colorMaker®

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January 15,, 2007

ORIGINAL

Robert L. Pooler
National Organic Program, AMS / USDA
STOP 0268 – Room 4008S
1400 Independence Avenue SW
Washington, DC 20250-0268

Re: Petitions for the Addition of
Non-Organic Agricultural Substances to the National List
Pursuant to Section 205.606 of the NOP

Dear Mr. Pooler:

Thank you for your letter, dated December 20, 2006, wherein you returned our original "combined" petition for fifteen natural colorants (dated October 16, 2006) and instructed us to file fifteen "separate" petitions, one for each colorant.

Pursuant to your instructions, please find enclosed with this letter fifteen (15) separate petitions, one for each natural colorant. We enclose an original and one copy of each petition for you to review. We ask the National Organic Standards Board (NOSB) to add onto the National List the following natural colorants:

***Anthocyanins***: (1) chokeberry juice, (2) black currant juice, (3) red cabbage extract, (4) purple carrot extract, (5) elderberry juice, (6) grape juice, (7) grape skin extract, (8) red radish extract; and

***Carotenoids***: (9) annatto seed extract, (10) beta-carotene from carrots, (11) lycopene, (12) paprika, (13) saffron; and

***Betalains***: (14) beet juice; and

***Other***: (15) turmeric.

You may recall that our original petition was organized by the four categories shown above. It may be prudent -- in the interest of time -- for the NOSB to consider the enclosed petitions in these same categories / groups.

DDWilliamson
Color With Confidence

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# Petition for the Addition of
# A Non-Organic Agricultural Substance to the National List
# Pursuant to Section 205.606 of the NOP

1. The substance's common name: Annatto seed extract, the extract from the seed coat of *bixaceae bixa orellana* (family, genus, species). This agricultural substance is also commonly known as "annatto."

2. The producer's name, address and telephone number: There are limited numbers of producers of annatto seed extract, among them are:

2.1 DD Williamson Colors, Inc.
815 Sunset Road
Port Washington, WI 53074
United States
(262) 268-7272

2.2 Kalsec, Inc.
PO Box 50511
Kalamazoo, MI 49005-0511
United States
(269) 349-9711



3. The intended or current use of the substance: Annatto seed extract is used as a natural color additive in baked goods, beverages, candies, condiments, dairy products, desserts, jams & gelatins, pet foods, snack foods, soups & sauces, and compressed tablets. Its usage as a color additive exempt from certification is permitted by the US Food & Drug Administration (the "FDA") under 21 CFR 73.

The use of annatto seed extract as a natural color additive supports and promotes the organic philosophy because an all-natural, agricultural product is being used to improve the visual appeal of organic food and beverage products, rather than an artificial color such as FD&C Red 40. Thus, annatto seed extract may advance the organic movement by helping organic producers present to consumers a wide variety of organic food and beverage products with dynamic visual appeal.

4. A list of handling activities for which the substance will be used: Annatto seed extract develops a deep orange to light yellow color in organic food and beverage products, depending upon its concentration. It is used as a natural color additive to enhance the visual appeal of organic products. The color, itself, results from very high concentrations of carotenoid molecules in the annatto seeds.

5. The source of the substance and a detailed description of its manufacturing or processing procedures: Annatto seeds grow on small trees in South America and Africa. The annatto seeds are collected, hulled, and crushed into small pieces, which are physically ground against one another in vegetable oil. The oil is filtered and concentrated, producing a dark, viscous, orange liquid concentrate composed of the same carotenoid molecules produced inside the annatto seeds.

6. A summary of any available previous reviews by State or private certification programs or other organizations of the petitioned substance: There are many government reviews of annatto seed extract, and carotenoids in general (particularly from carrots) have been used since antiquity to color human foods. Carotenoids are therefore Generally Regarded As Safe (GRAS).

7. Information regarding EPA, FDA, and State Regulations: FDA permits the use of annatto seed extract as a color additive exempt from certification. 21 CFR 73.30 Annatto. Annatto seed extract is also permitted as a natural color additive in foods in the European Union (E160(b)) and throughout Asia.

8. The Chemical Abstract Service (CAS) number: There exists a specific CAS Number for annatto seed extract. It is 1393-63-1. Further, carotenoids in general have been assigned CAS No. 7235-40-7.

