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OFFICE OF
FOOD ADDITIVE SAFETY

November 15, 2004

Division of Biotechnology and GRAS Review
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

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re: Notification of GRAS Determination of Chlorine Dioxide From Food-Contact Packaging Made With Engelhard's Controlled Sustained Release (CSR) Systems

Dear Sir or Madam:

On behalf of Engelhard Corporation, I am notifying you that Engelhard has determined that chlorine dioxide is generally recognized as safe ("GRAS") for use in food-contact packaging made with Engelhard's Controlled Sustained Release (CSR) Systems. Accordingly, chlorine dioxide generated in this manner is exempt from the premarket approval requirements of the Federal Food, Drug and Cosmetic Act ("FFDCA").

In support of this GRAS notification, the following information is being provided:

Name and Address of Notifier:
Eliot I. Harrison, Agent for Engelhard Corporation
Lewis & Harrison
122 C St., NW
Washington, DC 20012
202-393-3903 (tel) x 14
202-393-3906 (fax)
Eharrison@lewisharrison.com

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Name and Identity of GRAS Substance

The substance that is the subject of this GRAS determination is chlorine dioxide generated from either of two CSR Systems in food-contact packaging material. Engelhard's two CSR Systems are both powder mixtures composed of sodium chlorite and an activator phase which is stable in combination with sodium chlorite when dry and which release chlorine dioxide in a predictable, controlled and sustained manner when exposed to ambient humidity of at least 25% relative.

In either type of CSR System, the powder is encapsulated in a solid (organic polymer) matrix layer (i.e., a label or a patch) that is applied to packaging materials for fresh fresh and vegetables. As noted above, the CSR Systems generate low levels of chlorine dioxide when exposed to ambient humidity. The generated chlorine dioxide extends the shelf life of the produce contained within the package.

It should be noted that this GRAS Notice is very similar to the GRAS Notice (GRN No. 62) submitted by Bernard Technologies for their Microsphere® System. Both notices involve the generation of chlorine dioxide from sodium chlorite. The only difference between the CSR Systems and the Microsphere System is the activator materials that are used to generate chlorine dioxide from sodium chlorite.

Applicable Conditions of Use

The CSR Systems can be used in a variety of packaging materials for fresh fruits and vegetables. In a typical application, the back side of the patch containing one of the CSR Systems is coated with a contact adhesive and than adhered to the underside of the lid of the food packaging material. The polymer matrix, laminating material and adhesive will all be approved for use in food-contact packaging.

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Basis for GRAS Determination

Engelhard has based its GRAS determination for chlorine dioxide generated by the CSR Systems on scientific procedures. These procedures are presented in the attached GRAS Determination Document (3 copies). The procedures demonstrate that no detectable residues of the chlorite ion (the residue that results subsequent to chlorine dioxide contacting organic material such as food) remain on treated food. In addition, since fruits and vegetables will be likely be washed prior to use even if any chlorite ion remained on the treated food it would be removed prior to consumption.

GRAS Exemption Claim

Engelhard has determined that chlorine dioxide generated by the CSR Systems used in food-contact packaging is GRAS. Therefore, as specifically provided in the food additive regulations under 21 CFR §170.35, chlorine dioxide generated from the CSR Systems can be used as indicated.

The attached GRAS Determination Document provides detailed information about chlorine dioxide and sodium chlorite and the activators that are used in the CSR Systems. In addition, the GRAS Determination Document includes a residue study that shows no detectable residues of the chlorite ion on treated food.

Availability of Information

The data and information that are the basis for Engelhard's GRAS determination are available for FDA to review and copy at reasonable times at the offices of Eliot Harrison, Lewis & Harrison, 122 C St., NW, Suite 740, Washington, DC 20001; telephone: 202-393-3903, ext. 14.

If there are any questions about this submission, please contact me at the address of telephone number given above.

Sincerely,

(b)(6)

Eliot I. Harrison
Agent for Engelhard Corporation

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OFFICE OF
FOOD ADDITIVE SAFETY

GENERALLY RECOGNIZED AS SAFE DETERMINATION FOR CHLORINE DIOXIDE GENERATED BY THE CSR SYSTEM WHEN USED IN FOOD-CONTACT PACKAGING MATERIAL

Submitted by:
Engelhard Corporation
Iselin, NJ

November 15, 2004

000004.001

[redacted]

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I. GRAS EXEMPTION CLAIM

A. Name and Address of Notifier

Eliot Harrison
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122 C St., NW
Washington, DC 20012
Telephone: (202) 393-3903, ext. 14
Facsimile: (202) 393-3906
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B. Name of GRAS Substance

The substance that is the subject of this generally recognized as safe ("GRAS") determination is chlorine dioxide generated from either of two CSR Systems in food-contact packaging material. Engelhard's two CSR Systems are both powder mixtures composed of sodium chlorite and an activator phase which is stable in combination with sodium chlorite when dry, and which releases chlorine dioxide in a predictable, controlled, and sustained release (CSR) manner when exposed to ambient humidity of at least 25% relative.

In either system, the powder is encapsulated in a solid organic polymer matrix layer (i.e, a label or a patch) that is applied to packaging materials for fresh fruits and vegetables. When exposed to ambient humidity, each of the CSR Systems generates low levels of chlorine dioxide on a sustained basis. The generated chlorine dioxide extends the shelf life of the produce contained within the package.

C. **Intended Technical Effect**

The intended technical effect of the generated chlorine dioxide is to lengthen the shelf life and extend the freshness of fresh fruits and vegetables. The mechanism of action and the intended technical effects on food are described in Section III. Laboratory tests have shown that the storage life of blackberries and strawberries have increased when the CSR System is included with the packaging material.

D. **Proposed Use and Consumer Exposure**

The CSR System can be used in a variety of packaging materials for fresh fruits and vegetables. In a typical application, the back side of the patch containing the CSR System is coated with a contact adhesive and then adhered to the underside of the food-packaging material.

The polymer matrix and adhesive will all be approved for use in food-packaging. It is anticipated that no residual chlorine dioxide or chlorite ion will remain on the treated food since chlorine dioxide is rapidly converted into chlorite upon contact with organic matter and chlorite is quickly degraded into chloride. Any remaining chlorite will then be removed during washing of treated fruits or vegetables.

E. Basis for GRAS Determination

The basis for the GRAS determination is the lack of dietary exposure to either chlorine dioxide or the chlorite ion from the use of the CSR System in food-contact packaging of fruits and vegetables. It should also be noted that the toxicity of chlorine dioxide and the chlorite ion have been extensively evaluated by several expert bodies including the U.S. Environmental Protection Agency (USEPA)[1]. The USEPA derived a Reference Dose (RfD) of 0.03 mg/kg-day for chlorine dioxide and chlorite using the no-observed adverse-effect level ("NOAEL") of 3.0 mg/kg-day (from a 2-generation reproduction study) and applying a 100 fold uncertainty factor. This RfD can be considered an acceptable daily intake for chlorine dioxide and chlorite. Since the RfD is many times greater than the estimated daily intake (EDI) for both chlorine dioxide and the chlorite ion, the proposed use of chlorine dioxide generated from the CSR System in food-packaging can be considered GRAS.

[1] *Toxicological Review of Chlorine Dioxide and Chlorite*, In support of Summary Information on the Integrated Risk Information System (IRIS), U.S. Environmental Protection Agency, September, 2000.

II. DESCRIPTION OF SUBSTANCE

A. **Identity**

1. Common or Usual Names

The substance that is the subject of this GRAS Notice is chlorine dioxide, which can be generated, *in situ*, by either of Engelhard's two CSR Systems. The CSR Systems are both powder mixtures composed of sodium chlorite and an activator phase which is stable in combination with sodium chlorite when dry.

In one system (Type I), the activator phase consists of kaolin clay that has been heat treated to remove its waters of crystallization (a process known as calcination). Type 1 CSR materials release chlorine dioxide at a relatively constant rate for their design life (which can range from <5 to 60 days depending upon the amount of sodium chlorite in the system). In the second system (Type II), the activator phase consists of a mixture of calcium chloride and sulfated calcined kaolin clay.

Type II CSR materials initially release chlorine dioxide at a high rate for about 12-48 hours, which then decreases to about 20% of the peak rate for another 12 hours to 14 days, depending on the amount of sodium chlorite in the system.

2. Chemical Names

Chlorine dioxide, sodium chlorite, calcium chloride, kaolin clay, and sulfated kaolin clay, are commonly recognized as the names for these chemicals.

3. CAS Registry Numbers, Empirical Formulas and Other Properties

The Chemical Abstract Service (CAS) Registry Number, empirical formula, molecular weight and other physical properties of chlorine dioxide are presented in Table 1. The CAS Registry Numbers, empirical formulas, molecular weights and other physical properties of the components of the CSR Systems are presented in Table 2.

Table 1. Identity and Properties of Chlorine Dioxide

Property/Parameter	Value
Chlorine Dioxide	
CAS Registry Number	10049-04-4
Empirical Formula	ClO_2
Molecular Weight	67.46
Physical State	Gas (at 25°C)
Form	Green (in gaseous state) Yellow (in aqueous state)
Odor	Chlorine like
Boiling Point	9.9 - 11°C at 1 atm
Melting Point	-59.5°C
Density	1.765 g/cm^3 @ -56°C 1.62 g/cm^3 @ -11°C
Vapor Pressure	10 mmHg @ -59°C
Henry's Law Constant	10^{-5} atm m^3/mol
Solubility in Water	3.01 g/L @25°C
Solubility in Organic Solvents	Soluble in alcohol

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Table 2. Identity and Properties of Components of CSR System	
Property/Parameter	Value
Sodium Chlorite	
CAS Registry No.	7758-19-2
Empirical Formula	$NaClO_2$
Molecular Weight	90.44
Physical State @20°C	Solid
Form	White crystalline powder or flake
Odor	None
Melting Point	180-200°C (decomposes)
Vapor Pressure	Negligible
Solubility in Water	34 g/100 g solution at 5°C 55 g/100 g solution at 60°C
Calcium Chloride	
CAS Registry Number	68915-31-1
Empirical Formula	$CaCl_2$
Molecular Weight	110.98
Physical State	White crystalline powder or granule.
Density	2.15
Solubility in Water	74.5 g/100 cm^3 solution at 20 deg C

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Table 2: Identity and Properties of Components of CSR System (cont.)	
Property/Parameter	Value
Calcined Kaolin Clay	
CAS Registry Number	1332-58-7
Chemical Composition	SiO_2: 50%-54% Al_2O_3 42-46% Fe_2O_3 + TiO_2: Balance
Form	Off-white to tan fine powder.
Particle Size	2-5 microns
Bulk Density	0.3 gm/cm^3 (loose) 0.5 g/cm3 (tamped)
Sulfated Kaolin Clay	
CAS Registry Number	Not Applicable
Chemical Composition	SiO_2: 45-47% Al_2O_3: 38-40% SO_4 : 11-15% Fe_2O_3 + TiO_2: Balance
Form	Off-white to tan fine powder
Particle Size	2-5 microns
Bulk Density	0.3 g/cm^3 (loose) 0.5 g/cm^3 (tamped)

B. **Manufacturing Process**

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1. Overview of Process

The manufacturing process for the CSR Systems is a batch process. All the components used in manufacturing the CSR Systems are dried and blended together. The blended materials are then encapsulated into a polymer matrix or patch. No chemical reactions take place in the manufacture of the CSR Systems.

2. Components

The CSR Systems constituents are physically mixed at the ratios indicated below:

Type 1 CSR Material	
Component	Amount
Technical Sodium Chlorite	1.25-6.25 parts (depending upon the desired duration of chlorine dioxide release)
Calcined Kaolin Clay	Balance
Type II CSR Material	
Technical Sodium Chlorite	1.25-6.25 parts (depending upon the desired duration of chlorine dioxide release)
Calcium Chloride	10 Parts
Sulfated Kaolin Clay	Balance

3. Specific Manufacturing Steps

For small-scale production (<200 lb. batches), each of the components are separately dried as indicated below:

- Technical sodium chlorite is dried and, if necessary, milled in a ceramic mill with a ceramic media to reduce its average particle size.
- The sulfated kaolin clay, calcined kaolin clay and calcium chloride are dried at a relatively high temperature.

The appropriate weight of each constituent is added to a dry mixer and the constituents are blended together. Blending conditions are selected so that the components remain in a substantially dry condition.

For large-scale production (>500 lb batch size), the constituents are simultaneously dried and blended in a fluidized bed dryer as follows:

- Type I CSR Material - In a vertical, cylindrical vessel being fluidized by dry air, calcined kaolin clay is dried at a relatively high temperature. The dryer is then cooled and the appropriate amount of Technical Sodium Chlorite is added to the dryer. The dry blended product is cooled and unloaded from the dryer.
- Type II CSR Material - In a vertical, cylindrical vessel being fluidized by dry air, sulfated calcined kaolin clay is dried at a relatively high temperature. The appropriate amount of calcium chloride is added to the hot fluid bed and drying continues. The dryer is then cooled and the appropriate amount of Technical Sodium Chlorite is added to the dryer. The dry blended product is then cooled and unloaded from the dryer.

In all cases, the dry, blended product is transferred to sealed moisture barrier containers, such as polyethylene 5 gallon pails or 55 gallon drums for storage. Drums are either heavy walled high-density polyethylene or steel with a double liner of at least 3 mil polyethylene bags.

4. By-Products and Impurities

There are no chemical reactions in the manufacture of the CSR Systems. Accordingly, the production process does not lead to any by-products or impurities.

III. INTENDED TECHNICAL EFFECT

A. Sustained-Release of Chlorine Dioxide by the CSR System

1. Mechanism of Action

The CSR System is a patented formulation that is designed to produce low levels of chlorine dioxide on a continuous basis. The CSR System needs to be activated in order to generate chlorine dioxide. Activation is accomplished by having the CSR powder contact ambient moisture. Chlorine dioxide will continue to be generated until all available chlorite ion is consumed.

B. Mechanism of Action of Chlorine Dioxide

Chlorine dioxide is a highly effective antimicrobial agent against both bacteria and viruses. It works at low concentrations (0.1 ppm) and over a wide pH range. It mode of action appears to be interference with protein synthesis. Comparative studies have shown that chlorine dioxide is slightly less effective (at an equivalent dose) against bacteria but more effective against viruses than hypochlorous acid.

C. Laboratory Data

The efficacy of the CSR System, when incorporated into food-contact packaging, in extending the shelf-life of various fruits is shown in Appendix 1.

IV. USES, DIETARY EXPOSURE AND SAFETY ASSESSMENT

A. Historical and Current Uses

All of the components of the CSR Systems have current food-grade uses and regulatory approvals, as detailed below.

