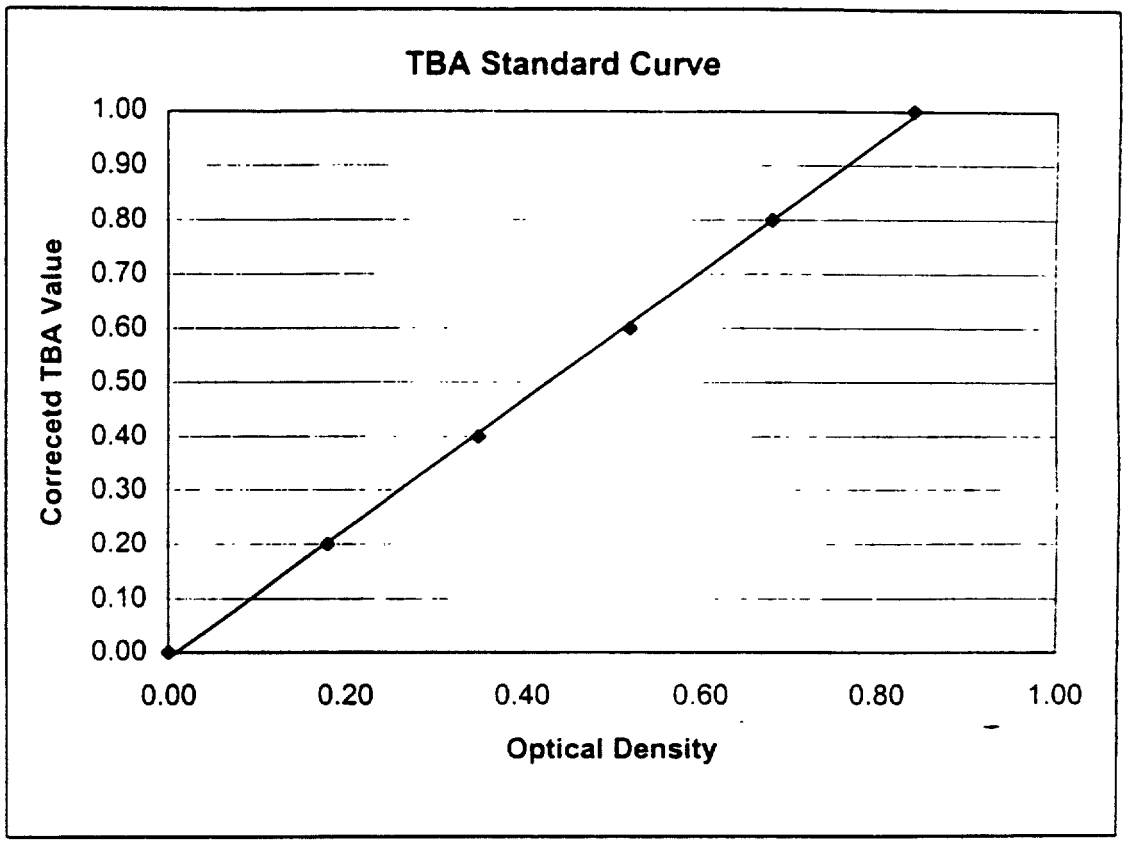
Calculated TBA Value: 0.08

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



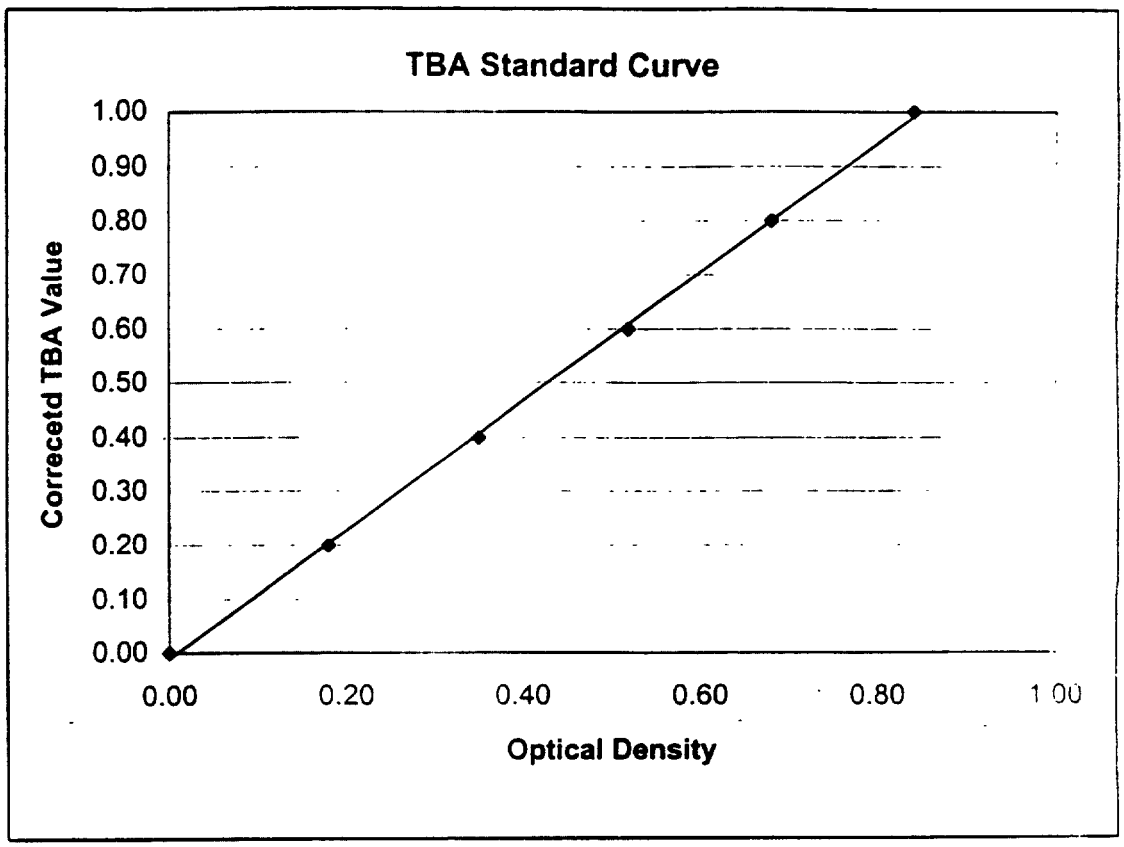
Sample #	KEY-R-MED-147 RAW WHOLE PIECES
Observed Optical Density:	0.10
Calculated TBA Value:	0.11

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



Sample # :	KEY-R-HI-292 RAW WHOLE PIECES
Observed Optical Density:	0.07
Calculated TBA Value:	0.07

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COVANCE
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00203865

BATCH NUMBER: 00203853

DATE ENTERED: 02/18/00

REPORT PRINTED: 02/25/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-R-Z-201, DAY 1
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

Covance Laboratories Inc.
P.O. Box 7545
Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00203866

BATCH NUMBER: 00203853

DATE ENTERED: 02/18/00

REPORT PRINTED: 02/25/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-R-Z-203, DAY 1
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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Tel: 608/241-4471 Fax: 608/241-7227

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00203853

BATCH NUMBER: 00203853

DATE ENTERED: 02/18/00

REPORT PRINTED: 02/25/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-R-LO-098, DAY 1
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00203854

BATCH NUMBER: 00203853

DATE ENTERED: 02/18/00

REPORT PRINTED: 02/25/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-R-LO-110, DAY 1
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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COVANCE.
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REPORT OF ANALYSIS

SAMPLE NUMBER: 00203857

BATCH NUMBER: 00203853

DATE ENTERED: 02/18/00

REPORT PRINTED: 02/25/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-R-MED-138, DAY 1
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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COVANCE.
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REPORT OF ANALYSIS

SAMPLE NUMBER: 00203858

BATCH NUMBER: 00203853

DATE ENTERED: 02/18/00

REPORT PRINTED: 02/25/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-R-MED-143, DAY 1
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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Tel: 608/241-4471 Fax: 608/241-7227

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REPORT OF ANALYSIS

SAMPLE NUMBER: 00203861

BATCH NUMBER: 00203853

DATE ENTERED: 02/18/00

REPORT PRINTED: 02/25/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-R-HI-280, DAY 1
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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Tel: 608/241-4471 Fax: 608/241-7227

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REPORT OF ANALYSIS

SAMPLE NUMBER: 00203862

BATCH NUMBER: 00203853

DATE ENTERED: 02/18/00

REPORT PRINTED: 02/25/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-R-HI-297, DAY 1
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
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REPORT OF ANALYSIS

SAMPLE NUMBER: 00204161

BATCH NUMBER: 00204149

DATE ENTERED: 02/18/00

REPORT PRINTED: 03/03/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-R-Z-202, DAY 2
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

Covance Laboratories Inc.
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Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00204162

BATCH NUMBER: 00204149

DATE ENTERED: 02/18/00

REPORT PRINTED: 03/03/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-R-Z-220, DAY 2
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

Covance Laboratories Inc.
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Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
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COVANCE.
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REPORT OF ANALYSIS

SAMPLE NUMBER: 00204149

BATCH NUMBER: 00204149

DATE ENTERED: 02/18/00

REPORT PRINTED: 03/03/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-R-LO-099, DAY 2
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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REPORT OF ANALYSIS

SAMPLE NUMBER: 00204150

BATCH NUMBER: 00204149

DATE ENTERED: 02/18/00

REPORT PRINTED: 03/03/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-R-LO-103, DAY 2
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00204153

BATCH NUMBER: 00204149

DATE ENTERED: 02/18/00

REPORT PRINTED: 03/03/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-R-MED-132, DAY 2
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00204154

BATCH NUMBER: 00204149

DATE ENTERED: 02/18/00

REPORT PRINTED: 03/03/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-R-MED-NO CODE, DAY 2
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

Covance Laboratories Inc.
P.O. Box 7545
Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00204157

BATCH NUMBER: 00204149

DATE ENTERED: 02/18/00

REPORT PRINTED: 03/03/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-R-HI-263, DAY 2
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

Covance Laboratories Inc.
P.O. Box 7545
Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00204158

BATCH NUMBER: 00204149

DATE ENTERED: 02/18/00

REPORT PRINTED: 03/03/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-R-HI-270, DAY 2
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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Madison, WI 53707-7545
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COVANCE
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00204177

BATCH NUMBER: 00204165

DATE ENTERED: 02/18/00

REPORT PRINTED: 03/03/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-R-Z-208, DAY 5
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

Covance Laboratories Inc.
P.O. Box 7545
Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

COVANCE
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00204178

BATCH NUMBER: 00204165

DATE ENTERED: 02/18/00

REPORT PRINTED: 03/03/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-R-Z-216, DAY 5
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

Covance Laboratories Inc.
P.O. Box 7545
Madison, WI 53707-7545
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COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00204165

BATCH NUMBER: 00204165

DATE ENTERED: 02/18/00

REPORT PRINTED: 03/03/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-R-LO-114, DAY 5
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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Tel: 608/241-4471 Fax: 608/241-7227

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00204166

BATCH NUMBER: 00204165

DATE ENTERED: 02/18/00

REPORT PRINTED: 03/03/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-R-LO-118, DAY 5
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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COVANCE
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00204169

BATCH NUMBER: 00204165

DATE ENTERED: 02/18/00

REPORT PRINTED: 03/03/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-R-MED-124, DAY 5
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00204170

BATCH NUMBER: 00204165

DATE ENTERED: 02/18/00

REPORT PRINTED: 03/03/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-R-MED-150, DAY 5
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
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COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00204173

BATCH NUMBER: 00204165

DATE ENTERED: 02/18/00

REPORT PRINTED: 03/03/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-R-HI-284, DAY 5
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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Tel: 608/241-4471 Fax: 608/241-7227

COVANCE
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00204174

BATCH NUMBER: 00204165

DATE ENTERED: 02/18/00

REPORT PRINTED: 03/03/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-R-HI-294, DAY 5
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

Covance Laboratories Inc.
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Tel: 608/241-4471 Fax: 608/241-7227

REPORT OF ANALYSIS

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00205049

BATCH NUMBER: 00205046

DATE ENTERED: 02/24/00

REPORT PRINTED: 03/09/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW WHOLE CHICKEN: KEY-R-Z-188, DAY 7
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

Covance Laboratories Inc.
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COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00205046

BATCH NUMBER: 00205046

DATE ENTERED: 02/24/00

REPORT PRINTED: 03/09/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW WHOLE CHICKEN: KEY-R-LO-093, DAY 7
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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Tel: 608/241-4471 Fax: 608/241-7227

REPORT OF ANALYSIS

COVANCE
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00205047

BATCH NUMBER: 00205046

DATE ENTERED: 02/24/00

REPORT PRINTED: 03/09/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW WHOLE CHICKEN: KEY-R-MED-137, DAY 7
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

Covance Laboratories Inc.
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Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

REPORT OF ANALYSIS

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00205048

BATCH NUMBER: 00205046

DATE ENTERED: 02/24/00

REPORT PRINTED: 03/09/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW WHOLE CHICKEN: KEY-R-HI-290, DAY 7
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

Covance Laboratories Inc.
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COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00205060

BATCH NUMBER: 00205057

DATE ENTERED: 02/24/00

REPORT PRINTED: 03/03/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW WHOLE PIECES CHICKEN: KEY-R-Z-185, DAY 9
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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Tel: 608/241-4471 Fax: 608/241-7227

COVANCE
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00205057

BATCH NUMBER: 00205057

DATE ENTERED: 02/24/00

REPORT PRINTED: 03/03/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW WHOLE PIECES CHICKEN: KEY-R-LO-105, DAY 9
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00205058

BATCH NUMBER: 00205057

DATE ENTERED: 02/24/00

REPORT PRINTED: 03/03/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW WHOLE PIECES CHICKEN: KEY-R-MED-146, DAY 9
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

Covance Laboratories Inc.
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Tel: 608/241-4471 Fax: 608/241-7227

COVANCE
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00205059

BATCH NUMBER: 00205057

DATE ENTERED: 02/24/00

REPORT PRINTED: 03/03/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW WHOLE PIECES CHICKEN: KEY-R-HI-293, DAY 9
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

Protocol: Key-R-1-0200b

A QUALITATIVE COMPARATIVE STUDY OF CUBED RAW POULTRY PRODUCTS STORED IN POLYETHYLENE BAGS CONTAINING EITHER NO ADDITIVES OR BTI PROPRIETARY TECHNOLOGY

Objective: To determine any differences in selected characteristics of raw poultry products packaged in polyethylene (LDPE) bags or LDPE bags containing BTI proprietary technology.

Materials:

Cubed raw poultry products prepared by Keystone Foods, Folcroft, PA.

1. Cubed pieces of raw poultry muscle meat, boneless & skinless breast meat

Packaging film/bags

1. LDPE bags without active additives
2. LDPE opaque bags containing BTI additive at low %
3. LDPE opaque bags containing BTI additive at medium %
4. LDPE opaque bags containing BTI additive at high %

Packages:

1. Approximately 7 ounces of cubed raw poultry pieces per package
2. Four (4) replicates per sample type (2 for Keystone, 2 for Covance)
3. Total Packages: 160 (4 types of film)*(4 replicates/type)*(5 storage periods)*(2 products)
4. Total Packages per Test Lab: 80
5. Packages containing samples will be stored in a refrigerated cooler at 4° to 8°C

Analyses as listed in attached chart (Page 2):

1. Food evaluation characteristics to be conducted by Keystone Foods; Project Contact is Jade Lau; Test and Analytical Protocols to be provided to BTI by Keystone Foods.
2. Chemical analyses to be conducted by Covance, Madison, WI; Project Contact is Wayne Ellefson; Protocols to be provided by Covance.

Schedule:

- Day 0 — Prepare raw poultry products/obtain baseline data
- Day 1 — Package in test bags with labels and store as indicated
- Day 1 — Ship bags containing product to Covance test labs via FedEx

Samples for Covance may be frozen and all shipped at once on Day 10

- Day 1 — Test Labs conduct analyses as DAY 1 samples
- Day 2 — Test Labs conduct analyses as DAY 2 samples
- Day 5 — Test Labs conduct analyses as DAY 5 samples

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PHONE 312.337.7007 · FACSIMILE 312.337.0505

- Day 7 — Test Labs conduct analyses as DAY 7 samples
- Day 9 — Test Labs conduct analyses as DAY 9 samples
- Day -- — Preliminary Report of Results
- Day -- — Final Reports from Covance and Keystone Foods

Analyses:

Samples from each treatment will be analyzed for color, odor, TBA and residual chlorite on each of the following days: 1, 2, 5, 7, and 9.

Color and Odor by Keystone Labs:

If possible, color and odor comments will be obtained for each sample prior to the rinse. Color change may be documented using a digital camera.

TBA Analyses:

Keystone Labs will conduct TBA analyses.

Residual Chlorite:

A duplicate samples will be shipped to a COVANCE lab for residual chlorite analysis.

Chlorine Dioxide and Chlorite Anions: Standard Methods for Water Analysis Method 4500-ClO2 B. Iodometric Method. APHA, AWWA, and WPCF. Washington, D. C. (17th ed.) Pages 4-54 (modified as Southwest Research Institute BTI-SOP# 99-02. Aieta, E. M., P. V. Roberts, and M. Hernandez, J. Am. Water Works Assoc., 76(1), 64-70 (1984).

TBA: See Methods section.

Day	
0	Collect baseline data on test and control products
1 - 10	Store samples at 38°F
1	Sample: color, odor, TBA, residual chlorite
2	Sample: color, odor, TBA, residual chlorite
5	Sample: color, odor, TBA, residual chlorite
7	Sample: color, odor, TBA, residual chlorite
9	Sample: color, odor, TBA, residual chlorite

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RESULTS

Sensory: The CRT did not alter the sensory characteristics of cubed raw poultry muscle meat as skinless pieces, including color and odor. At all storage times, the color, as seen in the photographs, of the low, medium, and high CRT packaging was similar to the control.

TBA: The TBA values either as a function of film type or duration of storage were within the normal limits of the untreated muscle meat. The values for the poultry muscle meat stored in the bags containing the CRT technology were either below the control film bags or within the normal limits of sample-to-sample variability.

Chlorite: Residual chlorite anions were not detected. The cooking process would destroy any residual chlorite.

CONCLUSION

Packaging materials containing the Microsphère® CRT at levels of loading up to 50% may be used for the storage of fresh poultry without demonstrating any negative qualitative effects.

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COMPANY	Keystone Foods	

TEST ARTICLE	Poultry-Raw Cubed Pieces

BTI SAMPLE FORM:	Microsphere® bags: zero, low, medium, high %

Temperature of Test:	30 to 38°C	

Day	Sample Evaluation					
	Color	Odor	Appearance		Chemical	
			(see photos)		TBA	Chlorite Residual
						ppm
Day 1						
Zero	Pink	Fresh	No discoloration		0.17	None Detected
Low	Pink	Fresh	No discoloration		0.24	None Detected
Medium	Pink	Fresh	No discoloration		0.19	None Detected
High	Pink	Fresh	No discoloration		0.11	None Detected
Day 2	pale yellow	slightly	No discoloration			
Zero	pale yellow	slightly	No discoloration		0.19	None Detected
Low	pale yellow	slightly	No discoloration		0.14	None Detected
Medium	pale yellow	slightly	No discoloration		0.14	None Detected
High	pale yellow	slightly	No discoloration		0.17	None Detected
Day 5	dk pink	moderate	film			
Zero	dk pink	moderate	film		0.18	None Detected
Low	dk pink	moderate	film		0.20	None Detected
Medium	dk pink	moderate	film		0.39	None Detected
High	dk pink	moderate	film		0.19	None Detected
Day 7	[illegible]	[illegible]	film/discolor			
Zero	dk pink	high	film/discolor		0.14	None Detected
Low	dk pink	high	film/discolor		0.14	None Detected
Medium	dk pink	high	film/discolor		0.18	None Detected
High	dk pink	high			0.20	None Detected
Day 9	very dk pink	Extremely	Heavy film			
Zero	very dk pink	Extremely	Heavy film		0.26	None Detected
Low	very dk pink	Extremely	Heavy film		0.12	None Detected
Medium	very dk pink	Extremely	Heavy film		0.12	None Detected
High	very dk pink	Extremely	Heavy film		0.12	None Detected

Sensory Tests
Name of Person Conducting Tests: _____
Signature: _____ Date: 5/11 [illegible]

TBA Analyses
Name of Person Conducting Tests: _____
Signature: _____ Date: 5-15-00

000499

Chlorite Analyses
Name of Person Conducting Tests: Conducted at Covance Labs - see Covance Report
Signature: _____ Date: _____

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PAGES

000500-000502

TBA VALUE
RAW POULTRY
WHOLE PIECES AND CUBED PIECES
(Conducted by Keystone Foods)

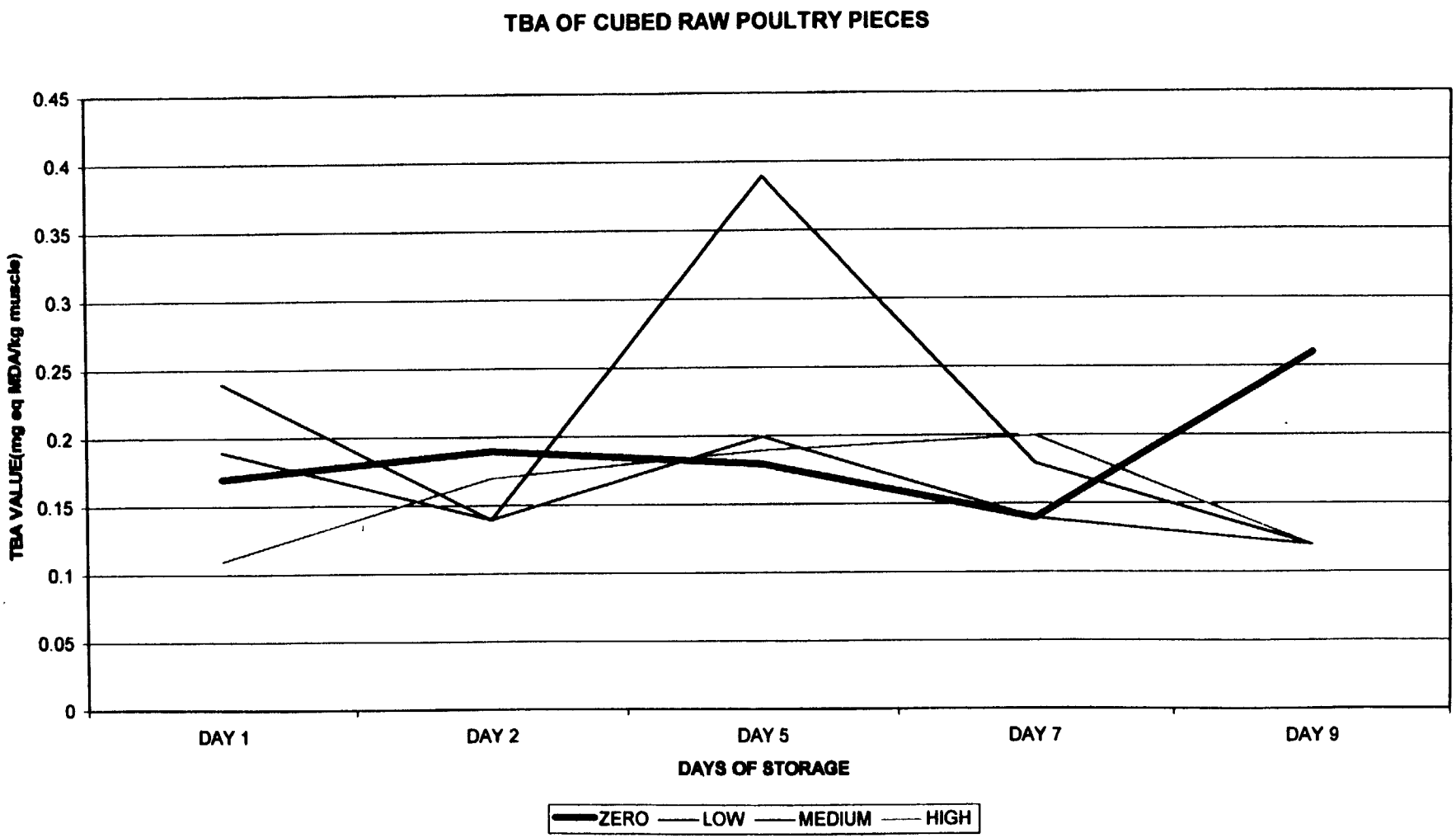
	WHOLE PIECES	CUBED PIECES
	ZERO	ZERO
DAY 1	0.22	0.17
DAY 2	0.18	0.19
DAY 5	0.19	0.18
DAY 7	0.08	0.14
DAY 9	0.19	0.26

	LOW	LOW
DAY 1	0.08	0.24
DAY 2	0.1	0.14
DAY 5	0.14	0.2
DAY 7	0.13	0.14
DAY 9	0.08	0.12

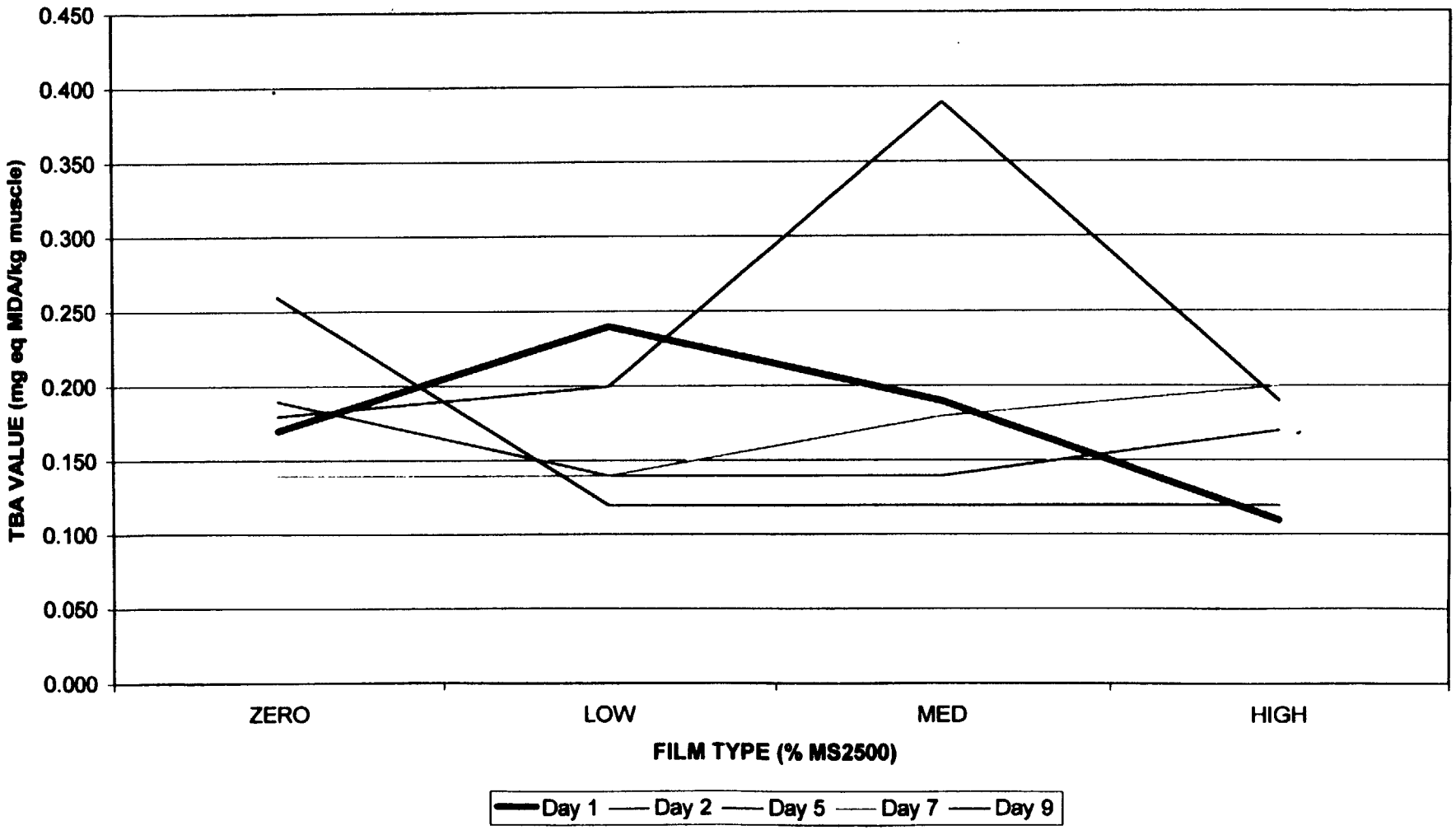
	MEDIUM	MEDIUM
DAY 1	0.17	0.19
DAY 2	0.07	0.14
DAY 5	0.16	0.39
DAY 7	0.11	0.18
DAY 9	0.11	0.12

	HIGH	HIGH
DAY 1	0.13	0.11
DAY 2	0.1	0.17
DAY 5	0.32	0.19
DAY 7	0.08	0.2
DAY 9	0.07	0.12

Pieces were packaged in polyethylene film film containing zero, low, medium and high % of BTI's proprietary chlorine dioxide generating product


TBA OF CUBED RAW POULTRY PIECES
TBA VALUE(mg eq MDA/kg muscle)
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
DAY 1
DAY 2
DAY 5
DAY 7
DAY 9
DAYS OF STORAGE
ZERO
LOW
MEDIUM
HIGH

TBA of CUBED RAW POULTRY PIECES
as a
FUNCTION of FILM for ANY GIVEN DAY of STORAGE



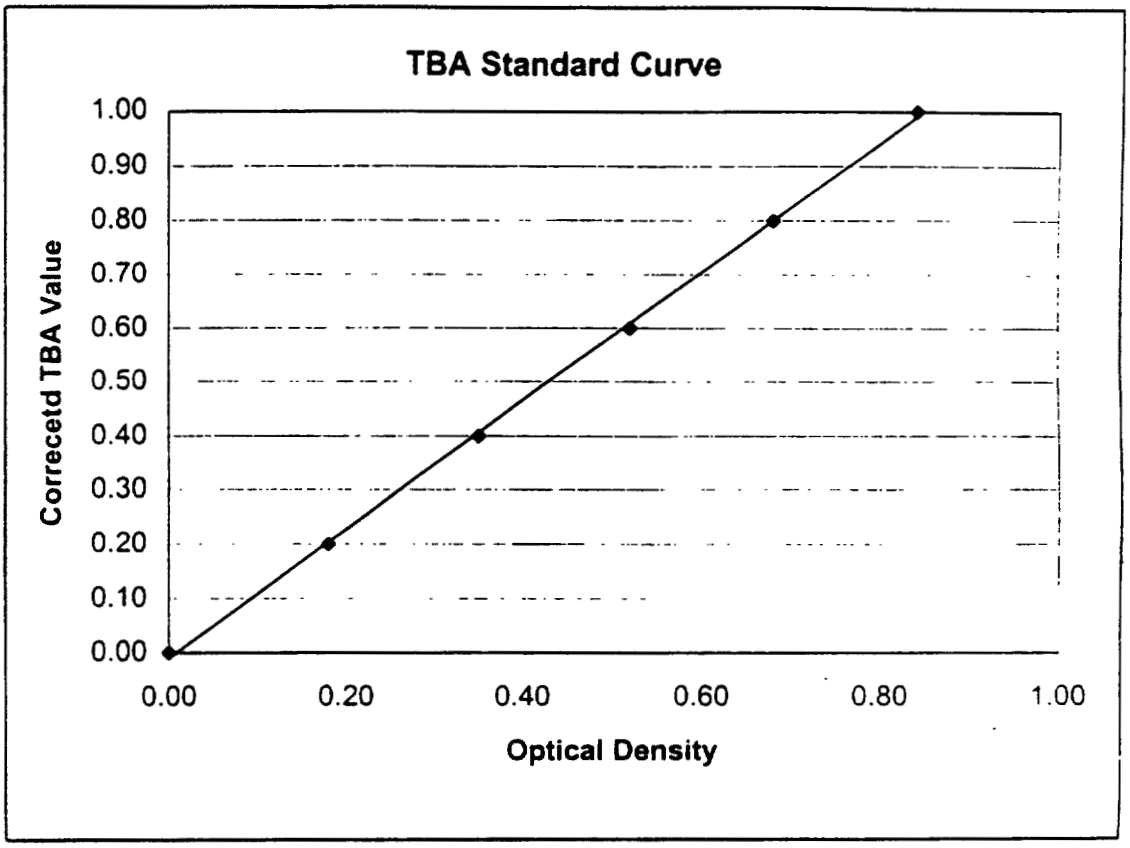
005305

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



Sample # :	KEY-RC-Z-186
Observed Optical Density:	0.15
Calculated TBA Value:	0.17

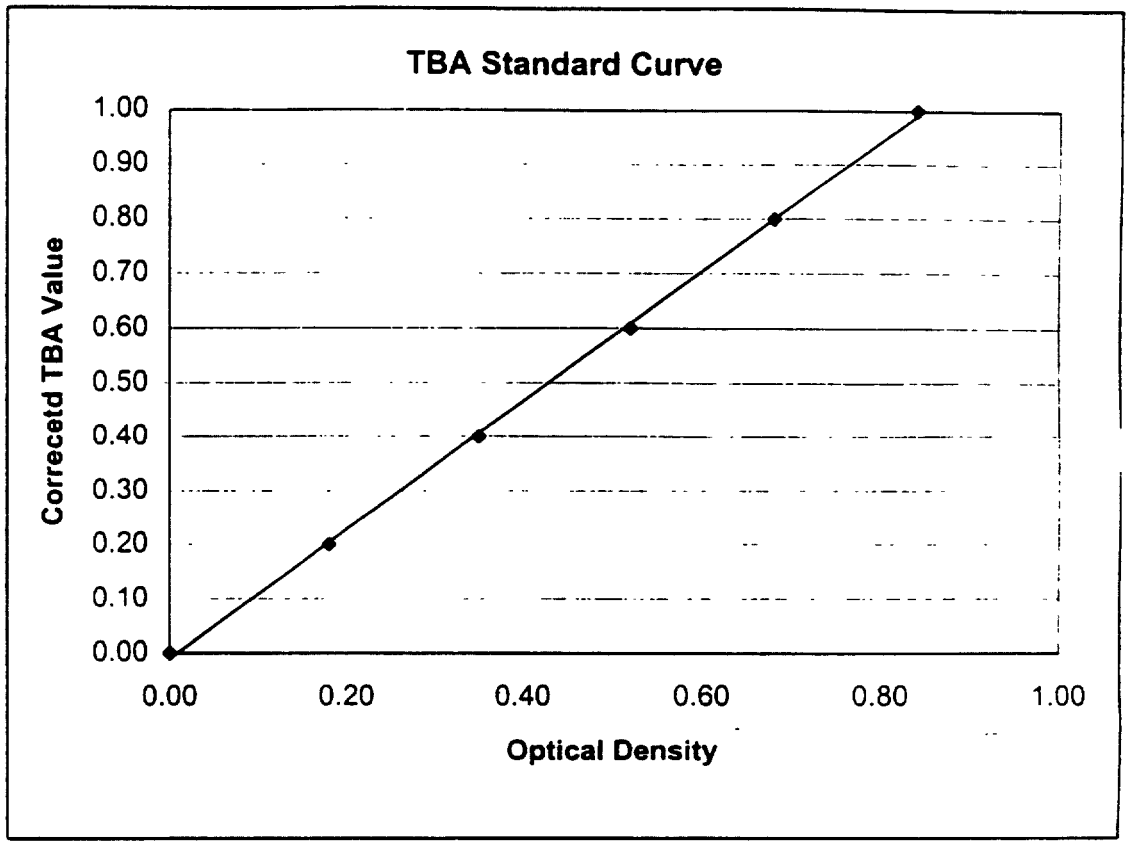
-Day 1 raw pieces

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



Sample # : KEY-RC-Z-183 CUBE PIECES – Day 2

Observed Optical Density: 0.17

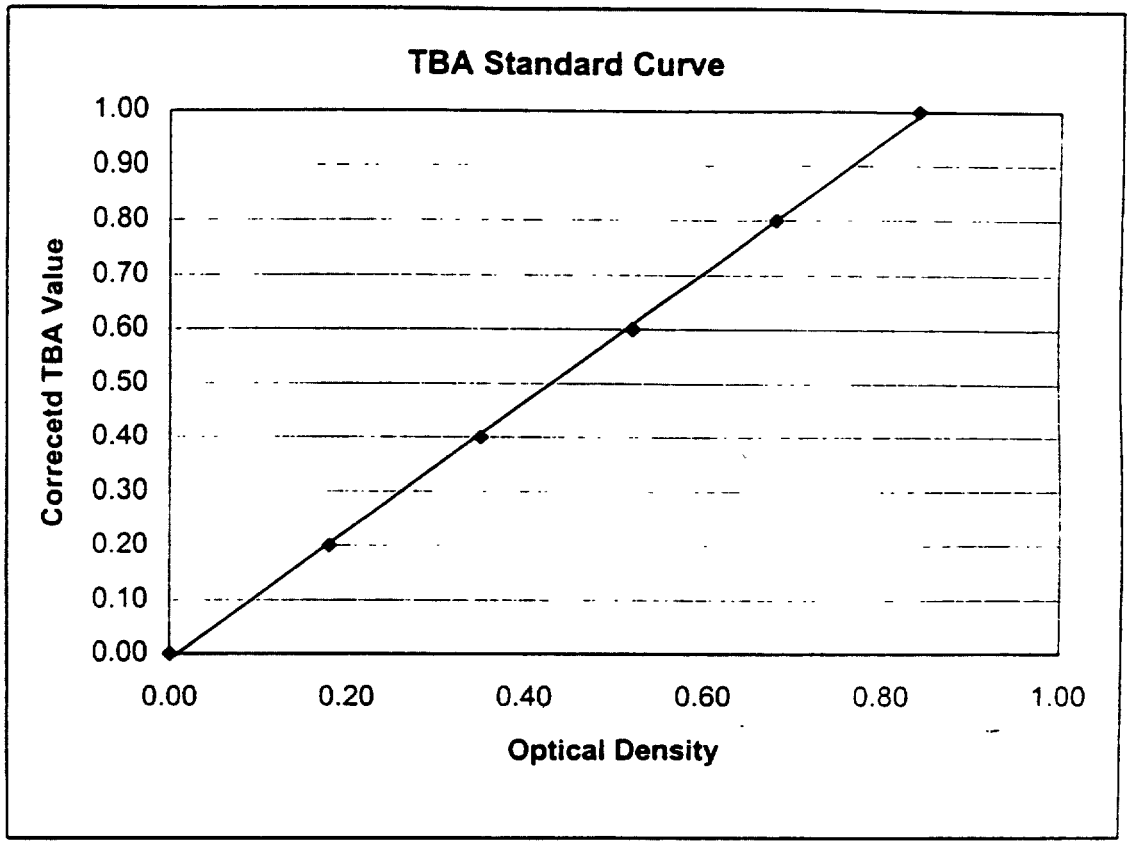
Calculated TBA Value: 0.19

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



Sample # : KEY-RC-Z-194 RAW CUBE PIECES - Day 5

Observed Optical Density: 0.16

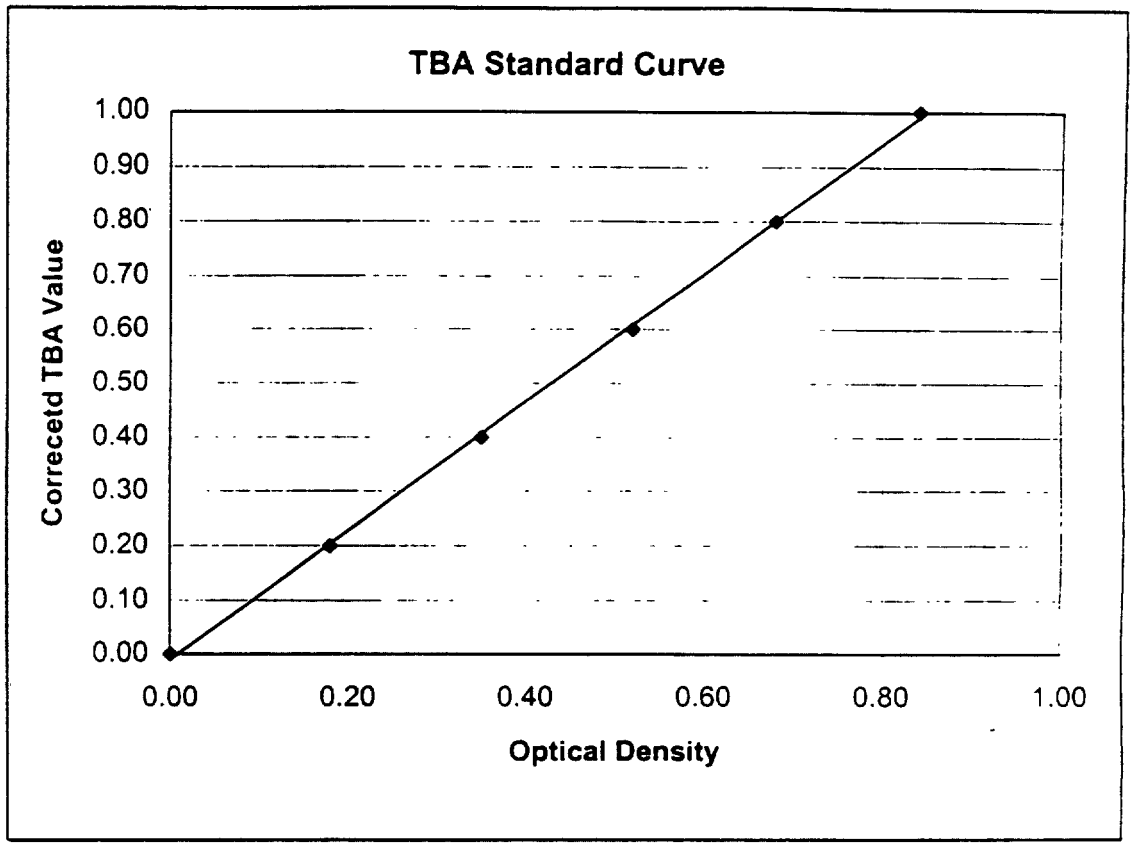
Calculated TBA Value: 0.18

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



Sample # : KEY-RC-Z-196 RAW CUBE PIECES -DAY 7

Observed Optical Density: 0.13

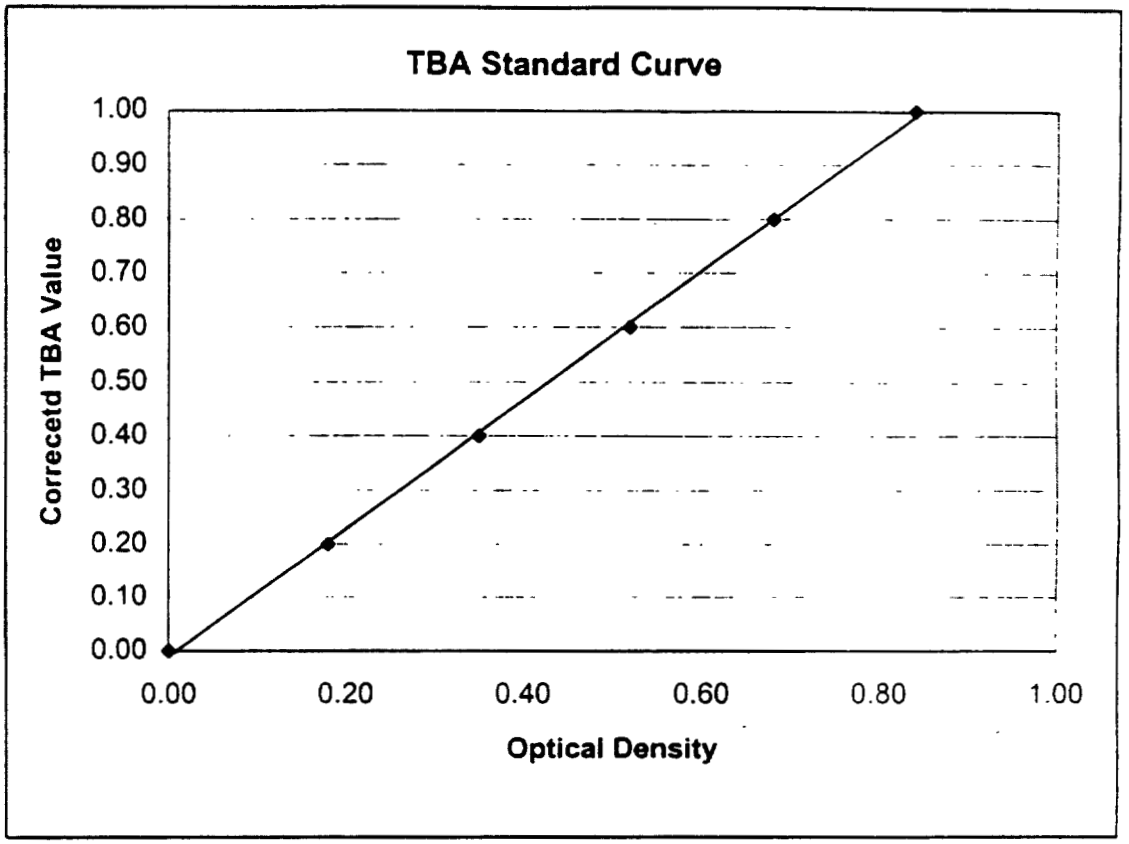
Calculated TBA Value: 0.14

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



Sample # : KEY-RC-Z-198 RAW CUBE PIECES - Day 1

Observed Optical Density: 0.23

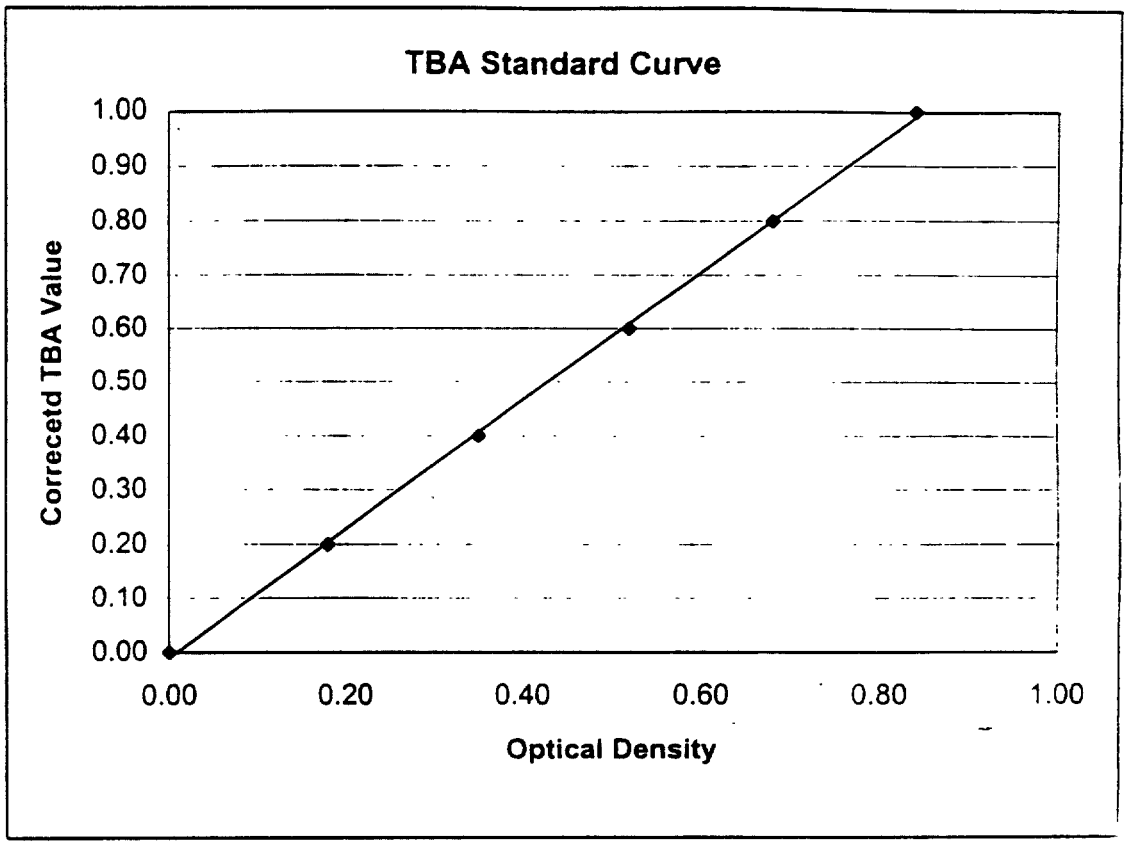
Calculated TBA Value: 0.26

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



Sample # : KEY-RC-LO-501 -Day1 raw pieces

Observed Optical Density: 0.21

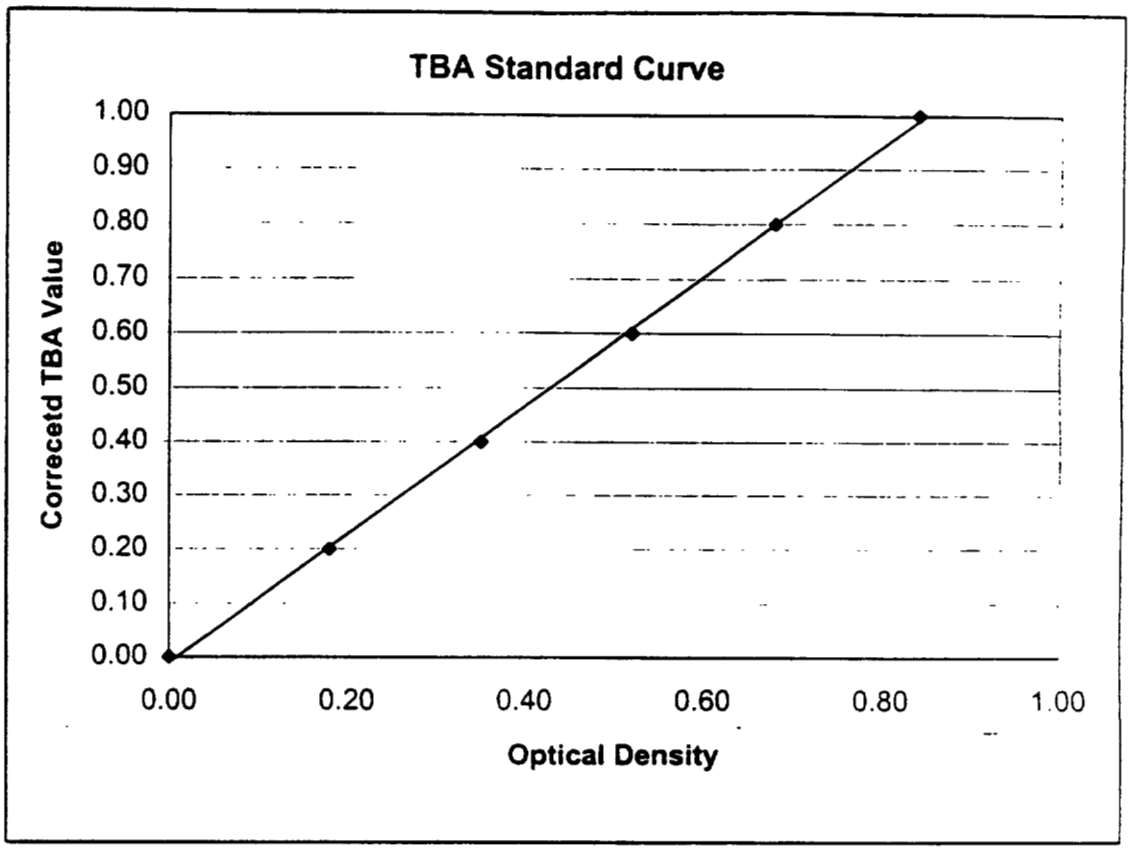
Calculated TBA Value: 0.24

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



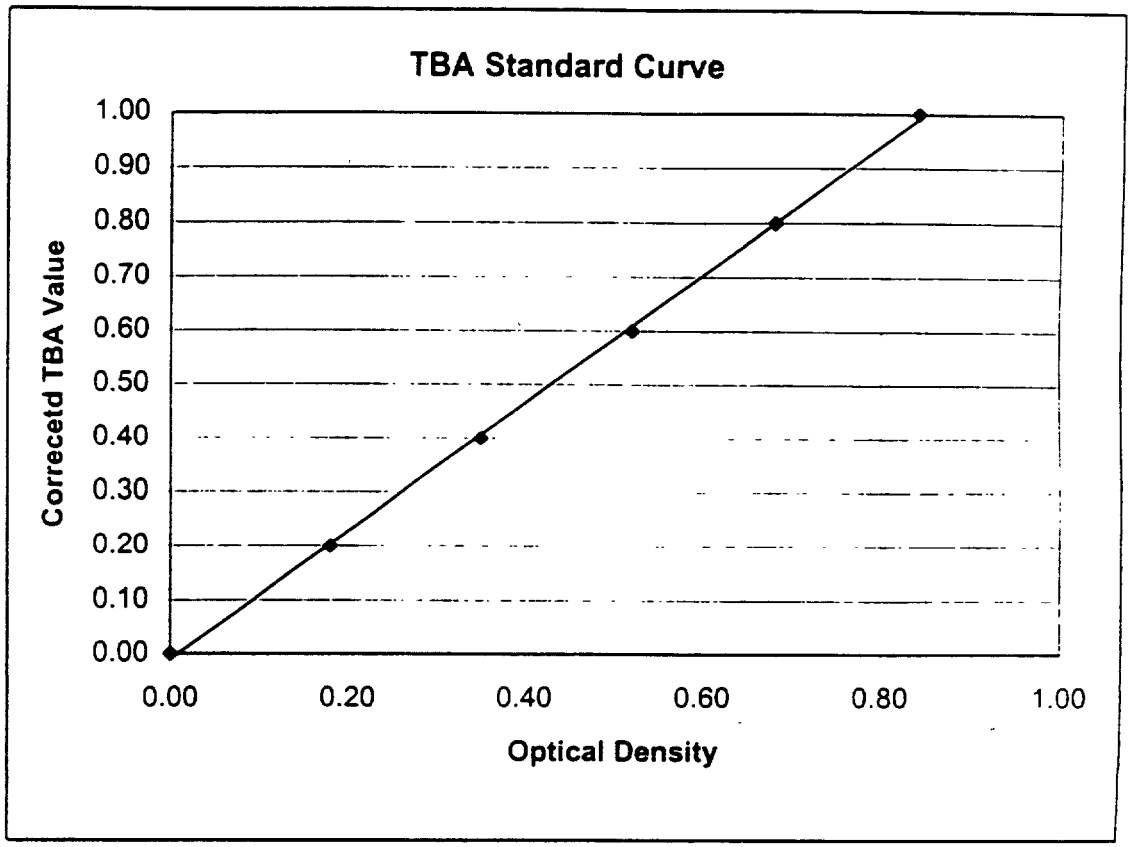
Sample # :	KEY-RC-LO-504 CUBE PIECES – Day 2
Observed Optical Density:	0.13
Calculated TBA Value:	0.14

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



Sample # : KEY-RC-LO-518 RAW CUBE PIECES - Day 5

Observed Optical Density: 0.18

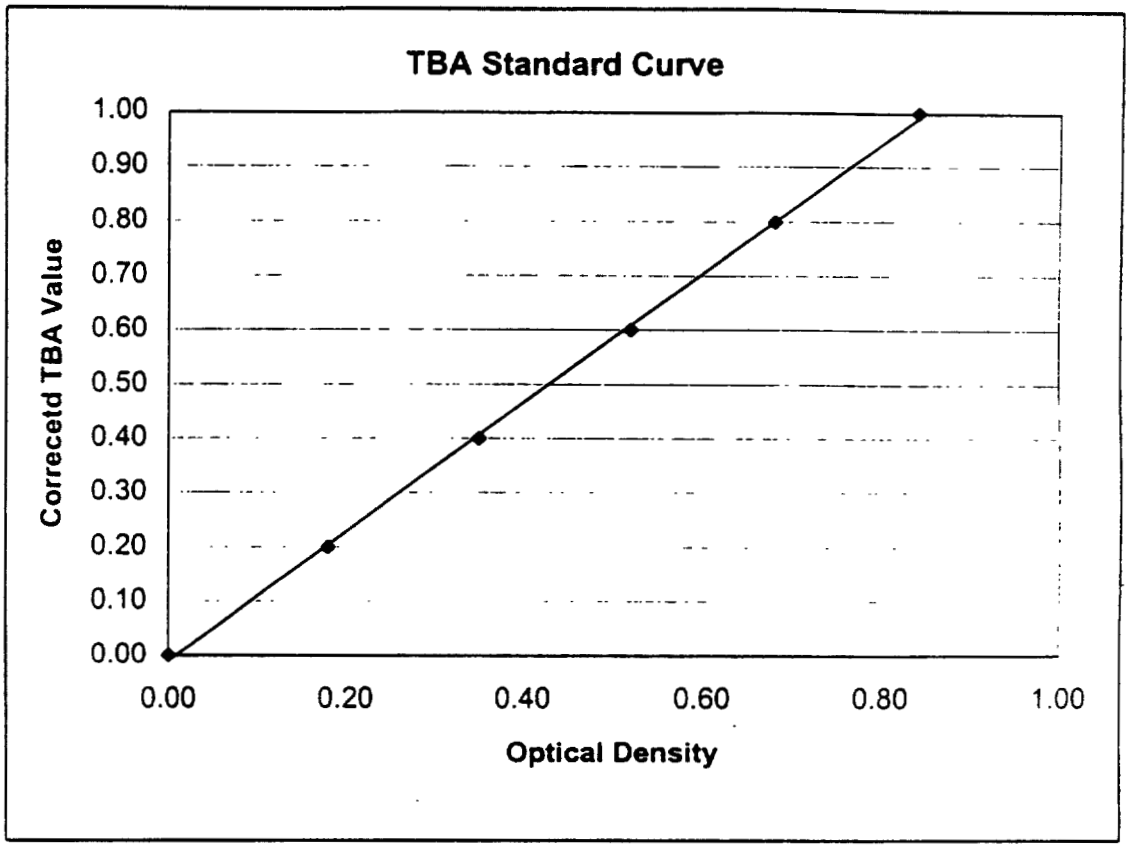
Calculated TBA Value: 0.20

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



Sample # : KEY-RC-LO-510 RAW CUBE PIECES -DAY 7

Observed Optical Density: 0.13

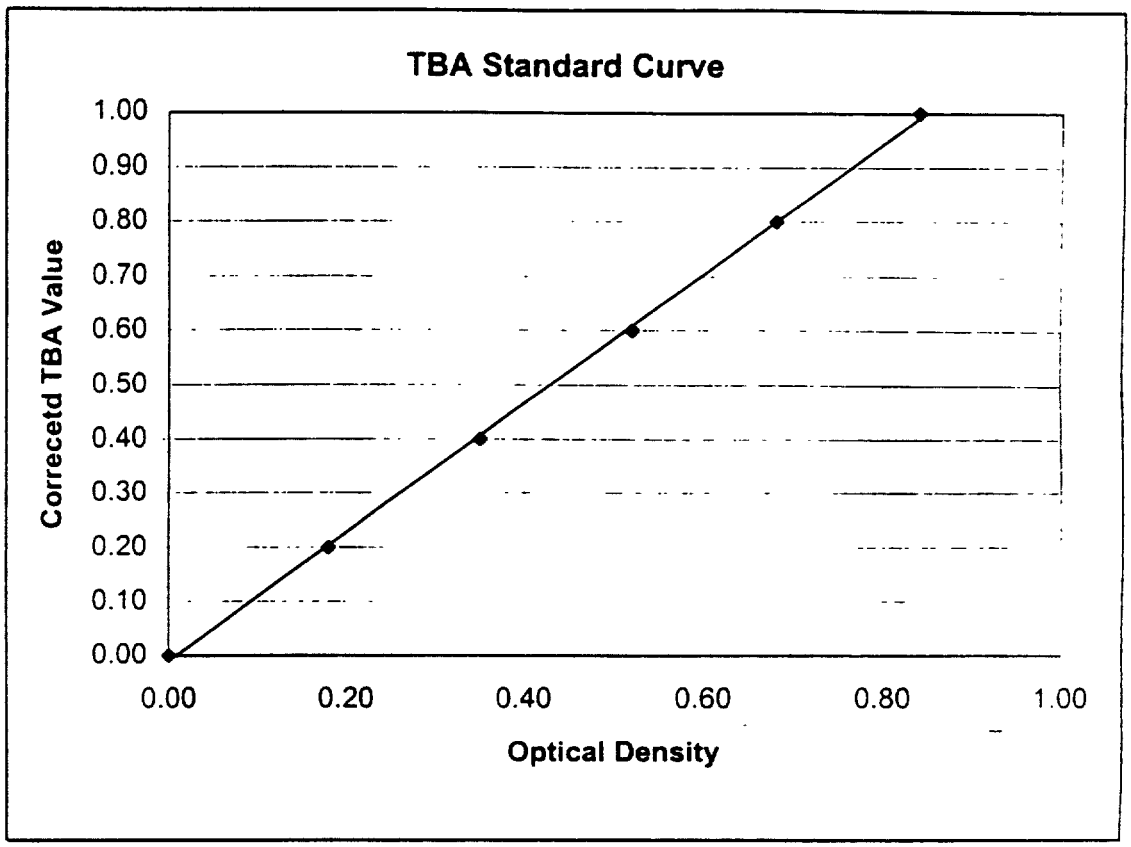
Calculated TBA Value: 0.14

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



Sample # : KEY-RC-MED-583 – Day 1 [illegible]

Observed Optical Density: 0.17

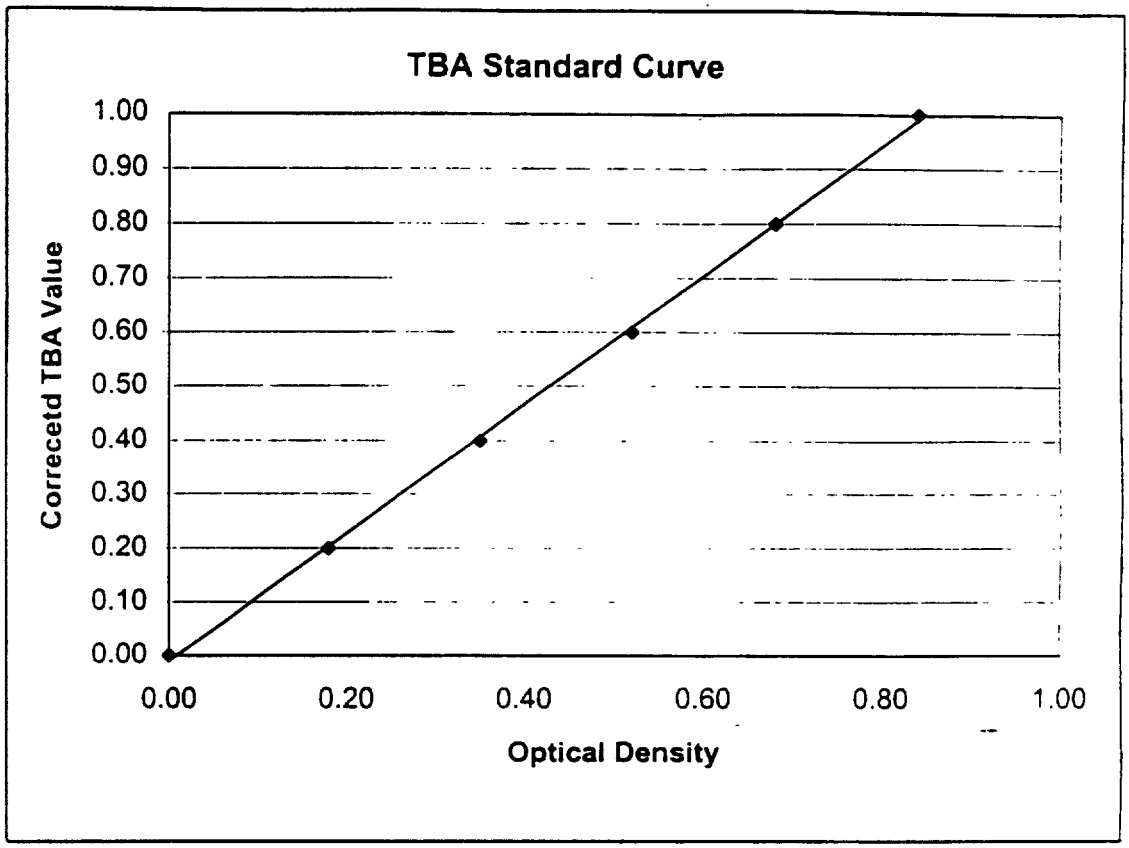
Calculated TBA Value: 0.19

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



Sample # : KEY-RC-MED-599 CUBE PIECES - Day 2

Observed Optical Density: 0.13

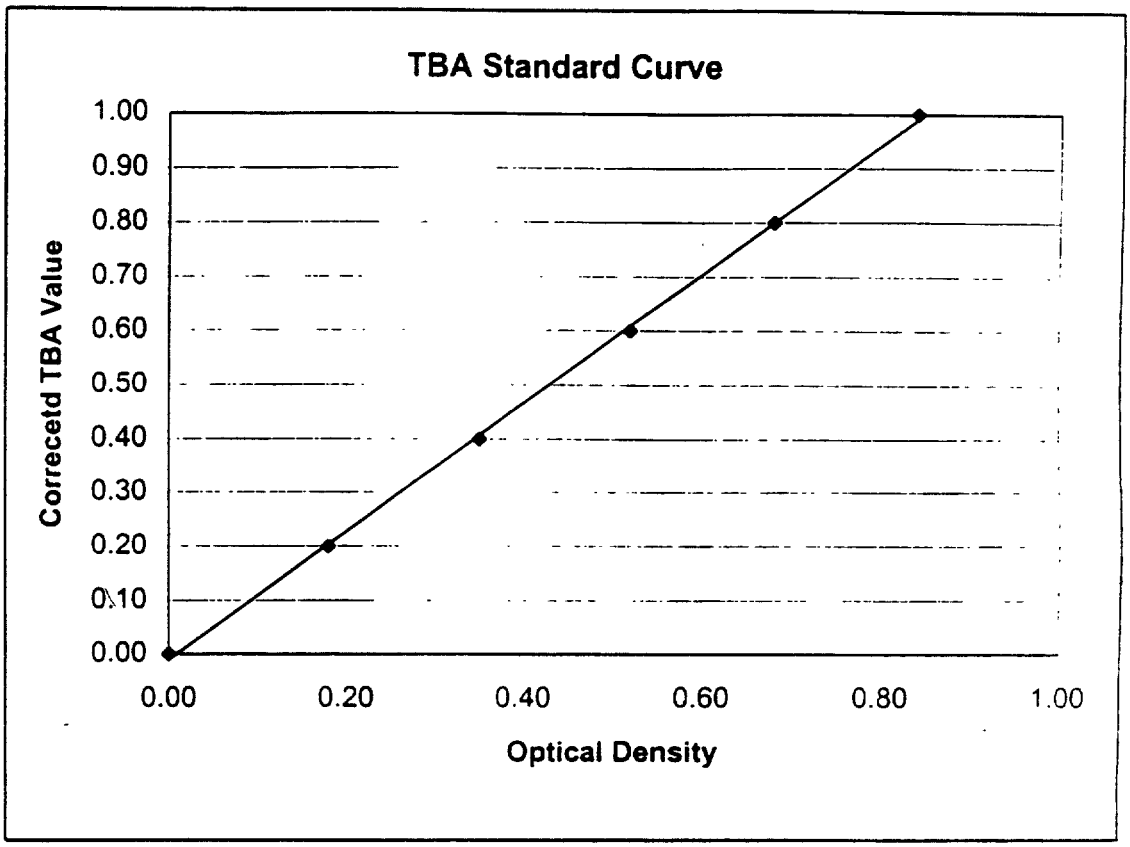
Calculated TBA Value: 0.14

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



Sample # : KEY-RC-MED-590 RAW CUBE PIECES – Day 5

Observed Optical Density: 0.34

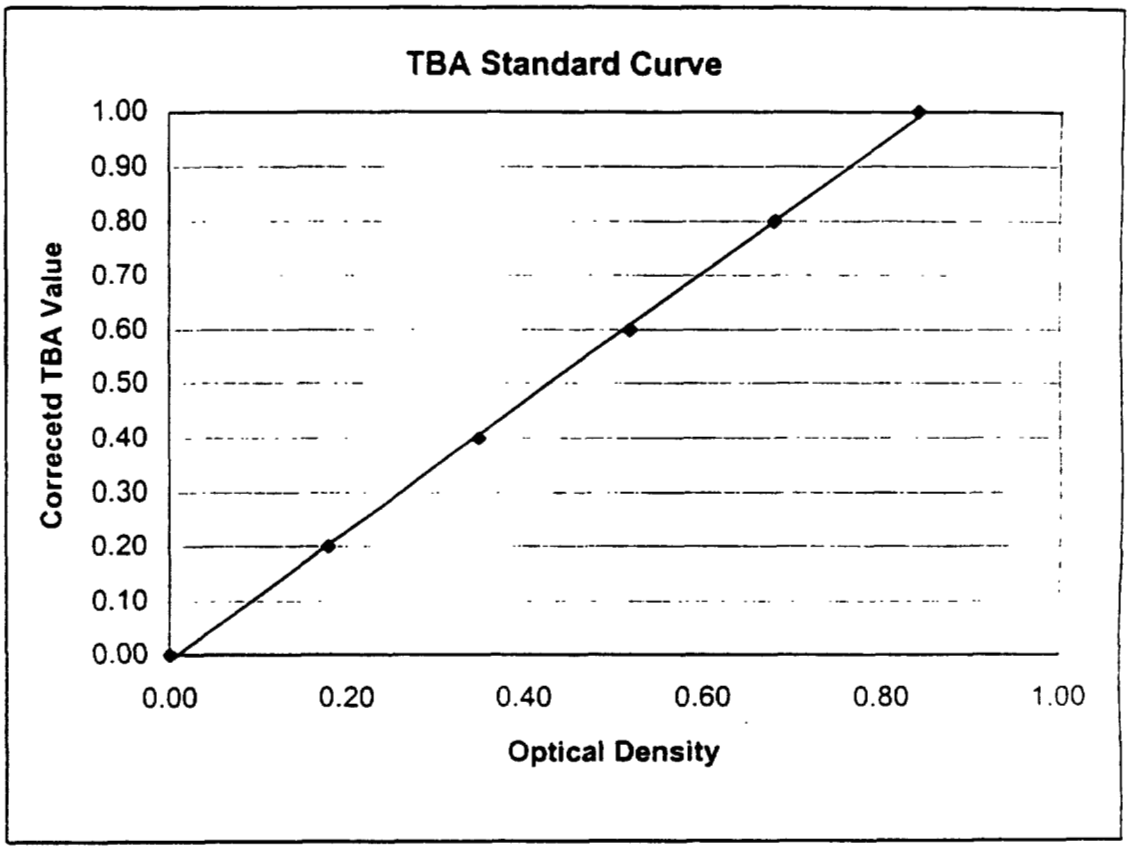
Calculated TBA Value: 0.39

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



Sample # : KEY-RC-MED-598 RAW CUBE PIECES - DAY 7

Observed Optical Density: 0.16

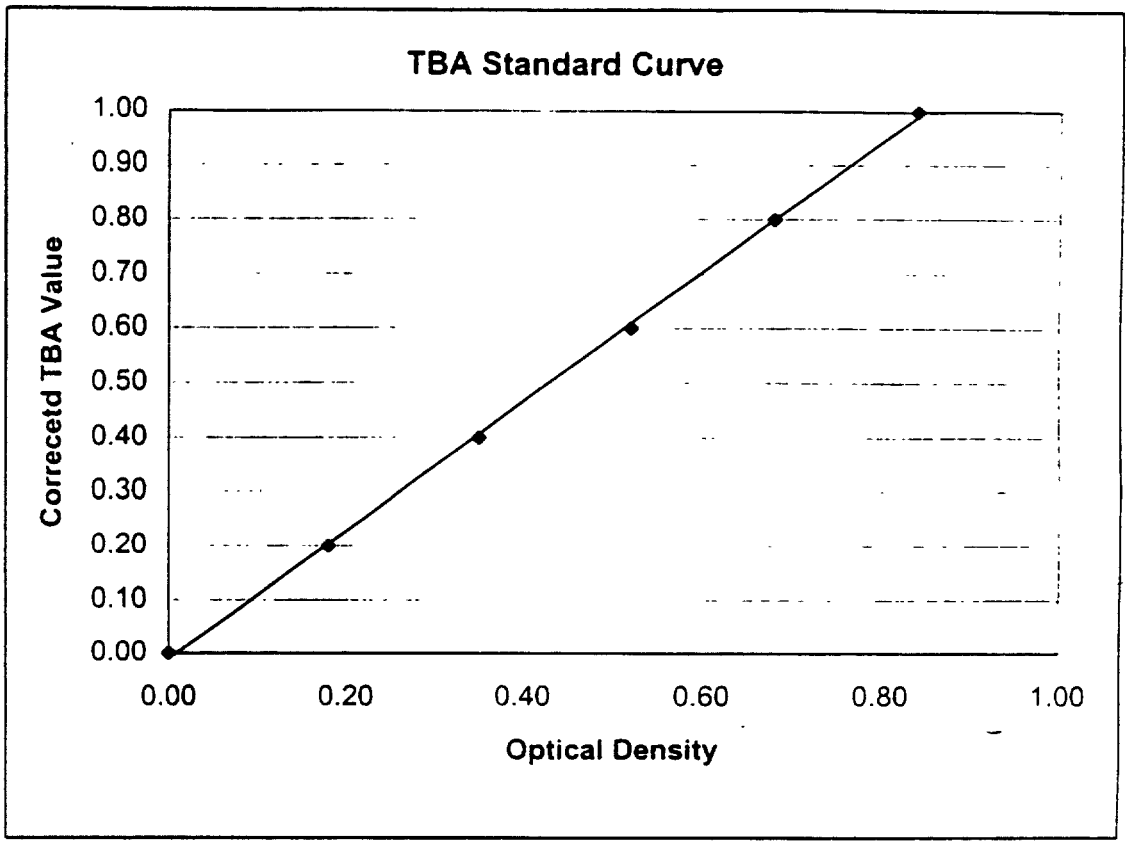
Calculated TBA Value: 0.18

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1 192



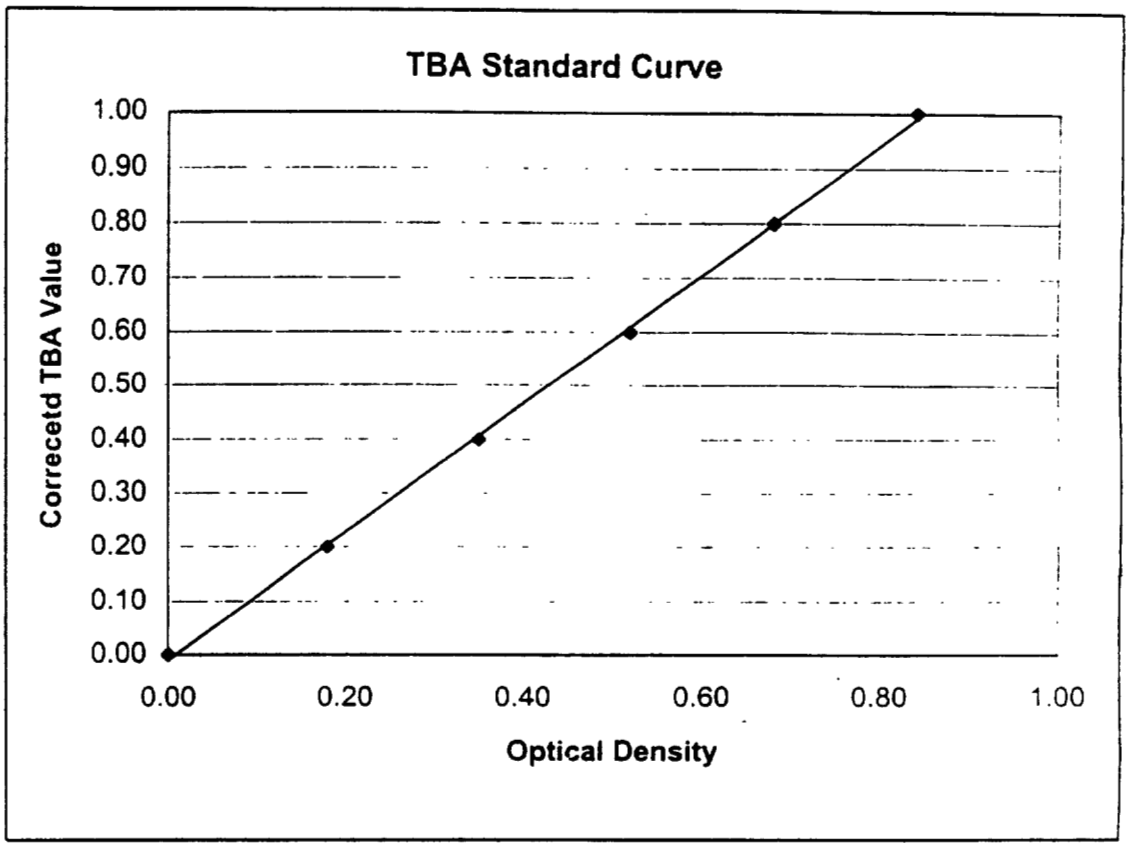
Sample # :	KEY-RC-MED-587 RAW CUBE PIECES
Observed Optical Density:	0.11
Calculated TBA Value:	0.12