9. The substance's physical properties and chemical mode of action: The carotenoids extracted from annatto seeds are distinct and unique molecules. They are different from anthocyanins and betalains (other molecules used as natural colorants). Carotenoids are sensitive to light and heat, degrading rapidly under high heat and/or direct sunlight. In addition, carotenoids display very strong antioxidant properties which now appear beneficial to human health. Beyond these unique properties, carotenoids do not interact with substances used in organic food production and have no impact on the environment.

Annatto seeds have been consumed for centuries and their growth and ultimate consumption has the exact same impact on the environment as organically grown, bio-degradable fruits and vegetables.

10. Safety information about the substance: Please see the attached Material Safety Data Sheet (MSDS). Annatto seeds, and the carotenoids extracted from annatto seeds, are GRAS.

11. Research information about the substance: See the attached Bibliography. There are several leading researchers on carotenoids in the US including Carol Locey (Kalsec, Inc.), Minhthy L. Nguyen (formerly of Ohio State University, Columbus), and Steven J. Schwartz (Ohio State University, Columbus).

12(G) Justification Statements:

*Enhanced Visual Appeal Using Natural Colorants*. Food safety dictates that processed foods must be fully cooked to assure low bacterial counts for extended shelf-life and broad geographical distribution. Many food and beverage processors also employ a low pH environment and/or low water activity and/or or low temperature distribution of the finished product (refrigeration or freezing) to further assure minimal bacterial counts. These processing parameters are challenging to colorants residing inside the "core food" (for example, chlorophyll inside florets of broccoli, beta-carotene inside cut carrots, or anthocyanins inside strawberry preserves).

The addition of natural colorants compensates for the "original" colorants destroyed by high temperature / low pH processing. In so doing, the finished organic food or beverage product presents to the consumer the same visual appeal it would have if it were fresh. The addition of natural colorants can also enhance an existing color, making the organic food or beverage even more appealing; or it may extend the shelf-life of an organic food or beverage, making it available to more consumers both over time and geographical distance.

Without the addition of natural colorants, organic food and beverage products might lack the visual appeal and attraction of their direct non-organic competition. Thus, natural colorants help organic processors compete.

In so doing, natural colorants advance the organic philosophy by (literally) displaying to consumers visually appealing organic food and beverage products brightly colored without artificial colors such as FD&C Yellow 5.

*Low Usage Levels of Natural Colorants*. Because natural colorants are concentrated and very strong, they are used in organic food and beverage products at very low levels, typically less than 1%. The inherent strength of natural colorants sets in motion or "triggers" two distinct events: (1) natural colorants always fall under the 95 / 5 rule where five percent of the ingredients in an organic product may be non-certified; and (2) the volume of natural colorants purchased is very small.

By way of example, a hypothetical organic dairy develops organic certified yogurt. First, new product developers add annatto seed extract at 0.5% of the formula. They do not actively seek out organic certified annatto seed extract because they know the ingredient easily falls under the 95 / 5 rule. Second, the dairy's new product is successful and within the first year it produces 500 tons of organic certified yogurts. Despite such success, the dairy would purchase only 833 lb of annatto seed extract per month. This low volume of natural colorant sales, combined with inclusion of natural colorants in the "five percent non-certified" portion of the formula, provides little or no economic incentive to certify natural colorants as organic.

In the future, we anticipate the total amount of organic food and beverage products to increase. We may reach a point in time where a strong economic incentive places natural colorant crops under organic systems of production. It should be noted that no ingredient may remain on the National List for more than five (5) years without review by the National Organic Standards Board (NOSB).

The NOSB must therefore review the status of natural colorants five years hence (roughly 2012) and, at that time, may discover that an adequate supply of natural colorants is available for use in organic foods and beverages.

*International Production of Natural Colorants*. Most natural colorants are derived from International fruit and vegetable crops grown in developing countries; there is little International acreage certified organic. Most international organic acreage is utilized for corn, sugar and grains. Further, organic certification of International acreage remains problematic, plagued by cultural, financial, and language difficulties. Moreover, most fruit and vegetable crops are typically consumed where they are grown. As a result, there is a limited supply of the requisite fruit and vegetable crops needed for the creation of natural colorants.