1. Chlorine Dioxide

Chlorine dioxide has been cleared by FDA for direct contact with food as follows:

- Under 21 CFR §173.000, chlorine dioxide has been approved for as an antimicrobial agent for use in poultry processing in an amount not to exceed 3 ppm residual chlorine dioxide and as an antimicrobial agent in water used to wash processed fruits and vegetables.
- Chlorine dioxide is approved for optional use as a bleaching agent for fluor under food standards (21 CFR §137), in quantities sufficient for bleaching and artificial aging effects.

2. Sodium Chlorite

Sodium chlorite has been approved by FDA for both direct and indirect contact with food as follows:

- Under 21 CFR §173.325, acidified sodium chlorite solutions have been approved as an antimicrobial agent for use in poultry processing, red meat and red meat part processing, seafood processing, the treatment of raw agricultural commodities and comminuted or formed meat products.
- Under 21 CFR §186.1750, sodium chlorite has been affirmed as GRAS for use as a slimicide in the manufacture of paper and paperboard.
- Under 21 CFR §172.892, sodium chlorite is approved as a bleaching agent for modified starch.

Calcium Chloride

Under 21 CFR §184.1193, calcium chloride has been affirmed by FDA as a multi-purpose GRAS substance.

Kaolin Clay/Sulfated Kaolin clay

Under 21 CFR §186.1256, kaolin clay has been affirmed by FDA as GRAS when used in manufacture of paper and paperboard that contacts food. Since sulfated kaolin clay is merely kaolin clay that has been calcined and surface treated with sulfate the clearance for kaolin clay is applicable to sulfated kaolin clay.

B. Dietary Exposure

Dietary exposure to either chlorine dioxide or the chlorite ion from the use of the CSR Systems in food-packaging will be negligible for the following reasons:

- It is well established in the published literature that chlorine dioxide is rapidly converted to the chlorite ion upon contact with organic material. Therefore, it is highly unlikely for any chlorine dioxide residue to occur in the packaged food.
- Residue data included with this submission (Appendix 2) clearly demonstrates that the chlorite ion residue will also quickly dissipate. Consequently, the remaining residue on fruits and vegetables from the use of the CSR System will be chloride ions.
- Even if unlikely occurrence that any chlorite residue did remain on the treated fruits and vegetables it should be removed prior to dietary consumption by washing.

It should also be noted that dietary exposure to the other constituents of the CSR Systems will not occur since they are enclosed in the packaging materials and, therefore, will not contact the packaged food.

C. Safety/GRAS Determination

Based on the information and data presented in this document and the previous determination made in GRAS Notice Number 62, Engelhard regards the use chlorine dioxide from food-contact packaging that incorporates the CSR Systems as GRAS. We believe that other qualified and competent scientists reviewing the same information would reach the same conclusion.

APPENDIX 1

INTENDED TECHNICAL EFFECT DATA

Blackberry clamshells exposed to ClO_2 gas
Room Temperature Batch

Avery Dennison
20 June 2003

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Blackberry/ClO_2 Trial

- Blackberries were picked, packed in clear plastic clamshell packaging, chilled and stored in Alma, GA on Thursday, June 12th.

- Type II CSR Patches and Type II CSR Tyvek sachets were applied on Thursday, June 12th. Patches and sachets contained either 0.5 or 1.0 gm of CSR material

- Ventilation openings were sealed with tape in ½ of the plastic clamshells, and left open in the remainder.

- The blackberries were packed in a cooler with frozen gel packs and taken to Clemson Univ. where the clamshells were separated into refrigerated and room temperature treatments.

- Room temperature blackberries were held at temperatures between 70 - 80°F.

- Refrigerated blackberries were stored at ~4°F until day 4 (Tuesday, June 17th) when they were placed at room temperature.

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Blackberry/ClO_2 Trial - Room Temp

Room Temperature Summary

Controls: Spoiled in 2-3 days

Trial 1, Red Star **ClO_2 level**: 0 **Sachet/Patch:** n/a **Vents:** open

Trial 11, Light Blue Tape **ClO_2 level**: 0 **Sachet/Patch:** n/a **Vents:** closed

Trial 1 was at a "probably would not buy" status at day 3 (Monday) because of the appearance and minimal mold growth started at day 4 with moderate mold growth by day 6

Trial 11 had mold started by day 2 (Sunday) and was at a "would not buy" status by day 3 (Monday) because of the appearance, odor and mold growth

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Blackberry/ClO_2 Trial - Room Temp

Room Temperature Summary

Two treatments resulted in buy or probably buy at day 4, and did not reach "would not buy" status until day 6:

Trial 5, Gold Star **ClO_2 level**: 1.0gm **Sachet/Patch**: patch **Vents**: closed

Trial 4, Green Star ClO_2 level: 0.5gm **Sachet/Patch**: patch **Vents**: open

Other treatments with questionable buy at day 4 include:

Trial 3, Silver Star ClO_2 level: 0.5gm **Sachet/Patch**: patch **Vents**: closed

Trial 6, Orange Tape **ClO_2 level**: 1.0gm **Sachet/Patch**: sachet **Vents**: closed

Trial 7a, Yellow Tape **ClO_2 level**: 1.0gm **Sachet/Patch**: patch **Vents**: open

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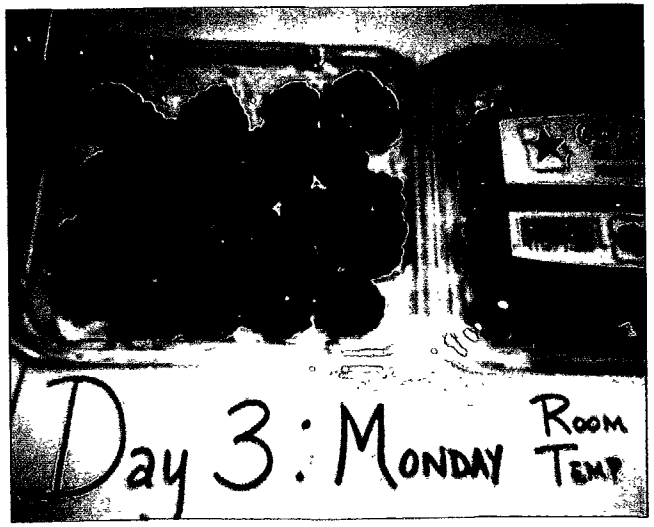
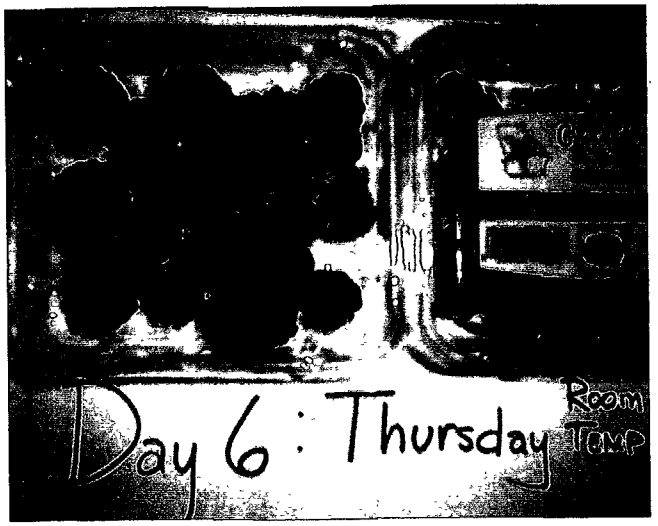
Blackberry/ClO_2 Trial - Room Temp.

Trial 1, Red Star ClO_2 level: 0 Sachet/Patch: n/a Vents: open





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Blackberry/ClO_2 Trial - Room Temp.

Trial 1, Red Star ClO_2 level: 0 **Sachet/Patch:** n/a **Vents:** open

5 clamshells

Day 2 (Sunday): berries look fresh; mild dehydration throughout; ripe odor; no mold *definitely buy*

Day 3 (Monday): mild dehydration; starting to look mushy w/ mild decay; beginning to mold; overripe odor *probably would not buy b/c of appearance*

Day 4 (Tuesday): mild to moderate dehydration; minimal mold growth in 4/5 clamshells; strong odor *probably would not buy b/c of appearance*

Day 6 (Thursday): moderate mold growth *would not buy*

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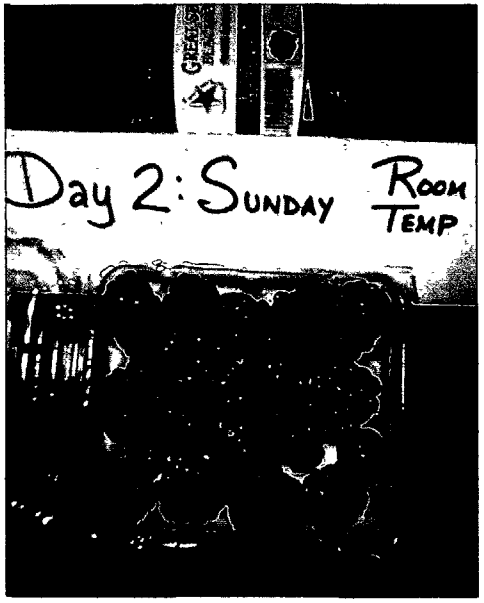
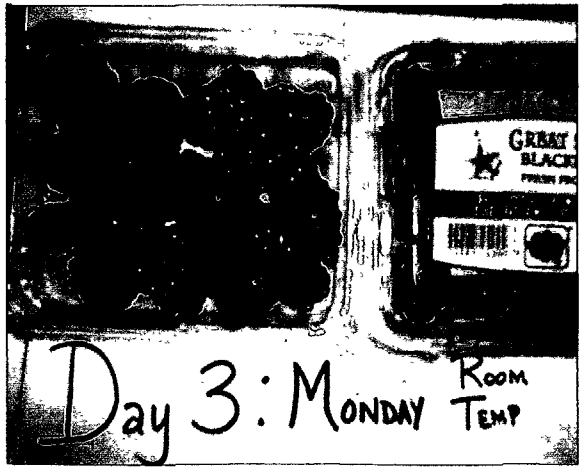
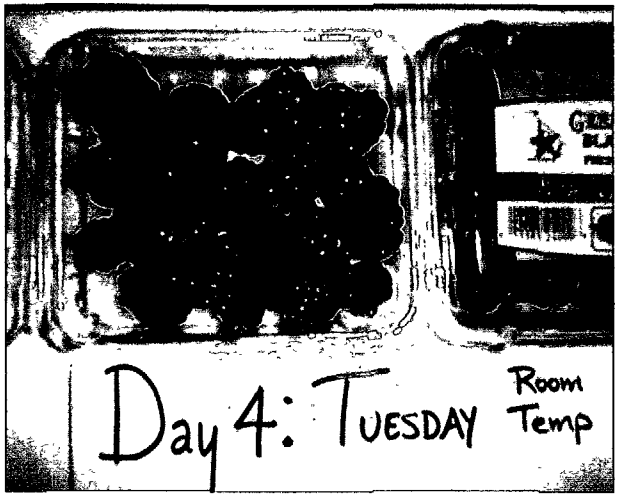
Blackberry/ClO_2 Trial - Room Temp.

Trial 3, Silver Star ClO_2 level: 0.5gm **Sachet/Patch:** patch **Vents:** closed







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Blackberry/ClO_2 Trial - Room Temp.

Trial 3, Silver Star ClO_2 level: 0.5gm **Sachet/Patch:** patch **Vents:** closed

2 clamshells

Day 2 (Sunday): most berries are plump and tender; few show signs of dehydration; one clamshell has one berry with mold; moderate berry odor *definitely buy*

Day 3 (Monday): most berries look plump and tender with very little dehydration; one clamshell has one berry with mold; moderate odor *would probably buy*

Day 4 (Tuesday): most berries look plump and tender with slight dehydration; moderate odor; 1 clamshell has minimal-moderate mold growth, other clamshell has no mold growth; *questionable buy*

Day 6 (Thursday): both clamshells have moderate *would not buy*

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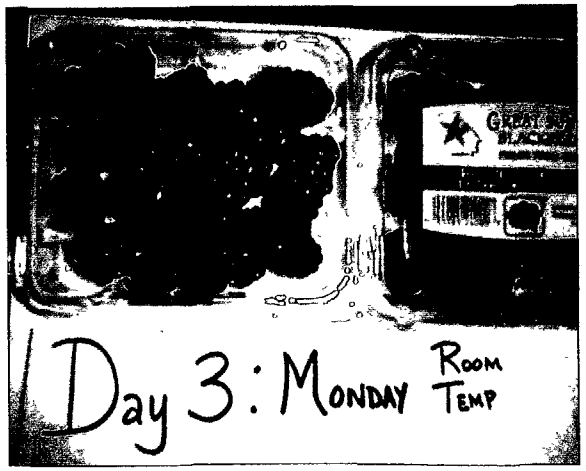
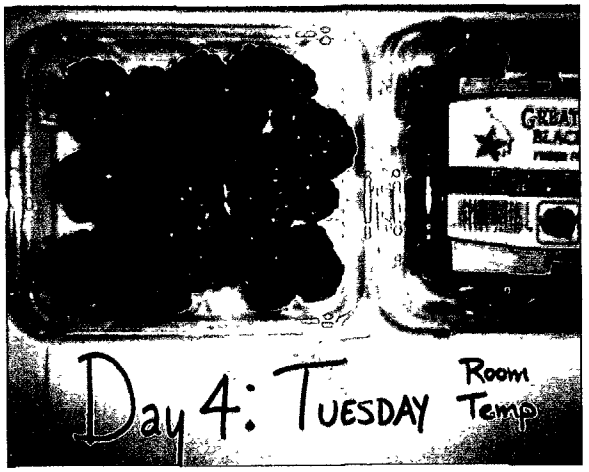
Blackberry/ClO_2 Trial - Room Temp.

Trial 4, Green Star ClO_2 level: 0.5gm **Sachet/Patch:** patch **Vents:** open





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Blackberry/ClO_2 Trial - Room Temp.