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



Sample # : KEY-RC-HI-576 - Day1 raw pieces

Observed Optical Density: 0.10

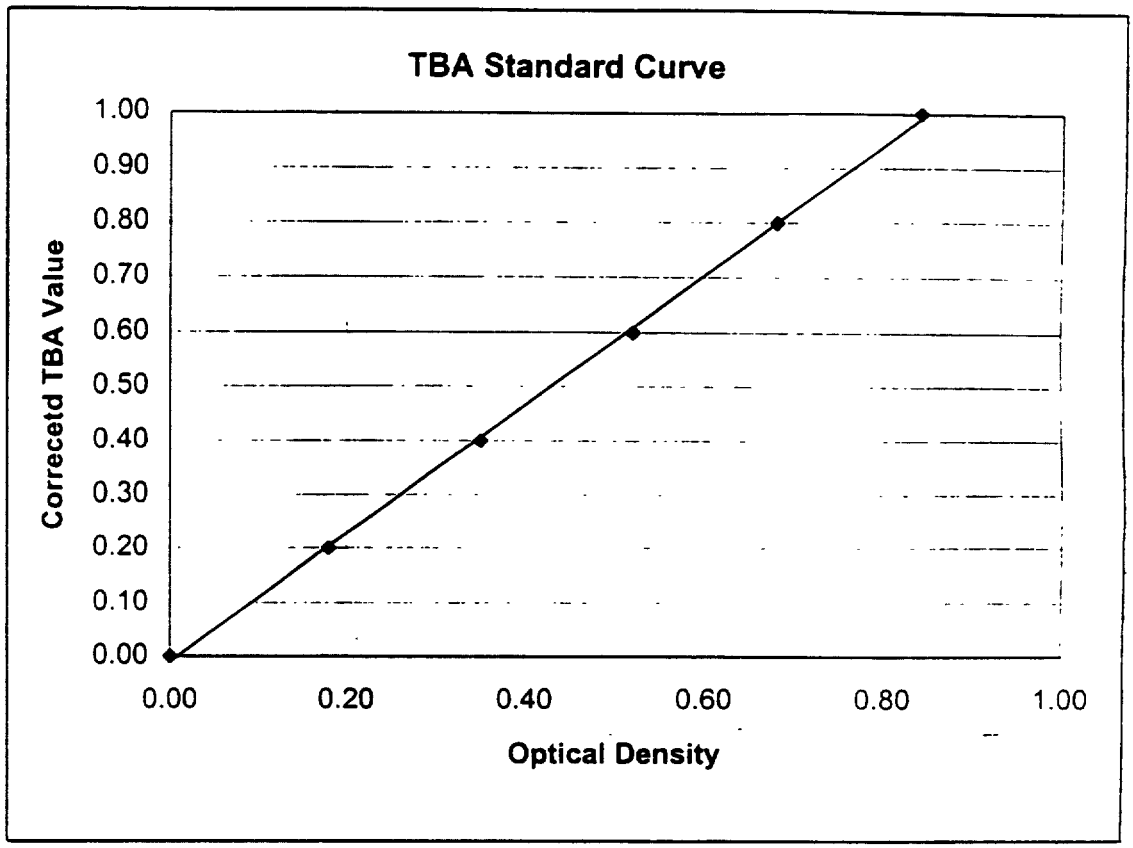
Calculated TBA Value: 0.11

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



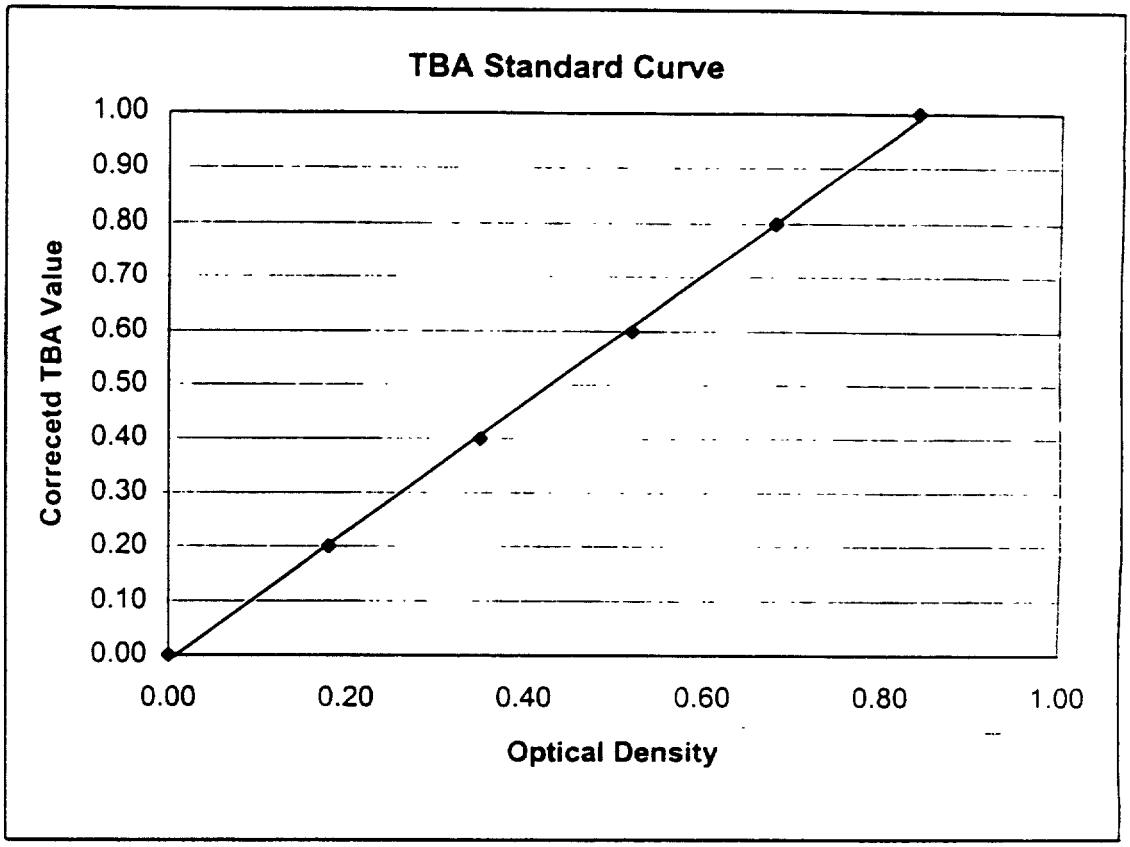
Sample # :	KEY-RC-H I-528 CUBE PIECES - Day 2
Observed Optical Density:	0.15
Calculated TBA Value:	0.17

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



Sample # : KEY-RC-HI-524 RAW CUBE PIECES - Day 5

Observed Optical Density: 0.17

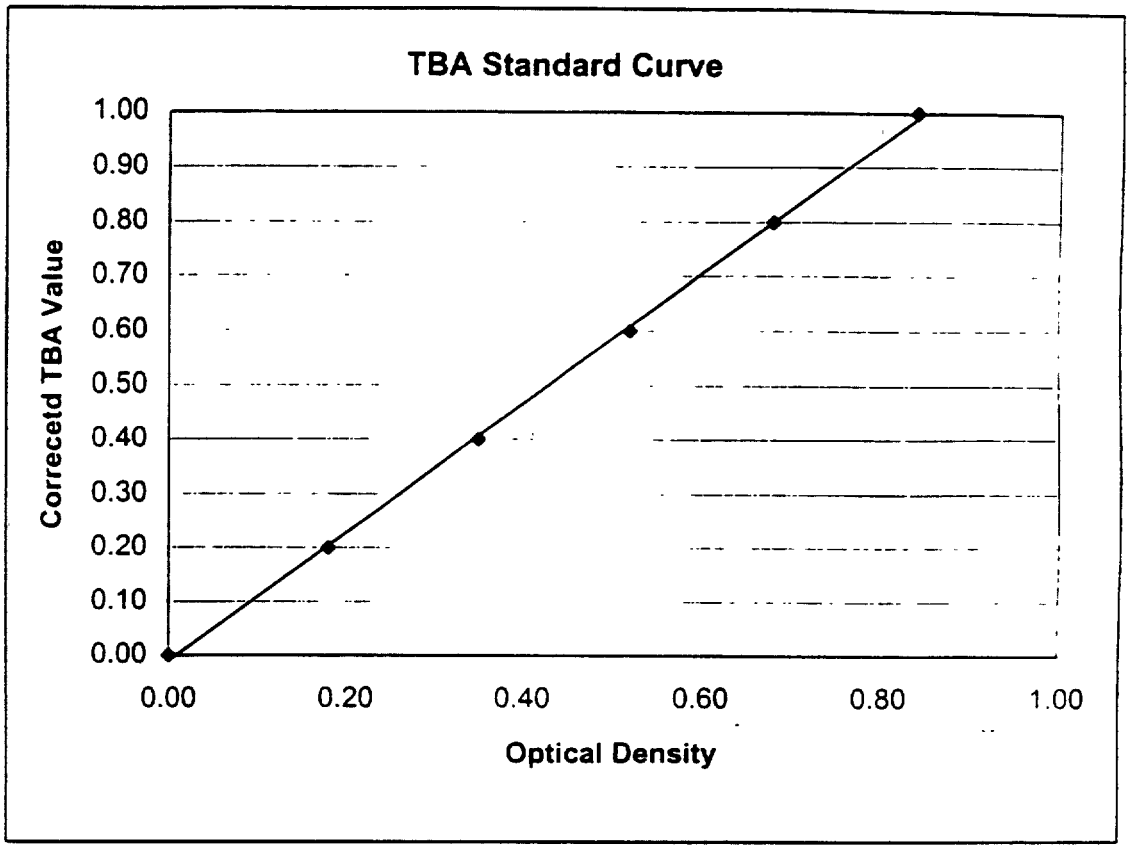
Calculated TBA Value: 0.19

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



Sample # : KEY-RC-HI-525 RAW CUBE PIECES - DAY 7

Observed Optical Density: 0.18

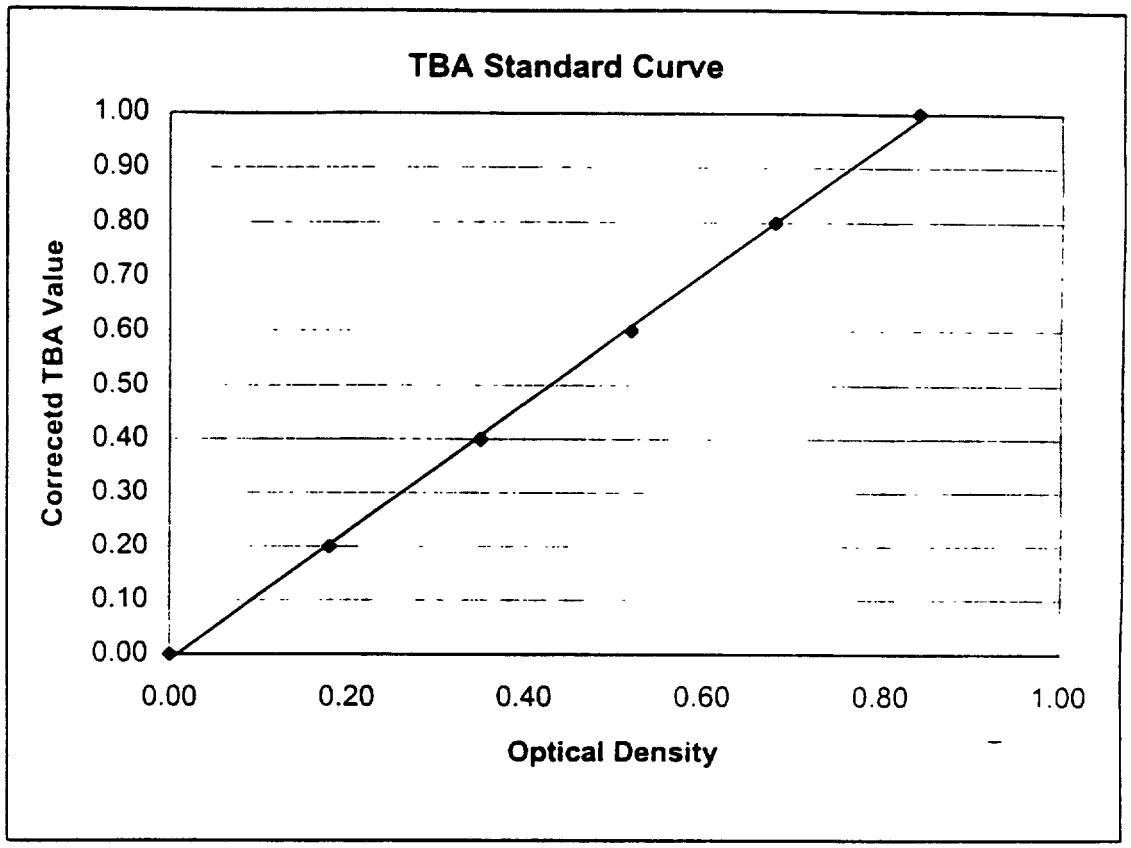
Calculated TBA Value: 0.20

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



Sample # : KEY-RC-HI-532 RAW CUBE PIECES- Day 4

Observed Optical Density: 0.11

Calculated TBA Value: 0.12

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COVANCE
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00203867

BATCH NUMBER: 00203853

DATE ENTERED: 02/18/00

REPORT PRINTED: 02/25/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-RC-Z-193, DAY 1
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

Covance Laboratories Inc.
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Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

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THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00203868

BATCH NUMBER: 00203853

DATE ENTERED: 02/18/00

REPORT PRINTED: 02/25/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-RC-Z-197, DAY 1
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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REPORT OF ANALYSIS

SAMPLE NUMBER: 00203855

BATCH NUMBER: 00203853

DATE ENTERED: 02/18/00

REPORT PRINTED: 02/25/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-RC-LO-511, DAY 1
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00203856

BATCH NUMBER: 00203853

DATE ENTERED: 02/18/00

REPORT PRINTED: 02/25/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-RC-LO-519, DAY 1
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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REPORT OF ANALYSIS

SAMPLE NUMBER: 00203860

BATCH NUMBER: 00203853

DATE ENTERED: 02/18/00

REPORT PRINTED: 02/25/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-RC-MED-596, DAY 1
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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REPORT OF ANALYSIS

SAMPLE NUMBER: 00203859

BATCH NUMBER: 00203853

DATE ENTERED: 02/18/00

REPORT PRINTED: 02/25/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-RC-MED-591, DAY 1
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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REPORT OF ANALYSIS

SAMPLE NUMBER: 00203863

BATCH NUMBER: 00203853

DATE ENTERED: 02/18/00

REPORT PRINTED: 02/25/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-RC-HI-521, DAY 1
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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Tel: 608/241-4471 Fax: 608/241-7227

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REPORT OF ANALYSIS

SAMPLE NUMBER: 00203864

BATCH NUMBER: 00203853

DATE ENTERED: 02/18/00

REPORT PRINTED: 02/25/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-RC-HI-525, DAY 1
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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REPORT OF ANALYSIS

SAMPLE NUMBER: 00204163

BATCH NUMBER: 00204149

DATE ENTERED: 02/18/00

REPORT PRINTED: 03/03/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
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CHICAGO, IL 60611

RAW CHICKEN: KEY-RC-Z-195, DAY 2
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

Covance Laboratories Inc.
P.O. Box 7545
Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

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REPORT OF ANALYSIS

SAMPLE NUMBER: 00204164

BATCH NUMBER: 00204149

DATE ENTERED: 02/18/00

REPORT PRINTED: 03/03/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-RC-Z-199, DAY 2
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
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REPORT OF ANALYSIS

SAMPLE NUMBER: 00204151

BATCH NUMBER: 00204149

DATE ENTERED: 02/18/00

REPORT PRINTED: 03/03/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
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CHICAGO, IL 60611

RAW CHICKEN: KEY-RC-LO-512, DAY 2
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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COVANCE.
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REPORT OF ANALYSIS

SAMPLE NUMBER: 00204152

BATCH NUMBER: 00204149

DATE ENTERED: 02/18/00

REPORT PRINTED: 03/03/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-RC-LO-516, DAY 2
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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P.O. Box 7545
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Tel: 608/241-4471 Fax: 608/241-7227

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00204155

BATCH NUMBER: 00204149

DATE ENTERED: 02/18/00

REPORT PRINTED: 03/03/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-RC-MED-585, DAY 2
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

Covance Laboratories Inc.
P.O. Box 7545
Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00204156

BATCH NUMBER: 00204149

DATE ENTERED: 02/18/00

REPORT PRINTED: 03/03/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-RC-MED-NO CODE, DAY 2
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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Madison, WI 53707-7545
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COVANCE
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00204159

BATCH NUMBER: 00204149

DATE ENTERED: 02/18/00

REPORT PRINTED: 03/03/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-RC-HI-573, DAY 2
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

Covance Laboratories Inc.
P.O. Box 7545
Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

COVANCE
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00204160

BATCH NUMBER: 00204149

DATE ENTERED: 02/18/00

REPORT PRINTED: 03/03/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-RC-HI-580, DAY 2
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

Covance Laboratories Inc.
P.O. Box 7545
Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00204179

BATCH NUMBER: 00204165

DATE ENTERED: 02/18/00

REPORT PRINTED: 03/03/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-RC-Z-188, DAY 5
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

Covance Laboratories Inc.
P.O. Box 7545
Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00204180

BATCH NUMBER: 00204165

DATE ENTERED: 02/18/00

REPORT PRINTED: 03/03/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-RC-Z-189, DAY 5
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00204167

BATCH NUMBER: 00204165

DATE ENTERED: 02/18/00

REPORT PRINTED: 03/03/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-RC-LO-508, DAY 5
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

Covance Laboratories Inc.
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Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

COVANCE
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00204168

BATCH NUMBER: 00204165

DATE ENTERED: 02/18/00

REPORT PRINTED: 03/03/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-RC-LO-515, DAY 5
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

Covance Laboratories Inc.
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Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

COVANCE
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00204171

BATCH NUMBER: 00204165

DATE ENTERED: 02/18/00

REPORT PRINTED: 03/03/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-RC-MED-581, DAY 5
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

Covance Laboratories Inc.
P.O. Box 7545
Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00204172

BATCH NUMBER: 00204165

DATE ENTERED: 02/18/00

REPORT PRINTED: 03/03/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-RC-MED-NO CODE, DAY 5
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

COVANCE
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00204175

BATCH NUMBER: 00204165

DATE ENTERED: 02/18/00

REPORT PRINTED: 03/03/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-RC-HI-527, DAY 5
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

Covance Laboratories Inc.
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COVANCE
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00204176

BATCH NUMBER: 00204165

DATE ENTERED: 02/18/00

REPORT PRINTED: 03/03/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CHICKEN: KEY-RC-HI-NO CODE, DAY 5
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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Tel: 608/241-4471 Fax: 608/241-7227

REPORT OF ANALYSIS

COVANCE
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00205053

BATCH NUMBER: 00205046

DATE ENTERED: 02/24/00

REPORT PRINTED: 03/09/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CUBED CHICKEN: KEY-RC-Z-191, DAY 7
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

Covance Laboratories Inc.
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COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00205050

BATCH NUMBER: 00205046

DATE ENTERED: 02/24/00

REPORT PRINTED: 03/09/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CUBED CHICKEN: KEY-RC-LO-505, DAY 7
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

Covance Laboratories Inc.
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Madison, WI 53707-7545
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COVANCE
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00205051

BATCH NUMBER: 00205046

DATE ENTERED: 02/24/00

REPORT PRINTED: 03/09/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CUBED CHICKEN: KEY-RC-MED-593, DAY 7
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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Tel: 608/241-4471 Fax: 608/241-7227

COVANCE
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00205052

BATCH NUMBER: 00205046

DATE ENTERED: 02/24/00

REPORT PRINTED: 03/09/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CUBED CHICKEN: KEY-RC-HI-523, DAY 7
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

Covance Laboratories Inc.
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Tel: 608/241-4471 Fax: 608/241-7227

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00205064

BATCH NUMBER: 00205057

DATE ENTERED: 02/24/00

REPORT PRINTED: 03/03/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CUBED CHICKEN: KEY-RC-Z-185, DAY 9
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

Covance Laboratories Inc.
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Tel: 608/241-4471 Fax: 608/241-7227

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00205061

BATCH NUMBER: 00205057

DATE ENTERED: 02/24/00

REPORT PRINTED: 03/03/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CUBED CHICKEN: KEY-RC-LO-520, DAY 9
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

Covance Laboratories Inc.
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Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00205062

BATCH NUMBER: 00205057

DATE ENTERED: 02/24/00

REPORT PRINTED: 03/03/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CUBED CHICKEN: KEY-RC-MED-597, DAY 9
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

Covance Laboratories Inc.
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Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

COVANCE
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00205063

BATCH NUMBER: 00205057

DATE ENTERED: 02/24/00

REPORT PRINTED: 03/03/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

RAW CUBED CHICKEN: KEY-RC-HI-529, DAY 9
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

TR BTI # 00-11(B)

Protocol Key-PCN-1-0200

A QUALITATIVE COMPARATIVE STUDY OF PARTIALLY COOKED POULTRY PRODUCTS STORED IN POLYETHYLENE BAGS CONTAINING EITHER NO ADDITIVES OR BTI PROPRIETARY TECHNOLOGY

1. Protocol
2. Table of Sensory Evaluations
3. Table of TBA Values
4. Graphs of TBA Values
5. Results of TBA Analyses
6. Results of Chlorite Anion Analyses

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PHONE 312.337.7007 · FACSIMILE 312.337.0505

Protocol Key-PCN-1-0200

A QUALITATIVE COMPARATIVE STUDY OF PARTIALLY COOKED POULTRY PRODUCTS STORED IN POLYETHYLENE BAGS CONTAINING EITHER NO ADDITIVES OR BTI PROPRIETARY TECHNOLOGY

Objective: To determine any differences in selected characteristics of partially cooked poultry products packaged in polyethylene (LDPE) bags or LDPE bags containing BTI proprietary technology.

Materials:

Partially-cooked poultry products prepared by Keystone Foods, Folcroft, PA. (filets of boneless and skinless breast meat)

Packaging film/bags

1. DPE opaque bags without active additives
2. LDPE opaque bags containing BTI additive at low %
3. LDPE opaque bags containing BTI additive at medium %
4. LDPE opaque bags containing BTI additive at high %

Packages:

1. 150 grams per package (2 pcs. per bag)
2. Four (4) replicates per sample (2 for Keystone, 2 for Covance)
3. Total Packages: 80 (4 types of film)*(4 replicates/type)*(5 storage periods)*(1 product)
4. Total Packages per Test Lab: 40
5. Packages to be stored in chest freezer at –5° to –10°C. All packages of each type were placed in a larger LDPE bag and placed in the freezer (4 large bags in the freezer).

Analyses as listed in attached chart (Page 2):

1. Food evaluation characteristics to be conducted by Keystone Foods; Project Contact is Jade Lau; Test and Analytical Protocols to be provided to BTI by Keystone Foods.
2. Chemical analyses to be conducted by Covance, Madison, WI; Project Contact is Wayne Ellefson; Protocols to be provided by Covance.

Schedule:

- Day 0 — Prepare partially cooked poultry products
- Day 0 — Package in test bags with labels and store as indicated
- Day 0 — Ship bags containing product to test labs via FedEx

Since samples are frozen, shipments via FedEx <u>must</u> be made to Covance one day prior to the sampling day.

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- Day 1 Test Labs conduct analyses as DAY 1 samples
- Day 9 Test Labs conduct analyses as DAY 10 samples
- Day 30 Test Labs conduct analyses as DAY 30 samples
- Day 60 Test Labs conduct analyses as DAY 60 samples
- Day -- Preliminary Report of Results as soon as each is done
- Day -- Final Reports from Covance and Keystone Foods

Analyses:

Samples from each treatment will be analyzed for, color, odor, TBA and residual chlorite on each of the following days: 1, 9, 30, 60

Color and Odor by Keystone Labs:

If possible, color and odor comments will be obtained for each sample prior to the rinse. Color change may be documented using a digital camera.

TBA Analyses:

TBA analyses will be conducted on duplicate samples by Keystone Labs

Residual Chlorite:

A duplicate samples will be shipped to a COVANCE lab for residual chlorite analysis.

Statistical Analysis:

Appropriate statistical analysis will be performed to detect any significant differences between treatments.

Chlorine Dioxide and Chlorite Anions: Standard Methods for Water Analysis Method 4500-ClO2 B. Iodometric Method. APHA, AWWA, and WPCF. Washington, D. C. (17th ed.) Pages 4-54 (modified as Southwest Research Institute BTI-SOP# 99-02. Aieta, E. M., P. V. Roberts, and M. Hernandez, J. Am. Water Works Assoc., 76(1), 64-70 (1984).

TBA:

Day	
0	Collect baseline data on test and control products
1 - 60	Store samples at 20°F
9	Sample: color, odor, TBA, residual chlorite

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30	Sample: color, odor, TBA, residual chlorite
60	Sample: color, odor, TBA, residual chlorite

RESULTS

Sensory: The CRT did not alter the sensory characteristics of partially cooked and frozen poultry muscle meat, including color and odor. At all storage times, the color, as seen in the photographs of the low, medium, and high CRT packaging was similar to the control.

TBA: The TBA values either as a function of film type or duration of storage were within the normal limits of the untreated muscle meat. The values for the poultry muscle meat stored in the bags containing the CRT technology were either below the control film bags or within the normal limits of sample-to-sample variability.

Chlorite: Residual chlorite anions were not detected. The cooking process would destroy any residual chlorite.

CONCLUSION

Packaging materials containing the Microsphère® CRT at levels of loading up to 50% may be used for the storage of partially cooked frozen poultry without demonstrating any negative qualitative effects for at least 30 days.

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TBA VALUE
PARTIALLY COOKED POULTRY
(Conducted by Keystone Foods)

ZERO

DAY 1	0.11
DAY 10	0.13
DAY 30	0.56
DAY 60	

LOW

DAY 1	0.11
DAY 10	0.12
DAY 30	0.7
DAY 60	

MEDIUM

DAY 1	0.11
DAY 10	0.24
DAY 30	0.48
DAY 60	

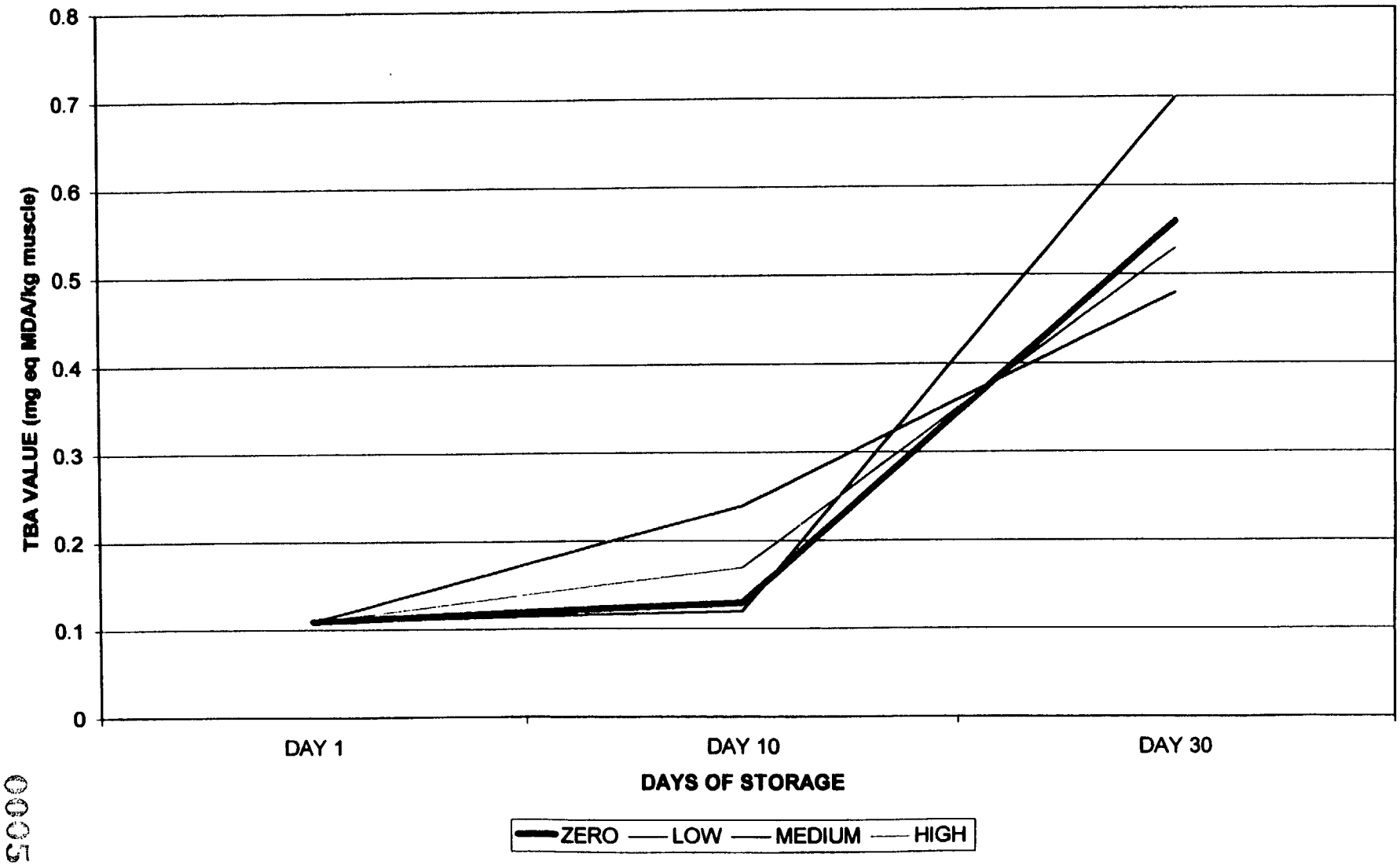
HIGH

DAY 1	0.11
DAY 10	0.17
DAY 30	0.53
DAY 60	

The DAY 1 value was determined only for the ZERO film and used as representative for the other films.

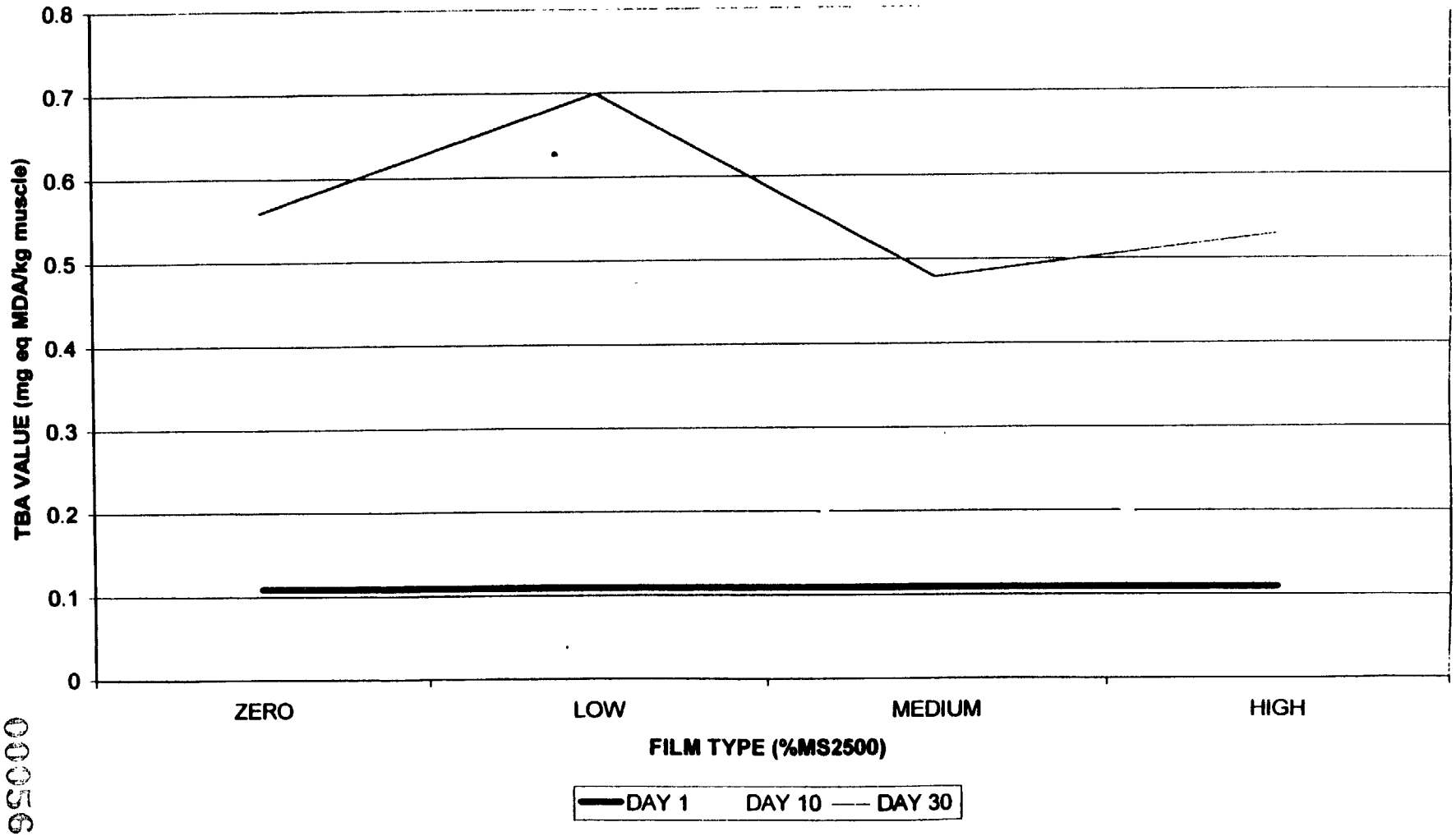
Pieces were packaged in polyethylene film containing zero, low, medium and high % of BTI's proprietary chlorine dioxide generating product. They were stored frozen until sampling.

TBA of PARTIALLY COOKED FROZEN POULTRY



000562

TBA of PARTIALLY COOKED POULTRY
as a
FUNCTION of FILM for ANY GIVEN DAY of STORAGE



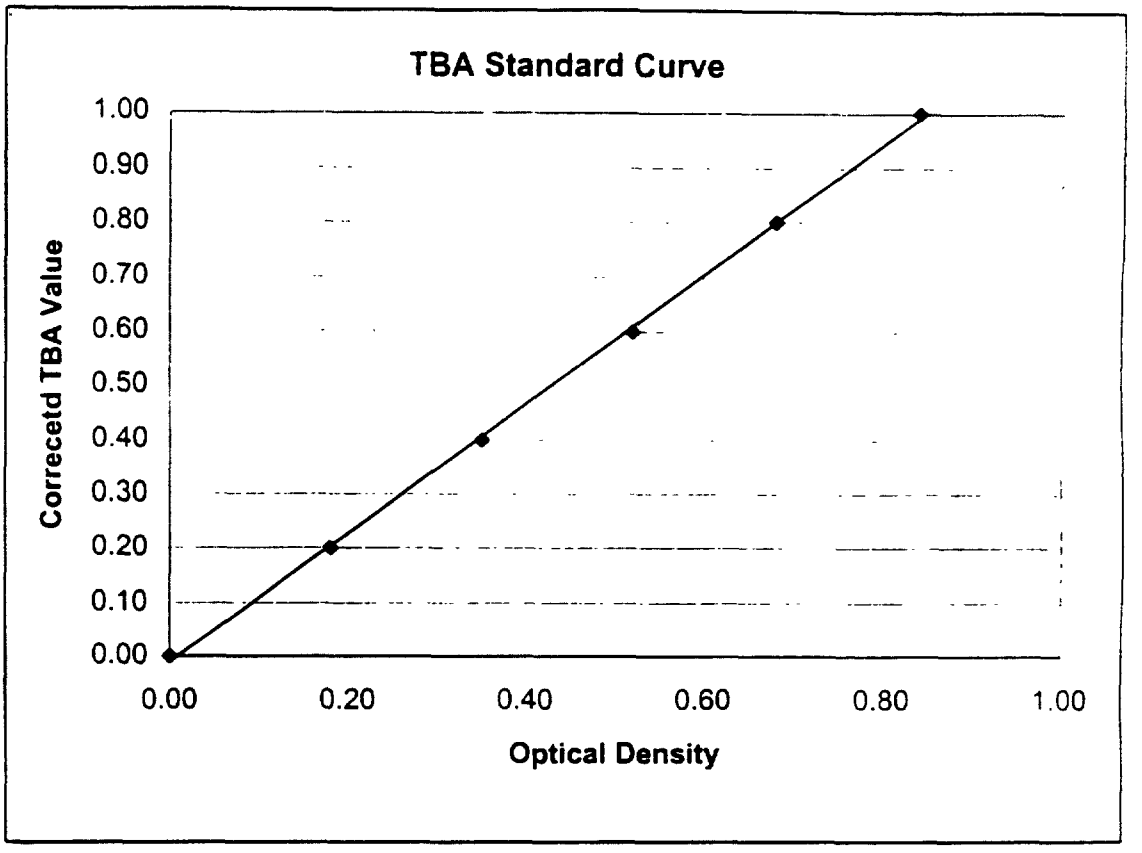
000563

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



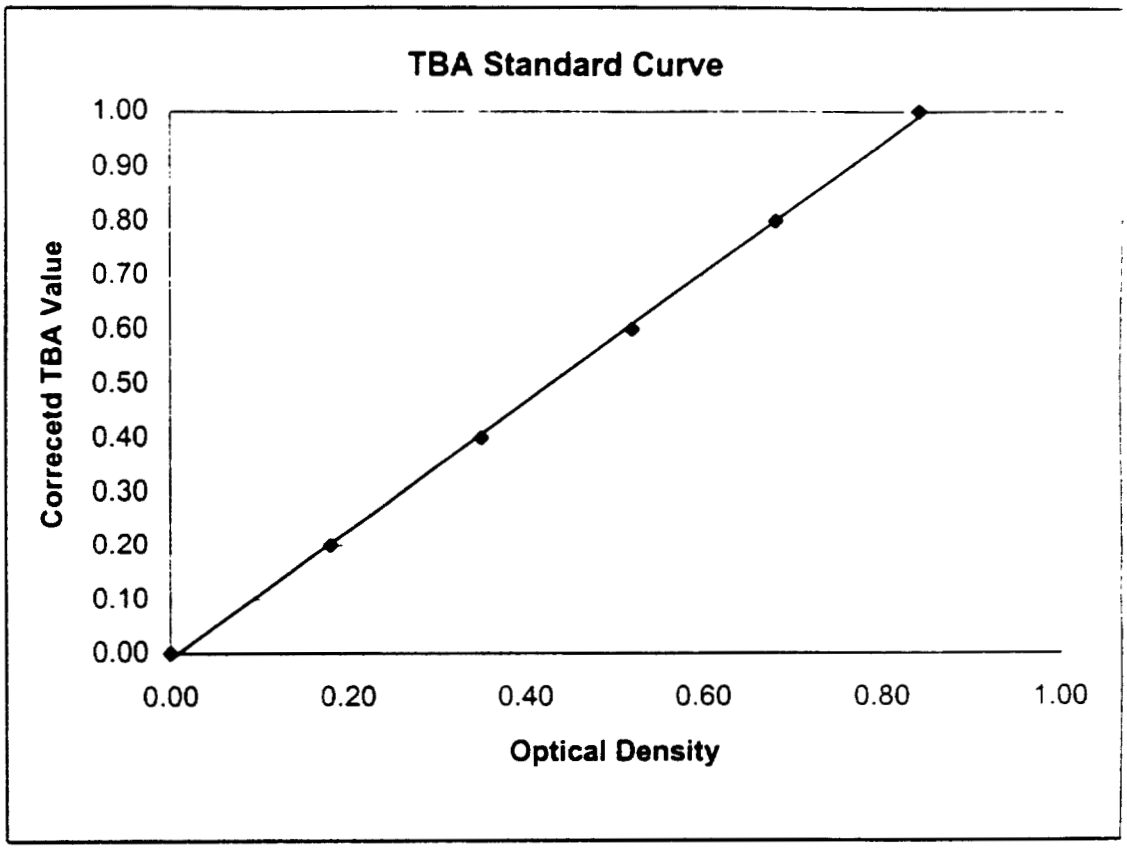
Sample # :	DAY ONE PARTIAL COOKED
Observed Optical Density:	0.10
Calculated TBA Value:	0.11

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



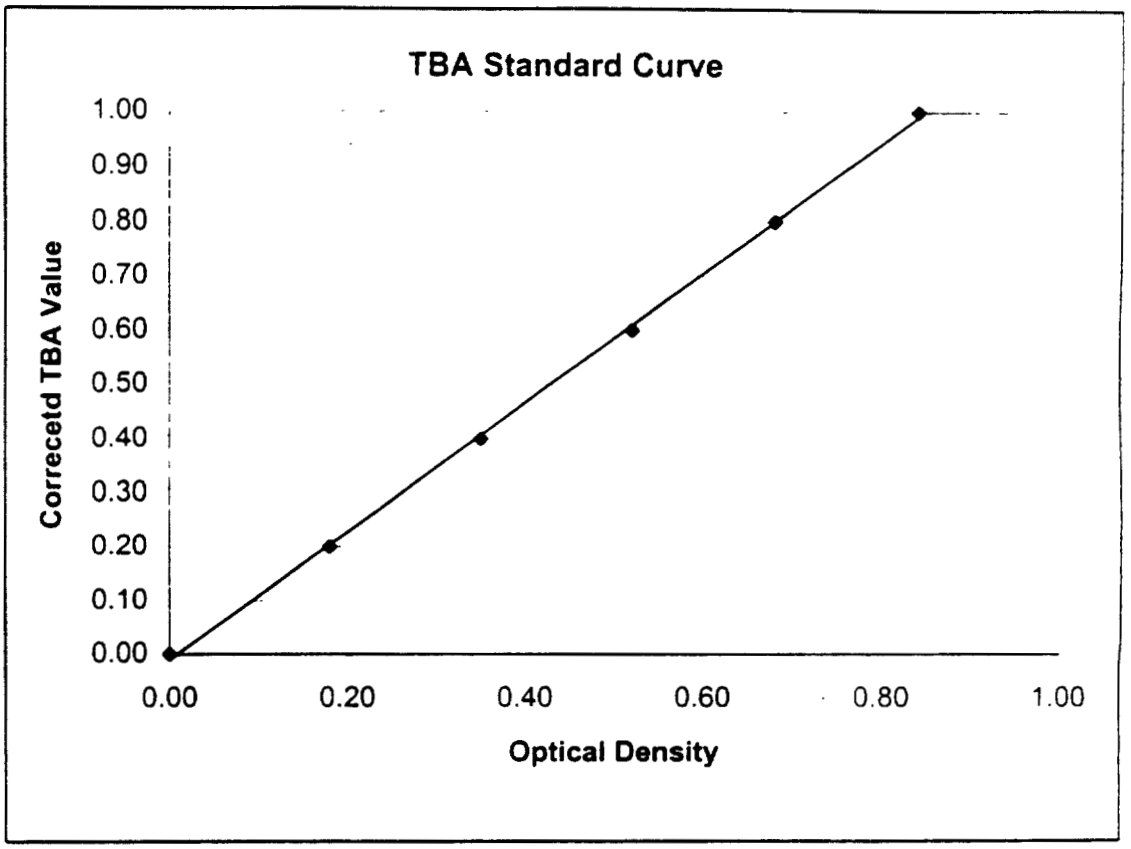
Sample # :	KEY-PCN-Z-792 FROZEN PIECES
Observed Optical Density:	0.12
Calculated TBA Value:	0.13

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0 52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



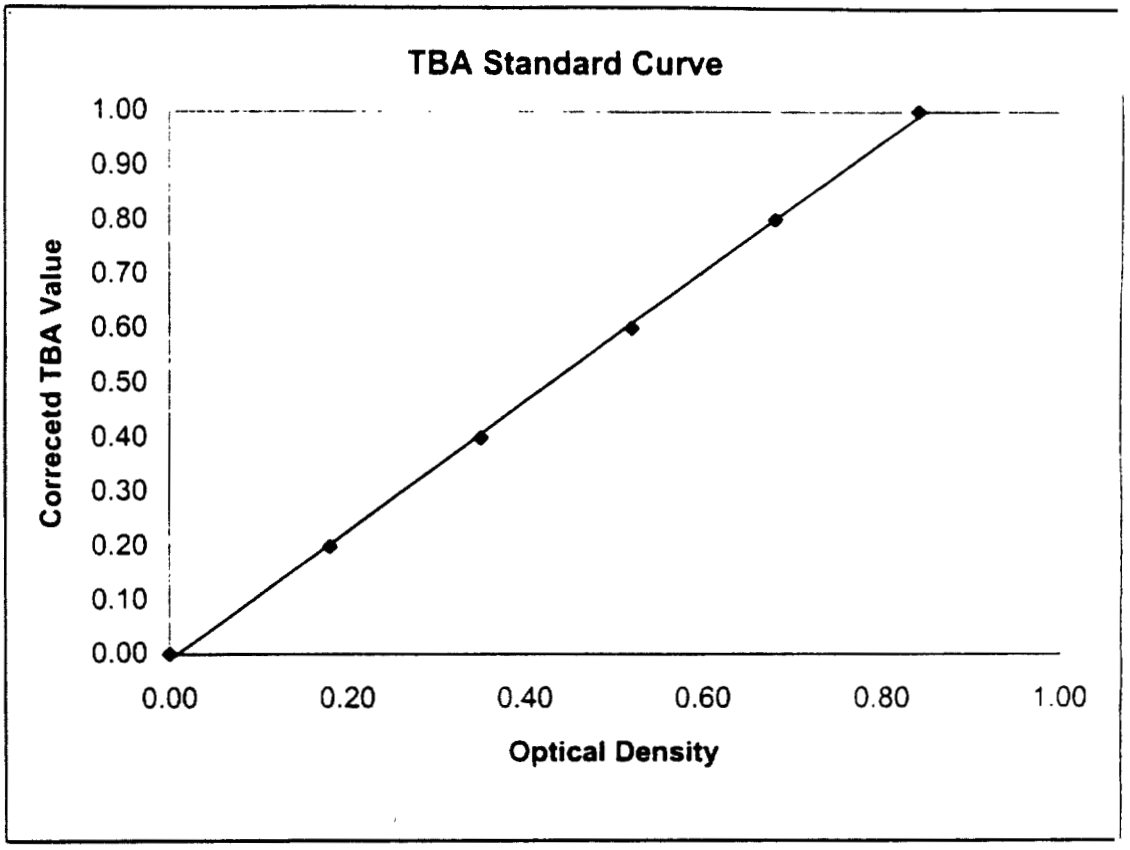
Sample # :	KEY-PCN-LO-785 FROZEN PIECES
Observed Optical Density:	0.11
Calculated TBA Value:	0.12

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



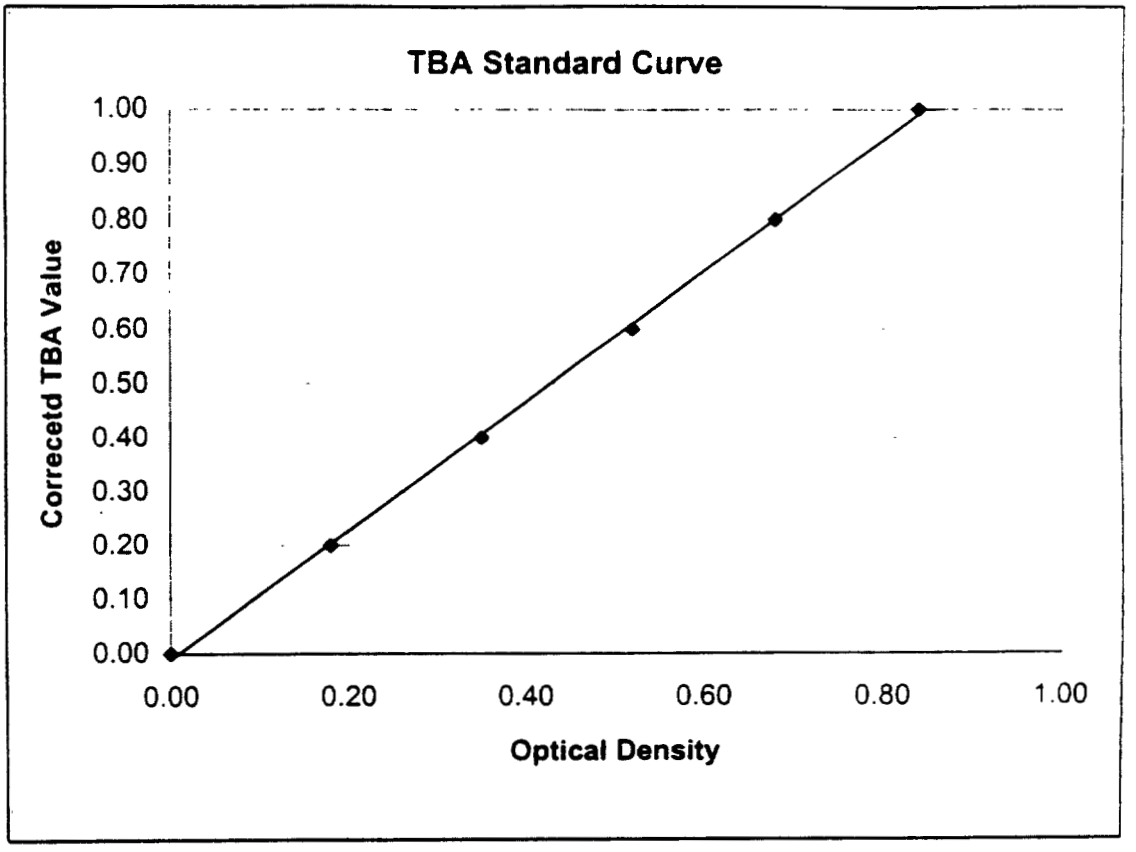
Sample # :	KEY-PCN-MED-743 FROZEN PIECES -
Observed Optical Density:	0.21
Calculated TBA Value:	0.24

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0 40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



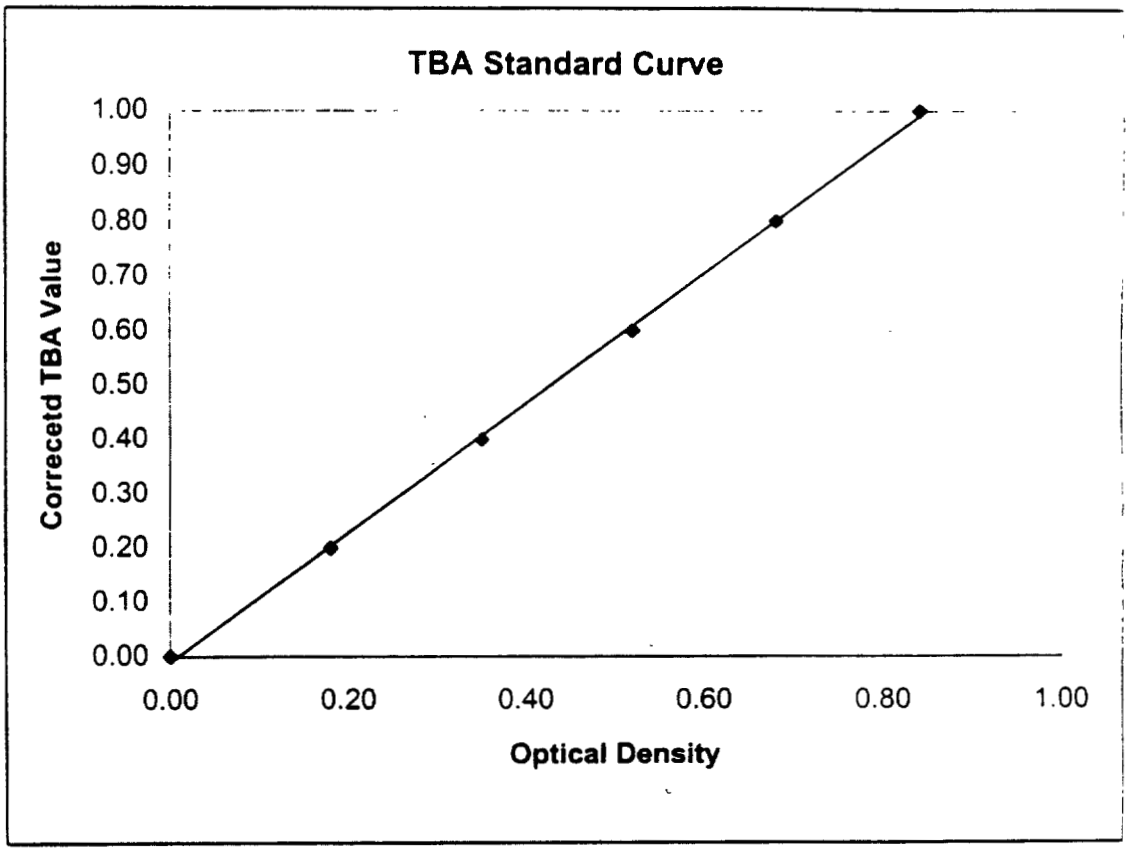
Sample # :	KEY-PCN-HI-724 FROZEN PIECES
Observed Optical Density:	0.15
Calculated TBA Value:	0.17

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0 20	0.18
0 40	0.35
0.60	0.52
0 80	0.68
1 00	0.84

y-Intercept =	-0.011
Slope =	1.192



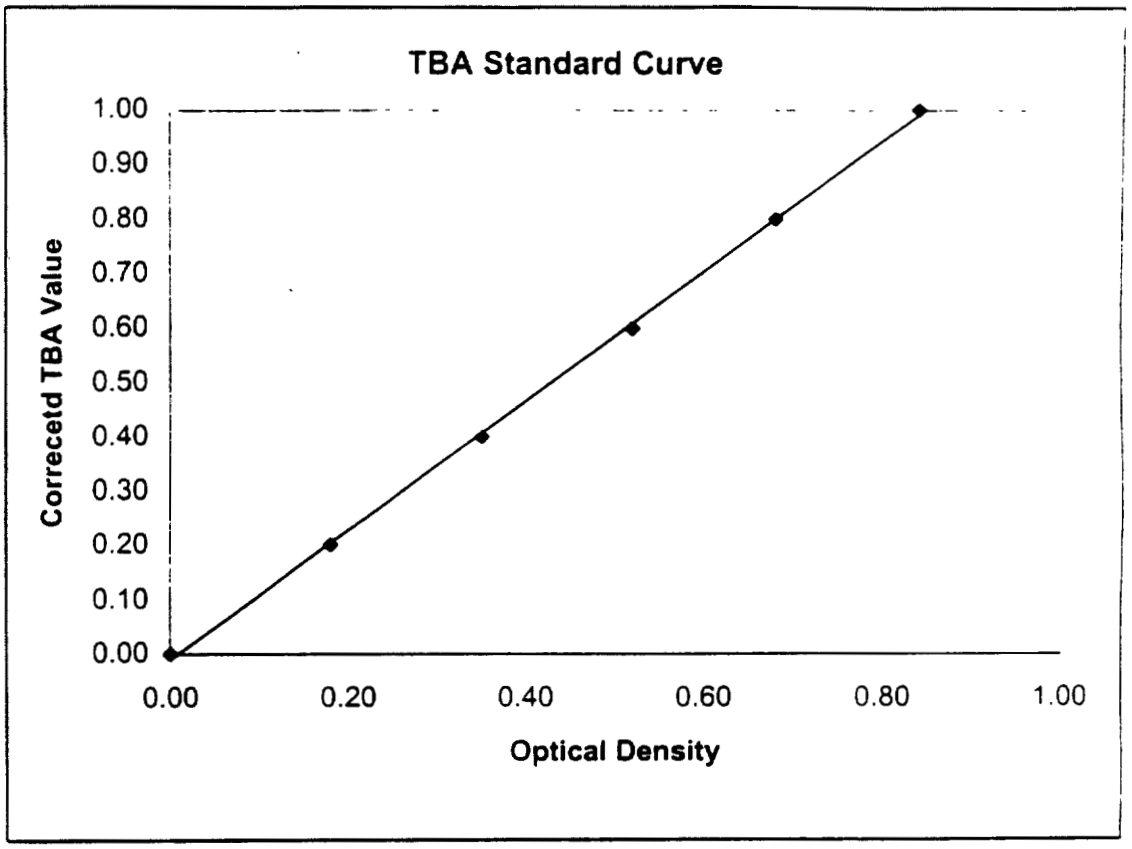
Sample # :	KEY-PCN-Z-781 FZ PIECES - Partially Ckd Bag 50
Observed Optical Density:	0.48
Calculated TBA Value:	0.56

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



Sample # : KEY-PCN-60-780 FZ PIECES - Partially ckd. Day 30

Observed Optical Density: 0.60

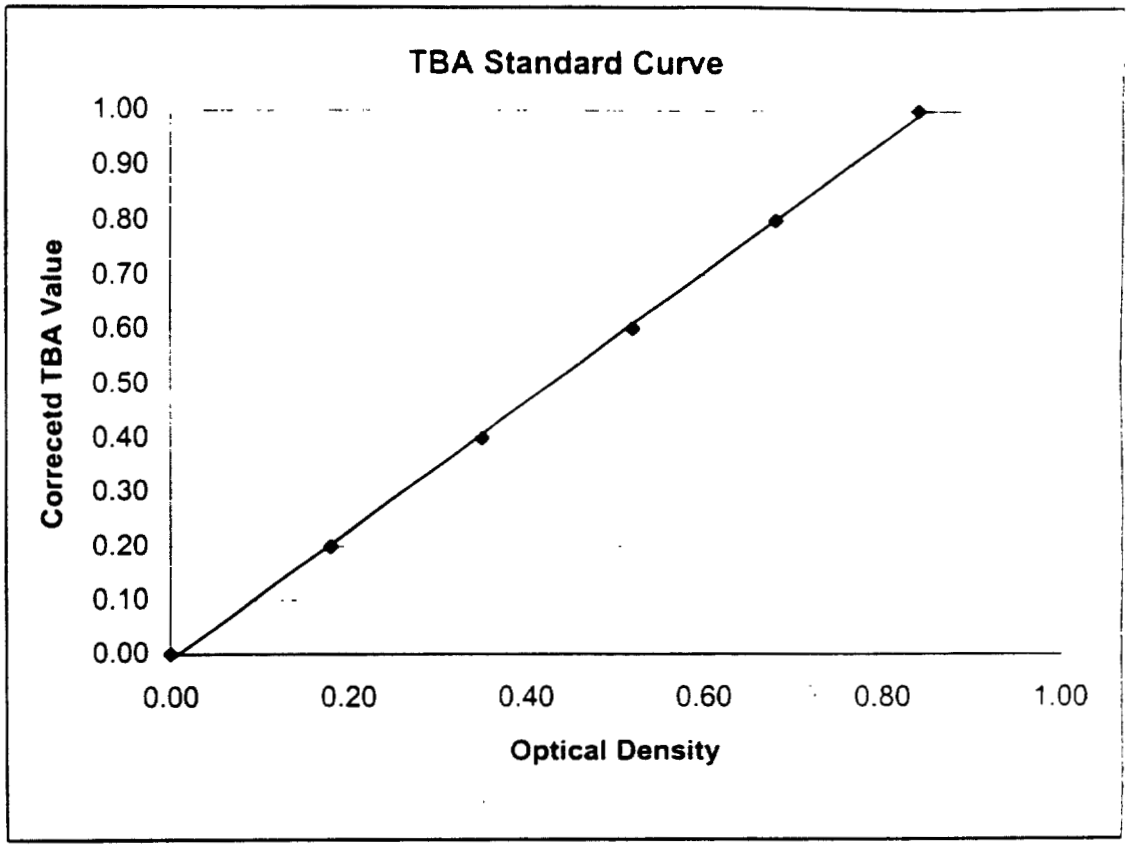
Calculated TBA Value: 0.70

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



Sample # : KEY-PCN-MED-752 FZ PIECES - Partially Ckd - [illegible]

Observed Optical Density: 0.41

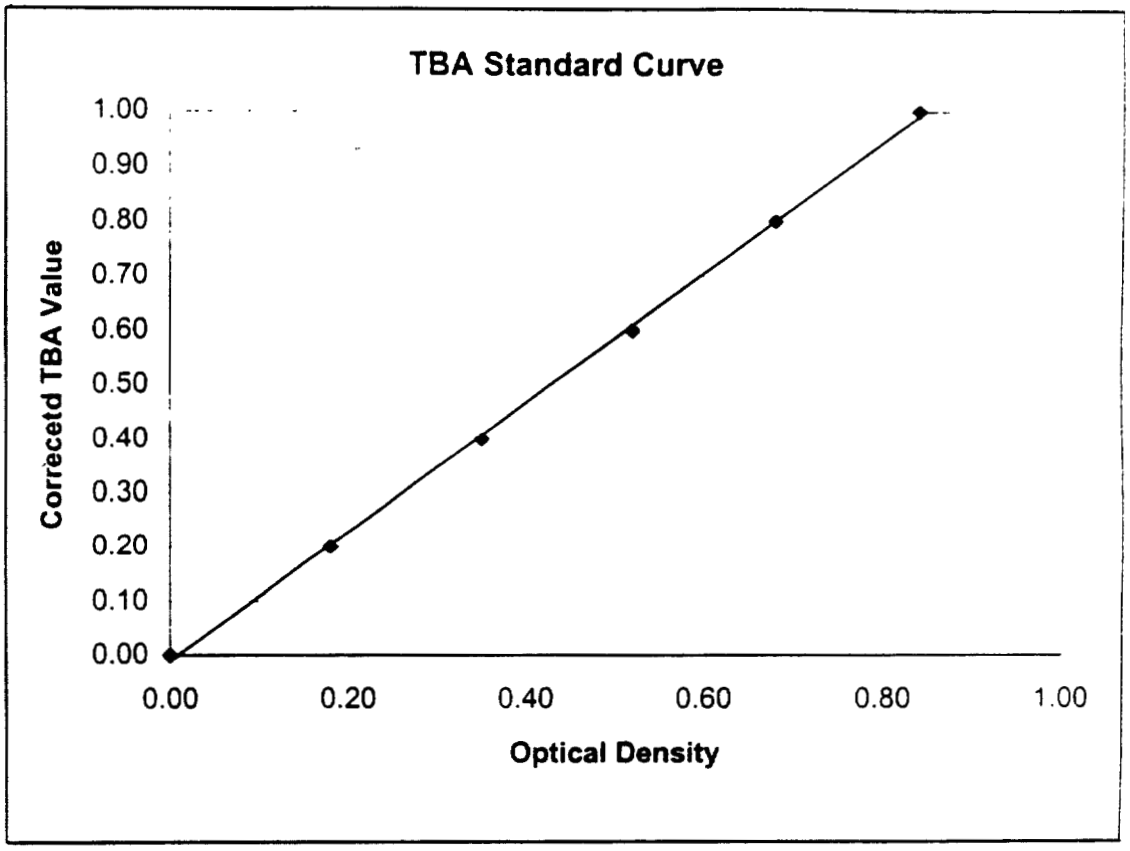
Calculated TBA Value: 0.48

TBA Values in Meat

TBA Standard Curve

Corrected TBA	Optical Density
0.00	0.00
0.20	0.18
0.40	0.35
0.60	0.52
0.80	0.68
1.00	0.84

y-Intercept =	-0.011
Slope =	1.192



Sample # : KEY-PCN-HI-736 FZ PIECES - Partially Ckd - Day 20

Observed Optical Density: 0.45

Calculated TBA Value: 0.53

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COVANCE
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00203839

BATCH NUMBER: 00203833

DATE ENTERED: 02/18/00

REPORT PRINTED: 02/25/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

COOKED CHICKEN: KEY-PCN-Z-800, DAY 1
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

Covance Laboratories Inc.
P.O. Box 7545
Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
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COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00203840

BATCH NUMBER: 00203833

DATE ENTERED: 02/18/00

REPORT PRINTED: 02/25/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

COOKED CHICKEN: KEY-PCN-Z-793, DAY 1
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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Madison, WI 53707-7545
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Tel: 608/241-4471 Fax: 608/241-7227

COVANCE
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00203833

BATCH NUMBER: 00203833

DATE ENTERED: 02/18/00

REPORT PRINTED: 02/25/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

COOKED CHICKEN: KEY-PCN-LO-782, DAY 1
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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Madison, WI 53707-7545
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COVANCE
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00203834

BATCH NUMBER: 00203833

DATE ENTERED: 02/18/00

REPORT PRINTED: 02/25/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

COOKED CHICKEN: KEY-PCN-LO-NO CODE, DAY 1
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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Madison, WI 53707-7545
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Tel: 608/241-4471 Fax: 608/241-7227

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00203835

BATCH NUMBER: 00203833

DATE ENTERED: 02/18/00

REPORT PRINTED: 02/25/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

COOKED CHICKEN: KEY-PCN-MED-749, DAY 1
(COMPOSITE OF 2)REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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Madison, WI 53707-7545
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Tel: 608/241-4471 Fax: 608/241-7227

COVANCE
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00203836

BATCH NUMBER: 00203833

DATE ENTERED: 02/18/00

REPORT PRINTED: 02/25/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

COOKED CHICKEN: KEY-PCN-MED-NO CODE, DAY 1
(COMPOSITE OF 2)REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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Madison, WI 53707-7545
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COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00203837

BATCH NUMBER: 00203833

DATE ENTERED: 02/18/00

REPORT PRINTED: 02/25/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

COOKED CHICKEN: KEY-PCN-HI-721, DAY 1
(COMPOSITE OF 2)REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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Madison, WI 53707-7545
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COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00203838

BATCH NUMBER: 00203833

DATE ENTERED: 02/18/00

REPORT PRINTED: 02/25/00

PETER N. GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

COOKED CHICKEN: KEY-PCN-HI-729, DAY 1
(COMPOSITE OF 2)REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00205068

BATCH NUMBER: 00205065

DATE ENTERED: 02/24/00

REPORT PRINTED: 03/09/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

PARTIALLY COOKED CHICKEN: KEY-PCN-Z-788, DAY 10
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

Covance Laboratories Inc.
P.O. Box 7545
Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00205065

BATCH NUMBER: 00205065

DATE ENTERED: 02/24/00

REPORT PRINTED: 03/09/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

PARTIALLY COOKED CHICKEN: KEY-PCN-LO-786, DAY 10
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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COVANCE
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00205066

BATCH NUMBER: 00205065

DATE ENTERED: 02/24/00

REPORT PRINTED: 03/09/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

PARTIALLY COOKED CHICKEN: KEY-PCN-MED-742, DAY 10
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

Covance Laboratories Inc.
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REPORT OF ANALYSIS

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00205067

BATCH NUMBER: 00205065

DATE ENTERED: 02/24/00

REPORT PRINTED: 03/09/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

PARTIALLY COOKED CHICKEN: KEY-PCN-HI-728, DAY 10
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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REPORT OF ANALYSIS

COVANCE
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00303917

BATCH NUMBER: 00303917

DATE ENTERED: 03/17/00

REPORT PRINTED: 04/12/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

PARTIALLY COOKED CHICKEN: KEY-PCN-Z-784
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

REPORT OF ANALYSIS

COVANCE
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00303918

BATCH NUMBER: 00303917

DATE ENTERED: 03/17/00

REPORT PRINTED: 04/12/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

PARTIALLY COOKED CHICKEN: KEY-PCN-Z-785
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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REPORT OF ANALYSIS

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00303919

BATCH NUMBER: 00303917

DATE ENTERED: 03/17/00

REPORT PRINTED: 04/12/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

PARTIALLY COOKED CHICKEN: KEY-PCN-LO-705
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

000587

[redacted]

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REPORT OF ANALYSIS

COVANCE
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00303920

BATCH NUMBER: 00303917

DATE ENTERED: 03/17/00

REPORT PRINTED: 04/12/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

PARTIALLY COOKED CHICKEN: KEY-PCN-LO-709
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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REPORT OF ANALYSIS

COVANCE
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00303921

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

BATCH NUMBER: 00303917

DATE ENTERED: 03/17/00

REPORT PRINTED: 04/12/00

PARTIALLY COOKED CHICKEN: KEY-PCN-MED-745
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA (COMPOSITE OF 2)

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

Covance Laboratories Inc.
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Tel: 608/241-4471 Fax: 608/241-7227

REPORT OF ANALYSIS

COVANCE
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00303922

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

BATCH NUMBER: 00303917

DATE ENTERED: 03/17/00

REPORT PRINTED: 04/12/00

PARTIALLY COOKED CHICKEN: KEY-PCN-MED-755
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA (COMPOSITE OF 2)

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

Covance Laboratories Inc.
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Tel: 608/241-4471 Fax: 608/241-7227

REPORT OF ANALYSIS

COVANCE
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00303923

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

BATCH NUMBER: 00303917
DATE ENTERED: 03/17/00
REPORT PRINTED: 04/12/00

PARTIALLY COOKED CHICKEN: KEY-PCN-HI-727
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA (COMPOSITE OF 2)

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

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Tel: 608/241-4471 Fax: 608/241-7227

REPORT OF ANALYSIS

COVANCE
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00303924

BATCH NUMBER: 00303917

DATE ENTERED: 03/17/00

REPORT PRINTED: 04/12/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

PARTIALLY COOKED CHICKEN: KEY-PCN-HI-734
REC'D FROM KEYSTONE FOODS R & D, FOLCROFT, PA (COMPOSITE OF 2)

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

Method listed above with results.

TR BTI # 00-11(C)

Protocol EB-2-0200

A QUALITATIVE COMPARATIVE STUDY OF HAMBURGER BUNS STORED IN POLYETHYLENE BAGS CONTAINING EITHER NO ADDITIVES OR BTI PROPRIETARY TECHNOLOGY

1. Protocol
2. Tables of Sensory Evaluations
3. Table of Thiamin Hydrochloride Values
4. Graphs of Thiamin Hydrochloride Values
5. Table of Riboflavin Values
6. Graph of Riboflavin Values
7. Results of Chlorite Anion Analyses

919 NORTH MICHIGAN AVENUE, SUITE 1710 · CHICAGO, ILLINOIS · 60611
PHONE 312.337.7007 · FACSIMILE 312.337.0505

Protocol EB-2-0200

A QUALITATIVE COMPARATIVE STUDY OF HAMBURGER BUNS STORED IN POLYETHYLENE BAGS CONTAINING EITHER NO ADDITIVES OR BTI PROPRIETARY TECHNOLOGY

Objective: To determine any differences in selected characteristics of hamburger buns packaged in polyethylene (LDPE) bags or LDPE bags containing BTI proprietary technology.

Materials:

Hamburger buns prepared by East Balt, Inc., Chicago Illinois

1. Hamburger buns containing calcium propionate as preservative
2. Hamburger bums without calcium propionate

Packaging film/bags

1. LDPE bags without active additives (Base)
2. LDPE opaque bags containing BTI additive at low % (Low)
3. LDPE opaque bags containing BTI additive at medium % (Medium)
4. LDPE opaque bags containing BTI additive at high % (High)

Packages:

1. Nine (9) buns per package
2. Four (4) replicates per sample type (2 for Merlin, 2 for Covance)
3. Total Packages: 128 (4 types of film)*(4 replicates/type)*(4 periods)*(2 bun types)
4. Total Packages per Test Lab: 64

   Covance and Merlin will maintain buns in packages under normal storage conditions at room temperature, withdrawing samples as needed.

Analyses as listed in attached chart:

1. Food evaluation characteristics to be conducted by Merlin Development, Plymouth, MN; Project Contact is Martha Porter; Protocols to be provided by Merlin Development
2. Chemical analyses to be conducted by Covance, Madison, WI; Project Contact is Wayne Ellefson; Protocols to be provided by Covance

Schedule:

- Day 1 — Bake both types of buns
- Day 1 — Package in test bags with labels and store as indicated
- Day 1 — Ship bags containing buns to test labs

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- Day 3 — Test Labs conduct analyses as DAY 3 samples
- Day 6 — Test Labs conduct analyses as DAY 6 samples
- Day 9 — Test Labs conduct analyses as DAY 9 samples
- Day 13 — Test Labs conduct analyses as DAY 13 samples
- Day 16 — Observe for visible mold – no other tests
- Day -- — Preliminary Report of Results
- Day -- — Final Reports from Covance and Merlin Development

Analyses to be performed by Merlin Development

- Visual assessment for mold (all buns in remaining bagged samples).
- Odor in bag (single evaluator, sensory description vs. control for two bags to be opened at each sample interval).
- Odor of raw bun (6 evaluators, open ended description and off-odor scale).
- Texture of raw bun (moistness, softness, freshness, resiliency, gumminess, off-flavor scales).
- Hardness of bun measured by TXTA2 texture analyzer, per AACC method.
- % Moisture, whole bun to confirm that any changes seen are not attributable to moisture differences.
- Dipix color of raw bun (average values for 6 samples, outer crown and heel crusts and crumb on crown and heel) to determine if raw bun appearance is affected.
- Dipix color of Antunes rapid toasted bun (average value for 6 samples, crown and heel crumb color, post toast height dimensions) to determine if bun toasting characteristics are affected.

Analyses to be performed by Covance

- Residual chlorite in the buns
- Thiamin HCl
- Riboflavin

Chlorine Dioxide and Chlorite Anions: Standard Methods for Water Analysis Method 4500-ClO2 B. Iodometric Method. APHA, AWWA, and WPCF. Washington, D. C. (17th ed.) Pages 4-54 (modified as Southwest Research Institute BTI-SOP# 99-02. Aieta, E. M., P. V. Roberts, and M. Hernandez, J. Am. Water Works Assoc., 76(1), 64-70 (1984).

Riboflavin: AOAC International, 940.33 Riboflavin (Vitamin B2) in Vitamin Preparations (modified), Official Methods of Analysis, (ed.) Patricia Cuniff, Sixteenth Wd., Vol. 2, AOAC International: Gaithersburg, Maryland (1995).

Thiamin Hydrochloride: Official Methods of Analysis, 16th Edition, Method 942.23, Thiamine (Vitamin B1) in Foods, 953.17, Thiamine (Vitamin B1) in Grain Products, and

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95.7.17, Thiamine (Vitamin B1) in Bread, modified, Chapter 45, AOAC, Gaithersburg, Maryland (1995).

RESULTS

Sensory: Sensory characteristics such as off-odors, moistness, freshness, off flavor and overall acceptance were not affected when compared to control packaging.

Nutrients: The CRT did not alter the amounts of thiamin and riboflavin in fresh buns stored for 13 days.

Chlorite: Chlorite residues were below the limits of detection.

CONCLUSION

Packaging materials containing the Microsphère® CRT at levels of loading up to 50% may be used for the storage of fresh baked goods, such as bread-type products, without demonstrating any negative qualitative effects for at least 13 days. Results were similar for the buns containing or not containing propionate as a preservative.