Thus, natural colorants are not available in the appropriate quantity from International sources to meet the needs of organic processors.

*Domestic Production of Natural Colorants / The Current State of the US Organic Industry*. Certified organic cropland and pasture accounted for about 0.5% of total US farmland in 2005. Only a small percentage of top US field crops – corn (0.2%), soybeans (0.2%), and wheat (0.5%) – were grown under certified organic farming systems. Organic carrots (6% of the US carrot acreage), organic lettuce (4% of US lettuce acreage), and organic apples (3% of US apple acreage) were more commonly grown organic.

Markets for organically grown fruits and vegetables have been developing for decades in the US, and fresh produce is still the top-selling organic category in retail sales. Organic livestock was beginning to catch up with produce in 2005, with 1% of US dairy cows and 0.6% of the layer hens managed under certified organic systems. After decades of strong growth, the US organic marketplace is a bountiful "Farmers' Market" for consumers, but it does not supply the appropriate quantity of natural colorants for organic food processors.

Because there is no current supply of organic certified natural colorants from International sources, and because there is no current supply of organic certified natural colorants from US sources, and because natural colorants at levels below 5% greatly improve the visual appearance of organic foods and beverages, this Petition seeks the addition of natural colorants to the National List.

13. This Petition respectfully seeks the addition of annatto seed extract, a.k.a. "annatto seed," to the National List as a non-organic agricultural product under Section 205.606 of the NOP.

Respectfully Submitted,

COLORMAKER, INC.,
a California corporation

By: ______________________

Stephen D. Lauro
(Name & Title)

DD WILLIAMSON, INC.
a Kentucky corporation

By: Margaret A. Lawson

Margaret A. Lawson
(Name & Title)
VP Science & Innovation

ColorMaker, Inc.
3309 East Miraloma Ave., Suite 105
Anaheim, California 92806
(714) 572-0444
(714) 572-0999 *fax*

*inquire@colormaker.com*

**Hazard rating at a glance**
0-least, 1-slight, 2-moderate, 3-high, 4-extreme

| | |
|---|---|
| HEALTH | 0 |
| FLAMMABILITY | 0 |
| REACTIVITY | 0 |

# MATERIAL SAFETY DATA SHEET

## 1. *Product Identification:*

1.1 Product Name: Standard Annatto Seed Extract

1.2 Product Number: 2741

1.3 Ingredient Statement: Annatto

1.4 Description of Product: An orange liquid designed to color and function in food and beverage products. Specific formulation is withheld as a trade secret pursuant to 21 CFR 20.61. The characterizing principles and/or other components of this color blend are approved and are in compliance with 21 CFR 73. None of the ingredients appear on the list of hazardous items established under California's Proposition 65.

## 2. *Hazardous Ingredients and Exposure Limits:*

2.1 It is our opinion that the above named product does not meet the definition of a "Hazardous Chemical" as defined in 21 CFR 1910.1200. This MSDS is provided as general information for health and safety reasons.

## 3. *Health Hazard Data*

| | | |
|---|---|---|
| 3.1 | Carcinogenic | None known. |
| 3.2 | Acute Toxicity | None known. |
| 3.3 | Oral LD50 | Not determined. |
| 3.4 | Dermal LD50 | Not determined. |
| 3.5 | Ingestion | None known. |
| 3.6 | Skin Contact | None known. |
| 3.7 | Irritation (skin) | None known. |
| 3.8 | Irritation (eye) | May cause slight irritation. |

## 4. *First Aid Measures*

| | | |
|---|---|---|
| 4.1 | Eye Contact | Remove contact lenses and flush eyes with copious amount of water for at least fifteen minutes. Contact physician if irritation persists. |
| 4.2 | Skin Contact | No significant health hazard. Wash exposed skin with soap and water for at least fifteen minutes. If irritation persists, consult a doctor. |
| 4.3 | Ingestion | Administer 1 - 2 glasses of water or milk to dilute. DO NOT INDUCE VOMITING. Seek medical attention if it seems advisable. |

## 5. *Fire Fighting Measures*

| | | |
|---|---|---|
| 5.1 | Flash Point (method used) | Not determined. |
| 5.2 | Flammable Limits | Not determined. |
| 5.3 | Unusual Fire & Explosion Hazard | None known. |
| 5.4 | Extinguishing Media | Carbon dioxide, dry chemical, foam, and water spray. |

## 6. *Spill, Leak, and Waste Disposal*

6.1 Absorb spills on vermiculite or other absorbent materials. Remove to approved disposal containers. Use rag and mop to clean small spots or dilute with large amounts of water. Colorant is biodegradable.