Trial 4, Green Star ClO_2 level: 0.5gm **Sachet/Patch:** patch **Vents:** open

2 clamshells

Day 2 (Sunday): berries are plump and tender; no signs of dehydration; look really good; moderate berry odor *definitely buy*

Day 3 (Monday): berries look great; plump and tender; no dehydration or mold; gentle-moderate berry odor *definitely buy*

Day 4 (Tuesday): berries look plump and tender with some dehydration; small (tiny) amount of mold on 2 berries in 1 clamshell, none in the other clamshell; moderate to strong berry odor *most likely buy*

Day 6 (Thursday): 1 clamshell has minimal to moderate mold (Day 6 picture), the other clamshell has minimal mold *would not buy*

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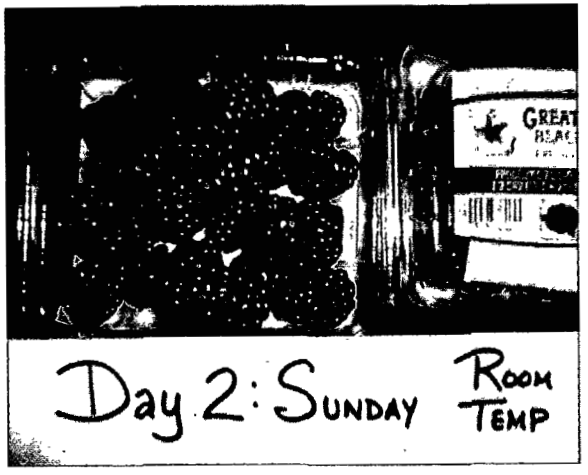
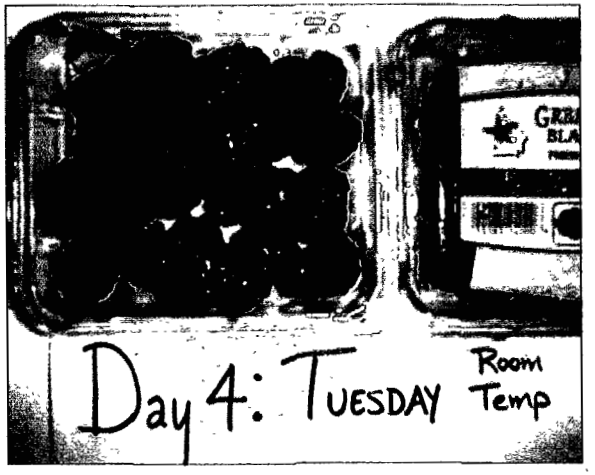
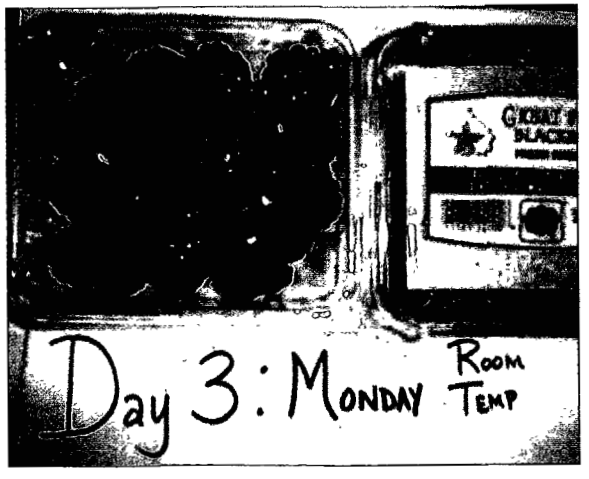
Blackberry/ClO_2 Trial - Room Temp.

Trial 5, Gold Star **ClO_2 level:** 1.0gm **Sachet/Patch:** patch **Vents:** closed







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Blackberry/ClO_2 Trial - Room Temp.

Trial 5, Gold Star **ClO_2 level**: 1.0gm **Sachet/Patch**: patch **Vents**: closed

3 clamshells

Day 2 (Sunday): most berries are plump but slightly moist; a few show signs of dehydration; no mold; moderate berry odor *definitely buy*

Day 3 (Monday): most berries look plump and tender with very little dehydration but still look good; no mold; moderate berry odor *definitely buy*

Day 4 (Tuesday): berries look generally good with slight dehydration; 2 of 3 clamshells have minimal (tiny) mold on 1 berry; moderate berry odor – smells good; *definitely buy*

Day 6 (Thursday): all 3 clamshells have moderate mold *would not buy*

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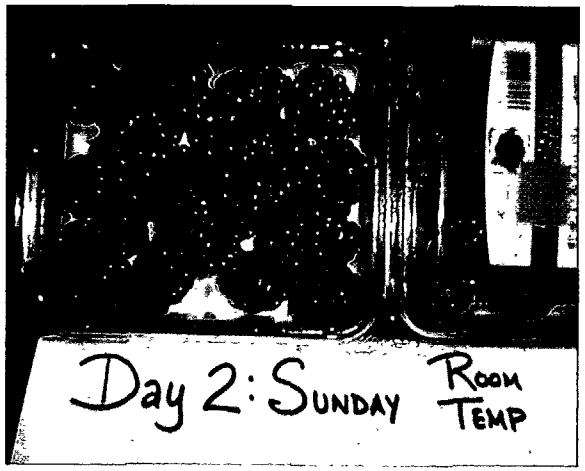
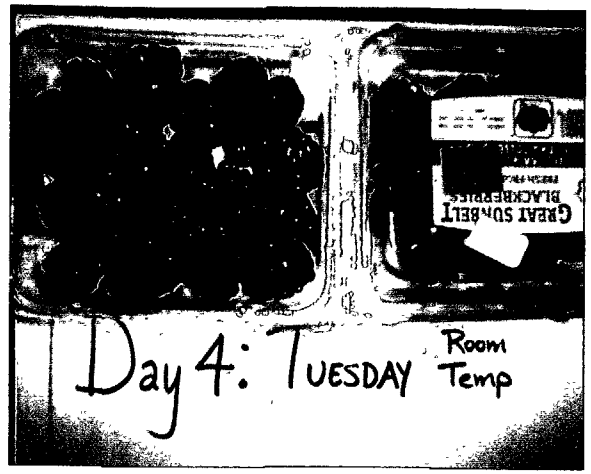
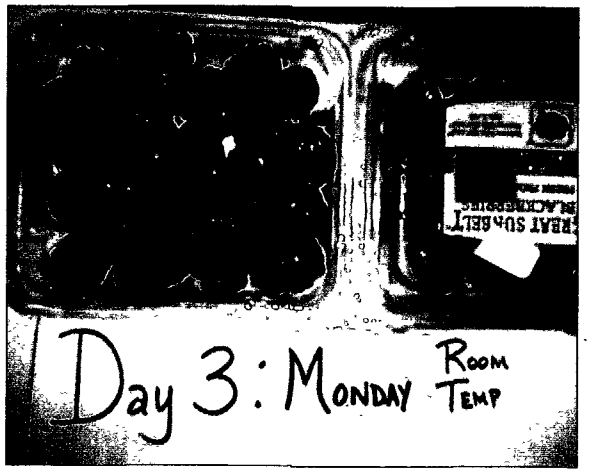
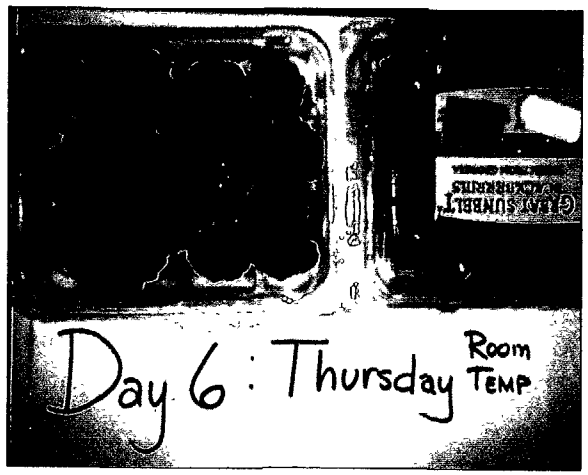
Blackberry/ClO_2 Trial - Room Temp.

Trial 6, Orange Tape **ClO_2 level:** 1.0gm **Sachet/Patch:** sachet **Vents:** closed









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Blackberry/ClO_2 Trial - Room Temp.

Trial 6, Orange Tape **ClO_2 level:** 1.0gm **Sachet/Patch:** sachet **Vents:** closed

5 clamshells

Day 2 (Sunday): most berries are plump and tender; few show signs of dehydration; 2 clamshells have one berry with a little mold; mild berry odor *definitely buy*

Day 3 (Monday): most berries look good - plump and tender; some are dehydrated; 2 of 5 clamshells have minimal mold growth on 1-2 berries; moderate berry odor *questionable buy*

Day 4 (Tuesday): berries beginning to look dehydrated and puckered but some still look good; moderate berry odor; 2 of 5 clamshells have minimal to moderate mold, 1 of 5 has minimal mold and 2 of 5 have no mold *questionable buy*

Day 6 (Thursday): all 5 clamshells have moderate to major mold growth *would not buy*

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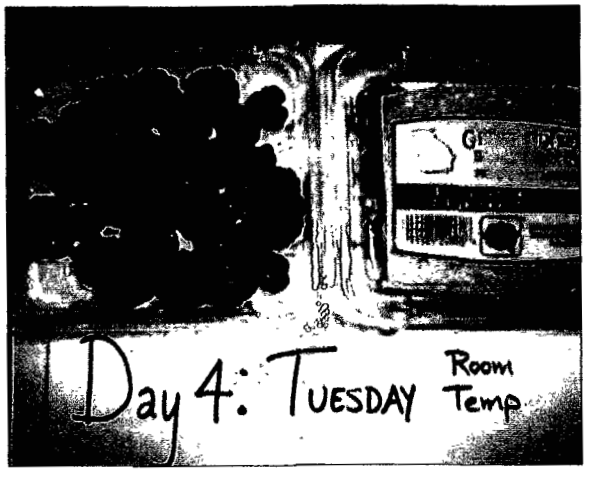
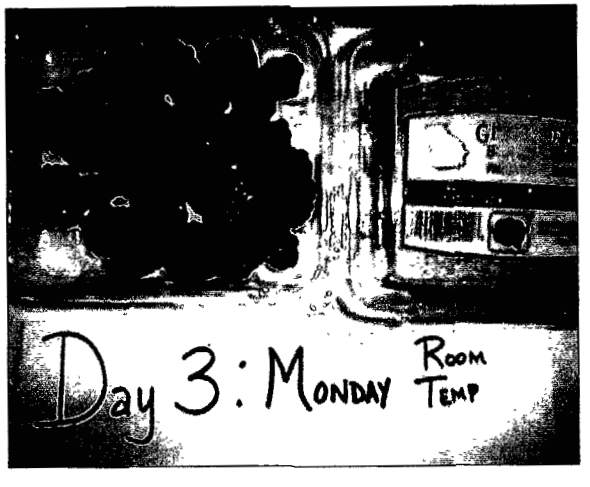
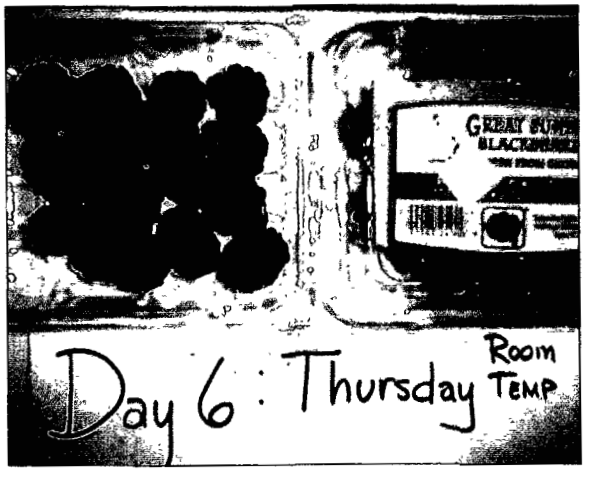
Blackberry/ClO_2 Trial - Room Temp.

Trial 7a, Yellow Tape **ClO_2 level:** 1.0gm **Sachet/Patch:** patch **Vents:** open







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Blackberry/ClO_2 Trial - Room Temp.

Trial 7a, Yellow Tape **ClO_2 level**: 1.0gm **Sachet/Patch**: patch **Vents**: open

2 clamshells

Day 2 (Sunday): most berries are plump and tender; no dehydration; no mold; slight chlorine odor *definitely buy*

Day 3 (Monday): berries look ok; mild dehydration, moderate berry odor; 1 berry in 1 clamshell has mold beginning *most likely buy*

Day 4 (Tuesday): berries about 30% dehydrated; 1 berry with minimal mold in 1 clamshell; moderate berry odor but also Chlorine smell *questionable buy*

Day 6 (Thursday): 1 clamshell has minimal mold; 1 clamshell has moderate mold (picture) *would not buy*

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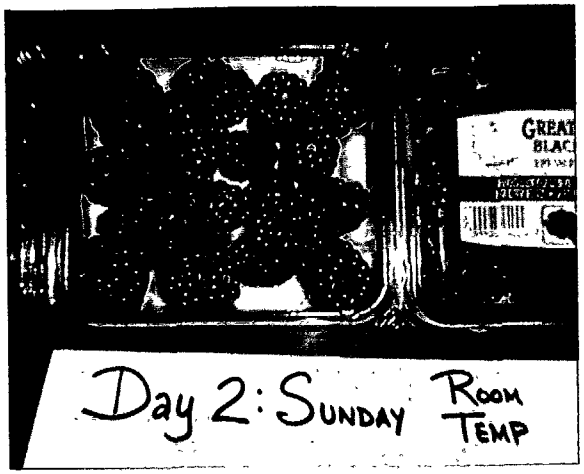
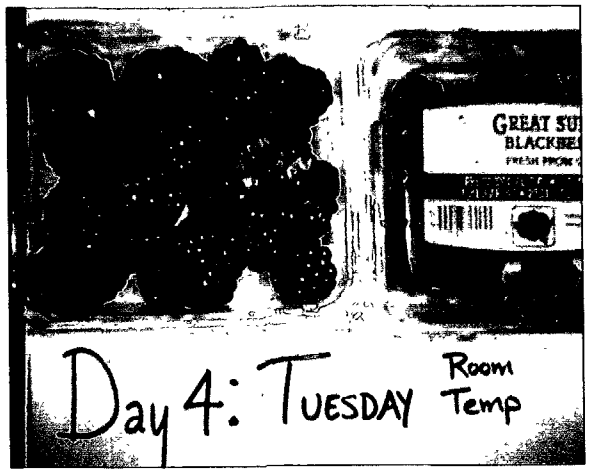
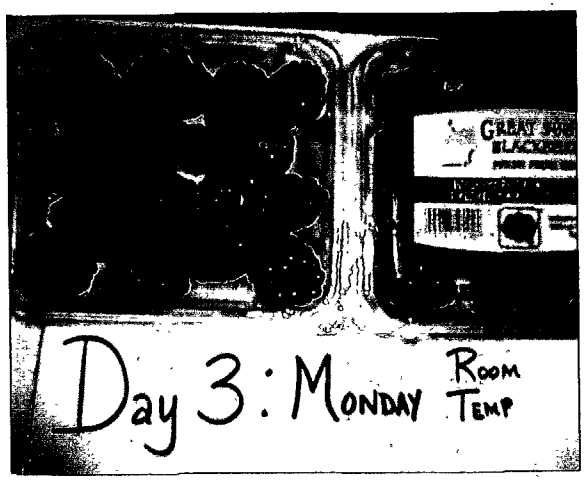
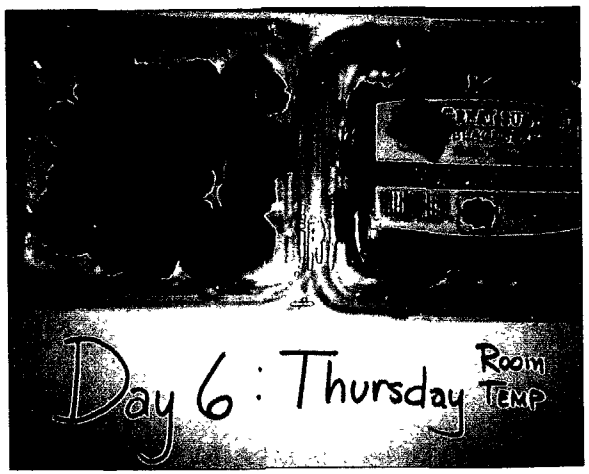
Blackberry/ClO_2 Trial - Room Temp.