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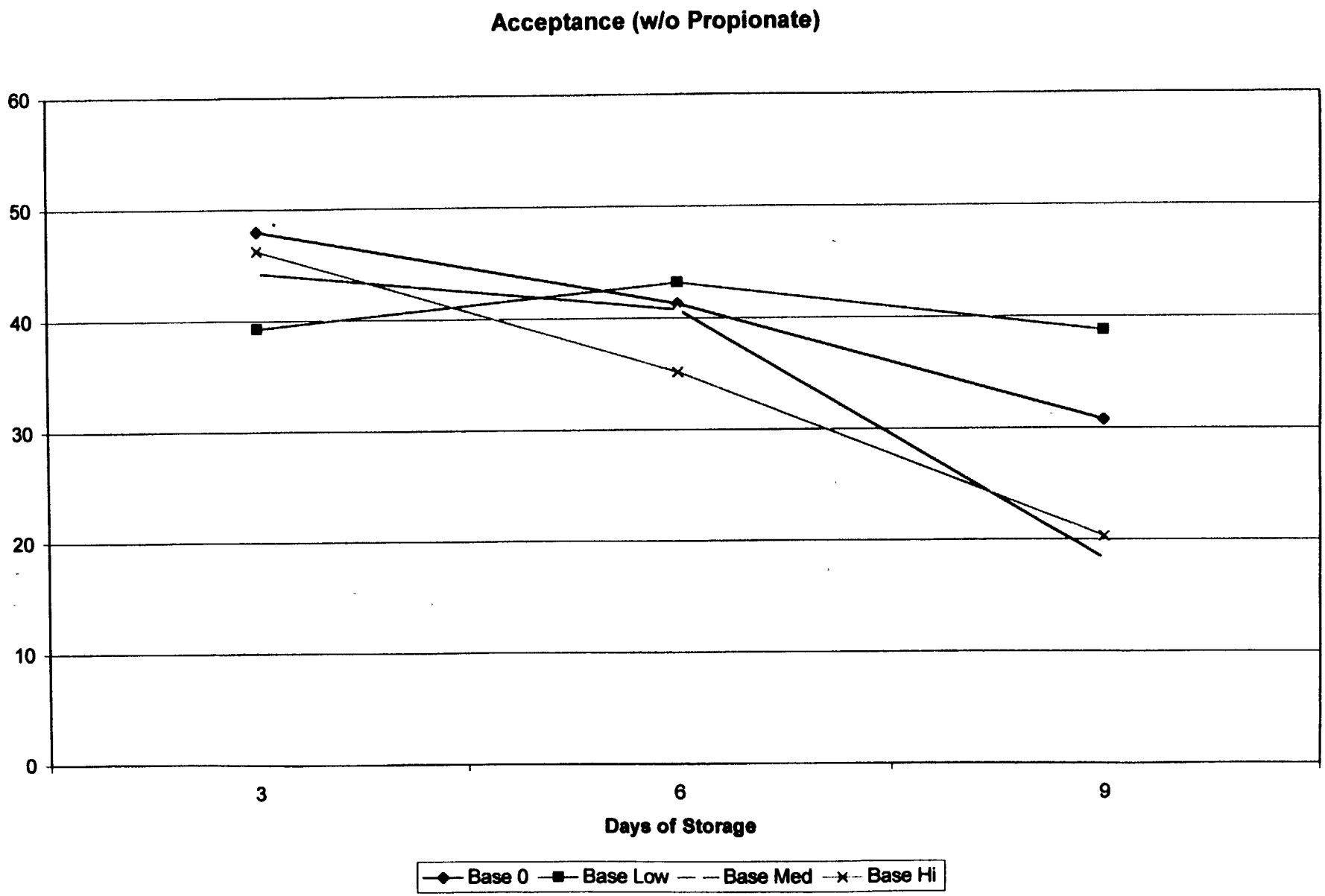
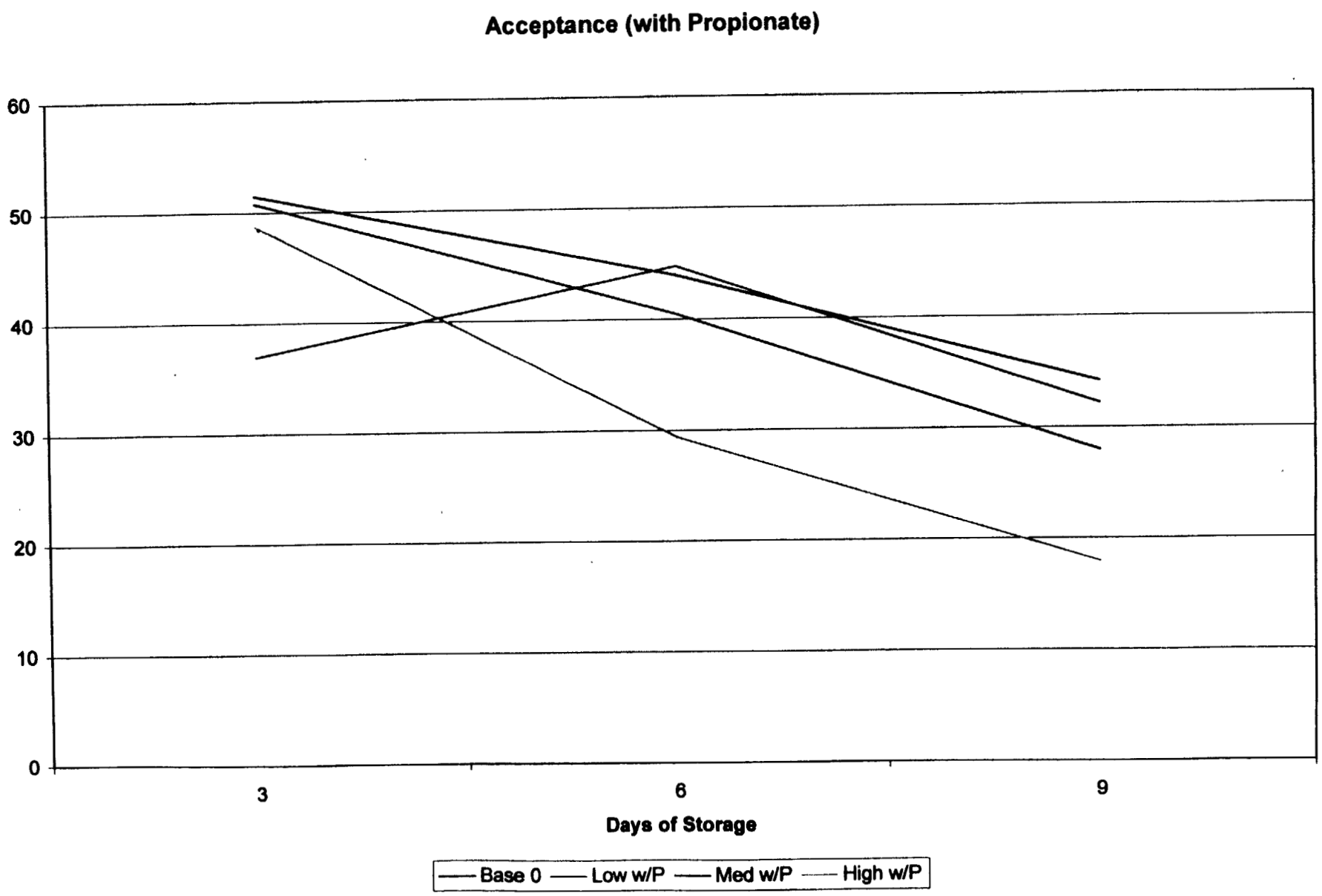
SENSORY CHARACTERISITCS OF BUNS

Off odors								
		0=without propionate			**1=with propionate**			
	Base 0	Low 0	Med 0	High 0	Base 1	Low 1	Med 1	High 1
Day 3	10.8	20.3	7.5	7.8	5	15.3	5	5
Day 6	9.2	7.2	17.2	16.2	9.4	8.6	12.4	5.6
Day 9	14.6	16.4	26.2	21	14.8	16.2	18.5	12.2
Moistness								
	Base 0	Low 0	Med 0	High 0	Base 1	Low 1	Med 1	High 1
Day 3	37.3	38.8	37.8	39.3	40	28.8	32.5	36.3
Day 6	29.6	27	31.6	28.4	27.2	27.6	26.2	22.6
Day 9	27.4	31.2	23	23.8	24	27	23.8	15.4
Freshness								
	Base 0	Low 0	Med 0	High 0	Base 1	Low 1	Med 1	High 1
Day 3	39.5	40.8	39.8	41.5	41	29.3	36.3	35.5
Day 6	33.6	31.8	34.2	31.6	29.8	29	29	23.2
Day 9	26.8	32	19.4	19.6	30.8	26.4	26.8	18
Off Flavor								
	Base 0	Low 0	Med 0	High 0	Base 1	Low 1	Med 1	High 1
Day 3	4	16	17.3	7.5	4.3	16.8	3.8	5.5
Day 6	10.8	9.8	13	23.2	4.6	8.6	12.8	21
Day 9	17.8	19.2	25	21.6	14.6	10.8	15.8	22.8
Overall Acceptance								
	Base 0	Low 0	Med 0	High 0	Base 1	Low 1	Med 1	High 1
Day 3	48	39.3	44.3	46.3	51.5	37	50.8	48.8
Day 6	41.3	43.2	40.8	35.2	44	44.8	40.5	29.4
Day 9	30.8	38.8	18.4	20.4	34.2	32.2	28	18


Acceptance (w/o Propionate)
60
50
40
30
20
10
0
3
6
9
Days of Storage
Base 0
Base Low
Base Med
Base Hi

000598


Acceptance (with Propionate)
60
50
40
30
20
10
0
3
6
9
Days of Storage
Base 0
Low w/P
Med w/P
High w/P

000599

Anova: Two-Factor Without Replication

SUMMARY	Count	Sum	Average	Variance
Day 3	8	366	45.75	27.64286
Day 6	8	319.2	39.9	26.74
Day 9	8	220.8	27.6	61.31429
Base 0	3	120.1	40.03333	75.16333
Low 0	3	121.3	40.43333	5.803333
Med 0	3	103.5	34.5	197.47
High 0	3	101.9	33.96667	168.8433
Base 1	3	129.7	43.23333	75.26333
Low 1	3	114	38	40.44
Med 1	3	119.3	39.76667	130.3633
High 1	3	96.2	32.06667	242.4933

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	1373.16	2	686.58	19.28131	9.50948E-05	3.738890086
Columns	311.36	7	44.48	1.249137	0.341304939	2.764195983
Error	498.52	14	35.60857			
Total	2183.04	23				

Day of age is significant

Significant differences seen between day 9 and days 3 and 6.
Acceptability decreased with time.

Anova: Two-Factor Without Replication

SUMMARY	Count	Sum	Average	Variance
Day 3	8	76.7	9.5875	31.36411
Day 6	8	85.8	10.725	17.42786
Day 9	8	139.9	17.4875	19.37554
Base 0	3	34.6	11.53333	7.693333
Low 0	3	43.9	14.63333	45.24333
Med 0	3	50.9	16.96667	87.46333
High 0	3	45	15	44.64
Base 1	3	29.2	9.733333	24.09333
Low 1	3	40.1	13.36667	17.24333
Med 1	3	35.9	11.96667	45.70333
High 1	3	22.8	7.6	15.96

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	291.8275	2	145.9138	7.186542	0.007121155	3.738890086
Columns	192.92	7	27.56	1.357385	0.296019224	2.764195983
Error	284.2525	14	20.30375			
Total	769	23				

Day of Age is significant

Off odor increases with bun age. Most significant changes occur between day 9 and other days.

Anova: Two-Factor Without Replication

SUMMARY	Count	Sum	Average	Variance
Day 3	8	290.8	36.35	14.69429
Day 6	8	220.2	27.525	6.867857
Day 9	8	195.6	24.45	20.83143
Base 0	3	94.3	31.43333	27.02333
Low 0	3	97	32.33333	35.77333
Med 0	3	92.4	30.8	55.24
High 0	3	91.5	30.5	63.37
Base 1	3	91.2	30.4	71.68
Low 1	3	83.4	27.8	0.84
Med 1	3	82.5	27.5	20.19
High 1	3	74.3	24.76667	112.7233

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	610.5233	2	305.2617	26.19362	1.85483E-05	3.738890086
Columns	133.5983	7	19.08548	1.637669	0.204538999	2.764195983
Error	163.1567	14	11.65405			
Total	907.2783	23				

Day of age is significant.

Difference between day 3 and other days is significant. Day 6 and 9 are not significantly different. Moistness decreases with age.

Anova: Two-Factor Without Replication

SUMMARY	Count	Sum	Average	Variance
Day 3	8	303.7	37.9625	17.05696
Day 6	8	242.2	30.275	12.01071
Day 9	8	199.8	24.975	28.65643
Base 0	3	99.9	33.3	40.39
Low 0	3	104.6	34.86667	26.41333
Med 0	3	93.4	31.13333	111.0933
High 0	3	92.7	30.9	120.27
Base 1	3	101.6	33.86667	38.41333
Low 1	3	84.7	28.23333	2.543333
Med 1	3	92.1	30.7	24.73
High 1	3	76.7	25.56667	80.76333

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	682.3008	2	341.1504	23.0805	3.69565E-05	3.738890086
Columns	197.1363	7	28.16232	1.905319	0.144279879	2.764195983
Error	206.9325	14	14.78089			
Total	1086.37	23				

Day of age is significant

All days are significantly different from each other. Buns reach unacceptablity between day 6 and day 9.

Anova: Two-Factor Without Replication

SUMMARY	Count	Sum	Average	Variance
Day 3	8	75.2	9.4	38.01143
Day 6	8	103.8	12.975	39.01071
Day 9	8	147.6	18.45	21.87143
Base 0	3	32.6	10.86667	47.61333
Low 0	3	45	15	22.84
Med 0	3	55.3	18.43333	36.96333
High 0	3	52.3	17.43333	74.64333
Base 1	3	23.5	7.833333	34.36333
Low 1	3	36.2	12.06667	18.01333
Med 1	3	32.4	10.8	39
High 1	3	49.3	16.43333	90.46333

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	332.4233	2	166.2117	5.885434	0.013963455	3.738890086
Columns	296.8783	7	42.41119	1.501749	0.244659635	2.764195983
Error	395.3767	14	28.24119			
Total	1024.678	23				

Day of age is signficant

Day 3 is significantly different from day 9.
Off-flavor increases with age.
There was a general trend in the data that showed base variables had a lower rate of off-flavor development. The HIGH variables had the highest off-flavor scores but the differences were not statistically significant.

ANALYTICAL CHARACTERISTICS OF BUNS

Analytical Data Analysis								
		0=without propionate			1=with propionate			
Weight								
	Base 0	Low 0	Med 0	High 0	Base 1	Low 1	Med 1	High 1
Day 3	53.35	52.98	53.05	52.91	52.47	52.59	52.9	52.63
Day 6	52.95	53.26	52.9	52.8	52.62	52.43	52.62	51.98
Day 9	52.6	52.88	52.53	52.35	52.32	52.4	51.9	51.59
Day 13	52.57	52.62	51.66	52.18	52.27	52.29	51.99	51.73
Crown Height								
	Base 0	Low 0	Med 0	High 0	Base 1	Low 1	Med 1	High 1
Day 3	29.71	31.35	27.66	28.71	28.07	26.82	28.93	29
Day 6	30.82	31.62	29.02	30.19	27.4	29.35	27.98	29.44
Day 9	30.1	31.07	28.01	30.03	27.87	27.26	27.05	27.36
Day 13	27.91	29.97	28.47	31.69	25.45	28.08	28.51	28.12
Heel Height								
	Base 0	Low 0	Med 0	High 0	Base 1	Low 1	Med 1	High 1
Day 3	15.08	15.58	14.51	14.63	15.1	14.95	15.92	14.85
Day 6	15.5	14.77	15.79	14.16	14.69	15.89	14.73	15.16
Day 9	14.2	14.79	16.35	16.75	14.89	15.51	14.48	14.74
Day 13	14.92	15.05	14.44	16.12	14.24	15.27	14.91	15.09
% Moisture								
	Base 0	Low 0	Med 0	High 0	Base 1	Low 1	Med 1	High 1
Day 3	30.72	31.97	31.92	31.66	31.12	31.05	31.33	31.19
Day 6	32.28	32.43	31.71	31.94	30.85	31.22	31.08	31.28
Day 9	32.28	31.62	30.95	30.96	31.15	31.47	30.79	30.63
Day 13	32.3	32.31	32.41	30.83	31.47	32.31	29.88	29.46
Crown Color								
	Base 0	Low 0	Med 0	High 0	Base 1	Low 1	Med 1	High 1
Day 3	17.54	16.34	18.11	15.18	12.32	13.06	12.53	13.05
Day 6	16.76	15.98	17.49	15.7	12.28	12.34	11.76	11.21
Day 9	18.35	16.6	15.79	18.14	11.8	13.54	13.16	11.89
Day 13	16.18	17.27	16.89	16.81	13.54	13.52	11.97	11.56
Heel Color								
	Base 0	Low 0	Med 0	High 0	Base 1	Low 1	Med 1	High 1
Day 3	25.79	24.92	24.54	23.17	18.73	15.2	15.94	20.28
Day 6	26.41	22.54	20.97	23.69	16.64	16.76	16.92	15.34
Day 9	25.38	25.46	23.22	27.07	17.52	15.51	16.2	15.68
Day 13	23.87	24.75	22.53	25.09	16.94	16.38	16.7	14.75

Anova: Two-Factor Without Replication

SUMMARY	Count	Sum	Average	Variance
Day 3	8	422.88	52.86	0.0818
Day 6	8	421.56	52.695	0.147143
Day 9	8	418.57	52.32125	0.16467
Day 13	8	417.31	52.16375	0.124684
Base 0	4	211.47	52.8675	0.133225
Low 0	4	211.74	52.935	0.069967
Med 0	4	210.14	52.535	0.388033
High 0	4	210.24	52.56	0.122867
Base 1	4	209.68	52.42	0.025
Low 1	4	209.71	52.4275	0.015358
Med 1	4	209.41	52.3525	0.235825
High 1	4	207.93	51.9825	0.212358

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	2.497925	3	0.832642	15.75303	1.35218E-05	3.072472055
Columns	2.5181	7	0.359729	6.805829	0.000276462	2.487581696
Error	1.109975	21	0.052856			
Total	6.126	31				

Buns with propionate were heavier than buns without propionate.
HIGH with propionate was significantly different (lighter) from all other bags.
There was a significant difference between days 3&6 and days 9&13.
Buns lost weight during the 13 days of shelf life.

Anova: Two-Factor Without Replication

SUMMARY	Count	Sum	Average	Variance
Day 3	8	230.25	28.78125	1.877955
Day 6	8	235.82	29.4775	1.955107
Day 9	8	228.75	28.59375	2.430313
Day 13	8	228.2	28.525	3.186343
Base 0	4	118.54	29.635	1.5339
Low 0	4	124.01	31.0025	0.524225
Med 0	4	113.16	28.29	0.346867
High 0	4	120.62	30.155	1.487033
Base 1	4	108.79	27.1975	1.436092
Low 1	4	111.51	27.8775	1.236292
Med 1	4	112.47	28.1175	0.657558
High 1	4	113.92	28.48	0.858667

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	4.557162	3	1.519054	1.620552	0.214682768	3.072472055
Columns	46.46329	7	6.637612	7.081114	0.000212199	2.487581696
Error	19.68474	21	0.937368			
Total	70.70519	31				

Generally crowns with propionate are shorter than buns without propionate but there is no specific trend in the data with regard to specific bag types.

Note: Buns were "squished" during shipping and this may have affected crown height in a random manner.

Anova: Two-Factor Without Replication

SUMMARY	Count	Sum	Average	Variance
Day 3	8	120.62	15.0775	0.221879
Day 6	8	120.69	15.08625	0.365684
Day 9	8	121.71	15.21375	0.830541
Day 13	8	120.04	15.005	0.320486
Base 0	4	59.7	14.925	0.293433
Low 0	4	60.19	15.0475	0.142292
Med 0	4	61.09	15.2725	0.901092
High 0	4	61.66	15.415	1.490167
Base 1	4	58.92	14.73	0.134733
Low 1	4	61.62	15.405	0.157167
Med 1	4	60.04	15.01	0.399133
High 1	4	59.84	14.96	0.039133

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	0.180662	3	0.060221	0.120547	0.946987315	3.072472055
Columns	1.679337	7	0.239905	0.480232	0.838081698	2.487581696
Error	10.49079	21	0.499561			
Total	12.35079	31				

No significant differences between bag types or days of age variables.

Anova: Two-Factor Without Replication

SUMMARY	Count	Sum	Average	Variance
Day 3	8	250.96	31.37	0.195429
Day 6	8	252.79	31.59875	0.336955
Day 9	8	249.85	31.23125	0.28907
Day 13	8	250.97	31.37125	1.411927
Base 0	4	127.58	31.895	0.6137
Low 0	4	128.33	32.0825	0.133025
Med 0	4	126.99	31.7475	0.368692
High 0	4	125.39	31.3475	0.288892
Base 1	4	124.59	31.1475	0.064425
Low 1	4	126.05	31.5125	0.312425
Med 1	4	123.08	30.77	0.400733
High 1	4	122.56	30.64	0.701533

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	0.555984	3	0.185328	0.480819	0.699109786	3.072472055
Columns	7.539372	7	1.077053	2.79433	0.032030409	2.487581696
Error	8.094291	21	0.385442			
Total	16.18965	31				

Slightly significant differences between bag types.

Buns without propionate have a higher % moisture than bun with propionate. No other trends between bag types were noted.

Anova: Two-Factor Without Replication

SUMMARY	Count	Sum	Average	Variance
Day 3	8	118.13	14.76625	5.48057
Day 6	8	113.52	14.19	6.405
Day 9	8	119.27	14.90875	7.089841
Day 13	8	117.74	14.7175	5.44165
Base 0	4	68.83	17.2075	0.890625
Low 0	4	66.19	16.5475	0.296625
Med 0	4	68.28	17.07	0.976267
High 0	4	65.83	16.4575	1.720292
Base 1	4	49.94	12.485	0.5505
Low 1	4	52.46	13.115	0.3161
Med 1	4	49.42	12.355	0.393633
High 1	4	47.71	11.9275	0.637092

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	2.372362	3	0.790787	1.109244	0.367577828	3.072472055
Columns	155.9484	7	22.27834	31.25002	1.00591E-09	2.487581696
Error	14.97104	21	0.712907			
Total	173.2918	31				

Significant differences between bag types.

Very clear difference between buns with and without propionate. Differences within those groups were not significant. Buns with propionate are darker.

Anova: Two-Factor Without Replication

SUMMARY	Count	Sum	Average	Variance
Day 3	8	168.57	21.07125	17.20361
Day 6	8	159.27	19.90875	16.43133
Day 9	8	166.04	20.755	24.85171
Day 13	8	161.01	20.12625	18.66306
Base 0	4	101.45	25.3625	1.169292
Low 0	4	97.67	24.4175	1.658292
Med 0	4	91.26	22.815	2.2083
High 0	4	99.02	24.755	3.0393
Base 1	4	69.83	17.4575	0.853092
Low 1	4	63.85	15.9625	0.532158
Med 1	4	65.76	16.44	0.201867
High 1	4	66.05	16.5125	6.456092

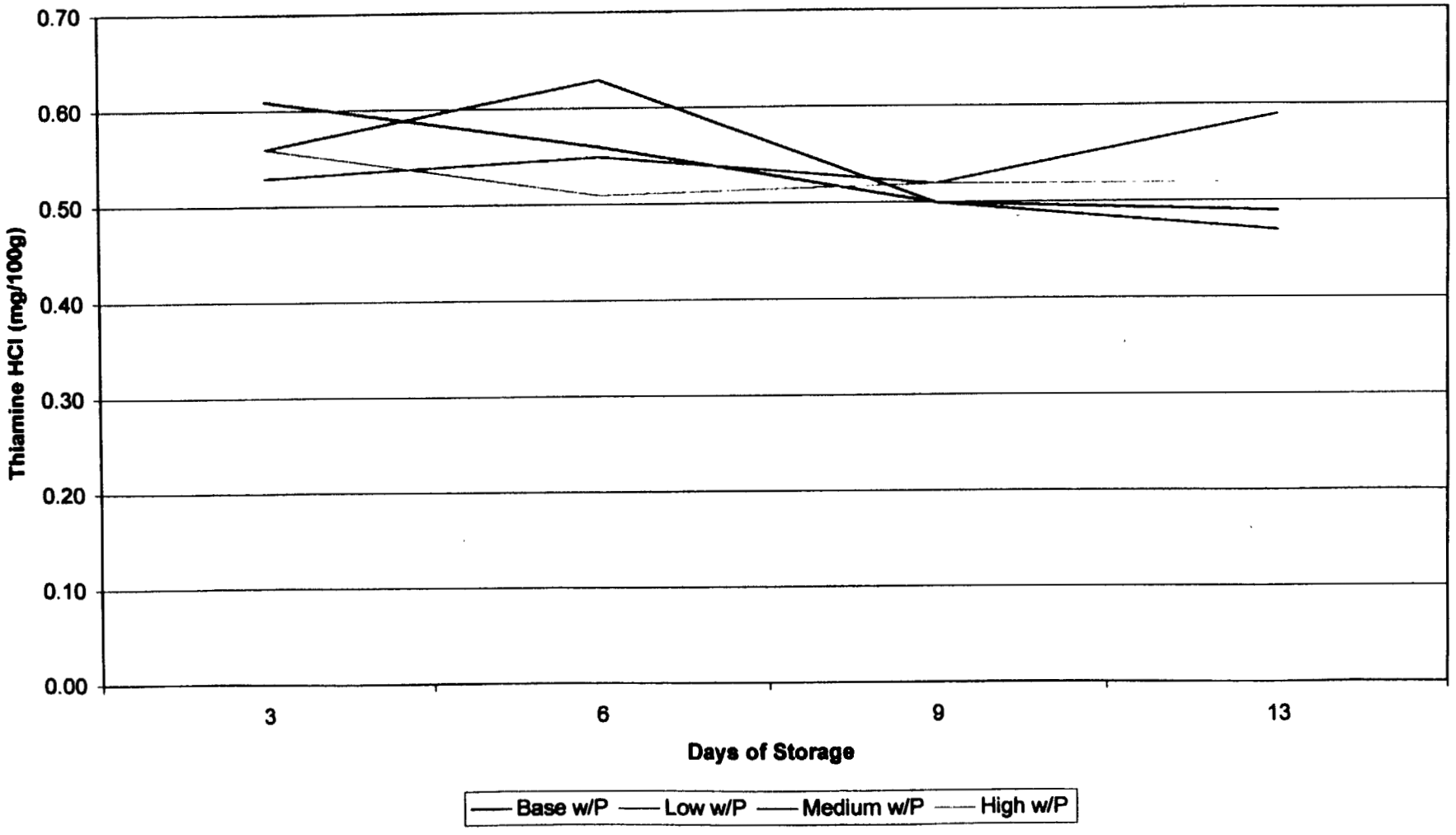
ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	7.006434	3	2.335478	1.186131	0.338985438	3.072472055
Columns	498.6992	7	71.24275	36.18242	2.51677E-10	2.487581696
Error	41.34874	21	1.968988			
Total	547.0544	31				

Significant differences between bag types

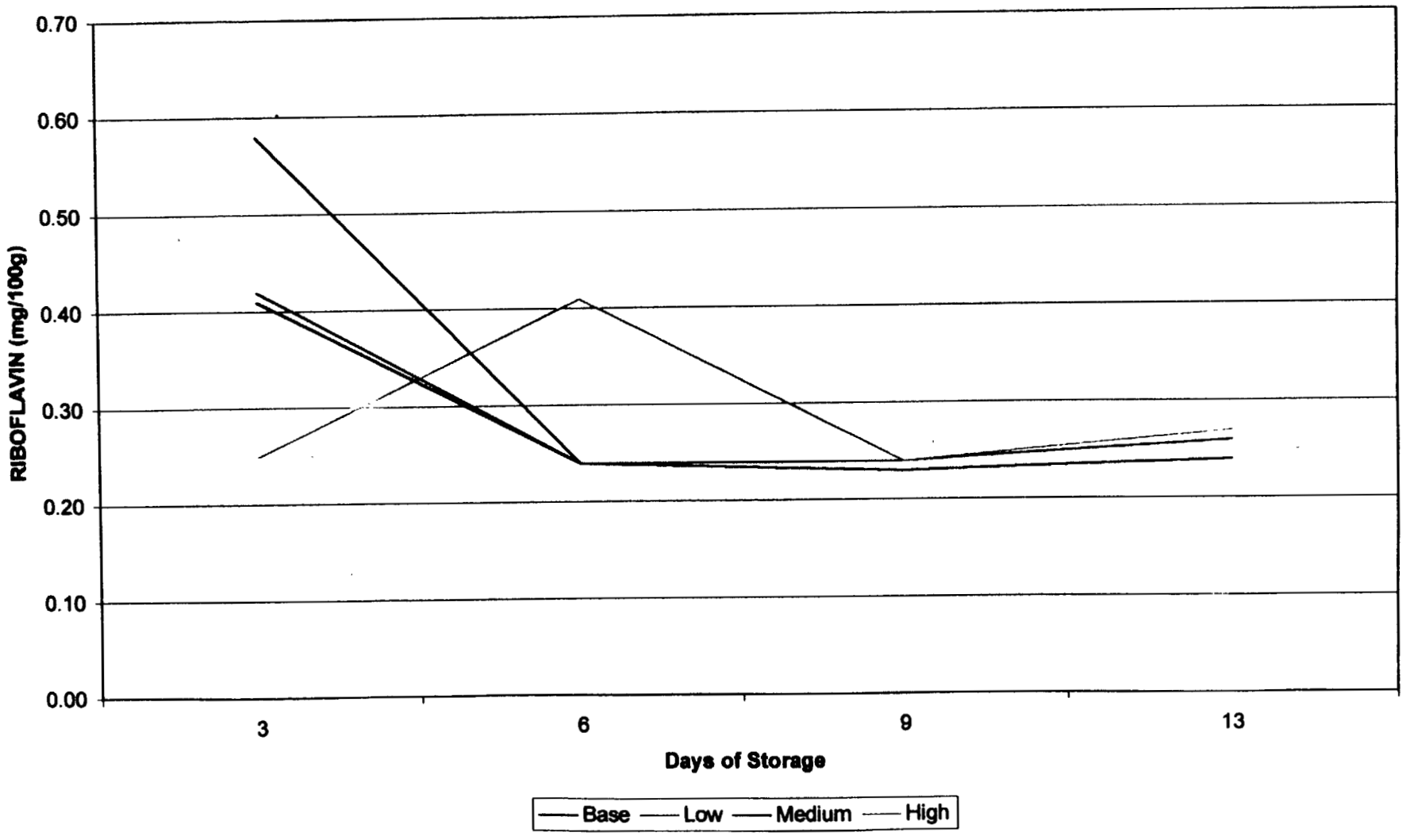
Similar to Crown color. Clear differences between buns with and without propionate. Buns with propionate have signifciantly darker heels. There is a trend in the data that shows the test packages (low, med, hi) had buns with darker heels when compared to base but these differences were not statistically significant.

EAST BALT BUN STUDY No. 2
THIAMINE HYDROCHLORIDE
(Buns with Propionate)

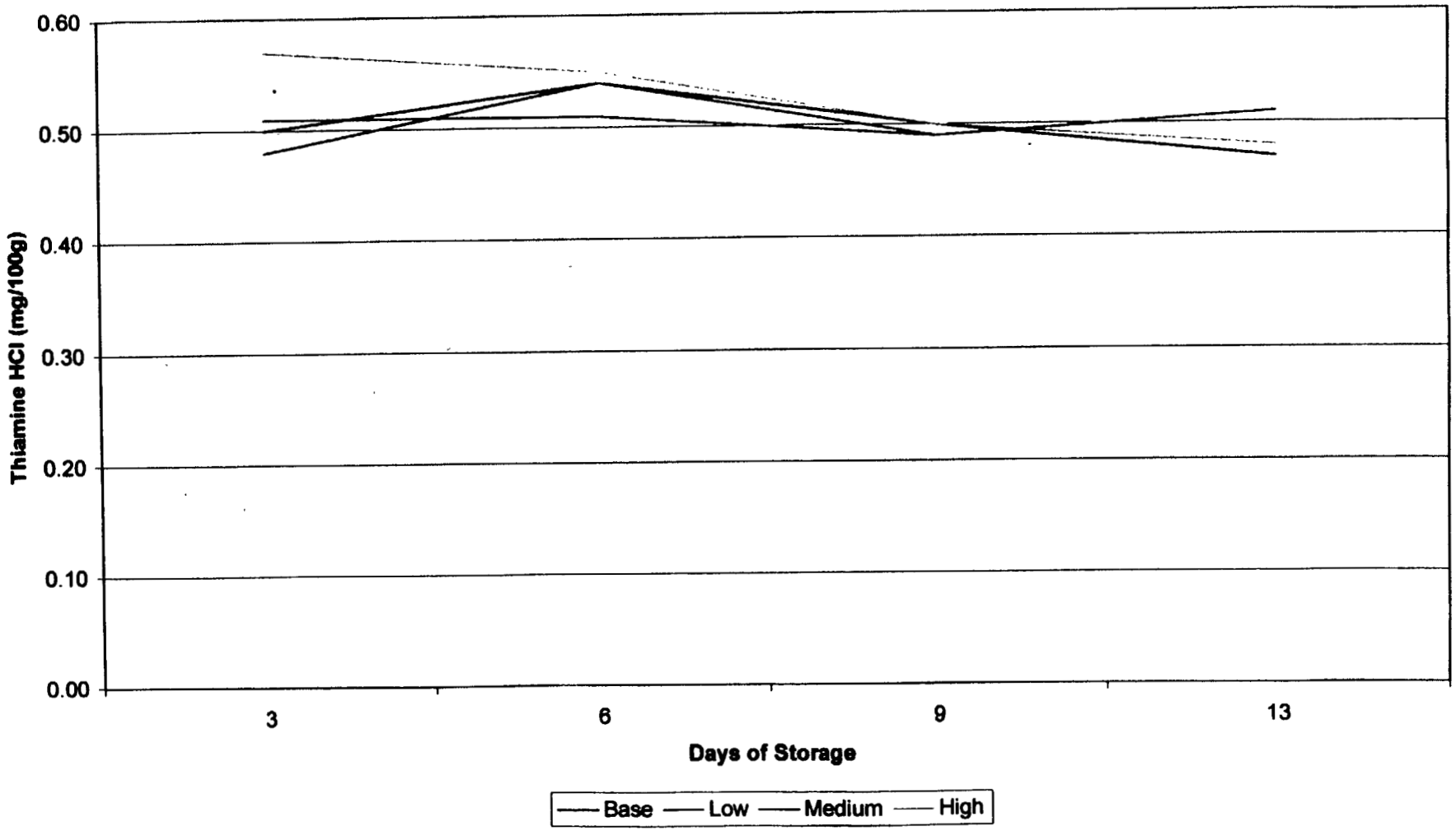


EAST BALT BUN STUDY No. 2

RIBOFLAVIN
(Buns with Propionate)

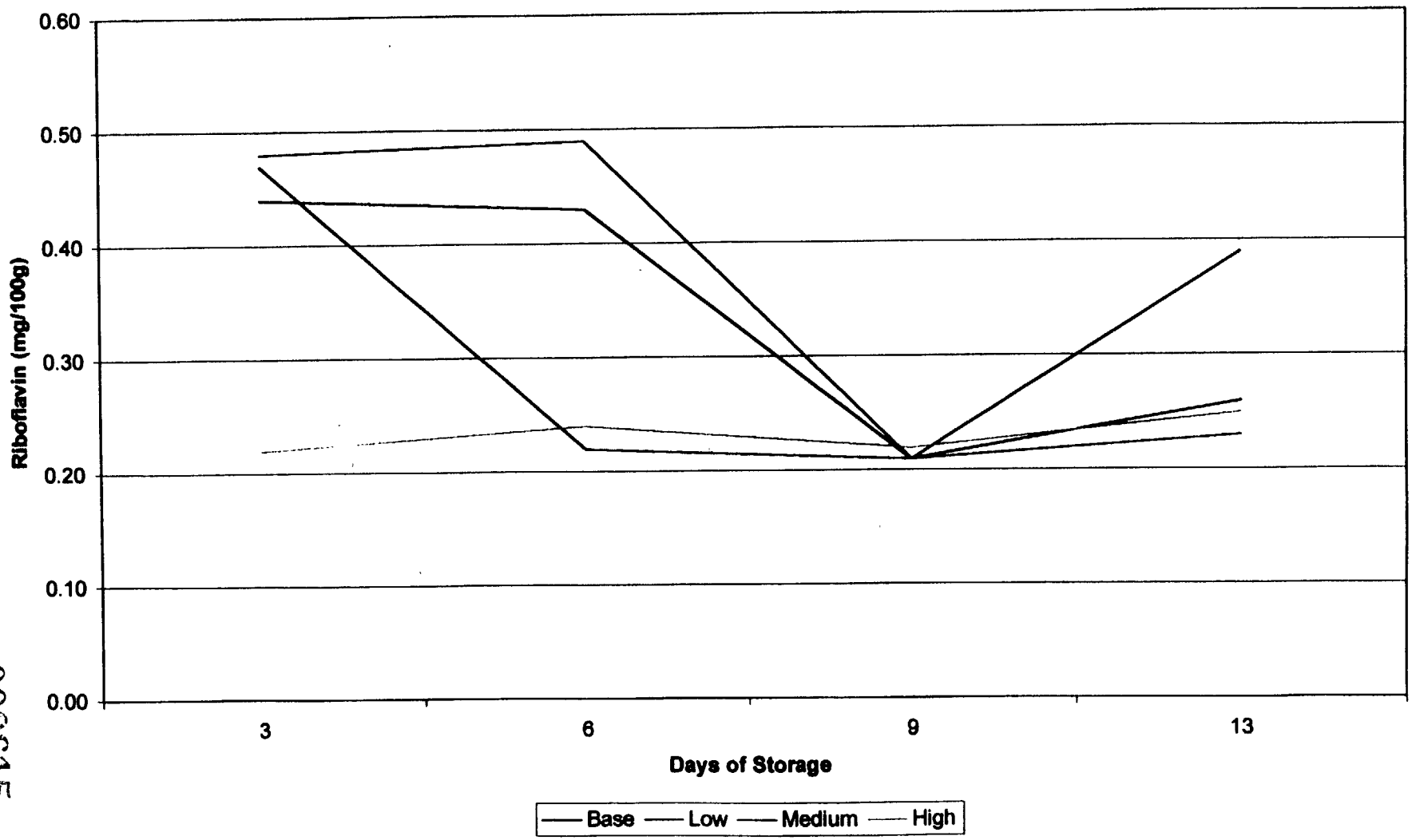


EAST BALT BUN STUDY No. 2
THIAMINE HYDROCHLORIDE
(Buns without Propionate)



EAST BALT BUN STUDY No. 2

RIBOFLAVIN
(Buns without Propionate)



000615

Covance Laboratories Inc.
P.O. Box 7545
Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

COVANCE
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300715

BATCH NUMBER: 00300715

DATE ENTERED: 03/02/00

REPORT PRINTED: 03/10/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS CONTAINING CALCIUM PROPIONATE: EB BUNS-BASE, DAY 3
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 3)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.61	MG/100 G
RIBOFLAVIN	.41	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

Covance Laboratories Inc.
P.O. Box 7545
Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

COVANCE
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300717

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

BATCH NUMBER: 00300715
DATE ENTERED: 03/02/00
REPORT PRINTED: 03/10/00

HAMBURGER BUNS CONTAINING CALCIUM PROPIONATE: EB BUNS-LOW, DAY 3
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 3)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.53	MG/100 G
RIBOFLAVIN	.58	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

Covance Laboratories Inc.
P.O. Box 7545
Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

COVANCE
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300719

BATCH NUMBER: 00300715

DATE ENTERED: 03/02/00

REPORT PRINTED: 03/10/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS CONTAINING CALCIUM PROPIONATE: EB BUNS-MED, DAY 3
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 3)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.56	MG/100 G
RIBOFLAVIN	.42	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

Covance Laboratories Inc.
P.O. Box 7545
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Tel: 608/241-4471 Fax: 608/241-7227

COVANCE
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300721

BATCH NUMBER: 00300715

DATE ENTERED: 03/02/00

REPORT PRINTED: 03/10/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS CONTAINING CALCIUM PROPIONATE: EB BUNS-HIGH, DAY 3
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 3)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.56	MG/100 G
RIBOFLAVIN	.25	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300757
BATCH NUMBER: 00300757
DATE ENTERED: 03/02/00
REPORT PRINTED: 03/21/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS CONTAINING CALCIUM PROPIONATE: EB BUNS-BASE, DAY 6
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 6)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.56	MG/100 G
RIBOFLAVIN	.24	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

000620

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300759

BATCH NUMBER: 00300757

DATE ENTERED: 03/02/00

REPORT PRINTED: 03/21/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS CONTAINING CALCIUM PROPIONATE: EB BUNS-LOW, DAY 6
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 6)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.55	MG/100 G
RIBOFLAVIN	.24	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300761
BATCH NUMBER: 00300757
DATE ENTERED: 03/02/00
REPORT PRINTED: 03/21/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS CONTAINING CALCIUM PROPIONATE: EB BUNS-MED, DAY 6
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 6)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.63	MG/100 G
RIBOFLAVIN	.24	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300763
BATCH NUMBER: 00300757
DATE ENTERED: 03/02/00
REPORT PRINTED: 03/21/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS CONTAINING CALCIUM PROPIONATE: EB BUNS-HIGH, DAY 6
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 6)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.51	MG/100 G
RIBOFLAVIN	.41	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300765
BATCH NUMBER: 00300765
DATE ENTERED: 03/02/00
REPORT PRINTED: 03/21/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS CONTAINING CALCIUM PROPIONATE: EB BUNS-BASE, DAY 9
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 9)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.50	MG/100 G
RIBOFLAVIN	.23	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

000624

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300767
BATCH NUMBER: 00300765
DATE ENTERED: 03/02/00
REPORT PRINTED: 03/21/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS CONTAINING CALCIUM PROPIONATE: EB BUNS-LOW, DAY 9
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 9)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.52	MG/100 G
RIBOFLAVIN	.24	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300769

BATCH NUMBER: 00300765

DATE ENTERED: 03/02/00

REPORT PRINTED: 03/21/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS CONTAINING CALCIUM PROPIONATE: EB BUNS-MED, DAY 9
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 9)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.50	MG/100 G
RIBOFLAVIN	.24	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300771
BATCH NUMBER: 00300765
DATE ENTERED: 03/02/00
REPORT PRINTED: 03/21/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS CONTAINING CALCIUM PROPIONATE: EB BUNS-HIGH, DAY 9
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 9)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.52	MG/100 G
RIBOFLAVIN	.24	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

000627

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300777
BATCH NUMBER: 00300777
DATE ENTERED: 03/02/00
REPORT PRINTED: 03/21/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS CONTAINING CALCIUM PROPIONATE: EB BUNS-BASE, DAY 13
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 13)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.49	MG/100 G
RIBOFLAVIN	.24	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300779
BATCH NUMBER: 00300777
DATE ENTERED: 03/02/00
REPORT PRINTED: 03/21/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS CONTAINING CALCIUM PROPIONATE: EB BUNS-LOW, DAY 13
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 13)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.59	MG/100 G
RIBOFLAVIN	.26	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300781
BATCH NUMBER: 00300777
DATE ENTERED: 03/02/00
REPORT PRINTED: 03/21/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS CONTAINING CALCIUM PROPIONATE: EB BUNS-MED, DAY 13
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 13)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.47	MG/100 G
RIBOFLAVIN	.26	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

000630

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300783

BATCH NUMBER: 00300777

DATE ENTERED: 03/02/00

REPORT PRINTED: 03/21/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS CONTAINING CALCIUM PROPIONATE: EB BUNS-HIGH, DAY 13
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 13)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.52	MG/100 G
RIBOFLAVIN	.27	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

000631

Covance Laboratories Inc.
P.O. Box 7545
Madison, WI 53707-7545
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Tel: 608/241-4471 Fax: 608/241-7227

REPORT OF ANALYSIS

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00300757

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

BATCH NUMBER: 00300757

DATE ENTERED: 03/02/00

REPORT PRINTED: 03/17/00

HAMBURGER BUNS CONTAINING CALCIUM PROPIONATE: EB BUNS-BASE, DAY 6
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 6)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.56	MG/100 G
RIBOFLAVIN	.24	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

Covance Laboratories Inc.
P.O. Box 7545
Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

REPORT OF ANALYSIS

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00300759

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

BATCH NUMBER: 00300757
DATE ENTERED: 03/02/00
REPORT PRINTED: 03/17/00

HAMBURGER BUNS CONTAINING CALCIUM PROPIONATE: EB BUNS-LOW, DAY 6
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 6)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.55	MG/100 G
RIBOFLAVIN	.24	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

Covance Laboratories Inc.
P.O. Box 7545
Madison, WI 53707-7545
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REPORT OF ANALYSIS

COVANCE
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00300761

BATCH NUMBER: 00300757

DATE ENTERED: 03/02/00

REPORT PRINTED: 03/17/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS CONTAINING CALCIUM PROPIONATE: EB BUNS-MED, DAY 6
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 6)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.63	MG/100 G
RIBOFLAVIN	.24	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

Covance Laboratories Inc.
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COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300763

BATCH NUMBER: 00300757

DATE ENTERED: 03/02/00

REPORT PRINTED: 03/17/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS CONTAINING CALCIUM PROPIONATE: EB BUNS-HIGH, DAY 6
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 6)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.51	MG/100 G
RIBOFLAVIN	.41	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

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COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300765

BATCH NUMBER: 00300765

DATE ENTERED: 03/02/00

REPORT PRINTED: 03/17/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS CONTAINING CALCIUM PROPIONATE: EB BUNS-BASE, DAY 9
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 9)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.50	MG/100 G
RIBOFLAVIN	.23	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

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COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300767

BATCH NUMBER: 00300765

DATE ENTERED: 03/02/00

REPORT PRINTED: 03/17/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS CONTAINING CALCIUM PROPIONATE: EB BUNS-LOW, DAY 9
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 9)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.52	MG/100 G
RIBOFLAVIN	.24	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

Covance Laboratories Inc.
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Tel: 608/241-4471 Fax: 608/241-7227

REPORT OF ANALYSIS

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00300769

BATCH NUMBER: 00300765

DATE ENTERED: 03/02/00

REPORT PRINTED: 03/17/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS CONTAINING CALCIUM PROPIONATE: EB BUNS-MED, DAY 9
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 9)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.50	MG/100 G
RIBOFLAVIN	.24	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

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REPORT OF ANALYSIS

COVANCE
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00300771

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

BATCH NUMBER: 00300765

DATE ENTERED: 03/02/00

REPORT PRINTED: 03/17/00

HAMBURGER BUNS CONTAINING CALCIUM PROPIONATE: EB BUNS-HIGH, DAY 9
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 9)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.52	MG/100 G
RIBOFLAVIN	.24	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

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COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300777

BATCH NUMBER: 00300777

DATE ENTERED: 03/02/00

REPORT PRINTED: 03/17/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS CONTAINING CALCIUM PROPIONATE: EB BUNS-BASE, DAY 13
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 13)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.49	MG/100 G
RIBOFLAVIN	.24	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

Covance Laboratories Inc.
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Madison, WI 53707-7545
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COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300779

BATCH NUMBER: 00300777

DATE ENTERED: 03/02/00

REPORT PRINTED: 03/17/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS CONTAINING CALCIUM PROPIONATE: EB BUNS-LOW, DAY 13
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 13)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.59	MG/100 G
RIBOFLAVIN	.26	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

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COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300781

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

BATCH NUMBER: 00300777

DATE ENTERED: 03/02/00

REPORT PRINTED: 03/17/00

HAMBURGER BUNS CONTAINING CALCIUM PROPIONATE: EB BUNS-MED, DAY 13
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 13)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.47	MG/100 G
RIBOFLAVIN	.26	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

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REPORT OF ANALYSIS

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00300783

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

BATCH NUMBER: 00300777

DATE ENTERED: 03/02/00

REPORT PRINTED: 03/17/00

HAMBURGER BUNS CONTAINING CALCIUM PROPIONATE: EB BUNS-HIGH, DAY 13
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 13)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.52	MG/100 G
RIBOFLAVIN	.27	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

Covance Laboratories Inc.
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COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300716

BATCH NUMBER: 00300715

DATE ENTERED: 03/02/00

REPORT PRINTED: 03/10/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS WITH/OUT CALCIUM PROPIONATE: EB BUNS-BASE, DAY 3
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 3)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.50	MG/100 G
RIBOFLAVIN	.44	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

Covance Laboratories Inc.
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Madison, WI 53707-7545
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Tel: 608/241-4471 Fax: 608/241-7227

COVANCE
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300718

BATCH NUMBER: 00300715

DATE ENTERED: 03/02/00

REPORT PRINTED: 03/10/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS WITH/OUT CALCIUM PROPIONATE: EB BUNS-LOW, DAY 3
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 3)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.48	MG/100 G
RIBOFLAVIN	.48	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

Covance Laboratories Inc.
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Tel: 608/241-4471 Fax: 608/241-7227

REPORT OF ANALYSIS

COVANCE
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00300720

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

BATCH NUMBER: 00300715

DATE ENTERED: 03/02/00

REPORT PRINTED: 03/10/00

HAMBURGER BUNS WITH/OUT CALCIUM PROPIONATE: EB BUNS-MED, DAY 3
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 3)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.51	MG/100 G
RIBOFLAVIN	.47	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

Covance Laboratories Inc.
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Tel: 608/241-4471 Fax: 608/241-7227

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300722

BATCH NUMBER: 00300715

DATE ENTERED: 03/02/00

REPORT PRINTED: 03/10/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS WITH/OUT CALCIUM PROPIONATE: EB BUNS-HIGH, DAY 3
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 3)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.57	MG/100 G
RIBOFLAVIN	.22	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300758
BATCH NUMBER: 00300757
DATE ENTERED: 03/02/00
REPORT PRINTED: 03/21/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS WITH/OUT CALCIUM PROPIONATE: EB BUNS-BASE, DAY 6
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 6)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.54	MG/100 G
RIBOFLAVIN	.43	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

000650

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300760
BATCH NUMBER: 00300757
DATE ENTERED: 03/02/00
REPORT PRINTED: 03/21/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS WITH/OUT CALCIUM PROPIONATE: EB BUNS-LOW, DAY 6
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 6)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.54	MG/100 G
RIBOFLAVIN	.49	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

000651

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300762
BATCH NUMBER: 00300757
DATE ENTERED: 03/02/00
REPORT PRINTED: 03/21/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS WITH/OUT CALCIUM PROPIONATE: EB BUNS-MED, DAY 6
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 6)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.51	MG/100 G
RIBOFLAVIN	.22	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

000652

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300764
BATCH NUMBER: 00300757
DATE ENTERED: 03/02/00
REPORT PRINTED: 03/21/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS WITH/OUT CALCIUM PROPIONATE: EB BUNS-HIGH, DAY 6
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 6)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.55	MG/100 G
RIBOFLAVIN	.24	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300766
BATCH NUMBER: 00300765
DATE ENTERED: 03/02/00
REPORT PRINTED: 03/21/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS WITH/OUT CALCIUM PROPIONATE: EB BUNS-BASE, DAY 9
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 9)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.50	MG/100 G
RIBOFLAVIN	.21	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

000654

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300768
BATCH NUMBER: 00300765
DATE ENTERED: 03/02/00
REPORT PRINTED: 03/21/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS WITH/OUT CALCIUM PROPIONATE: EB BUNS-LOW, DAY 9
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 9)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.49	MG/100 G
RIBOFLAVIN	.21	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

000655

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300770
BATCH NUMBER: 00300765
DATE ENTERED: 03/02/00
REPORT PRINTED: 03/21/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS WITH/OUT CALCIUM PROPIONATE: EB BUNS-MED, DAY 9
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 9)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.49	MG/100 G
RIBOFLAVIN	.21	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

000656

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300772
BATCH NUMBER: 00300765
DATE ENTERED: 03/02/00
REPORT PRINTED: 03/21/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS WITH/OUT CALCIUM PROPIONATE: EB BUNS-HIGH, DAY 9
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 9)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.50	MG/100 G
RIBOFLAVIN	.22	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

000657

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300778
BATCH NUMBER: 00300777
DATE ENTERED: 03/02/00
REPORT PRINTED: 03/21/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS WITH/OUT CALCIUM PROPIONATE: EB BUNS-BASE, DAY 13
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 13)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.47	MG/100 G
RIBOFLAVIN	.26	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

000658

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300780
BATCH NUMBER: 00300777
DATE ENTERED: 03/02/00
REPORT PRINTED: 03/21/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS WITH/OUT CALCIUM PROPIONATE: EB BUNS-LOW, DAY 13
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 13)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.51	MG/100 G
RIBOFLAVIN	.23	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300782
BATCH NUMBER: 00300777
DATE ENTERED: 03/02/00
REPORT PRINTED: 03/21/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS WITH/OUT CALCIUM PROPIONATE: EB BUNS-MED, DAY 13
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 13)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.51	MG/100 G
RIBOFLAVIN	.39	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

000660

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300784
BATCH NUMBER: 00300777
DATE ENTERED: 03/02/00
REPORT PRINTED: 03/21/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS WITH/OUT CALCIUM PROPIONATE: EB BUNS-HIGH, DAY 13
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 13)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.48	MG/100 G
RIBOFLAVIN	.25	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

000661

Covance Laboratories Inc.
P.O. Box 7545
Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

REPORT OF ANALYSIS

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00300758

BATCH NUMBER: 00300757

DATE ENTERED: 03/02/00

REPORT PRINTED: 03/17/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS WITH/OUT CALCIUM PROPIONATE: EB BUNS-BASE, DAY 6
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 6)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.54	MG/100 G
RIBOFLAVIN	.43	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

Covance Laboratories Inc.
P.O. Box 7545
Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300760

BATCH NUMBER: 00300757

DATE ENTERED: 03/02/00

REPORT PRINTED: 03/17/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS WITH/OUT CALCIUM PROPIONATE: EB BUNS-LOW, DAY 6
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 6)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.54	MG/100 G
RIBOFLAVIN	.49	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

Covance Laboratories Inc.
P.O. Box 7545
Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300762

BATCH NUMBER: 00300757

DATE ENTERED: 03/02/00

REPORT PRINTED: 03/17/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS WITH/OUT CALCIUM PROPIONATE: EB BUNS-MED, DAY 6
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 6)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.51	MG/100 G
RIBOFLAVIN	.22	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

Covance Laboratories Inc.
P.O. Box 7545
Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300764

BATCH NUMBER: 00300757

DATE ENTERED: 03/02/00

REPORT PRINTED: 03/17/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS WITH/OUT CALCIUM PROPIONATE: EB BUNS-HIGH, DAY 6
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 6)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.55	MG/100 G
RIBOFLAVIN	.24	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

Covance Laboratories Inc.
P.O. Box 7545
Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300766

BATCH NUMBER: 00300765

DATE ENTERED: 03/02/00

REPORT PRINTED: 03/17/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS WITH/OUT CALCIUM PROPIONATE: EB BUNS-BASE, DAY 9
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 9)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.50	MG/100 G
RIBOFLAVIN	.21	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

Covance Laboratories Inc.
P.O. Box 7545
Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

REPORT OF ANALYSIS

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00300768

BATCH NUMBER: 00300765

DATE ENTERED: 03/02/00

REPORT PRINTED: 03/17/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS WITH/OUT CALCIUM PROPIONATE: EB BUNS-LOW, DAY 9
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 9)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.49	MG/100 G
RIBOFLAVIN	.21	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

Covance Laboratories Inc.
P.O. Box 7545
Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

REPORT OF ANALYSIS

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00300770

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

BATCH NUMBER: 00300765

DATE ENTERED: 03/02/00

REPORT PRINTED: 03/17/00

HAMBURGER BUNS WITH/OUT CALCIUM PROPIONATE: EB BUNS-MED, DAY 9
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 9)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.49	MG/100 G
RIBOFLAVIN	.21	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

Covance Laboratories Inc.
P.O. Box 7545
Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300772

BATCH NUMBER: 00300765

DATE ENTERED: 03/02/00

REPORT PRINTED: 03/17/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS WITH/OUT CALCIUM PROPIONATE: EB BUNS-HIGH, DAY 9
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 9)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.50	MG/100 G
RIBOFLAVIN	.22	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

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REPORT OF ANALYSIS

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00300778

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

BATCH NUMBER: 00300777
DATE ENTERED: 03/02/00
REPORT PRINTED: 03/17/00

HAMBURGER BUNS WITH/OUT CALCIUM PROPIONATE: EB BUNS-BASE, DAY 13
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 13)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.47	MG/100 G
RIBOFLAVIN	.26	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

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COVANCE
THE DEVELOPMENT SERVICES COMPANY

REPORT OF ANALYSIS

SAMPLE NUMBER: 00300780

BATCH NUMBER: 00300777

DATE ENTERED: 03/02/00

REPORT PRINTED: 03/17/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

HAMBURGER BUNS WITH/OUT CALCIUM PROPIONATE: EB BUNS-LOW, DAY 13
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 13)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.51	MG/100 G
RIBOFLAVIN	.23	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

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REPORT OF ANALYSIS

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00300782

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

BATCH NUMBER: 00300777

DATE ENTERED: 03/02/00

REPORT PRINTED: 03/17/00

HAMBURGER BUNS WITH/OUT CALCIUM PROPIONATE: EB BUNS-MED, DAY 13
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 13)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.51	MG/100 G
RIBOFLAVIN	.39	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

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REPORT OF ANALYSIS

COVANCE
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00300784

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

BATCH NUMBER: 00300777

DATE ENTERED: 03/02/00

REPORT PRINTED: 03/17/00

HAMBURGER BUNS WITH/OUT CALCIUM PROPIONATE: EB BUNS-HIGH, DAY 13
REC'D FROM EAST BALT INC., CHICAGO, IL (COMPOSITE OF 9)(START DATE MARCH 13)

ASSAY	ANALYSIS	UNITS
THIAMIN HYDROCHLORIDE	.48	MG/100 G
RIBOFLAVIN	.25	MG/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

THIAMIN HYDROCHLORIDE
Official Methods of Analysis, 16th Edition, Method 942.23, 'Thiamine (Vitamin B1) in Foods', 953.17, 'Thiamine (Vitamin B1) in Grain Products', and 95.7.17, 'Thiamine (Vitamin B1) In Bread' (modified), Chapter 45, AOAC, Gaithersburg, MD (1995).

RIBOFLAVIN
AOAC International, '940.33 Riboflavin (Vitamin B2) in Vitamin Preparations' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Gaithersburg, MD (1995).

Method listed above with results.

Beginning Formula #:	925	Revision #:	0	Item Code:	925-0	FormulaNumberID:	925
Date:	3/2/00						

Item Name:	Regulars-No Cal Prop	Bakery Name:	Chicago
Main Item Catagory:	Buns-4"	Customer Name:	
Sub Item Catagory:		Description:	4" Regular Hamburger bun without Calcium Propionate. For packaging film study with Bernard Technologies / McDonald's
Sub Item Catagory1:			
Reason for test:	Analytical Testing - Spc Packaging		
Variations of product:			

Next Steps 1 (from Cutting Form):

Total Weight:	1,593.45
Total Flour:	870.00
ABS:	54.60%

Sponge Weight:	914.7	Bakers %:
Flour:	560.00	64.37%
	0.00	0.00%
	0.00	0.00%
	0.00	0.00%
	0.00	0.00%
	0.00	0.00%
Water:	300.00	34.48%
Yeast Type:	Creamed	
Yeast:	45.00	5.17%
Gluten:	0.00	0.00%
Yeast Food	3.20	0.37%
Star Plex 90	3.20	0.37%
W 52	2.20	0.25%
SSL	1.10	0.13%
	0.00	0.00%
	0.00	0.00%
	0.00	0.00%
	0.00	0.00%
	0.00	0.00%
sp Pills: A Tablets	0	0.000%
sp Pills: P Tablets	18	2.069%
sp Pills:	0	0.000%
sp Pills:	0	0.000%

Dough Weight:		678.75	Bakers %
Flour:		310.000	35.63%
		0.000	0.00%
		0.000	0.00%
		0.000	0.00%
		0.000	0.00%
		0.000	0.00%
Water:		45	6.32%
Yeast Type:		Creamed	
Yeast:		9.	1.61%
Sugar Type:	HFCS 42	Solids:	50.00%
Sugar:		240.000	13.79%
Shortening Type:		Soybean	
Shortening:		35.000	4.02%
Salt:		17.500	2.01%
Gluten:		0.000	0.00%
Calcium Propionate		0.000	0.00%
Calcium Sulfate		7.000	0.80%
McZyme		0.250	0.03%
		0.000	0.0000%
		0.000	0.00%
		0.000	0.00%
		0.000	0.00%
		0.000	0.00%
		0.000	0.00%
do Pills:	ADA	8.0000	0.920%
do Pills:	P Tablets	4	0.460%
do Pills:		0	0
do Pills		0.0000	0.000%

Special Instructions::
-REGULARS WITH OUT CALCIUM PROPIONATE
-Pull 15 trays of product ~~and run 100 of the dough~~
~~Remix the rest of the dough into the sponges.~~
DISCARD THE BUNS
AFTER GETTING 15 TRAYS

Test Results::

L-WAHAY

000674

Beginning Formula #:	925	Revision #:	0
Item Code:	925-0		
Item Name:	Regulars-No Cal Prop		
Main Item Catagory:	Buns-4"		
Sub Item Catagory:			
Sub Item Catagory1:			
Test Reason	Analytical Testing - Spc Packaging		
Variations of product:			

Date:	3/2/00
Bakery Name:	Chicago
Customer Name:	
FormulaNumberID:	925
Description:	4" Regular Hamburger bun without Calcium Propionate For packaging film study with Bernard Technologies / McDonald's

Process Method:	Sponge & Dough	Proof Time (Min):	58		
Sp Mix Time Low:	1	Proof Dry Bulb:	102	Proof Wet Bulb:	99
Sp Mix Time High:	5	☐ Spray After Proof		Water Spray Setting:	0
Sp Beg pH:	0	☐ Topping		Topping Type:	
Sp Beg TTA:	0	☐ Crown Cuts		Number:	0
Sp Set Temp:	76	Oven Type:		Continous	
Sp Back Temp:	86	☐ Steam	# of Headers/Seconds:	0	
Sp Fermentaion Time:	4	Bake Time:	7.5	Zone4:	0
Do Mix Time Low:	1	Zone1:	0	Zone5:	0
Do Mix Time High:	0	Zone2:	0	Zone6:	0
Do Temp:	78	Zone3:	0	Internal Temp:	0
Floor Time:	0	☐ Spray After Baking		Spray Type:	
Scaling Weight:	721	Spray Dilution (xx/Water):			
Divider Type:		Sprayer Setting:	0	Cooling Time:	23
Rest (Floor Time):	0	Slice Height/Thickness:		5/8"	

Tray / Box Type:	Tray	Product On Dollies or Skids:	Dollies
Items/Pillow or Bag:	30	# Trays/Boxes per Skid:	
# Pillows/Bags per Tray/Box:	2	Film Color:	Clear
# Trays/Boxes per Stack:		Code Date:	Normal

Special Instructions:

Pull 15 trays of the test product 925-0
PULL 15 TRAYS OF CONTROL PRODUCT

Processing Comments.

Beginning Formula #	925	Revision #:	0
Item Code:	925-0		
Item Name:	Regulars-No Cal Prop		
Main Item Catagory:	Buns-4"		
Sub Item Catagory:			
Sub Item Catagory1:			
Test Reason	Analytical Testing - Spc Packaging		
Variations of product:			

Date:	3/2/00
Bakery Name:	Chicago
Customer Name:	
FormulaNumberID:	925
Description:	4" Regular Hamburger bun without Calcium Propionate For packaging film study with Bernard Technologies / McDonald's

Previous Cutting Results: Previous Cutting Date:

Cutting Results: Cutting Date:

Next Steps:

Expected outcome::

TR BTI # 00-11 (D)

Protocol TACS-2-0300

A QUALITATIVE COMPARATIVE STUDY OF CARROTS STORED IN POLYETHYLENE BAGS CONTAINING EITHER NO ADDITIVES OR BTI PROPRIETARY TECHNOLOGY

1. Protocol
2. Tables of Sensory Evaluations
3. Table of Beta-Carotene Values
4. Graphs of Beta-Carotene Values
5. Table of Vitamin A Values
6. Graph of Vitamin A Values
7. Results of Chlorite Anion Analyses

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Protocol TACS-2-0300

A QUALITATIVE COMPARATIVE STUDY OF CARROTS STORED IN POLYETHYLENE BAGS CONTAINING EITHER NO ADDITIVES OR BTI PROPRIETARY TECHNOLOGY

Objective: To determine any differences in selected characteristics of shredded carrots packaged in polyethylene (LDPE) bags or LDPE bags containing BTI proprietary technology.

Materials:

Shredded Carrots prepared by Salad Time Farms, Tanimura & Antle, Salinas, CA.

Packaging film/bags

1. LDPE bags without active additives (0%)
2. LDPE opaque bags containing BTI additive at low %
3. LDPE opaque bags containing BTI additive at medium %
4. LDPE opaque bags containing BTI additive at high %

Packages:

1. 10 oz of shredded carrots per package
2. Six (6) replicates per sample type (4for T & A, 2 for Covance)
3. Total Packages: 144 (4 types of film)*(6 replicates/type)*(6 periods)*(1 carrot type)

Storage Conditions:

1. Pack bags with test food at 36°F to 38°F
2. Store packed bags at 36°F to 42°F
3. Ship samples to Covance via FedEx with cool packs in the boxes
4. Covance to refrigerate, *not freeze*, samples until each time of analysis

Analyses as listed below:

1. Food evaluation characteristics to be conducted by Tanimura & Antle, Salinas, CA;
2. Chemical analyses to be conducted by Covance, Madison, WI

Schedule:

- Day 1 - Prepare Shredded Carrots
- Day 1 - Package in test bags with labels and store as indicated
- Day 1 - Ship bags containing carrots to test labs
- Day 4 - Test Labs conduct analyses as DAY 4 samples
- Day 11 - Test Labs conduct analyses as DAY 11 samples

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- Day 18 - Test Labs conduct analyses as DAY 18 samples
- Day 24 - Test Labs conduct analyses as DAY 24 samples
- - Preliminary Report of Results
- - Final Reports from Covance and Tanimura & Antle

Analyses to be performed by Tanimura & Antle

- Visual assessment
- Odor of raw carrots
- Texture
- Color

Analyses to be performed by COVANCE

- Residual chlorite
- Beta-carotene

Beta-Carotene and Vitamin A from Beta-Carotene: Determined by HPLC using AOAC International, Official Methods of Analysis (modified), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, Virginia (1995). Quackenbush, F. W., Journal of Liquid Chromatography (modified), 10:643-653 (1987).

Chlorine Dioxide and Chlorite Anions: Standard Methods for Water Analysis Method 4500-ClO2 B. Iodometric Method. APHA, AWWA, and WPCF. Washington, D. C. (17th ed.) Pages 4-54 (modified as Southwest Research Institute BTI-SOP# 99-02. Aieta, E. M., P. V. Roberts, and M. Hernandez, J. Am. Water Works Assoc., 76(1), 64-70 (1984).

Activities:

Shredded carrots will be prepared by Salad Time Farms (T&A) Day 1 in the morning
10 oz. of shredded carrots will be placed in each bag, air pressed from bag and the bag sealed
All bags completed by 11:00 a.m.
Duplicate samples will be sent to COVANCE Laboratories via Federal Express on Day 1

Carrots received by COVANCE on Day 2
Carrots stored refrigerated until analyzed.

The express carrier lost part of the first shipment of carrots to Covance Laboratories during the transportation. This necessitated a second packaging of carrots in the types of bags that were lost in transit. This second batch was treated the same as in the initial shipment, although the specific source of carrots was different from the first.

RESULTS

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Sensory: There were no differences in sensory characteristics such as color, taste, and odor when CRT packaging was compared to control packaging for 24 days.

Nutrients: The CRT did not alter the concentrations of endogenous beta-carotene or vitamin A from beta-carotene in fresh shredded carrots stored for 24 days.

Chlorite: Chlorite residues were below the limit of detection.

CONCLUSIONS

Packaging materials containing the Microsphère® CRT at levels of loading up to 50% may be used for the storage of fresh vegetables and fruits, as exemplified by fresh carrots, without demonstrating any negative qualitative effects for at least 24 days.

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Salad Time Farms, Inc.
1400 Schilling Place
Salinas, CA 93901

To: Gurmail Mudahar
From: Daniel Mesa
Subject: Carrot Study
Date: May 12, 2000

Report of Study Conducted in Collaboration with Bernàrd Technologies, Inc.

Protocol Number: TACS-2-0300

SMELL, TASTE, AND VISUAL OBSERVATION:

Tanimura & Antle using their standard methods conducted the evaluation of shredded carrots packaged as presented in Protocol Number TACS-2-0300 (see attached). Evaluations were made of the treated samples as compared to the non-treated samples. Any differences of note would have been reported.

In the smell, taste, odor, and visual observation all of these factors were similar to the control carrots until the 24th day. At the 24th day the taste on the carrots was not as fresh and the flavor was almost gone. There were no obvious negative differences between control and "treated" carrots.

Day	Date	Sample	Item #	Smell/Odor	Taste	Color
4	3/9/00	1	TACS-2-Zero	Fresh	Satisfactory	Standard
4	3/9/00	2	TACS-2-Low	Fresh	Satisfactory	Standard
4	3/9/00	3	TACS-2-Med	Fresh	Satisfactory	Standard
4	3/9/00	4	TACS-2-High	Fresh	Satisfactory	Standard
11	3/16/00	1	TACS-2-Zero	Acceptable	Satisfactory	Standard
11	3/16/00	2	TACS-2-Low	Acceptable	Satisfactory	Standard
11	3/16/00	3	TACS-2-Med	Acceptable	Satisfactory	Standard
11	3/16/00	4	TACS-2-High	Acceptable	Satisfactory	Standard
18	3/23/00	1	TACS-2-Zero	Acceptable	Satisfactory	Standard
18	3/23/00	2	TACS-2-Low	Acceptable	Satisfactory	Standard
18	3/23/00	3	TACS-2-Med	Acceptable	Satisfactory	Standard
18	3/23/00	4	TACS-2-High	Acceptable	Satisfactory	Standard
24	3/29/00	1	TACS-2-Zero	Acceptable	Little Flavor	Standard
24	3/29/00	2	TACS-2-Low	Acceptable	Little Flavor	Standard
24	3/29/00	3	TACS-2-Med	Acceptable	Little Flavor	Standard
24	3/29/00	4	TACS-2-High	Acceptable	Little Flavor	Standard

Report submitted by: Daniel Mesa
Printed Name

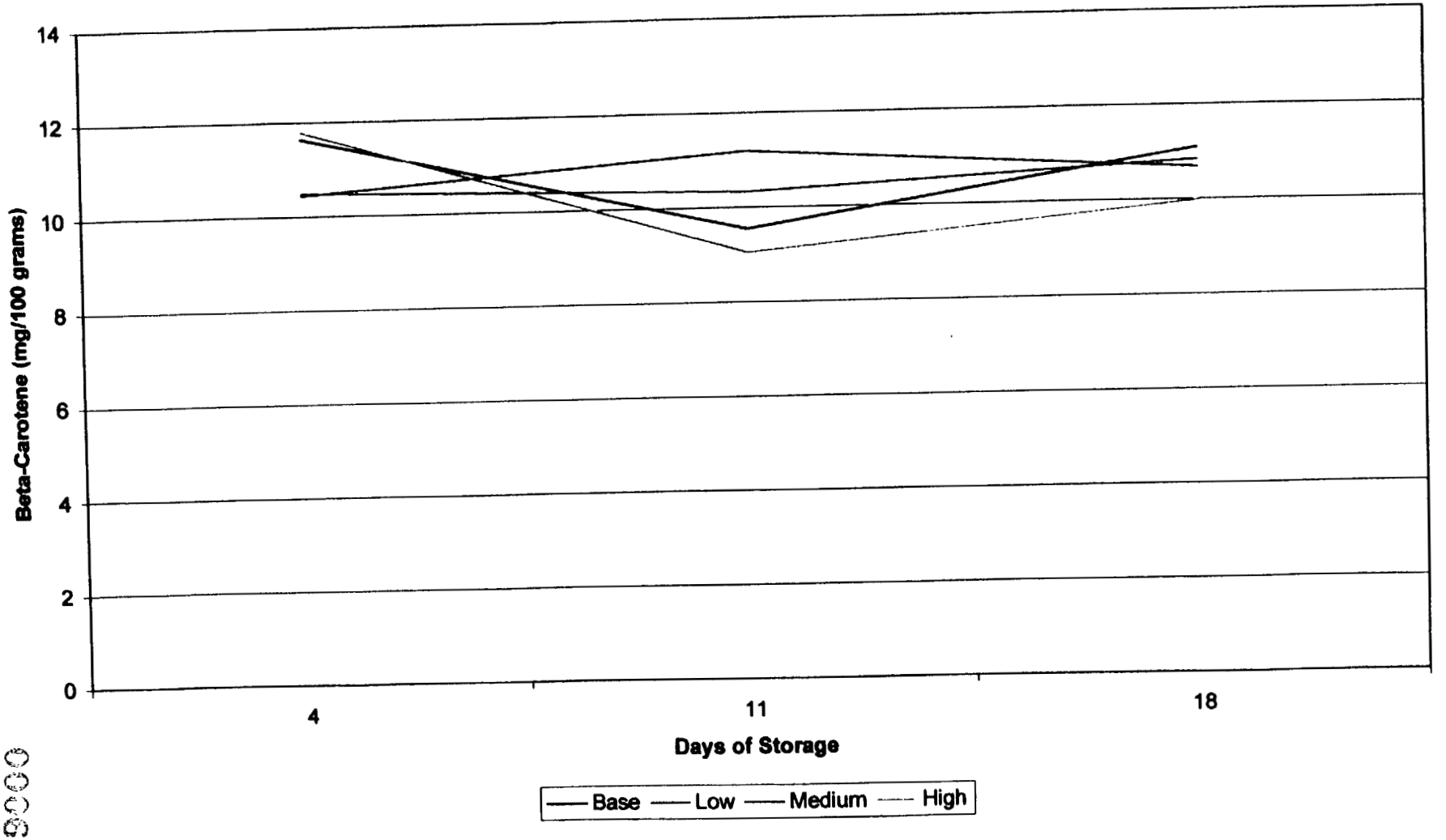
SHREDDED CARROTS
Salad Time Farms TACS-2
BETA-CAROTENE

TACS-2 Shredded Carrots

Beta-Carotene by HPLC **mg/100 G**
2each by 10 OZ. BAGS

DAY	Zero-A	Zero-B	Zero-C	Zero-D	Zero-Avg.	Low-A	Low-B	Low-Avg.	Med-A	Med-B	Med-Avg.	High-A	High-B	High-Avg.
4	10.50	10.70	12.30	13.10	11.65	10.60	10.40	10.50	10.30	10.60	10.45	12.30	11.30	11.80
11	10.60	10.20	8.88	8.56	9.56	9.97	10.70	10.34	11.70	10.70	11.20	8.99	9.12	9.06
18	11.00	11.00	10.90	11.50	11.10	11.70	10.00	10.85	10.40	11.00	10.70	10.20	9.77	9.99
24	-	-	10.30	10.60	10.45	-	-	-	-	-	-	10.80	10.00	10.40

SHREDDED CARROTS
Salad Time Farms TACS-2
BETA-CAROTENE



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SHREDDED CARROTS
Salad Time Farms TACS-2
VITAMIN A

TACS-2 Shredded Carrots

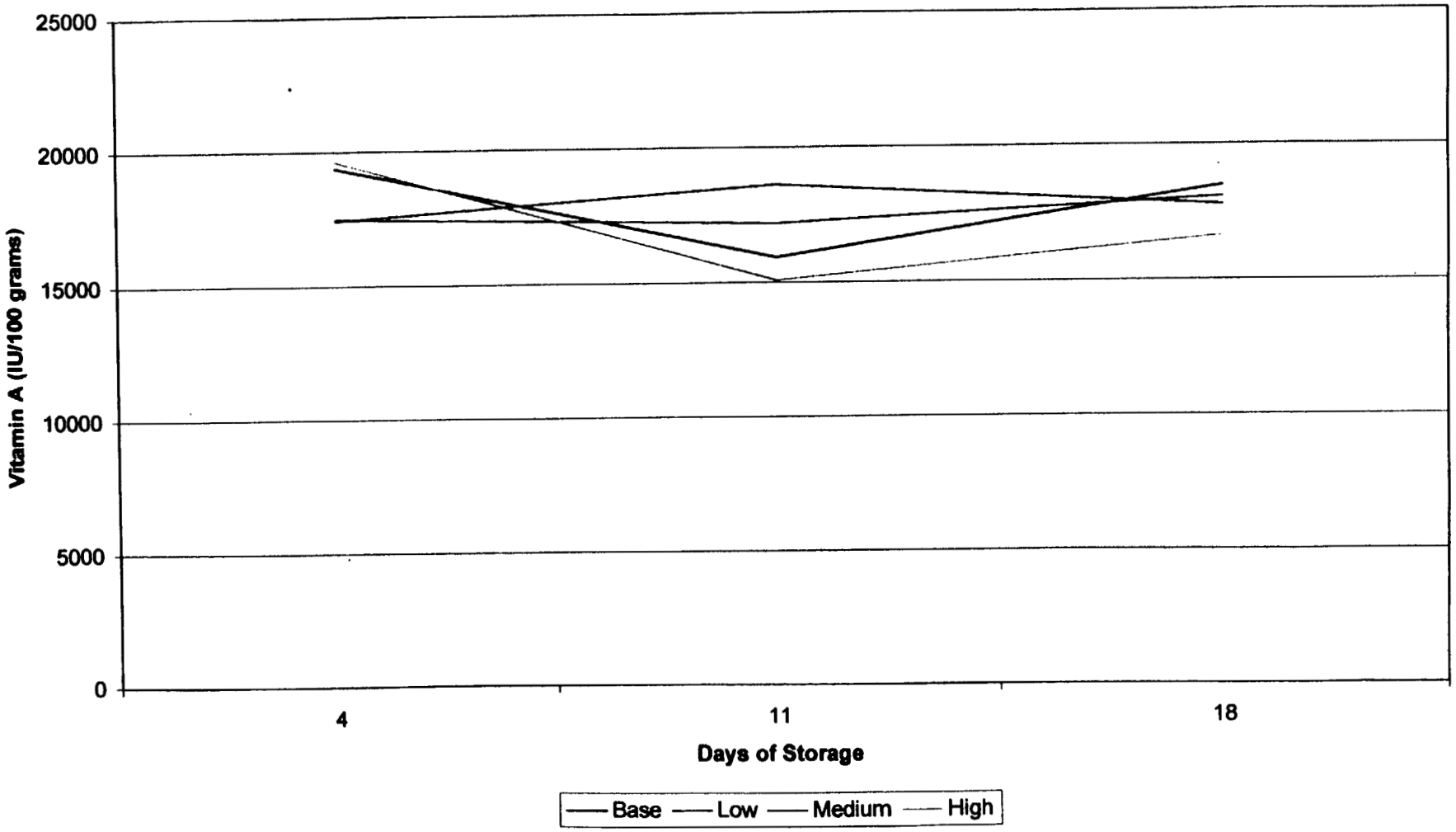
VITAMIN A FROM CAROTENE **IU/100 GRAMS**

2each by 10 OZ. BAGS

DAY	Zero-A	Zero-B	Zero-C	Zero-D	Zero-Avg.	Low-A	Low-B	Low-Avg.	Med-A	Med-B	Med-Avg.	High-A	High-B	High-Avg.
4	17500.00	17800.00	20500.00	21800.00	19400.00	17700.00	17300.00	17500.00	17200.00	17700.00	17450.00	20500.00	18800.00	19650.00
11	17700.00	17000.00	14800.00	14300.00	15950.00	16600.00	17800.00	17200.00	19500.00	17800.00	18650.00	15000.00	15200.00	15100.00
18	18300.00	18300.00	18200.00	19200.00	18500.00	19500.00	16700.00	18100.00	17300.00	18300.00	17800.00	17000.00	16300.00	16650.00
24	-	-	17200.00	17700.00	17450.00	-	-	-	-	-	-	18000.00	16700.00	17350.00

000584

SHREDDED CARROTS
Salad Time Farms TACS-2
VITAMIN A



000585

Covance Laboratories Inc.
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REPORT OF ANALYSIS

COVANCE
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00301983

BATCH NUMBER: 00301981

DATE ENTERED: 03/09/00

REPORT PRINTED: 03/20/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

CARROTS: TACS CONTROL, DAY 4, "A"
REC'D FROM SALD TIME FARMS, SALINAS, CA (START DATE 3/10)

ASSAY	ANALYSIS	UNITS
BETA CAROTENE BY HPLC	12.3	MG/100 G
VITAMIN A FROM CAROTENE	20500.	IU/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

BETA CAROTENE BY HPLC
AOAC International, Official Methods of Analysis (modified), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, VA (1995).
Quackenbush, F. W., Journal of Liquid Chromatography (modified), 10: 643-653 (1987).