## 7. *Handling and Storage*

7.1 Store in a cool dry area. The wearing of rubber gloves and safety glasses to prevent skin and eye contact is recommended. Store in tightly closed containers.

## *8. Exposure Protection*

| | | |
|---|---|---|
| 8.1 | Respiratory | No special equipment under normal conditions of use. |
| 8.2 | Skin | Skin protection appropriate to use conditions. |
| 8.3 | Eye | Safety glasses must be worn at all times |
| 8.4 | Hand | Suitable gloves. |
| 8.5 | Other | None |

## *9. Physical / Chemical Characteristics*

| | | |
|---|---|---|
| 9.1 | Appearance | Orange liquid |
| 9.2 | Boiling Point | Not established |
| 9.3 | Vapor Pressure | Not established |
| 9.4 | pH value | 7.0 |
| 9.5 | Solubility in Water | Complete |
| 9.6 | Specific Gravity | To be established |

## *10. Stability and Reactivity*

| | | |
|---|---|---|
| 10.1 | Stability | Stable. |
| 10.2 | Incompatibility | Avoid strong oxidizing agents. |
| 10.3 | Hazardous Decomposition | Not known. |
| 10.4 | Hazardous Polymerization | Not known. |

## *11. Toxicological Health Hazards*

11.1 None known. Colorant is naturally derived and biodegradable.

## *12. Ecological Effects*

12.1 None known. Colorant is naturally derived and biodegradable

## *13. Disposal Considerations*

13.1 Incineration. Observe local, State, and Federal regulations concerning health and the environment. Do not incinerate in sealed containers.

*The information contained herein is based upon data considered accurate and reliable. Nevertheless, an independent investigation and verification of this information should be made by the user. No warranty is made, expressed or implied, regarding the accuracy or correctness of these data. The use of this information and this product are beyond the control of ColorMaker, Inc. Therefore, it is the sole responsibility of the user to determine the conditions necessary for the safe use of this product.*

# Bibliography

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Dean, Jenny, Wild Color; Watson – Guptill, Publishers, Copyright 1999.

Delgado-Vargas, Francisco, and Paredes-Lopez, Octavio, Natural Colorants for Food and Nutraceutical Uses; CRC Press, Publishers; Copyright 2003.

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Greenfield, Amy Butler, A Perfect Red; Harper Collins, Publishers; Copyright 2005.

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Marmion, Daniel M., Handbook of US Colorants; John Wiley & Sons, Publishers; Copyright 1991.

**EVALUATION CRITERIA FOR SUBSTANCES ADDED TO THE NATIONAL LIST**

**Category 1. Adverse impacts on humans or the environment?** Substance – ANNATTO SEED EXTRACT

| Question | Yes | No | N/A | Documentation (TAP; petition; regulatory agency; other) |
|---|---|---|---|---|
| 1. Are there adverse effects on environment from manufacture, use, or disposal? [§205.600 b.2] | | X | | Petition; FDA regulations |
| 2. Is there environmental contamination during manufacture, use, misuse, or disposal? [§6518 m.3] | | X | | Petition; FDA regulations |
| 3. Is the substance harmful to the environment? [§6517c(1)(A)(i);6517(c)(2)(A)i] | | X | | Petition; FDA Regulations |
| 4. Does the substance contain List 1, 2, or 3 inerts? [§6517 c (1)(B)(ii); 205.601(m)2] | | | X | |
| 5. Is there potential for detrimental chemical interaction with other materials used? [§6518 m.1] | | X | | Petition; FDA Regulations |
| 6. Are there adverse biological and chemical interactions in agroecosystem? [§6518 m.5] | | X | | Petition; FDA Regulations |
| 7. Are there detrimental physiological effects on soil organisms, crops, or livestock? [§6518 m.5] | | X | | Petition; FDA Regulations |
| 8. Is there a toxic or other adverse action of the material or its breakdown products? [§6518 m.2] | | | X | |
| 9. Is there undesirable persistence or concentration of the material or breakdown products in environment?[§6518 m.2] | | X | | Petition; FDA Regulations |
| 10. Is there any harmful effect on human health? [§6517 c (1)(A)(i) ; 6517 c(2)(A)i; §6518 m.4] | | X | | Petition; FDA Regulations |
| 11. Is there an adverse effect on human health as defined by applicable Federal regulations? [205.600 b.3] | | X | | Petition; FDA Regulations |