Trial 11, Light Blue Tape **ClO_2 level:** 0 **Sachet/Patch:** n/a **Vents:** closed









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Blackberry/ClO_2 Trial - Room Temp.

Trial 11, Light Blue Tape **ClO_2 level**: 0 **Sachet/Patch**: n/a **Vents**: closed

5 clamshells

Day 2 (Sunday): most berries are plump and tender; slight dehydration; moderate berry odor; all 5 clamshells have mold starting *questionable buy*

Day 3 (Monday): most berries are plump and tender with some dehydration; strong overripe odor; all clamshells have minimal mold growth *would not buy b/c mold*

Day 4 (Tuesday): all 5 clamshells have mild to moderate growth; berries still look plump and tender with little dehydration; *no buy*

Day 6 (Thursday): 4 clamshells have super major mold and 1 clamshell has minimal mold *would not buy*

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Blackberry clamshells exposed to ClO_2 gas
Refrigerated Batch

Avery Dennison
20 June 2003

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Blackberry/ClO_2 Trial - Refrigerated

Refrigerated Summary

Controls: Questionable to Would Not Buy by Day 6

Trial 1, Red Star **ClO_2 level**: 0 **Sachet/Patch**: n/a **Vents**: open

Trial 11, Light Blue Tape **ClO_2 level**: 0 **Sachet/Patch**: n/a **Vents**: closed

Trial 1 was at a "questionable buy" status at day 6 (Thursday) because of 3 of the 5 clamshells had minimal growth beginning. By day 7 (Friday) all 5 clamshells had minimal to moderate growth and were "would not buy".

Trial 11 had mild to moderate mold by day 6 (Thursday) and was at a "would not buy" status by this time.

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Blackberry/ClO_2 Trial - Refrigerated

Refrigerated Summary

Treated packages: At day 6 (Thursday), the following treatments were definite buy, and at least questionable buy on day 7:

Trial 5, Gold Star **ClO_2 level**: 1.0 **Sachet/Patch**: patch **Vents**: closed
Trial 4, Green Star ClO_2 level: 0.5 **Sachet/Patch**: patch **Vents**: open
Trial 13, Lt Blue Tape w/ Black lines **ClO_2 level**: 1.0 **Sachet/Patch**: patch **Vents**: open
Trial 16, White Tape ClO_2 level: 1.0 **Sachet/Patch**: patch **Vents**: open

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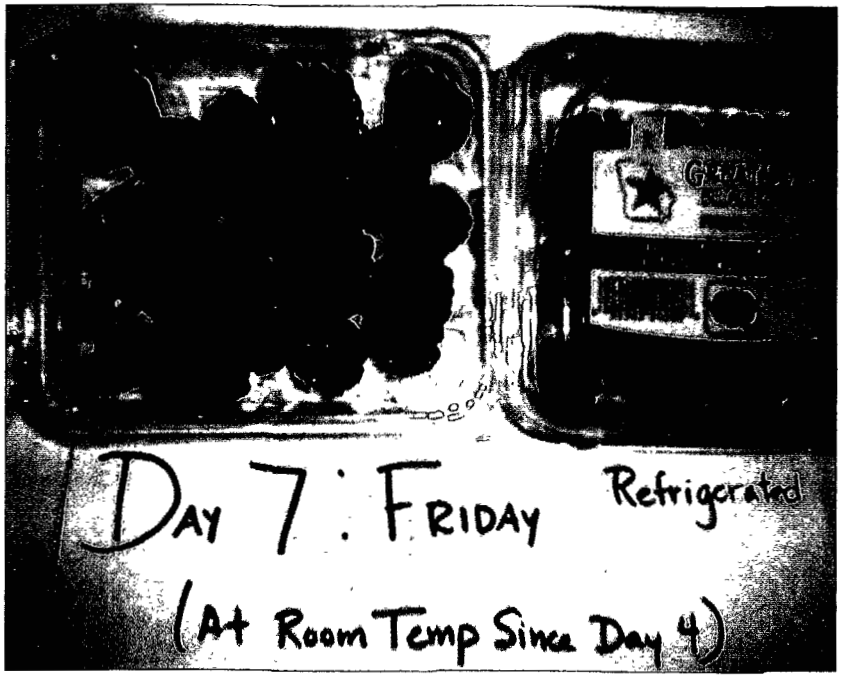
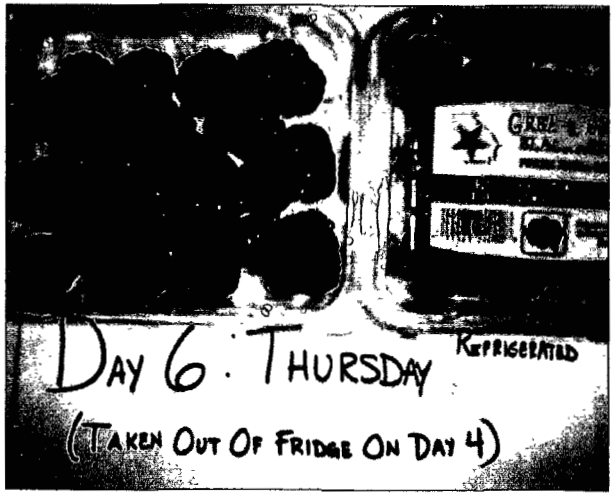
Blackberry/ClO_2 Trial - Refrigerated

Trial 1, Red Star ClO_2 level: 0 **Sachet/Patch:** n/a **Vents:** open





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Blackberry/ClO_2 Trial - Refrigerated

Trial 1, Red Star ClO_2 level: 0 **Sachet/Patch:** n/a **Vents:** open

4 clamshells

Day 2 (Sunday): berries look fresh – plump and tender; moist on surface, no berry smell; no mold *definitely buy*

Day 3 (Monday): same as Sunday *definitely buy*

Day 4 (Tuesday): same as Sunday *definitely buy* ***removed from refrigerator***

Day 6 (Thursday): berries look mild to moderate dehydrated; 3 of 5 have very mild mold; moderate odor *questionable buy*

Day 7 (Friday): all 5 minimal to moderate mold growth *would not buy*

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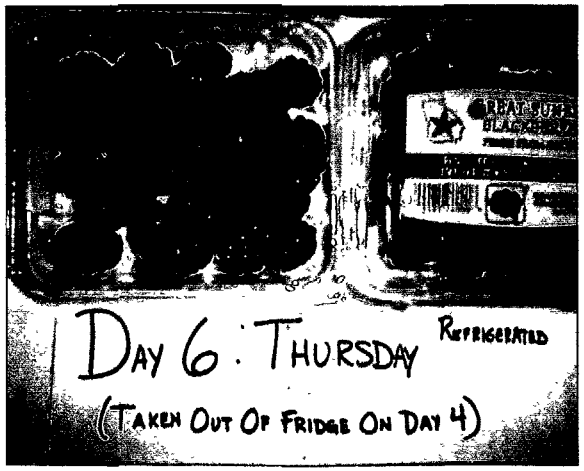
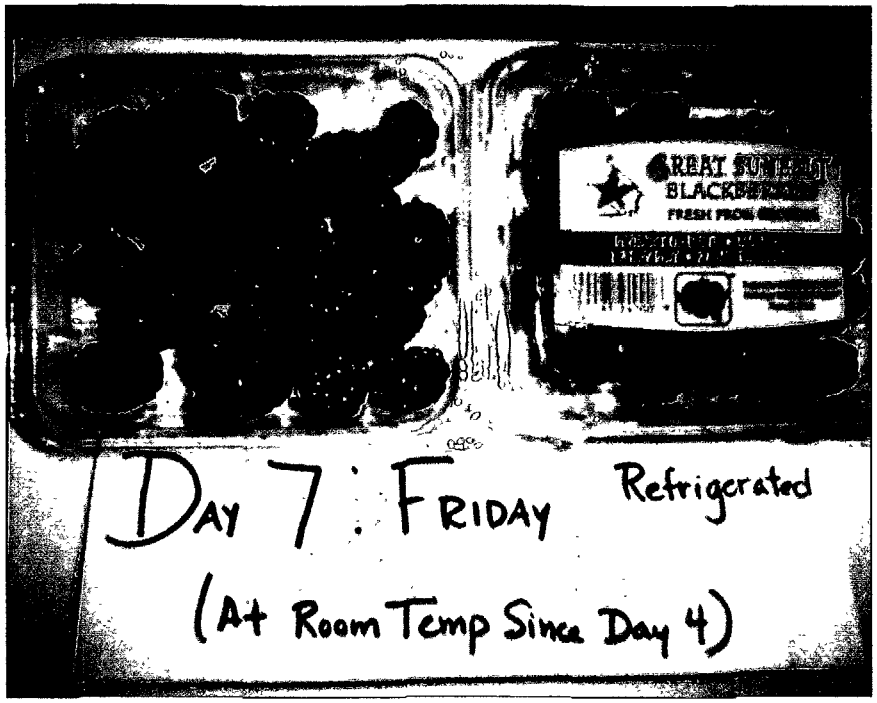
Blackberry/ClO_2 Trial - Refrigerated

Trial 4, Green Star ClO_2 level: 0.5gm **Sachet/Patch:** patch **Vents:** open





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Blackberry/ClO_2 Trial - Refrigerated

Trial 4, Green Star ClO_2 level: 0.5gm **Sachet/Patch:** patch **Vents:** open

3 clamshells

Day 2 (Sunday): berries look fresh – plump and tender; moist on surface, no berry smell; no mold *definitely buy*

Day 3 (Monday): same as Sunday *definitely buy*

Day 4 (Tuesday): same as Sunday *definitely buy* ***removed from refrigerator***

Day 6 (Thursday): berries look plump and tender with very little dehydration; 1 berry in 1 clamshell has minimal mold *definitely buy*

Day 7 (Friday): 2 of 3 clamshells have minimal mold; 1 clamshell has minimal mold (picture); all 3 have slight dehydration *all are questionable buy*

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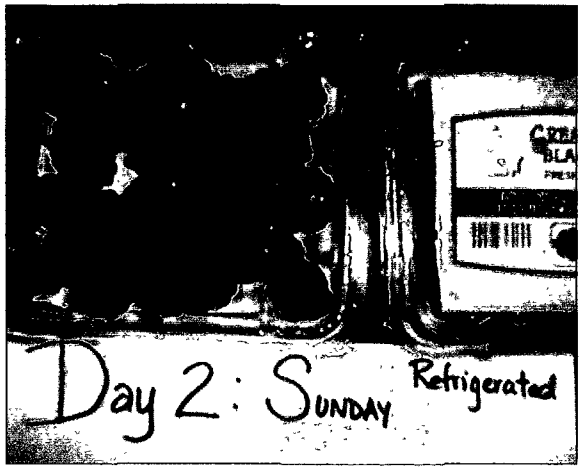
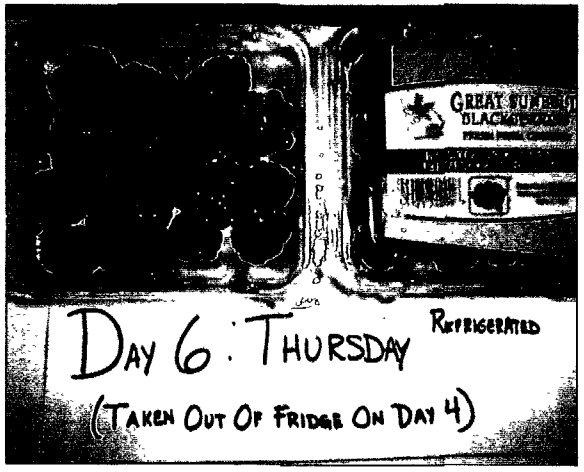
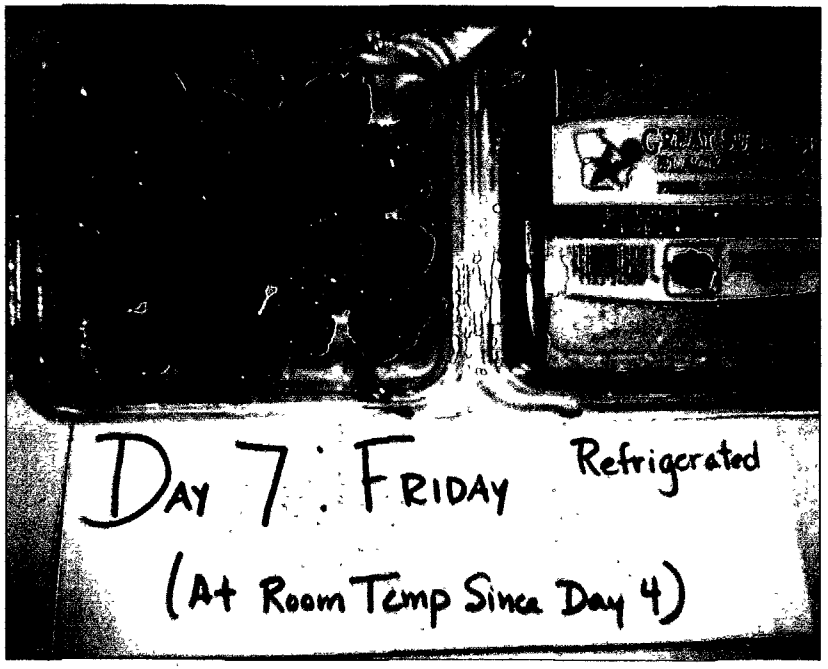
Blackberry/ClO_2 Trial - Refrigerated

Trial 5, Gold Star **ClO_2 level:** 1.0gm **Sachet/Patch:** patch **Vents:** closed







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Blackberry/ClO_2 Trial - Refrigerated

Trial 5, Gold Star **ClO_2 level**: 1.0gm **Sachet/Patch:** patch **Vents:** closed

3 clamshells

Day 2 (Sunday): berries look fresh – plump and tender; moist on surface, no berry smell; no mold *definitely buy*

Day 3 (Monday): same as Sunday *definitely buy*

Day 4 (Tuesday): same as Sunday *definitely buy* ***removed from refrigerator***

Day 6 (Thursday): berries look mostly plump and tender with mild and moderate dehydration; 1 berry in 1 clamshell has mold *definitely buy*

Day 7 (Friday): 2 of 3 look really good – minimal to moderate dehydration; no mold (picture); 1 clamshell has minimal to moderate *1 clamshell is a questionable buy and 1 clamshell would not buy*