Method listed above with results.

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REPORT OF ANALYSIS

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00301984

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

BATCH NUMBER: 00301981

DATE ENTERED: 03/09/00

REPORT PRINTED: 03/20/00

CARROTS: TACS CONTROL, DAY 4, "B"
REC'D FROM SALAD TIME FARMS, SALINAS, CA (START DATE 3/10)

ASSAY	ANALYSIS	UNITS
BETA CAROTENE BY HPLC	13.1	MG/100 G
VITAMIN A FROM CAROTENE	21800.	IU/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

BETA CAROTENE BY HPLC
AOAC International, Official Methods of Analysis (modified), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, VA (1995).
Quackenbush, F. W., Journal of Liquid Chromatography (modified), 10: 643-653 (1987).

Method listed above with results.

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REPORT OF ANALYSIS

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00306659

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

BATCH NUMBER: 00306659
DATE ENTERED: 03/30/00
REPORT PRINTED: 04/12/00

CARROTS: TACS-2-ZERO, DAY 4, "A"
REC'D FROM SALAD TIME FARMS, SALINAS, CA (START DATE 3/30/00)

ASSAY	ANALYSIS	UNITS
BETA CAROTENE BY HPLC	10.5	MG/100 G
VITAMIN A FROM CAROTENE	17500.	IU/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

BETA CAROTENE BY HPLC
AOAC International, Official Methods of Analysis (modified), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, VA (1995).
Quackenbush, F. W., Journal of Liquid Chromatography (modified), 10: 643-653 (1987).

Method listed above with results.

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REPORT OF ANALYSIS

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00306660

BATCH NUMBER: 00306659

DATE ENTERED: 03/30/00

REPORT PRINTED: 04/12/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

CARROTS: TACS-2-ZERO, DAY 4, "B"
REC'D FROM SALAD TIME FARMS, SALINAS, CA (START DATE 3/30/00)

ASSAY	ANALYSIS	UNITS
BETA CAROTENE BY HPLC	10.7	MG/100 G
VITAMIN A FROM CAROTENE	17800.	IU/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

BETA CAROTENE BY HPLC
AOAC International, Official Methods of Analysis (modified), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, VA (1995).
Quackenbush, F. W., Journal of Liquid Chromatography (modified), 10: 643-653 (1987).

Method listed above with results.

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REPORT OF ANALYSIS

COVANCE
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00306661

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

BATCH NUMBER: 00306659
DATE ENTERED: 03/30/00
REPORT PRINTED: 04/12/00

CARROTS: TACS-2-LOW, DAY 4, "A"
REC'D FROM SALAD TIME FARMS, SALINAS, CA (START DATE 3/30/00)

ASSAY	ANALYSIS	UNITS
BETA CAROTENE BY HPLC	10.6	MG/100 G
VITAMIN A FROM CAROTENE	17700.	IU/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

BETA CAROTENE BY HPLC
AOAC International, Official Methods of Analysis (modified), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, VA (1995).
Quackenbush, F. W., Journal of Liquid Chromatography (modified), 10: 643-653 (1987).

Method listed above with results.

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REPORT OF ANALYSIS

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00306662

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

BATCH NUMBER: 00306659
DATE ENTERED: 03/30/00
REPORT PRINTED: -/12/00

CARROTS: TACS-2-LOW, DAY 4, "B"
REC'D FROM SALAD TIME FARMS, SALINAS, CA (START DATE 3/30/00)

ASSAY	ANALYSIS	UNITS
BETA CAROTENE BY HPLC	10.4	MG/100 G
VITAMIN A FROM CAROTENE	17300.	IU/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

BETA CAROTENE BY HPLC
AOAC International, Official Methods of Analysis (modified), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, VA (1995).
Quackenbush, F. W., Journal of Liquid Chromatography (modified), 10: 643-653 (1987).

Method listed above with results.

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REPORT OF ANALYSIS

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00306663

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

BATCH NUMBER: 00306659
DATE ENTERED: 03/30/00
REPORT PRINTED: 04/12/00

CARROTS: TACS-2-MEDIUM, DAY 4, "A"
REC'D FROM SALAD TIME FARMS, SALINAS, CA (START DATE 3/30/00)

ASSAY	ANALYSIS	UNITS
BETA CAROTENE BY HPLC	10.3	MG/100 G
VITAMIN A FROM CAROTENE	17200.	IU/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

BETA CAROTENE BY HPLC
AOAC International, Official Methods of Analysis (modified), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, VA (1995).
Quackenbush, F. W., Journal of Liquid Chromatography (modified), 10: 643-653 (1987).

Method listed above with results.

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REPORT OF ANALYSIS

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00306664

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

BATCH NUMBER: 00306659

DATE ENTERED: 03/30/00

REPORT PRINTED: 04/12/00

CARROTS: TACS-2-MEDIUM, DAY 4, "B"
REC'D FROM SALAD TIME FARMS, SALINAS, CA (START DATE 3/30/00)

ASSAY	ANALYSIS	UNITS
BETA CAROTENE BY HPLC	10.6	MG/100 G
VITAMIN A FROM CAROTENE	17700.	IU/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

BETA CAROTENE BY HPLC
AOAC International, Official Methods of Analysis (modified), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, VA (1995).
Quackenbush, F. W., Journal of Liquid Chromatography (modified), 10: 643-653 (1987).

Method listed above with results.

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REPORT OF ANALYSIS

COVANCE
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00301981

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

BATCH NUMBER: 00301981

DATE ENTERED: 03/09/00

REPORT PRINTED: 03/20/00

CARROTS: TACS HIGH, DAY 4, "A"
REC'D FROM SALAD TIME FARMS, SALINAS, CA (START DAY 3/10)

ASSAY	ANALYSIS	UNITS
BETA CAROTENE BY HPLC	12.3	MG/100 G
VITAMIN A FROM CAROTENE	20500.	IU/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

BETA CAROTENE BY HPLC
AOAC International, Official Methods of Analysis (modified), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, VA (1995).
Quackenbush, F. W., Journal of Liquid Chromatography (modified), 10: 643-653 (1987).

Method listed above with results.

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REPORT OF ANALYSIS

COVANCE
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00301982

BATCH NUMBER: 00301981

DATE ENTERED: 03/09/00

REPORT PRINTED: 03/20/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

CARROTS: TACS HIGH, DAY 4, "B"
REC'D FROM SALAD TIME FARMS, SALINAS, CA (START DATE 3/10)

ASSAY	ANALYSIS	UNITS
BETA CAROTENE BY HPLC	11.3	MG/100 G
VITAMIN A FROM CAROTENE	18800.	IU/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

BETA CAROTENE BY HPLC
AOAC International, Official Methods of Analysis (modified), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, VA (1995).
Quackenbush, F. W., Journal of Liquid Chromatography (modified), 10: 643-653 (1987).

Method listed above with results.

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REPORT OF ANALYSIS

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00303476

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

BATCH NUMBER: 00303474

DATE ENTERED: 03/16/00

REPORT PRINTED: 04/12/00

CARROTS: TACS CONTROL, DAY 11, "A"
REC'D FROM SALAD TIME FARMS, SALINAS, CA (START DAY 3/16/00)

ASSAY	ANALYSIS	UNITS
BETA CAROTENE BY HPLC	8.88	MG/100 G
VITAMIN A FROM CAROTENE	14800.	IU/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

BETA CAROTENE BY HPLC
AOAC International, Official Methods of Analysis (modified), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, VA (1995).
Quackenbush, F. W., Journal of Liquid Chromatography (modified), 10: 643-653 (1987).

Method listed above with results.

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REPORT OF ANALYSIS

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00303477

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

BATCH NUMBER: 00303474

DATE ENTERED: 03/16/00

REPORT PRINTED: 04/12/00

CARROTS: TACS CONTROL, DAY 11, "B"
REC'D FROM SALAD TIME FARMS, SALINAS, CA (START DAY 3/16/00)

ASSAY	ANALYSIS	UNITS
BETA CAROTENE BY HPLC	8.56	MG/100 G
VITAMIN A FROM CAROTENE	14300.	IU/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

BETA CAROTENE BY HPLC
AOAC International, Official Methods of Analysis (modified), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, VA (1995).
Quackenbush, F. W., Journal of Liquid Chromatography (modified), 10: 643-653 (1987).

Method listed above with results.

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REPORT OF ANALYSIS

COVANCE
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00400711

BATCH NUMBER: 00400707

DATE ENTERED: 04/05/00

REPORT PRINTED: 04/13/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

CARROTS: TACS ZERO, DAY 11, "A"
REC'D FROM SALAD TIME FARMS, SALINAS, CA (START DAY 4/06)

ASSAY	ANALYSIS	UNITS
BETA CAROTENE BY HPLC	10.6	MG/100 G
VITAMIN A FROM CAROTENE	17700.	IU/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

BETA CAROTENE BY HPLC
AOAC International, Official Methods of Analysis (modified), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, VA (1995).
Quackenbush, F. W., Journal of Liquid Chromatography (modified), 10: 643-653 (1987).

Method listed above with results.

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REPORT OF ANALYSIS

COVANCE
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00400712

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

BATCH NUMBER: 00400707

DATE ENTERED: 04/05/00

REPORT PRINTED: 04/13/00

CARROTS: TACS ZERO, DAY 11, "B"
REC'D FROM SALAD TIME FARMS, SALINAS, CA (START DAY 4/06)

ASSAY	ANALYSIS	UNITS
BETA CAROTENE BY HPLC	10.2	MG/100 G
VITAMIN A FROM CAROTENE	17000.	IU/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

BETA CAROTENE BY HPLC
AOAC International, Official Methods of Analysis (modified), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, VA (1995).
Quackenbush, F. W., Journal of Liquid Chromatography (modified), 10: 643-653 (1987).

Method listed above with results.

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REPORT OF ANALYSIS

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00400707

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

BATCH NUMBER: 00400707

DATE ENTERED: 04/05/00

REPORT PRINTED: 04/13/00

CARROTS: TACS LOW, DAY 11, "A"
REC'D FROM SALAD TIME FARMS, SALINAS, CA (START DAY 4/06)

ASSAY	ANALYSIS	UNITS
BETA CAROTENE BY HPLC	9.97	MG/100 G
VITAMIN A FROM CAROTENE	16600.	IU/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

BETA CAROTENE BY HPLC
AOAC International, Official Methods of Analysis (modified), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, VA (1995).
Quackenbush, F. W., Journal of Liquid Chromatography (modified), 10: 643-653 (1987).

Method listed above with results.

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REPORT OF ANALYSIS

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00400708

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

BATCH NUMBER: 00400707

DATE ENTERED: 04/05/00

REPORT PRINTED: 04/13/00

CARROTS: TACS LOW, DAY 11, "B"
REC'D FROM SALAD TIME FARMS, SALINAS, CA (START DAY 4/06)

ASSAY	ANALYSIS	UNITS
BETA CAROTENE BY HPLC	10.7	MG/100 G
VITAMIN A FROM CAROTENE	17800.	IU/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

BETA CAROTENE BY HPLC
AOAC International, Official Methods of Analysis (modified), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, VA (1995).
Quackenbush, F. W., Journal of Liquid Chromatography (modified), 10: 643-653 (1987).

Method listed above with results.

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REPORT OF ANALYSIS

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00400709

BATCH NUMBER: 00400707

DATE ENTERED: 04/05/00

REPORT PRINTED: 04/20/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

CARROTS: TACS MEDIUM, DAY 11, "A"
REC'D FROM SALAD TIME FARMS, SALINAS, CA (START DAY 4/06)

ASSAY	ANALYSIS	UNITS
BETA CAROTENE BY HPLC	11.7	MG/100 G
VITAMIN A FROM CAROTENE	19500.	IU/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

BETA CAROTENE BY HPLC
AOAC International, Official Methods of Analysis (modified), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, VA (1995).
Quackenbush, F. W., Journal of Liquid Chromatography (modified), 10: 643-653 (1987).

Method listed above with results.

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REPORT OF ANALYSIS

COVANCE
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00400710

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

BATCH NUMBER: 00400707
DATE ENTERED: 04/05/00
REPORT PRINTED: 04/13/00

CARROTS: TACS MEDIUM, DAY 11, "B"
REC'D FROM SALAD TIME FARMS, SALINAS, CA (START DAY 4/06)

ASSAY	ANALYSIS	UNITS
BETA CAROTENE BY HPLC	10.7	MG/100 G
VITAMIN A FROM CAROTENE	17800.	IU/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

BETA CAROTENE BY HPLC
AOAC International, Official Methods of Analysis (modified), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, VA (1995).
Quackenbush, F. W., Journal of Liquid Chromatography (modified), 10: 643-653 (1987).

Method listed above with results.

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REPORT OF ANALYSIS

COVANCE
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00303474

BATCH NUMBER: 00303474

DATE ENTERED: 03/16/00

REPORT PRINTED: 04/12/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

CARROTS: TACS HIGH, DAY 11, "A"
REC'D FROM SALAD TIME FARMS, SALINAS, CA (START DAY 3/16/00)

ASSAY	ANALYSIS	UNITS
BETA CAROTENE BY HPLC	8.99	MG/100 G
VITAMIN A FROM CAROTENE	15000.	IU/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

BETA CAROTENE BY HPLC
AOAC International, Official Methods of Analysis (modified), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, VA (1995).
Quackenbush, F. W., Journal of Liquid Chromatography (modified), 10: 643-653 (1987).

Method listed above with results.

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REPORT OF ANALYSIS

COVANCE
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00303475

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

BATCH NUMBER: 00303474
DATE ENTERED: 03/16/00
REPORT PRINTED: 04/12/00

CARROTS: TACS HIGH, DAY 11, "B"
REC'D FROM SALAD TIME FARMS, SALINAS, CA (START DAY 3/16/00)

ASSAY	ANALYSIS	UNITS
BETA CAROTENE BY HPLC	9.12	MG/100 G
VITAMIN A FROM CAROTENE	15200.	IU/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

BETA CAROTENE BY HPLC
AOAC International, Official Methods of Analysis (modified), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, VA (1995).
Quackenbush, F. W., Journal of Liquid Chromatography (modified), 10: 643-653 (1987).

Method listed above with results.

Covance Laboratories Inc.
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Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

REPORT OF ANALYSIS

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00304987

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

BATCH NUMBER: 00304985
DATE ENTERED: 03/23/00
REPORT PRINTED: 04/12/00

CARROTS: TACS CONTROL, DAY 18, "A"
REC'D FROM SALAD TIME FARMS, SALINAS, CA (START DATE 3/23)

ASSAY	ANALYSIS	UNITS
BETA CAROTENE BY HPLC	10.9	MG/100 G
VITAMIN A FROM CAROTENE	18200.	IU/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

BETA CAROTENE BY HPLC
AOAC International, Official Methods of Analysis (modified), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, VA (1995).
Quackenbush, F. W., Journal of Liquid Chromatography (modified), 10: 643-653 (1987).

Method listed above with results.

Covance Laboratories Inc.
P.O. Box 7545
Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

REPORT OF ANALYSIS

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00304988

BATCH NUMBER: 00304985

DATE ENTERED: 03/23/00

REPORT PRINTED: 04/12/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

CARROTS: TACS CONTROL, DAY 18, "B"
REC'D FROM SALAD TIME FARMS, SALINAS, CA (START DATE 3/23)

ASSAY	ANALYSIS	UNITS
BETA CAROTENE BY HPLC	11.5	MG/100 G
VITAMIN A FROM CAROTENE	19200.	IU/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

BETA CAROTENE BY HPLC
AOAC International, Official Methods of Analysis (modified), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, VA (1995).
Quackenbush, F. W., Journal of Liquid Chromatography (modified), 10: 643-653 (1987).

Method listed above with results.

REPORT OF ANALYSIS

SAMPLE NUMBER: 00402438
BATCH NUMBER: 00402434
DATE ENTERED: 04/13/00
REPORT PRINTED: 04/27/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

CARROTS: TACS ZERO, DAY 18, "A"
REC'D FROM SALAD TIME FARMS, SALINAS, CA (START DATE 4/13)

ASSAY	ANALYSIS	UNITS
BETA CAROTENE BY HPLC	11.0	MG/100 G
VITAMIN A FROM CAROTENE	18300.	IU/100 G

THIS IS A PARTIAL REPORT.
WHEN ALL ANALYSES ARE COMPLETED, YOU WILL RECEIVE A COMPLETE REPORT.
THE FOLLOWING ANALYSES ARE NOT COMPLETE:

METHOD REFERENCES

BETA CAROTENE BY HPLC
AOAC International, Official Methods of Analysis (modified), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, VA (1995).
Quackenbush, F. W., Journal of Liquid Chromatography (modified), 10: 643-653 (1987).

000708

REPORT OF ANALYSIS

SAMPLE NUMBER: 00402439
BATCH NUMBER: 00402434
DATE ENTERED: 04/13/00
REPORT PRINTED: 04/27/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

CARROTS: TACS ZERO, DAY 18, "B"
REC'D FROM SALAD TIME FARMS, SALINAS, CA (START DATE 4/13)

ASSAY	ANALYSIS	UNITS
BETA CAROTENE BY HPLC	11.0	MG/100 G
VITAMIN A FROM CAROTENE	18300.	IU/100 G

THIS IS A PARTIAL REPORT.
WHEN ALL ANALYSES ARE COMPLETED, YOU WILL RECEIVE A COMPLETE REPORT.
THE FOLLOWING ANALYSES ARE NOT COMPLETE:

METHOD REFERENCES

BETA CAROTENE BY HPLC
AOAC International, Official Methods of Analysis (modified), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, VA (1995).
Quackenbush, F. W., Journal of Liquid Chromatography (modified), 10: 643-653 (1987).

REPORT OF ANALYSIS

SAMPLE NUMBER: 00402434
BATCH NUMBER: 00402434
DATE ENTERED: 04/13/00
REPORT PRINTED: 04/27/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

CARROTS: TACS LOW, DAY 18, "A"
REC'D FROM SALAD TIME FARMS, SALINAS, CA (START DATE 4/13)

ASSAY	ANALYSIS	UNITS
BETA CAROTENE BY HPLC	11.7	MG/100 G
VITAMIN A FROM CAROTENE	19500.	IU/100 G

THIS IS A PARTIAL REPORT.
WHEN ALL ANALYSES ARE COMPLETED, YOU WILL RECEIVE A COMPLETE REPORT.
THE FOLLOWING ANALYSES ARE NOT COMPLETE:

METHOD REFERENCES

BETA CAROTENE BY HPLC
AOAC International, Official Methods of Analysis (modified), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, VA (1995).
Quackenbush, F. W., Journal of Liquid Chromatography (modified), 10: 643-653 (1987).

REPORT OF ANALYSIS

SAMPLE NUMBER: 00402435
BATCH NUMBER: 00402434
DATE ENTERED: 04/13/00
REPORT PRINTED: 04/27/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

CARROTS: TACS LOW, DAY 18, "B"
REC'D FROM SALAD TIME FARMS, SALINAS, CA (START DATE 4/13)

ASSAY	ANALYSIS	UNITS
BETA CAROTENE BY HPLC	10.0	MG/100 G
VITAMIN A FROM CAROTENE	16700.	IU/100 G

THIS IS A PARTIAL REPORT.
WHEN ALL ANALYSES ARE COMPLETED, YOU WILL RECEIVE A COMPLETE REPORT.
THE FOLLOWING ANALYSES ARE NOT COMPLETE:

METHOD REFERENCES

BETA CAROTENE BY HPLC
AOAC International, Official Methods of Analysis (modified), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, VA (1995).
Quackenbush, F. W., Journal of Liquid Chromatography (modified), 10: 643-653 (1987).

REPORT OF ANALYSIS

SAMPLE NUMBER: 00402436
BATCH NUMBER: 00402434
DATE ENTERED: 04/13/00
REPORT PRINTED: 04/27/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

CARROTS: TACS MEDIUM, DAY 18, "A"
REC'D FROM SALAD TIME FARMS, SALINAS, CA (START DATE 4/13)

ASSAY	ANALYSIS	UNITS
BETA CAROTENE BY HPLC	10.4	MG/100 G
VITAMIN A FROM CAROTENE	17300.	IU/100 G

THIS IS A PARTIAL REPORT.
WHEN ALL ANALYSES ARE COMPLETED, YOU WILL RECEIVE A COMPLETE REPORT.
THE FOLLOWING ANALYSES ARE NOT COMPLETE:

METHOD REFERENCES

BETA CAROTENE BY HPLC
AOAC International, Official Methods of Analysis (modified), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, VA (1995).
Quackenbush, F. W., Journal of Liquid Chromatography (modified), 10: 643-653 (1987).

REPORT OF ANALYSIS

SAMPLE NUMBER: 00402437
BATCH NUMBER: 00402434
DATE ENTERED: 04/13/00
REPORT PRINTED: 04/27/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

CARROTS: TACS MEDIUM, DAY 18, "B"
REC'D FROM SALAD TIME FARMS, SALINAS, CA (START DATE 4/13)

ASSAY	ANALYSIS	UNITS
BETA CAROTENE BY HPLC	11.0	MG/100 G
VITAMIN A FROM CAROTENE	18300.	IU/100 G

THIS IS A PARTIAL REPORT.
WHEN ALL ANALYSES ARE COMPLETED, YOU WILL RECEIVE A COMPLETE REPORT.
THE FOLLOWING ANALYSES ARE NOT COMPLETE:

METHOD REFERENCES

BETA CAROTENE BY HPLC
AOAC International, Official Methods of Analysis (modified), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, VA (1995).
Quackenbush, F. W., Journal of Liquid Chromatography (modified), 10: 643-653 (1987).

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REPORT OF ANALYSIS

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00304985

BATCH NUMBER: 00304985

DATE ENTERED: 03/23/00

REPORT PRINTED: 04/12/00

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
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CHICAGO, IL 60611

CARROTS: TACS HIGH, DAY 18, "A"
REC'D FROM SALAD TIME FARMS, SALINAS, CA (START DATE 3/23)

ASSAY	ANALYSIS	UNITS
BETA CAROTENE BY HPLC	10.2	MG/100 G
VITAMIN A FROM CAROTENE	17000.	IU/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

BETA CAROTENE BY HPLC
AOAC International, Official Methods of Analysis (modified), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, VA (1995).
Quackenbush, F. W., Journal of Liquid Chromatography (modified), 10: 643-653 (1987).

Method listed above with results.

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REPORT OF ANALYSIS

COVANCE
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00304986

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

BATCH NUMBER: 00304985

DATE ENTERED: 03/23/00

REPORT PRINTED: 04/12/00

CARROTS: TACS HIGH, DAY 18, "B"
REC'D FROM SALAD TIME FARMS, SALINAS, CA (START DATE 3/23)

ASSAY	ANALYSIS	UNITS
BETA CAROTENE BY HPLC	9.77	MG/100 G
VITAMIN A FROM CAROTENE	16300.	IU/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

BETA CAROTENE BY HPLC
AOAC International, Official Methods of Analysis (modified), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, VA (1995).
Quackenbush, F. W., Journal of Liquid Chromatography (modified), 10: 643-653 (1987).

Method listed above with results.

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Tel: 608/241-4471 Fax: 608/241-7227

REPORT OF ANALYSIS

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00306654

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

BATCH NUMBER: 00306652

DATE ENTERED: 03/30/00

REPORT PRINTED: 04/12/00

CARROTS: TACS CONTROL, DAY 24, "A"
REC'D FROM SALAD TIME FARMS, SALINAS, CA (START DATE 3/30/00)

ASSAY	ANALYSIS	UNITS
BETA CAROTENE BY HPLC	10.3	MG/100 G
VITAMIN A FROM CAROTENE	17200.	IU/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

BETA CAROTENE BY HPLC
AOAC International, Official Methods of Analysis (modified), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, VA (1995).
Quackenbush, F. W., Journal of Liquid Chromatography (modified), 10: 643-653 (1987).

Method listed above with results.

Covance Laboratories Inc.
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Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

REPORT OF ANALYSIS

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00306655

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

BATCH NUMBER: 00306652
DATE ENTERED: 03/30/00
REPORT PRINTED: 04/12/00

CARROTS: TACS CONTROL, DAY 24, "B"
REC'D FROM SALAD TIME FARMS, SALINAS, CA (START DATE 3/30/00)

ASSAY	ANALYSIS	UNITS
BETA CAROTENE BY HPLC	10.6	MG/100 G
VITAMIN A FROM CAROTENE	17700.	IU/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

BETA CAROTENE BY HPLC
AOAC International, Official Methods of Analysis (modified), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, VA (1995).
Quackenbush, F. W., Journal of Liquid Chromatography (modified), 10: 643-653 (1987).

Method listed above with results.

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Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

REPORT OF ANALYSIS

COVANCE
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00306652

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

BATCH NUMBER: 00306652

DATE ENTERED: 03/30/00

REPORT PRINTED: 04/12/00

CARROTS: TACS HIGH, DAY 24, "A"
REC'D FROM SALAD TIME FARMS, SALINAS, CA (START DATE 3/30/00)

ASSAY	ANALYSIS	UNITS
BETA CAROTENE BY HPLC	10.8	MG/100 G
VITAMIN A FROM CAROTENE	18000.	IU/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

BETA CAROTENE BY HPLC
AOAC International, Official Methods of Analysis (modified), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, VA (1995).
Quackenbush, F. W., Journal of Liquid Chromatography (modified), 10: 643-653 (1987).

Method listed above with results.

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Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

REPORT OF ANALYSIS

COVANCE
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 00306653

PETER GRAY
BERNARD TECHNOLOGIES, INCORPORATED
919 NORTH MICHIGAN AVENUE
SUITE 1710
CHICAGO, IL 60611

BATCH NUMBER: 00306652

DATE ENTERED: 03/30/00

REPORT PRINTED: 04/12/00

CARROTS: TACS HIGH, DAY 24, "B"
REC'D FROM SALAD TIME FARMS, SALINAS, CA (START DATE 3/30/00)

ASSAY	ANALYSIS	UNITS
BETA CAROTENE BY HPLC	10.0	MG/100 G
VITAMIN A FROM CAROTENE	16700.	IU/100 G

CHLORITE

NO CHLORITE DETECTED AT 0.1 %

METHOD REFERENCE

CLIENT SUPPLIED METHOD.

METHOD REFERENCES

BETA CAROTENE BY HPLC
AOAC International, Official Methods of Analysis (modified), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, VA (1995).
Quackenbush, F. W., Journal of Liquid Chromatography (modified), 10: 643-653 (1987).

Method listed above with results.

TR BTI # 00-11 (E)

Protocol IBP-RCB-1-0400

A QUALITATIVE COMPARATIVE STUDY OF PRIMAL CUTS OF BEEF STORED IN POLYETHYLENE BAGS CONTAINING EITHER NO ADDITIVES OR BTI PROPRIETARY TECHNOLOGY

1. Protocol
2. Report from Beef Processor
3. Table of TBA Values (within report)
4. Graphs of TBA Values
5. Results of Chlorite Anion Analyses

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PHONE 312.337.7007 · FACSIMILE 312.337.0505

Protocol IBP-RCB-1-0400

A QUALITATIVE COMPARATIVE STUDY OF PRIMAL CUTS OF BEEF STORED IN POLYETHYLENE BAGS CONTAINING EITHER NO ADDITIVES OR BTI PROPRIETARY TECHNOLOGY

Objective: To determine any differences in selected characteristics of primal cuts of beef packaged in polyethylene (LDPE) bags or LDPE bags containing BTI proprietary technology.

Materials:

Primal cuts of beef prepared by IBP

1. Whole pieces of raw primal cuts of beef

Packaging film/bags (approx. 12" x 15")

1. LDPE bags without active additives
2. LDPE bags containing BTI additive at low %
3. LDPE bags containing BTI additive at medium %
4. LDPE bags containing BTI additive at high %

Packages:

1. One piece (approx. 1 pound) per package for chlorite analyses
2. Seven core samples per package for TBA and sensory testing
3. Storage Periods: 0 (8 hours), 4, 8, 16 days
4. Test bags heat sealed and placed in larger over-bags.

Analyses as listed in attached chart (Page 2):

1. Food evaluation characteristics to be conducted by IBP; Project Contact is Miyuki Castillo; Test and Analytical Protocols to be provided to BTI by IBP.
2. Chemical analyses to be conducted by Covance, Madison, WI; Project Contact is Wayne Ellefson.

Schedule:

- Day 0 — Prepare raw primal cuts of beef
- Day 0 — Package in test bags with labels and store as indicated

Samples for Covance may be frozen and all shipped at once after Day 16.

- Day 0 — Test Labs conduct analyses as DAY 0 samples
- Day 4 — Test Labs conduct analyses as DAY 4 samples
- Day 8 — Test Labs conduct analyses as DAY 8 samples
- Day 16 — Test Labs conduct analyses as DAY 16 samples
- Day -- — Final Reports from IBP and Covance

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Analyses:

Samples from each treatment will be analyzed for, color, odor, TBA and residual chlorite on each of the following days: 0, 4, 8,16.

Color and Odor by IBP Labs:

If possible, color and odor comments will be obtained for each sample prior to the collection for. Color change may be documented using a digital camera.

TBA Analyses:

TBA analyses will be conducted on duplicate samples by IBP Labs

Residual Chlorite:

Samples will be shipped frozen to COVANCE lab for residual chlorite analysis.

Statistical Analysis:

Appropriate statistical analysis will be performed to detect any significant differences between treatments.

Chlorine Dioxide and Chlorite Anions: Standard Methods for Water Analysis Method 4500-ClO2 B. Iodometric Method. APHA, AWWA, and WPCF. Washington, D. C. (17th ed.) Pages 4-54 (modified as Southwest Research Institute BTI-SOP# 99-02. Aieta, E. M., P. V. Roberts, and M. Hernandez, J. Am. Water Works Assoc., 76(1), 64-70 (1984).

TBA: AOAC Method, see Methods section.

Day	
0	Collect baseline data on test and control products
1 - 16	Store samples at 38°F
4	Sample: color, odor, TBA, residual chlorite
8	Sample: color, odor, TBA, residual chlorite
16	Sample: color, odor, TBA, residual chlorite

RESULTS

Sensory: The CRT did not alter the sensory characteristics of raw beef muscle meat and associated fat, including color and odor for bags containing the low and medium levels of

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CRT. The meat packaged with the "high" bags showed a greenish tint after a few days, indicating oxidation of the endogenous myoglobin. The CRT did not alter the "spoiled" odor after 8 days. This odor is important to consumers in order to make appropriate buying decisions.

Nutrients: The TBA values, a test for fat oxidation and rancidity, were comparable to the control packaged red muscle meat.

Chlorite: Chlorite residues were not detected. The cooking process would destroy any residual chlorite.

CONCLUSIONS

Packaging materials containing the Microsphère® CRT at levels of loading up to 40% may be used for the storage of fresh red muscle meat without demonstrating any negative qualitative effects for at least 8 days.

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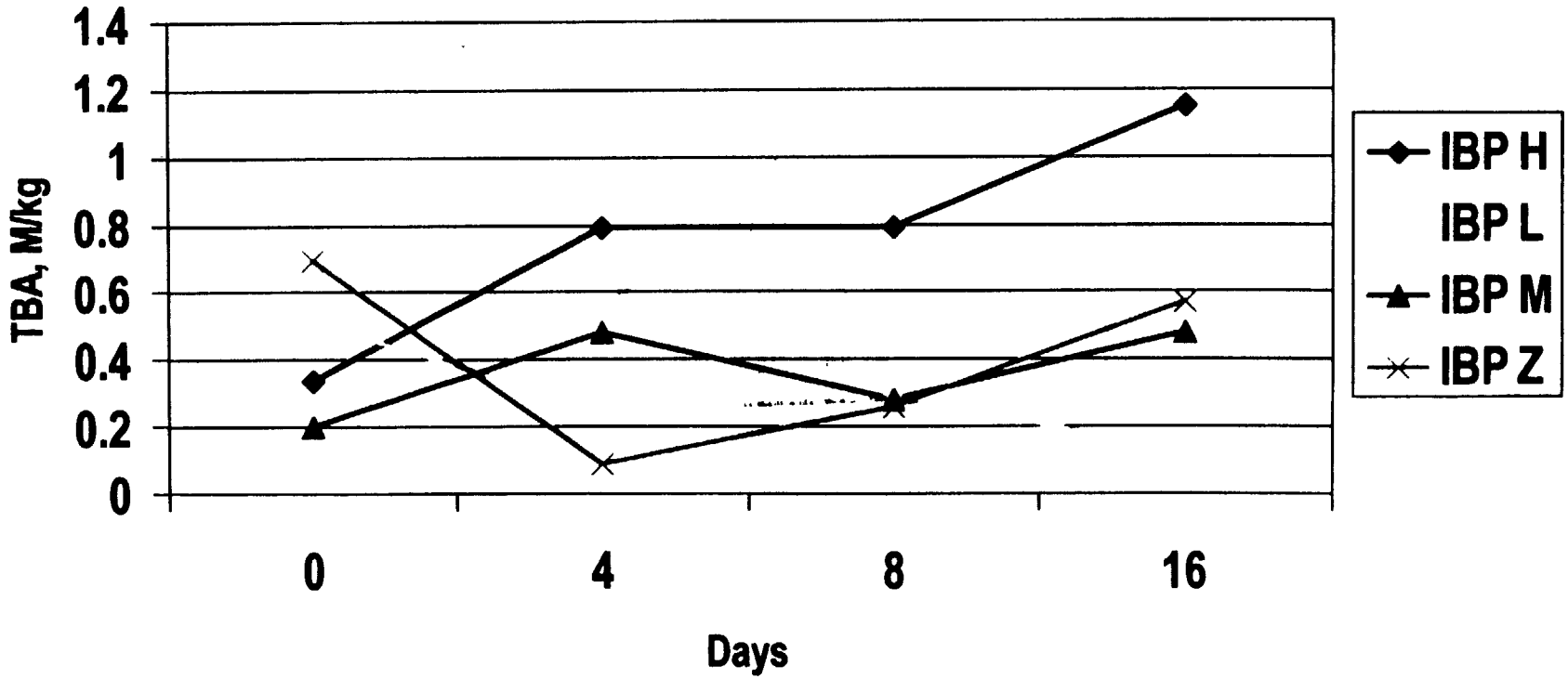
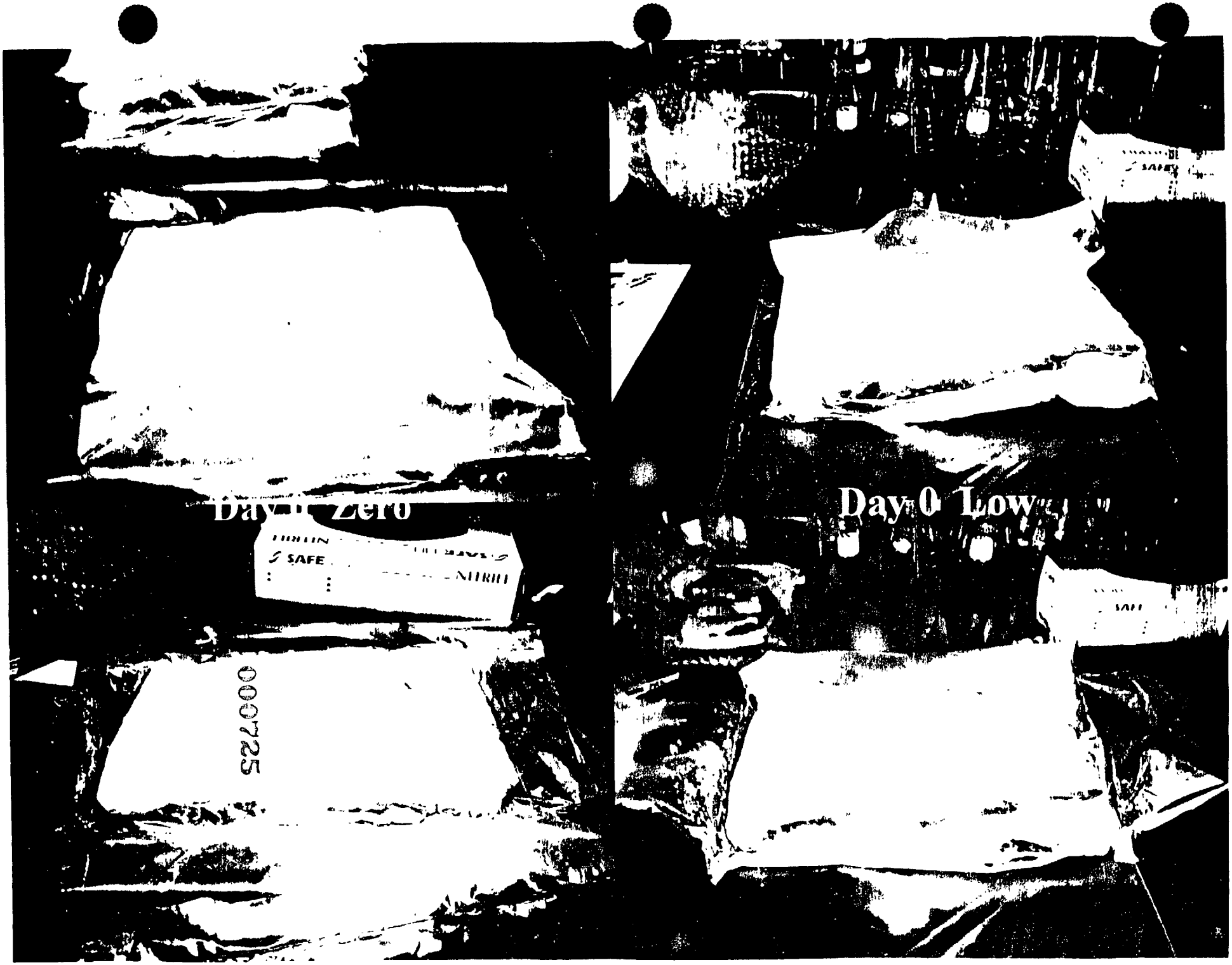
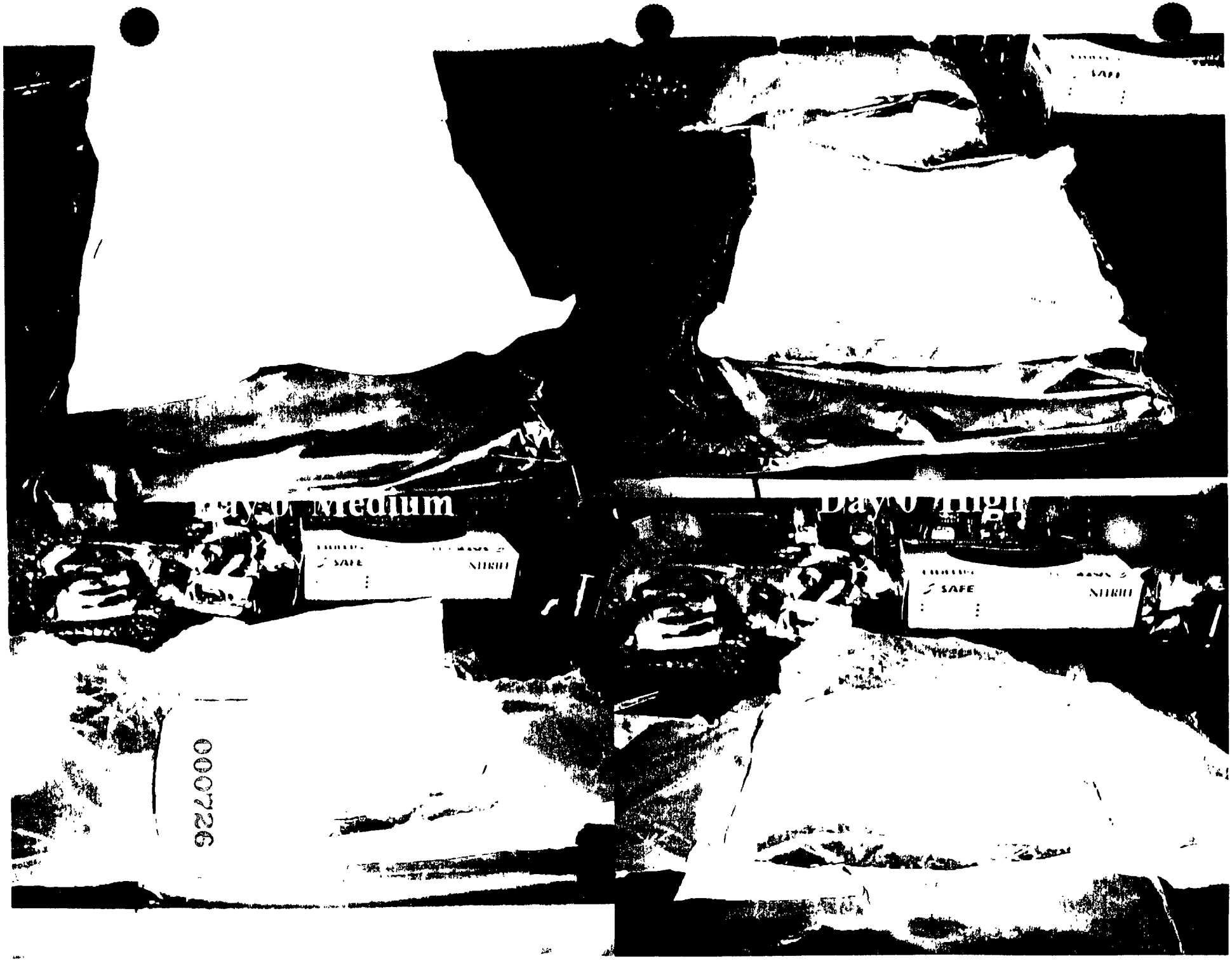
QUALITATIVE COMPARATIVE STUDY OF BEEF PRIMAL CUT (PLATE) STORED IN POLYETHYLENE BAGS CONTAINING EITHER NO ADDITIVE OR BTI PROPERTIES.



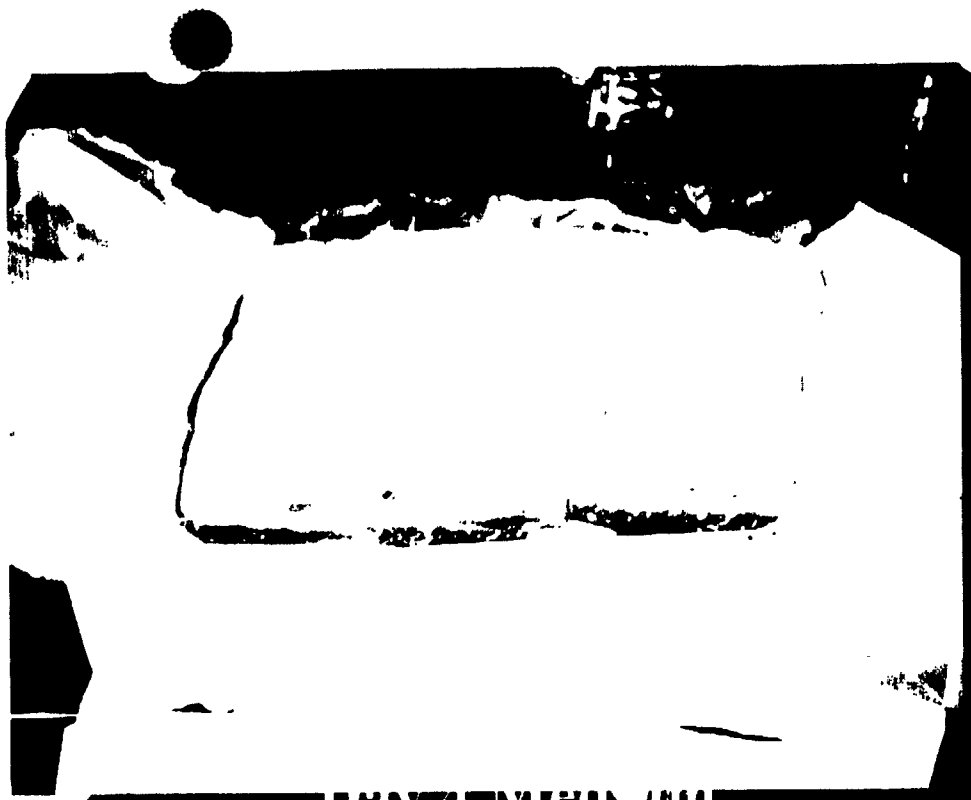
Confidential, Proprietary, Trade
Secret of IBP, Inc.

0007224

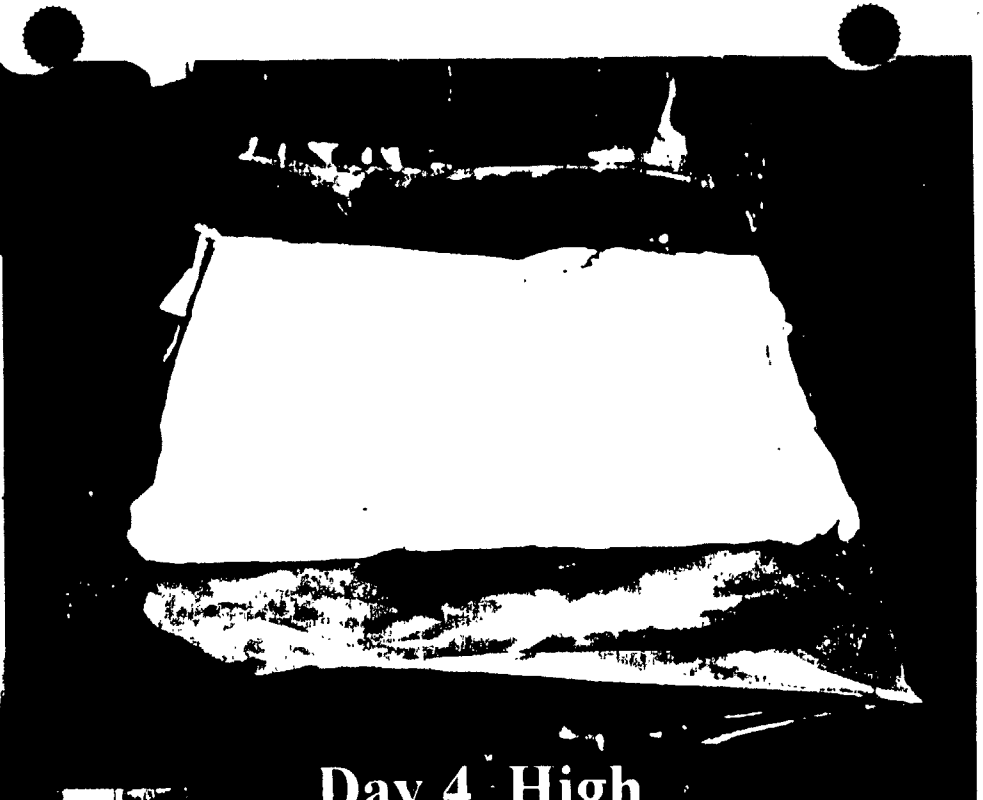

Day 0 Zero
SAFE
0007725
Day 0 Low


Medium
SAFE
NIGHT
000726
SAFE
NIGHT

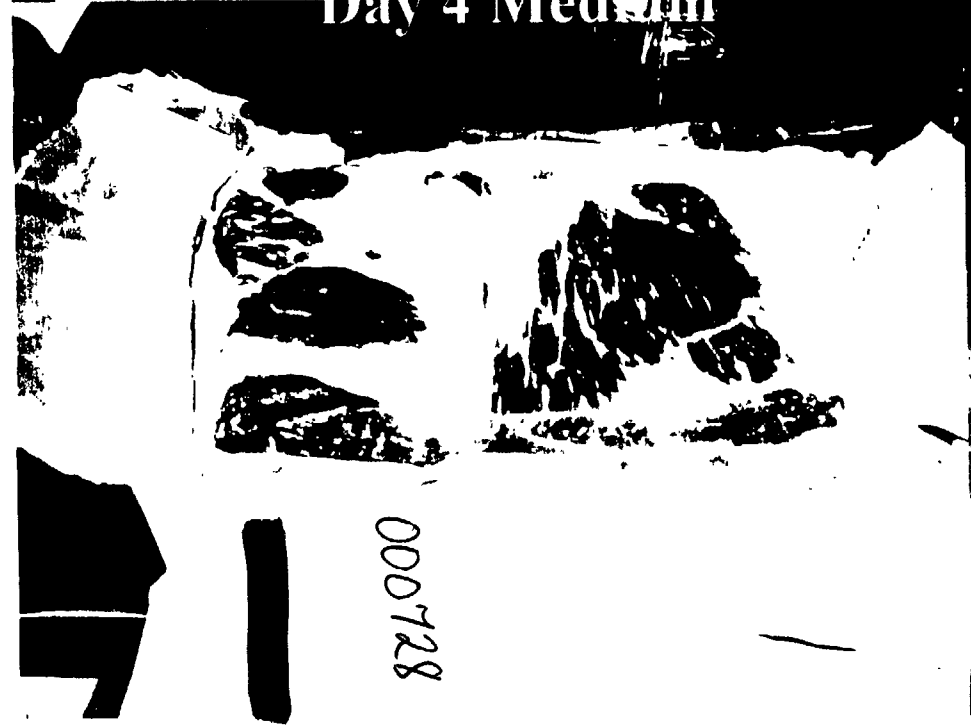
4 Low
0007227

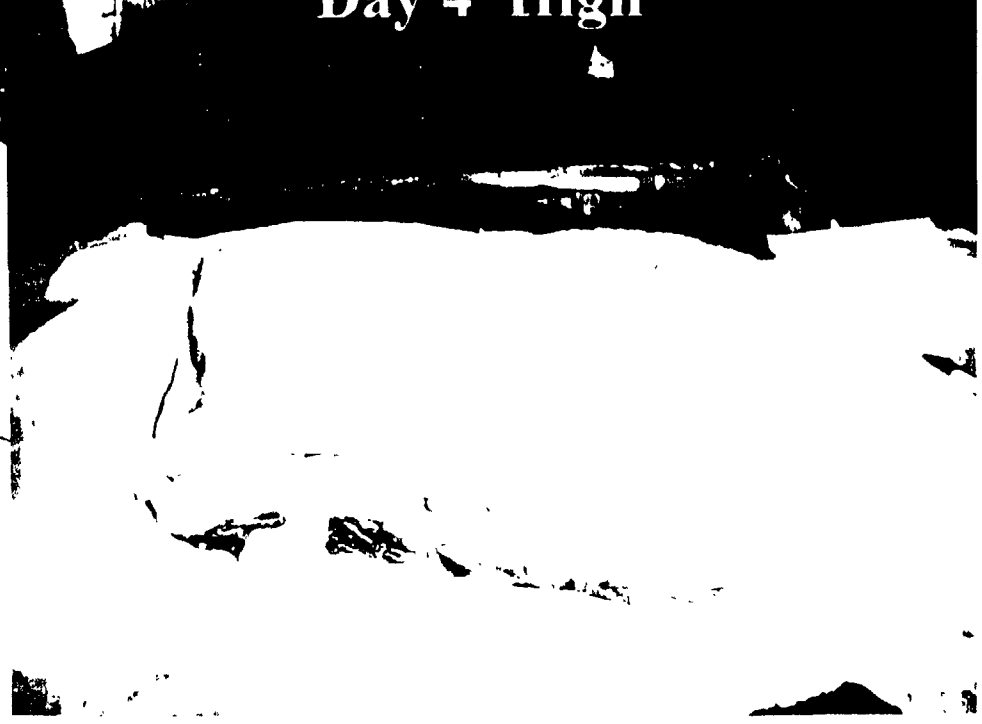


Day 4 Medium



Day 4 High





000728

000729

000730

Fresh Meats
Food Safety ♦ QA ♦ Regulatory

IBP, Inc.
World Headquarters
800 Stevens Port Dr.
Dakota Dunes, SD 57409

Phone: 605-235-3780
Fax: 605-235-3642
Email: miyuki.castillo@ibpinc.com

DATE: May 17, 2000

TO: Sharon Beals, Dean Danilson

FROM: Miyuki Y. Castillo

SUBJECT: Qualitative Comparative Study of Beef Primal Cut (Plate) Stored in Polyethylene Bags Containing Either No Additives or BTI Properties Summary.

The test sampling was conducted April 6 – 22. The test was conducted over 16 days.

Protocol Summary

- Randomly select 6 whole plates of fresh beef and cut into 16 pieces approximately equal in weight and size.
- Vacuum-seal bags and place in an outer bag
- Sample product will be held at temperature of 36-38°F.
- Evaluate color and odor. If any color change occurs, change may be documented by using a digital camera.
- Sterile gloves are to be used and must be changed between each sample. Core sampler must be sanitized using either a 180°F sterilizer or a chemical sanitizer.
- Seven cores will be collected, 2 for sensory observations and 5 for TBA analysis, and placed in separate identified whirl-pak bags.
- Half-pound samples will be collected for the residual chlorite analysis and place in individual identified whirl-pak bags. Residual chlorite samples will be pulled on the corresponding dates and frozen until shipping to Covance Laboratories in Wisconsin.

Materials:

- LPDE bags without active additives, LPDE bags containing BTI additives at low %, LPDE bags containing BTI medium % and LPDE bags containing BTI additives at high %. (Bag size: 12" x 15"). (The particular dosages were not made known to the test administrator.
- Whole pieces of raw primal cut of beef (Plate)
- Sterile gloves
- Sterile sample collection bags (Whirlpak)
- Hook, core sampler (11 cm^2) and disposal scalpels.

000731

Ethanol Torch

Laboratory Procedure: Each sampling day, the individual packaged beef cuts were aseptically opened and the beef tissues excised and analyzed in an IBP laboratory. AOAC Analytical Methods were used to conduct TBA analyses.

Data Summary

Bag ID IBP H	TBA M/kg
Day 0	0.34
Day 4	0.79
Day 8	0.79
Day 16	1.15
Std. Dev.	0.3317

Bag ID IBP L	TBA M/kg
Day 0	0.51
Day 4	0.25
Day 8	0.28
Day 16	0.13
Std. Dev.	0.1588

Bag ID IBP M	TBA M/kg
Day 0	0.20
Day 4	0.48
Day 8	0.28
Day 16	0.48
Std. Dev.	0.1424

Bag ID IBP Z	TBA M/kg
Day 0	0.70
Day 4	0.09
Day 8	0.26
Day 16	0.57
Std. Dev.	0.2796

Z= Zero or Control Bag, H = High, M = Medium, L = Low
Day 0 is approximately eight hours following packaging.

Physical Observation Summary

Day 0
- Stored at 38°F, chlorite samples being stored at –11°F
- M & H Bags were harder to handle and were harder to seal.
- No color change (see pictures), no odors
- M and H Bags, contained more purge (juice) than the other two bags.

Day 4
- Stored at 38°F, chlorite samples stored at –11°F
- H and M Bags areas were slightly discolored having a light greenish appearance.
- H and M Bags overall surface of the meat appeared slightly slimy
- H Bag gave a faint chlorine-type odor at opening. No odors were detected in the other samples.
- Z Bags meat did not show any differences from normal experiences.

Day 8
- Stored at 38°F, chlorite samples stored at–11°F
- H and M Bags areas had a slightly greenish tint; surface lean was darker than the interior lean.
- H and M Bags overall surface of the meat appeared slimy.
- H, M, L Bags exhibited purge in the bag.
- No odors were detected from the H Bag or the other samples.
- Z Bags displayed slight lean discoloration. (No green tones)

Day 16
- Stored at 38°F, chlorite samples stored at –11°F
- Z and L Bags upon opening, gave out a sour smell, typical of usual aging and spoilage
- Z Bag displayed discoloration. (No green tone)
- L Bag overall surface of the meat was slimy.
- H, M, L Bags exhibited purge in the bag.
- H an M Bags had a faint sour smell, but less than the Z and L Bags
- H and M Bags displayed extreme discoloration, surface lean/fat was greenish
- L Bags meat was starting to develop a greenish tint.

Conclusion

The objective of this test was to treat beef plate cuts with three different treated polyethylene bags and one control. The film was designed to deliver different dosage rates of chlorine dioxide when in contact with the meat. The particular dosages were not made known to the test administrator.

During packing of the plate cuts, problems were experienced obtaining an acceptable seal on some bags marked H and M. Numerous attempts were made until finally obtaining an acceptable seal. Test bags and control bags were subsequently bagged into non-treated bags that were not sealed.

Test storage during this test was kept at a constant 38°F for the sixteen day period. The TBA values increased during storage but remained below 1.0 in spite of the fat content of the beef cut and all were considered to be within the normal range of acceptable levels.

The color in the beef cuts began to deteriorate after 2 days of storage in the packages labeled H and M. The changes in color were determined by comparing the control sample to the treated samples. The green color that formed on the surface of the beef cut was common to the fat and lean. As storage time increased a slimy appearance along with the green coloration was noted to increase. There was a sour odor experienced, albeit at different degrees at the last day of storage. This can be attributed to the growth of spoilage bacteria. A faint odor of chlorine was experienced during day 4 in the bag labeled H; however, all other bags and subsequent samples of the H Bags did not yield any chlorine – type odors.

PROJECT REPORT

PROTOCOL NUMBER: TACS-1-1199

A QUALITATIVE COMPARATIVE STUDY OF SHREDDED CARROTS STORED IN POLYETHYLENE BAGS CONTAINING EITHER NO ADDITIVIE OR BTI PROPRIETARY TECHNOLOGY

Prepared by: Peter N. Gray, Ph.D.

Bernàrd Technologies, Inc.
919 North Michigan Avenue
Chicago, IL 60611

A QUALITATIVE COMPARATIVE STUDY OF CARROTS STORED IN POLYETHYLENE BAGS CONTAINING EITHER NO ADDITIVES OR BTI PROPRIETARY TECHNOLOGY

Objective: To determine any differences in selected characteristics of carrots packaged in polyethylene (LDPE) bags or LDPE bags containing BTI proprietary technology.

Materials:

Shredded Carrots prepared by Salad Time Farms, Tanimura & Antle

Packaging film/bags incorporating BTI's Microlite™ technology.

1. LDPE opaque bags without active additives (0%)
2. LDPE opaque bags containing BTI additive at low % (2%)
3. LDPE opaque bags containing BTI additive at medium % (5%)
4. LDPE opaque bags containing BTI additive at high % (10%)

Packages:

1. 10 oz of shredded carrots per package
2. Six (6) replicates per sample type (4for T & A, 2 for Covance)
3. Total Packages: 144 (4 types of film)*(6 replicates/type)*(6 periods)*(1 carrot type)

Storage Conditions:

1. Pack bags with test food at 36°F to 38°F
2. Store packed bags at 36°F to 38°F
3. Ship samples to Covance via FedEx with cool packs in the boxes
4. Covance to freeze samples until analyses performed

Analyses as listed in attached chart:

1. Food evaluation characteristics to be conducted by Tanimura & Antle, Salinas, CA;
2. Chemical analyses to be conducted by Covance, Madison, WI

Schedule:

- NOV 5 FRIDAY Prepare Shredded Carrots
- NOV 5 FRIDAY Package in test bags with labels and store as indicated
- NOV 8 MONDAY Ship bags containing carrots to test labs
- NOV 9 TUESDAY Test Labs conduct analyses as DAY 4 samples
- NOV 16 TUESDAY Test Labs conduct analyses as DAY 11 samples
- NOV 23 TUESDAY Test Labs conduct analyses as DAY 18 samples
- NOV 29 MONDAY Test Labs conduct analyses as DAY 24 samples
- DEC 1 WEDNESDAY Preliminary Report of Results
- DEC 3 FRIDAY Final Reports from Covance and Tanimura & Antle

Tests to be performed by Tanimura & Antle

- Visual assessment
- Odor of raw carrots
- Texture
- Color
- Total Plate Count (TPC)
- Coliform Bacteria

Tests to be performed by COVANCE

- Residual chlorite
- Beta-carotene

Activities:

Shredded carrots were prepared by Salad Time Farms (T&A) 9:30 a.m.
10 oz. of shredded carrots were placed in each bag, air pressed from bag and the bag sealed
All bags completed by 11:00 a.m.
Bags were placed on edge, one level deep in plastic trays
The trays were placed on the top shelf of racks in the QC area of the plant
Temperature was 36 to 38 degrees F
Lighting came from ceiling fixtures approximately 12 feet above the top shelf
Light meter readings were approximately 30 milliwatts
Bag were exposed on edge for 24 hours and then turned over
Bags were exposed on reverse side for 48 hours
Duplicate samples were sent to COVANCE Laboratories via Federal Express on Monday, Nov. 8

T & A will provide freshly shredded carrots on Monday and include them in the package for Covance to provide a Day 1 baseline sample.

Carrots received by COVANCE on Tuesday, November 9th.
First samples analyzed.
Remaining carrots stored in freezer.

Contacts:

Company	Person	Telephone	Facsimile	Email
McDonald's	Bruce Feinberg	630-623-3120	630-623-5521	Bruce.feinberg@mcd.com
BTI	Peter N. Gray	312-337-7007	312-337-0505	btipng@ibm.net
Tanimura & Antle	Leonard Batti Gurmail Mudahar	831-455-2950	831-455-3994	Leonard@taproduce.com Gurmail@taproduce.com
COVANCE	Wayne Ellefson	608-241-7213	608-241-7227	wayne.ellefson@covance.com

Tanimura & Antle
Salad Time Farms, Inc.
1400 Schilling Place
Salinas, CA 93901

To: Gurmail Mudahar, Jim Lipman
From: Daniel Mesa
Subject: Carrot Study
Date:12/28/99

Report of Study Conducted in Collaboration with Bernàrd Technologies, Inc.

Protocol Number: TACS-1-1199

SMELL, TASTE, ODOR AND VISUAL OBSERVATION:

Tanimura & Antle using their standard methods conducted the evaluation of shredded carrots packaged as presented in Protocol Number TACS-1-1199 (see attached). Evaluations were made of the treated samples as compared to the non-treated samples. Any differences of note would have been reported.

In the smell, taste, odor, and visual observation all of this factors were ok until the 21st day. At the 21st day the taste on the carrots was not as fresh and the flavor was almost gone.

Report submitted by: _____ _____
Signature Printed Name

Tanimura & Antle
Salad Time Farms, Inc.
1400 Schilling Place
Salinas, CA 93901

To: Gurmail Mudahar, Jim Lipman
From: Daniel Mesa
Subject: Carrot Study
Date:12/28/99

Report of Study Conducted in Collaboration with Bernàrd Technologies, Inc.

Protocol Number: TACS-1-1199

MICROBIOLOGICAL EVALUATION OF SAMPLES FROM PROTOCOL NUMBER: TACS-1-1199:

Shredded carrots packaged as described in Protocol Number TACS-1-1199 were sampled on the test days described in the Table below. Replicate samples were plated using the standard microbiological methods used in the Tanimura & Antle laboratories.

The amount of material analyzed from each sample was _____ grams.

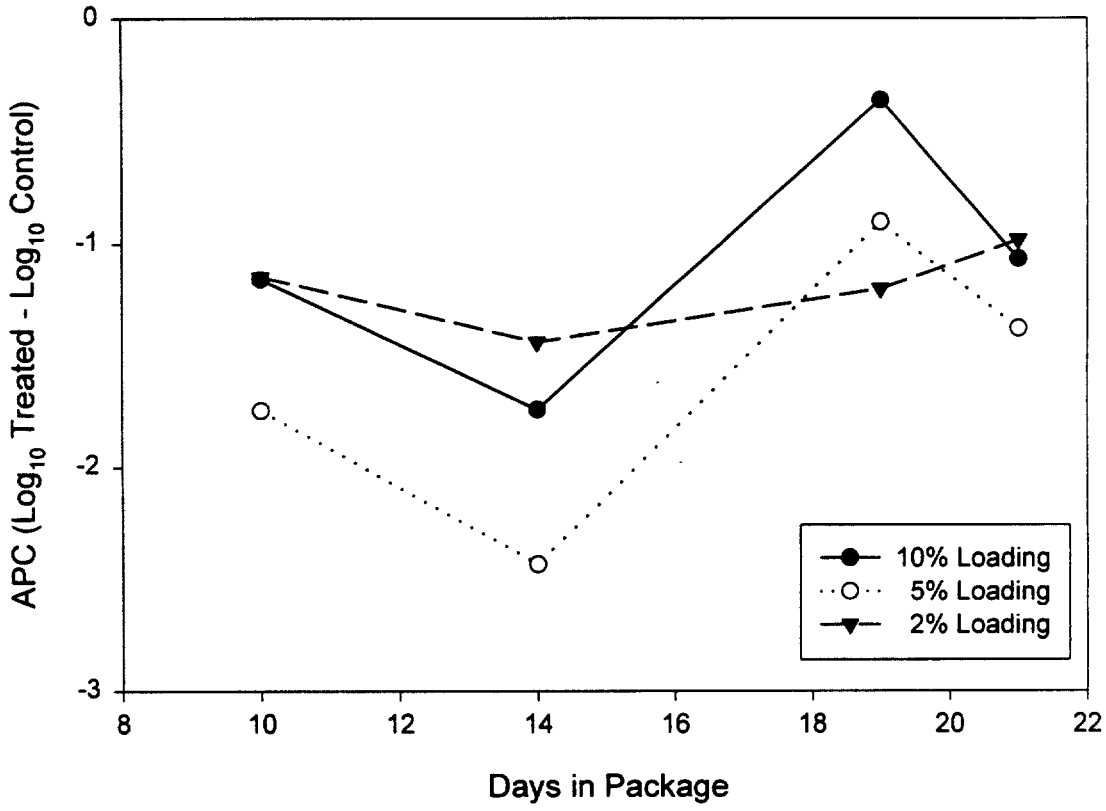
The following information was determined by microbiological testing during the products' shelf life:

Day	Date	Sample	Item #	APC	Coliforms	E. *coli*
				CFU/ Sample	CFU/ Sample	CFU/ Sample
10	11/15/99	1	TACS-27-064	142,000	0	0
10	11/15/99	2	TACS-17-154	138,000	0	0
10	11/15/99	3	TACS-22-115	36,000	0	0
10	11/15/99	4	TACS-29-024	58,000	0	0
10	11/15/99	5	Control	2,000,000	0	0
14	11/19/99	1	TACS-29-017	700,000	160	0
14	11/19/99	2	TACS-22-120	100,000	400	0
14	11/19/99	3	TACS-17-158	500,000	200	0
14	11/19/99	4	TACS-27-053	1,000,000	0	0
14	11/19/99	5	Control	27,400,000	0	0
19	11/24/99	1	TACS-29-036	2,000,000	10	0
19	11/24/99	2	TACS-17-122	21,000,000	70	0
19	11/24/99	3	TACS-27-053	3,000,000	200	0
19	11/24/99	4	TACS-22-095	6,000,000	100	0
19	11/24/99	5	Control	48,000,000	400	0
21	11/27/99	1	TACS-29-003	10,000,000	300	0
21	11/27/99	2	TACS-22-091	2,000,000	0	0
21	11/27/99	3	TACS-27-044	5,000,000	0	0
21	11/27/99	4	TACS-17-35	4,100,000	0	0

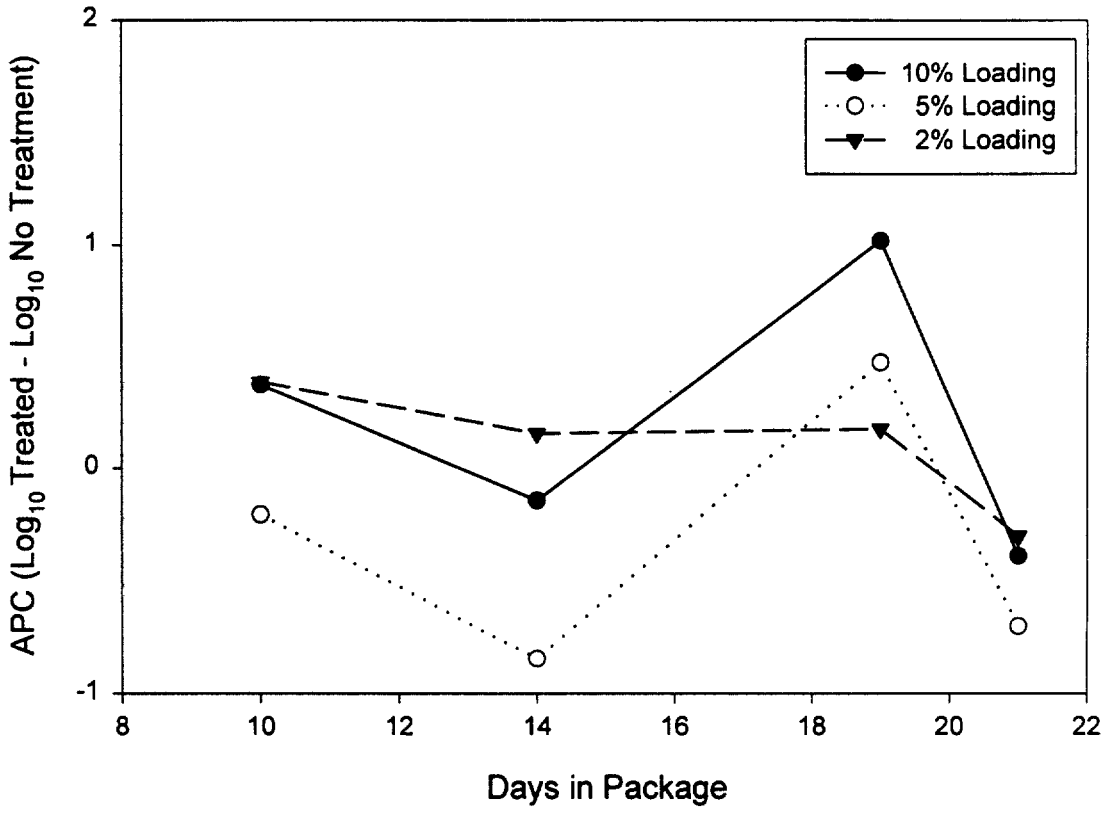
See the attached graphs for analysis of the results.

Report submitted by: _____ _____
Signature Printed Name

REDUCTION OF AEROBIC PLATE COUNT IN SHREDDED CARROTS BY ClO_2 GENERATING MICROLÌTE™ POLYMER BAGS COMPARED TO STANDARD POLYMER BAGS



REDUCTION OF AEROBIC PLATE COUNT IN SHREDDED CARROTS BY ClO_2 GENERATING MICROLÌTE™ POLYMER BAGS COMPARED TO SIMILAR BAGS WITHOUT ADDITIVE



CHEMICAL ANALYSES OF SHREDDED CARROTS PACKAGED WITH A ClO_2 GENERATING PACKAGING SYSTEM

A study was conducted on shredded carrots under Protocol Number TACS-1-1199 to determine whether there were any differences between carrots when packaged in normal commercial bags as compared to bags containing a ClO_2 generating packaging system.

The chemical analyses were conducted by Covance Laboratories, Inc., Madison, Wisconsin on samples prepared by Tanimura & Antle Salad Time Farms, Salinas, California.

The samples were analyzed for Beta Carotene, Vitamin A from Beta Carotene, and residual chlorite.

Methods References:

1. Beta Carotene by HPLC; AOAC International, Official Methods of Analysis (modified), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, Virginia.
2. Vitamin A from Beta Carotene; Quackenbush, F.W., Journal of Liquid Chromatography (modified), 10:643-653 (1987).
3. Iodometric Titration for the Analysis of ClO_2 and Chlorite Anion; Standard Methods for Water Analysis Method 4500-ClO_2 B as reported by APHA, AWWA, and WPCF (modified), SOP BTI#99-01.

Results:

1. Beta Carotene; there were no differences in the levels of Beta Carotene between the different packages tested. Results are presented in Table 1 and Figure 1.
2. Vitamin A from Beta Carotene; there were no differences in the levels of Vitamin A from Beta Carotene between the different packages tested. Results are presented in Table 2 and Figure 2.
3. Residual chlorite anion; no chlorite anion was detected in any of the samples. Results are presented in Table 3.

Conclusions:

1. The ClO_2 generating packaging material did not cause any change in the nutrient, Beta Carotene, concentrations of carrots after 24 days of exposure.
2. No residual chlorite anion was detected in the carrots after 24 days of exposure to ClO_2 generating packaging.