| | | | | |
|---|---|---|---|---|
| 12. Is the substance GRAS when used according to FDA's good manufacturing practices? [§205.600 b.5] | X | | | Petition; FDA Regulations |
| 13. Does the substance contain residues of heavy metals or other contaminants in excess of FDA tolerances? [§205.600 b.5] | | X | | Petition; FDA Regulations |

1 If the substance under review is for crops or livestock production, all of the questions from 205.600 (b) are N/A—not applicable.

**Category 2. Is the Substance Essential for Organic Production?** Substance – ANNATTO SEED EXTRACT

| Question | Yes | No | N/A | Documentation (TAP; petition; regulatory agency; other) |
|---|---|---|---|---|
| 1. Is there a natural source of the substance? [§205.600 b.1] | | | X | |
| 2. Is there an organic substitute? [§205.600 b.1] | | X | | Petition |
| 3. Is the substance essential for handling of organically produced agricultural products? [§205.600 b.6] | | | X | |
| 4. Is there a wholly natural substitute product? [§6517 c (1)(A)(ii)] | | | X | |
| 5. Is the substance used in handling not synthetic, but not organically produced? [§6517 c (1)(B)(iii)] | X | | | Petition; FDA Regulations |
| 6. Is there any alternative substances? [§6518 m.6] | | X | | Petition; FDA Regulations |
| 7. Is there another practice that would make the substance unnecessary? [§6518 m.6] | | | X | |

1 If the substance under review is for crops or livestock production, all of the questions from 205.600 (b) are N/A—not applicable.

**Category 3. Is the substance compatible with organic production?** Substance – ANNATTO SEED EXTRACT

| Question | Yes | No | N/A | Documentation (TAP; petition; regulatory agency; other) |
|---|---|---|---|---|
| 1. Is the substance compatible with organic handling? [§205.600 b.2] | | | X | |
| 2. Is the substance consistent with organic farming and handling? [§6517 c (1)(A)(iii); 6517 c (2)(A)(ii)] | X | | | Petition; FDA Regulations |
| 3. Is the substance compatible with a system of sustainable agriculture? [§6518 m.7] | X | | | Petition; FDA Regulations |
| 4. Is the nutritional quality of the food maintained with the substance? [§205.600 b.3] | | | X | |
| 5. Is the primary use as a preservative? [§205.600 b.4] | | X | | |
| 6. Is the primary use to recreate or improve flavors, colors, textures, or nutritive values lost in processing (except when required by law, e.g., vitamin D in milk)? [205.600 b.4] | | | X | |
| 7. Is the substance used in production, and does it contain an active synthetic ingredient in the following categories: | | | | |
| a. copper and sulfur compounds; | | | X | |
| b. toxins derived from bacteria; | | | X | |
| c. pheromones, soaps, horticultural oils, fish emulsions, treated seed, vitamins and minerals? | | | X | |
| d. livestock parasiticides and medicines? | | | X | |
| e. production aids including netting, tree wraps and seals, insect traps, sticky barriers, row covers, and equipment cleaners? | | | X | |

1 If the substance under review is for crops or livestock production, all of the questions from 205.600 (b) are N/A—not applicable.

SENSIENT®

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December 21, 2006

National Organic Standards Board
c/o Robert Pooler,
Agricultural Marketing Specialist
USDA/AMS/TM/NOP
Room 4008-So., Ag Stop 0268
P.O. Box 96456
Washington, D.C. 20090-6456

Dear Mr. Pooler,

Please accept our petition for Annatto Extract, Water Soluble to be added to National List Section 205.606 for review by the National Organic Program (NOP) and the National Organic Standards Board (NOSB). Enclosed are two copies each of two versions, one with Confidential Business Information included and one with Confidential Business Information deleted.