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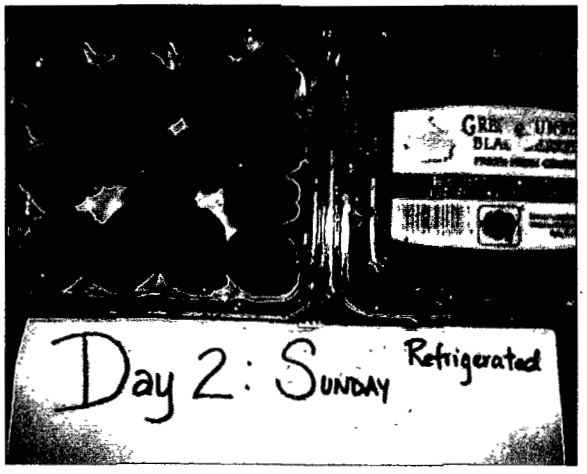
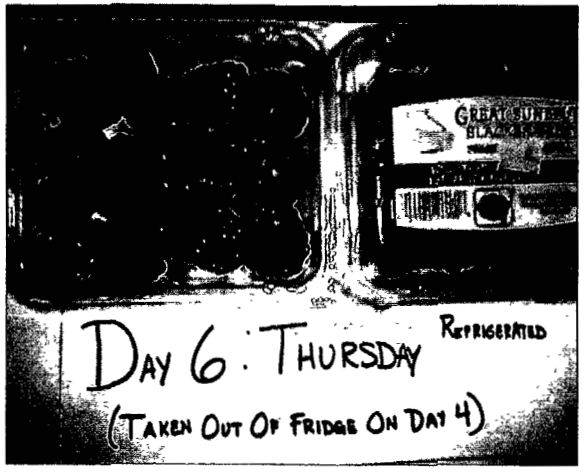
Blackberry/ClO_2 Trial - Refrigerated

Trial 11, Light Blue Tape **ClO_2 level:** 0 **Sachet/Patch:** n/a **Vents:** closed





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Blackberry/ClO_2 Trial - Refrigerated

Trial 11, Light Blue Tape **ClO_2 level:** 0 **Sachet/Patch:** n/a **Vents:** closed

5 clamshells

Day 2 (Sunday): berries look fresh – plump and tender; moist on surface, no berry smell; no mold *definitely buy*

Day 3 (Monday): same as Sunday *definitely buy*

Day 4 (Tuesday): same as Sunday *definitely buy* ***removed from refrigerator***

Day 6 (Thursday): all 5 clamshells have a mild to moderate mold *would not buy*

Day 7 (Friday): all 5 clamshells have moderate to major mold *would not buy*

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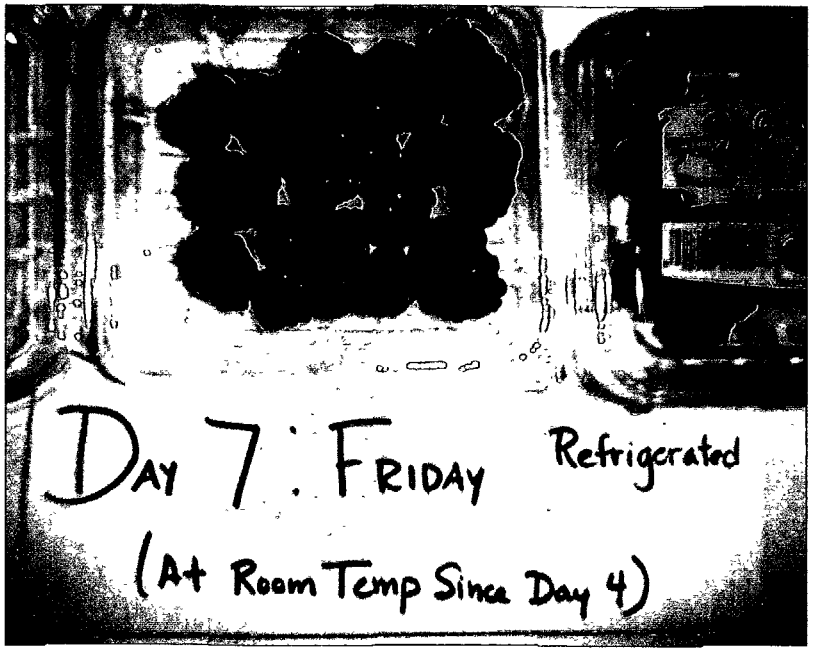
Blackberry/ClO_2 Trial - Refrigerated

Trial 13, Lt Blue Tape w/Black lines **ClO_2 level:** 1.0g **Sachet/Patch:** patch **Vents:** open



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Blackberry/ClO_2 Trial - Refrigerated

Trial 13, Lt Blue Tape w/Black lines **ClO_2 level:** 1.0g **Sachet/Patch:** patch **Vents:** open

2 clamshells

Day 2 (Sunday): berries look fresh – plump and tender; moist on surface, no berry smell; no mold *definitely buy*

Day 3 (Monday): same as Sunday *definitely buy*

Day 4 (Tuesday): same as Sunday *definitely buy* ***removed from refrigerator***

Day 6 (Thursday): berries are plump and tender with very little dehydration; no mold *definitely buy*

Day 7 (Friday): both clamshells have moderate dehydration and 1 berry with slight mold; *probably would not buy*

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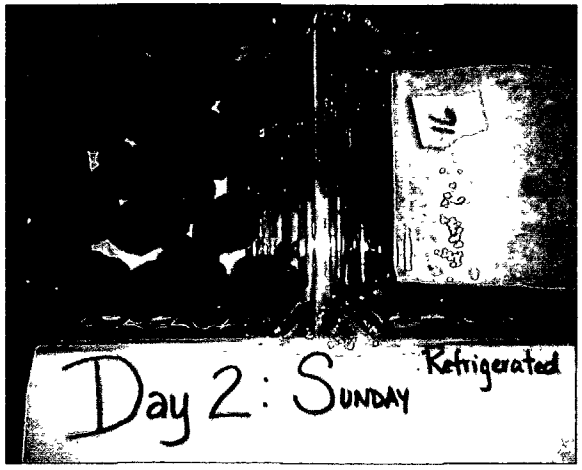
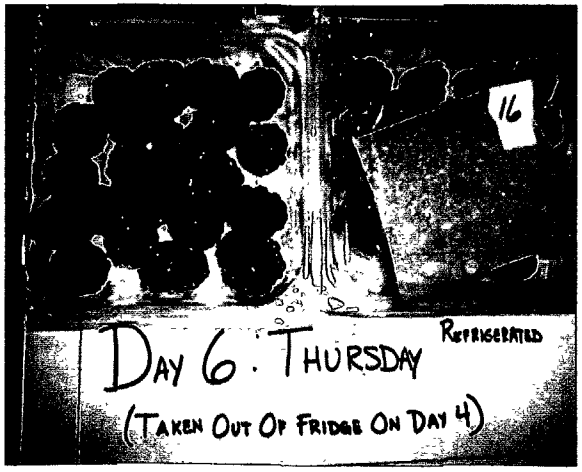
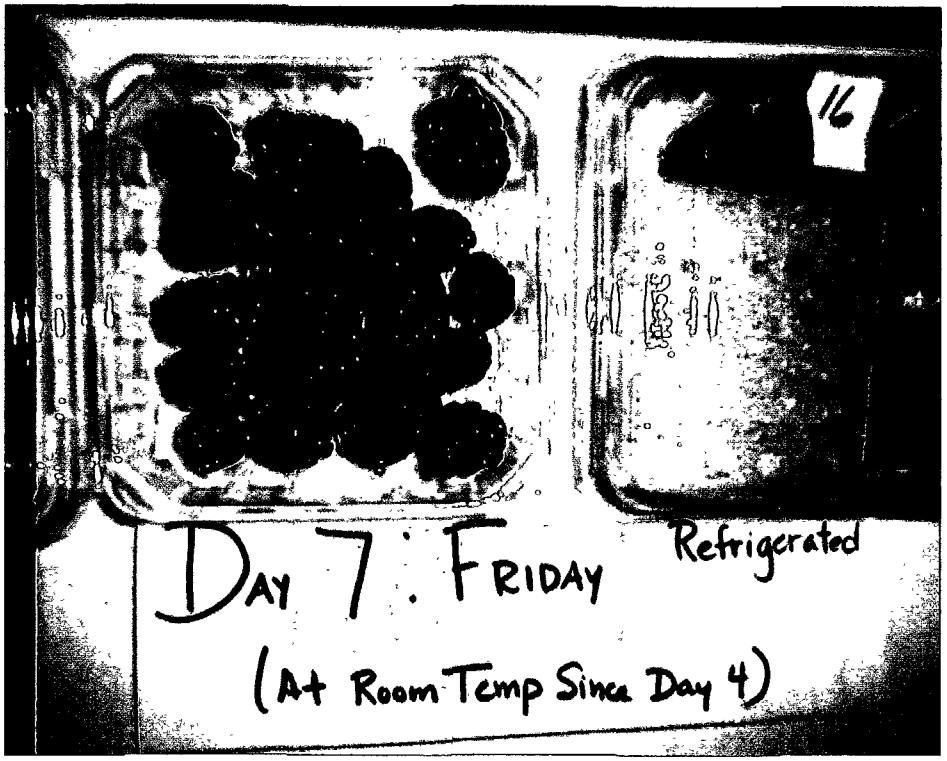
Blackberry/ClO_2 Trial - Refrigerated

Trial 16, White Tape ClO_2 level: 1.0gm **Sachet/Patch:** patch **Vents:** open







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Blackberry/ClO_2 Trial - Refrigerated

Trial 16, White Tape ClO_2 level: 1.0gm **Sachet/Patch:** patch **Vents:** open

2 clamshells

Day 2 (Sunday): berries look fresh – plump and tender; moist on surface, no berry smell; no mold *definitely buy*

Day 3 (Monday): same as Sunday *definitely buy*

Day 4 (Tuesday): same as Sunday *definitely buy* ***removed from refrigerator***

Day 6 (Thursday): both clamshells look plump and tender with slight dehydration; 1 berry in 1 clamshell has slight mold *definitely buy*

Day 7 (Friday): mold is just starting in both clamshells; berries look slightly dehydrated but still ok *questionable buy*

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STRAWBERRY CLAMSHELLS EXPOSED TO ClO_2 GAS

REFRIGERATED BATCH

Strawberry Trial - Refrigerated

Clear plastic clamshell containers were filled with topped (Calyx removed) strawberries (variety Lanai)

Half of the containers had a 2 cm diameter patch adhered to the underside of its lid (0.25 gram of Type II CSR material)

Remainder left untreated

Strawberries held at 35°F for 3 days, and then at 45°F for another 5 days

Results:

Evaluation Day	Mold		Decay	
	Conrol	CSR Patch	Control	CSR Patch
5	None	None	None	None
6	None	None	None	None
7	None	None	Slight	None
8	Moderate	None	Slight	Slight

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APPENDIX 2

RESIDUE DATA

Technical Report

REPORT TITLE
The Reaction of Chlorite Ion with Raspberry Extract

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DATE
December 10, 2003

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1 Summary of Study and Results

Three concentrations of dilute raspberry extract were reacted with three chlorite ion concentrations in analytical vials. Nine reaction mixtures were prepared. The reaction mixtures were analyzed for chlorite ion using ion chromatography. Each reaction mixture was analyzed twice. For each mixture, the analyses were performed within 38 to 67 min. for the first analysis and about five hours after preparation for the second analysis.

The results were expressed as the percentage of the initial chlorite ion remaining. The table below presents the values obtained.

Raspberry extract Concentration (%)	0.1		0.5		1.0	
	Percent Chlorite Ion Remaining					
Nominal Chlorite Ion Concentration (ppb)	First Analysis	Second Analysis	First Analysis	Second Analysis	First Analysis	Second Analysis
100	98.6	84.1	102.4	97.3	102.4	78.8
300	102.8	92.5	82.0	15.2	62.0	0.0
500	88.0	78.1	90.2	45.5	69.5	8.6

The results showed:

a) that there was a reaction between chlorite anion and raspberry extract, which lowered the chlorite ion concentration.
b) that the amount of reaction depended on the time and concentration of the reactants.
c) that at the highest concentrations of both reactants the reaction was about 91.3% complete in about five hours.

It is expected that as the concentration of raspberry extract increases the reaction will proceed faster. The consequence of these results will be that low recoveries (possibly undetectable) will be obtained when raspberry samples are spiked with chlorite ion for residue study purposes.

2 Introduction

Engelhard Corporation will be pursuing regulatory agency approval of an antimicrobial product that extends the shelf life of raspberries at the point of sale. Approval is required by both FDA and EPA since the 1998 amendments (Antimicrobial Regulation Technical Corrections Act or "ARTCA") gave FDA jurisdiction for dietary residues that result from antimicrobials used in food-packaging and EPA jurisdiction for product registration.

The product is patches in clamshell containers used for the retail packaging of raspberries. The patches contain sodium chlorite and give a controlled release of chlorine dioxide gas. The gas in turn reduces spoilage bacteria on the berry. The key issue are dietary residues that may result from chlorine dioxide use.

The major residue produced by the treatment of organic material with chlorine dioxide, under most conditions, has been well established in published literature as the chlorite ion, ClO_2^-. Accordingly, chlorite ion has been considered the residue of concern by FDA in its evaluation of chlorine dioxide use in process water associated with fruits and vegetables, poultry and seafood.

This study provides data showing that chlorite ion reacts with components in raspberries. The result of such reactions will be that any chlorite ion formed in the fruits will be substantially reduced and that the conventional requirement for high recovery levels for spiked samples will probably not be met.

3 Objectives of the Study

The primary objective of the study was to show that chlorite ion reacts with components in raspberries with a consequent reduction of chlorite ion levels. A secondary objective was to determine the approximate time scale for these reactions.

4 Study Administration

4.1 Testing Facilities

The study was conducted at:
The Department of Biological and Physical Sciences
Chapman University
One University Drive
Orange, CA 92866

4.2 Personnel

Chapman University
Contributing Scientist Richard L. Pilling, D. Phil.

4.3 Sponsor

Engelhard Corporation
101 Wood Avenue
Iselin, NJ 08830

4.4 Sponsor Coordinator

Barry K. Speronello, Ph. D.

5 Overview of Experimental Study Design

The experimental design involved the reaction of three concentrations of chlorite ion (100, 300 and 500 ppb) with three concentrations of raspberry extract (0.1, 0.5 and 1.0%) in all combinations. The nine reaction solutions were analyzed for chlorite ion after two time intervals, using ion chromatography. The results are expressed as the percentage of chlorite ion remaining in each sample. Consumption of chlorite ion demonstrated the presence of a reaction.