TABLE 1

Beta-Carotene (mg/100 g)

DAY	0% Film	2% Film	5% Film	10% Film	Comm'l Film
4	5.31	4.74	4.93	4.79	5.01
11	4.10	3.92	4.50	4.22	4.69
17	4.40	3.75	3.94	4.34	4.53
24	3.63	4.82	4.32	4.24	4.14

Composite results from 3 x 10 oz. bags of shredded carrots/assay

TABLE 2

Vitamin A from Beta Carotene (IU/100 g)

DAY	0% Film	2% Film	5% Film	10% Film	Comm'l Film
4	8850.00	7900.00	8220.00	7980.00	8350.00
11	6830.00	6530.00	7500.00	7030.00	7820.00
17	7330.00	6250.00	6570.00	7230.00	7550.00
24	6050.00	8030.00	7200.00	7070.00	6900.00

Composite results from 3 x 10 oz. bags of shredded carrots/assay

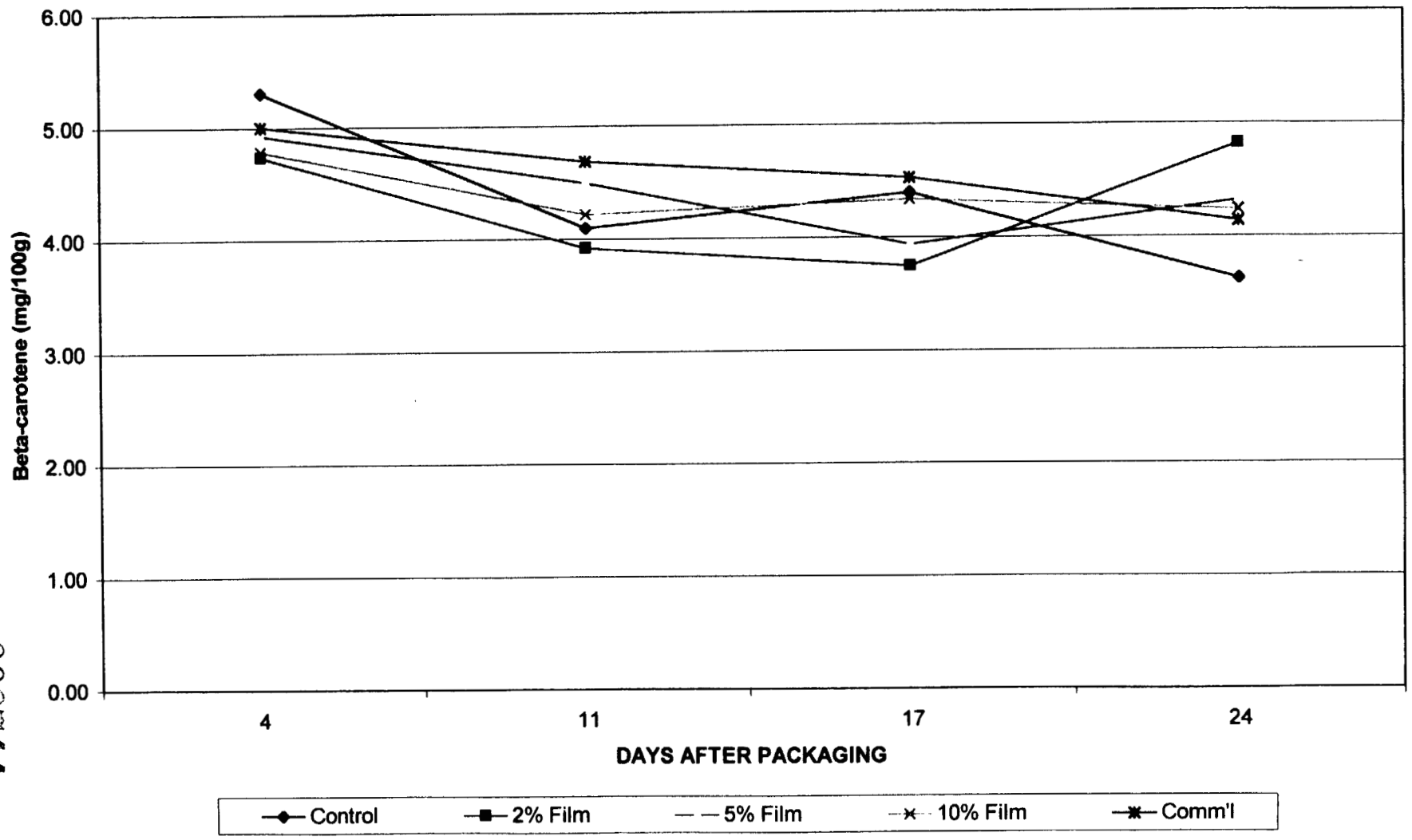
TABLE 3

Chlorite Anion (mg/100g)

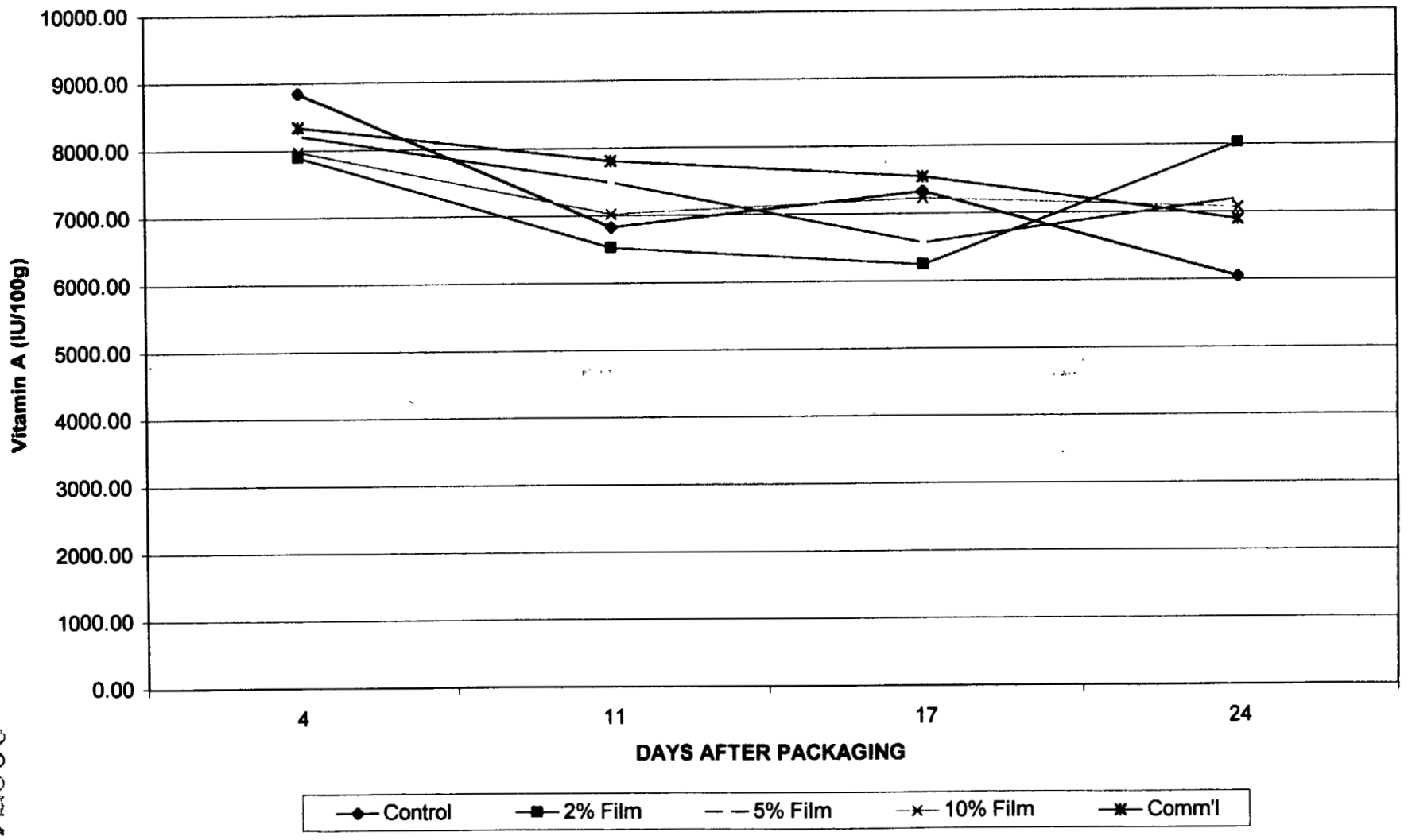
DAY	0% Film	2% Film	5% Film	10% Film	Comm'l Film
4	n.d.	n.d.	n.d.	n.d.	n.d.
11	n.d.	n.d.	n.d.	n.d.	n.d.
17	n.d.	n.d.	n.d.	n.d.	n.d.
24	n.d.	n.d.	n.d.	n.d.	n.d.

Composite results from 3 x 10 oz. bags of shredded carrots/assay

BETA-CAROTENE LEVELS IN FRESH SHREDDED CARROTS FOLLOWING EXPOSURE TO ClO2 GENERATING PACKAGING



VITAMIN A FROM B-CAROTENE IN FRESH SHREDDED CARROTS FOLLOWING EXPOSURE TO ClO2 GENERATING PACKAGING



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TECHNICAL REPORT TR BTI # 00-11

SUMMARY OF QUALITATIVE STUDIES OF CHLORINE DIOXIDE GENERATING PACKAGING COMPONENTS:

METHODS

a. Oxidative Rancidity Test (TBA) – Keystone

b. Determination of Detection Limit for the Analysis of Chlorite by Iodometric Titration Chlorite – Southwest Research Institute

c. Iodometric Titration for the Analysis of ClO_2 and Chlorite Anion Concentration – Bernard Technologies, Inc. SOP BTI # 99-01

OXIDATIVE RANCIDITY TEST (TBA)

PURPOSE / OBJECTIVE:

Determination of oxitative rancicity in meat is done by colorimetric analysis. The amount of red color is formed when an aques suspension of meat is acidified and heated with 2-thiobarbituric acid depends on the degree of fat oxidation

EQUIPMENT:

1. Calibrated analytical balance.
2. 100 degree constatnt temerature bath, or boling water bath.
3. Ice bath.
4. Spectrophotometer, Baush & Lomb Spectronic 20.
5. Vortex - Genie Mixer
6. Centrifuge
7. Fume Hood

MATERIALS:

1. 40 ml glass centrifuge tubes
2. Glass stirring rods
3. Para film or corks (black rubber stoppers cannot be used)
4. Spatulas
5. Pipets - one, two, three, four, five, ten, and fifteen
6. Nitril gloves
7. Saftey glasses / goggles
8. Respirator

REAGENTS:

1. TBA Reagent - (0.01 M Thiobarabituric Acid) Dissolve 1.4415 grams acid (M.W 144.15) in 350 ml. hot distilled water.Wash into a 1 liter volumertic flask, cool, and add distilled water ot mark
2. TCA Reagent - (Phosphoric Acid) Measure 184 grams of phosphoric acid into distilled water and make to 800 mls. add 200 grams of Trichloracetic Acid. Stir.
3. Isoopentyl Alcohol - (Pyridine Reagent) 2 volumes of isopentyl alcohol to 1 volume pyridine. * Isopentyl alchol (3- methyl - 1- butanol) is also known as Isoamyl alchol and Isobutyl Carbinol.
4. TMP 1, 1, 3, 3, - Tetramethoxupropane or malonaldehyde bis (dimethyl acetal) obtained from Aldrich Chemical Co. 100 gram size.

ASSOCIATED MATERIALS:

1. Graph paper or linear régression program (TBA values) in the computer.
2. Sample log book.

NOTES:

1. Samples should be analyzed immediately upon receival to prevent misinterpretation of results.
2. If samples are not analyzed immediately upon arrival, hold in a refrigerator at 31 - 33 degrees F (Max 12 hrs)
3. Ground and unground samples should be ground with a 1/16 inch plate prior to analysis, mixing well

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PROCEDURE:

1. Weigh out 5 grams of sample into a glass centrifuge tube.
2. Add 10 mls of 0.01 mls TBA reagent.
3. Add 5 mls of TCA (Phosphoric Acid) Reagent.
4. Stir to mix samples with reagents with glass stir rod, leaving rod in the tube.
5. Place tube in 100 degree constant temperature apparatus for 30 min. * under fume hood
6. Place in a ice water bath for 10 - 20 minutes until fat hardens at top of centrifuge tube
7. Remove any particles of fat with glass rod, Q - tip applicators or stainless steel spatulas.
8. Add 15 mls of Isopentyl Alchols - Pyridine Reagent.
9. Seal top of tubes wiht para - film or cork, mix on vortex for 30 seconds.
10. Place tube in the centrifuge until cloudiness had dissapeared. Avoid exess centrifuging (approximately 10 min. at 2200 rpm is sufficient).
11. Withdraw 5 mls of top extract using a 5 ml pipette into a curvet tube or pour extract into curvet tube.
12. Place curvet into spectrophotometer at 538 nm, read optical density and convert to corrected TBA by means of the standard curve. (graph or computer)
    * To zero the spectrophotometer use a blank following steps 2 thru 11
13. Two samples of the standard should be run when doing samples to check the curve. If the curve is not verfied corrective action must be instituted.

PREPARATION OF STANDARD:

1. Weigh .220 grams of TMP into 30 ml beaker containing 5 mls of distilled water.
2. Transfer quantitatively into a 1 liter volumetric flask and fill to volume.
3. Mix Well and pipette 20 mls of this solution into another liter volumetric flask, bring to volume with distilled water and mix well. This solution contains 2 x 10 to the minus 8 moles TMP per ml . This solution should be stored to about 40 degrees F and warmed to room temperature just prior to use.

STANDARD CURVE

1. Pipette one, two, three, four and five mls of the 2 x 10 minus 8 moles TMP solution into five 40 ml individual centrifuge tubes. This will result in 2, 4, 6, 8 and 10 moles x 10 minus 8 of TMP respectively
2. Pipette distilled water into each tube to bring the total volume of each tube to 5 mls
3. Follow stteps 2 thru 11.
4. Feed data into a linear regression program using ordinate as moles x 10 to minus 7 TMP and including the coordinate (0.0). The equation of a line should be used to calculate the TBA values in place of standard curve.
5. If graph paper is to be used - plot moles x 10 minus 7 of TMP versus optical density (abscissa) as the standard curve.
   * Note the values should contain values 0, 0.2, 0.4, 0.6, 0.8, 1.0 moles x 10 minus 7

KEYSTONE FOODS
North America

Keystone Foods, L.L.C.
Corporate Laboratory

600 -A Kaiser Drive
Folcroft, PA 19032

Phone: 610 534-5316
Fax: 610 586 6669

DAILY COMPLETED REPORT

Date Completed 03/20/2000

UPDATED
3/20/00
2:49:59PM

PLANT SAMPLEID	TEST LIST	PRODUCT	MFG HOUR	CODE DATE	ENV LOCATION	ANALYSIS	RESULT	
Plant Code	**KEY RD**	**Plant Name**	Keystone R&D Division					
EQR&D00046	TBATEST			00077		TBA	Sample Wt	5.0193
EQR&D00046	TBATEST			00077		TBA	Optical Density	45
EQR&D00046	TBATEST			00077		TBA	TBA Value	53
EQR&D00047	TBATEST			00077		TBA	Sample Wt	5.0520
EQR&D00047	TBATEST			00077		TBA	Optical Density	41
EQR&D00047	TBATEST			00077		TBA	TBA Value	48
EQR&D00048	TBATEST			00077		TBA	Sample Wt	5.0894
EQR&D00048	TBATEST			00077		TBA	Optical Density	60
EQR&D00048	TBATEST			00077		TBA	TBA Value	70
EQR&D00049	TBATEST			00077		TBA	Sample Wt	5.1065
EQR&D00049	TBATEST			00077		TBA	Optical Density	48
EQR&D00049	TBATEST			00077		TBA	TBA Value	56

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SOUTHWEST RESEARCH INSTITUTE

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Division of Chemistry and Chemical Engineering
Department of Microcapsules, Coatings and Polymers

March 29, 2000

Joel Kampa
Peter Gray
Bernard Technologies
919 N Michigan Ste 1710
Chicago IL 60611

Subject: SwRI Project 03470 Release of Chlorine Dioxide
Determination of Detection Limit for the Analysis of Chlorite by Iodometric Titration

Dear Drs. Kampa and Gray:

Attached is a report on the *Determination of Detection Limit for the Analysis of Chlorite by Iodometric Titration*. If you have any questions, please feel free to call me at 210 522-3325.

Sincerely yours,

David Herrera
Scientist

APPROVED:

Michael G. MacNaughton, Ph.D., P.E.
Vice President

/mal
Encl:Report

Determination of Detection Limit for the Analysis of Chlorite by Iodometric Titration

Abstract: A series of stock chlorite solutions were prepared covering a range of 0.1 to 1000 ppm $NaClO_2$. Iodometric titration, as described in Bernard Technologies SOP-99-02, was used to analyze the solutions. The measured concentrations were calculated and plotted against the theoretical concentrations. The detection limit for chlorite anion was determined to be 0.3 ppm.

Introduction: Bernard Technologies currently uses iodometric titration for the analysis of the active ingredient, chlorite, in many of its product formats. The methods described in BTI SOP-99-02 have been adapted from known methods for application with BTI proprietary materials. The procedure is also written to minimize the time required for analysis and simplified for use in the field at production and customer facilities. The detection limit for the procedure as written were determined. A series of chlorite solutions ranging in concentration from 0.1 to 1000 ppm sodium chlorite were analyzed by BTI SOP-99-02. Results were reviewed to determine at what level does the method fail to give reliable measurement.

Experimental:

A. Sodium Chlorite Stock Solutions

An ~1000 ppm sodium chlorite solution was prepared by dissolving 1.25g sodium chlorite (tech., 80%) in 1000ml water in a volumetric flask. This solution was then used to directly prepare each of the subsequent dilutions. All dilutions were made using volumetric glassware. Table 1 describes the series of dilutions made from the "1000 ppm" solution.

Table 1. Series Of Dilutions Made From The "100 ppm" Solution

Vol. 1000 ppm solution (ml)	Final Vol. (ml)	Final conc. (ppm)
10	100	100
1	100	10
1	1000	1
0.5	1000	0.5
0.1	1000	0.1

B. Standardization of Sodium Thiosulfate Titrant

Two concentrations of sodium thiosulfate (NTS) solution were required for the analysis of the stock chlorite solutions over the range required. The titrant solutions were prepared from a commercially available ~0.1N NTS (Labchem) by 1:10 and 1:100 dilution to ~0.01 and 0.001N NTS, respectively. These solutions were then standardized as described in BTI SOP-99-02 using 0.002083 M potassium iodate/iodide (Labchem) as the primary standard.

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C. Sample Preparation and Titration

The procedures described in BTI SOP-99-02 were followed with minor changes due to the difference in sample form. Stock chlorite solutions are substituted for the powder samples and no sample digestion time was required. Titration was done immediately after completely mixing the reagents. The procedure was summarized as follows:

1. Weigh 1.0 +/- 0.1g KI into an Erlenmeyer flask containing a magnetic stir bar.
2. Add the desired amount of stock chlorite solution, mix.
3. Add ~5ml 5% HF solution to the sample, mix.
4. Titrate the sample, stirring constantly. When the color lightens, add starch indicator to visualize the endpoint and continue titrating.

Results: The exact concentration for the NTS solutions used for titration of the samples was determined by titration of the primary standard. Table 2 contains the experimental data along with the calculated concentrations, averaged for three trials.

Table 2. Experimental Data With Calculated Concentrations

Vol. Iodate (ml, 0.002083M)	Vol. NTS (ml)	Conc. NTS (N)	Avg. conc. (M)
5.0	6.46	0.00967	
5.0	6.26	0.00998	
5.0	6.41	0.00975	**0.0098**
5.0	64.47	0.00097	
1.0	13.03	0.00096	
1.0	13.07	0.00096	**0.00096**

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Table 3 summarizes the titration of the stock chlorite solutions using these NTS solutions. The final two columns shown in the table are the calculated concentrations of the stock chlorite solutions based on the experimental data. Concentrations are expressed in ppm $NaClO_2$ and ppm ClO_2^- for convenience.

Table 3. Titration of Stock Chlorite Solutions--Summary

Std. Conc. (ppm)	Vol. Std. (ml)	Conc. NTS (N)	Vol. NTS (ml)	Measured Conc. (ppm $NaClO_2$)	Measured conc. (ppm $Cl\ O_2$-)
1000	10	0.0098	45.1	999.47	745.41
1000	1	0.0098	4.77	1057.09	788.38
1000	1	0.0098	4.63	1026.07	465.24
100	10	0.0098	4.32	95.74	71.40
100	10	0.0098	4.08	90.42	67.43
100	10	0.0098	4.31	95.52	71.24
10	10	0.0098	0.7	15.51	11.57
10	10	0.0098	0.49	10.86	8.10
10	10	0.0098	0.51	11.30	8.43
10	10	0.00096	4.8	10.44	7.78
10	10	0.00096	4.71	10.24	7.61
10	10	0.00096	4.76	10.35	7.72
1	10	0.00096	0.6	1.30	0.97
1	10	0.00096	0.57	1.24	0.92
1	10	0.00096	0.64	1.39	1.04
0.5	50	0.00096	1.15	0.50	0.37
0.5	50	0.00096	1.02	0.44	0.33
0.5	50	0.00096	1.13	0.49	0.37
0.1	50	0.00096	--	Not Detected	Not Detected
0.1	50	0.00096	--	Not Detected	Not Detected
0.1	50	0.00096	--	Not Detected	Not Detected

The above data is shown graphically below in Chart 1. The data is linear over 5 orders of magnitude with good precision. The 0.1ppm solution did not produce a measurable result. The detection limit of the method based on the current data is 0.3 ppm chlorite anion.

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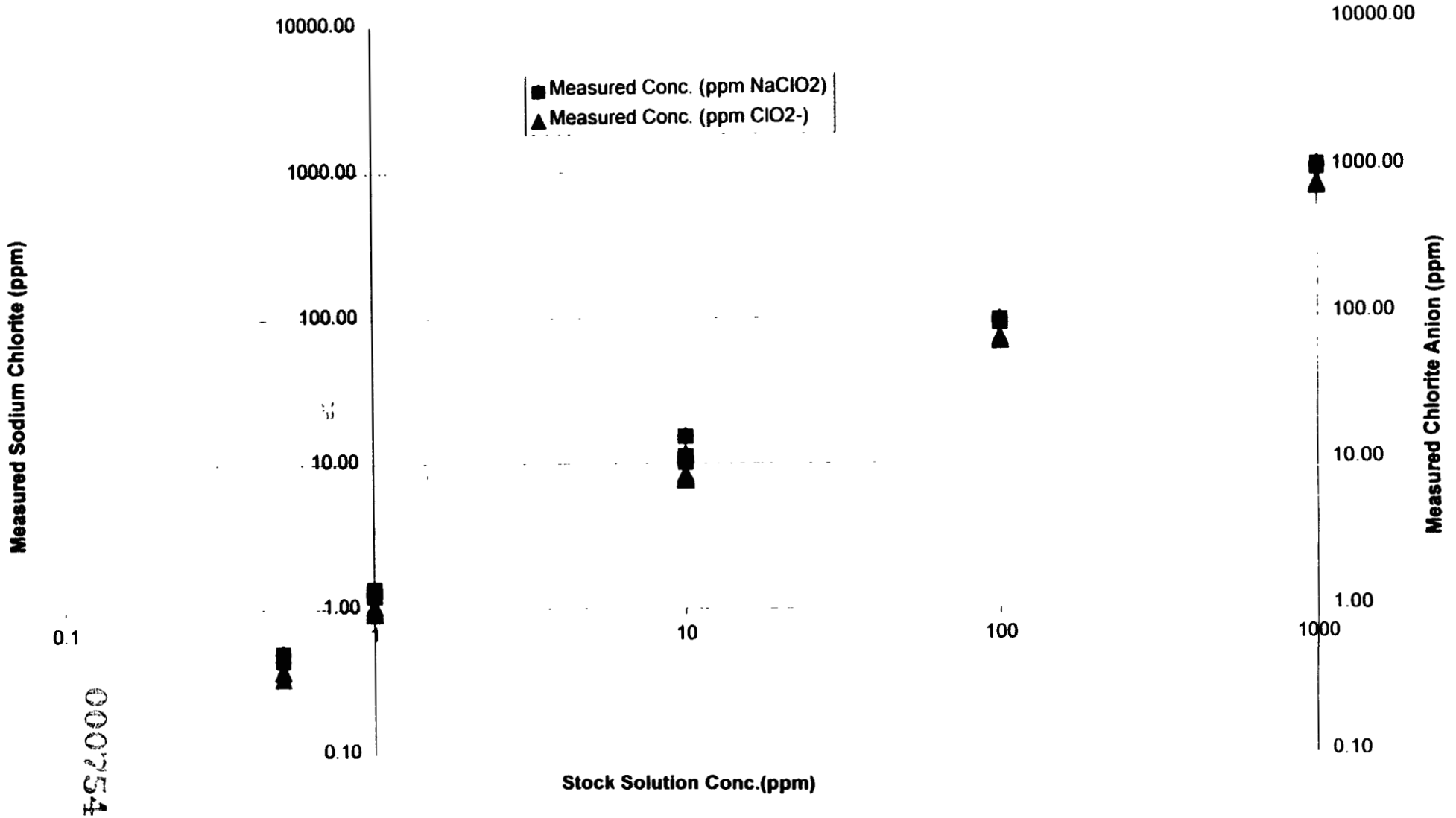
Iodometric Titration of Chlorite



000754

BERNÀRD TECHNOLOGIES, INC.

A Health and Food Safety Technology Company

STANDARD OPERATING PROCEDURE
SOP BTI # 99-01

Iodometric Titration for the Analysis of ClO_2 and Chlorite Anion Concentration

This Standard Operating Procedure for the analysis of chlorine dioxide and chlorite anion in aqueous solution and solid systems is based on the Standard Methods for Water Analysis Method 4500-ClO2 B as reported by the APHA, AWWA, and WPCF. This SOP is PROPRIETARY and CONFIDENTIAL to BERNÀRD TECHNOLOGIES, INC.

A. Reagents and Equipment:
   1. Reagents:
      a) Potassium Iodide (KI) – granular, minimum 99%
      b) pH 7.0 phosphate buffer
      c) 2.5M HCl – prepared from concentrated HCl (12M) by diluting 104ml of the12M to 500ml in a volumetric flask.
      d) 0.01N Sodium thiosulfate solution – standardized. Dilution for more accurate assay of low concentrations is most easily done by dilution of the appropriate quantity of the 0.01N NTS into a 500ml volumetric flask.
      e) De-ionized water.
      f) 1% soluble starch indicator solution.

   2. Equipment:
      a) Analytical balance, 0.001g resolution minimum.
      b) 500ml volumetric flask with stopper.
      c) Graduated pipet or cylinder.
      d) Purge gas – 20-40cc/min nitrogen scrubbed through 5% KI solution. Discard scrubbing solution at the first sign of color. The outlet should be fitted with a gas diffusion tube.
      e) Erlenmeyer flask with stopper.
      f) Disposable pipets with bulb.
      g) 5ml repeating pipet.
      h) Burette
      i) De-ionized water squirt bottle.

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j) Magnetic stir plate and stir bar.

A. Sample Preparation: All samples should be handled to minimize the loss of I_2 through evaporation. All vessels containing samples should be tightly stoppered between steps. A blank should also be carried though all steps. The results of the blank titration will be used in the final calculations.

1. ClO_2 in Aqueous Solution: In the case of aqueous solutions, it is possible to measure both ClO_2 and chlorite concentration. ClO_2 is analyzed at neutral pH. Low pH is required to quantify the chlorite anion concentration.
   a) Place 50-100ml water and a magnetic stir bar in a 250ml Erlenmeyer flask so that the final volume after addition of the sample is 100-200ml.
   b) Add 1ml pH 7.0 phosphate buffer.
   c) Add ~1g potassium iodide (KI).
   d) Carefully measure (S_1), using the graduated pipet or cylinder, and transfer an aliquot of the solution to be analyzed to the Erlenmeyer flask. Formation of a yellow to brown color indicates the formation of I_2.
   e) Stopper the flask and let stand ~2 minutes in the dark.
   f) Titrate.

2. Chlorite anion in Aqueous Solution: To analyze chlorite only, the ClO_2 must first be removed by purging the sample. Once the volatile gas is removed, the chlorite is quantified at low pH
   a) Place 50-100ml water and a magnetic stir bar in a 250ml Erlenmeyer flask so that the final volume after addition of the sample is 100-200ml.
   b) Carefully measure (S_2), using the graduated pipet or cylinder, and transfer an aliquot of the solution to be analyzed to the Erlenmeyer flask.
   c) Purge the sample for approximately 15 minutes.
   d) Add ~1g potassium iodide (KI).
   e) Acidify the sample by adding ~2ml 2.5M HCl.
   f) Stopper the flask and let stand ~5 minutes in the dark.
   g) Titrate.

3. Chlorite anion in solid powder mixtures: Solids containing sodium chlorite such as Microlite™ or Microsphère®, and its precursor core material are analyzed in the following manner. In this case, a more aggressive sample preparation solution is used along with an extended extraction period to facilitate the analysis of chlorite which may be held within an insoluble phase. The blank will yield a non-trivial value due to air oxidation of the iodide with the higher acid concentration.
   a) Weigh 0.05 - 0.2 grams (M_3) of the solid to be analyzed into a test tube.

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b) Add ~1g KI.
c) Add ~5ml 2.5M HCl. Use the repeating pipet if multiple samples are to be analyzed.
d) Cap the test tube tightly and shake.
e) Let the sample stand in the dark for >30 minutes for extraction of the chlorite from the solid material.
f) After the extraction period, transfer the sample to a 250ml Erlenmeyer flask containing ~50-100ml de-ionized H_2O and a magnetic stir bar.
g) Use a de-ionized water squirt bottle to rinse the sample from the test tube into the Erlenmeyer flask. Rinse the cap also.
h) Titrate.

4. Chlorite anion in polymer matrices: In this case , the chlorite is typically difficult to extract. Consequently, the extraction period is much longer. The blank is again quite important due to air oxidation of iodide.
   a) The polymer should first be cut into small pieces. For samples <0.5mm thick, 1 mm^2 is a typical size, however sample density, or porosity should be considered. If possible, the polymer composite can be powdered by cryogenic grinding to improve the extraction efficiency.
   b) Weigh 0.1 - 0.2 grams (M_4) of the solid to be analyzed into a test tube.
   c) Add ~1g KI.
   i) Add ~5ml 2.5M HCl. Use the repeating pipet if multiple samples are to be analyzed.
   d) Cap the test tube tightly and shake.
   e) Let the sample stand in the dark 24-30 hours for extraction of the chlorite from the solid polymeric material.
   f) After the extraction period, transfer the sample to a 250ml Erlenmeyer flask containing ~50-100ml de-ionized H_2O and a magnetic stir bar.
   j) Use a de-ionized water squirt bottle to rinse the sample from the test tube into the Erlenmeyer flask. Rinse the cap also.
   k) Titrate

B. Titration: The iodine generated by the oxidation if iodide is titillated using sodium thiosulfate (NTS).
   1. Method: The following is a list of guidelines to prevent some of the most common errors which effect the accuracy of the titration.
      a) The concentration of the NTS should be chosen so that a total of 2-20ml of titrant are required for each analysis. A concentration of 0.01N is typical for the masses used above.
      b) All titrant additions should be well stirred.
      c) The flask walls should be rinsed occasionally to avoid loss of reactants from splashing.

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d) The volume of the titrant should be read (avoiding meniscus and parallax errors) and recorded carefully.
e) If the burette must be refilled during a titration, make sure all initial and final volumes are recorded.
f) Starch indicator should be added after most of the iodine has been reduced. The starch-I_2 complex is decomposed in the presence of excess iodine.
g) A white background should be place under the titration flask to assist in the detection of the end-point.
h) Samples should be kept stoppered at all times unless adding reagents or transferring.

2. Procedure:
   a) Record the initial volume of NTS titrant.
   b) Titrate the I_2 in the sample until only a faint yellow remains.
   c) Add ~ 1ml starch indicator solution. An intense blue (purple or black) color will develop.
   d) Continue the titration until the starch-iodine complex color is no longer visible.
   e) Record the final volume of NTS. Record the total volume of NTS consumed (V_1).

C. Calculations: The calculations are based on the stoichiometries described in the following equations. Equations 1 and 2 show the oxidation of iodide by ClO_2 and chlorite anion, respectively. Equation 3 shows the reduction of iodine by the thiosulfate titrant. Measured volumes and masses are used throughout the calculations are in milliliters and grams, respectively.

At pH ~7: $2\ ClO_2 + 2\ I^- \rightarrow I_2 + 2\ ClO_2^-$ [Eqn. 1]

At pH <2: $ClO_2^- + 4\ I^- + 4\ H^+ \rightarrow 2\ I_2 + Cl^- + 2\ H_2O$ [Eqn. 2]

$2\ Na_2S_2O_3 + I_2 \rightarrow 2\ I^- + Na_2S_4O_6 + 2\ Na^+$ [Eqn. 3]

1. ClO_2 in Aqueous Solution: For liquid samples, results are typically reported in ppm, or mg/kg, or in the specific case of water, mg/l.

$$ClO_2\ (ppm) = (V_1 - B) * N * 67442 / S_1$$

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V_1 is the titrant volume, B is the corresponding blank titrant volume, S_1 the sample volume, N the normality of the NTS solution (equivalents/liter), and the molecular weight of ClO_2 is 67442 mg/mole.

2. Chlorite anion in Aqueous Solution: Again, results are calculated on a ppm basis. It should be pointed out that results indicate a concentration of chlorite anion and not sodium chlorite.

$$ClO_2^- \text{ (ppm)} = (V_1 - B) * N * 16863 / S_2$$

V_1 is the titrant volume, B is the blank titrant volume, and S_2 is the sample volume. The constant 16863 is the molecular weight of the chlorite anion expressed in mg/mole divided by the 4 electrons (equivalents) per mole transferred in the reduction to chloride ion.

3. Chlorite anion in solid powder mixtures: The concentration of chlorite in solid samples is reported here in percent of the total solid. Again, this result is a concentration of chlorite anion.

$$ClO_2^- \text{ (\%)} = (V_1 - B) * N * 1.6863 / M_3$$

V_1 is the volume of titrant, B is the blank titrant volume for this procedure, and M_3 is the mass of the sample. The constant in this case is the molecular weight expressed in g/mole, divided by four equivalents/mole, and again by ten to report the result in percent.

4. Chlorite anion in polymer matrices: As in the previous section, results are reported as percent chlorite anion. The calculation is also the same as the previous section.

$$ClO_2^- \text{ (\%)} = (V_1 - B) * N * 1.6863 / M_4$$

D. <u>References:</u> Similar methods can be found in references 1 and 2 below. Reference 3 contains information on iodometric titration in general and information on the chemistry and application of the method.

1. *Standard Methods for the Examination of Water and Wastewater. 4500-ClO2 B. Iodometric Method.* APHA, AWWA, and WPCF. Washington, D.C. (17th ed.,) pg. 4-54.

2. E. M. Aieta, P. V. Roberts, and M. Hernandez, *J. Am. Water Works Assoc.* **76**(1), 64-70 (1984).

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3. *Fundamentals of Analytical Chemistry.* D. A. Skoog and D. M. West, 4th ed., CBS College Publishing, 1982, pp.356-389, 764-773.

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COVANCE
THE DEVELOPMENT SERVICES COMPANY

Sponsor:

Bernard Technologies, Inc.
Chicago, Illinois

FINAL REPORT

Study Title:

Analysis of Chlorite, Thiamine, Riboflavin, Beta Carotene, and Vitamin A from Carotene in Various Foods

Author:

David S. Levin

Date:

November 7, 2000

Performing Laboratory:

Covance Laboratories Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

000761

STUDY IDENTIFICATION

Purpose
The purpose of this study was to determine levels of chlorite, thiamine, riboflavin, beta-carotene, and vitamin A from carotene in various food samples provided by the sponsor.

Sponsor
Bernard Technologies, Inc.
919 North Michigan Avenue
Chicago, Illinois 60611

Study Monitor
Peter N. Gray, Ph.D.
Bernard Technologies, Inc.
Telephone No.: 312.337.7007
Facsimile No.: 312.337.0505

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Madison, WI 53704

Principal Analytical Investigator
David S. Levin
Covance Laboratories Inc.
Telephone No.: 608.242.2712 ext.2585
Facsimile No.: 608.242.2732

Study Timetable

Analytical Start Date:	November 1999
Analytical End Date:	April 2000
Study Completion Date:	November 7, 2000

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KEY PERSONNEL

Food and Drug Analysis

David S. Levin
Principal Analytical Investigator
Manager

James R. Wehrmann
Associate Director

Dennis R. LaChance
Analyst

Dawn M. Ostrowski
Analyst

Ted Pritchard
Supervisor

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Morgan Petty
Supervisor

Sherri Buchholz
Analyst

Amy Jerde
Analyst

CONTENTS

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INTRODUCTION

Samples were obtained from the sponsor and entered into the Covance Laboratory Information Management System (LIMS) with unique LIMS numbers. Each sample identification was matched with the LIMS information. Food matrices included raw poultry pieces, partially cooked poultry, buns with preservatives, buns without preservatives, and shredded carrots. The samples were analyzed for one or more of the following compounds: chlorite, thiamine, riboflavin, beta-carotene, and vitamin A from carotene. The analysis of the chlorite anion by iodometric titration was performed in accordance with a method supplied by the sponsor. Reference attached client SOP BTI # 99-02 in Appendix A. The samples were also analyzed by in-house methods for thiamine (BIDE), riboflavin (B2FV), beta-carotene (BCLC), and vitamin A from carotene (BCAV).

REGULATORY COMPLIANCE

All aspects of this study were carried out in accordance with Covance standard operating procedures (SOPs). This study was not performed within the scope of the Good Laboratory Practice (GLP) or current Good Manufacturing Practice (cGMP) Regulations.

QUALITY CONTROL

Management personnel in accordance with Covance SOPs reviewed the data and final report. Control and method validation data were performed in accordance with Covance SOP OP-NC 8 titled "Analytical Quality Control Procedures".

STATISTICAL METHODS

No statistical analysis of the data was performed at Covance.

MAINTENANCE OF RAW DATA AND RECORDS

All data relating to or generated by the project, including (if applicable) protocol, protocol amendments, a copy of the final report, results, magnetically encoded records, laboratory notebooks, applicable standard operating procedures (SOPs) lists and any other information or records relating to the project will be retained in the archives of Covance in accordance with 40 CFR Part 160. Ten years after signing of the final report, all of the aforementioned materials will be sent to the sponsor.

The supporting records retained at Covance, but not archived with the study data, include the following items:

Storage area temperature records
Instrument calibration and maintenance records
Employee training records

RESULTS

A summary of the results is presented in Table 1.

SIGNATURE

_____	11-7-00
David S. Levin Principal Analytical Investigator Food and Drug Analysis Covance Laboratories Inc.	Date
_____	11-7-00
James R. Wehrmann Associate Director Food and Drug Analysis Covance Laboratories Inc.	Date

TABLE
Sample Analysis Data

Matrix	Day	Covance LIMS Number	Beta Carotene (mg/100 g)	Vitamin A (iu/100 g)	Chlorite
		Shredded Carrots			
TACS-17-121, 124, 129	Stability, 4	91101858	4.79	7980	ND[a]
TACS-22-080, 083, 085	Stability, 4	91101859	4.93	8220	ND[a]
TACS-27-041, 042, 046	Stability, 4	91101860	4.74	7900	ND[a]
TACS-29-001, 004, 005	Stability, 4	91101861	5.31	8850	ND[a]
50641 00092	Stability, 4	91101862	5.01	8350	ND[a]
TACS-17-132, 133, 136	Stability, 11	91101870	4.22	7030	ND[a]
TACS-22-086, 094, 097	Stability, 11	91101871	4.50	7500	ND[a]
TACS-27-049, 055, 057	Stability, 11	91101872	3.92	6530	ND[a]
TACS-29-010, 014, 018	Stability, 11	91101873	4.10	6830	ND[a]
50641 00092	Stability, 11	91101874	4.69	7820	ND[a]
TACS-17-137, 140, 145	Stability, 17	91101889	4.34	7230	ND[a]
TACS-22-099, 104, 106	Stability, 17	91101890	3.94	6570	ND[a]
TACS-27-061, 066, 068	Stability, 17	91101891	3.75	6250	ND[a]
TACS-29-019, 023, 027	Stability, 17	91101892	4.40	7330	ND[a]
50641 00092	Stability, 17	91101893	4.53	7550	ND[a]
TACS-17-156, 157, 160	Stability, 24	91101896	4.24	7070	ND[a]
TACS-22-107, 108, 111	Stability, 24	91101897	4.32	7200	ND[a]
TACS-27-069, 073, 077	Stability, 24	91101898	4.82	8030	ND[a]
TACS-29-029, 032, 035	Stability, 24	91101899	3.63	6050	ND[a]
50641 00092	Stability, 24	91101900	4.14	6900	ND[a]

a No chlorite was detected by the method supplied by the client.

Table (Continued)
Sample Analysis Data

Matrix	Day	Sample Number	Chlorite
Cooked Chicken (Received from Keystone Foods R&D, Folcroft, Pennsylvania)			
KEY-PCN-LO-782	1	00203833	ND[b]
KEY-PCN-LO-NO CODE	1	00203834	ND[b]
KEY-PCN-MED-749	1	00203835	ND[b]
KEY-PCN-MED-NO CODE	1	00203836	ND[b]
KEY-PCN-HI-721	1	00203837	ND[b]
KEY-PCN-HI-729	1	00203838	ND[b]
KEY-PCN-Z-800	1	00203839	ND[b]
KEY-PCN-Z-793	1	00203840	ND[b]
Raw Chicken (Received from Keystone Foods R&D, Folcroft, Pennsylvania)			
KEY-R-LO-098	1	00203853	ND[b]
KEY-R-LO-110	1	00203854	ND[b]
KEY-RC-LO-511	1	00203855	ND[b]
KEY-RC-LO-519	1	00203856	ND[b]
KEY-R-MED-138	1	00203857	ND[b]
KEY-R-MED-143	1	00203858	ND[b]
KEY-RC-MED-591	1	00203859	ND[b]
KEY-RC-MED-596	1	00203860	ND[b]
KEY-R-HI-280	1	00203861	ND[b]
KEY-R-HI-297	1	00203862	ND[b]
KEY-RC-HI-521	1	00203863	ND[b]
KEY-RC-HI-525	1	00203864	ND[b]
KEY-R-Z-201	1	00203865	ND[b]
KEY-R-Z-203	1	00203866	ND[b]
KEY-RC-Z-193	1	00203867	ND[b]
KEY-RC-Z-197	1	00203868	ND[b]
KEY-R-LO-099	2	00204149	ND[b]
KEY-R-LO-103	2	00204150	ND[b]
KEY-RC-LO-512	2	00204151	ND[b]
KEY-RC-LO-516	2	00204152	ND[b]
KEY-R-MED-132	2	00204153	ND[b]
KEY-R-MED-NO CODE	2	00204154	ND[b]
KEY-RC-MED-585	2	00204155	ND[b]
KEY-RC-MED-NO CODE	2	00204156	ND[b]
KEY-R-HI-263	2	00204157	ND[b]
KEY-R-HI-270	2	00204158	ND[b]
KEY-RC-HI-573	2	00204159	ND[b]
KEY-RC-HI-580	2	00204160	ND[b]

b No chlorite was detected at 0.1%.

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Table (Continued)
Sample Analysis Data

Matrix	Day	Sample Number	Chlorite
Raw Chicken (continued)			
(Received from Keystone Foods R&D, Folcroft, Pennsylvania)			
KEY-R-Z-202	2	00204161	ND[b]
KEY-R-Z-220	2	00204162	ND[b]
KEY-RC-Z-195	2	00204163	ND[b]
KEY-RC-Z-199	2	00204164	ND[b]
KEY-R-LO-114	5	00204165	ND[b]
KEY-R-LO-118	5	00204166	ND[b]
KEY-RC-LO-508	5	00204167	ND[b]
KEY-RC-LO-515	5	00204168	ND[b]
KEY-R-MED-124	5	00204169	ND[b]
KEY-R-MED-150	5	00204170	ND[b]
KEY-RC-MED-581	5	00204171	ND[b]
KEY-RC-MED-NO CODE	5	00204172	ND[b]
KEY-R-HI-284	5	00204173	ND[b]
KEY-R-HI-294	5	00204174	ND[b]
KEY-RC-HI-527	5	00204175	ND[b]
KEY-RC-HI-NO CODE	5	00204176	ND[b]
KEY-R-Z-208	5	00204177	ND[b]
KEY-R-Z-216	5	00204178	ND[b]
KEY-RC-Z-188	5	00204179	ND[b]
KEY-RC-Z-189	5	00204180	ND[b]
Raw Whole Chicken			
(Received from Keystone Foods R&D, Folcroft, Pennsylvania)			
KEY-R-LO-093	7	00205046	ND[b]
KEY-R-MED-137	7	00205047	ND[b]
KEY-R-HI-290	7	00205048	ND[b]
KEY-R-Z-188	7	00205049	ND[b]
Raw Cubed Chicken			
(Received from Keystone Foods R&D, Folcroft, Pennsylvania)			
KEY-RC-LO-505	7	00205050	ND[b]
KEY-RC-MED-593	7	00205051	ND[b]
KEY-RC-HI-523	7	00205052	ND[b]
KEY-RC-Z-191	7	00205053	ND[b]
Raw Whole Pieces Chicken			
(Received from Keystone Foods R&D, Folcroft, Pennsylvania)			
KEY-R-LO-105	9	00205057	ND[b]
KEY-R-MED-146	9	00205058	ND[b]
KEY-R-HI-293	9	00205059	ND[b]
KEY-R-Z-185	9	00205060	ND[b]

b No chlorite was detected at 0.1%.

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Table (Continued)
Sample Analysis Data

Matrix	Day	Sample Number	Chlorite
Raw Cubed Chicken (Received from Keystone Foods R&D, Folcroft, Pennsylvania)			
KEY-RC-LO-520	9	00205061	ND[b]
KEY-RC-MED-597	9	00205062	ND[b]
KEY-RC-HI-529	9	00205063	ND[b]
KEY-RC-Z-185	9	00205064	ND[b]
Partially Cooked Chicken (Received from Keystone Foods R&D, Folcroft, Pennsylvania)			
KEY-PCN-LO-786	10	00205065	ND[b]
KEY-PCN-MED-742	10	00205066	ND[b]
KEY-PCN-HI-728	10	00205067	ND[b]
KEY-PCN-Z-788	10	00205068	ND[b]

b No chlorite was detected at 0.1%.

Table (Continued)
Sample Analysis Data

Matrix	Day	Sample Number	Thiamin Hydrochloride (mg/100 g)	Riboflavin (mg/100 g)	Chlorite
Hamburger Buns (Received from East Balt Inc., Chicago, Illinois)					
EB BUNS-BASE (with calcium propionate)	3	00300715	0.61	0.41	ND[b]
EB BUNS-BASE (without calcium propionate)	3	00300716	0.50	0.44	ND[b]
EB BUNS-LOW (with calcium propionate)	3	00300717	0.53	0.58	ND[b]
EB BUNS-LOW (without calcium propionate)	3	00300718	0.48	0.48	ND[b]
EB BUNS-MED (with calcium propionate)	3	00300719	0.56	0.42	ND[b]
EB BUNS-MED (without calcium propionate)	3	00300720	0.51	0.47	ND[b]
EB BUNS-HIGH (with calcium propionate)	3	00300721	0.56	0.25	ND[b]
EB BUNS-HIGH (without calcium propionate)	3	00300722	0.57	0.22	ND[b]
EB BUNS-BASE (with calcium propionate)	6	00300757	0.56	0.24	ND[b]
EB BUNS-BASE (without calcium propionate)	6	00300758	0.54	0.43	ND[b]
EB BUNS-LOW (with calcium propionate)	6	00300759	0.55	0.24	ND[b]
EB BUNS-LOW (without calcium propionate)	6	00300760	0.54	0.49	ND[b]

b No chlorite was detected at 0.1%.

Table (Continued)
Sample Analysis Data

Matrix	Day	Sample Number	Thiamin Hydrochloride (mg/100 g)	Riboflavin (mg/100 g)	Chlorite
Hamburger Buns (Received from East Balt Inc., Chicago, Illinois)					
EB BUNS-MED (with calcium propionate)	6	00300761	0.63	0.24	ND[b]
EB BUNS-MED (without calcium propionate)	6	00300762	0.51	0.22	ND[b]
EB BUNS-HIGH (with calcium propionate)	6	00300763	0.51	0.41	ND[b]
EB BUNS-HIGH (without calcium propionate)	6	00300764	0.55	0.24	ND[b]
EB BUNS-BASE (with calcium propionate)	9	00300765	0.50	0.23	ND[b]
EB BUNS-BASE (without calcium propionate)	9	00300766	0.50	0.21	ND[b]
EB BUNS-LOW (with calcium propionate)	9	00300767	0.52	0.24	ND[b]
EB BUNS-LOW (without calcium propionate)	9	00300768	0.49	0.21	ND[b]
EB BUNS-MED (with calcium propionate)	9	00300769	0.50	0.24	ND[b]
EB BUNS-MED (without calcium propionate)	9	00300770	0.49	0.21	ND[b]
EB BUNS-HIGH (with calcium propionate)	9	00300771	0.52	0.24	ND[b]
EB BUNS-HIGH (without calcium propionate)	9	00300772	0.50	0.22	ND[b]
EB BUNS-BASE (with calcium propionate)	13	00300777	0.49	0.24	ND[b]
EB BUNS-BASE (without calcium propionate)	13	00300778	0.47	0.26	ND[b]
EB BUNS-LOW (with calcium propionate)	13	00300779	0.59	0.26	ND[b]
EB BUNS-LOW (without calcium propionate)	13	00300780	0.51	0.23	ND[b]
EB BUNS-MED (with calcium propionate)	13	00300781	0.47	0.26	ND[b]
EB BUNS-MED (without calcium propionate)	13	00300782	0.51	0.39	ND[b]
EB BUNS-HIGH (with calcium propionate)	13	00300783	0.52	0.27	ND[b]
EB BUNS-HIGH (without calcium propionate)	13	00300784	0.48	0.25	ND[b]

b No chlorite was detected at 0.1%.

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Table (Continued)
Sample Analysis Data

Matrix	Day	Sample Number	Beta Carotene by HPLC (mg/100 g)	Vitamin A (iu/100 g)	Chlorite
Carrots (Received from Salad Time Farms, Salinas, California)					
TACS HIGH, "A"	4	00301981	12.3	20500	ND[b]
TACS HIGH, "B"	4	00301982	11.3	18800	ND[b]
TACS HIGH, "A"	4	00301983	12.3	20500	ND[b]
TACS CONTROL, "B"	4	00301984	13.1	21800	ND[b]
TACS HIGH, "A"	11	00303474	8.99	15000	ND[b]
TACS HIGH, "B"	11	00303475	9.12	15200	ND[b]
TACS CONTROL, "A"	11	00303476	8.88	14800	ND[b]
TACS CONTROL, "B"	11	00303474	8.56	14300	ND[b]

b No chlorite was detected at 0.1%.

Matrix	Day	Sample Number	Chlorite
Partially Cooked Chicken (Received from Keystone Foods R&D, Folcroft, Pennsylvania)			
KEY-PCN-Z-784	NA	00303917	ND[b]
KEY-PCN-Z-785	NA	00303918	ND[b]
KEY-PCN-LO-705	NA	00303919	ND[b]
KEY-PCN-LO-709	NA	00303920	ND[b]
KEY-PCN-MED-745	NA	00303921	ND[b]
KEY-PCN-MED-755	NA	00303922	ND[b]
KEY-PCN-HI-727	NA	00303923	ND[b]
KEY-PCN-HI-734	NA	00303924	ND[b]

b No chlorite was detected at 0.1%.

NA Not applicable.

000774

Table (Continued)
Sample Analysis Data

Matrix	Day	Sample Number	Beta Carotene by HPLC (mg/100 g)	Vitamin A (iu/100 g)	Chlorite
Carrots (Received from Salad Time Farms, Salinas, California)					
TACS HIGH, "A"	18	00304985	10.2	17000	ND[b]
TACS HIGH, "B"	18	00304986	9.77	16300	ND[b]
TACS CONTROL, "A"	18	00304987	10.9	18200	ND[b]
TACS CONTROL, "B"	18	00304988	11.5	19200	ND[b]
TACS HIGH, "A"	24	00306652	10.8	18000	ND[b]
TACS HIGH, "B"	24	00306653	10.0	16700	ND[b]
TACS CONTROL, "A"	24	00306654	10.3	17200	ND[b]
TACS CONTROL, "B"	24	00306655	10.6	17700	ND[b]
TACS-2-ZERO, "A"	4	00306659	10.5	17500	ND[b]
TACS-2-ZERO, "B"	4	00306660	10.7	17800	ND[b]
TACS-2-LOW, "A"	4	00306661	10.6	17700	ND[b]
TACS-2-LOW, "B"	4	00306662	10.4	17300	ND[b]
TACS-2-MEDIUM, "A"	4	00306663	10.3	17200	ND[b]
TACS-2-MEDIUM, "B"	4	00306664	10.6	17700	ND[b]
TACS LOW, "A"	11	00400707	9.97	16600	ND[b]
TACS LOW, "B"	11	00400708	10.7	17800	ND[b]
TACS MEDIUM, "A"	11	00400709	11.7	19500	ND[b]
TACS MEDIUM, "B"	11	00400710	10.7	17800	ND[b]
TACS ZERO, "A"	11	00400711	10.6	17700	ND[b]
TACS ZERO, "B"	11	00400712	10.2	17000	ND[b]
TACS LOW, "A"	18	00402434	11.7	19500	ND[b]
TACS LOW, "B"	18	00402435	10.0	16700	ND[b]
TACS MEDIUM, "A"	18	00402436	10.4	17300	ND[b]
TACS MEDIUM, "B"	18	00402437	11.0	18300	ND[b]
TACS ZERO, "A"	18	00402438	11.0	18300	ND[b]
TACS ZERO, "B"	18	00402439	11.0	18300	ND[b]
TACS-LOW, "A"	24	00403620	11.4	19000	NA
TACS LOW, "B"	24	00403621	12.0	20000	NA
TACS MEDIUM, "A"	24	00403622	11.0	18300	NA
TACS MEDIUM, "B"	24	00403623	11.6	19300	NA
TACS ZERO, "A"	24	00403624	11.7	19500	NA
TACS ZERO, "B"	24	00403625	11.1	18500	NA

b No chlorite was detected at 0.1%.

NA Not applicable.

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APPENDIX A

SOP BTI # 99-02

Analyte: Chlorite Anion

BERNÀRD TECHNOLOGIES, INC.

A Health and Food Safety Technology Company

STANDARD OPERATING PROCEDURE
SOP BTI # 99-02

Iodometric Titration for the Analysis of Chlorite Anion Concentration in Inorganic Solids

This Standard Operating Procedure for the analysis of chlorite anion in solid inorganic materials is based on the Standard Methods for Water Analysis Method 4500-ClO2 B as reported by the APHA, AWWA, and WPCF. This SOP is PROPRIETARY and CONFIDENTIAL to BERNÀRD TECHNOLOGIES, INC.

A. Reagents and Equipment:

1. Reagents:
   a) Potassium Iodide (KI) – granular, minimum 99%
   b) 5% HF – prepared from concentrated HF (48-50%) by diluting 10:1 with DI water.
   c) 0.01N Sodium thiosulfate (NTS) solution.
   d) Potassium Iodate solution – 0.002-0.005M certified primary standard.
   e) De-ionized (DI) water.
   f) 1% soluble starch indicator solution.

1. Equipment:
   a) Analytical balance, 0.001g resolution minimum.
   b) 5ml Graduated pipet and bulb.
   c) Chemical spatula
   d) Non-glass, disposable test tube with tight fitting cap. (Polystyrene with polypropylene cap recommended.)
   e) 1-2ml Disposable pipet and bulb.
   f) 125-250 ml Erlenmeyer flask.
   g) Burette, 0.01ml resolution minimum (Digital preferred).
   h) De-ionized water squirt bottle.
   i) 5% HF squirt bottle.
   j) Magnetic stir plate and stir bar.

A. Standardization of Sodium Thiosulfate Solution.

Prior to using the ~0.01N NTS solution for titration of samples, its exact concentration must first be determined. This procedure should be performed at the beginning of each day or upon refilling the titrant burette reservoir. Potassium iodate solution is used as the primary standard.

1. Weigh 1.0 +/- 0.1g KI into an Erlenmeyer flask containing a magnetic stir bar.
2. Add ~5ml 5% HF.
3. Accurately measure 5ml of Potassium iodate primary standard into the flask using the graduated pipet. Record the concentration (C_1) and exact volume added (V_1).
4. Immediately titrate (See section D.) and record the volume (V_2) of NTS used.
5. Repeat three times.
6. Calculate the exact concentration of the NTS (C_2)using the following equation:

$$C_1 * V_1 * 6 / V_2 = C_2$$

A. Sample Preparation: Chlorite anion in solid inorganic powder mixtures: Solids containing sodium chlorite such as Microlite™ or Microsphère®, and its precursor core material are analyzed in the following manner. An aggressive sample digestion solution is used to facilitate the analysis of chlorite that may be held within an insoluble phase.

All samples should be handled to minimize the loss of I_2 through evaporation. All vessels containing samples should be tightly closed between steps. A blank containing all reagents except the sample should be carried though all steps. The results of the blank titration will be used in the final calculations, Sec E.

1. Weigh 0.05 - 0.2 grams of the inorganic powder to be analyzed into a test tube and record the exact mass (M_1).
2. Add 1.0 +/- 0.1g KI.
3. Add ~5ml 5% HF. Cap the test tube tightly and shake. Let stand 5 minutes in the dark.**
4. Transfer the sample to a 250ml Erlenmeyer flask containing a magnetic stir bar.
5. Use DI water to completely rinse the sample from the test tube into the Erlenmeyer flask. Rinse the cap also.
6. Immediately titrate the sample using the standardized NTS. (See Sec D.).
7. Repeat the titration using a blank that contains all reagents except the sample, and has been treated in the same manner as all other samples.

** If several samples are to be analyzed, no more than 6 samples (which can all be titrated within a 20 minute period) should be acidified at a time to minimize the deviation in times until each sample is titrated. Each set should have a blank titrated which is representative of the average digestion period of the samples.

A. Titration: The iodine generated by the oxidation if iodide is titrated using sodium thiosulfate (NTS). General Method: The following is a list of guidelines to prevent some of the most common errors that effect the accuracy of the titration.

1. The concentration of the NTS should be chosen so that a total of 2-20ml of titrant is required for each analysis. A concentration of 0.01N is typical for the masses used above.
2. All titrant and indicator additions should be well stirred.
3. Care should be taken to minimize splashing and evaporation.
4. The flask walls should be rinsed with DI water occasionally to avoid loss of reactants from splashing.
5. The volume of the titrant should be read and recorded carefully.
6. Starch indicator should be added only after most of the iodine has been reduced. The starch-I_2 complex is decomposed in the presence of excess iodine.
7. A white background should be place under the titration flask to assist in the detection of the end-point.
8. Samples should be kept stoppered at all times unless adding reagents or transferring.

Procedure:

1. Stir the sample solution.
2. Titrate the I_2 in the sample using the standardized NTS (from Sec. B.) until only a faint yellow remains.
3. Add ~1ml starch indicator solution. An intense blue (purple or black) color will develop due to the formation of a starch-iodine complex.
4. Continue the titration until the starch-iodine complex color is no longer visible, rinsing the walls of the flask as necessary.
5. Record the total volume of NTS consumed (V_3).
6. Record the volume of NTS used on the blank (B).

A. Calculations: The calculations are based on the stoichiometries described in the following equations. Equation 1 shows the oxidation of iodide to iodine by chlorite anion. Equation 2 shows the reduction of iodine back to iodide by the thiosulfate titrant.

At pH <2: $ClO_2^- + 4\,I^- + 4\,H^+ \rightarrow 2\,I_2 + Cl^- + 2\,H_2O$ [Eqn. 1]

$2\,Na_2S_2O_3 + I_2 \rightarrow 2\,I^- + Na_2S_4O_6 + 2\,Na^+$ [Eqn. 2]

The concentration of chlorite in the inorganic powder is reported here in mg ClO_2^-/g powder. This result is a concentration of chlorite anion only.

$$\text{mg } ClO_2^-/g = (V_3 - B) * C_2 * 67.442 / 4 / M_1$$

V_3 is the volume of titrant used on the sample, in milliliters; B is the volume of titrant used on the blank, in milliliters; C_2 is the concentration of the NTS, calculated in Sec B.6.; and M_1 is the mass of the sample, in grams. The molecular weight of the chlorite anion is 67.442 g/mole, and four equivalents of iodide are oxidized per mole of chlorite.

B. References: Similar methods can be found in references 1 and 2 below. Reference 3 contains information on iodometric titration in general and information on the chemistry and application of the method.

1. *Standard Methods for the Examination of Water and Wastewater. 4500-ClO2 B. Iodometric Method.* APHA, AWWA, and WPCF. Washington, D.C. (17th ed.,) pg. 4-54.

2. E. M. Aieta, P. V. Roberts, and M. Hernandez, *J. Am. Water Works Assoc.* **76**(1), 64-70 (1984).

3. *Fundamentals of Analytical Chemistry.* D. A. Skoog and D. M. West, 4th ed., CBS College Publishing, 1982, pp.356-389, 764-773.

Written By: Joel J. Kampa, Ph.D.
Date: 10 Sept 99
File Name & Path:

APPENDIX B

Covance method summaries

Analyte: Thiamine (Vitamin B1)

Mnemonic(s): BIDE

SOP: MP-BIDE-MA

Scope: This method is applicable to food products, animal feeds, and synthetic products.

Principle: The sample is autoclaved under weak acid conditions to extract the thiamine. The resulting solution is incubated with a buffered enzyme solution to release any bound thiamine. The solution is purified on an ion-exchange column. An aliquot is taken and reacted with potassium ferricyanide to convert thiamine to thiochrome. The thiochrome is extracted into isobutyl alcohol and read on a fluorometer against a known standard.

Control:

Matrix	LOD	CV
Most foods	0.01 mg/100g	N/A
Egg Noodle	0.01 mg/100g	2.48%
Infant formula	0.01 mg/100g	1.76%

Sample Required: 25 g

Turnaround Time: 10 days

Method References(s): Official Methods of Analysis (1995), 16th Edition, Methods 942.23, 953.17, "Thiamine (Vitamin B1) in Foods", 953.17 "Thiamine (Vitamin B1) in Grain Products" and 95.7.17 "Thiamine (Vitamin B1) in Bread", Chapter 45, pp 6-9, AOAC, Arlington, Virginia, (modified).

Date: 5/6/97

Analyte: Riboflavin (Vitamin B2)

Mnemonic(s): B2FV

SOP: MP-B2FV-MA

Scope: This method is applicable to foods, some finished feeds, and infant formulas.

Principle: The sample is hydrolyzed with dilute hydrochloric acid (HCl) and the pH is adjusted to remove interferences. The amount of riboflavin is determined by comparing the growth response of the sample, using the bacteria Lactobacillus casei, with the growth response of riboflavin standard. This growth response is measured turbidimetrically.

Control:

Matrix	LOD	CV
Most foods	<0.2 mcg/g	N/A
Infant formula powder	<0.2 mcg/g	7.0%

Sample Required: 50 g

Turnaround Time: 10 days

Method References(s): AOAC International, Official Methods of Analysis (1995), 16th Edition, Methods 45.2.01 and 45.2.06 (Turbidimetric), AOAC, Arlington, Virginia, (modified).

The United States Pharmacopeia, Volume 23, PP 1749-1750 (1990), (modified).

Date: 5/7/97

Analyte: Alpha and Beta Carotene

Mnemonic(s): ACLC, BCLC

SOP: MP-ACLC, BCLC-MA

Scope: This method is applicable to the determination of alpha and beta carotene in food products and food additives.

Principle: The sample is extracted with alcohol. An aliquot is de-esterified with potassium hydroxide and partitioned with hexane. The sample is cleaned up, evaporated to dryness and diluted with methonal/chloroform. The sample is then injected on a reverse phase high-performance liquid chromatography (HPLC) system and compared to a standard curve.

Control:

Matrix	LOD	CV
Infant Formula at 0.07mg/100g	0.01 mg/100g	10.8 %
Pureed vegetable at 1.20 mg/100g	0.01 mg/100g	4.3 %

Sample Required: 10 grams

Turnaround Time: 10 working days

Method References(s): AOAC International, Official Methods of Analysis (1995), 16th Edition, Volume 2, Method 45.1.03, AOAC, Arlington, Virginia, (modified).

Quackenbush, F. W., Journal of Liquid Chromatography, 10: 643-653, (1987), (modified).

Date: 5/9/97

Analyte: Vitamin A from Carotene

Mnemonic(s): BCAV

Scope: This is a calculation mnemonic used to convert MG of carotene to IU of vitamin A.

Principle: 1 MG of alpha carotene is equivalent to 833.5 IU of vitamin A.
1 MG of beta carotene is equivalent to 1667 IU of vitamin A.

Control: Not applicable

Sample Required: Not applicable

Turnaround Time: Not applicable

Method References(s): Recommended Dietary Allowances, 10th Edition, pp. 80-81, National Research Council, 1989.

Date: *10/23/00*

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APPENDICES

Volume 3 of 3

GENERALLY RECOGNIZED AS SAFE DETERMINATION FOR CHLORINE DIOXIDE GENERATED BY THE MICROSPHÈRE® SYSTEM IN LOW-DENSITY POLYETHYLENE FILM FOR FOOD-CONTACT PACKAGING MATERIAL

Prepared for

Bernàrd Technologies, Inc.
Chicago, Illinois

Prepared by

ENVIRON International Corporation
Arlington, Virginia

November 27, 2000

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APPENDIX I

TOXICOLOGY REVIEW OF CHLORINE DIOXIDE AND CHLORITE

CONTENTS OF APPENDIX I

1. **IRIS Monograph for Chlorine Dioxide**

2. **Iris Monograph for Chlorite**

3. **Toxicology Review of Chlorine Dioxide and Chlorite**

Category (section)	Status	Last Revised
Oral RfD Assessment (I.A.)	On-line	10/12/00
Inhalation RfC Assessment (I.B.)	On-line	10/12/00
Carcinogenicity Assessment (II.)	On line	10/12/00

I. CHRONIC HEALTH HAZARD ASSESSMENTS FOR NONCARCINOGENIC EFFECTS

I.A. REFERENCE DOSE FOR CHRONIC ORAL EXPOSURE (RfD)

Chlorine dioxide
CASRN -- 10049-04-4
Last Revised -- 10/12/00

The oral reference dose (RfD) is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. Please refer to the Background Document for an elaboration of these concepts. RfDs can also be derived for the noncarcinogenic health effects of substances that are also carcinogens. Therefore, it is essential to refer to other sources of information concerning the carcinogenicity of this substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

I.A.1. ORAL RfD SUMMARY

Critical Effect	Experimental Doses*	UF	MF	RfD
Neurodevelopmental effects	NOAEL: 3 mg/kg-day (35 ppm sodium chlorite)	100	1	3×10^{-2} mg/kg-day
Two-generation rat drinking water study	LOAEL: 6 mg/kg-day (70 ppm sodium chlorite)			

CMA, 1996
*Conversion Factors and Assumptions -- MW of sodium chlorite = 90.5; MW of chlorite = 67.5. Doses (mg sodium chlorite/kg-day) were estimated by the study authors using measured water consumption and body weight data. To express doses as the chlorite ion, the estimated doses were multiplied by the molecular weight ratio of sodium chlorite to chlorite.

I.A.2. PRINCIPAL AND SUPPORTING STUDIES (ORAL RfD)

Chlorine dioxide (ClO_2) in drinking water rapidly degrades to chlorite (ClO_2^-), chlorate (ClO_3^-), and chloride ion (Cl^-). In an epidemiology study by Michael et al. (1981), chlorine dioxide rapidly disappeared from the stored water (within 2-4 hours) and water chlorite concentrations concomitantly increased. Once absorbed, chlorine dioxide and chlorite are cleared from the blood at similar rates and are similarly distributed throughout the body (Abdel-Rahman et al., 1979, 1982). Additionally, chloride is the major in vivo degradation product for chlorine dioxide, chlorite, and chlorate. The available data suggest that chlorine

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dioxide and chlorite have similar targets of toxicity and potencies. Therefore, the toxicity information for chlorite is relevant to deriving an RfD for chlorine dioxide. See also the oral RfD entry in the IRIS summary for chlorite.

CMA (Chemical Manufacturers Association). (1996) Sodium chlorite: drinking water rat two-generation reproductive toxicity study. Quintiles Report Ref. CMA/17/96.

CMA (1996) conducted a two-generation study to examine reproductive, developmental neurotoxicity, and hematological endpoints in rats exposed to sodium chlorite. Thirty male and 30 female Sprague-Dawley rats (F0) generation received drinking water containing 0, 35, 70, or 300 ppm sodium chlorite for 10 weeks and were then paired for mating. Males were exposed throughout mating and then were sacrificed. Exposure for the females continued through mating, pregnancy, and lactation until necropsy following weaning of their litters. Twenty-five males and females from each of the first 25 litters to be weaned in a treatment group were chosen to produce the F1 generation. The F1 pups were continued on the same treatment regimen as their parents. At approximately 14 weeks of age, they were mated to produce the F2a generation. Owing to a reduced number of litters in the 70 ppm F1-F2a generation, the F1 animals were remated following weaning of the F2a to produce the F2b generation. Pregnant F1 females were allowed to litter and rear the F2a and F2b generations until weaning at postnatal day (PND) 21. Using water consumption and body weight data, the study authors calculated doses (adjusted for molecular weight) of 0, 3.0, 5.6, and 20.0 mg chlorite/kg-day for F_0 males; 0, 3.8, 7.5, and 28.6 mg chlorite/kg-day for F_0 females; 0, 2.9, 5.9, and 22.7 mg chlorite/kg-day for F_1 males; and 0, 3.8, 7.9, and 28.6 mg chlorite/kg-day for the F_1 females. Numerous parameters were measured or calculated, including body weight, food and water consumption, estrus cycle in the F0 and F1, hematology and T3 and T4 levels in the F1 (blood samples collected from 1 male and 1 female from the first 20 F1 litters at age PND 25 and another group at 13 weeks). Additional parameters were reproductive/developmental toxicity parameters (i.e., gestation duration, litter size, pup body weight, pup developmental landmarks), total caudal sperm number and percent motile, sperm morphology in the F0 and F1, and organ weight and histopathological examination (brain, pituitary gland, liver, adrenal, spleen, thymus, kidneys, and reproductive organs) of all F0 and F1 controls and high-dose animals. An additional group of F1 pups was chosen for neurohistopathology on PND 11 (examination of the brain and spinal cord) or PND 60 (sensory ganglia, dorsal and ventral nerve roots, and several peripheral nerves and muscles).

Another group of F1 rats was examined for neurotoxicological endpoints (motor activity in a "Figure 8" Activity System and neuropathology on PND 60, auditory startle in the SR-Screening System, learning and memory retention in a water E-maze). A functional observational battery (FOB) was also conducted on the pups undergoing the auditory and learning assessments. This group was composed of 2 males and 2 females from 20 litters, and exposure was discontinued after weaning. Reevaluation of the auditory startle response was conducted in 20 males and 20 females in the F2a and F2b generations.

Reductions occurred in water consumption, food consumption, and body weight gain in both sexes in all generations at various times throughout the experiment, primarily in the 70 and 300 ppm groups. The authors attributed these reductions to lack of palatability of the drinking water solution, but did not show data to support this contention. Significant alterations related to treatment at 300 ppm include reductions in absolute and relative liver weight in F0 females and F1 males and females, reduced pup survival (increase in number of pups found dead and/or killed prematurely during lactation) and reduced body weight at birth and throughout lactation in F1 and F2, lower thymus and spleen weight in both generations, lowered incidence of pups exhibiting a normal righting reflex and with eyes open on PND 15, alteration in clinical condition in F2 animals chosen for neurotoxicity, decreases in absolute brain weight for F1 males and F2 females, delays in sexual development in males (preputial

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separation) and females (vaginal opening) in F1 and F2, and lower red blood cell parameters in F1. It is possible that the reported alterations in pup sexual maturation measures may be due to reduced pup body weight, but a definitive conclusion cannot be drawn. In the 70 ppm groups, reduced absolute and relative liver weight in F0 females and F1 males was observed. In addition, a significant decrease in maximum response to an auditory startle stimulus was noted in the 70 and 300 ppm groups on PND 24 but not on PND 60. Minor, statistically significant changes in hematological data at the 35 and 70 ppm concentrations (generally 1%-7%) in the F1 appear to be within normal ranges based on historical data and are, therefore, not considered clinically or biologically significant or adverse. The no-observed-adverse-effects level (NOAEL) for this study is 35 ppm (3 mg chlorite/kg-day) and the lowest-observed-adverse-effects level (LOAEL) is 70 ppm (6 mg chlorite/kg-day), based on lowered auditory startle amplitude and altered liver weights in two generations.

___I.A.3. UNCERTAINTY AND MODIFYING FACTORS (ORAL RfD)

UF = 100.

The composite uncertainty factor (UF) of 100 includes a factor of 10 to account for uncertainties associated with interspecies extrapolation and a factor of 10 for intrahuman variability. Because the critical effect is developmental toxicity in a database that includes chronic studies, it is not necessary to use an uncertainty factor to account for use of a less-than-lifetime study.

MF = 1.

___I.A.4. ADDITIONAL STUDIES/COMMENTS (ORAL RfD)

The short-term toxicity of chlorine dioxide was assessed in two human studies conducted by Lubbers and associates (Lubbers et al., 1981, 1982, 1984; Bianchine et al., 1981). These studies found no alterations in hematological or urine chemistry or in physical symptoms in human volunteers administered up to 0.34 mg/kg chlorine dioxide in drinking water for 1 day or administered 0.036 mg/kg-day of chlorine dioxide or chlorite in drinking water for 84 days.

Michael et al. (1981), Tuthill et al. (1982), and Kanitz et al. (1996) have examined communities with chlorine dioxide-disinfected water. In an epidemiological study of a community using chlorite as a drinking water disinfectant, adult exposures ranged from 0 to 39.4 mg/day for chlorite for 10 weeks, and no consistent alterations in hematological parameters were reported (Michael et al., 1981). Tuthill et al. (1982) retrospectively compared morbidity and mortality data for a community that had utilized high levels of chlorine dioxide as a drinking water disinfectant with data from a neighboring community and found a greater postnatal weight loss in infants from the exposed community and no increase in the proportion of premature births when the age of the mother was controlled. The authors reported average monthly levels of 0.32 ppm of sodium chlorite added post-treatment, but did not report total chlorine dioxide levels in the treated water. Kanitz et al. (1996) followed 598 births to women who lived in a community with filtered water disinfected with chlorine dioxide, sodium hypochlorite, or both, and 128 births to women living in a community with well water that did not undergo disinfection treatment. Levels of chlorine dioxide in the water immediately after treatment were less than 0.3 mg/L, while chlorine residue was less than 0.4 mg/L. The authors concluded that infants of women who consumed drinking water treated with chlorine compounds during pregnancy were at higher risk for neonatal jaundice, cranial circumference $\leq$ 35 cm, and body length $\leq$ 49.5 cm. However, the epidemiological studies were limited by methodological problems such as lack of characterization of exposure to other agents in the drinking water, lack of drinking water consumption data, atypical baseline comparison data (in the Kanitz et al. study), and lack of

control of potential confounding factors (such as nutritional and smoking habits, and age distribution).

Subchronic/chronic toxicity of chlorine dioxide in animals was investigated by Daniel et al. (1990) and Haag (1949). In the Daniel et al. (1990) study, groups of Sprague-Dawley rats (10/sex) were administered chlorine dioxide in drinking water for 90 days at concentrations of 0, 25, 50, 100, or 200 mg/L (0, 2, 4, 6, or 12 mg/kg-day for males and 0, 2, 5, 8, and 15 mg/kg-day for females). Significant increases in incidence of nasal lesions (goblet cell hyperplasia and inflammation of nasal turbinates) were found in the exposed animals. However, the study authors postulated that these lesions were most likely from inhalation of chlorine dioxide vapors at the drinking water sipper tube or from off-gassing of the vapors after drinking rather than ingestion of the drinking water. Thus, the 2 mg/kg-day dose group could be described as a LOAEL, but the toxicological significance of the findings was inconclusive because the lesions were not reported in any other studies and may possibly be an artifact of treatment.

Haag (1949) exposed groups of seven male and seven female rats to 0, 0.5, 1, 5, 10, or 100 mg/L chlorine dioxide in drinking water (0.07, 0.13, 0.7, 1.3, or 13 mg/kg-day) for 2 years. Survival in the 100 mg/L group was significantly decreased. No chlorine dioxide-related alterations were observed in the histopathological examination of representative animals from each group. Thus, a NOAEL of 10 mg/L (1.3 mg/kg-day) and a Frank Effect Level (FEL) (based on decreased survival) of 100 mg/L (13 mg/kg-day) can be identified from this study. Renal pathology, characterized by distention of the glomerular capsule and appearance of a pinkish staining material in the renal tubules, was observed in rats exposed to 100 or 1,000 mg/L chlorite in drinking water for 2 years (9.3 or 81 mg/kg-day).

Numerous animal studies have examined neurodevelopmental toxicity of chlorine dioxide and chlorite. These studies consistently show a LOAEL of 14 mg/kg-day and NOAEL of 3 mg/kg-day for multiple neurodevelopmental endpoints. Decreases in locomotor activity on PND 18-19, but not on days 15-17 or day 20, were observed in Sprague-Dawley rat pups administered gavage doses of 14 mg/kg-day chlorine dioxide on PND 5-20 (Orme et al., 1985). In in utero-exposed pups (dams exposed to 100 mg/L chlorine dioxide in drinking water [14 mg/kg-day] for 2 weeks prior to mating and throughout gestation and lactation), there was a consistent decrease in locomotor activity, but the activity was not statistically significantly lower than controls. Triiodothryonine (T3) and thyroxine (T4) were significantly decreased in the in utero-exposed pups and T4 levels were decreased in the postnatally exposed pups. No significant alterations in locomotor activity or T3 or T4 levels were observed in the offspring of rats exposed to 2 or 20 mg/L (1 or 3 mg/kg-day; exposure protocol the same as 100 mg/L group). However, there was a significant correlation between T4 levels and locomotor activity in all groups. Thus, this study identifies a NOAEL of 3 mg/kg-day and a LOAEL of 14 mg/kg-day.

Mobley et al. (1990) found decreases in exploratory activity on postconception days 36-39, but not on days 39-41 in offspring of Sprague-Dawley rats exposed to 100 ppm chlorine dioxide in the drinking water (14 mg/kg-day) for 10 days prior to mating with unexposed males and during the gestation and lactation periods. A significant decrease in litter weight was also observed. Mobley et al. also found significant decreases in exploratory activity on PND 36-39, but not on days 39-41, in the offspring of Sprague-Dawley rats exposed to 40 ppm chlorine dioxide in the drinking water (6 mg/kg-day) for 10 days prior to mating and during gestation and lactation. T3 and T4 levels were not significantly altered. A slight decrease in activity was also observed in the offspring of rats exposed to 20 ppm (3 mg/kg-day). This study identifies a NOAEL of 3 mg/kg-day and a LOAEL of 14 mg/kg-day.

Decreases in exploratory activity (PND 60) were also observed by Taylor and Pfohl (1985) in offspring of Sprague-Dawley rats exposed to 100 ppm chlorine dioxide in the drinking water (14 mg/kg-day) for 14 days prior to breeding and throughout gestation and

lactation. A nonsignificant decrease in locomotor activity was noted in PND 10-20. Decreases in home cage or wheel running activity occurred on PND 10 and 18-19 in pups (not exposed in utero) administered gavage doses of 14 mg/kg-day on PND 5-20. In addition to the decreases in motor activity, decreases in brain weight (primarily due to a decrease in cerebellar weight) and total cell numbers in the cerebellum were observed in the in utero-exposed pups. A LOAEL of 14 mg/kg-day was identified in this study; a NOAEL was not identified.

Toth et al. (1990) found decreases in forebrain weight, accompanied by decreases in protein content, on PND 21 and 35 in Long-Evans hooded rat pups receiving gavage doses of 14 mg/kg-day on PND 1-20. Dendritic spine counts in Krieg's area 18 (a visual association region of the cortex) were also significantly decreased. No gross lesions, loss of myelin, or changes in cells staining positive for Nissl substance in the forebrain, cerebellum, or brainstem were observed. T3, T4, and free T4 index were not significantly altered on PND 11, 21, and 35. The 14 mg/kg-day dose is a LOAEL for neurodevelopmental effects.