The petition has been completed compiling the best available instructional information and guidance, using the NOP instructions for filing a petition published in the Federal Register, July 13, 2000, additional information as provided by NOP in the Federal Register notice of June 7, 2006, as well as insight based on the October 19, 2006 NOSB recommendation for the determination of commercial availability. We will be glad to provide any additional information required that is not already included in this petition.

Thank you for your consideration and please do not hesitate to contact us with any questions of content or completeness at the address listed above or by phone at 800-325-8110, ext. 7316, or by e-mail at sueann.mcavoy@sensient-tech.com.

Sincerely,

SueAnn Bobcock McAvoy

Sue Ann McAvoy
Manager, Regulatory Compliance
Sensient Colors Inc.

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2006 DEC 22 A 11: 21

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## CBI Deleted Version Notes:

This version contains:

CBI Deleted Version of the Petition for Annatto Extract, Water Soluble

CBI Deleted version of List of Attachments

CBI Attachments #1 through #8, #11 and #12

Please note: Attachments #1 through #8, #11 and #12 are included as part of the CBI Deleted version.

Attachments #9 and #10 are included with the CBI Copy version.

Thank you for your understanding.

# CBI Deleted Version

**Petition to the National Organic Program and**
**National Organic Standards Board**
**for Annatto Extract, Water Soluble**
**to be Added to National List Section 205.606**

**Information to be included in a National List petition is based on the Federal Register notice dated July 13, 2000.**

## Item A

This is a petition to amend the National List to include **Annatto Extract, Water Soluble** as an nonorganically produced agricultural product allowed in or on processed products labeled as "organic" §205.606.

## Item B

**1. Common Name**: Annatto Extract, Water Soluble

Other Names: Alkaline Annatto Extract
Norbixin Annatto Extract
Sensient Annatto Extract Liquid W.S.



**2. Manufacturers' Name, Address and Telephone**

Sensient Colors, Inc.
2526 Baldwin Street
St. Louis, MO 63106

Contact: Sue Ann McAvoy
Manager, Regulatory Compliance
Email: SueAnn.McAvoy@sensient-tech.com

**3. The intended or current use of the substance**

Annatto Extract, Water Soluble is currently used as a color additive in a variety of organic food products, allowed as "Colors, non-synthetic sources only", an allowed non-synthetic ingredient as listed on the National List §205.605(a)(5).

As a result of the National Organic Standards Board recommendation made October 19, 2006, to not renew Colors after the sunset of the National List in October 2007, Annatto Extract, Water Soluble must now be petitioned for placement on the National List as an individual color. Annatto Extract meets the current definition of an agricultural substance therefore this petition is to place Annatto Extract as an allowed non-organic agricultural ingredient under §205.606 until such time as an organic form of this product becomes commercially available in the form, quality and quantity needed to fulfill the demands of the organic industry.

Annatto Extract, Water Soluble is used in many organic products including dairy, baked goods, beverages, cereals, fruit products and more. Annatto Extract provides the characteristic yellow-orange color in cheddar and colby cheeses. It also provides color in peach yogurts and fruit fillings and a golden to egg shade in some baked goods such as cookies, crackers and cereal. Annatto Extract is commonly used in ice cream to provide a range of colors from French vanilla to egg custard. It also provides the characteristic color of cheddar cheese in many macaroni and cheese products.

The usage levels of Annatto Extract, Water Soluble range from 0.005% to 0.1% in food products. The typical usage rate is 0.01%. The lower percentage range would be representative of the amount of color to produce French vanilla while the higher range for cheddar.

There is no restriction for the rate of Annatto Extract usage set by FDA as long as it is considered Good Manufacturing Practices and does not exceed rates needed to provide the intended coloring effect.

**4. A list of the crop, livestock or handling activities for which the substance will be used. If used for handling (including processing), the substance's mode of action must be described.**

Annatto Extract, Water Soluble is used in handling as a food color ingredient. It is used to color baked goods, cheese, cereal, ice cream, ice cream cones, sausage casing, spice blends, soups, dessert products, beverages, fats, oils, butter, processed cheeses, snack foods, and confectionery products.