6 Description of Materials

Name	Form	Supplier/Source	Lot Number
Raspberries	Fresh fruit	Stater Bros Market, Fullerton, CA.	NA
Helium	Colorless gas >99.999% purity	Oxygen Services Co.	NA
Reagent Water	Colorless liquid	Barnstead Nanopure Infinity Ultrapure water system	NA
1.0 N Sodium Carbonate Solution	Colorless aqueous solution	GFS Chemicals	L032019
Sodium Chlorite	White solid, 95% ±1.5% purity	GFS Chemicals	L031939
Nitric Acid, 70%	Colorless liquid, redistilled, >99.999% purity	Sigma-Aldrich	L 133662
Potassium Bromide	White crystalline solid, ACS Reagent Grade	GFS Chemicals	L 819016
o-Dianisidine dihydrochloride salt (ODA)	White powder	Sigma-Aldrich	40 K 5316
Methanol	Colorless volatile liquid, spectrophotometric grade	Sigma-Aldrich	CO 0505830

7 Description of Equipment

7.1 Ion Chromatograph

Dionex IC 500 DX Ion Chromatograph equipped with:

7.1.1 Autosampler: Dionex AS50 Autosampler with a temperature-controlled sample tray held at 6°C and a 200µL sample loop
7.1.2 Chromatography compartment: Dionex AS50 Chromatography Compartment
7.1.3 Detector: Dionex AD25 Absorbance Detector operating with a wavelength setting of 450nm
7.1.4 Post Column Heater: Dionex PCH-2 Post Column Heater set at 100°C and allowed to come to temperature for at least 30 minutes
7.1.5 Pump: Dionex GS50 Gradient Pump set to a flow rate of 1.0 mL/min
7.1.6 Column: IonPac AS9-HC 4mm Anion Exchange column with an IonPac AG9-HC 4mm Guard Column
7.1.7 Pneumatic Controller: Dionex PC 10 Pneumatic Controller, to control the post-column reagent, set to give a flow rate of 0.5 to 0.7 mL/min
7.1.8 Software: Dionex PeakNet 6
7.1.9 Sample Vials: Dionex 2mL sample vials with split septum

7.2 Analytical Balance

Denver Instrument Co. A 200 DS analytical balance reading to ±0.0001g

7.3 Standard Laboratory Equipment

Pipettes, volumetric flasks, filters, syringe filters.

8 Description of Methods

8.1 Preparation of Sodium Chlorite Solutions

A stock solution containing a nominal level of 100 ppm of chlorite ion was prepared by dissolving an accurately measured amount of sodium chlorite solid in reagent water. Standard and calibration solutions were prepared from the stock solution by making quantitative direct or sequential dilutions of the stock solution using pipettes and volumetric flasks.

8.2 Preparation of Raspberry Extract

Raspberry extract was prepared from the fresh fruit. A portion of the fruit was put in a glass beaker and reagent water was added. The fruit was squeezed against the side of the flask with a spatula until all the juice had been released. The clear

liquid was decanted from the seeds and other solid material and was filtered twice through filter paper and once through a 0.45µm syringe filter.

8.3 Preparation of Reaction Solutions

Reaction solutions were prepared in 2mL sample vials. An Eppendorf pipette was used to add measured amounts of water and chlorite solution to the vial. The reaction was started by using an Eppendorf pipette to add a measured amount of raspberry extract to the vial containing the water and chlorite ion.

8.4 Chlorite Ion Analysis

8.4.1 Analytical Method

The solutions were analyzed for chlorite anion using an ion chromatograph. The method used was based on EPA method 317 and is detailed in Appendix A. In brief, the anions were separated on an anion exchange column using sodium carbonate solution as eluant. A post column reagent (PCR) was introduced into the eluant flow after the column. The PCR contained nitric acid, potassium bromide and o-dianisidine dihydrochloride. A post column heater was used to increase the rate of reaction between the chlorite anion and the PCR. The color developed in the reaction was detected by an absorbance detector set at 450 nm. The ion chromatograph results were recorded by the PeakNet software and the peak area was used as a quantitative measure of the chlorite ion concentration. The system was calibrated using solutions of known chlorite ion concentration.

8.4.2 Sequence of Samples.

The system was calibrated before running the unknown samples using solutions with nominal concentrations of 10, 50, 100, 200, 300, and 500 ppb.

The unknown samples were in a sequence of 37 runs. Samples were grouped in sets of five so that the three solutions with the same initial chlorite ion concentration were run together and they were preceded and followed by a standard solution containing the same initial chlorite concentration. The groups of five were bracketed by water blanks. For example the first seven runs of the sequence were:

1. Water blank
2. Nominal 100 ppb Chlorite ion
3. Unknown sample RR-2-1
4. Unknown sample RR-2-2
5. Unknown sample RR-2-3
6. Nominal 100 ppb Chlorite ion
7. Water blank

The three unknown samples had a nominal initial chlorite ion concentration of 100 ppb.
Sample chromatograms were printed. Retention time and peak area data were recorded. The time for each sample run was ten minutes.

8.5 Calculation of Percent Remaining Chlorite Anion

The percent of remaining chlorite anion was calculated directly from the peak areas. Using the set of seven runs above as an example:
The average peak area for nominal 100ppb chlorite was found from runs 2 and 6 using:

$$\text{Average peak area}_{(100ppb)} = (\text{Pk area run 2} + \text{Pk Area Run 6})/2$$

The percent of remaining chlorite for the unknown samples with initial nominal chlorite ion concentration of 100 ppb was calculated using:

$$\% \text{ remaining chlorite} = 100 \times (\text{Pk area of unknown})/\text{Average peak area}_{(100\ ppb)}.$$

9 RESULTS

9.1 Results Of Preparation Of Sodium Chlorite Solutions.

9.1.1 Results Of Preparation Of Stock Sodium Chlorite Solution

The stock sodium chlorite solution was prepared by dissolving 0.1420 g of solid sodium chlorite in 1 L of reagent water in a volumetric flask. Purity of the sodium chlorite solid had previously been found to be 95%±1.5%. The level of chlorite ion in the solution was calculated using:

$$g \text{ of } ClO_2^-/L = (g\ NaClO_2) \times (FW_{(ClO2-)}/FW_{(NaClO2)} \times \text{purity of } NaClO_2/\text{liters of solution.}$$

$$g \text{ of } ClO_2^-/L = 0.1420\ (g) \times 67.4515/90.4412 \times 0.95/1.00(L)\ \ g/L$$
$$= 0.1006\ g/l$$

The solution was labeled "100 ppm ClO_2^- stock solution 11/5/03". The estimated actual value of the concentration was 100.6 ppm. No correction was made for the 0.6% difference between the actual and nominal concentration and nominal concentrations were used throughout the study.

9.1.2 Results of Preparation Of Calibration And Standard Solutions Of Sodium Chlorite

Sodium chlorite solutions used as standards and for calibration were prepared from the above stock solution. The experimental details are summarized in the table below:

Solution used	Volume used (pipetted) (mL)	Final Volume (Volumetric Flask) (mL)	Solution label
100 ppm ClO_2^- stock solution 11/5/03	1.00	100.0	1ppm, (1000ppb) ClO_2^- 11/5/03
1ppm, (1000ppb) ClO_2^- 11/5/03	6.00	10.0	600 ppb ClO_2^- 11/5/03
1ppm, (1000ppb) ClO_2^- 11/5/03	4.00	10.0	400 ppb ClO_2^- 11/5/03
1ppm, (1000ppb) ClO_2^- 11/5/03	1.00	10.0	100 ppb ClO_2^- 11/5/03
1ppm, (1000ppb) ClO_2^- 11/5/03	0.50	10.0	50 ppb ClO_2^- 11/5/03
1ppm, (1000ppb) ClO_2^- 11/5/03	0.10	10.0	10 ppb ClO_2^- 11/5/03

The nominally 1000, 600 and 400 ppb ClO_2^- solutions were diluted 1:1 with reagent water in analytical vials by adding 0.8mL of the appropriate solution to a vial and then adding 0.80 mL of water to the vial, which was capped and shaken. The nominal concentrations of these solutions were 500, 300 and 200 ppb ClO_2^-.

9.2

9.2.1 Results Of Preparation Of Raspberry Extract

Three raspberries weighing about 8.2 g, were placed in a small beaker with 4mL of reagent water. The fruits were squeezed against the sides of the beaker with a spatula until no unbroken juice sacs could be seen. The liquid was decanted from the solid portion and was filtered twice through P5 filter paper and once through a 0.45 µm nylon syringe filter. The yield was about 3 mL of clear red liquid labeled "Raspberry extract 11/4/03". The extract was sealed in a small container and was placed in a refrigerator for storage overnight.

9.2.2 Results of Preparation Of Raspberry Extract Dilutions

The "Raspberry extract 11/4/03" was diluted on 11/5/03 using reagent water to obtain three dilute solutions. The experimental details are presented in the table below:

Solution used	Volume used (pipetted) (mL)	Final Volume (Volumetric Flask) (mL)	Solution label
Raspberry extract 11/4/03	1.00	50.0	RE-2-2%
RE-2-2%	5.00	10.0	RE-2-1%
RE-2-2%	1.00	10.0	RE-2-0.2%

9.3 Results Of Preparation Of Reaction Solutions

Nine reaction solutions were prepared in 2 mL analytical vials. The time of addition of the dilute raspberry extract solution was recorded. A total of 1.60 mL of each solution was prepared. The experimental details are presented in the table below:

Nominal conc. of chlorite ion solution used (ppb)	Volume of chlorite ion solution used (mL)	Dilute raspberry extract solution used	Volume of dilute raspberry extract solution used (mL)	Volume of reagent water used (mL)	Time of addition of dilute raspberry extract on 11/5/03	Reaction solution label
400	0.40	RE-2-0.2%	0.80	0.40	11:35	RR-2-1
400	0.40	RE-2-1%	0.80	0.40	11:36	RR-2-2
400	0.40	RE-2-2%	0.80	0.40	11:37	RR-2-3
600	0.80	RE-2-0.2%	0.80	0.00	13:03	RR-2-4
600	0.80	RE-2-1%	0.80	0.00	13:05	RR-2-5
600	0.80	RE-2-2%	0.80	0.00	13:06	RR-2-6
1000	0.80	RE-2-0.2%	0.80	0.00	14:32	RR-2-7
1000	0.80	RE-2-1%	0.80	0.00	14:33	RR-2-8
1000	0.80	RE-2-2%	0.80	0.00	14:34	RR-2-9

The initial concentrations of the reaction solutions were calculated using:

Init. conc. = Conc. of soln. added x mL of soln. added/total final mL of soln.

The nominal initial concentrations of the reaction solutions are given in the table below:

Reaction solution label	Nominal chlorite ion concentration (ppb)	Nominal concentration of raspberry extract (%)
RR-2-1	100	0.10
RR-2-2	100	0.50
RR-2-3	100	1.00
RR-2-4	300	0.10
RR-2-5	300	0.50
RR-2-6	300	1.00
RR-2-7	500	0.10
RR-2-8	500	0.50
RR-2-9	500	1.00

9.4 Results Chlorite Ion Analysis

9.4.1 Results Of Calibration of Ion Chromatograph

The ion chromatography system was calibrated by analyzing chlorite ion standards containing nominal chlorite ion concentrations of 10, 50, 100, 200, 300 and 500 ppb. The calibration runs were made between 9:36 a.m. and 11:15 a.m. on 11/5/03. The retention times and peak areas obtained are presented in Table 1. The calibration curve is presented graphically in Figure 1. The results showed that the instrument response was linear over the range from 10 to 500 ppb with an R^2 value of 0.997, and that the response could be represented by the expression:

Peak area (AU*min) = 2.098×10^{-5} x(ppb chlorite ion) + 4.494×10^{-4}.

9.4.2 Results Of Analysis Of Reaction Solutions

The analyses were grouped in sets of five. Each set consisted of three samples with the same initial chlorite ion concentration, and a standard of the same concentration run before and after them. For each group the three reaction solutions were placed in the autosampler chamber together just before analysis and were removed together. Each group of three reaction solutions was analyzed approximately 30 to 70 minutes after preparation and again 5 to 5½ hours after preparation. Details of the timings are presented in Table 2. Each sample had a different time exposure to the room temperature (22°C) and to the autosampler chamber temperature (6°C). These times are also summarized in Table 2.

The peak areas and retention times for the analyses of the reaction solutions are shown in Table 3. The reaction solutions were run in a sequence, which included standards and water blanks. No peaks were detected in any of the water blanks and these are omitted from the table. The average retention times for the chlorite ion peaks was 4.48 min with a standard deviation of 0.01 min. Peak area results for the standards, which were run before and after each set of three reaction solutions agreed within ±2½ % of their average value. The good agreement and the consistent retention times confirmed that the equipment response was stable and that the data were reliable. The peak areas for the reaction solutions and the bracketing standard solutions were used to calculate the percentage of chlorite ion remaining at the time of the analysis. These results are also presented in Table 3, and as bar charts in Figures 2, 3, and 4.

10 Discussion

The bar charts displayed in Figures 2, 3 and 4 showed that there was a reaction between raspberry extract and chlorite anion, and that the reaction consumed chlorite anion.

These reaction features were most clearly shown at the highest initial chlorite ion concentration (Fig. 4). Here the reaction was fast enough for the drop in chlorite ion level to be detected at the time of the first analysis, and the amount of chlorite ion consumed was clearly dependent on the raspberry extract concentration. Indeed, for the sample with the highest raspberry extract concentration (RR-2-9) 91% of the chlorite anion had been consumed after 5 hr 20 min.

At the lowest initial chlorite ion concentration (Fig 2) the reaction was considerably slower and there was no significant change in the chlorite ion levels for the first analysis. However, by the time of the second analysis the chlorite ion level had been reduced by about 20%.

The results displayed in Figure 3 showed similar time and concentration trends as Figures 2 and 4. However the results presented in figures 3 and 4 are not fully consistent. The initial chlorite ion concentration in Figure 3 was 300 ppb compared with 500 ppb in Figure 4. The lower concentration would be expected to give a slower reaction, whereas it appears to give a faster reaction. Reasons for this are not apparent.

Overall the results are consistent with a reaction between chlorite ion and raspberry extract, which is dependent on the concentrations of the two reactants.

11 Conclusions

- Raspberry extract reacts with chlorite ion and reduces its concentration.
- The reaction proceeds faster when either the chlorite ion or extract concentration is increased.
- The samples in this study used a maximum extract concentration of 1%. In fresh fruit where the concentration is 100% the reaction can be expected to be faster than this study demonstrates.
- Recovery levels of chlorite ion spiked into fresh raspberries are anticipated to be poor because of the reaction demonstrated in this technical report.