___I.A.5. CONFIDENCE IN THE ORAL RfD

Study -- Medium
Database -- High
RfD -- Medium-to-High

The overall confidence in this RfD assessment is medium-to-high. Confidence in the CMA (1996) principal study is medium. Although the study design and analytical approaches are consistent with EPA testing guidelines, some limitations in the design and conduct of the study exist. These limitations include: (1) lack of pair-watered and -fed controls, which confounds the results and precludes definitive conclusions on whether the alterations in food and water consumption and body weight are related to water palatability or to a direct toxic effect of the agent; (2) developmental landmarks (e.g., vaginal opening in F2a group) were not reported for all groups; (3) grip strength and landing foot splay were not included in the FOB; and (4) discontinuation of exposure for the animals undergoing neurotoxicity testing minimizes the likelihood of finding a positive effect and precludes comparison of the data with those of other rats with continued exposure. Discontinuation of exposure after weaning reduces the opportunity to detect neurological effects from continuous or lifetime exposures similar to those expected from lifetime drinking water exposure in humans. Confidence in the database is high, because there are studies in multiple species, chronic duration studies in males and females, reproductive/developmental toxicity studies, and a multigenerational study. The threshold for adverse effects is consistently defined among the animal studies.

___I.A.6. EPA DOCUMENTATION AND REVIEW OF THE ORAL RfD

Source Document -- This assessment is presented in the Toxicological Review of Chlorine Dioxide and Chlorite (CAS No. 10049-04-4 and 7758-19-2) (U.S. EPA, 2000).

This assessment was peer reviewed by external scientists. Their comments have been evaluated carefully and incorporated in finalization of this IRIS summary. A record of these comments is included as an appendix to U.S. EPA, 2000.

Agency Consensus Date – 9/20/00

___I.A.7. EPA CONTACTS (ORAL RfD)

Please contact the Risk Information Hotline for all questions concerning this assessment or IRIS, in general, at (513)569-7254 (phone), (513)569-7159 (FAX), or RIH.IRIS@EPAMAIL.EPA.GOV (Internet address).

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I.B. REFERENCE CONCENTRATION FOR CHRONIC INHALATION EXPOSURE (RfC)

Chlorine dioxide
CASRN -- 10049-04-4
Last Revised -- 10/12/00

The inhalation reference concentration (RfC) is analogous to the oral RfD and is likewise based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis. The inhalation RfC considers toxic effects for the respiratory system (portal-of-entry) and effects peripheral to the respiratory system (extrarespiratory effects). It is generally expressed in units of mg/m^3. In general, the RfC is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily inhalation exposure of the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. Inhalation RfCs were derived according to the Interim Methods for Development of Inhalation Reference Doses (EPA/600/8-88/066F August 1989) and subsequently according to Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (EPA/600/8-90/066F October 1994). RfCs can also be derived for the noncarcinogenic health effects of substances that are carcinogens. Therefore, it is essential to refer to other sources of information concerning carcinogenicity of this substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

I.B.1. INHALATION RfC SUMMARY

Critical Effect	Experimental Doses*	UF	MF	RfC
Vascular congestion and peribronchial edema	NOAEL: None	3,000	1	2×10^{-4} mg/m^3
60-day rat inhalation study Paulet and Desbrousses, 1972	LOAEL: 2.76 mg/m^3 (1 ppm) $LOAEL_{ADJ}$: 0.41 mg/m^3 $LOAEL_{HEC}$: 0.64 mg/m^3			
Hemorrhagic alveoli and congested capillaries the lungs	NOAEL: None			
45-day rabbit inhalation study Paulet and Desbrousses, 1970	LOAEL: 6.9 mg/m^3 (2.5 ppm) $LOAEL_{ADJ}$: 0.82 mg/m^3 $LOAEL_{HEC}$: 0.49 mg/m^3			

*Conversion Factors and Assumptions -- MW = 67.46.

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Paulet and Desbrousses, 1972: Assuming 25° C and 760 mm Hg, LOAEL (mg/m^3) = 1.0 ppm × 67.46/24.45 = 2.76 mg/m^3. $LOAEL_{ADJ}$ = 2.76 mg/m^3 × 5 hours/24 hours × 5 days/7 days = 0.41 mg/m^3. The $LOAEL_{HEC}$ was calculated for a gas:respiratory effect in the thoracic region. MV_a = 0.17 m^3, MV_h = 20 m^3, Sa_{TH} = 3,461.6 cm^2. Sh_{TH} = 640.581 cm^2. $RGDR_{TH}$ = $(MV_a/Sa_{TH}) / (MV_h/Sh_{TH})$ = 1.57. $LOAEL_{HEC}$ = $LOAEL_{ADJ}$ × RGDR = 0.64 mg/m^3.

Paulet and Desbrousses, 1970: Assuming 25° C and 760 mm Hg, LOAEL (mg/m^3) = 2.5 ppm × 67.46/24.45 = 6.9 mg/m^3. $LOAEL_{ADJ}$ = 6.9 mg/m^3 × 4 hours/24 hours × 5 days/7 days = 0.82 mg/m^3. The $LOAEL_{HEC}$ was calculated for a gas:respiratory effect in the thoracic region. MV_a = 1.10 m^3, Sa_{TH} = 59,100 cm^2. $RGDR_{TH}$ = $(MV_a/Sa_{TH}) / (MV_h/Sh_{TH})$ = 0.596. $LOAEL_{HEC}$ = $LOAEL_{ADJ}$ × RGDR = 0.49 mg/m^3.

___I.B.2. PRINCIPAL AND SUPPORTING STUDIES (INHALATION RfC)

Paulet, G; Desbrousses, S. (1970) On the action of ClO_2 at low concentrations on laboratory animals. Arch Mal Prof Med Trav Secur Soc 31(3):97-106.

Paulet, G; Desbrousses, S. (1972) On the toxicology of chlorine dioxide. Arch Mal Prof Med Trav Secur Soc 33(1-2):59-61.

Paulet and Desbrousses (1970) conducted 4 studies to investigate the toxicity of inhaled chlorine dioxide in rats and rabbits (strains not specified): (a) 5 male and 5 female rats were exposed to 10 ppm (28 mg/m^3) 2 hours/day for 30 days; (b) 10 male rats, 10 female rats, and 5 rabbits were exposed to 5 ppm chlorine dioxide (14 mg/m^3) 2 hours/day for 30 days; (c) 10 male and 10 female rats were exposed to 2.5 ppm (6.9 mg/m^3) 7 hours/day for 30 days; and (d) 8 rabbits were exposed to 2.5 ppm chlorine dioxide (6.9 mg/m^3) 4 hours/day for 45 days. The weekly exposure frequency was not reported; presumably it was 5 days/week. Control groups with equal numbers of animals were used for each study. The following adverse effects were observed at 10 ppm: nasal discharge and red eyes, localized bronchopneumonia with desquamation of the alveolar epithelium, and significantly increased blood erythrocyte and leukocyte levels. Similar, but less severe, respiratory tract effects were observed at 5 ppm; there were no alterations in erythrocyte or leukocyte levels at this concentration. Lymphocytic infiltration of the alveolar spaces, alveolar vascular congestion, hemorrhagic alveoli, epithelial erosions, and inflammatory infiltrations of the bronchi were observed in the rats exposed to 2.5 ppm. The study authors noted that body weight gain was "slightly slowed" (data not presented), and the erythrocyte and leukocyte levels were 85% and 116% of controls, respectively (statistical analysis not reported), in the rats exposed to 2.5 ppm. In rabbits exposed to 2.5 ppm chlorine dioxide, hemorrhagic alveoli and congested capillaries were observed in the lungs. Body weight gain was not adversely affected and erythrocyte and leukocyte levels were 80% and 116% of control (statistical analysis not reported, study authors state that the cell counts "changed very little"). Another group of rats and rabbits was sacrificed 15 days after termination of the 2.5 ppm exposure regimens. Recovery from the pulmonary lesions was evident in these animals. The liver was not adversely affected in the rats or rabbits following exposure to 2.5, 5, or 10 ppm chlorine dioxide. This study identifies a LOAEL of 2.5 ppm (6.9 mg/m^3) for thoracic effects (alveolar congestion and hemorrhage and bronchial inflammation) in rats (7 hours/day for 30 days) and pulmonary effects (alveolar hemorrhage and capillary congestion) in rabbits (4 hours/day for 45 days).

In a followup study by Paulet and Desbrousses (1972), groups of eight Wistar rats (sex not reported) were exposed to 1 ppm chlorine dioxide (2.8 mg/m^3) 5 hours/day, 5 days/week,

for 2 months. The study authors noted that weight gain and erythrocyte and leukocyte levels were not affected, but did not present concurrent control data. Vascular congestion and peribronchiolar edema were observed in the lungs of chlorine dioxide-exposed rats; no alterations in the epithelium or parenchyma were observed. This subchronic study identifies a LOAEL of 1 ppm (2.8 mg/m^3) for respiratory effects in rats.

___I.B.3. UNCERTAINTY AND MODIFYING FACTORS (INHALATION RfC)

UF = 3,000.
MF = 1.

The uncertainty factor of 3,000 comprises a factor of 10 to account for extrapolation of a chronic RfC from a subchronic study, 3 for interspecies extrapolation using dosimetric adjustments, 10 for intrahuman variability, and 10 to account for extrapolation from a LOAEL for mild effects and for the lack of inhalation developmental and reproductive toxicity studies. EPA's policy is to limit the size of the composite UF to 3,000 in recognition of the lack of independence of these factors. (U.S. EPA, 1994). The LOAEL-to-NOAEL and database uncertainties are therefore coalesced into one UF of 10. The composite UF for this RfC is therefore 3,000.

___I.B.4. ADDITIONAL STUDIES/COMMENTS (INHALATION RfC)

Several case reports of accidental inhalation exposure to chlorine dioxide have been reported in the literature. Elkins (1959) described the case of a bleach tank worker who died after being exposed to 19 ppm chlorine dioxide (52 mg/m^3) for an unspecified amount of time; another worker who was exposed at the same time survived. Elkins also stated that 5 ppm (14 mg/m^3) was definitely irritating to humans. In a case reported by Exner-Freisfeld et al. (1986), a woman experienced coughing, pharyngeal irritation, and headache after inhaling an unknown amount of chlorine dioxide inadvertently generated while bleaching flowers. Seven hours after exposure, the woman was hospitalized with cough, dyspnea, tachypnea, tachycardia, rales on auscultation, and marked leukocytosis; a decrease in lung function (reduced vital capacity and 1-second forced expiratory volume) was also reported. Most of these symptoms were alleviated with corticosteroid treatment.

Meggs et al. (1996) examined 13 individuals (1 man and 12 women) 5 years after they were occupationally exposed to chlorine dioxide from a leak in a water purification system pipe. The long-term effects of the accident included development of sensitivity to respiratory irritants (13 subjects), disability with loss of employment (11 subjects), and chronic fatigue (11 subjects). Nasal abnormalities (including injection, telangectasia, paleness, cobblestoning, edema, and thick mucus) were found in all 13 individuals. Nasal biopsies taken from the subjects revealed chronic inflammation, with lymphocytes and plasma cells present within the lamina propria in 11 of the 13 subjects; the inflammation was graded as mild in 2 subjects, moderate in 8 subjects, and severe in 1 subject. Nasal biopsies from three control individuals showed chronic inflammation in one person. The average inflammation grading was statistically higher in the exposed subjects as compared with the controls. The number of nerve fibers in the biopsies was higher in the subjects (rare fibers in three subjects, moderate fibers in two subjects, and many fibers in three subjects) than controls, but the difference was not statistically significant.

Gloemme and Lundgren (1957), Ferris et al. (1967), and Kennedy et al. (1991) examined male workers occasionally exposed to high concentrations of chlorine dioxide that resulted from equipment failure. Concurrent exposure to chlorine gas and, in some cases, sulfur dioxide confounds interpretation of the results of these studies. Gloemme and Lundgren examined the respiratory health of 12 workers employed at a sulfite-cellulose production facility. Under normal working conditions, the atmospheric chlorine content was

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less than 0.1 ppm (chlorine dioxide levels were not measured); however, occasional equipment leakages would result in high levels of chlorine dioxide, chlorine, and/or sulfur dioxide. The workers reported respiratory (breathlessness, wheezing, irritant cough) and ocular (conjunctivitis and "halo phenomena") discomfort in connection with these leakage exposures. A slight, nonspecific chronic bronchitis was diagnosed in 7 of the 12 men.

In the Ferris et al. (1967) study, no significant alterations in pulmonary function (forced vital capacity, maximum expiratory flow, forced expiratory flow, and forced expiratory volume) were observed in 147 men employed at a pulp mill (length of employment not reported), compared with 124 men employed at a paper mill. The pulp mill workers were exposed to sulfur dioxide or chlorine dioxide and chlorine; the chlorine dioxide concentrations ranged from trace amounts to 2.0 ppm (average concentrations ranged from trace amounts to 0.25 ppm) and chlorine concentrations ranged from trace amounts to 64 ppm (average concentrations ranged from trace amounts to 7.4 ppm). When the pulp mill workers were divided into those exposed to sulfur dioxide and those exposed to chlorine or chlorine dioxide, significantly higher incidences of shortness of breath and excess phlegm were found in the chlorine/chlorine dioxide workers.

In the Kennedy et al. (1991) study of 321 kraft pulp mill workers exposed to chlorine and chlorine dioxide, significant increases in the incidence of wheezing, wheezing accompanied by breathlessness, and work-related wheezing were observed, compared with 37 workers at a rail maintenance yard. Personal time-weighed average (TWA) exposure concentration for chlorine at the pulp mill ranged from 5 to 14 ppm, whereas TWA for chlorine dioxide was below 0.1 ppm. However, 60% of the pulp mill workers reported one or more chlorine or chlorine dioxide "gassing" incidents. No significant differences in tests of pulmonary function were observed between the two groups. The pulp mill workers were divided into two groups based on self-reported accidental exposures to high levels of chlorine/chlorine dioxide gas ("gassing"). In the workers reporting one or more incidents of gassing, prevalence of wheezing and missed work because of chest illness was higher than in the pulp mill workers not reporting gassing incidents. Additionally, incidence of airflow obstruction (measured by a decrease in midmaximal flow rate and the ratio of 1-second forced expiratory volume to forced vital capacity) was higher in nonsmokers and former smokers reporting gassing incidents compared with smokers also reporting gassing incidents.

In a second series of animal studies conducted by Paulet and Desbrousses (1974), groups of 10-15 rats (sex and strain not reported) were exposed to 5, 10, or 15 ppm chlorine dioxide (41, 28, or 14 mg/m^3) for 15-minute periods 2 or 4 times/day for 1 month. Control groups were similarly exposed to room air. At 15 ppm, 1/10 and 1/15 rats exposed for 2 or 4 times/day, respectively, died; body weight loss was observed in both groups. Histological alterations observed at this exposure level included nasal and ocular inflammation and discharge, bronchitis, catarrhous lesions of the alveoli with peribronchiolar infiltrations (more pronounced in the four times/day group). The alveolar lesions were reversible; 15 days after exposure termination, the lung histology was similar to controls. No histological alterations were observed in the liver. At 10 ppm, alveolar irritation and decreases in body weight gain were observed. No adverse effects on clinical signs, body weight gain, or histopathology of the lungs were observed at 5 ppm. Exposure to chlorine dioxide did not adversely affect hematological parameters. This study identified a NOAEL of 5 ppm (6.9 mg/m^3) and LOAEL of 10 ppm (28 mg/m^3) for lung damage following intermittent exposure for 15-minute periods, 2 or 4 times/day for 4 weeks.

Dalhamn (1957) conducted a series of inhalation studies to assess the toxicity of chlorine dioxide in the rat (sex and strain not reported). In the first study, a group of three rats was exposed once a week for 3 minutes to decreasing concentrations of chlorine dioxide (3,400 ppm [9,400 mg/m^3] in week 1, 1,100 ppm [3,000 mg/m^3] in week 2, and 800 ppm [2,200 mg/m^3] in week 3); a second group of three rats served as controls. Respiratory

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distress and decreased body weight were observed in the chlorine dioxide-exposed rats. Bronchopneumonia and hyperemia of the renal corticomedullary junction were observed in two out of three rats; the renal hyperemia was also observed in the control group (2/3). In the second study, exposure to 260 ppm (720 mg/m^3) chlorine dioxide for 2 hours resulted in ocular discharge, epistaxis, death (1/4 rats), pulmonary edema, and circulatory engorgement. In the third study, groups of five rats were exposed to 0 or approximately 10 ppm chlorine dioxide (28 mg/m^3) 4 hours/day for 9 days in a 13-day period. Death (3/5 rats), rhinorrhea, "embarrassed respiration," and weight loss were observed in the chlorine dioxide-exposed rats. Respiratory infection with acute renal and hepatic congestion was also observed. The fourth study involved exposure of groups of 5 rats to 0 or approximately 0.1 ppm chlorine dioxide (0.28 mg/m^3) 5 hours/day for 10 weeks (the number of exposure days per week was not reported). No effects on body weight gain were observed and no histological alterations were observed in the lungs, kidneys, or liver. The Dalhamn (1957) studies identified a NOAEL of 0.1 ppm (0.28 mg/m^3) in rats exposed 5 hours/day for 10 weeks and a LOAEL of 10 ppm (28 mg/m^3) for respiratory tract irritation in rats exposed 4 hours/day for approximately 2 weeks. Note that the RfC value is the same as previously available on IRIS.

___I.B.5. CONFIDENCE IN THE INHALATION RfC

Study -- Low
Database -- Low
RfC -- Low

The overall confidence in this RfC assessment is low. Confidence in the coprincipal studies by Paulet and Desbrousses (1970, 1972) is low. These studies identified only a LOAEL in rats and rabbits for adverse lung effects in 60- and 45-day studies and are lacking in experimental detail. Confidence in the database is also low. There were no adequate subchronic or chronic inhalation studies that examined nonlung effects and no acceptable developmental or reproductive studies for inhaled chlorine dioxide.

___I.B.6. EPA DOCUMENTATION AND REVIEW OF THE INHALATION RfC

Source Document -- This assessment is presented in the Toxicological Review of Chlorine Dioxide and Chlorite (CAS No. 10049-04-4 and 7758-19-2) (U.S. EPA, 2000)

This assessment was peer reviewed by external scientists. Their comments have been evaluated carefully and incorporated in finalization of this IRIS summary. A record of these comments is included as an appendix to U.S. EPA (2000).

Agency Consensus Date -- 9/20/00

___I.B.7. EPA CONTACTS (INHALATION RfC)

Please contact the Risk Information Hotline for all questions concerning this assessment or IRIS, in general, at (513) 569-7254 (phone), (513) 569-7159 (fax), or RIH.IRIS@EPAMAIL.EPA.GOV (Internet address).

_II. CARCINOGENICITY ASSESSMENT FOR LIFETIME EXPOSURE

Chlorine dioxide
CASRN -- 10049-04-4
Last Revised -- 10/12/00

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Section II provides information on three aspects of the carcinogenic assessment for the substance in question: the weight-of-evidence judgment of the likelihood that the substance is a human carcinogen, and quantitative estimates of risk from oral exposure and from inhalation exposure. The quantitative risk estimates are presented in three ways. The slope factor is the result of application of a low-dose extrapolation procedure and is presented as the risk per (mg/kg)/day. The unit risk is the quantitative estimate in terms of either risk per µg/L drinking water or risk per µg/m^3 air breathed. The third form in which risk is presented is a concentration of the chemical in drinking water or air associated with cancer risks of 1 in 10,000, 1 in 100,000, or 1 in 1,000,000. The rationale and methods used to develop the carcinogenicity information in IRIS are described in the Risk Assessment Guidelines of 1986 (EPA/600/8-87/045) and in the IRIS Background Document. IRIS summaries developed since the publication of EPA's more recent Proposed Guidelines for Carcinogen Risk Assessment also utilize those Guidelines where indicated (Federal Register 61(79):17960-18011, April 23, 1996). Users are referred to Section I of this IRIS file for information on long-term toxic effects other than carcinogenicity.

II.A. EVIDENCE FOR HUMAN CARCINOGENICITY

II.A.1. WEIGHT-OF-EVIDENCE CHARACTERIZATION

Under the current guidelines (U.S. EPA, 1986), chlorine dioxide is classified as Group D; not classifiable as to human carcinogenicity because of inadequate data in humans and animals. Under the draft Carcinogen Assessment Guidelines (U.S. EPA, 1996), the human carcinogenicity of chlorine dioxide cannot be determined because no satisfactory human or animal studies assessing the chronic carcinogenic potential of chlorine dioxide have been located. Concentrates prepared from drinking water treated with chlorine dioxide did not increase the incidence of lung adenomas in Strain A mice, the skin tumor frequency in mice, or the incidence of gamma-glutamyl transpeptidase positive foci (a measure of preneoplastic changes) in rat livers (Miller et al., 1986). However, chlorine dioxide did induce a hyperplastic response in the mouse skin (Robinson et al., 1986). Both positive and negative results have been found in genotoxicity studies.

II.A.2. HUMAN CARCINOGENICITY DATA

None

II.A.3. ANIMAL CARCINOGENICITY DATA

None

II.A.4. SUPPORTING DATA FOR CARCINOGENICITY

Miller et al. (1986), using three short-term assays, tested the carcinogenic potential of drinking water disinfected with chlorine dioxide. Following disinfection with chlorine dioxide, the water samples (containing 0.5 mg/L chlorine dioxide residue) were concentrated 2,000 or 4,000 × with a macroreticular resin process. In a mouse initiation-promotion assay, groups of 14-34 SENCAR mice (sex not specified) were orally administered 0.5 mL of the 4,000 × concentrate in 2% emulphor 3 times/week for 2 weeks followed by topical exposure to 1.0 µg 12-tetradecanylphorbal-13-acetate (TPA) in acetone applied to the dorsal skin 3 times/week for 20 weeks, and were then sacrificed. No significant increases, compared with vehicle controls, in the number of skin tumors or the number of tumors per animal were observed.

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In a lung adenoma assay (Miller et al.. 1986), groups of 20 male and 20 female Strain A mice received 0.25 mL gavage doses of 2.000 × or 4.000 × concentrates in 2% emulphor 3 times/week for 8 weeks followed by a 16-week observation period. The number of animals with lung adenomas and the number of adenomas per animal were not significantly altered compared with vehicle controls.

Miller et al. (1986) also examined the development of liver foci in rats in a short-term assay. In this study, groups of partially hepatectomized rats received a single dose of concentrated water (chlorine dioxide concentration not reported) in 2% emulphor followed (1 week later) by administration of 500 ppm sodium phenobarbital in drinking water for 56 days; animals were sacrificed on day 70. A control group received nondisinfected water. No significant increases in the incidence of γ-glutamyltranspeptidase foci were observed.

The potential for chlorine dioxide to induce proliferative epidermal hyperplasia was examined by Robinson et al. (1986). Groups of five dorsally shaved female SENCAR mice were placed in chambers filled with 0, 1, 10, 100, 300, or 1,000 ppm liquid chlorine dioxide; the chambers were designed to prevent the head from getting wet and to prevent inhalation of vapors. The animals were exposed 10 minutes/day for 4 days. A significant increase in interfollicular epidermal thickness was observed in the 1,000-ppm group but not at the lower concentrations. Increases in total cell numbers and basal cell numbers in skin sections were observed in both the 300 and the 1,000 ppm groups. In a second study, groups of 40 mice were immersed in 0 or 1,000 ppm chlorine dioxide for 10 minutes; animals (5/group) were killed 1, 2, 3, 4, 5, 8, 10, or 12 days post-exposure. A significant increase in interfollicular epidermis thickness was observed at all time periods, with the highest values at 10 and 12 days post-exposure. The authors concluded that even short-term dermal exposure to high concentrations of chlorine dioxide is capable of inducing hyperplastic responses in the mouse skin.

Both positive and negative results have been found in in vitro genotoxicity studies. Chlorine dioxide did not increase occurrence of chromosome aberrations in Chinese hamster fibroblast cells (Ishidate et al., 1984) but did increase occurrence of reverse mutation in *Salmonella typhimurium* (with activation) (Ishidate et al., 1984). However, water samples disinfected with chlorine dioxide did not induce reverse mutations in *S. typhimurium* with or without activation (Miller et al.. 1986). In vivo micronucleus and bone marrow chromosomal aberration assays in Swiss CD-1 mice administered 0.1-0.4 mg via gavage for 5 consecutive days and sperm-head abnormality assay in B6C3F1 mice administered 0.1-0.4 mg via gavage for 5 consecutive days (0, 3.2, 8, and 16 mg/kg-day) were negative (Meier et al., 1985). Hayashi et al. (1988) reported positive results in the micronucleus assay in ddY mice following a single intraperitoneal injection of 3.2-25 mg/kg.

II.B. QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM ORAL EXPOSURE

None

II.C. QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM INHALATION EXPOSURE

None

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__II.D. EPA DOCUMENTATION, REVIEW, AND CONTACTS (CARCINOGENICITY ASSESSMENT)

___II.D.1. EPA DOCUMENTATION

Source Document -- This assessment is presented in the Toxicological Review of Chlorine Dioxide and Chlorite (CAS No. 10049-04-4 and 7758-19-2) (U.S. EPA, 2000).

This assessment was peer reviewed by external scientists. Their comments have been evaluated carefully and incorporated in finalization of this IRIS summary. A record of these comments is included as an appendix to U.S. EPA, 2000.

___II.D.2. EPA REVIEW (CARCINOGENICITY ASSESSMENT)

Agency Consensus Date -- 9/20/00

___II.D.3. EPA CONTACTS (CARCINOGENICITY ASSESSMENT)

Please contact the Risk Information Hotline for all questions concerning this assessment or IRIS, in general, at (513) 569-7254 (phone), (513) 569-7159 (fax), or RIH.IRIS@EPAMAIL.EPA.GOV (Internet address)

___III. [reserved]
___IV. [reserved]
___V. [reserved]

_VI. BIBLIOGRAPHY

Chlorine dioxide
CASRN -- 10049-04-4
Last Revised – 10/12/00

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VII. REVISION HISTORY

Chlorine dioxide
CASRN -- 10049-04-4

Date	Section	Description
11/01/1990	I.B	Inhalation RfC summary on-line
11/01/1990	VI.	Bibliography on-line
01/01/1992	IV.	Regulatory Action section on-line
09/01/1992	I.A	Oral RfD now under review
05/01/1993	I.A	Work group review date added
05/01/1993	II.	Carcinogenicity assessment now under review
12/01/1993	I.A.	Work group review date added
01/01/1994	I.A.	Work group review date added
08/01/1995	I.A., II.	EPA's RfD/RfC and CRAVE

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		workgroups were discontinued in May, 1995. Chemical substance reviews that were not completed by September 1995 were taken out of IRIS review. The IRIS Pilot Program replaced the workgroup functions beginning in September, 1995.
11/01/1995	II.	Carcinogenicity assessment on-line
11/01/1995	VI.C	Carcinogenicity references on-line
04/01/1997	III., IV., V.	Drinking Water Health Advisories, EPA Regulatory Actions, and V. Supplementary Data were removed from IRIS on or before April 1997. IRIS users were directed to the appropriate EPA Program Offices for this information.
10/12/2000	I., II. VI	Oral RfD on-line, revised RfC and carcinogenicity assessment on-line

_VIII. SYNONYMS

Chlorine dioxide
CASRN -- 10049-04-4
Last Revised – 10/12/00

10049-04-4
Chlorine oxide
Alcide
Anthium Dioxcide
Caswell No. 179A
Chlorine dioxide
Chlorine peroxide
CHLORINE (IV) OXIDE
Chloroperoxyl
CHLORYL RADICAL
Dioxide de cloro [Spanish]
Dioxide de chlore [French]
Doxcide 50
EPA Pesticide Chemical Code 020503
HSDB 517

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Last updated: **12 October 2000**
URL: http://www.epa.gov/iris/subst/0496.htm

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EPA United States Environmental Protection Agency

IRIS

Integrated Risk Information System

Chlorite (sodium salt)

CASRN 7758-19-2

10/12/2000

Contents

I.A. REFERENCE DOSE FOR CHRONIC ORAL EXPOSURE (RfD)

I.B. REFERENCE CONCENTRATION FOR CHRONIC INHALATION EXPOSURE (RfC)

II. CARCINOGENICITY ASSESSMENT FOR LIFETIME EXPOSURE

VI. BIBLIOGRAPHY

VII. REVISION HISTORY

VIII. SYNONYMS

Note: ***A TOXICOLOGICAL REVIEW*** is available for this chemical in Adobe* PDF format (56 Pages, 144K).
Similar documents can be found in the List of Available IRIS Toxicological Reviews

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Chlorite (sodium salt): CASRN 7758-19-2; 10/12/00

Health assessment information on a chemical substance is included in IRIS only after a comprehensive review of chronic toxicity data by U.S. EPA health scientists from several Program Offices, Regional Offices, and the Office of Research and Development. The summaries presented in Sections I and II represent a consensus reached in the review process. Background information and explanations of the methods used to derive the values given in IRIS are provided in the Background Documents.

STATUS OF DATA FOR Chlorite

File First On-Line 11/01/95

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Category (section)	Status	Last Revised
Oral RfD Assessment (I.A.)	On-line	10/12/00
Inhalation RfC Assessment (I.B.)	On-line	10/12/00
Carcinogenicity Assessment (II.)	On line	10/12/00

I. CHRONIC HEALTH HAZARD ASSESSMENTS FOR NONCARCINOGENIC EFFECTS

I.A. REFERENCE DOSE FOR CHRONIC ORAL EXPOSURE (RfD)

Chlorite (sodium salt)
CASRN -- 7758-19-2
Last Revised -- 10/12/00

The oral reference dose (RfD) is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. Please refer to the Background Document for an elaboration of these concepts. RfDs can also be derived for the noncarcinogenic health effects of substances that are also carcinogens. Therefore, it is essential to refer to other sources of information concerning the carcinogenicity of this substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

I.A.1. ORAL RfD SUMMARY

Critical Effect	Experimental Doses*	UF	MF	RfD
Neurodevelopmental effects	NOAEL: 3 mg/kg-day (35 ppm sodium chlorite)	100	1	3×10^{-2} mg/kg-day
Two-generation rat drinking water study	LOAEL: 6 mg/kg-day (70 ppm sodium chlorite)			

CMA, 1996

*Conversion Factors and Assumptions -- MW of sodium chlorite = 90.5; MW of chlorite = 67.5. Doses (mg sodium chlorite/kg-day) were estimated by the study authors using measured water consumption and body weight data. To express doses as the chlorite ion, the estimated doses were multiplied by the molecular weight ratio of sodium chlorite to chlorite.

I.A.2. PRINCIPAL AND SUPPORTING STUDIES (ORAL RfD)

CMA (Chemical Manufacturers Association). (1996) Sodium chlorite: drinking water rat two-generation reproductive toxicity study. Quintiles Report Ref. CMA/17/96.

CMA (1996) conducted a two-generation study to examine reproductive, developmental neurotoxicity, and hematological endpoints in rats exposed to sodium chlorite. Thirty male and 30 female Sprague-Dawley rats (F0) generation received drinking water containing 0, 35, 70, or 300 ppm sodium chlorite for 10 weeks and were then paired for mating. Males were exposed throughout mating and then were sacrificed. Exposure for the females continued through mating, pregnancy, and lactation until necropsy following

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weaning of their litters. Twenty-five males and females from each of the first 25 litters to be weaned in a treatment group were chosen to produce the F1 generation. The F1 pups were continued on the same treatment regimen as their parents. At approximately 14 weeks of age, they were mated to produce the F2a generation. Because of a reduced number of litters in the 70 ppm F1-F2a generation, the F1 animals were remated following weaning of the F2a to produce the F2b generation. Pregnant F1 females were allowed to litter and rear the F2a and F2b generations until weaning at postnatal day (PND) 21. Using water consumption and body weight data, the study authors calculated doses (adjusted for molecular weight) of 0, 3.0, 5.6, and 20.0 mg chlorite/kg-day for F_0 males: 0, 3.8, 7.5, and 28.6 mg chlorite/kg-day for F_0 females: 0, 2.9, 5.9, and 22.7 mg chlorite/kg-day for F_1 males; and 0, 3.8, 7.9, and 28.6 mg chlorite/kg-day for F_1 females. Numerous parameters were measured or calculated, including body weight, food and water consumption, estrus cycle in the F0 and F1, hematology and T3 and T4 levels in the F1 (blood samples collected from one male and one female from the first 20 F1 litters at age PND 25 and another group at 13 weeks), reproductive/developmental toxicity parameters (i.e., gestation duration, litter size, pup body weight, pup developmental landmarks), total caudal sperm number and percent motile, sperm morphology in the F0 and F1, and organ weight and histopathological examination (brain, pituitary gland, liver, adrenal, spleen, thymus, kidneys, and reproductive organs) of all F0 and F1 controls and high-dose animals. An additional group of F1 pups was chosen for neurohistopathology on PND 11 (examination of the brain and spinal cord) or PND 60 (sensory ganglia, dorsal and ventral nerve roots, and several peripheral nerves and muscles). Another group of F1 rats was examined for neurotoxicological endpoints (motor activity in a "Figure 8" Activity System and neuropathology on PND 60, auditory startle in the SR-Screening System, learning and memory retention in a water E-maze). A functional observational battery (FOB) was also conducted on the pups undergoing the auditory and learning assessments. This group was composed of 2 males and 2 females from 20 litters, and exposure was discontinued after weaning. A reevaluation of the auditory startle response was conducted in 20 males and 20 females in the F2a and F2b generations.

There were reductions in water consumption, food consumption, and body weight gain in both sexes in all generations at various times throughout the experiment, primarily in the 70 and 300 ppm groups. The authors attributed these reductions to a lack of palatability of the drinking water solution, but did not show data to support this contention. Significant alterations related to treatment at 300 ppm include reductions in absolute and relative liver weight in F0 females and F1 males and females, reduced pup survival (increase in number of pups found dead and/or killed prematurely during lactation) and reduced body weight at birth and throughout lactation in F1 and F2, lower thymus and spleen weight in both generations, lowered incidence of pups exhibiting a normal righting reflex and with eyes open on PND 15, alteration in clinical condition in F2 animals chosen for neurotoxicity, decreases in absolute brain weight for F1 males and F2 females, delays in sexual development in males (preputial separation) and females (vaginal opening) in F1 and F2, and lower red blood cell parameters in F1. It is possible that the reported alterations in pup sexual maturation measures may be due to reduced pup body weight, but a definitive conclusion cannot be drawn. In the 70 ppm groups, reduced absolute and relative liver weight in F0 females and F1 males was observed. Minor, statistically significant changes in hematological data at the 35 and 70 ppm concentrations (generally 1%-7%) in the F1 appear to be within normal ranges based on historical data and are, therefore, not considered clinically or biologically significant or adverse. In addition, a significant decrease in maximum response to an auditory startle stimulus was noted in the 70 and 300 ppm groups on PND 24, but not on PND 60. The NOAEL for this study is 35 ppm (3 mg chlorite/kg-day) and the LOAEL is 70 ppm (6 mg chlorite/kg-day) based on lowered auditory startle amplitude and altered liver weights in two generations.

I.A.3. UNCERTAINTY AND MODIFYING FACTORS (ORAL RfD)

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UF = 100.

The composite uncertainty factor (UF) of 100 includes a factor of 10 to account for uncertainties associated with interspecies extrapolation and a factor of 10 for intrahuman variability. Because the critical effect is developmental toxicity in a database that includes chronic studies, it is not necessary to use an additional uncertainty factor to account for use of a less-than-lifetime study.

MF = 1.

I.A.4. ADDITIONAL STUDIES/COMMENTS (ORAL RfD)

Lubbers et al. (1981, 1982, 1984a) examined the toxicity of chlorite in normal healthy adults. In the single-exposure study (Lubbers et al., 1981, 1982), 10 male adults consumed two (separated by 4 hours) 500 mL solutions containing 2.4 mg/L chlorite (0.034 mg/kg, assuming a reference body weight of 70 kg). In a 12-week study (Lubbers et al., 1984a), groups of 10 men drank 500 mL solutions of 0 or 5 mg/L chlorite (0.04 mg/kg-day assuming a 70 kg body weight). No physiologically relevant alterations in general health (observations and physical examination), vital signs, hematological (including erythrocyte and total and differential leukocyte counts, hemoglobin, hematocrit, and methemoglobin) or serum clinical chemistry (including glucose, electrolytes, calcium, urea nitrogen, enzyme levels, and cholesterol) parameters, or serum T3 or T4 levels were found in either study.

In a companion study, three healthy glucose-6-phosphate dehydrogenase (G6PD) deficient male subjects were given deionized water containing 5 mg/L chlorite (0.04 mg/kg-day, assuming a reference body weight of 70 kg) for 12 weeks (Lubbers et al., 1984b). Compared with the control group in Lubbers et al. (1984a), the chlorite exposure did not alter general health, vital signs, hematological parameters, or serum clinical chemistry parameters.

Michael et al. (1981), Tuthill et al. (1982), and Kanitz et al. (1996) examined communities with chlorine dioxide-disinfected water. Michael et al. (1981) found that chlorine dioxide in drinking water rapidly disappeared from the stored water (within 2-4 hours) and chlorite levels concomitantly increased. In an epidemiological study of a community using chlorite as a drinking water disinfectant, adult exposures ranged from 0 to 39.4 mg/day for chlorite for 10 weeks, and no consistent alterations in hematological parameters were reported (Michael et al., 1981). Tuthill et al. (1982) retrospectively compared morbidity and mortality data for a community that had utilized high levels of chlorine dioxide as a drinking water disinfectant with data from a neighboring community and found a greater postnatal weight loss in infants from the exposed community and no increase in the proportion of premature births when the age of the mother was controlled. The authors reported average monthly levels of 0.32 ppm of chlorine dioxide added post-treatment, but did not report total chlorine dioxide levels in the treated water. Kanitz et al. (1996) followed 598 births to women who lived in a community with filtered water disinfected with chlorine dioxide, sodium hypochlorite, or both, and 128 births to women living in a community with well water that did not undergo disinfection treatment. Levels of chlorine dioxide in the water immediately after treatment were less than 0.3 mg/L, while chlorine residue was less than 0.4 mg/L. The study authors concluded that infants of women who consumed drinking water treated with chlorine compounds during pregnancy were at higher risk for neonatal jaundice, cranial circumference < 35 cm, and body length < 49.5 cm. However, these studies as a whole are limited by methodological problems such as lack of characterization of exposure to other agents in the drinking water, drinking water consumption data, and control of potential confounding factors.

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The subchronic/chronic toxicity of chlorite was investigated by Harrington et al. (1995)

and Haag et al. (1949). Harrington et al. (1995) administered via gavage 0, 10, 25, or 80 mg sodium chlorite/kg-day (0, 7.4, 19, or 60 mg chlorite/kg-day) to Sprague-Dawley rats for 13 weeks. At the highest dose, gross effects included increased adrenal, spleen, liver, and kidney weight. Hematological alterations included decreased erythrocyte counts, hemoglobin levels, and hematocrit; increased methemoglobin levels (males) and decreased methemoglobin levels (females). Histologic alterations of the stomach consisted of squamous epithelial hyperplasia, hyperkeratosis, ulceriation, chronic inflammation, and edema. At 19 mg/kg-day, stomach lesions (similar to those in the high-dose group) and increases in absolute and relative spleen weights and relative adrenal weights were observed. No effects were observed at 7.4 mg/kg-day.

In the Haag (1949) study, renal pathology, characterized by distention of the glomerular capsule and appearance of a pinkish staining material in the renal tubules, was observed in rats exposed to 100 or 1,000 mg/L chlorite in drinking water for 2 years (9.3 or 81 mg/kg-day). These effects were also observed in a group of animals administered sodium chlorite at a concentration equimolar to 1,000 mg sodium chlorite/L. No other effects were observed. The study was limited because there was an insufficient number of animals tested per group, pathology was conducted on a small number of animals, and it did not provide adequate evaluations of more sensitive parameters, which would have been more useful in the overall assessment of chronic toxicity.

Numerous animal studies have examined neurodevelopmental toxicity of chlorine dioxide and chlorite. These studies consistently show a LOAEL of 14 mg/kg-day and NOAEL of 3 mg/kg-day for multiple neurodevelopmental endpoints. Decreases in locomotor activity on PND 18-19, but not on days 15-17 or day 20, were observed in Sprague-Dawley rat pups administered gavage doses of 14 mg/kg-day chlorine dioxide on PND 5-20 (Orme et al., 1985). In in utero-exposed pups (dams exposed to 100 mg/L chlorine dioxide in drinking water [14 mg/kg-day] for 2 weeks prior to mating and throughout gestation and lactation), there was a consistent decrease in locomotor activity, but the activity was not statistically significantly lower than controls. Triiodothryonine (T3) and thyroxine (T4) were significantly decreased in the in utero-exposed pups and T4 levels were decreased in the postnatally exposed pups. No significant alterations in locomotor activity or T3 or T4 levels were observed in the offspring of rats exposed to 2 or 20 mg/L (1 or 3 mg/kg-day; exposure protocol the same as 100 mg/L group). However, there was a significant correlation between T4 levels and locomotor activity in all groups. Thus, this study identifies a NOAEL of 3 mg/kg-day and LOAEL of 14 mg/kg-day.

Mobley et al. (1990) found decreases in exploratory activity on postconception days 36-39, but not on days 39-41, in offspring of Sprague-Dawley rats exposed to 100 ppm chlorine dioxide in the drinking water (14 mg/kg-day) for 10 days prior to mating with unexposed males and during the gestation and lactation periods. A significant decrease in litter weight was also observed. Mobley et al. also found significant decreases in exploratory activity on PND 36-39, but not on days 39-41, in the offspring of Sprague-Dawley rats exposed to 40 ppm chlorine dioxide in the drinking water (6 mg/kg-day) for 10 days prior to mating and during gestation and lactation. T3 and T4 levels were not significantly altered. A slight decrease in activity was also observed in the offspring of rats exposed to 20 ppm (3 mg/kg-day). This study identifies a NOAEL of 3 mg/kg-day and LOAEL of 14 mg/kg-day.

Decreases in exploratory activity (PND 60) were also observed by Taylor and Pfohl (1985) in offspring of Sprague-Dawley rats exposed to 100 ppm chlorine dioxide in the drinking water (14 mg/kg-day) for 14 days prior to breeding and throughout gestation and lactation. A nonsignificant decrease in locomotor activity was noted in PND 10-20. Decreases in home cage or wheel-running activity occurred on PND 10 and 18-19 in pups (not exposed in utero) administered gavage doses of 14 mg/kg-day on PND 5-20. In addition to the decreases in motor activity, decreases in brain weight (primarily due to a decrease in cerebellar weight) and total cell numbers in the cerebellum were observed in the in utero-

exposed pups. A LOAEL of 14 mg/kg-day was identified in this study; a NOAEL was not identified.

Toth et al. (1990) found decreases in forebrain weight, accompanied by decreases in protein content, on PND 21 and 35 in Long-Evans hooded rat pups receiving gavage doses of 14 mg/kg-day on PND 1-20. Dendritic spine counts in Krieg's area 18 (a visual association region of the cortex) were also significantly decreased. No gross lesions, loss of myelin, or changes in cells staining positive for Nissl substance in the forebrain, cerebellum, or brainstem were observed. T3, T4, and free T4 index were not significantly altered on PND 11, 21, and 35. The 14 mg/kg-day dose is a LOAEL for neurodevelopmental effects.

___I.A.5. CONFIDENCE IN THE ORAL RfD

Study -- Medium
Database -- High
RfD -- Medium-to-High

The overall confidence in this RfD assessment is medium-to-high. Confidence in the CMA (1996) principal study is medium. Although the study design and analytical approaches are consistent with EPA testing guidelines, some limitations in the design and conduct of the study exist. These limitations include (1) lack of pair-watered and -fed controls, which confounds the results and precludes definitive conclusions on whether the alterations in food and water consumption and body weight are related to water palatability or are a direct toxic effect of the agent; (2) developmental landmarks (e.g., vaginal opening in F2a group) were not reported for all groups; (3) grip strength and landing foot splay were not included in the FOB; and (4) discontinuation of exposure for the animals undergoing neurotoxicity testing minimizes the likelihood of finding a positive effect and precludes comparison of the data with those of other rats with continued exposure. Discontinuation of exposure after weaning reduces the opportunity to detect neurological effects from continuous or lifetime exposures similar to those expected from lifetime drinking water exposure in humans. Confidence in the database is high because there are studies in multiple species, chronic duration studies in males and females, reproductive/developmental toxicity studies, and a multigenerational study. The threshold for adverse effects is consistently defined among the animal studies.

___I.A.6. EPA DOCUMENTATION AND REVIEW OF THE ORAL RfD

Source Document -- This assessment is presented in the Toxicological Review of Chlorine Dioxide and Chlorite (CAS No. 10049-04-4 and 7758-19-2) (U.S. EPA, 2000).

This assessment was peer reviewed by external scientists. Their comments have been evaluated carefully and incorporated in finalization of this IRIS summary. A record of these comments is included as an appendix to U.S. EPA, 2000.

Agency Consensus Date -- 9/20/00

___I.A.7. EPA CONTACTS (ORAL RfD)

Please contact the Risk Information Hotline for all questions concerning this assessment or IRIS, in general, at (513)569-7254 (phone), (513)569-7159 (FAX), or RIH.IRIS@EPAMAIL.EPA.GOV (Internet address).

__I.B. REFERENCE CONCENTRATION FOR CHRONIC INHALATION EXPOSURE (RfC)

000812

Chlorite (sodium salt)
CASRN -- 7758-19-2
Last Revised -- 10/12/00

The inhalation reference concentration (RfC) is analogous to the oral RfD and is likewise based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis. The inhalation RfC considers toxic effects for the respiratory system (portal-of-entry) and effects peripheral to the respiratory system (extrarespiratory effects). It is generally expressed in units of mg/m^3. In general, the RfC is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily inhalation exposure of the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious effects during a lifetime. Inhalation RfCs were derived according to the Interim Methods for Development of Inhalation Reference Doses (EPA/600/8-88/066F August 1989) and subsequently, according to Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (EPA/600/8-90/066F October 1994). RfCs can also be derived for the noncarcinogenic health effects of substances that are carcinogens. Therefore, it is essential to refer to other sources of information concerning the carcinogenicity of this substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

___I.B.1. INHALATION RfC SUMMARY

An RfC for chlorite is not recommended at this time. No human or animal studies examining the toxicity of inhaled chlorite were located. Although the available human and animal data on inhaled chlorine dioxide support the derivation of an RfC for this chemical, these data cannot be used to derive an RfC for chlorite. Under ambient conditions, airborne chlorite is likely to exist as a particulate, whereas inhalation exposure to chlorine dioxide is as a gas. On the basis of their physical and chemical properties, it is anticipated that inhaled chlorine dioxide and chlorite would have very different modes of exposure, and the potential hazard associated with exposure to these two chemicals is also very different. In the absence of data demonstrating parallels in pharmacokinetic behavior following inhalation exposure, as is present following oral exposure, derivation of an RfC for chlorite from the available data for chlorine dioxide is not recommended.

___I.B.2. PRINCIPAL AND SUPPORTING STUDIES (INHALATION RfC)

None

___I.B.3. UNCERTAINTY AND MODIFYING FACTORS (INHALATION RfC)

Not applicable.

___I.B.4. ADDITIONAL STUDIES/COMMENTS (INHALATION RfC)

Not applicable.

___I.B.5. CONFIDENCE IN THE INHALATION RfC

Not applicable.

___I.B.6. EPA DOCUMENTATION AND REVIEW OF THE INHALATION RfC

000813

Source Document -- U.S. EPA, 2000

This assessment was peer reviewed by external scientists. Their comments have been evaluated carefully and incorporated in finalization of this IRIS summary. A record of these comments is included as an appendix to U.S. EPA, 2000.

Agency Consensus Date -- 9/20/00

___I.B.7. EPA CONTACTS (INHALATION RfC)

Please contact the Risk Information Hotline for all questions concerning this assessment or IRIS, in general, at (513) 569-7254 (phone), (513) 569-7159 (fax), or RIH.IRIS@EPAMAIL.EPA.GOV (Internet address).

_II. CARCINOGENICITY ASSESSMENT FOR LIFETIME EXPOSURE

Chlorite (sodium salt)
CASRN -- 7758-19-2
Last Revised -- 10/12/00

Section II provides information on three aspects of the carcinogenic assessment for the substance in question, the weight-of-evidence judgment of the likelihood that the substance is a human carcinogen, and quantitative estimates of risk from oral exposure and from inhalation exposure. The quantitative risk estimates are presented in three ways. The slope factor is the result of application of a low-dose extrapolation procedure and is presented as the risk per (mg/kg)/day. The unit risk is the quantitative estimate in terms of either risk per µg/L drinking water or risk per µg/m^3 air breathed. The third form in which risk is presented is a concentration of the chemical in drinking water or air associated with cancer risks of 1 in 10,000, 1 in 100,000, or 1 in 1,000,000. The rationale and methods used to develop the carcinogenicity information in IRIS are described in the Risk Assessment Guidelines of 1986 (EPA/600/8-87/045) and in the IRIS Background Document. IRIS summaries developed since the publication of EPA's more recent Proposed Guidelines for Carcinogen Risk Assessment also utilize those Guidelines where indicated (Federal Register 61(79):17960-18011, April 23, 1996). Users are referred to Section I of this IRIS file for information on long-term toxic effects other than carcinogenicity.

__II.A. EVIDENCE FOR HUMAN CARCINOGENICITY

___II.A.1. WEIGHT-OF-EVIDENCE CHARACTERIZATION

Under the current guidelines (U.S. EPA, 1986), chlorite is classified as Group D; not classifiable as to human carcinogenicity because of inadequate data in humans and animals. Under the draft Carcinogen Assessment Guidelines (U.S. EPA, 1996), the human carcinogenicity of chlorite cannot be determined because of a lack of human data and limitations in animal studies. Chronic oral studies in rats showed no evidence of carcinogenic activity of chlorite (Kurokawa et al., 1986). The short exposure duration (85 weeks) and high incidence of Sendai viral infection in control and exposed rats limit the use of this study to assess carcinogenicity. The mouse studies (Kurokawa et al., 1986; Yokose et al., 1987) showed an increase in liver and lung tumors in treated male mice. However, relatively short exposure duration (80 weeks) and the high incidence of early mortality in the concurrent control males from excessive fighting make statistical comparisons between concurrent controls and treated animals difficult to interpret. No increases in tumor incidence were seen

in female mice in this study. Chlorite did not act as a complete carcinogen in a 51-week dermal carcinogenicity assay in mice (Kurokawa et al., 1984). In the same study, chlorite induced skin tumors following initiation by DMBA, but the increase was not statistically significant. Chlorite has shown both positive and negative results in in vitro and in vivo genotoxicity assays.

___II.A.2. HUMAN CARCINOGENICITY DATA

None

___II.A.3. ANIMAL CARCINOGENICITY DATA

Inadequate. Kurokawa et al. (1986) exposed groups of 50 male and 50 female F344 rats to 0, 300, or 600 ppm sodium chlorite in drinking water for 85 weeks. Using water consumption and body weight data, the study authors estimated the doses to be 18 and 32 mg/kg-day in male rats and 28 and 41 mg/kg-day in female rats. All groups of rats were infected with the Sendai virus. No adverse effect on survival was observed. A slight dose-related decrease in body weight gain was observed (body weight gain in the high-dose group was within 10% of controls). No chlorite-related increases in tumor incidence were observed.

Kurokawa et al. (1986) also exposed groups of 50 male and 50 female B6C3F1 mice to 0, 250, and 500 ppm sodium chlorite in the drinking water for 80 weeks followed by a 5-week recovery period. The results of this study are also presented in Yokose et al. (1987). Daily doses of 0, 48, and 95 mg sodium chlorite/kg-day (0, 36, and 71 mg chlorite/kg-day) were calculated by U.S. EPA (1994). In the mice, there were no significant chlorite-related alterations in survival or body weight gain; increased mortality observed in the male control group was attributed to severe fighting. Significant increases in liver and lung tumors were observed in the male mice. The incidence of hyperplastic nodules in the liver was significantly increased in the low- and high-dose groups relative to controls (3/35 [reported as 6/35 in Yokose et al., 1987], 14/47, 11/43, in the control, low-, and high-dose groups, respectively) and the combined incidence of liver hyperplastic nodules and hepatocellular carcinoma was increased in the low-dose group (7/35, 22/47, and 17/43, respectively). The incidences of lung adenoma (0/35, 2/47, and 5/43, respectively) and the combined incidence for lung adenoma and adenocarcinoma (0/35, 3/47, and 7/43, respectively) were significantly increased in the high-dose group when compared with the controls. The study authors noted that the incidences of liver hyperplastic nodules and lung adenomas in the treated animals were within the range of historical controls in their laboratory and in the National Toxicology Program laboratories. The high mortality in the control males due to fighting may have contributed to the low tumor incidence in the concurrent control group. In the female mice, the only significant alteration in tumor incidence was a significantly lower incidence of malignant lymphoma/leukemia in the high-dose group (7/47, 5/50, 1/50, respectively).

___II.A.4. SUPPORTING DATA FOR CARCINOGENICITY

Kurokawa et al. (1984) also conducted dermal carcinogenicity studies. In a study to assess the ability of chlorite to act as a complete carcinogen, groups of 20 female SENCAR mice were exposed twice weekly for 51 weeks to 20 mg sodium chlorite/mL in acetone. The solution (0.2 mL; 100 mg sodium chlorite/kg per application) was applied to the shaved backs of the mice. The sodium chlorite exposure did not result in increased tumor incidence. To test the ability of chlorite to act as a tumor promoter, a single initiating dose of 20 μM of dimethylbenzanthracene (DMBA) was applied to the skin of 20 SENCAR mice. The DMBA application was followed by a 51-week exposure to sodium chlorite (as described for the complete carcinogen study). The tumor incidence was 6/20 (30%) compared with 0/20 in mice that received DMBA followed by acetone treatments for 51 weeks. Squamous cell carcinomas were observed in 5/20 animals in the chlorite group. However, these changes

failed to reach statistical significance.

The genotoxicity of chlorite has been assessed in several in vitro and in vivo assays. In in vitro assays, chlorite induced reverse mutations in *Salmonella typhimurium* (with activation) and chromosome aberrations in Chinese hamster fibroblast cells (Ishidate et al., 1984). In general, the results of the in vivo assays have been negative. In the micronucleus assays, negative results were found in ddY mice following an oral gavage dose of 37.5-300 mg/kg single injection (Hayashi et al., 1988) and in Swiss CD-1 mice administered 0.25-1 mg via gavage for 5 consecutive days (0, 8, 20, and 40 mg/kg-day) (Meier et al., 1995). Using the same dosages, Meier et al. (1985) also reported negative results in the bone marrow chromosomal aberration assay in Swiss CD-1 mice and in the sperm-head abnormality assay in B6C3F1 mice. Positive results were found in the micronucleus assay in ddY mice when the chlorite was administered via intraperitoneal injection (7.5-60 mg/kg) (Hayashi et al., 1988).

II.B. QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM ORAL EXPOSURE

None

II.C. QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM INHALATION EXPOSURE

None

II.D. EPA DOCUMENTATION, REVIEW, AND CONTACTS (CARCINOGENICITY ASSESSMENT)

II.D.1. EPA DOCUMENTATION

Source Document -- U.S. EPA, 2000

This assessment was peer reviewed by external scientists. Their comments have been evaluated carefully and incorporated in finalization of this IRIS summary. A record of these comments is included as an appendix to U.S. EPA, 2000.

II.D.2. EPA REVIEW (CARCINOGENICITY ASSESSMENT)

Agency Consensus Date -- 9/20/00

II.D.3. EPA CONTACTS (CARCINOGENICITY ASSESSMENT)

Please contact the Risk Information Hotline for all questions concerning this assessment or IRIS, in general, at (513) 569-7254 (phone), (513)569-7159 (fax), or RIH.IRIS@EPAMAIL.EPA.GOV (Internet address)

III. [reserved]
IV. [reserved]

000816

___V. [reserved]

_VI. BIBLIOGRAPHY

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CASRN -- 7758-19-2
Last Revised – 10/12/00

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_VII. REVISION HISTORY

Chlorine dioxide
CASRN -- 10049-04-4

Date	Section	Description
09/01/1992	I.A	Oral RfD now under review
05/01/1993	All	CASRN corrected
05/01/1993	I.A	Work group review date added
05/01/1993	II.	Carcinogenicity assessment now under review
07/01/1993	I.A	Work group review date added
12/01/1993	I.A	Work group review date added
01/01/1994	I.A.	Work group review date added
08/01/1995	I.A., II.	EPA's RfD/RfC and CRAVE workgroups were discontinued in May. 1995. Chemical substance reviews that were not completed by September 1995 were taken out of IRIS review. The IRIS Pilot Program replaced the workgroup functions beginning in September, 1995.
11/01/1995	II.	Carcinogenicity assessment on-line
11/01/1995	VI.C.	Carcinogenicity references on-line
04/01/1997	III., IV., V.	Drinking Water Health Advisories, EPA Regulatory Actions, and V. Supplementary Data were removed from IRIS on or before April 1997. IRIS users were directed to the appropriate EPA Program Offices for this information.
10/12/2000	I.,II,VI	Oral RfD on-line, revised carcinogenicity assessment

_VIII. SYNONYMS

000819

Chlorite (sodium salt)
CASRN -- 7758-19-2
Last Revised – 10/12/00

14998-27-7
Chlorite
Chlorous acid, ion (1-)
Clorito [Spanish]

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Last updated: **12 October 2000**
URL: http://www.epa.gov/iris/subst/0648.htm

000820

EPA

EPA/635/R-00/007

TOXICOLOGICAL REVIEW

OF

CHLORINE DIOXIDE

AND

CHLORITE

(CAS Nos. 10049-04-4 and 7758-19-2)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

September 2000

U.S. Environmental Protection Agency
Washington, DC

000321

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FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic exposure to chlorine dioxide and chlorite. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of chlorine dioxide and chlorite.

In Section 6, EPA has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose response. Matters considered in this characterization include knowledge gaps, uncertainties, quality of data, and scientific controversies. This characterization is presented in an effort to make apparent the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's Risk Information Hotline at 513-569-7254.

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Reviewers

This document and summary information on IRIS have received peer review both by EPA scientists and by independent scientists external to EPA. Subsequent to external review and incorporation of comments, this assessment has undergone an Agencywide review process whereby the IRIS Program Manager has achieved a consensus approval among the Office of Research and Development; Office of Air and Radiation; Office of Prevention, Pesticides, and Toxic Substances; Office of Solid Waste and Emergency Response; Office of Water; Office of Policy, Planning, and Evaluation; and the Regional Offices.

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Summaries of the external peer reviewers' comments and the disposition of their recommendations are in the Appendix.

000826

1. INTRODUCTION

This document presents background and justification for the hazard and dose-response assessment summaries in the U.S. Environmental Protection Agency (EPA) Integrated Risk Information System (IRIS). IRIS summaries may include an oral reference dose (RfD), inhalation reference concentration (RfC), and a carcinogenicity assessment.

The RfD and RfC provide quantitative information for noncancer dose-response assessments. The RfD is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis but may not exist for other toxic effects such as some carcinogenic responses. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer effects during a lifetime. The inhalation RfC is analogous to the oral RfD, but it provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for the respiratory system (portal of entry) and for effects peripheral to the respiratory system (extra respiratory or systemic effects). It is generally expressed in units of mg/m^3.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral exposure and inhalation exposure. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates are presented in three ways. The *slope factor* is the result of application of a low-dose extrapolation procedure and is presented as the risk per mg/kg-day. The *unit risk* is the quantitative estimate in terms of either risk per μg/L drinking water or risk per $\mu g/m^3$ air breathed. Another form in which risk is presented is a drinking water or air concentration providing cancer risks of 1 in 10,000, 1 in 100,000, or 1 in 1,000,000.

Development of these hazard identification and dose-response assessments for chlorine dioxide and chlorite has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines that were used in the development of this assessment may include the following: *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1986a); *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986b); *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986c); *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991); *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a); *Proposed Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1996a); *Reproductive Toxicity Risk Assessment Guidelines* (U.S. EPA, 1996b); *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988); (proposed) *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a); *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b); *Peer Review and Peer Involvement at the U.S. Environmental Protection Agency* (U.S. EPA, 1994c); *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995); *Science Policy Council Handbook: Peer Review* (U.S.

EPA, 1998b); and a memorandum from EPA Administrator, Carol Browner, dated March 21, 1995, Subject: Guidance on Risk Characterization.

Literature search strategies employed for these compounds were based on the CASRN and at least one common name. At a minimum, the following databases were searched: RTECS, HSDB, TSCATS, CCRIS, GENETOX, EMIC, EMICBACK, DART, ETICBACK, TOXLINE, CANCERLINE, MEDLINE, and MEDLINE backfiles. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

Chlorine dioxide (ClO_2; CASRN 10049-04-4) is a yellow to reddish-yellow gas at room temperature that is stable in the dark but is unstable in light. It is a strong oxidizing agent tha under oxidant demand conditions is readily reduced to chlorite (ClO_2^-; CASRN 7758-19-2), another strong oxidizing agent. The *Drinking Water Criteria Document on Chlorine Dioxide, Chlorite, and Chlorate* (U.S. EPA, 1994d) provides the relevant information concerning dissociation byproducts of chlorine dioxide in water. The strong oxidizing ability of chlorine dioxide makes it useful as a drinking water disinfectant. Other uses of chlorine dioxide include bleaching textiles and wood pulp for paper manufacturing, antimicrobial applications, and reducing loads of adsorbable organic halogenated compounds in industrial effluents. Chlorite is also used for etching printed circuit boards. The physical and chemical properties of chlorine dioxide and chlorite are presented in Table 1.

Chlorine dioxide and chlorite are characterized together in this report because studies conducted with chlorite, the predominant degradation product of chlorine dioxide, are likely relevant to characterizing the toxicity of chlorine dioxide. In addition, studies conducted with chlorine dioxide may be relevant to characterizing the toxicity of chlorite. Chlorine dioxide is fairly unstable and rapidly dissociates, predominantly into chlorite and chloride, and to a lesser extent, chlorate. There is a ready interconversion among these species in water (before administration to animals) and in the gut (after ingestion) (U.S. EPA, 1994d). Therefore, what exists in water or the stomach is a mixture of these chemical species (i.e., chlorine dioxide, chlorite, chlorate) and possibly their reaction products with the gastrointestinal contents.

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Table 1. Physical and chemical properties of chlorine dioxide and chlorite

Properties	Chlorine dioxide	Chlorite (sodium salt)
CAS registry number	10049-04-4	7758-19-2
Molecular formula	ClO_2	$NaClO_2$
Molecular weight	67.46	90.45
Melting point, °C	-59	decomposes at 180–200
Boiling point, °C	11	no data
Water solubility, g/L	3.0 at 25°C and 34 mmHg	39 at 30°C
Specific gravity	1.642 at 0°C	no data

Source: Budavari et al., 1989.

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

3.1. ABSORPTION

3.1.1. Gastrointestinal Absorption

3.1.1.1. *Chlorine Dioxide*

After ingestion, chlorine dioxide is rapidly absorbed from the gastrointestinal tract. Levels of radioactive chlorine in plasma peaked 1 hour after Sprague-Dawley rats were administered a single gavage dose of 100 mg/L $^{36}ClO_2$ (approximately 1.4 mg/kg) (Abdel-Rahman et al., 1979a). Peak plasma levels were achieved 2 hours after Sprague-Dawley rats received a gavage dose of 300 mg/L $^{36}ClO_2$ after a 15-day exposure to 100 mg/L chlorine dioxide in drinking water (Abdel-Rahman et al., 1979a). Approximately 30% of the 100 mg/L single gavage dose was excreted in the urine after 72 hours, indicating that at least 30% of the dose was absorbed (Abdel-Rahman et al., 1979a); the absorption rate constant and half time were 3.77/hour and 0.18 hours, respectively (Abdel-Rahman et al., 1982). Since total radioactivity was measured rather than identification of individual chemical entities, it was not clear from these reports whether the parent chlorine dioxide itself or the chlorite, chlorate, or chloride ion degradation products were absorbed.

3.1.1.2. *Chlorite*

Chlorite is also rapidly absorbed from the gastrointestinal tract. Peak plasma levels of radiolabeled chlorine were reached 2 hours after administration of a single gavage dose of 10 mg/L $^{36}ClO_2^-$ (approximately 0.13 mg/kg) to Sprague-Dawley rats. Using 72-hour urinary

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excretion data, it can be assumed that at least 35% of the initial dose was absorbed (Abdel-Rahman et al., 1984a). The absorption rate constant and half-time were 0.198/hour and 3.5 hours, respectively (Abdel-Rahman et al., 1982). Since total radioactivity was measured rather than identification of individual chemical entities, it was not clear from these reports whether the parent chlorine dioxide itself or the chlorite, chlorate, or chloride ion degradation products were absorbed.

3.1.2. Respiratory Tract Absorption

No data were located on respiratory tract absorption of chlorine dioxide or chlorite.

3.1.3. Dermal Absorption

Scatina et al. (1984) reported on the dermal absorption of Alcide, an antimicrobial compound consisting of solutions of sodium chlorite and lactic acid, which when mixed immediately before use result in the formation of chlorine dioxide. 0.6 g ^{36}Cl-labeled sodium chlorite as part of the Alcide was used to monitor absorption following application to the shaved backs of 10 female Sprague-Dawley rats. Maximum absorption of ^{36}Cl into plasma was observed after 72 hours, where a plasma concentration of 69.4 µg% ^{36}Cl was reached. The absorption half-life was calculated to be 22.1 hours, which corresponds to a rate constant of 0.0314 hr^{-1}.

3.2. DISTRIBUTION

3.2.1. Chlorine Dioxide

Following a single 100 mg/L gavage dose of $^{36}ClO_2$, the ^{36}Cl was slowly cleared from the blood; the rate constant and half-time for elimination from blood were 0.0156/hour and 43.9 hours, respectively (Abdel-Rahman et al., 1982). Elimination from blood was shortened in Sprague-Dawley rats exposed to chlorine dioxide in drinking water for 2 weeks prior to receiving the 300 mg/L gavage dose of $^{36}ClO_2$; the rate constant and half time were 0.022/hour and 31.0 hours, respectively (Abdel-Rahman et al., 1979a). After removal from the blood, the radiolabel appeared to be widely distributed throughout the body, although the highest concentrations were found in the blood, stomach, and small intestines. The lung, kidney, liver, testes (assessed only in the 300 mg/L group), spleen, thymus, and bone marrow also had high concentrations of radiolabel 72 hours after dosing with 100 mg/L (single dose) or 300 mg/L (with 2-week drinking water exposure to 100 mg/L) (Abdel-Rahman et al., 1979a). Seventy-two hours after a single gavage dose of 100 mg/L $^{36}ClO_2$, most of the ^{36}Cl label in the plasma was in the form of chloride ion (Cl^-) and chlorite; the ratio of chloride to chlorite was 4 to 1 (Abdel-Rahman et al., 1979b).

3.2.2. Chlorite

Removal of chlorite from the blood is slow; the rate constant and half-time for elimination of ^{36}Cl from the blood were 0.0197/hour and 35.2 hours in Sprague-Dawley rats receiving a single gavage dose of 10 mg/L $^{36}ClO_2^-$ (Abdel-Rahman et al., 1982). Seventy-two hours after dosing, the highest concentrations of radiolabel were found in the blood, stomach,

testes, skin, lung, kidneys, small intestine, carcass, spleen, brain, bone marrow, and liver (Abdel-Rahman et al., 1982, 1984a).

3.3. METABOLISM

3.3.1. Chlorine Dioxide

Chloride ion is the ultimate metabolite of chlorine dioxide. Approximately 87% and 80% of radiolabeled chlorine in the urine (collected 0–72 hours after administration) and plasma (collected 72 hours after administration), respectively, are in the form of chloride ion following administration of a single gavage dose of 100 mg/L $^{36}ClO_2$ in rats (Abdel-Rahman et al., 1979b). Chlorite was a major metabolite, accounting for approximately 11% and 21% of urine and plasma ^{36}Cl, respectively; approximately 2% of the urinary ^{36}Cl was in the form of chlorate. An in vivo recovery study by Bercz et al. (1982) suggests that ingested chlorine dioxide is rapidly reduced in the stomach to nonoxidizing species (presumably chloride). Five minutes after chlorine dioxide was instilled into the stomach of a monkey, only 8% of the total oxidizing capacity equivalents of chlorine dioxide was recovered. Bercz et al. (1982) also reported that in vitro chlorine dioxide was rapidly reduced to chloride ion by saliva obtained from anesthetized monkeys.

3.3.2. Chlorite

Although fewer data are available on metabolism of chlorite, it is likely that metabolism of chlorite is similar to that of chlorine dioxide. Approximately 85% of the ^{36}Cl recovered in the urine of Sprague-Dawley rats 0–72 hours after administration of a single gavage of 10 mg/L $^{36}ClO_2^-$ was in the form of chloride; the remaining 15% was present as chlorite (Abdel-Rahman et al., 1984a).

3.4. ELIMINATION

3.4.1. Chlorine Dioxide

The radioactive chlorine label was primarily excreted in the urine of rats administered a single gavage dose of 100 mg/L $^{36}ClO_2$ (Abdel-Rahman et al., 1979a). During the first 24 hours after dosing, 18% of the label was excreted in the urine and 4.5% in the feces. Seventy-two hours after dosing, 31% and 10% of the label were excreted in the urine and feces, respectively; the label was not detected in expired air. The parent compound was not detected in the urine; most of the label was in the form of chloride, with smaller amounts as chlorite. The ratio of $^{36}Cl^-$ to $^{36}ClO_2^-$ was 5 to 1 during the first 24 hours and 4 to 1 during the first 72 hours (Abdel-Rahman et al., 1979b).

3.4.2. Chlorite

Urine was the primary route of excretion in rats administered a single gavage dose of 10 mg/L $^{36}ClO_2^-$. Twenty-four hours after dosing, 14% of the label was excreted in the urine and

0.9% in the feces; 35% and 5% of the label were excreted in the urine and feces, respectively, 72 hours after dosing (Abdel-Rahman et al., 1984a). Approximately 90% of the excreted label was in the form of chloride.

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS—EPIDEMIOLOGY, CASE REPORTS, CLINICAL CONTROLS

4.1.1. Oral Exposure

4.1.1.1. *Chlorine Dioxide*

The short-term toxicity of chlorine dioxide was assessed in two human studies conducted by Lubbers and associates (Lubbers et al., 1981, 1982, 1984a; Bianchine et al., 1981). In the first study (Lubbers et al., 1981; also published as Lubbers et al., 1982), a group of 10 healthy male adults drank 1,000 mL (divided into two 500 mL portions, separated by 4 hours) of a 0 or 24 mg/L chlorine dioxide solution (0.34 mg/kg, assuming a 70 kg reference body weight). In the second study (Lubbers et al., 1984a), groups of 10 adult males were given 500 mL distilled water containing 0 or 5 mg/L chlorine dioxide (0.04 mg/kg-day assuming a reference body weight of 70 kg) for 12 weeks. Neither study found any physiologically relevant alterations in general health (observations and physical examination), vital signs (blood pressure, pulse rate, respiration rate, and body temperature), serum clinical chemistry parameters (including glucose, urea nitrogen, phosphorus, alkaline phosphatase, and aspartate and alanine aminotransferases), serum triiodothyronine (T3) and thyroxine (T4) levels, or hematologic parameters.

4.1.1.2. *Chlorite*

Lubbers et al. (1981, 1982, 1984a) also examined the toxicity of chlorite in normal healthy adults in studies that were run concurrently with the chlorine dioxide studies. In the single exposure study (Lubbers et al., 1981, 1982), 10 male adults consumed two 500 mL (separated by 4 hours) solutions containing 2.4 mg/L chlorite (0.034 mg/kg assuming a reference body weight of 70 kg). In a 12-week study (Lubbers et al., 1984a), groups of 10 men drank 500 mL solutions of 0 or 5 mg/L chlorite (0.04 mg/kg-day assuming a 70 kg body weight). No physiologically relevant alterations in general health (observations and physical examination), vital signs, hematologic (including erythrocyte and total and differential leukocyte counts, hemoglobin, hematocrit, and methemoglobin) or serum clinical chemistry (including glucose, electrolytes, calcium, urea nitrogen, enzyme levels, and cholesterol) parameters, or serum T3 or T4 levels were found in either study.

In a companion study, three healthy glucose-6-phosphate dehydrogenase deficient male subjects were given deionized water containing 5 mg/L chlorite (0.04 mg/kg-day assuming a reference body weight of 70 kg) for 12 weeks (Lubbers et al., 1984b). Compared with the

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control group in Lubbers et al. (1984a), the chlorite exposure did not alter general health, vital signs, hematologic parameters (including erythrocyte and total and differential leukocyte counts, hemoglobin, hematocrit, and methemoglobin) or serum clinical chemistry (including glucose, electrolytes, calcium, urea nitrogen, enzyme levels, and cholesterol) parameters.

4.1.1.3. *Chlorine Dioxide–Disinfected Water*

Michael et al. (1981), Tuthill et al. (1982), and Kanitz et al. (1996) have examined communities with chlorine dioxide disinfected water. The focus of the Tuthill et al. (1982) and Kanitz et al. (1996) studies was developmental toxicity. Michael et al. (1981) measured hematologic (erythrocyte, leukocyte, and reticulocyte counts, hemoglobin and methemoglobin levels, hematocrit, mean corpuscular volume, and osmotic fragility) and serum chemistry (blood urea nitrogen and total bilirubin levels) parameters in 198 individuals 1 week before the community initiated the chlorine dioxide water treatment program and 10 weeks after initiation. Blood samples were collected at the same times from a control group of 118 individuals not exposed to chlorine dioxide–treated drinking water. The water treatment facility operated only 8 hours/day; water was drawn from storage tanks for the rest of the day. Chlorine dioxide rapidly disappeared from the stored water (within 2–4 hours), and chlorite levels concomitantly increased. Weekly average concentrations (presumably measured during plant operation hours) of chlorine dioxide ranged from 0.25 to 1.11 ppm, and chlorite concentrations ranged from 3.19 to 6.96 ppm (daily mean chlorite concentration was 5.21 ppm). Using measured water consumption rates (1.98 L/day), the study authors estimated that daily chlorite intakes ranged from 0 to 39.4 mg/day (0–0.56 mg/kg-day assuming a 70 kg reference body weight); the mean intake was 10.3 mg/day (0.15 mg/kg-day). The difference between pre- and posttreatment blood urea nitrogen levels was lower in the community with chlorine dioxide–disinfected water than in the control community. However, the study authors noted that this difference was probably because mild dehydration had occurred in the control community, the postinitiation sample was taken during extremely hot weather, and more individuals in the control group had active, outdoor jobs. No other hematologic or serum chemistry alterations were found.

Tuthill et al. (1982) retrospectively compared infant morbidity and mortality data for a community that had utilized "high" levels of chlorine dioxide as a drinking water disinfectant in the 1940s with data of a neighboring community using conventional drinking water chlorination practices. The authors reported average monthly levels of 0.32 ppm of sodium chlorite added post-treatment, but they did not report chlorine dioxide levels in the treated water. Exposure to chlorine dioxide–treated water did not adversely affect fetal, neonatal, postneonatal, or infant mortality, nor did it affect birthweight, sex ratio, or birth condition. Incidence of newborns judged premature by physician assessment was significantly higher in the community with chlorine dioxide–treated water. In reviewing this study, EPA (1994d) concluded there was no increase in the proportion of premature infants when the age of the mother was controlled and that there was a greater postnatal weight loss in infants from the exposed community.

Kanitz et al. (1996) followed 548 births at Galliera Hospital, Genoa, and 128 births at Chiavari Hospital, Chiavari, Italy, during 1988–1989. Data on infant birthweight, body length, cranial circumference, and neonatal jaundice and on maternal age, smoking, alcohol

consumption, education, and preterm delivery were collected from hospital records. Women in Genoa were exposed to filtered water disinfected with chlorine dioxide, sodium hypochlorite, or both; trihalomethane levels varied from 8 to 16 ppb in sodium hypochlorite-treated water and 1 to 3 ppb in chlorine dioxide–disinfected water. Levels of chlorine dioxide in the water immediately after treatment were less than 0.3 mg/L, while chlorine residue was less than 0.4 mg/L. Women residing in Chiavari used water pumped from wells, without any disinfection treatment, and served as the comparison group (controls). Odds ratios were determined for the somatic parameters by comparison of groups exposed to chlorine dioxide, sodium hypochlorite, or both with controls and adjusted for maternal education level, income, age, and smoking and for sex of the child. Neonatal jaundice occurred more frequently (odds ratio [OR] = 1.7; 95% confidence interval [CI] = 1.1–3.1) in infants whose mothers resided in the area where surface water was disinfected with chlorine dioxide, when compared with infants with mothers using nondisinfected well water. Infants born to mothers residing in areas where surface water was disinfected had smaller cranial circumference (≤ 35 cm) (OR = 2.2, 95% CI = 1.4–3.9 for chlorine dioxide; OR = 3.5, 95% CI = 2.1–8.5 for sodium hypochlorite vs. untreated well water; OR = 2.4, 95% CI = 1.6–5.3 for both vs. untreated well water). In addition, these infants had a smaller body length (≤ 49.5 cm) (OR = 2.0, 95% CI = 1.2–3.3 for chlorine dioxide vs. untreated well water; OR = 2.3, 95% CI = 1.3–4.2 for sodium hypochlorite vs. untreated well water). Risks for low-birthweight infants (≤ 2,500 g) were reported to be increased in mothers residing in areas using water disinfected with chlorite and chlorine dioxide, but these associations were not statistically significant. For preterm delivery (≤ 37 weeks), small but not statistically significant increased risks were found among mothers residing in the area using chlorine dioxide. The study authors concluded that infants of women who consumed drinking water treated with chlorine compounds during pregnancy were at higher risk for neonatal jaundice, cranial circumference ≤ 35 cm, and body length ≤ 49.5 cm.

Interpretability of the results of Kanitz et al. (1996) is limited by lack of consideration of exposure and potential confounding variables such as quantity of water consumed during pregnancy, lack of quantitative exposure information, exposure to other chemicals in the water, and nutritional and smoking habits and age distribution of the women. In addition, baseline values for the infant sex ratio and percentage of low-weight births for the comparison group deviate from values presented by the World Health Organization for Italy. For example, the sex ratio (male/female live births * 100) used in the study for the comparison group was 86, but most recent data (for 1996, as cited in WHO, 2000) for Italy indicate a sex ratio value of 113. Although the percentage of low-weight births in the control group for the Kanitz et al. (1996) study was 0.8%, the percentage of low-weight births (< 2,500 g) in Italy for 1994 is 6%. The quality of the untreated well water is not known (i.e., whether it contained any chemical or biological contaminants). The atypical baseline data raise concerns about the control population selected for this study and render any comparison to them by the exposed group difficult to interpret, thereby precluding the ability to draw conclusions (Selevan, 1997).

4.1.2. Inhalation Exposure

4.1.2.1. *Chlorine Dioxide*

Several case reports of accidental inhalation exposure to chlorine dioxide have been reported in the literature. Elkins (1959) described the case of a bleach tank worker who died after being exposed to 19 ppm chlorine dioxide (52 mg/m^3) for an unspecified amount of time; another worker exposed at the same time survived. Elkins also stated that 5 ppm (14 mg/m^3) was definitely irritating to humans. In a case reported by Exner-Freisfeld et al. (1986), a woman experienced coughing, pharyngeal irritation, and headache after inhaling an unknown amount of chlorine dioxide inadvertently generated while bleaching flowers. Seven hours after exposure, the woman was hospitalized with cough, dyspnea, tachypnea, tachycardia, rales on auscultation, and marked leukocytosis; a decrease in lung function (reduced vital capacity and 1-second forced expiratory volume) was also reported. Most of these symptoms were alleviated with corticosteroid treatment.

Meggs et al. (1996) examined 13 individuals (1 man and 12 women) 5 years after they were occupationally exposed to chlorine dioxide from a leak in a water purification system pipe. The long-term effects of the accident included development of sensitivity to respiratory irritants (13 subjects), disability with loss of employment (11 subjects), and chronic fatigue (11 subjects). Nasal abnormalities (including injection, telangectasia, paleness, cobblestoning, edema, and thick mucus) were found in all 13 individuals. Nasal biopsies taken from the subjects revealed chronic inflammation with lymphocytes and plasma cells present within the lamina propria in 11 of the 13 subjects; the inflammation was graded as mild in 2 subjects, moderate in 8 subjects, and severe in 1 subject. Nasal biopsies from three control subjects showed chronic inflammation in one subject. The average inflammation grading was statistically higher in the subjects compared with the controls. The number of nerve fibers in the biopsies was higher in the subjects (rare fibers in three subjects, moderate fibers in two subjects, and many fibers in three subjects) than controls, but the difference was not statistically significant.

Gloemme and Lundgren (1957), Ferris et al. (1967), and Kennedy et al. (1991) examined workers occasionally exposed to high concentrations of chlorine dioxide that resulted from equipment failure. Concurrent exposure to chlorine gas and, in some cases, sulfur dioxide confounds interpretation of the results of these studies. Gloemme and Lundgren (1957) examined the respiratory health of 12 workers employed at a sulfite-cellulose production facility. Under normal working conditions, the atmospheric chlorine content was less than 0.1 ppm (chlorine dioxide levels were not measured); however, occasional equipment leakages would result in high levels of chlorine dioxide, chlorine, and/or sulfur dioxide. The workers reported respiratory discomfort (breathlessness, wheezing, irritant cough) and ocular discomfort (conjunctivitis and "halo phenomena") connected with these leakage exposures. A slight, nonspecific chronic bronchitis was diagnosed in 7 of the 12 men. An earlier-observed bronchitis disappeared in one case, suggesting to the study authors that improved working conditions might entail reversal of this disorder.

In the Ferris et al. (1967) study, no significant alterations in pulmonary function (forced vital capacity, maximum expiratory flow, forced expiratory flow, and forced expiratory volume) were observed in 147 men employed (length of employment not reported) at a pulp mill, compared with 124 men employed at a paper mill. The pulp mill workers were exposed to sulfur dioxide or chlorine dioxide and chlorine; the chlorine dioxide concentrations ranged from trace amounts to 2 ppm (average concentrations ranged from trace amounts to 0.25 ppm), and chlorine concentrations ranged from trace amounts to 64 ppm (average concentrations ranged from trace amounts to 7.4 ppm). When the pulp mill workers were divided into workers exposed to sulfur dioxide and those exposed to chlorine or chlorine dioxide, significantly higher incidences of shortness of breath and excess phlegm were found in the chlorine/chlorine dioxide workers.

In the Kennedy et al. (1991) study of 321 pulp mill workers exposed to chlorine and chlorine dioxide, significant increases in the incidence of wheezing, wheezing accompanied by breathlessness, and work-related wheezing were observed, compared with 237 workers at a rail maintenance yard. Personal time-weighed average (TWA) exposure concentration for chlorine at the pulp mill ranged from 5 to 14 ppm, whereas TWA for chlorine dioxide was below 0.1 ppm. However, 60% of the pulp mill workers reported one or more chlorine or chlorine dioxide "gassing" incidents. No significant differences in tests of pulmonary function were observed between the two groups. The pulp mill workers were divided into two groups based on self-reported accidental exposures to high levels of chlorine/chlorine dioxide gas ("gassing"). In the workers reporting one or more incidents of gassing, the prevalence of wheezing and missed work because of chest illness was higher than in the pulp mill workers not reporting gassing incidents. Additionally, the incidence of airflow obstruction (as measured by a decrease in midmaximal flow rate and the ratio of 1-second forced expiratory volume to forced vital capacity) was higher in nonsmokers and former smokers reporting gassing incidents compared with smokers also reporting gassing incidents.

4.1.2.2. *Chlorite*

No human inhalation exposure data for chlorite were located.

4.2. PRECHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION

4.2.1. Oral Exposure

4.2.1.1. *Chlorine Dioxide*

Although the subchronic/chronic toxicity of chlorine dioxide has been investigated in a number of studies, only Daniel et al. (1990) and Haag (1949) examined a wide range of endpoints. The other studies (Bercz et al., 1982; Abdel-Rahman et al., 1984b; Couri and Abdel-Rahman, 1980; Moore and Calabrese, 1982) focused on the hematologic system. To date, no studies have examined the carcinogenic potential of chlorine dioxide.

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Daniel et al. (1990) exposed groups of 10 male and 10 female Sprague-Dawley rats to chlorine dioxide in drinking water for 90 days at concentrations of 0, 25, 50, 100, or 200 mg/L. These concentrations correspond to administered doses of 0, 2, 4, 6, or 12 mg/kg-day chlorine dioxide for males and 0, 2, 5, 8, or 15 mg/kg-day chlorine dioxide for females (calculated by the study authors using water consumption and body weight data). No exposure-related deaths were reported. Exposure to 200 mg/L resulted in significant reductions in terminal body weights and body weight gain (26%–29% lower than controls). Significant reductions in water consumption were observed in the males exposed to ≥ 50 mg/L and in females exposed to ≥ 25 mg/L; decreases in food consumption were also observed in the 200 mg/L males. Absolute liver weights were decreased in males at ≥ 50 mg/L, and absolute spleen weights were decreased in females at ≥ 25 mg/L. No consistent alterations in hematologic parameters (erythrocyte and total and differential leukocyte counts, hemoglobin levels, hematocrit, and mean corpuscular volume measured) were observed. Serum lactate dehydrogenase and aspartate aminotransferase levels were decreased and serum creatinine levels were increased in the males exposed to 100 or 200 mg/L; no other alterations in serum chemistry parameters were consistently found. A significant increase in incidence of nasal lesions (goblet cell hyperplasia and inflammation of nasal turbinates) was found in males exposed to ≥ 25 mg/L and in females at ≥100 mg/L. The study authors postulated that these lesions were likely caused by inhalation of chlorine dioxide vapors at the drinking water sipper tube or from off-gassing of the vapors after drinking rather than ingestion of the drinking water. Thus, 25 mg/L (2 mg/kg-day) can be described as a lowest-observed-adverse-effect level (LOAEL), but the toxicological significance of the nasal lesions is not known. Respiratory tract pathologies have not been reported in other oral studies and the effect may possibly be an artifact of treatment.

In a chronic toxicity study by Haag (1949), groups of seven male and seven female rats were exposed to 0, 0.5, 1, 5, 10, or 100 mg/L chlorine dioxide in drinking water (0.07, 0.13, 0.7, 1.3, or 13 mg/kg-day as calculated by U.S. EPA, 1994d) for 2 years. Survival in the 100 mg/L group was significantly decreased. No chlorine dioxide-related alterations were observed in the histopathologic examination of representative animals (2–6/sex) from each group. Thus, a no-observed-adverse-effect level (NOAEL) of 10 mg/L (1.3 mg/kg-day) and a frank effect level (FEL) (based on decreased survival) of 100 mg/L (13 mg/kg-day) can be identified from this study.

The Bercz et al. (1982) study used a rising-dose design in which each animal served as its own control. Five male and seven female adult African green monkeys (*Cercopithecus aethiops*) were exposed to 0, 30, 100, and 200 mg/L chlorine dioxide for 4–6 weeks. The study authors estimated chlorine dioxide administered doses to be 3.5 and 9.5 mg/kg-day in the 30 and 100 mg/L groups, respectively. Exposure to 200 mg/L resulted in erythema and ulceration of the oral mucosa, mucous nasal discharge, and avoidance of drinking water; exposure to 200 mg/L was terminated after 1 week because some of the animals showed signs of dehydration. No significant alterations in hematologic clinical chemistry (erythrocyte, total and differential leukocyte, and reticulocyte counts, hemoglobin levels, hematocrit, osmotic fragility, and methemoglobin levels) or serum clinical chemistry (creatinine, blood urea nitrogen [BUN], alkaline phosphatase, lactate dehydrogenase, and alanine and aspartate aminotransferase) parameters or body weight gain were observed. Serum T4 levels were significantly decreased in

the 100 mg/L chlorine dioxide-exposed monkeys after 6 weeks of exposure. Thus, this study identifies a NOAEL of 30 mg/L (3.5 mg/kg-day) and a LOAEL of 100 mg/L (9.5 mg/kg-day) for alterations in thyroid hormone levels in monkeys exposed to chlorine dioxide in the drinking water for 4–6 weeks.

Abdel-Rahman et al. (1984b) exposed groups of four male Sprague-Dawley rats to 0, 1, 10, 100, or 1,000 mg/L chlorine dioxide in the drinking water 20 hours/day for 11 months (doses of 0.10, 1, 10, and 100 mg/kg-day are estimated using a reference body weight of 0.523 kg and reference water intake of 0.062 L/day and adjusting for intermittent exposure). Significant reductions in body weight gain were observed in the 1,000 mg/L group at 2, 5, 7, 10, and 11 months and in all groups during months 10 and 11. A number of statistically significant hematologic alterations were observed; however, the magnitude of the alterations does not appear to be dose related. Osmotic fragility was decreased in the 100 and 1,000 mg/L groups after 2, 4, 7, or 9 months of exposure and in the 10 mg/L group only after 9 months of exposure. Erythrocyte counts were decreased in the 1 and 1,000 mg/L groups after 9 months of exposure, but not after 7 months. Reduced hematocrit and hemoglobin levels were observed in all groups at 9 months; hematocrit levels were significantly increased the 100 and 1,000 mg/L groups at 7 months. Mean corpuscular hemoglobin concentrations were increased in the 100 and 1,000 mg/L groups after 9 months. Blood glutathione levels were significantly reduced in the 1, 10, and 1,000 mg/L groups at 2 months; the 1 and 10 mg/L groups after 4 months; the 1 mg/L group after 7 months; and the 100 mg/L group after 9 months. DNA synthesis (assessed using ^{3}H-thymidine incorporation) was significantly reduced in the kidneys of rats exposed to 100 mg/L, decreased in the testes of rats in the 10 and 100 mg/L groups, and increased in the intestinal mucosa of rats exposed to 10 or 100 mg/L chlorine dioxide; thymidine incorporation was not significantly altered in the liver. The lack of a consistent relationship between dose and hematologic alterations and the small number of animals (four males/group) confound interpretation of the study.

Couri and Abdel-Rahman (1980) found significant increases in blood glutathione reductase levels in Sprague-Dawley rats (four males/group) exposed to 10, 100, or 1,000 mg/L chlorine dioxide in drinking water 20 hours/day, 7 days/week for up to 1 year (0, 0.1, 1, 10, or 100 mg/kg-day using reference body weights and drinking water intakes of 0.523 kg and 0.062 L/day, respectively, and adjusting for intermittent exposure). After 12 months of exposure, the erythrocyte glutathione reductase levels in rats exposed to 1, 10, 100, or 1,000 mg/L were similar to those of controls, but the levels of erythrocyte glutathione peroxidase were significantly increased at 100 and 1,000 mg/L. Erythrocyte glutathione concentrations were significantly decreased at 1, 10, and 100 mg/L after 6 months and at 1,000 mg/L after 12 months of exposure. Erythrocyte catalase levels were increased in the 1,000 mg/L group after 6 and 12 months of exposure and decreased in the 1 and 10 mg/L groups after 6 months of exposure.

In similarly exposed Swiss Webster mice (six males/group) (estimated doses of 0.18, 1.8, 18, and 180 mg/kg-day [as calculated by U.S. EPA, 1994d] for 1, 10, 100, and 1,000 mg/L chlorine dioxide, respectively, drinking water concentrations), glutathione peroxidase levels were decreased at 100 mg/L and increased at 1,000 mg/L after 12 months of exposure, and glutathione levels were decreased at 10 and 100 mg/L after 12 months (Couri and Abdel-Rahman, 1980).

Catalase levels were increased in the 10, 100, and 1,000 mg/L groups after 12 months of exposure. As with the Abdel-Rahman et al. (1984b) study, the inconsistent relationship between the dose and the magnitude of the alterations in the glutathione-dependent system makes interpretation of the results of this study difficult; additionally, it is not clear if these effects are biologically significant, precluding determination of a NOAEL and LOAEL for these studies.

Moore and Calabrese (1982) exposed groups of 10 A/J or C57L/J mice (sex not specified) to 0 or 100 ppm chlorine dioxide in drinking water for 30 days (0 or 19 mg/kg-day using a reference body weight of 0.0316 kg and water intake of 0.0078 L/day). No significant alterations in hematologic parameters (complete blood count, reticulocyte count, glucose-6-phosphate activity, and osmotic fragility) were observed in either mouse strain.

4.2.1.2. *Chlorite*

The database for chlorite subchronic/chronic systemic toxicity consists of the Harrington et al. (1995a) subchronic study, the Haag (1949) chronic study, and the Bercz et al. (1982), Abdel-Rahman et al. (1984b), Couri and Abdel-Rahman (1980), and Moore and Calabrese (1982) studies, which examined a limited number of endpoints. Kurokawa et al. (1986) is the only study that examined the carcinogenic potential of ingested chlorite.

Harrington et al. (1995a) administered doses of 0, 10, 25, or 80 mg/kg-day sodium chlorite (equivalent to 0, 7.4, 19, or 60 mg chlorite/kg-day, respectively) via gavage to Crl:CD (SD) BR rats (15/sex/group) for 13 weeks. In the 60 mg/kg-day group, four animals died during treatment and both sexes exhibited salivation, significantly decreased erythrocyte counts, and decreased total serum protein levels. The males receiving 60 mg/kg-day exhibited significantly decreased hematocrit and hemoglobin levels and increased methemoglobin and neutrophil levels, whereas in the females, methemoglobin levels were significantly decreased. Possible reasons for the decrease in methemoglobin in females, which is unexpected considering the known oxidative effects of sodium chlorite, were not discussed by the study authors. The following observations were also noted in the 60 mg/kg-day group: morphological changes in erythrocytes in some animals of both sexes, significant increases in relative adrenal and spleen weights in the males, increases in absolute and relative spleen and adrenal weight in females, and increases in relative liver and kidney weights in the females. Body weight and food consumption were not affected by treatment. Histopathologic alterations in the 60 mg/kg-day group included squamous epithelial hyperplasia, hyperkeratosis, ulceration, chronic inflammation, and edema in the stomachs of seven males and eight females. At 19 mg/kg-day, the following alterations were reported: occasional salivation in two males, hematologic alterations in males (increased methemoglobin levels and neutrophil count, decreased lymphocyte count), increases in absolute and relative spleen and adrenal weights in females, and histologic alterations in the stomach of two males, similar to those seen in the high-dose group. The increase in absolute splenic weight was attributed to morphological alterations in erythrocytes, but no explanation was provided for alterations in absolute adrenal weight. The NOAEL in this study is determined to be 7.4 mg/kg-day, and the LOAEL is 19 mg/kg-day for stomach lesions and increases in spleen and adrenal weights in rats subchronically treated with sodium chlorite.

In a chronic study by Haag (1949), groups of rats (seven/sex/group) were exposed to 0, 1, 2, 4, 8, 100, or 1,000 mg/L chlorite in the drinking water (0, 0.09, 0.18, 0.35, 0.7, 9.3, or 81 mg/kg-day, as calculated by U.S. EPA, 1994d) for 2 years. Animals exposed to chlorite concentrations of 100 or 1,000 mg/L exhibited treatment-related renal pathology, characterized by distention of the glomerular capsule and appearance of a pale pinkish staining material in the renal tubules. These effects were also observed in a group of animals administered sodium chloride at a concentration equimolar to 1,000 mg sodium chlorite/L. The study author concluded that the renal pathology was a nonspecific salt effect, but this observation does not alter the observation that concentrations of 100 mg/L or higher led to adverse effects. Based on renal effects, this study identifies a NOAEL of 8 mg/L (0.7 mg/kg-day) and a LOAEL of 100 mg/L (9.3 mg/kg-day). The study was limited because an insufficient number of animals were tested per group, pathology was conducted on a small number of animals, and it did not provide adequate evaluations of more sensitive parameters, which would have been more useful in the overall assessment of chronic toxicity.

Two similarly designed studies, by Abdel-Rahman et al. (1984b) and Couri and Abdel-Rahman (1980), tested the hematotoxicity of chlorite in rats. Groups of four male Sprague-Dawley rats were exposed to 0, 10, or 100 mg/L chlorite in drinking water 20 hours/day, 7 days/week for up to 1 year (0, 1, or 10 mg/kg-day using a reference body weight of 0.523 kg and water intake rate of 0.062 L/day) and adjusting for intermittent exposure. At all measuring periods (after 2, 5, 7, 10, and 11 months of exposure), there were significant decreases in body weight gain in the 100 mg/L group; body weight gain also was decreased in the 10 mg/L group at 10 and 11 months. The study authors do not note whether water consumption was affected. No consistent alterations in erythrocyte count, hematocrit, or hemoglobin levels were observed. Mean corpuscular hemoglobin concentration was increased at both exposure levels after 7 months of exposure, but not after 9 months. Osmotic fragility was significantly decreased at 10 and 100 mg/L after 7 and 9 months of exposure. DNA synthesis (as measured by ^{3}H-thymidine incorporation) was decreased in the liver and testes at 10 and 100 mg/L, decreased in the intestinal mucosa at 100 mg/L, and increased in the intestinal mucosa at 10 mg/L. Blood glutathione reductase activity was significantly increased at 10 and 100 mg/L after 6 months of exposure and decreased at 10 mg/L after 12 months. Blood glutathione peroxidase was not altered after 6 months of exposure, but after 12 months it was decreased in both groups. Significant decreases in blood glutathione levels were observed in both groups. Blood catalase activity was decreased after 6 months of exposure in the 10 and 100 mg/L groups and increased in the 10 mg/L groups after 12 months. The lack of a consistent dose-effect relationship, small numbers of animals, and small magnitude of effects complicate interpretation of the results.

Moore and Calabrese (1982) also examined the hematotoxicity of chlorite. In this study, groups of 11–23 A/J or C57L/J mice (sex not specified) were exposed to 0, 1, 10, or 100 ppm sodium chlorite (0, 0.75, 7.5, or 75 ppm chlorite) in drinking water for 30 days. Significant increases in mean corpuscular volume, osmotic fragility, and glucose-6-phosphate activity were observed in both strains of mice exposed to 100 ppm; no other alterations in hematologic parameters were observed. This study identifies a NOAEL of 10 ppm sodium chlorite (1.9 mg/kg-day chlorite using a reference body weight of 0.0316 kg and water intake of 0.0078 L/day)

and a LOAEL of 100 ppm sodium chlorite (19 mg/kg-day) for hematologic effects in mice exposed to chlorite in drinking water for 30 days.

Using a rising-dose study protocol, Bercz et al. (1982) examined the effects of subchronic exposure to sodium chlorite in drinking water on hematologic and serum clinical chemistry parameters. Five male and seven female adult African green monkeys (*C. aethiops*) were exposed to 0, 25, 50, 100, 200, or 400 mg/L chlorite in drinking water for 4–6 weeks; the study authors estimated the dose for the 400 mg/L group to be 58.4 mg/kg-day. Each animal served as its own control. A number of statistically significant, dose-related alterations in hematologic and serum clinical chemistry parameters were observed. These included decreases in erythrocyte levels and cell indices, increases in aspartate aminotransferase (increases were subclinical), slight decreases in hemoglobin levels, and slight increases in reticulocyte count and methemoglobin levels. The data were not presented in a manner that would allow identification of threshold doses for the hematologic alterations. Other hematologic and clinical chemistry parameters and body weight were not affected. Serum T4 levels were significantly reduced in the 400 mg/L group.

To assess the renal toxicity of sodium chlorite, Moore and Calabrese (1982) exposed groups of 55–60 male C57L/J mice to 0, 4, 20, or 100 ppm sodium chlorite (0, 3, 15, or 75 ppm chlorite) in the drinking water for 30, 90, or 190 days. No significant alterations in body weight gain, absolute or relative kidney weights, water consumption, or kidney histology were observed.

In an oral carcinogenicity study conducted by Kurokawa et al. (1986) (mouse data were also presented in Yokose et al., 1987), groups of male and female F344 rats and B6C3F1 mice (50/sex/species/group) were exposed to sodium chlorite in the drinking water for 85 or 80 weeks (with a 5-week recovery period) (Yokose et al., 1987). The sodium chlorite concentrations were 0, 300, or 600 ppm for rats and 0, 250, or 500 ppm for mice. Using water consumption and body weight data, the study authors estimated the doses to be 18 and 32 mg/kg-day in male rats and 28 and 41 mg/kg-day in female rats. All groups of rats were infected with the Sendai virus. No adverse effect on survival was observed in the rats. A slight dose-related decrease in body weight gain was observed (body weight gain in the high-dose group was within 10% of controls). No chlorite-related increases in tumor incidence were observed in the rats.

For mice, daily doses of 0, 48, and 95 mg sodium chlorite/kg-day (0, 36, and 71 mg chlorite/kg-day) were calculated by EPA (1994d). In the mice, there were no significant chlorite-related alterations in survival or body weight gain; increased mortality was observed in the male control group, which was attributed to severe fighting. Significant increases in liver and lung tumors were observed in the male mice. Incidence of hyperplastic nodules in the liver was significantly increased in the low- and high-dose groups relative to controls (3/35 [reported as 6/35 in Yokose et al., 1987], 14/47, 11/43, in the control, low-, and high-dose groups, respectively) and combined incidence of liver hyperplastic nodules and hepatocellular carcinoma was increased in the low-dose group (7/35, 22/47, and 17/43, respectively). Incidence of lung adenoma (0/35, 2/47, and 5/43, respectively) and combined incidence for lung adenoma and adenocarcinoma (0/35, 3/47, and 7/43, respectively) were significantly increased in the high-dose group compared with controls. The study authors noted that incidences of liver hyperplastic

nodules and lung adenomas in the treated animals were within the range of historical controls in their laboratory and in the National Toxicology Program laboratories. The high mortality in the control males because of fighting may have contributed to the low tumor incidence in the concurrent control group, making statistical comparisons between concurrent controls and treated animals difficult to interpret. In the female mice, the only significant alteration in tumor incidence was a significantly lower incidence of malignant lymphoma/leukemia in the high-dose group (7/47, 5/50, 1/50, respectively). This study is considered inadequate for assessing carcinogenicity because of the relatively short exposure duration (80 weeks) and the high incidence of early mortality in the concurrent control males from excessive fighting.

4.2.2. Inhalation Exposure

4.2.2.1. *Chlorine Dioxide*

Paulet and Desbrousses (1970) conducted four studies to investigate toxicity of inhaled chlorine dioxide in rats and rabbits (strains not specified): (1) 5 male and 5 female rats were exposed to 10 ppm chlorine dioxide (28 mg/m^3) 2 hours/day for 30 days; (2) 10 male rats, 10 female rats, and 4 rabbits were exposed to 5 ppm chlorine dioxide (14 mg/m^3) 2 hours/day for 30 days; (3) 10 male and 10 female rats were exposed to 2.5 ppm chlorine dioxide (6.9 mg/m^3) 7 hours/day for 30 days; and (4) 8 rabbits were exposed to 2.5 ppm chlorine dioxide (6.9 mg/m^3) 4 hours/day for 45 days. The weekly exposure frequency was not reported—presumably it was 5 days/week. Control groups with equal numbers of animals were used for each study. The following adverse effects were observed at 10 ppm: nasal discharge and red eyes, localized bronchopneumonia with desquamation of the alveolar epithelium, and significantly increased blood erythrocyte and leukocyte levels. Similar, but less severe, respiratory tract effects were observed at 5 ppm; there were no alterations in erythrocyte or leukocyte levels at this concentration. Lymphocytic infiltration of the alveolar spaces, alveolar vascular congestion, hemorrhagic alveoli, epithelial erosions, and inflammatory infiltrations of the bronchi were observed in the rats exposed to 2.5 ppm. The study authors noted that body weight gain was "slightly slowed" (data not presented) and the erythrocyte and leukocyte levels were 85% and 116% of controls, respectively (statistical analysis not reported), in the rats exposed to 2.5 ppm. In rabbits exposed to 2.5 ppm chlorine dioxide, hemorrhagic alveoli and congested capillaries were observed in the lungs. Body weight gain was not adversely affected, and erythrocyte and leukocyte levels were 80% and 116% of controls (statistical analysis not reported; the study authors state that the cell counts "changed very little"). Another group of rats and rabbits were sacrificed 15 days after termination of the 2.5 ppm exposure regimens. Recovery from the pulmonary lesions was evident in these animals. The liver was not adversely affected in the rats or rabbits following exposure to 2.5, 5, or 10 ppm chlorine dioxide. This study identifies a LOAEL of 2.5 ppm (6.9 mg/m^3) for thoracic effects (alveolar congestion and hemorrhage and bronchial inflammation) in rats (7 hours/day for 30 days) and pulmonary effects (alveolar hemorrhage and capillary congestion) in rabbits (4 hours/day for 45 days).

In a follow-up study by Paulet and Desbrousses (1972), groups of eight Wistar rats (sex not reported) were exposed to 1 ppm chlorine dioxide (2.8 mg/m^3) 5 hours/day, 5 days/week for 2 months. The study authors noted that weight gain and erythrocyte and leukocyte levels were

not affected, but they did not present concurrent control data. Vascular congestion and peribronchiolar edema were observed in the lungs of chlorine dioxide-exposed rats; no alterations in the epithelium or parenchyma were observed. This subchronic study identifies a LOAEL of 1 ppm (2.8 mg/m^3) for respiratory effects in rats.

In a second series of studies conducted by Paulet and Desbrousses (1974), groups of 10–15 rats (sex and strain not reported) were exposed to 5, 10, or 15 ppm chlorine dioxide (14, 28, or 41 mg/m^3) for 15-minute periods two or four times/day for 1 month. Control groups were similarly exposed to room air. At 15 ppm, 1/10 and 1/15 rats exposed two or four times/day, respectively, died; body weight loss was observed in both groups. Histologic alterations observed at this exposure level included nasal and ocular inflammation and discharge, bronchitis, and catarrhous lesions of the alveoli with peribronchiolar infiltrations (more pronounced in the four times/day group). The alveolar lesions were reversible; 15 days after exposure termination, the lung histology was similar to that of controls. No histologic alterations were observed in the liver. At 10 ppm, alveolar irritation and decreases in body weight gain were observed. No adverse effects on clinical signs, body weight gain, or histopathology of the lungs were observed at 5 ppm. Exposure to chlorine dioxide did not adversely affect hematologic parameters. This study identifies a NOAEL of 5 ppm (14 mg/m^3) and LOAEL of 10 ppm (28 mg/m^3) for lung damage following intermittent exposure for 15-minute periods, two or four times/day for 4 weeks.

Dalhamn (1957) conducted a series of inhalation studies to assess toxicity of chlorine dioxide in the rat (sex and strain not reported). In the first study, a group of three rats was exposed once a week for 3 minutes to decreasing concentrations of chlorine dioxide (3,400 ppm [9,500 mg/m^3] in week 1, 1,100 ppm [3,000 mg/m^3] in week 2, and 800 ppm [2,200 mg/m^3] in week 3); a second group of three rats served as controls. Respiratory distress and decreased body weight were observed in the chlorine dioxide-exposed rats. Bronchopneumonia and hyperemia of the renal corticomedullary junction were observed in two of three rats; the renal hyperemia was also observed in the control group (2/3). In the second study, exposure to 260 ppm (720 mg/m^3) chlorine dioxide for 2 hours resulted in ocular discharge, epistaxis, death (1/4 rats), pulmonary edema, and circulatory engorgement. In the third study, groups of five rats were exposed to 0 or approximately 10 ppm chlorine dioxide (28 mg/m^3) 4 hours/day for 9 days in a 13-day period. Death (3/5 rats), rhinorrhea, "embarrassed respiration," and weight loss were observed in the chlorine dioxide-exposed rats. Respiratory infection with acute renal and hepatic congestion also were observed. The fourth study involved exposure of groups of five rats to 0 or approximately 0.1 ppm chlorine dioxide (0.28 mg/m^3) 5 hours/day for 10 weeks (frequency of exposure not reported). No effects on body weight gain were observed and no histologic alterations were observed in the lungs, kidneys, or liver. The Dalhamn studies identified a NOAEL of 0.1 ppm (0.28 mg/m^3) in rats exposed 5 hours/day for 10 weeks and a LOAEL of 10 ppm (28 mg/m^3) for respiratory tract irritation in rats exposed 4 hours/day for approximately 2 weeks.

4.2.2.2. *Chlorite*

No animal inhalation or intratracheal installation data were located for chlorite.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

4.3.1. Chlorine Dioxide

Carlton et al. (1991) administered daily gavage doses of 0, 2.5, 5, or 10 mg/kg chlorine dioxide in deionized water to groups of 12 male Long-Evans rats for 56 days prior to mating and throughout the 10-day mating period. Groups of 24 female rats received the same gavage doses for 14 days prior to mating, during the mating period, and throughout gestation and lactation. No significant alterations in mortality, clinical signs, fertility rates, sperm parameters, length of gestation, prenatal deaths, mean litter size, or mean pup weights were observed. A statistically significant delay in the day of eye opening was observed in pups from the 10 mg/kg-day group, but the study authors did not consider this effect to be biologically significant because it was not dose related (16.70, 15.59, 16.26, and 15.95 days in the 0, 2.5, 5, and 10 mg/kg-day groups, respectively). No significant alterations in reproductive tract organ weights were observed in the F1 male rats. In the F1 female rats, there were statistically significant decreases in absolute and relative vagina weights in the 10 mg/kg-day group, but no differences in terminal body weights or uterine and ovarian weights. No consistent chlorine dioxide-related alterations in T3 or T4 levels were measured in the F0 male and female rats and F1 male rats (hormone levels measured on postnatal days 17, 28, and 40). This study identifies a NOAEL of 10 mg/kg-day for reproductive effects in rats receiving gavage doses of chlorine dioxide.

In a developmental toxicity study by Suh et al. (1983), groups of six to eight female Sprague-Dawley rats were administered 0, 1, 10, or 100 mg/L chlorine dioxide in the drinking water (0, 0.1, 1, and 10 mg/kg-day using a reference body weight of 0.35 kg and water intake of 0.046 L/day) for 2.5 months prior to mating with unexposed males and during gestational days 0–20; the dams were killed on gestational day 20. A slight, nonsignificant decrease in maternal body weight gain was observed in the 10 and 100 mg/L groups. There was a statistically significant trend for decreasing number of implants per litter and number of live fetuses per dam. Total fetal weights and male fetal weights were significantly increased in the 100 mg/L group compared with controls; crown-rump length was not significantly affected. Incidences of skeletal anomalies did not significantly differ between groups. This study identifies a NOAEL of 10 mg/L (1 mg/kg-day) and LOAEL of 100 ppm (10 mg/kg-day) for developmental effects in the offspring of rats exposed to chlorine dioxide in the drinking water.

Toth et al. (1990) examined the neurodevelopmental toxicity of chlorine dioxide in the postnatally exposed Long-Evans hooded rats. Groups of four male and four female pups per litter received daily gavage doses of 0 or 14 mg/kg chlorine dioxide on postnatal days 1–20. The chlorine dioxide pups weighed significantly less than controls at ages 11, 21, and 35 days. No significant alterations in cerebellum or olfactory bulb weights were observed on postnatal days 11, 21, or 35. Forebrain weights were significantly lower in the chlorine dioxide-exposed pups on postnatal days 21 and 35. This reduction in forebrain weight was accompanied by reductions

in protein content on postnatal days 21 and 35 and reduced DNA content on postnatal day 35. The ratio of protein content to forebrain weight was decreased on postnatal days 11, 21, and 35; the protein content to cerebellum weight was increased on postnatal day 35. The ratio of DNA content to brain part weight was not significantly affected in the chlorine dioxide-exposed pups. No alterations in counts of branches of apical dendrites of cerebral cortical layer 5 pyramidal cells were observed, but dendritic spine counts in the Krieg's area 18 (a visual association region of the cortex) were significantly decreased. No gross lesions, loss of myelin, or changes in cells staining positive for Nissl substance in the forebrain, cerebellum, or brainstem were observed in the brains of chlorine dioxide-exposed pups. No significant alterations in T3 or T4 levels or free T4 index were observed on postnatal days 11, 21, and 35. This study identifies a LOAEL of 14 mg/kg-day for altered brain development (decreased forebrain weight and protein content) in postnatally exposed rats.

Mobley et al. (1990) exposed groups of 12 female Sprague-Dawley rats to 0 or 100 ppm chlorine dioxide in the drinking water (0 or 14 mg/kg-day using a reference body weight of 0.35 kg and water intake of 0.046 L/day) for 10 days prior to mating with unexposed males and during the gestation and lactation periods (until postconception days 35–42). No significant alterations in litter size were observed. At birth, the litter weight of the chlorine dioxide-exposed group was significantly lower than that of controls. Chlorine dioxide exposure significantly decreased exploratory activity on postconception days 36–39, but not on days 39–41. Although serum T3 and T4 levels were not significantly altered in the chlorine dioxide-exposed pups (assessed on postconception days 37 and 38), a significant decrease in T3 uptake was observed. Free T3 and T4 levels were lower in the chlorine dioxide group, but the difference was not statistically significant. On postconception day 42, there were no significant alterations in total T3 or T4, free T4, or T3 uptake. The day of eye opening was not significantly affected by chlorine dioxide exposure. Thus, 100 ppm (14 mg/kg-day) is a LOAEL for decreased litter weight and exploratory activity.

In a study conducted by Orme et al. (1985) designed to assess toxicity of chlorine dioxide on the thyroid, groups of female Sprague-Dawley rats were exposed to 0, 2, 20, or 100 mg/L in the drinking water (doses of 0, 1, 3, and 14 mg/kg-day were estimated by U.S. EPA, 1994d) for 2 weeks prior to mating and throughout gestation and lactation. In a companion study, groups of 5-day-old Sprague-Dawley pups (dams were not exposed) received gavage doses of 0 or 14 mg/kg-day chlorine dioxide on postnatal days 5–20. No significant alterations in pup weight were observed in the pups exposed in utero; the postnatally exposed pups weighed significantly less than controls on postnatal days 14–21. Age of eye opening was not affected by chlorine dioxide exposure. Locomotor activity was consistently decreased in the 100 mg/L group, but the decrease was not statistically significant. In the 14 mg/kg-day gavaged group, activity was significantly decreased on postnatal days 18–19; on days 15–17 and 20, activity levels were similar to controls. In the 100 mg/L group, there was a significant decrease in T3 and T4 levels; T4 levels were also significantly decreased in the 14 mg/kg-day group. In all groups, there was a significant correlation between T4 levels and locomotor activity. T4 levels were not significantly altered in the chlorine dioxide–exposed dams. This study identifies a NOAEL of 20 mg/L (3 mg/kg-day) and a LOAEL of 100 mg/L (14 mg/kg-day) for neurobehavioral effects (decreased

T3 and T4 levels and delayed development) in the offspring of rats exposed to chlorine dioxide in drinking water.

Taylor and Pfohl (1985) exposed groups of 13–16 female Sprague-Dawley rats to 0 or 100 ppm chlorine dioxide in drinking water (0 or 14 mg/kg-day calculated using a reference body weight of 0.35 kg and water intake of 0.046 L/day) for 14 days prior to breeding and throughout gestation and lactation. Groups of male pups from unexposed dams were administered 0 or 14 mg/kg chlorine dioxide via gavage from postnatal days 5 to 20. No significant alterations in maternal or pup body weights were observed in the group receiving 100 ppm in the drinking water. A significant decrease in whole brain weight, primarily because of a decrease in cerebellar weight, was observed in the 21-day-old offspring of dams receiving 100 ppm in the drinking water. A decrease in cerebellar total DNA content also was observed; the difference was caused by a decrease in total number of cells rather than in cell density. A nonsignificant decrease in locomotor activity (assessed at 10–20 days of age) was observed in the 100 ppm offspring. A significant decrease in exploratory behavior was observed in the 100 ppm offspring at 60 days of age. In the pups receiving gavage doses of chlorine dioxide, significant decreases in body weight, absolute and relative whole brain and forebrain weights, and forebrain DNA content and total cell number were observed in the 21-day-old pups; cerebellum and forebrain DNA content and total cell number were also significantly decreased in the 11-day-old pups. Significant decreases in home cage and wheel-running activity at ages 18–19 and 10 days, respectively, also were observed in the pups receiving gavage doses of chlorine dioxide. Thus, the LOAEL for neurobehavioral effects, decreased brain weight, and cell number in the offspring of rats exposed to chlorine dioxide in drinking water and in rats postnatally exposed to chlorine dioxide via gavage is 14 mg/kg-day.

4.3.2. Chlorite

The Chemical Manufacturers Association (CMA) conducted a two-generation study to examine reproductive, developmental neurotoxicity, and hematologic endpoints in rats exposed to sodium chlorite (CMA, 1996). Thirty male and 30 female Sprague-Dawley rats of the OFA(SD)IOPS-Caw strain (F0) generation received drinking water containing 35, 70, or 300 ppm sodium chlorite (concentrations of sodium chlorite in the drinking water were apparently adjusted to compensate for the 81.4% purity of the test material) for 10 weeks and were then paired (1M:1F) for mating. A similar group received purified water and served as controls. Males were exposed throughout mating and then were sacrificed. Exposure for the females continued through mating, pregnancy, and lactation until necropsy following weaning of their litters. Sodium chlorite concentrations were adjusted downward during lactation to offset increases in the volume of water consumed so that a constant intake (mg/kg-day) could be maintained. Twenty-five males and females from each of the first 25 litters to be weaned in a treatment group were chosen to produce the F1 generation. The F1 pups were continued on the same treatment regimen as their parents. At approximately 14 weeks of age, they were mated to produce the F2a generation. Because of a reduced number of litters in the 70 ppm F1-F2a generation, the F1 animals were remated following weaning of the F2a to produce the F2b generation. Pregnant F1 females were allowed to litter and rear the F2a and F2b generations until weaning at postnatal day 21. Based on sodium chlorite intake (in mg/kg-day) calculated by

the study authors from measured water consumption and body weight, and adjusting for the molecular weight of sodium in sodium chlorite, doses for the F0 animals were 0, 3, 5.6, and 20 and 0, 3.8, 7.5, and 28.6 mg/kg-day chlorite for males and females, respectively. For the F1 animals, doses were 0, 2.9, 5.9, and 22.7 mg/kg-day chlorite for the males and 0, 3.8, 7.9, and 28.6 mg/kg-day chlorite for the females. Numerous parameters were measured or calculated, including body weight, food and water consumption, estrus cycle in the F0 and F1 rats, and hematology and T3 and T4 levels in the F1 rats (blood samples collected from 1 male and 1 female from the first 20 F1 litters at age 25 days and another group at 13 weeks). Other parameters measured were gestation duration, litter size, pup sex, pup body weight, pup developmental landmarks, number alive/dead pups in the F1 and F2 generations, total caudal sperm number and percent motile, morphology by computer-assisted sperm motility analysis in the F0 and F1 rats, and organ weight and histopathologic examination of the brain, pituitary gland, liver, adrenal gland, spleen, thymus, kidneys, and reproductive organs of all F0 and F1 controls and high-dose animals. An additional group of F1 pups was chosen for neurohistopathology on postnatal day 11 (examination of the brain and spinal cord) or postnatal day 60 (sensory ganglia, dorsal and ventral nerve roots, and several peripheral nerves and muscles). Another group of F1 rats was examined for neurotoxicological endpoints (motor activity in a "Figure 8" Activity System and neuropathology on postnatal day 60, auditory startle in the SR-Screening System, learning and memory retention in a water E-maze). A functional observational battery (FOB) was also conducted on the pups undergoing auditory and learning assessments. This group was composed of 2 males and 2 females from 20 litters, and exposure was discontinued after weaning. Reevaluation of the auditory startle response was conducted in 20 males and 20 females in the F2a and F2b generations.

There were reductions in water consumption, food consumption, and body weight gain in both sexes in all generations at various times throughout the experiment (e.g., during premating, pregnancy, gestation, postweaning), primarily in the 70 and 300 ppm groups. The authors attributed these reductions to lack of palatability of the drinking water solution, but did not show data to support this contention. Significant alterations related to treatment at 300 ppm include reduced absolute and relative liver weight in F0 females and F1 males and females, reduced pup survival (increase in number of pups found dead and/or killed prematurely during lactation) and reduced body weight at birth and throughout lactation in F1 and F2 rats, lower thymus and spleen weight in both generations, lowered incidence of pups exhibiting normal righting reflex and with eyes open on postnatal day 15, alteration in clinical condition in F2 animals chosen for neurotoxicity, decrease in absolute brain weight for F1 males and F2 females, delay in sexual development in males (preputial separation) and females (vaginal opening) in F1 and F2 rats, and lower red blood cell parameters in F1 rats. The reported alterations in pup sexual maturation measures might be due to reduced pup body weight, but a definitive conclusion cannot be drawn. In the 70 ppm groups, reduced absolute and relative liver weight in F0 females and F1 males was observed. Minor, statistically significant changes in hematologic data at the 35 and 70 ppm concentrations (generally 1%–7%) in the F1 rats appear to be within normal ranges based on historical data and are therefore not considered clinically or biologically significant or adverse. In addition, a significant decrease in maximum response to an auditory startle stimulus was noted in the 70 and 300 ppm groups on postnatal day 24, but not on postnatal day 60. Analysis of the

E-maze data by EPA personnel indicated possible alterations in learning behavior in the 70 ppm group, but the differences from the conclusions of the report could not be resolved.

The CMA (1996) study is adequate in that it was conducted with sufficient numbers of animals of both sexes and examined numerous endpoints. The study is acceptable and consistent with EPA testing guidelines that were in effect at the time of the study (U.S. EPA, 1991). However, there are several limitations to this study. Lack of pair-watered and pair-fed control animals confounds the results and precludes definitive conclusions as to whether the alterations in food and water consumption and body weight are related to water palatability or a direct toxic effect of the agent. Developmental landmarks (e.g., vaginal opening in F2a group) were not reported for all groups. Grip strength and landing foot splay were not included in the FOB. Discontinuation of exposure for the animals undergoing neurotoxicity testing minimizes the likelihood of finding a positive effect and precludes comparison of the data with those of other rats with continued exposure. Although the study employed an exposure regimen consistent with testing guidelines and should potentially detect adverse effects on the developing nervous system, discontinuation of exposure after weaning reduces the opportunity to detect neurological effects from continuous or lifetime exposures similar to those expected from lifetime drinking water exposure in humans.

Interpretation of the neurobehavioral tests is limited. The report lacks detailed descriptions of experimental methods (e.g., size of the arena, length of observations) and positive control data (including estimates of variability) for the FOB. Positive control studies for the motor activity and E-maze studies used high doses of the validation chemicals, were not adequate to show the sensitivity of the methods, and showed only that effects of the chemicals at maximally toxic doses could be recognized. Variability in the startle response data was high. The high variability and problems in calibrating and operating the automated startle apparatus (as presented in the report) would tend to decrease the sensitivity of the test to detect a difference between control and treated groups, because differences in startle amplitude would have to be larger to attain statistical significance. In some cases, inappropriate statistical analyses were applied. For example, repeated-measures techniques were apparently not used to account for the fact that the rats were tested repeatedly, and it is not clear how nonparametric rank data were analyzed or why a log transformation was applied to the learning data. The NOAEL for this study is 35 ppm (2.9 mg/kg-day chlorite) and the LOAEL is 70 ppm (5.9 mg/kg-day chlorite) based on lowered auditory startle amplitude and altered liver weights in two generations.

Groups of 12 male Long-Evans rats were exposed to 0, 1, 10, or 100 ppm sodium chlorite (0, 0.7, 7, and 70 ppm chlorite) in the drinking water for 56 days prior to mating and throughout a 10-day mating period (Carlton and Smith, 1985; Carlton et al., 1985, 1987). Groups of 24 female rats were exposed to the same sodium chlorite drinking water concentrations for 14 days prior to mating, during the mating period, and throughout gestation and lactation. Doses of 0, 0.075, 0.75, and 7.5 mg/kg-day were estimated by EPA (1994d). No significant alterations in body weight gain or water consumption were observed. There was a wide degree of variability among fertility rates for the different groups (67%–96%), but no dose-related alterations in fertility rates were observed. No significant alterations in litter survival rates, median day of eye opening, or median day of observed vaginal patency were observed. Additionally, no alterations

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were observed in gross and histopathologic examination of reproductive tract tissues. hematologic parameters, or testis, epididymis, and caudal epididymis weights. No significant alterations in sperm count or percentage of sperm mobility were observed. A trend toward decreased sperm mean progressive movement was observed in the 100 ppm group, but the velocity was not significantly different from controls. The percentage of abnormal sperm in sodium chlorite-exposed rats did not differ from controls. No significant alterations in T3 and T4 hormone levels were observed in the F0 males or females. T3 and T4 levels were measured in the F1 males and females on postnatal days 17 (males only), 21, and 40; significant decreases in hormone levels were consistently observed at 100 ppm at days 21 and 40. This study identifies a NOAEL of 10 ppm (0.75 mg/kg-day) and a LOAEL of 100 ppm (7.5 mg/kg-day) for altered thyroid hormone levels in the offspring of rats exposed to sodium chlorite in drinking water.

Carlton and Smith (1985) and Carlton et al. (1985, 1987) conducted two follow-up studies to further investigate the effect of sodium chlorite on sperm parameters. In these studies, groups of 12 male rats received drinking water containing 0, 100, or 500 ppm sodium chlorite (0, 70, and 370 ppm chlorite) or 0, 1, 10, or 100 ppm sodium chlorite (0, 0.7, 7, and 70 ppm chlorite) for 72–76 days. Water consumption was significantly decreased (28%) in the 500 ppm group; in other groups, water consumption was similar to that of controls. Estimated doses of 0.075, 0.75, 7.5, and 27 mg/kg-day were calculated for the 1, 10, 100, and 500 ppm groups, respectively. No significant alterations in body weight gain were observed in the sodium chlorite-exposed rats. As in the first experiment, there were no significant alterations in sperm count, percentage of sperm mobility, or mean progressive movement. However, there was a trend toward decreased progressive movement in the 100 and 500 ppm groups. When the three experiments were combined, there was a statistically significant reduction of direct progressive movement at 100 and 500 ppm. A significant increase in abnormal sperm was observed in the 100 and 500 ppm groups; the most common morphological abnormalities were frayed tails, open hooks, and amorphous sperm heads. Collectively, these studies identify a NOAEL of 10 ppm (0.75 mg/kg-day) and LOAEL of 100 ppm (7.5 mg/kg-day) for reproductive effects in rats exposed to sodium chlorite in drinking water.

Couri et al. (1982) exposed groups of 7–13 pregnant Sprague-Dawley rats to 0%, 0.1%, 0.5%, or 2% sodium chlorite (0%, 0.07%, 0.4%, and 1.5% chlorite) in the drinking water during gestational days 8–15. The litters were either delivered at term or by cesarean section on gestational day 22. Using the daily doses of 0, 34, 163, and 212 mg sodium chlorite/rat/day calculated by the study authors and an estimated body weight (midpoint of gestation day 1 body weights [0.280 kg] plus one-half of the body weight gain), doses of 0, 95, 590, and 820 mg/kg-day sodium chlorite (0, 70, 440, and 610 mg/kg-day chlorite) were calculated. Another group of four pregnant rats received daily gavage doses of 200 mg/kg sodium chlorite on gestational days 8–15. Profuse vaginal and urethral bleeding and 100% mortality were observed in the rats receiving 200 mg/kg gavage doses. No deaths were observed in the rats receiving sodium chlorite via drinking water. Weight loss and decreases in food and water consumption were observed at the 0.5% and 2% concentrations; decreased water consumption was also observed in the 0.1% group. Irregular blood cells, ruptured cells, and hemolysis were observed in the 2% and 200 mg/kg-day groups. Significant decreases in crown-rump length were observed in litters

term-delivered in the 0.1%, 0.5%, and 2% groups and in the 0.5% group cesarean-delivered on gestational day 22. Fetal weights were not adversely affected. An increase in the number of resorbed and dead fetuses was observed in litters delivered on gestational day 22 in the 0.1%, 0.5%, and 2% groups; two litters out of five were totally resorbed in the 2% group. Postnatal growth and the incidences of soft tissue and skeletal malformations were not adversely affected by in utero exposure to sodium chlorite. This study identifies a FEL of 0.1% for resorbed and dead fetuses and decreases in crown–rump length in the offspring of rats exposed to 0.1% sodium chlorite (70 mg/kg-day chlorite) in drinking water.

Groups of six to nine female Sprague-Dawley rats were administered 0, 1, or 10 mg/L chlorite in the drinking water (0, 0.1, or 1 mg/kg-day calculated using a reference body weight of 0.35 kg and water intake of 0.046 L/day) for 2.5 months prior to mating with unexposed males and during gestational days 0–20; the dams were killed on gestational day 20 (Suh et al., 1983). No significant alterations in general appearance or maternal body weight gain were observed. No significant alterations in number of implants (total and per dam), resorptions, or dead fetuses were observed. No difference in fetal body weights was observed. Crown-rump length was significantly higher in the 10 mg/L group compared with controls, but the difference was very small and is probably not biologically significant. Chlorite exposure did not significantly alter incidence of skeletal anomalies. This study identifies a NOAEL of 10 mg/L (1 mg/kg-day) for developmental toxicity in the offspring of rats exposed to chlorite in the drinking water.

Mobley et al. (1990) exposed groups of 12 female Sprague-Dawley rats to 0, 20, or 40 ppm chlorite in the drinking water (doses of 0, 3, and 6 mg/kg-day were estimated by U.S. EPA, 1994d) for 10 days prior to mating with unexposed males and during gestation and lactation until postnatal days 42–53. Chlorite exposure did not adversely affect litter size or pup weight gain. Significant, consistent decreases in exploratory activity were observed in the 40 ppm group on postnatal days 36–39, but not on days 39–41. In the 20 ppm group, there were significant decreases in activity on days 36 and 37, but not on days 38–40. No significant alterations in serum T3 or T4 levels were observed in the 37–38- or 42-day-old postconception pups. However, the free T4 levels were significantly increased in the 40 ppm group. The day of eye opening in the 20 and 40 ppm groups was similar to that of controls. A review of the results of this study relative to the findings of the newer developmental studies in the database suggests that the NOAEL for neurodevelopmental behavioral effects in rats exposed to chlorite in drinking water for this study is 20 ppm (3 mg/kg-day) and the LOAEL is 40 ppm (6 mg/kg-day).

Moore et al. (1980) (data also presented in Moore and Calabrese, 1982) exposed groups of pregnant female A/J mice to 0 or 100 ppm sodium chlorite in drinking water throughout gestation and lactation; 21 control and 12 exposed dams had litters. EPA (1994d) estimated that the 100 ppm sodium chlorite (75 ppm chlorite) concentration corresponds to a dose of 22 mg/kg-day. A decrease in the conception rate (number of females positive for vaginal plug/number of females producing litters) was observed in the chlorite group (39% vs. 56% in controls); the statistical significance was not reported. No statistically significant alterations in gestation length, litter size, number of pups dead at birth, and number of pups alive at weaning were observed. Pup growth was adversely affected, as shown by significant decreases in average pup weaning weight and birth to weaning growth rate. This study identifies a LOAEL of 100 ppm

(22 mg/kg-day) for developmental effects in the offspring of mice exposed to chlorite in the drinking water.

Harrington et al. (1995b) treated groups of 16 female New Zealand white rabbits with technical-grade sodium chlorite (80.6% purity) via their drinking water at levels of 0, 200, 600, or 1,200 ppm from gestation days 7–20, followed by terminal sacrifice at day 28. Water concentrations were maintained at the same levels throughout pregnancy and were not adjusted for changes in volume of water consumed. Based on measured water consumption, the study authors calculated a mean daily intake of approximately 0, 10, 26, or 40 mg/kg-day chlorite (corrected for purity and adjusted by the weight of the salt). Clinical condition, maternal body weight, and food and water consumption were measured daily. At necropsy, gravid uterine weights, number of corpora lutea, number of implantation sites, and live and dead fetuses were recorded. Live fetuses were weighed, examined for external abnormalities, sexed, and dissected, and a gross visceral examination was performed. Skeletal examinations were also performed. Abnormalities were categorized as minor or major, and the latter were thought to impair survival or fitness. Commonly observed variations were also recorded. The study authors did not state which malformations fell into each of these categories. There was no mortality, although two rabbits (one from each of the control and 26 mg/kg-day groups) were sacrificed in extremis because of a clinical condition unrelated to treatment. A significant decrease in water consumption during the treatment period was observed in the 26 and 40 mg/kg-day groups, and a decrease during treatment days 16–20 of pregnancy was observed in the 10 mg/kg-day group. The study authors attributed the decreases in consumption to lack of palatability of the drinking water solution, although no supporting data were presented. Food consumption was decreased in the 26 and 40 mg/kg-day groups during days 7–11 of pregnancy. Body weight gain of treated animals was decreased on days 7–19, although by day 26 these groups showed no differences from controls in body weight gain.

The authors concluded there were no treatment-related effects on pregnancy incidence, number of implantations, number of preimplantation losses, fetal sex ratio, number of live fetuses, or fetal visceral or structural abnormalities. Data for specific malformations and variations were not shown; instead, data were presented as the number or mean percentage of fetuses with major or minor external and visceral or skeletal abnormalities. The number and mean percentage of major external and visceral and skeletal abnormalities were increased in the 26 and 40 mg/kg-day groups (external/visceral: 6.6% and 2.9%, respectively, vs. 1.5% in controls; skeletal: 5.4% and 0%, respectively, vs. 0% in controls). Mean fetal weights in the 26 and 40 mg/kg-day groups were slightly decreased (< 9% relative to controls). In the 26 and 40 mg/kg-day groups, the incidence of minor skeletal abnormalities (13.9 and 14.2 for the 26 and 40 mg/kg-day groups, respectively, vs. 7.7% in controls) and skeletal variants related to incomplete fetal bone ossification (such as of the pubis and sternebrae) was higher than for controls. The authors state in their discussion that these alterations in fetal body weight and delayed ossification indicate embryonic growth retardation. The NOAEL for this study is 200 ppm (10 mg/kg-day chlorite) and the LOAEL is 600 ppm (26 mg/kg-day chlorite) based on decreased fetal weight and delayed skeletal ossification, decreased food and water consumption in the dams, and decreased body weight gain in the dams. Although this study employed sufficient numbers of animals and administered chlorite by a route relevant to human exposure, uncertainties exist in

interpretation of the results because of inadequate reporting of the number and types of specific abnormalities and variations. There is additional uncertainty as to whether the decreases in food and water consumption and body weight gain in the dams are caused by unpalatability or a direct toxic effect of the chlorite.

4.4. OTHER STUDIES

4.4.1. Other Carcinogenicity Studies

4.4.1.1. *Chlorine Dioxide*

The potential for chlorine dioxide to induce proliferative epidermal hyperplasia was examined by Robinson et al. (1986). Groups of five dorsally shaved female SENCAR mice were placed in chambers filled with 0, 1, 10, 100, 300, or 1,000 ppm liquid chlorine dioxide; the chambers were designed to prevent the head from getting wet and to prevent inhalation of vapors. The animals were exposed 10 minutes/day for 4 days. A significant increase in interfollicular epidermal thickness was observed in the 1,000 ppm group, but not at the lower concentrations. Increases in total cell numbers and basal cell numbers in skin sections were observed in both the 300 and 1,000 ppm groups. In a second study, groups of 40 mice were immersed in 0 or 1,000 ppm chlorine dioxide for 10 minutes; animals (5/group) were killed 1, 2, 3, 4, 5, 8, 10, or 12 days postexposure. A significant increase in interfollicular epidermis thickness was observed at all time periods, with the highest values at 10 and 12 days postexposure. The authors concluded that even short-term dermal exposure to high concentrations of chlorine dioxide is capable of inducing hyperplastic responses in the mouse skin.

Miller et al. (1986) tested the carcinogenic potential of drinking water disinfected with chlorine dioxide using three short-term assays. Following disinfection with chlorine dioxide, the water samples (containing 0.5 mg/L chlorine dioxide residue) were concentrated 2,000× or 4,000× using a macroreticular resin process. In a mouse initiation-promotion assay, groups of 14–34 SENCAR mice (sex not specified) were orally administered 0.5 mL of the 4000× concentrate in 2% emulphor 3 times/week for 2 weeks followed by topical exposure to 1.0 μg 12-tetradecanylphorbal-13-acetate (TPA) in acetone applied to the dorsal skin 3 times/week for 20 weeks and then sacrificed. No significant increases, compared with vehicle controls, in the number of skin tumors or the number of tumors per animal were observed.

In a lung adenoma assay (Miller et al., 1986), groups of 20 male and 20 female Strain A mice received 0.25 mL gavage doses of 2000× or 4000× concentrates in 2% emulphor 3 times/week for 8 weeks followed by a 16-week observation period. The number of animals with lung adenomas and the number of adenomas per animal were not significantly altered compared with vehicle controls.

Miller et al. (1986) also examined the development of liver foci in rats in a short-term assay. In this study, groups of partially hepatectomized rats received a single dose of concentrated water (chlorine dioxide concentration not reported) in 2% emulphor followed 1 week later by administration of 500 ppm sodium phenobarbital in drinking water for 56 days;

animals were sacrificed on day 70. A control group received nondisinfected water. No significant increases in incidence of γ-glutamyltranspeptidase foci were observed.

4.4.1.2. *Chlorite*

Kurokawa et al. (1984) also conducted dermal carcinogenicity studies. In a study to assess the ability of chlorite to act as a complete carcinogen, groups of 20 female SENCAR mice were exposed twice weekly for 51 weeks to 20 mg/mL sodium chlorite in acetone. The solution (0.2 mL; 100 mg/kg sodium chlorite per application) was applied to the shaved backs of the mice. The sodium chlorite exposure did not result in increased tumor incidence. To test the ability of chlorite to act as a tumor promoter, a single initiating dose of 20 μmol of dimethylbenzanthracene (DMBA) was applied to the skin of 20 SENCAR mice. The DMBA application was followed by a 51-week exposure to sodium chlorite (as described for the complete carcinogen study). Tumor incidence was 6/20 (30%) compared with 0/20 in mice that received DMBA followed by acetone treatments for 51 weeks. Squamous cell carcinomas were observed in 5/20 animals in the chlorite group. However, the results were not statistically significant.

4.4.2. Genotoxicity Studies

4.4.2.1. *Chlorine Dioxide*

Both positive and negative results have been found in in vitro genotoxicity studies. Chlorine dioxide did not increase chromosome aberrations in Chinese hamster fibroblast cells but did increase reverse mutation in *Salmonella typhimurium* (with activation) (Ishidate et al., 1984). However, water samples disinfected with chlorine dioxide did not induce reverse mutations in *S. typhimurium* with or without activation (Miller et al., 1986). In vivo micronucleus and bone marrow chromosomal aberration assays in Swiss CD-1 mice administered 0.1–0.4 mg chlorine dioxide via gavage for 5 consecutive days were negative, as was a sperm-head abnormality assay in B6C3F1 mice administered 0.1–0.4 mg via gavage for 5 consecutive days (0, 3.2, 8, and 16 mg/kg-day) (Meier et al., 1985). Hayashi et al. (1988) reported positive results in the micronucleus assay in ddY mice following a single intraperitoneal injection of 3.2–25 mg/kg chlorine dioxide.

4.4.2.2. *Chlorite*

Genotoxicity of chlorite was assessed in several in vitro and in vivo assays. In in vitro assays, chlorite induced reverse mutations in *S. typhimurium* (with activation) and chromosome aberrations in Chinese hamster fibroblast cells (Ishidate et al., 1984). In general, the results of the in vivo assays were negative. In the micronucleus assays, negative results were found in ddY mice following an oral gavage dose of 37.5–300 mg/kg chlorite single injection (Hayashi et al., 1988) and in Swiss CD-1 mice administered 0.25–1 mg chlorite via gavage for 5 consecutive days (0, 8, 20, and 40 mg/kg-day) (Meier et al., 1985). Using the same dosages, Meier et al. also reported negative results in the bone marrow chromosomal aberration assay in Swiss CD-1 mice and in the sperm-head abnormality assay in B6C3F1 mice. Positive results were found in the

micronucleus assay in ddY mice when the chlorite was administered via intraperitoneal injection (7.5–60 mg/kg) (Hayashi et al., 1988).

4.4.3. Mechanistic Studies

EPA (1994d) has extensively discussed the mechanism of action whereby chlorine dioxide and chlorite produce hematologic and systemic effects. The mechanisms are still incompletely understood. Oxidative damage to the erythrocyte and production of methemoglobin are most likely related to their properties as oxidants (U.S. EPA, 1994d). Chlorite is thought to be the intermediate species responsible in many of the hematologic effects of chlorine dioxide because of its more efficient production of methemoglobin, depletion of red blood cell (RBC) glutathione, and alteration of erythrocyte fragility.

In a series of experiments, Bercz and co-workers (1982, 1986); and Harrington et al. (1986) suggested that chlorine dioxide increases binding of dietary iodide to gastrointestinal tissue and contents, producing a functional iodide deficiency. Bercz et al. (1982) found decreased levels of circulating thyroxine in monkeys drinking water containing > 9.5 mg/kg-day chlorine dioxide, but not 44 mg/kg-day chlorite, for 4–6 weeks. In a follow-up study, Harrington et al. (1986) demonstrated increases in thyroid iodide uptake and a rebound in thyroxine levels in monkeys 1 year after an 8-week exposure to approximately 5 mg/kg-day chlorine dioxide in drinking water. Unlike monkeys, rats showed dose-related declines in thyroxine levels and no alteration in thyroid iodide uptake following an 8-week exposure to 10 mg/kg-day chlorine dioxide in drinking water.

Whether either or both of these mechanisms are operable in inducing reproductive, developmental, and neurodevelopmental effects is not known. One could also speculate that hypothyroidism, induced by chlorine dioxide alteration of iodide uptake in the gastrointestinal tract, might contribute to alterations in maternal or neonatal behavior. Alternative, as yet unknown mechanisms are also plausible because few definitive mechanistic data are available. Additional research is needed to understand whether the parent chlorine dioxide and/or its oxychlorine degradation products induce delays and alterations in fetal/neonatal neurodevelopment and behavior through disturbance in maternal thyroid function or directly within the embryo itself.

4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS AND MODE OF ACTION (IF KNOWN)—ORAL AND INHALATION

4.5.1. Oral Exposure

4.5.1.1. *Chlorine Dioxide*

The subchronic/chronic toxicity of chlorine dioxide has not been adequately assessed. The Haag (1949) chronic drinking water study reported decreases in survival in rats exposed to 13 mg/kg-day chlorine dioxide for 2 years, but the cause of death was not reported and no effects were observed at lower concentrations. The small number of animals tested and the limited

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number and lack of sensitive endpoints examined make interpretation of this study difficult. Daniel et al. (1990) found increases in incidence of nasal lesions in rats exposed to ≥ 25 mg/L chlorine dioxide (2 mg/kg-day) in drinking water for 90 days; no other adverse effects were observed. However, it is not known if the nasal lesions resulted from inhaling chlorine dioxide vapors at the drinking water sipper tube or from off-gassing of the vapors after drinking. No other studies have reported similar effects. Other subchronic/chronic studies primarily examined hematologic parameters. Bercz et al. (1982) found significant decreases in serum T4 levels in monkeys exposed to 9.5 mg/kg-day chlorine dioxide in the drinking water for 4–6 weeks. Adverse hematologic effects could not be discerned in Abdel-Rahman et al. (1984b) because there was no consistent dose-effect relationship. Additionally, Daniel et al. (1990), Bercz et al. (1982), and Moore and Calabrese (1982) did not find hematologic alterations in rats, monkeys, or mice, respectively. Abdel-Rahman et al. (1984b) and Couri and Abdel-Rahman (1980) reported alterations in the glutathione-dependent system, in particular, decreases in erythrocyte glutathione levels, increases in glutathione peroxidase activity, and increases in erythrocyte catalase levels. However, as with the hematologic effects this group found, consistent relationships between dose and magnitude of the alterations were lacking.

A number of studies have consistently found developmental effects following in utero exposure or postnatal gavage administration of 14 mg/kg-day chlorine dioxide. The effects include altered brain development (decreases in forebrain and/or cerebellum DNA content, ratio of protein content to forebrain weight, and dendritic spine counts in a visual association area of the cerebral cortex) (Toth et al., 1990; Taylor and Pfohl, 1985), decreased locomotor or exploratory activity (Orme et al., 1985; Taylor and Pfohl, 1985), and increased T3 uptake (Mobley et al., 1990). Orme et al. (1985) found decreases in T3 and T4 levels in in utero and postnatally exposed pups; however, other studies did not find alterations in T3 and T4 levels in similarly exposed animals (Toth et al., 1990; Carlton et al., 1991).

The available data indicate that the critical effect of chlorine dioxide is neurodevelopmental toxicity.

4.5.1.2. *Chlorite*

A number of studies have examined the subchronic/chronic toxicity of chlorite; however, only the Harrington et al. (1995a) study examined a wide range of endpoints. This study identified a NOAEL and LOAEL of 7.4 and 19 mg/kg-day, respectively, for stomach lesions and alterations in spleen and adrenal weights in rats receiving gavage doses of sodium chlorite. The bolus administration of sodium chlorite might have contributed to the stomach lesions; these effects might not have been observed if the sodium chlorite had been administered in the drinking water. Haag (1949) found renal effects in rats drinking 9.3 mg/kg-day chlorite (NOAEL of 0.7 mg/kg-day); interpretation of the results of this study is limited by the small numbers of animals that underwent pathological examination and the limited number of endpoints examined. Abdel-Rahman et al. (1984b) and Couri and Abdel-Rahman (1980) found decreases in osmotic fragility, blood glutathione levels, and blood catalase activity in rats exposed to 1 and 10 mg/kg-day chlorite in drinking water. It is unclear, however, if these effects are statistically or biologically significant. In contrast, Moore et al. (1980) and Moore and Calabrese (1982) found

increases in osmotic fragility in mice exposed to 22 mg/kg-day chlorite in drinking water. Bercz et al. (1982) found decreases in erythrocyte and hemoglobin levels and decreases in T4 levels in monkeys exposed to 58.4 mg/kg-day chlorite.

As with chlorine dioxide, developmental toxicity appears to be the most sensitive effect of oral chlorite exposure. At exposure levels of 3 mg/kg-day and 6 mg/kg-day, Mobley et al. (1990) found significant decreases in exploratory activity in rat pups exposed to chlorite in utero. The changes at 3 mg/kg-day were small, whereas changes observed at 6 mg/kg-day were more consistent with findings from several other studies. Similarly, lowered auditory startle response and reduced liver weight were observed at 6 mg/kg-day, but not at 3 mg/kg-day, in rats in a two-generation study (CMA, 1996). At higher concentrations (19–28 mg/kg-day), decreases in fetal/pup body weight have been observed in mice and rabbits (Moore et al., 1980; Moore and Calabrese, 1982; Harrington et al., 1995b). Data from Carlton and Smith (1985) and Carlton et al. (1987) suggest that sperm may be a sensitive target of toxicity. Reductions in sperm progressive movement and increases in abnormal sperm have been observed in rats exposed to 7.5 mg/kg-day chlorite in drinking water for 72–76 days. However, the CMA (1996) two-generation study did not find any alterations in reproductive performance in rats exposed to 22.7 mg/kg-day chlorite in drinking water.

4.5.2. Inhalation Exposure

4.5.2.1. *Chlorine Dioxide*

Several human studies have examined the toxicity of inhaled chlorine dioxide (Gloemme and Lundgren, 1957; Elkins, 1959; Ferris et al., 1967; Exner-Freisfeld et al., 1986; Kennedy et al., 1991; Meggs et al., 1996). Despite the limitations of these studies (including poor exposure assessment, small number of subjects, and concomitant exposure to chlorine and/or sulfur dioxide), they consistently demonstrate that the respiratory tract is a very sensitive target of toxicity.

A series of studies by Paulet and Desbrousses (1970, 1972, 1974) and Dalhamn (1957) examined the acute and subchronic toxicity of chlorine dioxide in rats and rabbits. As with the human studies, the respiratory tract is the most sensitive target of toxicity. The effects include alveolar congestion and hemorrhage, bronchial inflammation, and peribronchiolar edema. A NOAEL for these effects has not been identified; the lowest LOAEL is 1 ppm (2.8 mg/m^3) in rats exposed to chlorine dioxide 5 hours/day, 5 days/week for 2 months (Paulet and Desbrousses, 1972).

4.5.2.2. *Chlorite*

No data are available on the toxicity of inhaled chlorite.

4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION—SYNTHESIS OF HUMAN, ANIMAL, AND OTHER SUPPORTING EVIDENCE, CONCLUSIONS ABOUT HUMAN CARCINOGENICITY, AND LIKELY MODE OF ACTION

4.6.1. Chlorine Dioxide

Under the current guidelines (U.S. EPA, 1986a), chlorine dioxide is classified as Group D, not classifiable as to human carcinogenicity because of inadequate data in humans and animals. Under the draft Carcinogen Assessment Guidelines (U.S. EPA, 1996a), the human carcinogenicity of chlorine dioxide cannot be determined because no satisfactory human or animal studies assessing the chronic carcinogenic potential of chlorine dioxide were located.

No human or animal studies assessing the carcinogenic potential of chlorine dioxide were located. The carcinogenic potential of concentrates prepared from drinking water treated with chlorine dioxide was tested by Miller et al. (1986). The concentrates did not increase incidence of lung adenomas in Strain A mice, skin tumor frequency in mice, or incidence of gamma-glutamyl transpeptidase positive foci (a measure of preneoplastic changes) in rat livers. Robinson et al. (1986) found significant increases in skin thickness in SENCAR mice immersed in chlorine dioxide, suggesting that high concentrations of chlorine dioxide are capable of inducing hyperplastic responses in the mouse skin.

Both positive and negative results have been found in genotoxicity studies of chlorine dioxide. Exposure to chlorine dioxide did not induce chromosomal aberrations in vitro, but it did increase occurrence of reverse mutations (Ishidate et al., 1984). In vivo assays did not find increases in micronucleus induction, chromosomal aberrations, or sperm-head abnormalities following oral exposure (Meier et al., 1985), but they did find increases in micronuclei induction after intraperitoneal injection (Hayashi et al., 1988).

4.6.2. Chlorite

Under the current guidelines (U.S. EPA, 1986a), chlorite is classified as Group D, not classifiable as to human carcinogenicity because of inadequate data in humans and animals. Under the draft Carcinogen Assessment Guidelines (U.S. EPA, 1996a), the human carcinogenicity of chlorite cannot be determined because of a lack of human data and limitations in animal studies.

No human studies assessing the carcinogenic potential of chlorite were located. Chlorite was tested for potential carcinogenicity in rat and mouse drinking water studies (Kurokawa et al., 1986; Yokose et al., 1987). These studies do not provide sufficient evidence to draw conclusions as to the carcinogenic potential of chlorite in humans. In the rat study (Kurokawa et al., 1986), exposure to sodium chlorite did not significantly increase the incidence of tumors. The short exposure duration (85 weeks) and high incidence of Sendai viral infection in control and exposed rats limit the use of this study to assess carcinogenicity.

In the mouse drinking water study (Kurokawa et al., 1986; Yokose et al., 1987), significant increases in liver and lung tumors were observed in male mice. Combined incidence of hepatocellular nodules and hepatocellular carcinomas was increased in the low-dose group, and combined incidence of lung adenomas and adenocarcinomas was elevated in the high-dose group relative to concurrent controls. However, these tumor incidences were within the range of values of historical controls in the study laboratory and in the National Toxicology Program laboratories (Kurokawa et al., 1986). This study is considered inadequate for assessing carcinogenicity because of the relatively short exposure duration (80 weeks) and the high incidence of early mortality in the concurrent control males from excessive fighting, making statistical comparisons between concurrent controls and treated animals difficult to interpret. No increases in tumor incidence were seen in female mice in this study.

Chlorite has been shown to be mutagenic in in vitro assays for reverse mutations and chromosome aberrations (Ishidate et al., 1984) and in an in vivo assay of micronucleus induction in which mice received an intraperitoneal injection of sodium chlorite (Hayashi et al., 1988). In vivo assays for micronucleus induction, chromosome aberrations, and sperm-head abnormalities were negative in mice receiving gavage doses of chlorite for 5 days (Meier et al., 1985; Hayashi et al., 1988).

4.7. SUSCEPTIBLE POPULATIONS

4.7.1. Possible Childhood Susceptibility

4.7.1.1. *Chlorine Dioxide and Chlorite*

Developmental delays have been observed in animal studies following in utero and postnatal exposure to ingested chlorine dioxide or chlorite, suggesting that infants and children may be more likely than adults to experience adverse effects following exposure to these chemicals, although the reasons for this increased sensitivity are not fully understood. It is well recognized that neurological development continues after birth and that gastrointestinal uptake of many nutrients and chemicals is greater in the neonate than the adult.

4.7.2. Possible Gender Differences

4.7.1.2. *Chlorine Dioxide and Chlorite*

No data are available to suggest there are gender differences in the toxicity of chlorine dioxide or chlorite.

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5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

5.1.1. Choice of Principal Study and Critical Effect—With Rationale and Justification

In general, human studies have not found adverse effects in individuals consuming low concentrations (0.04–0.15 mg/kg-day) of chlorine dioxide or chlorite in experimental studies (Lubbers et al., 1981, 1982, 1984a) or consuming drinking water disinfected with chlorine dioxide (Michael et al., 1981; Tuthill et al., 1982). An epidemiology study by Kanitz et al. (1996) found increases in the risk of several developmental effects (neonatal jaundice, small cranial circumference, and shorter body length) in a community with chlorine dioxide-disinfected drinking water. However, the Kanitz et al. (1996) study has numerous limitations (including multiple chemical exposures; lack of exposure data; lack of control for smoking, age, and nutritional habits; and atypical control data), making it difficult to interpret the study findings.

In animals, the most sensitive effect following oral exposure to chlorine dioxide or chlorite is neurodevelopmental delay. In utero exposure to chlorine dioxide or postnatal gavage administration of chlorine dioxide has resulted in altered brain development (decreases in brain weight, protein content, and cell number) (Taylor and Pfohl, 1985; Toth et al., 1990) and decreased locomotor or exploratory activity (Orme et al., 1985; Taylor and Pfohl, 1985; Mobley et al., 1990). The LOAEL for these effects is 14 mg/kg-day chlorine dioxide (Orme et al., 1985; Taylor and Pfohl, 1985; Mobley et al., 1990; Toth et al., 1980); Orme et al. (1985) identified a NOAEL of 3 mg/kg-day.

Neurobehavioral effects (lowered auditory startle amplitude, decreased brain weight, and decreased exploratory activity) are also the most sensitive endpoints following oral exposure to chlorite (Mobley et al., 1990; CMA, 1996). The LOAEL identified in the Mobley et al. (1990) developmental toxicity study and the CMA (1996) two-generation developmental toxicity study is 6 mg/kg-day chlorite; Mobley et al. (1990) also found significant decreases in exploratory activity at 3 mg/kg-day, but the difference between activity in this group and the controls was small. Thus, the NOAEL for neurobehavioral effects is 3 mg/kg-day chlorite. At higher concentrations (22–28 mg/kg-day chlorite), decreases in fetal/pup body weight have also been observed in mice and rabbits (Moore and Calabrese, 1982; Moore et al., 1980; Harrington et al., 1995b).