12 Report Approvals

Signature	Title	Date
(b)(6)	Study Director	12/10/03
(b)(6)	Study Monitor	12-10-03

Table 1

Results of Analysis of Calibration Standards

Run No.	Sample Chlorite Ion Conc. (ppb)	Ret. Time (min)	Pk Area (AU*min)
2	10	4.493	0.000063
3	50	4.507	0.000616
4	100	4.487	0.001510
5	200	4.480	0.003542
6	300	4.513	0.005654
7	500	4.507	0.010260

Mean	4.498
Std. Dev.	0.013

Table 2

Sample History

Data		Clock time on 11 5 03								
	Sample No.	RR-2-1	RR-2-2	RR-2-3	RR-2-4	RR-2-5	RR-2-6	RR-2-7	RR-2-8	RR-2-9
	Time of Addition of Rasp Extract	11:35	11:36	11:37	13:03	13:05	13:06	14:32	14:33	14:34
First Analysis	Time into Autosampler (first)	11:49	11:49	11:49	13:14	13:14	13:14	14:39	14:39	14:39
	Time of Injection	12:16	12:30	12:44	13:41	13:55	14:09	15:05	15:20	15:34
	Time out of Autosampler (first)	13:14	13:14	13:14	14:24	14:24	14:24	15:58	15:58	15:58
Second Analysis	Time into Autosampler	15:58	15:58	15:58	17:20	17:20	17:20	18:31	18:31	18:31
	Time of Injection	16:36	16:50	17:04	18:01	18:15	18:29	19:26	19:40	19:54
	Time out of Autosampler	17:16	17:16	17:16	18:31	18:31	18:31	20:07	20:07	20:07

Calculations		Elapsed Time (hr:min)								
First Analysis	Time at Room Temp before putting into Autosampler	0:14	0:13	0:12	0:11	0:09	0:08	0:07	0:06	0:05
	Time in Autosampler before injection	0:27	0:41	0:55	0:27	0:41	0:55	0:26	0:41	0:55
	Time in Autosampler before removal	1:25	1:25	1:25	1:10	1:10	1:10	1:19	1:19	1:19
Second Analysis	Time at Room Temp before putting into Autosampler	2:44	2:44	2:44	2:56	2:56	2:56	2:33	2:33	2:33
	Time in Autosampler before injection	0:38	0:52	1:06	0:41	0:55	1:09	0:55	1:09	1:23
	Time in Autosampler before removal	1:18	1:18	1:18	1:11	1:11	1:11	1:36	1:36	1:36

First Analysis									
Time at Room Temperature	0:14	0:13	0:12	0:11	0:09	0:08	0:07	0:06	0:05
Time at Autosampler Temperature	0:27	0:41	0:55	0:27	0:41	0:55	0:26	0:41	0:55
Sum of times	0:41	0:54	1:07	0:38	0:50	1:03	0:33	0:47	1:00
Second Analysis									
Total Time at Room Temperature	2:58	2:57	2:56	3:07	3:05	3:04	2:40	2:39	2:38
Total Time at Autosampler Temperature (hr:min)	2:03	2:17	2:31	1:51	2:05	2:19	2:14	2:28	2:42
Sum of times	5:01	5:14	5:27	4:58	5:10	5:23	4:54	5:07	5:20

Note Autosampler chamber temperature was 6 deg C, Room Temperature was 22 deg C.

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Table 3

Results Of Analysis Of Reaction Solutions And Calculation Of Percent Chlorite Remaining

Run No (1)	Sample Name	Ret. Time (min)	Pk Area (AU*min)	% ClO2- Remaining	Run No	Sample Name	Ret. Time (min)	Pk Area (AU*min)	% ClO2- Remaining
2	100 ppb chlorite	4.497	0.001529		20	100 ppb chlorite	4.467	0.00164	
3	RR-2-1	4.507	0.001526	98.6%	21	RR-2-1	4.480	0.001360	84.1%
4	RR-2-2	4.487	0.001584	102.4%	22	RR-2-2	4.473	0.00128	79.3%
5	RR-2-3	4.483	0.001584	102.4%	23	RR-2-3	4.493	0.00127	78.8%
6	!00 ppb Chlorite	4.477	0.001566		24	!00 ppb Chlorite	4.470	0.00159	
8	300 ppb chlorite	4.470	0.005604		26	300 ppb chlorite	4.500	0.005731	
9	RR-2-4	4.500	0.005837	102.8%	27	RR-2-4	4.500	0.005189	92.5%
10	RR-2-5	4.477	0.004656	82.0%	28	RR-2-5	4.490	0.000852	15.2%
11	RR-2-6	4.503	0.003520	62.0%	29	RR-2-6	n.d.	0.000000	0.0%
12	300 ppb chlorite	4.467	0.005757		30	300 ppb chlorite	4.477	0.005493	
14	500 ppb chlorite	4.463	0.010415		32	500 ppb chlorite	4.473	0.009802	
15	RR-2-7	4.467	0.009220	88.0%	33	RR-2-7	4.483	0.007834	78.1%
16	RR-2-8	4.480	0.009445	90.2%	34	RR-2-8	4.507	0.004566	45.5%
17	RR-2-9	4.467	0.007277	69.5%	35	RR-2-9	4.487	0.000867	8.6%
18	500 ppb chlorite	4.460	0.010531		36	500 ppb chlorite	4.500	0.010270	

(1) Runs Nos 1, 7, 13, 19, 25, 31 or 37 were water blanks. No peaks were detected in any of them.

Mean	4.480	Mean	4.486
St dev	0.015	St dev	0.013

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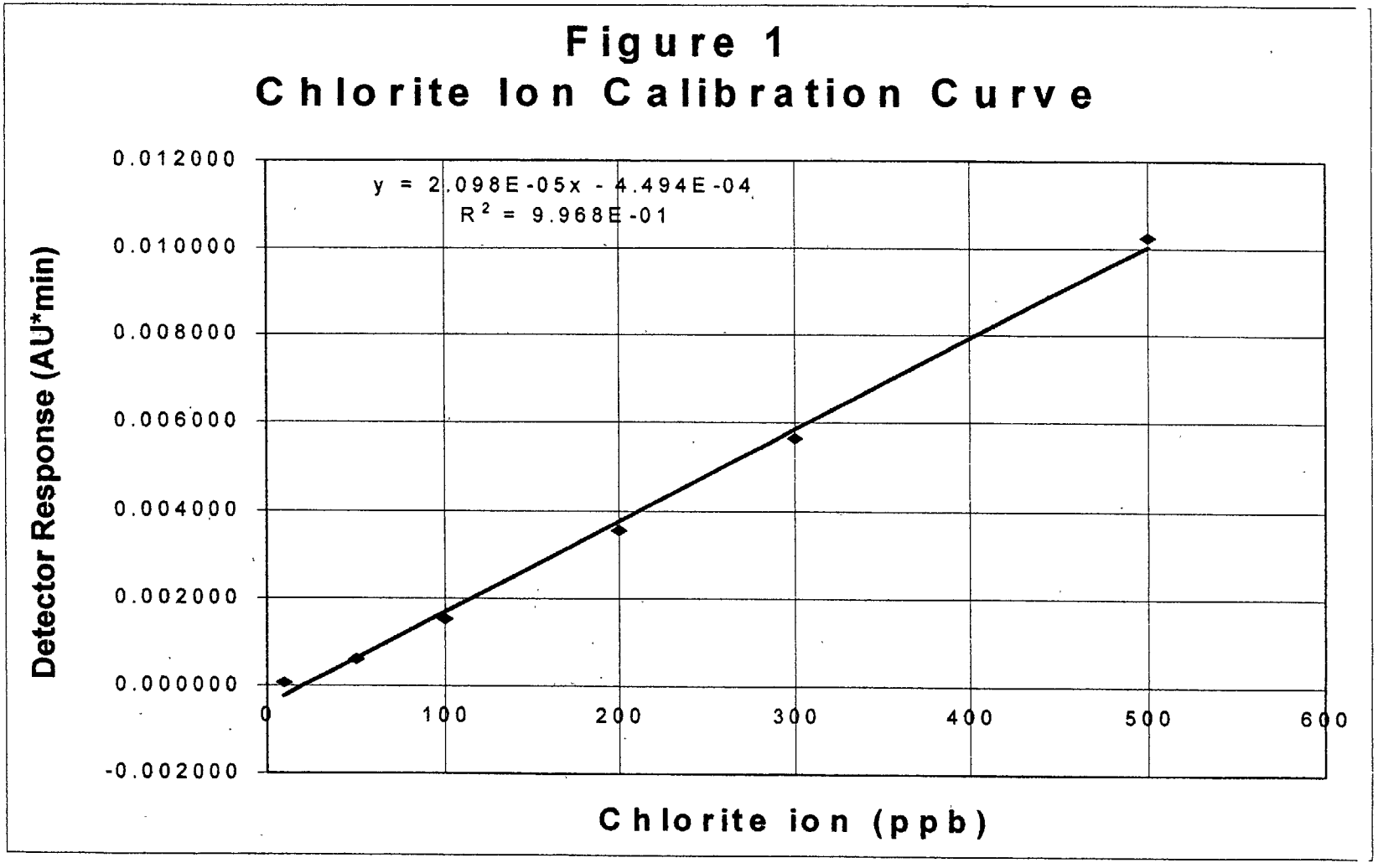
Figure 1
Chlorite Ion Calibration Curve
y = 2.098E-05x - 4.494E-04
R² = 9.968E-01
Detector Response (AU*min)
0.012000
0.010000
0.008000
0.006000
0.004000
0.002000
0.000000
-0.002000
0
100
200
300
400
500
600
Chlorite ion (ppb)

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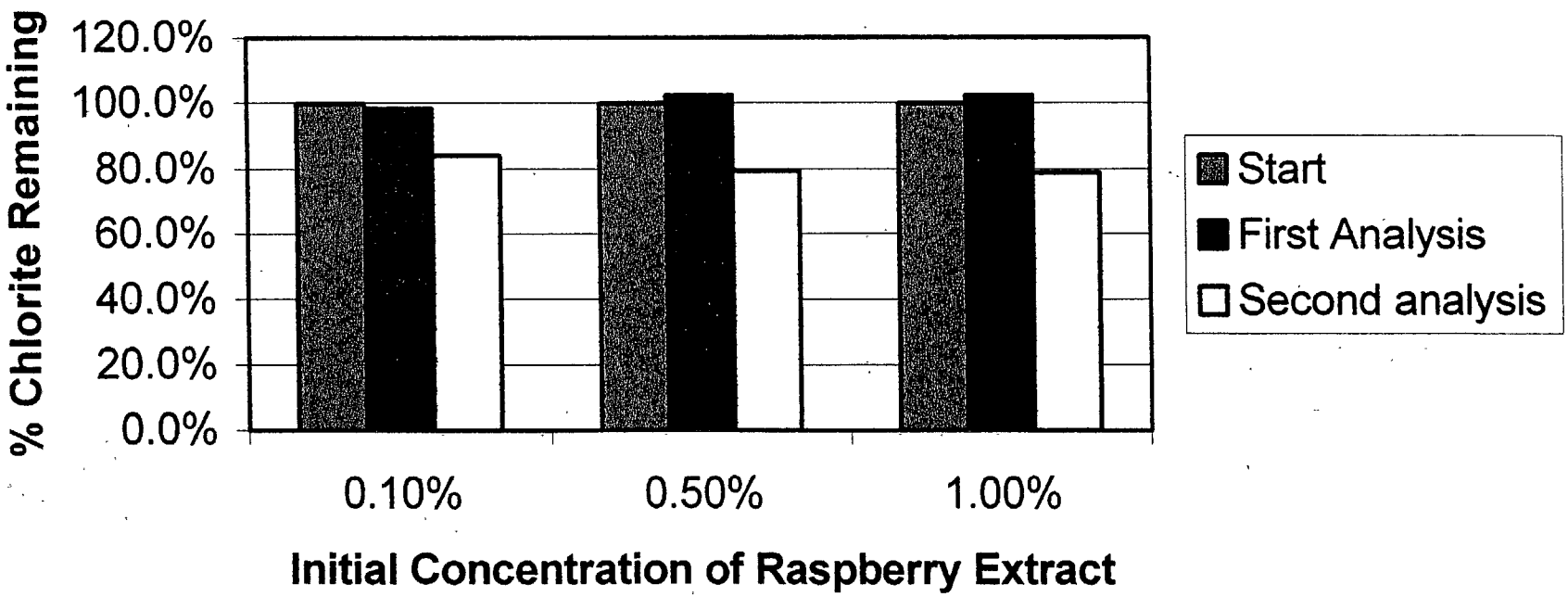
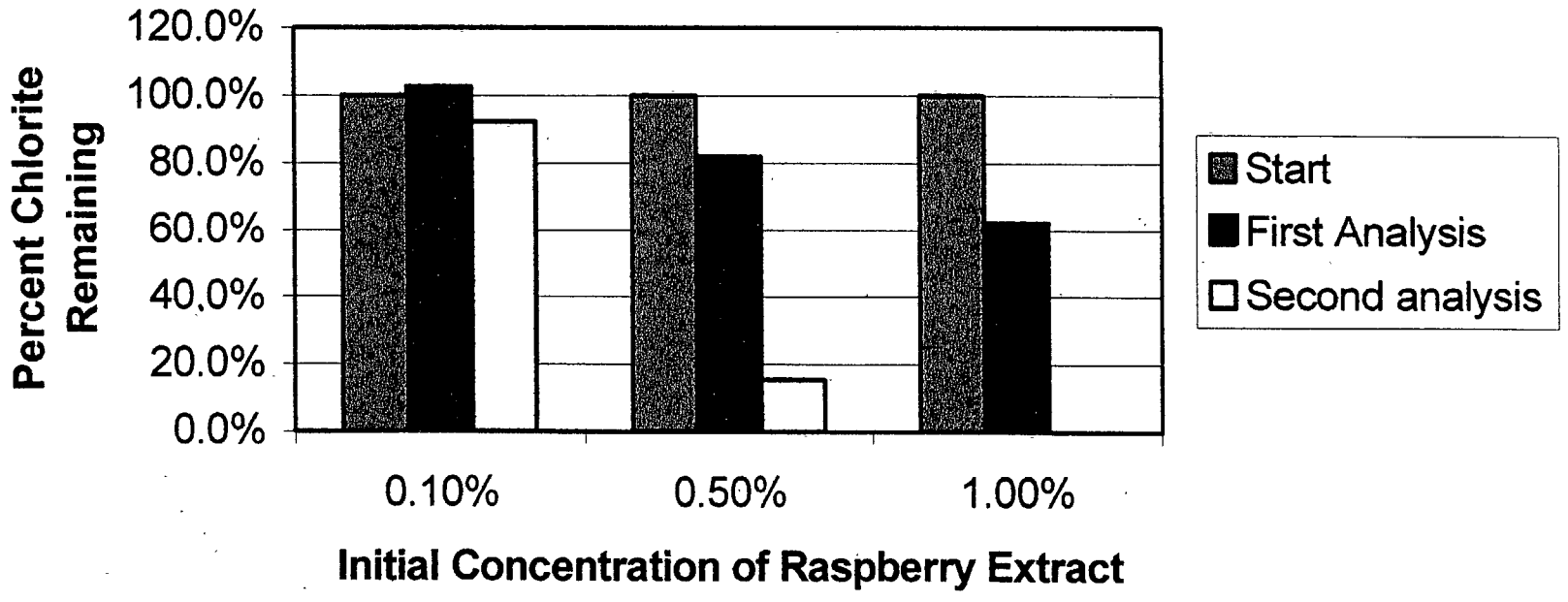
Figure 2
Percent Chlorite Remaining for Nominal Initial Chlorite Ion Concentration of 100 ppb