Chlorine dioxide in drinking water rapidly degrades to chlorite; in the Michael et al. (1981) study, chlorine dioxide rapidly disappeared from the stored water (within 2–4 hours) and chlorite levels concomitantly increased. Once absorbed, chlorine dioxide and chlorite are cleared from the blood at similar rates and are similarly distributed throughout the body (Abdel-Rahman et al., 1979b, 1982). Additionally, chloride is the major in vivo degradation product of both chlorine dioxide and chlorite. Available data suggest that chlorine dioxide and chlorite have similar targets of toxicity and potencies. Therefore, the toxicity information for chlorite is relevant to deriving an RfD for chlorine dioxide.

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The CMA (1996) two-generation study was selected as the critical study for the development of an RfD for both chlorine dioxide and chlorite. Both in its study report (CMA 1996) and in a later journal article (Gill et al., 2000), CMA reported that the study defined a NOAEL of 70 ppm (6 mg/kg-day chlorite) and a LOAEL of 300 ppm (28.6 mg/kg-day chlorite) based on hematologic toxicity. For the reasons outlined below, EPA disagrees with CMA's choice of NOAEL and LOAEL values. Alterations in multiple endpoints define the LOAEL-NOAEL boundary in the CMA study. Effects observed included statistically significant decreases in pup body weight, absolute brain weight, liver weight, and lowered startle amplitude at the 28.6 mg/kg-day dose. Statistically significant decreases in auditory startle amplitude (F1 and F2 generations) and absolute and relative liver weights (F0 and F1) occurred at 6 mg/kg-day. Although different responses were found for auditory startle (as indicated by measures of amplitude, latency, and habituation), this is not unexpected given that these measures examine different aspects of nervous system function and thus can be differently affected. Transient alterations in neurofunctional (or neurochemical) measures, such as in the auditory startle response, can occur without neuropathological changes and are considered of neurotoxic concern (U.S. EPA, 1998a). Some of effects observed at 6 mg/kg-day and 28.6 mg/kg-day occurred in both sexes and in more than one generation. These effects are considered toxicologically significant, which is consistent with EPA guidelines for reproductive, developmental, and neurotoxicity risk assessment (U.S. EPA, 1991, 1996b, 1998a). The NOAEL for this study is 3 mg/kg-day chlorite and the LOAEL is 6 mg/kg-day chlorite based on lowered auditory startle amplitude and decreased liver weight.

Although the CMA (1996) study is adequate, having been conducted with sufficient numbers of animals of both sexes at multiple dose levels showing a range of effects, and having examined numerous endpoints, there are several limitations. Lack of pair-watered and pair-fed control animals confounds the results and precludes making definitive conclusions as to whether the alterations in food and water consumption and body weight are related to water palatability or a direct toxic effect of the agent. Discontinuation of exposure for the animals undergoing neurotoxicity testing limits the likelihood of finding a positive effect, precludes comparison of the data with those of other rats with continued exposure, and does not reflect the expected lifetime exposure by humans to these chemicals in drinking water. In addition, a lack of detailed description of experimental methods and positive control data (including estimates of variability), and in some cases inappropriate statistical analysis, limits interpretation of the neurobehavioral tests.

The principal study is supported by the developmental studies by Orme et al. (1985), Taylor and Pfohl (1985), Mobley et al. (1990), and Toth et al. (1990), wherein rats administered chlorite or chlorine dioxide at similar dosages in drinking water also showed alterations in exploratory and locomotor behavior and reduced brain weights (NOAELs of 3 mg/kg-day; LOAELs of 14 mg/kg-day).

5.1.2. Methods of Analysis—Including Models (PBPK, BMD, etc.)

The NOAEL/LOAEL approach was used to derive RfDs for chlorine dioxide and chlorite. The RfD was derived using the NOAEL of 3 mg/kg-day identified in the CMA (1996)

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study. This dose was determined from the nominal water concentration based on measured water consumption and adjusted for the molecular weight of the salt, so that doses are expressed as the chlorite ion. (For example, males administered 35 ppm had intakes of sodium chlorite equivalent to 3.9 mg/kg-day. Adjusting for the molecular weight of sodium chlorite [MW = 90.5] relative to the chlorite ion [MW = 67.5] gives the NOAEL dose of 3 mg/kg-day chlorite.)

5.1.3. RfD Derivation—Including Application of Uncertainty Factors and Modifying Factors

The RfDs for chlorine dioxide and chlorite were derived by dividing the NOAEL of 3 mg/kg-day by an uncertainty factor of 100. This composite factor includes a factor of 10 to account for uncertainties associated with interspecies extrapolation and a factor of 10 for intrahuman variability. Because the critical effect is a developmental effect in a database that includes chronic studies, it is not necessary to use an uncertainty factor to account for use of a less-than-lifetime study. A default modifying factor of 1 is applied. The resultant RfD is 3×10^{-2} mg/kg-day:

$$\text{RfD} = 3 \text{ mg/kg-day} \div 100 = 3 \times 10^{-2} \text{ mg/kg-day.}$$

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

5.2.1. Choice of Principal Study and Critical Effect—With Rationale and Justification

5.2.1.1. *Chlorine Dioxide*

Human studies examining toxicity of inhaled chlorine dioxide are limited to several case reports (Elkins, 1959; Exner-Freisfeld et al., 1986; Meggs et al., 1996) and occupational exposure studies (Gloemme and Lundgren, 1957; Ferris et al., 1967; Kennedy et al., 1991) that involved concurrent exposure to chlorine and possibly sulfur dioxide. Although these studies cannot be used to establish risk assessment values, the results of these studies consistently demonstrate that the respiratory tract is a very sensitive target of chlorine dioxide toxicity.

A series of studies by Paulet and Desbrousses (1970, 1972, 1974) and Dalhamn (1957) examined the acute and subchronic toxicity of chlorine dioxide in rats and rabbits. The earliest Paulet and Desbrousses (1970) study identified a LOAEL of 2.5 ppm chlorine dioxide (6.9 mg/m^3) for thoracic effects (alveolar congestion and hemorrhage; bronchial inflammation) in rats exposed 7 hours/day (presumably 5 days/week) for 30 days and pulmonary effects (alveolar hemorrhage and capillary congestion) in rabbits exposed 4 hours/day (presumably 5 days/week) for 45 days; a NOAEL was not identified. A follow-up study by this group attempted to identify a threshold for respiratory effects (Paulet and Desbrousses, 1972). This study identified a LOAEL of 1 ppm (2.8 mg/m^3) for pulmonary effects (vascular congestion and peribronchiolar edema) in rats exposed 5 hours/day, 5 days/week for 2 months; a NOAEL was not identified. The Dalhamn (1957) study identified a NOAEL of 0.1 ppm chlorine dioxide (0.28 mg/m^3) for lung damage in rats exposed 5 hours/day (frequency of weekly exposure not reported) for 10

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weeks; a LOAEL of 10 ppm (28 mg/m^3) for respiratory tract irritation was identified in rats exposed 4 hours/day for 9 days in a 13-day period.

Collectively, the results of the human and animal studies suggest that the respiratory tract is the critical target. The Paulet and Desbrousses (1970, 1972) studies were selected as cocritical studies. The 1972 study identified the lowest LOAEL for a sensitive endpoint (respiratory tract effects); however, the study duration (2 months) is shorter than the typical subchronic study (approximately 90 days) and only one exposure concentration was tested. The 1970 study is used to support the identification of the critical effect and critical concentrations; this study tested several concentrations in two species for durations of 30 or 45 days.

5.2.1.2. *Chlorite*

An RfC for chlorite is not recommended at this time. No human or animal studies examining the toxicity of inhaled chlorite were located. Although the available human and animal data on inhaled chlorine dioxide support the derivation of an RfC for this chemical, these data cannot be used to derive an RfC for chlorite. Under ambient conditions, airborne chlorite is likely to exist as a particulate, whereas inhalation exposure to chlorine dioxide is as a gas. Based on their physical and chemical properties, it is anticipated that inhaled chlorine dioxide and chlorite would have very different modes of exposure. Therefore, the potential hazards associated with exposure to these two chemicals are also very different. In the absence of data demonstrating parallels in pharmacokinetic behavior following inhalation exposure—as are available following oral exposure—derivation of an RfC for chlorite from the available data for chlorine dioxide is not recommended.

5.2.2. Methods of Analysis—NOAEL/LOAEL

5.2.2.1. *Chlorine Dioxide*

The NOAEL/LOAEL approach was used to calculate the RfC for chlorine dioxide. A benchmark concentration (BMC) analysis could not be conducted because the report of the Paulet and Desbrousses (1970, 1972) studies did not include incidence data.

The RfC was derived using the Paulet and Desbrousses (1970, 1972) studies as co-critical studies. From the LOAEL of 1 ppm for pulmonary effects in rats identified in the Paulet and Desbrousses (1972) study, concentration in mg/m^3 was calculated using a molecular weight of 67.46 and the assumption of 25°C and 760 mmHg:

$$\text{LOAEL} = 1\ \text{ppm} \times 67.46/24.45 = 2.8\ \text{mg/m}^3\ \text{(Paulet and Desbrousses, 1972 - rats).}$$

The duration-adjusted LOAEL ($\text{LOAEL}_{\text{ADJ}}$) was calculated by multiplying the LOAEL by the daily exposure duration (5 hours/day) and the weekly exposure frequency (5 days/week):

$$\text{LOAEL}_{\text{ADJ}} = 2.8\ \text{mg/m}^3 \times 5\ \text{hours}/24\ \text{hours} \times 5\ \text{days}/7\ \text{days} = 0.41\ \text{mg/m}^3\ \text{(rat).}$$

The human equivalent concentration (HEC) for the LOAEL ($LOAEL_{HEC}$) was calculated by multiplying the $LOAEL_{ADJ}$ by the regional gas dose ratio for the thoracic region of the respiratory tract ($RGDR_{TH}$). The $RGDR_{TH}$ was calculated using the following equation:

$$RGDR_{TH} = \frac{\left[\frac{MV}{SA}\right]_A}{\left[\frac{MV}{SA}\right]_H}$$

where MV is the minute volume in rats (0.118 m^3/min; 0.17 m^3/day) and humans (13.8 m^3/min; 20 m^3/day) and SA is the surface area of the thoracic region in rats (3461.6 cm^2) and humans (640,581 cm^2).

$LOAEL_{HEC}$ = 0.41 mg/m^3 × [(0.118 m^3/min / 3461.6 cm^2) / (13.8 m^3/min / 640,581 cm^2);

$LOAEL_{HEC}$ = 0.41 mg/m^3 × 1.57 = 0.64 mg/m^3.

Similarly, for the Paulet and Desbrousses (1970) study, using values of 1.10 m^3/min for the minute volume and 59,100 cm^2 for the surface area of the thoracic region of rabbits, the calculation of the $LOAEL_{HEC}$ is as follows:

LOAEL = 2.5 ppm × 67.46/24.45 = 6.9 mg/m^3.

$LOAEL_{ADJ}$ = 6.9 mg/m^3 × 4 hours/24 hours × 5 days/7 days = 0.82 mg/m^3.

$LOAEL_{HEC}$ = 0.82 mg/m^3 × [(1.10 m^3/min / 59,100 cm^2) / (13.8 m^3/min / 640,581 cm^2);

$LOAEL_{HEC}$ = 0.82 mg/m^3 × 0.596 = 0.49 mg/m^3.

5.2.3. RfC Derivation—Including Application of Uncertainty Factors and Modifying Factors

5.2.3.1. *Chlorine Dioxide*

The RfC for chlorine dioxide is derived by dividing the $LOAEL_{HEC}$ thoracic effects by an uncertainty factor of 3,000. This uncertainty factor comprises a factor of 10 to account for extrapolation of a chronic RfC from a subchronic study, 3 for interspecies extrapolation using dosimetric adjustments, 10 for intrahuman variability, and 10 to account for extrapolation from a LOAEL for mild effects and for the lack of inhalation developmental and reproductive toxicity studies. EPA's policy is to limit the size of the composite uncertainty factor to 3,000 in recognition of the lack of independence of these factors (U.S. EPA, 1994b). The LOAEL to NOAEL and database uncertainties are therefore coalesced into one uncertainty factor of 10. The composite uncertainty factor for this RfC is therefore 3,000. No modifying factor is used for this assessment.

$$RfC = 0.64\ mg/m^3 \div 3{,}000 = 2 \times 10^{-4}\ mg/m^3.$$

or

$$RfC = 0.49\ mg/m^3 \div 3{,}000 = 2 \times 10^{-4}\ mg/m^3.$$

As can be seen, the same value for the RfC can be calculated using the LOAEL from either of the key studies. Note that this is the same value as was verified by the RfC workgroup in 1990, as no new data were available.

5.3. CANCER ASSESSMENT

5.3.1. Chlorine Dioxide

The oral and inhalation databases are inadequate to assess the carcinogenicity of chlorine dioxide in humans or animals; thus, derivation of an oral slope factor and inhalation unit risk level is precluded.

5.3.2. Chlorite

The oral and inhalation databases are inadequate to assess the carcinogenicity of chlorite in humans or animals; thus, derivation of an oral slope factor and inhalation unit risk level is precluded.

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

Chlorine dioxide and chlorite are strong oxidizing agents used as drinking water disinfectants and to bleach textile and wood pulp for paper manufacturing. Chlorine dioxide and chlorite are rapidly absorbed from the gastrointestinal tract and slowly cleared from the blood. Chlorine dioxide and chlorite, primarily in the form of chloride, are widely distributed throughout the body and predominantly excreted in the urine. Chloride is the major urinary "metabolite" for both chlorine dioxide and chlorite. No data are available on the pharmacokinetics of inhaled or dermally applied chlorine dioxide or chlorite.

In general, human ingestion studies have found no adverse effects in adults and neonates living in areas with chlorine dioxide-disinfected water. However, these studies are fraught with methodological problems, such as lack of characterization of exposure to other agents in the drinking water and control of potential confounding factors. These studies do little to confirm a possible association between exposure to chlorine dioxide and chlorite and adverse reproductive or developmental outcome in humans. Inhalation exposure to chlorine dioxide results in

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respiratory irritation in humans. However, these studies also poorly characterize exposure, and the occupational exposure studies involve concomitant exposure to chlorine and/or sulfur dioxide.

Animal toxicity databases for chlorine dioxide and chlorite is fairly comprehensive, composed of subchronic and chronic studies, reproductive and developmental studies, and toxicokinetic and mechanistic information. Multiple animal studies have shown similar alterations in neurodevelopmental endpoints, such as brain weight and behavioral measures. The majority of these studies have used sufficient numbers of animals and employed routes of exposure (gavage and drinking water) relevant to human exposure. The majority of the developmental studies have utilized rats and have shown a fairly consistent definition of the NOAEL/LOAEL.

Reproductive studies in male animals are not consistent in demonstrating alterations in spermatogenic indices, that is, abnormal morphology or motility; however, reported effects seem to appear at doses higher than the adverse developmental effects. Similarly, clinically or toxicologically significant alterations in hematologic parameters occur at higher doses.

The mode of action for induction of adverse neurodevelopmental effects is not known. It is also not known whether the rat is an adequate model for toxicity of chlorine dioxide and chlorite in humans. However, this species is widely used to characterize reproductive and developmental effects in humans.

Animal studies have demonstrated that the respiratory tract is the most sensitive target of toxicity following inhalation exposure to chlorine dioxide. No animal inhalation studies are available for chlorite.

No human studies assessing the carcinogenic potential of chlorine dioxide or chlorite were located. Chlorine dioxide carcinogenicity has not been tested in animal bioassays. Chlorite was not shown to increase tumor incidences in rats and mice; these studies are considered inadequate for assessing human carcinogenicity because the exposure was for less than a lifetime, a high incidence of Sendai virus was found in the rats, and mortality was high in the mouse control group because of excessive fighting.

Areas of scientific uncertainty in this assessment include the mode of action of chlorine dioxide and chlorite in producing adverse effects on multiple organ systems, including reproductive, developmental, and hematologic effects. Inherent in the uncertainty over the mode of action is identification of the susceptible populations or subgroups, and additional research in this area would help to better quantify the additional risk to these groups. Well-designed and conducted epidemiologic studies in communities with drinking water disinfected with these chemicals would decrease uncertainty in the utilization of animal models for determination of human health effects.

6.2. DOSE RESPONSE

Quantitative estimates of human risk as a result of low-level chronic chlorine dioxide or chlorite oral exposure are based on animal experiments, because no adequate human exposure data are available. Neurodevelopmental toxicity is the primary effect in offspring of rats exposed to chlorine dioxide or chlorite in drinking water. Quantitative estimates of human risk as a result of low-level chronic chlorine dioxide inhalation exposure are based on animal experiments, because no adequate human inhalation data are available. The respiratory tract appears to be the primary target of toxicity in human and animal studies.

The oral RfD for chlorine dioxide or chlorite is 3×10^{-2} mg/kg-day. This is 1/100 of the NOAEL, using neurodevelopmental toxicity in a two-generation rat study as the indicator of adverse effects. Overall confidence in this RfD assessment is medium to high. Confidence in the CMA (1996) principal study is medium. Although the study design and analytical approaches are consistent with EPA testing guidelines, some limitations in the design and conduct of the study exist. Confidence in the database is high because there are studies in multiple species, chronic duration studies in males and females, reproductive/developmental toxicity studies, and a multigenerational study. The threshold for adverse effects is consistently defined among the animal studies.

The inhalation RfC for chlorine dioxide is 2×10^{-4} mg/m^3. This concentration is 1/3,000 of the HEC for thoracic effects in rats (Paulet and Desbrousses, 1970, 1972). No human or animal data were located for chlorite that could be used to derive an RfC. Overall confidence in the RfC for chlorine dioxide is low. The studies by Paulet and Desbrousses (1970, 1972) identify only a LOAEL in rats and rabbits for adverse lung effects in 60- and 45-day studies and lack experimental detail. There were no adequate subchronic or chronic inhalation studies that examined extrarespiratory effects, and no acceptable developmental or reproductive studies on inhaled chlorine dioxide.

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APPENDIX A. EXTERNAL PEER REVIEW— SUMMARY OF COMMENTS AND DISPOSITION

The support document and IRIS summary for chlorine dioxide and chlorite have undergone both internal peer review performed by scientists within EPA and a more formal external review performed by scientists in accordance with EPA guidance on peer review (U.S. EPA, 1994c). Comments made by the internal reviewers were addressed prior to submitting the documents for external peer review and are not part of this appendix. The external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. A summary of significant comments made by the external reviewers and EPA's response to these comments follows.

Question 1. Are you aware of any other data/studies that are relevant (i.e., useful for hazard identification or dose-response assessment) for the assessment of the adverse health effects, both cancer and noncancer, of this chemical?

Comments: Two reviewers did not find any new relevant studies that would have any impact on the conclusions of this document. Four additional references were mentioned by the two other reviewers. One reviewer concurred that the results of the EPA evaluation agree with IARC (vol. 52, 1991), and there is inadequate evidence for the carcinogenicity of sodium chlorite in experimental animals. One reviewer commented on sensitive subgroups of the population and potential effects on blood chemistry parameters in renal dialysis patients when chlorine dioxide was used as a disinfectant. Also, one reviewer suggested a statement should be made on whether chlorite can be designated as a tumor promoter based on an initiation/promotion study on mouse skin (Kurokawa et al., 1984) and if the promoting activity is related to epidermal hyperplasia induction after topical exposure to sodium chlorite.

Response to Comments: The effects of chlorine dioxide and chlorite on human subjects and blood chemistry are described in the *Drinking Water Criteria Document on Chlorine Dioxide, Chlorite, and Chlorate* (U.S. EPA, 1994d) and in this Toxicological Review. All relevant ingestion studies, including the additional studies mentioned by the reviewers, have been evaluated in the drinking water criteria document, which was used in preparing this Toxicological Review. Changes seen in the tumor promoter study on mouse skin were not statistically significant.

Question 2. For RfD, RfC, and cancer, where applicable, have the most appropriate critical effects been chosen? For the cancer assessment, are the tumors observed biologically significant?

Comments: Two reviewers reiterated that it would appear that NOAELs around 3 mg/kg-day for the neurodevelopmental and behavioral effects are the most appropriate to develop the RfD for the oral exposure route, that the selection of the Paulet and Desbrousses (1972) study

for developing the RfC for chlorine dioxide is appropriate, and also that there is still no adequate evidence for the carcinogenicity of chlorine dioxide or chlorite. Other reviewers also stated that there are inadequate cancer data for risk assessment. One reviewer commented that an independent pathology group should review the histopathology diagnoses in the CMA (1996) study.

Response to Comments: The CMA (1996) study was vigorously subjected to independent peer review at EPA and by external reviewers. It was also reviewed by the stakeholders. Additional review of the histopathology diagnoses was not performed because the most sensitive endpoints (neurofunctional effects) were not histologic in nature.

Question 3. For RfD and RfC and cancer, have the appropriate studies been chosen as principal?

Comments: The external reviewers reiterated that appropriate studies were chosen for chlorine dioxide and chlorite. One reviewer stated that actual study reports cited were not available for review; the reviewer also suggested review of additional studies for irritating effects of chlorine dioxide in humans and questioned whether humans were more sensitive than rodents to chlorine dioxide.

Response to Comments: EPA cited the suggested studies as appropriate within the text. Studies describing irritating effects of chlorine dioxide in humans are described in the text. Data on the comparative sensitivity of rodents and humans to chlorine dioxide are not available. The 10-fold uncertainty factor for animal to human extrapolation was deemed an appropriate adjustment for this data gap.

Question 4. Studies included in the RfD and RfC and cancer under the heading "Supporting/Additional Studies" are meant to lend scientific justification for the designation of critical effect by including any relevant pathogenesis in humans, any applicable mechanistic information, any evidence corroborative of the critical effects, or to establish the comprehensiveness of the data base with respect to various endpoints. Should some studies be removed?

Comments: Reviewers indicated that additional and supporting studies cited for the RfD, RfC, and cancer assessments are appropriate and that no studies should be removed. One reviewer commented that he would question the quality and utility of studies that were conducted 50 years ago when quality assurance procedures and chemical production procedures and specifications were not what they are today. One reviewer asked whether any attempts were made to obtain histopathology slides from the unpublished Haag et al. (1949) studies.

Response to Comments: EPA agrees that no additional and supporting studies should be removed from this document. EPA did not attempt to acquire the histopathology slides from the unpublished Haag et al. (1949) chronic studies of chlorine dioxide in rats since they are older studies.

Question 5. Are there other data that should be considered in developing the uncertainty factors or the modifying factor? Do you consider that the data support use of different (default) values than those proposed?

Comments: One reviewer was unaware of any additional or other data that should be considered in developing the uncertainty factors for chlorine dioxide or chlorite. One reviewer questioned whether it would be useful to review/discuss the risk analysis that supports the use of chlorine dioxide and sodium chlorite as indirect food additives or as components of consumer products such as mouthwash or toothpaste. A comment was made that the report should compare lifetime animal and human oral exposures to chlorine dioxide or chlorite on the basis of mg/kg body weight and mg/mL body surface. One reviewer commented that patients on extracorporeal hemodialysis using home equipment may be potentially exposed to 70–90 times the residues exposed by adults who merely consume the water. A question was raised on the data available to support selection of an uncertainty factor that takes into account for those individuals with deficient glucose-6-phosphate dehydrogenase activity and neonates with sluggish methemoglobin reductase activity.

Response to Comments: EPA agrees with a reviewer that additional or other data are not warranted for this risk assessment. EPA followed the customary guideline for risk assessment for development of an RfD derivation. EPA did not examine chlorine dioxide or chlorite as indirect food additives or as components of consumer products such as mouthwash or toothpaste. EPA discussed individuals with deficiency in glucose-6-phosphate dehydrogenase and methemoglobin reductase as a potential susceptible subpopulation in the drinking water criteria document (U.S. EPA, 1994d). EPA thinks that an uncertainty factor of 100 is adequate to protect this group as well as the 80,000 Americans on renal dialysis.

Question 6. Do the confidence statements and weight-of-evidence statements present a clear rationale and accurately reflect the utility of the principal study and the comprehensiveness of the data? Do these statements make sufficiently apparent all the underlying assumptions and limitations of these assessments? If not, what needs to be added?

Comments: External reviewers indicated that the confidence and weight-of-evidence statements were clearly and rationally presented. One reviewer indicated that the comprehensiveness of the data was adequately presented and the underlying assumptions and limitations of the assessments were sufficently presented. One reviewer mentioned that the confidence statements for the RfC for chlorine dioxide should indicate whether humans are more susceptible to chlorine dioxide.

Response to Comments: Adequate information is not available to determine if humans are more susceptible. EPA has applied a 10-fold uncertainty factor for extrapolation from animals to humans to address this area of uncertainty.

Question 7. Is the weight of evidence for cancer assigned at the appropriate level (where applicable)?

Comments: External reviewers indicated that cancer assessment was not applicable for chlorine dioxide and chlorite, as the data are inadequate. One reviewer commented that a statement should be made concerning the designation of sodium chlorite as a tumor promoter in mouse skin under the conditions examined in the Kurokawa et al. (1984) study.

Response to Comments: The reviewers agreed that the cancer assessment was assigned at the appropriate level. EPA does not agree that such a statement should be made as the increased tumor incidence did not attain statistical significance.

Judi Douglass

From: tips@elementktips.com
Sent: Tuesday, November 07, 2000 11:45 AM
To: imatips@elementktips.com
Subject: IMATips: Easily view related fields in an Access query (95/97/2000)

Here's another useful Microsoft Access tip from Element K Journals, your number one source for software tips and techniques.

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********** THE TIP **********

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home	4140302	170	59.432239798	10.103480766

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APPENDIX J

ADDENDUM TO APPENDICES

CONTENTS OF APPENDIX J

1. **Analysis of Inorganic Constituents of Micronized MS2500**

2. ***Listeria monocytogenes* Inoculation of Cherry Tomatoes**

ANRESCO
INCORPORATED
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11 November 2000

File Number: 1100050

Bernard Technologies, Inc.
919 North Michigan Avenue, Suite 1710
Chicago, IL 60611-1601
Attn: Celeste Tabor, Chief Engineer FAX: 312-337-0505
cc: Peter N. Gray, Ph.D., E.V.P.

Re: Three lots of Micronized MS2500 Customer PO Number: 0000-1102-001
containing magnesium, sodium, sulfate, phosphate, chlorite & silicate
Lot Numbers: A - P0095-10-1 B - P0095-1-3 C - P0103-2-2

Rec'd via UPS Next Day Delivery on 11/03/00

Findings

Lot #	Lead	Heavy Metals	Arsenic	Fluoride	Selenium
P0095-10-1	nd	PASS	nd	nd	nd
P0095-1-3	nd	PASS	nd	nd	nd
P0103-2-2	nd	PASS	nd	nd	nd
Limit, ppm	1	5	0.5	10	2

Methods: as taken from Food Chemicals Codex, 4th edition thru the Second Supplement.

Notes: ppm = mg/kg
Heavy Metals expressed as Lead (Pb) using 4 grams samples compared to 20mcg Pb ion.
nd = none detected to the limit stated.
PASS = less than the stated limit based on comparison to standard solution.

Reported by
ANRESCO, Inc.

E. Jerry Oliveras
Laboratory Director

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November 1, 1999

Peter Gray, Ph.D.
Executive Vice President & CEO
Bernard Technologies Inc.
919 N. Michigan Ave.
Chicago, IL 60611

Report#8850

***Listeria monocytogenes* inoculation of cherry tomatoes to determine the effects of chlorine dioxide gas versus pathogen and spoilage organisms.**

Dear Peter:

Enclosed you will find the data for the Cherry Tomato Microbial Challenge Study initiated 10-14-99.

Protocol:

I. Culture Preparation:

Listeria monocytogenes cells ATCC 43249 and *Listeria innocua* cells (isolated from pork) were transferred from refrigerated stock cultures (Difco Trypticase Soy Agar Slants) to individual tubes containing Difco Brain Heart Infusion (BHI) Broth and incubated at 35°C for 24 hours. *Pseudomonas fluorescens* cells ATCC13525 and *Lactobacillus acidophilus* cells ATCC 2678 were transferred from refrigerated stock cultures (Difco All Purpose Tween Agar Slants) to individual tubes containing Difco All Purpose Tween broth. The *Pseudomonas fluorescens* tube was incubated at 25°C for 24 hours and the *Lactobacillus acidophilus* tube was incubated at 35°C for 96 hours under reduced oxygen in a CO2 system.

The incubated cell cultures were centrifuged in their respective media at 1450 X g for 30 minutes using a Clay Adams Dynac II centrifuge. The pellets were resuspended in 5 ml sterile 0.85% saline. The washed cells were diluted with sterile 0.85% saline to obtain the OD readings listed in Table 1, using a Milton Roy Spec 20 Spectrophotometer. These OD readings have been previously determined to result in an estimated cell concentration of approximately 1.0 X 10^8 cells per ml for each organism.

Table 1

Organism	OD reading @ 420 nm
Listeria monocytogenes	0.420
Listeria innocua	0.420
Pseudomonas fluorescens	0.450
Lactobacillus acidophilus	0.500

Bacterial cocktails were prepared by combining 5 ml from each of the desired OD suspensions into a sterile test tube and mixing on a vortex apparatus. The bacterial cocktails were appropriately dispensed into previously sterilized 500 ml Nalgene bottles containing 297 ml sterile Deionized water and mixed. A final inoculum volume of 300 ml was obtained with cell concentrations of approximately 1.0 x 10^6 cells per ml. Five inocula were prepared by combining the organisms into bacterial cocktails as listed in Table 2.

Table 2

Inoculum Number	*Listeria monocytogenes*[a]	*Listeria innocua*[a]	*Pseudomonas fluorescens*[a]	*Lactobacillus acidophilus*[a]	Deionized Water (ml)
1	-	-	+	+	297
2	+	-	-	-	297
3	+	-	+	+	297
4	+	+	-	-	297
5	-	-	-	-	300

[a]Organisms were cocktailed (5 ml per desired organism, previously diluted to OD in Table 1). The bacterial cocktail (3 ml) was added to sterile Deionized water to obtain the final inoculum volume of 300 ml with resulting cell concentrations of 1.0 x 10^6 cells per ml.
+ organism used in the cocktail
- organism not used in the cocktail

II. Experimental Design:

Desert Glory cherry tomatoes purchased from a local grocery store were used in this experiment. Stems and leaves were gently removed and the tomatoes rinsed for approximately one minute with potable water. Deionized water was used as a final rinse. The tomatoes were dried with paper towels, lightly misted with 70% Ethanol and allowed to air dry under a biologically controlled atmospheric hood for approximately 10 minutes.

Tomatoes were aseptically transferred, 10-20 at a time, to a 500 ml Nalgene bottle which was previously sterilized and the inoculum added. The Nalgene bottle was gently rotated for approximately 1 minute to insure contact of the entire surface of each tomato with the inoculum. Inoculated tomatoes were placed on a baker's rack, which was previously sanitized with 70% ethanol. The baker's rack with tomatoes was placed under a biologically controlled atmospheric hood for 30-45 minutes to allow the liquid portion of the inoculum to evaporate. Tomatoes were analyzed before and after drying to determine if the cell numbers decreased with the initial drying of the inoculum.

Three levels of MS2500 containing LDPE film (High, Low and No MS2500) were challenged with the inoculua prepared in section I. Inoculated tomatoes were packaged by aseptically transferring 6 tomatoes into the appropriate test bag (high concentration, low concentration or no MS2500) then placing the test bags inside a plastic bag. A heat sealer was used to seal the external plastic bag. Packages were incubated at room temperature for the test periods.

Exposed packaged tomatoes were analyzed at 24 hours and 96 hours. Once a package was opened and sampled, it was not used again. Tomatoes were analyzed by aseptically transferring two tomatoes into a 225 ml Nalgene bottle containing 10 ml sterile Difco DE Neutralizing Broth. The samples were gently shaken with rotation to insure the entire surface of each tomato was rinsed with the DE Neutralizing broth. Spread plate methodology was used with Difco Modified Oxford Agar Medium incubated at 35°C for 48 hours to analyze for total *Listeria*, Difco *Pseudomonas* Isolation Agar Medium incubated at 25°C for 48 hours to analyze for *Pseudomonas fluorescens*, Difco *Lactobacillus* MRS Agar Medium incubated at 35°C for 48 hours under a reduced oxygen atmosphere in a CO_2 system to analyze for *Lactobacillus acidophilus* and BCM *Listeria* incubated at 35°C for 48 hours to analyze for *Listeria monocytogenes* and *Listeria innocua*. Variables were analyzed in duplicate and plated in singlet. Results are in Table 3.

Report#8850

Sample #	Sample Time	MS2500 Level	Description	*Pseudomonas Fluorescens* CFU/2 tomatoes[a]	*Lactobacillus acidophilus* CFU/2 tomatoes[a]	*Listeria monocytogenes* CFU/2 tomatoes[a]	*Listeria innocua* CFU/ 2 tomatoes[a]
8850-1	Pre drying	---	Inoculum #1 Spoilage organisms only	191,000	20,900	NT	NT
8850-1	Time 0	---		3,400	3,090	NT	NT
8850-1	24 hours	High		<10	890	NT	NT
8850-1	96 hours	High		<10	35	NT	NT
8850-6	24 hours	Low	Inoculum #1 Spoilage organisms only	<10	175	NT	NT
8850-6	96 hours	Low		<10	775	NT	NT
8850-11	24 hours	None	Inoculum #1 Spoilage organisms only	56,000	375	NT	NT
8850-11	96 hours	None		3,100,000	130	NT	NT
8850-2	Pre drying	---	Inoculum #2 *Listeria monocytogenes*	NT	NT	476,000	NT
8850-2	Time 0	---		NT	NT	54,000	NT
8850-2	24 hours	High		NT	NT	<10	NT
8850-2	96 hours	High		NT	NT	<10	NT
8850-7	24 hours	Low	Inoculum #2 *Listeria monocytogenes*	NT	NT	<10	NT
8850-7	96 hours	Low		NT	NT	<10	NT
8850-12	24 hours	None	Inoculum #2 *Listeria monocytogenes*	NT	NT	7,450	NT
8850-12	96 hours	None		NT	NT	42,100	NT
8850-3	Pre drying	---	Inoculum #3 Spoilage Organisms and *Listeria monocytogenes*	260,000	8,950	169,000	NT
8850-3	Time 0	---		850	880	8,400	NT
8850-3	24 hours	High		<10	670	<10	NT
8850-3	96 hours	High		<10	5	<10	NT
8850-8	24 hours	Low	Inoculum #3 Spoilage Organisms and *Listeria monocytogenes*	<10	20	<10	NT
8850-8	96 hours	Low		<10	<10	<10	NT
8850-13	24 hours	None	Inoculum #3 Spoilage Organisms and *Listeria monocytogenes*	125,000	240	3,160	NT
8850-13	96 hours	None		126,000	230	410	NT

[a] Results recorded as average of duplicate samples plated in singlet

000386

Report#8850

Sample #	Sample Time	MS2500 Level	Description	*Pseudomonas Fluorescens* CFU/2 tomatoes[a]	*Lactobacillus acidophilus* CFU/2 tomatoes[a]	*Listeria monocytogenes* CFU/2 tomatoes[a]	*Listeria innocua* CFU/ 2 tomatoes[a]
8850-4	Pre drying	---	Inoculum #4 *Listeria monocytogenes* And *Listeria innocua*	NT	NT	218,000	201,000
8850-4	Time 0	---		NT	NT	50,000	72,000
8850-4	24 hours	High		NT	NT	<10	15
8850-4	96 hours	High		NT	NT	<10	<10
8850-9	24 hours	Low	Inoculum #4 *Listeria monocytogenes* And *Listeria innocua*	NT	NT	<10	<10
8850-9	96 hours	Low		NT	NT	<10	<10
8850-14	24 hours	None	Inoculum #4 *Listeria monocytogenes* And *Listeria innocua*	NT	NT	3,150*	3,150*
8850-14	96 hours	None		NT	NT	2,200	10,500
8850-5	Pre drying	---	Inoculum #5 Sterile Saline	<10	<10	<10	<10
8850-5	Time 0	---		<10	750	<10	<10
8850-5	24 hours	High		<10	<10	<10	<10
8850-5	96 hours	High		<10	<10	<10	<10
8850-10	24 hours	Low	Inoculum #5 Sterile Saline	<10	<10	<10	<10
8850-10	96 hours	Low		<10	<10	<10	<10
8850-15	24 hours	None	Inoculum #5 Sterile Saline	<10	<10	<10	<10
8850-15	96 hours	None		<10	<10	<10	<10

[a] Results recorded as average of duplicate samples plated in singlet

*BCM Listeria plate was destroyed (laboratory error), recorded count was from the MOX plate ie. total *Listeria* spp.

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Results and Discussion:

Sampling variation was substantial due to the inconsistency in tomato sizes used in the experiment. Two tomatoes were analyzed per sample and variables were run in duplicate to try and reduce the sampling variation. All organisms inoculated showed a decrease in cell numbers ranging from 0.44 log to 2.48 logs after drying on the tomato surface however, the cell numbers were still present in sufficient magnitude to determine if the test packaging affected the organism survival. All organisms inoculated onto the tomato surface were recovered from the positive control samples (tomatoes packaged in the "no MS2500" packaging). In all cases, the *Pseudomonas fluorescens* cell numbers and the *Listeria monocytogenes* cell numbers dropped below detection with the high and low test bags. *Listeria innocua* was isolated from the high test bag sample after 24 hours but not recovered at the 96-hour sampling period. *Lactobacillus acidophilus* cell numbers did not drop below detection with the high or low MS2500 concentrations, however; cell numbers did remain constant. A lactic acid bacterium was also recovered from one negative control sample.

If you have any questions or concerns regarding the above data please do not hesitate to call me at 630-293-4000.

Sincerely,

Signed/Sent Electronically

Dawn Allison, B.S.
Corporate Microbiologist

SUBMISSION END

000889

AM

BERNÀRD TECHNOLOGIES, INC.

A Health and Food Safety Technology Company

March 8, 2001

Via Facsimile (202) 418-3131 and Express Delivery

Linda S. Kahl, Ph.D.
Regulatory Policy Branch (HFS-206)
Division of Product Policy
Office of Premarket Approval
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street SW
Washington, DC 20204

RECEIVED
MAR 10 2001
OFFICE OF MARKET

Re: GRN No. 62

Dear Dr. Kahl:

On November 30, 2000, Bernàrd Technologies, Inc., submitted to FDA a notification of its determination of the generally recognized as safe ("GRAS") use of chlorine dioxide generated by the Microsphère® system that consists of chlorite and other inorganic salts contained in a low-density polyethylene ("PE") film for food-contact packaging materials. This GRAS notification was filed by the Agency on December 4, 2000 as GRN No. 62 ("the Notification").

Based on our discussions with you, including our meeting on February 27, 2001, we wish to amend the Notification; in particular, we request that the notification be amended to define the food-contact uses of the PE film containing the Microsphère® system (MS2500) as follows:

1. The packaging of meats, poultry, and seafood that will be cooked prior to being consumed. The meat may include beef, pork, and lamb; the poultry may include chicken, turkey, duck, geese, game birds; and seafood may include fish, shrimp, and other seafood that will be cooked before being consumed. (Packaging of these foods will be referred to as Use Group 1.)

2. The packaging of fresh fruits and vegetables and foods in transit or storage from producers to processors or institutional users that will be washed with a potable water rinse and further processed (*e.g.* cutting, blending, blanching,

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PHONE 312.337.7007 · FACSIMILE 312.337.0505

> canning) before being consumed. These foods may include fruits (*e.g.* strawberries, blueberries, melons, mangoes, papayas), vegetables (*e.g.* carrots, tomatoes, lettuce, broccoli), legumes, and potatoes being shipped from field to processing plants or that are being stored, shipped, or handled between the raw agricultural stage and institutional users such that the they will be washed with a potable water rinse and further processed (*e.g.*, cutting, blending) before being consumed. (Packaging of these foods will be referred to as Use Group 2.)

We wish to clarify some aspects of the Notification.

A. Polyethylene film containing MS2500 particles releases chlorine dioxide gas (not chlorite) into the package's containment atmosphere.

1. Notwithstanding ENVIRON's conservative (health protective) worst-case exposure assessment (as described in Section IV.C. of the Notification) that presents an evaluation as though it is chlorite that migrates (and at 100%) to the food and remains (that is, does not wash off and is not destroyed by processing, cooking, *etc.*), the intended migrant from the polyethylene (PE) film containing MS2500 particles is chlorine dioxide gas not chlorite or a salt of chlorite. The PE film containing MS2500 particles functions in the following way: (1) when humidity or moisture in the air is high, water molecules diffuse through and penetrate the surface of the polymer film; (2) water molecules diffuse through the polymer (PE continuous phase) and reach the MS2500 particles; (3) the water molecules react with the salts in the MS2500 particle and initiate the solid-state reaction that generates the chlorine dioxide gas; (4) the chlorine dioxide gas diffuses out of the MS2500 particle and through the PE polymer film; (5) the chlorine dioxide gas migrates out of the film and into the contained air space in the package of food. This process is shown diagrammatically in Exhibit 1 attached to this letter.

2. The pore size or void volume in the surface of the PE polymer film is too small for the MS2500 particles to pass out of the film; however, these spaces are large enough to permit small molecules such as water and chlorine dioxide gas to migrate freely into and out of the film. Thus, the intended migrant from the packaging system is chlorine dioxide. The chlorite

not available as free chlorite.

3. The MS2500 particles are embedded in the PE film. There is no free chlorite on the surface of the film that could contact an item packaged in the film. In the polymer extrusion industry, the generally accepted practice is to produce film where the thickness of the film is at least two times the diameter of any particulate additives. Exhibit 2 attached to this letter presents scanning electron micrographs ("SEMs") of the PE film containing MS2500 particles. Note that the film has a surface layer of the continuous phase that separates the outside of the film from the inside of the film where the MS2500 particles are contained.

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Thus, the polyethylene forms a continuous phase barrier around all surfaces of the film. The MS2500 particles are contained within the film (as described in Section II.B of the Notification) and are not able to move out of the film; however, the small molecules such as ambient moisture (H_2O) and chlorine dioxide gas (ClO_2) may penetrate the film surface.

4. Both thin and thick PE films have equal chlorine dioxide generating capacity per square inch of potential food-contact surface. As described in the Notification (Section IV.C. Proposed Use of MS2500-containing LDPE Film Packaging Products), because the thickness of food-contact PE film products may vary, the loading of the MS2500 in the PE film is specified in a manner that results in equal amounts of total chlorine dioxide releasing capacity per square inch of film. This is shown diagrammatically in Exhibit 3 attached to this letter. The permissible formulation curves generated by the governing equation to achieve this effect are shown in Figures 18 and 19 in the Notification for two possible MS2500 concentrations of chlorite salt (Tables 7 and 9 in the Notification):

- In a thin film (1mil film with 24% MS2500), the amount of MS2500 equals 24% of the total weight of the thin film with the remaining 76% being polyethylene. This formulation corresponds to the point, 1.0 mil film thickness and 0.24 g MS2500/g film, shown on the graph in Figure 18.

- In a thick film (2mil film with 12% MS2500), the same amount of MS2500 as in the thin film equals only 12% of the total weight of the film with the remaining 88% being polyethylene. This formulation corresponds to the point, 2.0 mil film thickness and 0.12 g MS2500/g film, shown on the graph in Figure 18.

B. <u>Cooking results in the degradation of chlorine dioxide and chlorite to chloride</u>.

1. Decomposition of solutions of chlorite produces chloride ion.[i, ii, iii] Sodium chlorite thermally decomposes at 175°C, even in the solid state, to sodium chloride, otherwise known as table salt (*i.e.*, $NaClO_2 = NaCl + O_2$). The instability of chlorite (and its accelerated degradation to chloride) is known to occur at cooking temperatures.[iv, v]

2. As discussed in Section IV.A. (page 38) of the Notification, the FDA regulation for the safe use of chlorine dioxide to control microbial populations in poultry process water (filed by Rio Linda Chemical) was published on March 3, 1995 (Fed. Reg. 60(42): 11899-11901). FDA's final rule provided the rationale that the residual would be converted to chloride:

> *Based on its evaluation of the information in the petition, the agency has concluded that no detectable residues of chlorine dioxide would remain on poultry carcasses, and that exposure to chlorite and chlorate as a result of this use of chlorine dioxide would be virtually nil. (No chlorite or chlorate could be detected on poultry (raw or cooked) at the limit of detection (50 ppb) for the method used.) The agency also concluded that the very low levels of*

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> *chlorite and chlorate that may be retained on poultry carcasses as a result of exposure to chlorine dioxide-containing process water would be converted to correspondingly low levels of chloride (a relatively innocuous substance, e.g., chloride in table salt) during cooking.*

FDA's final rule references work by Gordon *et al.*[iv] for evidence that cooking resulted in the chlorine dioxide degradation to chloride.

Therefore, Use Group 1 is asserted to be generally recognized as safe.

C. Washing and processing food reduces chlorite residual to below detectable levels.

Washing and further processing are generally known to lower any residual level of chlorite on the surface of foods to below detectable levels as evidenced by the Agency's discussion in its final rule for the safe use of chlorine dioxide as an antimicrobial agent in water used to wash certain fruits and vegetables published on July 20, 1998 (Fed. Reg. 63(138): 38746-38747). FDA's final rule provided the rationale that potable water rinse would reduce potential residuals to insignificant levels:

> *FDA has also considered the safety of chlorine dioxide breakdown products, i.e., chlorite and chlorate, and concludes that residues of these compounds would be removed from the treated produce if the treatment with chlorine dioxide is followed by a potable water rinse or by blanching, cooking or canning.*

FDA noted that foods undergoing a potable water rinse do not have any detectable residues of chlorite. In studies conducted on foods packaged in the PE film containing MS2500 (described in Appendix H of the Notification), no detectable chlorite residue was found on meat, poultry, or other foods.

Therefore, Use Group 2 is asserted to be generally recognized as safe.

If you have any further questions or comments at any time, please contact me directly at (312) 337-7007. Thank you.

Very truly yours....

/Peter N. Gray, Ph.D./
BERNARD TECHNOLOGIES, INC.

cc: V. H. Frankos

000897

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LIST OF EXHIBITS

Exhibit 1	MS2500-containing Polyethylene Film: Production and Release of Chlorine Dioxide Gas
Exhibit 2	Scanning Electron Micrographs of Polyethylene Film Containing MS2500 Particles
Exhibit 3	Examples of Thin and Thick Polyethylene Films with Equal Amounts of MS2500

---

[i] Buser, W. and H. Hanisch. 1952. Helv. Chim. Acta, 35:2547.

[ii] Taylor, M.C., J. F. White, G. P. Vincent, and G. L. Cunningham. 1940. Ind. Eng. Chem., 32:899.

[iii] White, J. F., M.C. Taylor and G. P. Vincent. 1942. Ind. Eng. Chem., 34:782.

[iv] Gordon, G., R. G. Kieffer, and D. H. Rosenblatt. 1972. "The Chemistry of Chlorine Dioxide" in *Progress in Inorganic Chemistry*, S.J. Lippard, ed., vol. 15, pp 201-286, Wiley-Interscience, NY, USA.

[v] Masschelein, W.J. 1979. *Chlorine Dioxide.* Ann Arbor Science Publishers, Ann Arbor, MI, USA.

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EXHIBIT 1

MS2500-CONTAINING POLYETHYLENE FILM: PRODUCTION & RELEASE OF CHLORINE DIOXIDE GAS

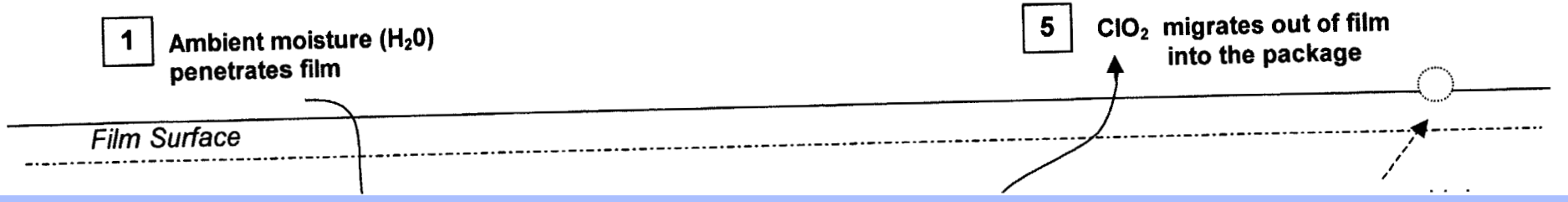

EXHIBIT 1

MS2500-CONTAINING POLYETHYLENE FILM: PRODUCTION & RELEASE OF CHLORINE DIOXIDE GAS

When humidity or moisture in the air is high, it diffuses into the MS2500 particles and initiates the solid-state reaction that produces chlorine dioxide gas. [redacted] This chlorine dioxide then diffuses out of the MS2500 particles and through the film. The outer portion of the MS2500 particle includes a rate-controlling matrix of other [redacted] such [redacted] that do not include chlorite.

These MS2500 particles are embedded in polyethylene film and there is no free chlorite on the surface of the film that would contact an item packaged in the film.

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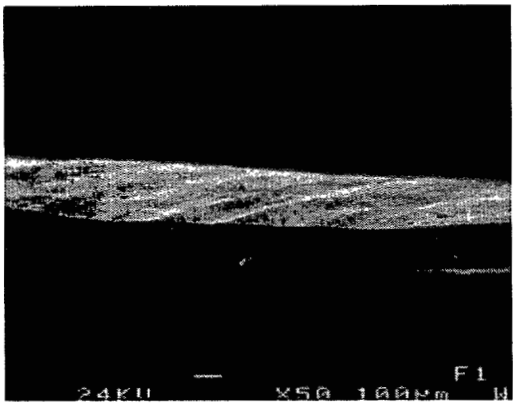
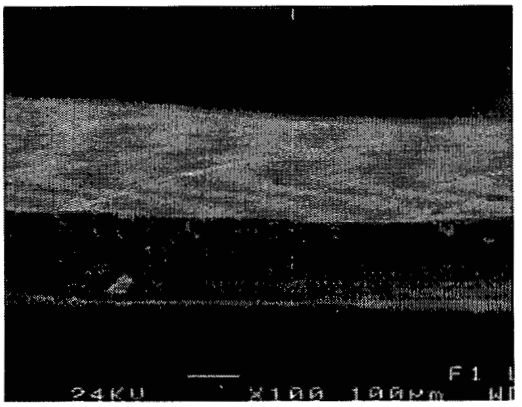
EXHIBIT 2

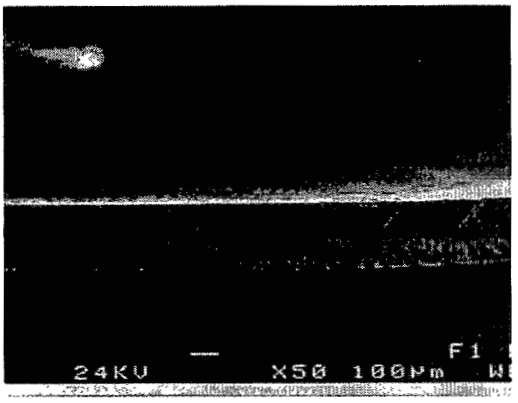
SCANNING ELECTRON MICROGRAPHS OF POLYETHYLENE FILM CONTAINING MS2500 PARTICLES



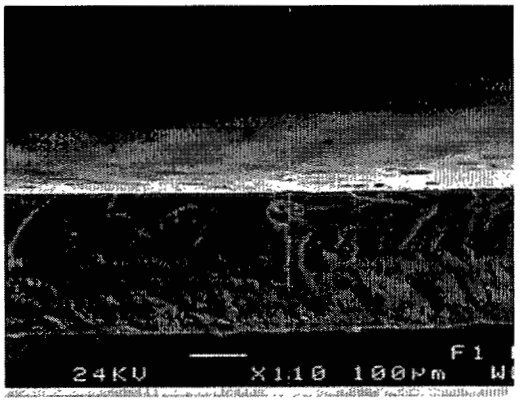
Control Film



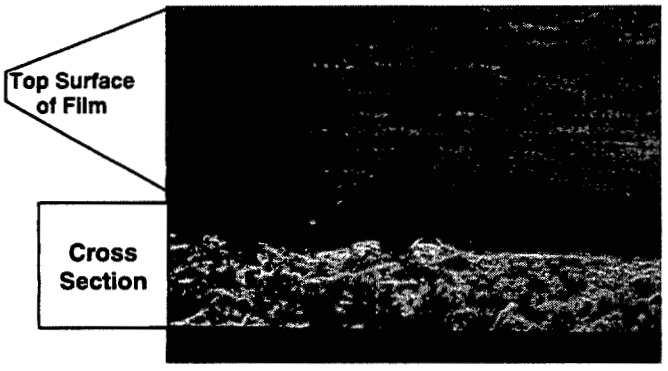
Control Film – High Magnification



MS2500-containing Film



MS2500-containing Film – High Mag.



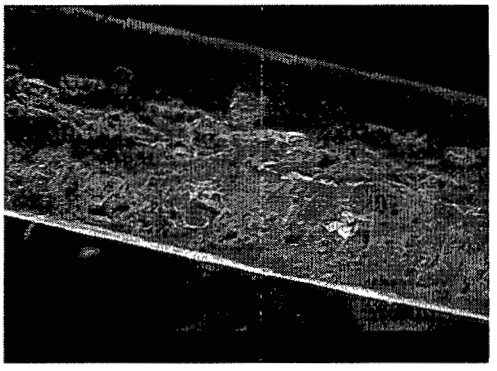


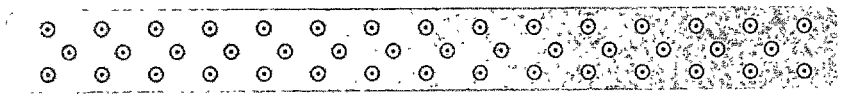
EXHIBIT 3

EXAMPLES OF THIN AND THICK POLYETHYLENE FILMS WITH EQUAL AMOUNTS OF MS2500

As described in GRN No. 62 (Section IV.C. Proposed Use of MS2500-containing LDPE Film Packaging Products), because the thickness of the food-contact polyethylene (PE) film products may vary, the loading of the MS2500 in the PE film is specified in a manner that results in equal amounts of total chlorine dioxide releasing capacity per square inch of film. The permissible formulation curves generated by the governing equation to achieve this effect are shown in Figures 18 and 19 for two possible MS2500 concentrations of [redacted] chlorite salt (Tables 7 and 9).

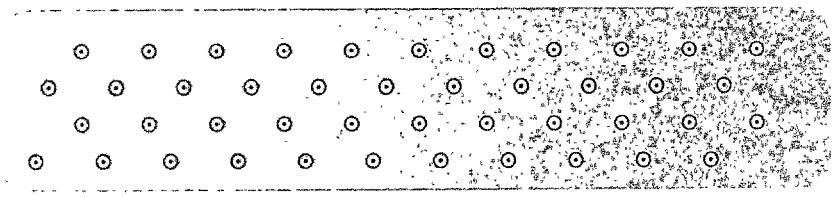
In the thin film shown diagrammatically below, the amount of MS2500 equals 24% of the total weight of the thin film with the remaining 76% being polyethylene. This formulation corresponds to the point (1.0 mil film thickness, 0.24 g MS2500/g film) on the graph shown as Figure 18.

Thin Film
(1mil Film with 24% MS2500)



In the thick film, the same amount of MS2500 as in the thin film equals only 12% of the total weight of the film with the remaining 88% being polyethylene. This formulation corresponds to the point (2.0 mil film thickness, 0.12 g MS2500/g film) on the graph shown as Figure 18.

Thick Film
(2mil Film with 12% MS2500)



⊙ **Represents a particle of MS2500 (*Discontinuous* Phase)**

Represents polyethylene (*Continuous* Phase)

EXHIBIT 3

EXAMPLES OF THIN AND THICK POLYETHYLENE FILMS WITH EQUAL AMOUNTS OF MS2500

Note:

Each film contains the same amount of MS2500 salt) per square inch of surface area (hence, the same chlorine dioxide generating capacity per square inch); however, the ratio of MS2500:polyethylene is different.

In the polymer extrusion industry the generally accepted practice is to produce film where the thickness of the film is at least two times the diameter of any particulate additives.

The polyethylene forms a continuous phase (barrier) around all surfaces of the film. The MS2500 particles are contained within the film and are not able to move out of the film; however, the film surface can be penetrated by ambient moisture (H_2O) and chlorine dioxide as a gas (ClO_2).

Pages 000904 - 000913 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000914 - 000915 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000916 - 000925 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

SU

Carlson, Susan

From:

Sent: Tuesday, April 29, 2003 1:03 PM

To: Carlson, Susan

Cc: 'PN GRAY'; Gavin Thompson; Bill Frankos

Subject: Consultation Meeting: April 17, 2003

Dr. Carlson:

As a follow-up to our consultation meeting, we prepared a brief discussion of our proposed approach and our understanding of the outscome from our meeting on April 17, 2003.

Emily Skowronek

Emily Skowronek
ENVIRON International Corporation
4350 North Fairfax Drive, Suite 300
Arlington, Virginia 22203
p. 703.516.2329
f. 703.516.2393

000959

ENVIRON

April 29, 2003

Susan J. Carlson, Ph.D.
OFAS/DBGNR
HFS-255
Harvey W. Wiley Federal Building
5100 Paint Branch Parkway
College Park, Maryland 20740-3835

Re: Consultation Regarding Proposed GRAS Notification for Chlorine Dioxide and Chlorite from Food-contact Packaging Made Using Low-density Polyethylene Film Containing the Microsphère® System: Additional Food Types

Dear Dr. Carlson:

Thank you for taking the time to have such a detailed and useful discussion with us during our meeting on 17 April 2003. This letter summarizes our understanding and proposal for the scope and approach for the GRN of additional uses for the Bernard Technologies, Microsphère® System.

A. Proposed GRAS Substances

The substance that is the subject of this generally recognized as safe ("GRAS") determination is chlorine dioxide generated by the Microsphère® System ("MS2500") in food-contact packaging material. The Microsphère® System is composed of a micronized powder that is a mixture of sodium phosphate, magnesium sulfate, sodium silicate, and sodium chlorite. The MS2500 is incorporated into a low-density polyethylene ("LDPE") film. This material, when combined with ambient humidity, generates a sustained release of chlorine dioxide. This GRAS substance and this food packaging system are the subject of a previous GRAS Notification, GRN 62[1].

[1] U.S. Food and Drug Administration (FDA). 2001. Agency Response Letter GRN 62. Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety (OFAS), June 19, 2001.

ENVIRON International Corporation
4350 North Fairfax Drive, Suite 300 • Arlington, VA 22203 • USA
Tel: (+1) (703) 516 2300 • Fax: (+1) (703) 516 2393

B. Proposed Applicable Conditions of Use

Bernard Technologies, Inc. ("BTI") intends to use MS2500 in LDPE films as a component of a variety of LDPE and multi-film layer products for packaging food. The applicable conditions of use of the notified substances, including the foods in which the substances are to be used, levels of use, and the purposes for which the substances are used are described in detail in GRN 62. A brief description of these issues is presented below.

Because there is a wide spectrum of packaging products that may be produced that deliver the same level of chlorine dioxide and may result in the same level of residual chlorite, we defined the products for this use based on estimating and limiting the potential consumer exposure. The methodology and the resulting definition of MS2500-containing LDPE film-based food-contact packaging products are described in detail in Section IV of the previous GRAS Determination Dossier[2]. Our assessment was based conservatively on standard default values for LDPE food-contact use conditions under the assumption that the MS2500-containing LDPE film-based products could replace all existing uses of LDPE film. That is, all foods currently packaged or otherwise contacting LDPE films would contact MS2500-containing LDPE films, including, but not limited to, fresh fruits and vegetables, breads and bakery goods, raw and processed meats. In addition, our assessment conservatively assumed that consumers would be exposed to 100 percent of the maximum chlorite in the Microsphère® System in the food packaging material. The governing relationship that limits the film thickness and loading of MS2500 in the LDPE film and, thus, limits the potential consumer exposure to the notified substances, is presented in Section IV of the previously submitted GRAS Determination Dossier.

The applications described in GRN 62 are:

1. For packaging meats, poultry, and seafood that will be cooked prior to being consumed, and
2. For packaging fresh fruits and vegetables and foods in transit or storage from producers to processors, or institutional users, that will be washed with a potable water rinse and further processed before being consumed.

[2] ENVIRON International Corporation (ENVIRON). 2000. Generally Recognized as Safe Determination for Chlorine Dioxide Generated by the Microsphère® System in Low-Density Polyethylene Film for Food-Contact Packaging Material. Arlington, Virginia. Submitted in support of GRN 62. November 27, 2000.

BTI has evaluated additional food-types for these same conditions of use. These additional applications are:

1. Fresh pasta products that will be cooked prior to being consumed;
2. Prepared dough products that will be baked, or otherwise cooked, prior to being consumed; and
3. Comminuted meats that will be cooked prior to being consumed.

Examples of these additional applications are:

1. Fresh, refrigerated, and frozen pasta products include but are not limited to cannelloni, cavatelli, fettuccini, gnocchi, lasagna, linguini, pasta dough, ravioli, rigatoni, spaghetti, spaghettini, and tortellini.
2. Prepared dough products include but are not limited to bagels, biscuits, bread, cookies, donuts, pastries, piecrust, pizza, rolls, and specialty dough products.
3. Comminuted meats include but are not limited to hot dogs, sausages, and bacon.

C. Proposed Basis for GRAS Determination

Based on our discussions with you, we understand that FDA concurs that it is generally recognized that chlorite is degraded by cooking (i.e., the chlorite is reduced to chloride). No residue of chlorine dioxide or chlorite has been detected on these foods. BTI's GRAS determination through scientific procedures is based on the lack of any residue of chlorine dioxide and chlorite in or on the foods. These uses fall under the guidelines stated in GRN 62 in that the foods will be cooked prior to consumption.

We previously submitted a GRAS Notification[3], which provides detailed information about the identities of the notified substances, including, their chemical names, Chemical Abstracts Service Registry Numbers, empirical formulae, structural formulae, quantitative compositions, method of manufacture, characteristic properties, any constituents of potential health concern, and specifications for food-grade materials. It also includes a comprehensive discussion of, and citations to, generally available and accepted scientific data, information, methods, or principles that BTI's has relied on to establish safety, including a consideration of the probable consumption of the substance and the probable consumption of any substance

[3] ENVIRON International Corporation (ENVIRON). 2000. Generally Recognized as Safe Determination for Chlorine Dioxide Generated by the Microsphère® System in Low-Density Polyethylene Film for Food-Contact Packaging Material. Arlington, Virginia. Submitted in support of GRN 62. November 27, 2000.

formed in or on food because of its use and the cumulative effect of the substance in the diet, taking into account any chemically or pharmacologically related substances in such diet. In addition, the GRAS Determination Dossier provides information on the levels of use and a detailed summary of the basis for BTI's determination that its particular use of the notified substances is exempt from the premarket approval requirements of the Act because such use is GRAS.

BTI has determined that chlorine dioxide generated by the Microsphère® System in LDPE film food-contact packaging material will not be present in or on the food after cooking. We propose that it is generally recognized that cooking degrades the chlorite derived from chlorine dioxide and thus no residual chlorine dioxide is present in or on the food and the use is GRAS. FDA has recognized the processes of blanching, cooking, and canning resulting in the removal of residual chlorite[4]. Thus, we intend to propose that these new uses for MS2500-containing LDPE films in fresh pasta, prepared dough, and comminuted meats that are to be cooked prior to consumption fall within the guidelines stated in GRN 62 and therefore, are also GRAS.

We invite and welcome your comments regarding this proposal and thank you for your attention to this matter.

Very truly yours,

Vasilios H. Frankos, Ph.D.
Principal

H:\0110710A Bernard GRAS Add Use\2003\letter to susan carlson - proposed GRN.doc

[4] U.S. Food and Drug Administration (FDA). 1999. Secondary direct food additives permitted in food for human consumption: Final rule. *Federal Register*, September 15, 1999, Vol. 64, No. 178, pp 49981-49982.

SU

Carlson, Susan

From:
Sent: Monday, May 19, 2003 5:30 PM
To: Carlson, Susan
Cc: THOMPSON, Gavin [Environ]; FRANKOS Vasilios H.
Subject: GRAS Notification Letter for Additional Uses GRN 62

GRAS Notification Letter-Addit...

Dr. Carlson:

As a follow-up to the outcome of our consultation meeting on April 17, 2003, we prepared the attached Notification of the GRAS Determination of Chlorine Dioxide and Chlorite from Food-contact Packaging Made using Low-density Polyethylene Film Containing the Microsphère® System for the additional food types discussed at the meeting.

A paper version also was mailed to your office.

Please let me know whether you have any questions.

Sincere regards,

Peter N. Gray, Ph.D.
BERNÀRD TECHNOLOGIES, INC.
Suite 500
75 East Wacker Drive
Chicago, Illinois 60601
(T) 312.337.7007 (F) 312.337.0505 (em) btipng@attglobal.net

000964

BERNÀRD TECHNOLOGIES, INC.

May 19, 2003

Susan J. Carlson, Ph.D.
FDA/CFSAN/OFAS/DBGNR
HFS-255
Harvey W. Wiley Federal Building
5100 Paint Branch Parkway
College Park, Maryland 20740-3835

Re: Notification of the GRAS Determination of Chlorine Dioxide and Chlorite from Food-contact Packaging Made Using Low-density Polyethylene Film Containing the Microsphère® System: Additional Food Types

---

Dear Dr. Carlson:

We wish to notify you that Bernàrd Technologies, Inc. ("BTI") has determined that chlorine dioxide and chlorite are generally recognized as safe ("GRAS") for use in food-contact packaging made using low-density polyethylene film containing BTI's Microsphère® System. Accordingly, chlorine dioxide and chlorite from the Microsphère® System are exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act ("the Act"). In accordance with our discussions with the Food and Drug Administration ("FDA"),[1] we are voluntarily submitting the attached summary of information upon which BTI relied in making its GRAS determination for specific additional food types.

A. Name and Address of Notifier:

Peter N. Gray, Ph.D.
Bernàrd Technologies, Inc.
75 East Wacker Drive, Suite 500
Chicago, IL 60601
Telephone: (312) 337-7007
Facsimile: (312) 337-0505

000965

[1] Consultation meeting with FDA on April 17, 2003.

B. Names and Identities of GRAS Substances:

The substance that is the subject of this generally recognized as safe ("GRAS") determination is chlorine dioxide generated by the Microsphère® System ("MS2500") in food-contact packaging material. The Microsphère® System is composed of a micronized powder that is a mixture of sodium phosphate, magnesium sulfate, sodium silicate, and sodium chlorite. The MS2500 is incorporated into a low-density polyethylene ("LDPE") film. This material, when activated, generates a sustained release of chlorine dioxide. This GRAS substance and this food packaging system are the subject of a previous GRAS Notification, GRN 62.[2]

C. Applicable Conditions of Use:

BTI intends to use MS2500 in LDPE films as a component of a variety of LDPE monolayer and multilayer film products for packaging food. The applicable conditions of use of the notified substances, including the foods in which the substances are to be used, levels of use, and the purposes for which the substances are used, are described in detail in GRN 62. A brief description of these issues is presented below.

Because a wide spectrum of packaging products may be produced that deliver the same level of chlorine dioxide and may result in the same level of residual chlorite, we defined the products for this use based on estimating and limiting the potential consumer exposure. The methodology and the resulting definition of MS2500-containing LDPE film-based food-contact packaging products are described in detail in Section IV of the previous GRAS Determination Dossier.[3] The assessment was based conservatively on standard default values for LDPE food-contact use conditions under the assumption that the MS2500-containing LDPE film-based products could replace all existing uses of LDPE film. That is, all foods currently packaged or otherwise contacting LDPE films would contact MS2500-containing LDPE films, including but not limited to fresh fruits and vegetables, breads and bakery goods, raw and processed meats. In addition, our assessment conservatively assumed that consumers would be exposed to 100 percent of the maximum chlorite in the Microsphère® System in the food packaging material. The governing relationship that limits the film thickness and loading of MS2500 in the LDPE film and thus limits the potential consumer exposure to the notified substances, is presented in Section IV of the previously submitted GRAS Determination Dossier (GRN 62).

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[2] U.S. Food and Drug Administration (FDA). 2001. Agency Response Letter GRN 62. Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety (OFAS), June 19, 2001.
[3] ENVIRON International Corporation (ENVIRON). 2000. Generally Recognized as Safe Determination for Chlorine Dioxide Generated by the Microsphère® System in Low-Density Polyethylene Film for Food-Contact Packaging Material. Arlington, Virginia. Submitted in support of GRN 62. November 27, 2000.

The applications described in GRN 62 are:

1. For packaging meats, poultry, and seafood that will be cooked prior to being consumed; and
2. For packaging fresh fruits and vegetables and foods in transit or storage from producers to processors, or institutional users, that will be washed with a potable water rinse and further processed before being consumed.

BTI has evaluated additional food-types for these same conditions of use. These additional applications are:

1. Fresh pasta products that will be cooked prior to being consumed;
2. Prepared dough products that will be baked, or otherwise cooked, prior to being consumed; and
3. Comminuted meats that will be cooked prior to being consumed.

Examples of these additional applications are:

1. Fresh, refrigerated, and frozen pasta products include but are not limited to cannelloni, cavatelli, fettuccini, gnocchi, lasagna, linguini, pasta dough, ravioli, rigatoni, spaghetti, spaghettini, and tortellini.
2. Prepared dough products include but are not limited to bagels, biscuits, bread, cookies, donuts, pastries, piecrust, pizza, rolls, and specialty dough products.
3. Comminuted meats include but are not limited to hot dogs, sausages, and bacon.

D. Basis for GRAS Determination:

It is generally recognized that cooking degrades chlorite (*i.e.*, the chlorite is reduced to chloride).[4] The FDA has recognized the processes of blanching, cooking, and canning resulting in the removal of residual chlorite.[5] In concurrence with these observations no residue of chlorine dioxide or chlorite is expected to be present on these food types after storage in Microsphère® System-containing packaging material and typical preparation (*e.g.*, cooking) for consumption. BTI's GRAS determination through scientific procedures is based on the lack of

[4] Yamamoto, A., A. Matsunaga, and M. Makino. 1985. Analysis of food additives by photometric ion chromatography. II. Chlorite in candied foods. *Journal of Hygienic Chemistry* 31(6):421-425.
[5] U.S. Food and Drug Administration (FDA). 1999. Secondary direct food additives permitted in food for human consumption: Final rule. *Federal Register*, September 15, 1999, Vol. 64, No. 178, pp 49981-49982.

any residue of chlorine dioxide and chlorite in or on these foods. These uses fall under the guidelines stated in GRN 62 in that the foods will be cooked prior to consumption.

Further details are provided in the previously submitted dossier (GRN 62) that includes a comprehensive discussion of, and citations to, generally available and accepted scientific data, information, methods, or principles that BTI has relied on to establish safety, including a consideration of the probable consumption of the substance and the probable consumption of any substance formed in or on food because of its use and the cumulative effect of the substance in the diet, taking into account any chemically or pharmacologically related substances in such diet. In addition, the dossier presents a consensus among experts qualified by scientific training and experience to evaluate the safety of substances added to food that there is reasonable certainty that the substance is not harmful under the intended conditions of use.

E. GRAS Exemption Claim:

Employing scientific procedures established under 21 CFR §170.30(b), BTI determined that chlorine dioxide generated by the Microsphère® System in low-density polyethylene film food-contact packaging material will not be present in or on the food after cooking, therefore the use is GRAS. Concurrently, cooking degrades the chlorite derived from chlorine dioxide and thus no residual chlorine dioxide is present in or on the food and the use is GRAS. Thus, as specifically provided in the food additive regulations under 21 CFR §170.35, these GRAS substances may be used in addition to the specific substances approved under the regulation.

We previously submitted a GRAS Notification that provides detailed information about the identities of the notified substances, including, their chemical names, Chemical Abstracts Service Registry Numbers, empirical formulae, structural formulae, quantitative compositions, method of manufacture, characteristic properties, any constituents of potential health concern, and specifications for food-grade materials. In addition, the GRAS Determination Dossier provides information on the levels of use and a detailed summary of the basis for BTI's determination that its particular use of the notified substances is exempt from the premarket approval requirements of the Act because such use is GRAS.

The new uses for Microsphère®-containing LDPE films for packaging of fresh pasta, prepared dough, and comminuted meats that are to be cooked prior to consumption are equivalent to the specifications for the uses defined in GRN 62 and, therefore, are also GRAS.

75 East Wacker Drive, Suite 500 · Chicago, Illinois · 60601
Phone 312.337.7007 · Facsimile 312.337.0505

F. Availability of Information:

The data and information that are the basis for BTI's GRAS determination are available for FDA to review and copy at reasonable times at the offices of Peter N. Gray, Ph.D., Bernàrd Technologies, Inc., 75 East Wacker Drive, Suite 500, Chicago, IL 60601; telephone: (312) 337-7007; facsimile: (312) 337-0505; or copies of the data and information will be sent to FDA upon request.

Very truly yours,

Peter N. Gray, Ph.D.
Chief Operating Officer
Bernàrd Technologies, Inc.

000969

SU

Carlson, Susan

From:
Sent: Friday, May 30, 2003 10:57 AM
To: Carlson, Susan; Kahl, Linda S
Cc: THOMPSON, Gavin [Environ]
Subject: Additional Uses for GRN-62

Dear Susan and Linda:

As we discussed in our telephonic conference on May 29, 2003, I am sending to you, attached to this email, a letter requesting additional uses for GRN-62.

Please call me with any questions you have.

Thank you very much for your attention.

Sincerely,

Peter

Peter N. Gray
BERNARD TECHNOLOGIES, INC.
Suite 500
75 East Wacker Drive
Chicago, Illinois 60601

(T) 312.337.7007 (F) 312.337.0505 (em) btipng@attglobal.net

000982

BERNÀRD TECHNOLOGIES, INC.

May 30, 2003

Susan J. Carlson, Ph.D.
FDA/CFSAN/OFAS/DBGNR
HFS-255
Harvey W. Wiley Federal Building
5100 Paint Branch Parkway
College Park, Maryland 20740-3835

Re: Notification of the GRAS Determination of Chlorine Dioxide and Chlorite from Food-contact Packaging Made Using Low-density Polyethylene Film Containing the Microsphère® System: Additional Food Types

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Dear Dr. Carlson:

We wish to notify you that Bernàrd Technologies, Inc. ("BTI") has determined that chlorine dioxide and chlorite are generally recognized as safe ("GRAS") for use in food-contact packaging made using low-density polyethylene film containing BTI's Microsphère® System. Accordingly, chlorine dioxide and chlorite from the Microsphère® System are exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act ("the Act"). In accordance with our discussions with the Food and Drug Administration ("FDA"),[1] we are voluntarily submitting the attached summary of information upon which BTI relied in making its GRAS determination for specific additional food types.

A. Name and Address of Notifier:

Peter N. Gray, Ph.D.
Bernàrd Technologies, Inc.
75 East Wacker Drive, Suite 500
Chicago, IL 60601
Telephone: (312) 337-7007
Facsimile: (312) 337-0505

[1] Consultation meeting with FDA on April 17, 2003.

000983

B. Names and Identities of GRAS Substances:

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1. Fresh pasta products that will be cooked prior to being consumed, and
2. Prepared dough products that will be baked, or otherwise cooked, prior to being consumed.

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2. Prepared dough products include but are not limited to bagels, biscuits, bread, cookies, donuts, pastries, piecrust, pizza, rolls, and specialty dough products.

D. Basis for GRAS Determination:

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[4] Yamamoto, A., A. Matsunaga, and M. Makino. 1985. Analysis of food additives by photometric ion chromatography. II. Chlorite in candied foods. *Journal of Hygienic Chemistry* 31(6):421-425.
[5] U.S. Food and Drug Administration (FDA). 1999. Secondary direct food additives permitted in food for human consumption: Final rule. *Federal Register*, September 15, 1999, Vol. 64, No. 178, pp 49981-49982.

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Technologies, Inc., 75 East Wacker Drive, Suite 500, Chicago, IL 60601; telephone: (312) 337-7007; facsimile: (312) 337-0505; or copies of the data and information will be sent to FDA upon request.

Very truly yours,

Peter N. Gray, Ph.D.
Chief Operating Officer
Bernàrd Technologies, Inc.

000987

Reference List for Industry Submission, GRN 000062

Pages	*Author*	*Title*	*Publish Date*	*Publisher*	*BIB_Info*
000207 - 000218	Goldblatt, Michael J.	Innovative Technology To Reduce Microbial Food Contamination	2001	Microbial Food Contamination	Chapter 2, pgs 5-15
000904 - 000913	Buser, W.; Hanisch, H.	Spektrophotometrische Untersuchung saurer Chloritlosungen (Original Article in German)	1952	Helvetica Chimica Acta	Volume 35, Issue 7, pgs 2547-2556
000914 - 000915	Taylor, M.C.; White, J.F.; Vincent, G.P.; Cunningham, G.L.	NA	1940	Industrial and Engineering Chemistry	Volume 32, Number 7, pgs 899-900
000916 - 000925	White, James F.; Taylor, Maurice, C.; Vincent, George P.	Chemistry of Chlorites	July 1942	Industrial and Engineering Chemistry	Volume 34, Number 7, pgs 782-792

NA- Not applicable