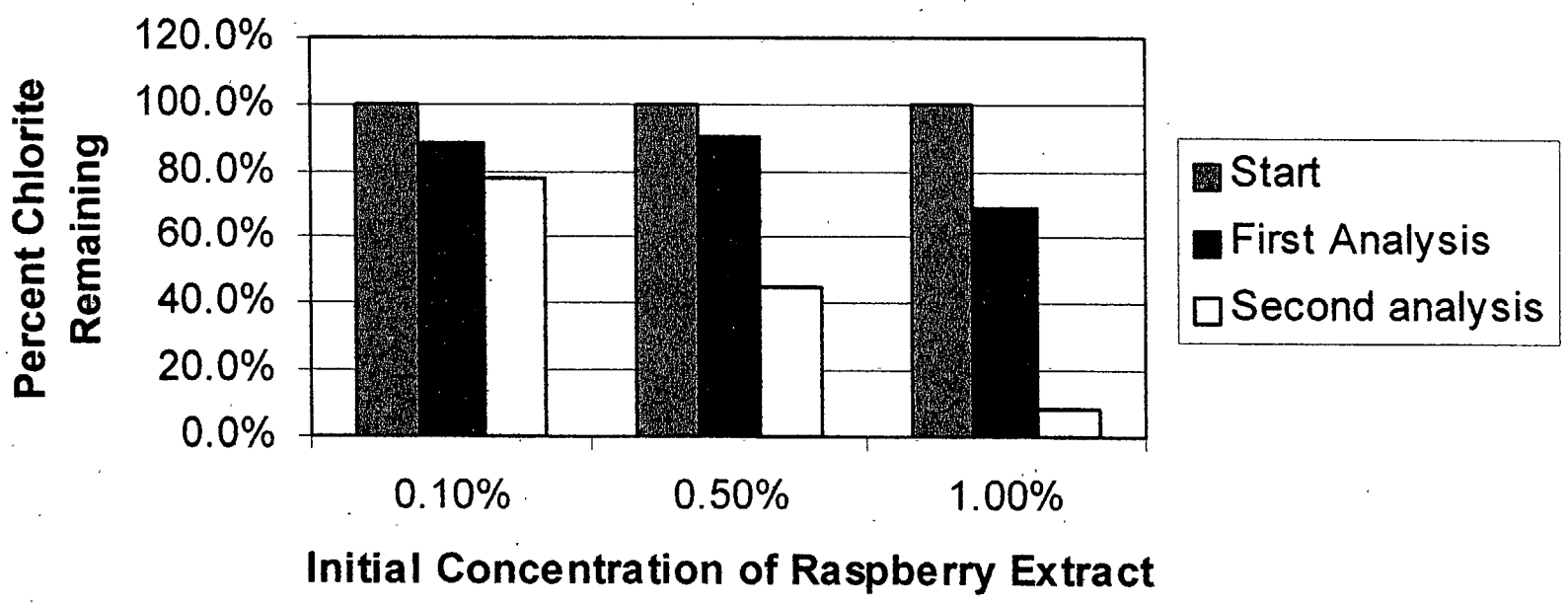
000071

Figure 3
Percent Chlorite Ion Remaining for Nominal Initial Chlorite Ion Concentration of 300 ppb



000072

Figure 4
Percent Chlorite Ion Remaining for Nominal Initial Chlorite Ion Concentration of 500 ppb



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APPENDIX A

Chlorite Ion In Aqueous Solutions By Ion Chromatography
SOP CUPS002.00

Analysis Of Chlorite Ion In Aqueous Solutions By Ion Chromatography

1. SCOPE

1.1 This procedure describes the determination of chlorite anion concentrations in aqueous solutions using ion chromatography. The method can detect chlorite anion concentrations in the range of 1 ppb to 1 ppm. The post column reagent, o-dianisidine, is used to increase the sensitivity of absorbance detection. The limits of detection and quantitation for the absorbance detector are 1.0 ppb and 3.0 ppb respectively.

2. METHOD SUMMARY

2.1 Liquid aliquots of the sample for analysis are placed into auto-sample vials and analyzed by ion chromatography for presence of chlorite ion.

3. REAGENTS

3.1 Helium, Ultra High Purity, >99.999% (CAS Number 7440-59-7, Oxygen Services Co. or equivalent).

3.2 Reagent Water, 17MΩ or better, (Barnstead Nanopure Infinity – Ultrapure Water System, or equivalent).

3.3 1.0 N (0.5 mol/L) Sodium Carbonate, ACS grade (CAS Number 497-19-8, GFS Chemicals, Item 2119, Catalog No. 25182, or equivalent).

3.4 Sodium Chlorite, ACS grade >99% purity (CAS Number 7758-19-2, GFS Chemicals, Item 2456 or equivalent). To be used for calibration. Store in dessicator.

3.5 70% Re-distilled Nitric Acid >99.999% purity, (CAS Number 7697-37-2, Sigma-Aldrich Catalog No. 22,571-1, or equivalent).

3.6 Potassium Bromide, ACS reagent grade (CAS Number 7758-02-3, GFS Chemicals, Item 729, Catalog No. 60401, or equivalent).

3.7 o-dianisidine, dihydrochloride salt (ODA), (CAS Number 30225-40-0, Sigma-Aldrich Catalog No. 19,124-8, or equivalent).

3.8 Methanol, Spectrophotometric grade, (CAS Number 67-56-1, Sigma-Aldrich Catalog No. 15,490-3, or equivalent).

4. APPARATUS AND EQUIPMENT

4.1 Dionex IC 500DX Ion Chromatograph, or equivalent, equipped with:

4.1.1 Sampler: Dionex AS50 Autosampler, or equivalent, equipped with a temperature controlled sample tray held at 6°C and a 200µL sample loop.

4.1.2 Chromatography Compartment: Dionex AS50 Chromatography Compartment, or equivalent.

4.1.3 Detector: Dionex AD25 Absorbance Detector, or equivalent.

4.1.4 Heater: Dionex PCH-2 Post Column Heater, or equivalent, set to 100°C. Allow at least 30 minutes to come up to temperature.

4.1.5 Pump: Dionex GS50 Gradient Pump, or equivalent, set to a flow rate of 1.0 mL/min.

4.1.6 Column: IonPac AS9-HC 4-mm Column, or equivalent, anion exchange column equipped with IonPac AG9-HC 4-mm guard column, or equivalent.

4.1.7 Detection Stabilizer: Dionex DS3 Detection Stabilizer, or equivalent. Requires a constant flow of reagent water ≥5 mL/min.

4.1.8 Pneumatic Controller: Dionex PC 10 Pneumatic Controller, or equivalent, used to control the flow rate of the post-column reagent, set to a flow rate of 0.5 mL/min.

4.2 Software: Dionex PeakNet 6, or equivalent.

4.3 Dessicator: Used to store solid $NaClO_2$.

4.4 Auto-sampler Vials: Dionex 2 mL sample vials (or equivalent) with split septa.

4.5 Graduated cylinders.

4.6 Volumetric pipettes.

4.7 Volumetric flasks.

4.8 Analytical Balance: ± 0.0001 g.

5. SAFETY CONSIDERATONS

5.1 The post column reagent, o-dianisidine, is listed as a potential human carcinogen. Minimize direct exposure to this reagent. Dispose of according to biohazardous waste procedures.

5.2 Headspace gas from ClO_2^- is known to be explosive at high concentrations (>5000ppm). Prepare all ClO_2^- solutions at concentrations below this limit.

6. PRELIMINARY PREPARATIONS

6.1 Eluent solution: Prepare by adding 18.0 mL of 1.0 N (0.5 M) sodium carbonate solution to a 1000 mL volumetric flask and diluting to the mark with helium purged reagent water. The resulting solution is 9.0 mM.

6.2 Post Column Reagent: Prepare by adding 40 mL of 70% re-distilled nitric acid to approximately 300 mL reagent water in a well-rinsed 500 mL volumetric flask, and adding 2.5 g KBr. Dissolve, while stirring, 250 mg o-dianisidine, dihydrochloride in 100 mL methanol. Add the o-dianisidine solution to the nitric acid/KBr mixture and dilute to 500 mL with reagent water. Store in dark for 24 hours prior to use. The reagent is stable for up to one month.

6.3 Check and refill the fluid levels of the 9 mM Na_2CO_3 eluent reservoirs and water reservoirs for the signal suppression device before running any samples for analysis.

6.4 Check reagent water. Replace with fresh at least monthly.

6.5 Check post-column reagent level. Replace with fresh if low.

6.6 Turn on the post-column heater at least 30 minutes before running samples requiring post-column reagent.

6.7 Check the baseline of the absorbance detector using water blanks to ensure that it is stable, and that there is no residue left in the system that may adversely contribute to instrument response.

6.8 The absorbance detector may require an hour or more of equilibration before an adequate baseline signal is produced.

6.9 Occasionally, it may be necessary to flush the system using 45mM Na_2CO_3 to remove impurities left on the column and prevent carry-over from previous samples. Three or more water blanks should be run and the results monitored after the flush to allow the system to re-equilibrate to normal operating conditions.

7. CALIBRATION PROCEDURE

7.1 Prepare chlorite ion calibration standards as follows:

7.1.1 Stock Standard Solution of 1000 ppm Chlorite Ion. Prepare by dissolving 0.1341 g of 99% pure sodium chlorite in reagent water and diluting to 100 mL in a volumetric flask.

7.1.2 Calibration Standards. Prepare from the stock standard solution by making dilutions and/or sequential dilutions. The table below indicates appropriate dilutions for 10, 100, 200, 500, and 1000 ppb solutions, others can be made as needed:

Tgt. Conc. (ppb)	Solution used	Vol. of Solution (mL)	Final Vol. (mL)	Dilution Factor
1000	Std. Stock Soln.	0.500	500	1:1000
500	Std. Stock Soln.	0.250	500	1:2000
200	1000 ppb	20.0	100	1:5
100	1000 ppb	10.0	100	1:10
10	1000 ppb	1.00	100	1:100

7.2 Analyze the calibration standards in duplicate and prepare a calibration curve based on the concentrations of the calibration standards and the chlorite ion peak areas. Duplicate measurements should agree within 10% and the r^2 value for a linear regression of the calibration data should be >0.99.

8. ANALYSIS OF SAMPLES

8.1 Fill 2 mL auto-sampler vials with at least 1 mL of sample or more, depending upon the number of injections to be made from the vial and the injection volume.

8.2 Prepare water blank samples to place between each unknown sample.

8.3 Once vials are filled and properly labeled, place them in the auto-sampler tray.

8.4 Prepare a Run Sequence for the batch of samples to be analyzed using the PeakNet 6 software package or equivalent. See software instruction manual for additional information.

9. PROCEDURE NOTES

9.1 Periodically check the flow rate of the column waste line during analyses to ensure that a steady, consistent flow rate is being produced. Fluctuations in waste flow may indicate flow problems in the column or post-column reagent lines, and may affect the results of the analysis.

9.2 Absorbance Detector: The limits of detection and quantitation for the chlorite ion are 1 ppb and 3.0 ppb respectively. These limits were established using standard ClO_2^- solutions in the range 1 to 100 ppb. With an eluent flow rate of 1.0 mL/min and a post column reagent flow rate of 0.5 mL/min, the retention time of the chlorite anion is 4.48 ± 0.05 min. Peak areas have been found to fluctuate near 7% for low concentration chlorite solutions (<5ppb). The ion chromatograph used here produces a very linear response (r^2 > 0.998) for the chlorite anion in the range of 1ppb to 1ppm.

10. CALCULATIONS

10.1 The chlorite ion concentration in ppb can be determined directly from the calibration curve.

10.2 The amount of chlorite ion in ng in a sample can be calculated using:

$$\text{Amount } ClO_2^- \text{ (ng)} = C * V / 1000$$

Where:

C = ppb of chlorite ion in sample (from calib. curve)

V = volume of sample in µL

11. INTERFERENCES

11.1 No interferences with chlorite anion were found during preliminary procedures. The chlorite anion has a consistent retention time and produces a well-defined peak with the post-column reagent / absorbance detector combination. Other strongly oxidizing or colored anions, if present, may interfere.

11.2 Potential anion contamination in the chromatographic system, such as carry-over from a preceding high concentration (>100 ppm) chlorite sample, may produce a false positive chlorite ion peak. Water blanks should be used prior to analysis to ensure the instrument produces a clean baseline response.

12. REFERENCES

12.1 U.S.EPA Method 317.0, Determination Of Inorganic Oxyhalide Disinfection By-products In Drinking Water Using Chromatography With The Addition Of A Postcolumn Reagent For Trace Bromate Analysis, Revision 1.0, May 2000

13. REVIEW AND APPROVAL

Reviewed and approved by:

Name	Title	Date
(b)(6)	Study Director	12/10/03
(b)(6)	Study Monitor	12-10-03

Ion Chromatograph Maintenance

Date	Initials	Checked Eluent	Replaced Eluent with Fresh	Checked Post Column Reagent (PCR)	Replaced PCR with Fresh	Checked Water Reservoirs	Refilled water Reservoirs	Checked Baseline	Flushed System with 45mM Na_2CO_3

000080

Ion Chromatography Reagent Preparation

Eluent preparation

Lot Number assigned:_____(mo/day/year - ER - #)*

Procedure	Batch No.____ Completed (check)	Batch No.____ Completed (check)
Add 18.0 mL of 0.5 M sodium carbonate to 1 L Vol. Flask		
Dilute to 1 L with He purged reagent water		
Label with name, concentration, storage and expiration date		
Date:	**Initials**	**Initials**

Post Column Reagent

Lot number assigned:_____(mo/day/year - PCR - #)*

Balance ID: _____Standardized before use?___yes___no.

Procedure		Batch No.____ Completed (check)	Batch No.____ Completed (check)
Fill 500 mL vol flask with 300 mL reagent water	Check		
Add 40 mL of 70% redistilled nitric acid	Check		
Add 2.5 g KBr	Enter wt.		
Dissolve with stirring, 250 mg of o-dianisidine dihydrochloride in 100 mL methanol	Enter wt.		
Add dianisidine/methanol to nitric acid/KBr mixture	Check		
Dilute to 500 mL with reagent water	Check		
Date:		**Initials:**	**Initials:**

Reagent Name	Manufacturer	Lot Number	Expiration Date
Nitric Acid			
Potassium Bromide			
o-dianisidine			

* Assign a lot number with the date of preparation. The # would be the batch made that day starting with 1.

000081

Submission End