**Mode of Action**

Annatto Extract, Water Soluble, adds a range of natural yellow to orange colors to the food products it is added to. The extract is soluble during the aqueous phase of formulation and mixes homogenously. The extract colors the aqueous phase of the food product replacing or enhancing the natural color of the product that may be lost in processing due to heat. The amount of Annatto Extract used is so small that no other intended or unintended quality results.

**5. The source of the substance and a detailed description of its manufacturing or processing procedures from the basic component(s) to the final product.**

Annatto Extract, Water Soluble is an extract from the seeds of the Bixa orellana L. shrub grown in tropical environments in Peru, Brazil, Costa Rica, Ivory Coast and other locations.

Its chemical formula is $C_{25}H_{30}O_4$.

The extracted color is found in the outer, resinous layer of the orange-red seeds. The color is naturally oil soluble.

Annatto Extract is exempt from certification by the Food and Drug Administration and its identity is standardized by FDA regulation found in CFR Title 21, §73.30, shown in detail in Item B, No. 7 below.

As with many color additives, Annatto Extract, Water Soluble is subject to Food and Drug Administration regulations under 21 CFR §73.30, which identifies substances allowed for use in extracting and preparing Annatto Extract, most of which are not permitted by the National

**CBI Deleted**

Organic Program. From that list, only those substances allowed on the National List would be used to produce the Annatto Extract, Water Soluble referred to by this petition.

**This petition is for Annatto Extract, Water Soluble that does not use any substances prohibited by NOP regulations.**

The manufacturing process begins post-harvest. The processes used are mechanical and are allowed by §205.270 of the National Organic Program Final Rule. In this example, the process is described for seeds grown in Peru.

**CBI Deleted**

**The Handbook of U.S. Colorants**, Third Edition, by Daniel M. Marmion, a Wiley-Interscience publication, published by John Wiley & Sons, Inc, includes a section on Annatto Extract with additional descriptive information, page 120-122. Please see Attachment #1.

**6. <u>A summary of any available previous reviews by State or private certification programs or other organizations of the petitioned substance.</u>**

Colors, non-synthetic sources only, were originally placed on the National List without review or NOSB recommendation in December 2000. In preparing for the National List sunset, the NOSB was provided with a technical advisory panel review dated October 2005, which is provided in attachment. Please see Attachment #2.

On October 19, 2006 the NOSB voted to allow Colors to sunset in October 2007. Consequently, it is necessary to submit this petition for the continued use of Annatto Extract, Water Soluble, as a previously allowed Color, non-synthetic sources only. There are no commercially available organic forms of Annatto Extract, Water Soluble that conform to NOP regulations as well as FDA regulations under 21 CFR §73.30.

Since there has never before been the requirement to determine commercial availability of Annatto Extract, Water Soluble, no certifiers have ever made such a determination.

Information about other forms of Annatto Extract sold as organic will be found in this petition, Item B No. 12, Petition Justification Statement.

Other reviews have been done of Annatto Extract, Water Soluble. They include the Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The evaluation of Annatto Extracts, January 8, 2005 may be found at:

http://jecfa.ilsi.org/evaluation.cfm?chemical=ANNATTO%20EXTRACTS&keyword=ANNATTO and is included. Please see Attachment #3.

JECFA also publishes an online Combined Compendium of Food Additive Specifications for Annatto Extract, Water Soluble and may be found at www.fao.org/ag/agn/jecfa-additives/details.html?id=860. Links are provided on this website to two monographs for Annatto Extracts (alkalai-processed norbixin, not acid-precipitated). Monograph 3 is the most recent, published at the $67^{th}$ JECFA meeting June 2006 in Rome. Monograph 3 is Attachment #4.

Canadian Organic Standards published September 2, 2006 include colors with the following condition:

§5.4.2.1 Colouring, natural, from non-synthetic sources only and shall not be produced using synthetic solvents and carrier systems or any artificial preservative.

The Official Journal of the European Union published on May 25, 2006

"amends Annex VI to Council Regulation (EEC) NO. 2092/91 on organic production of agricultural products and indication referring thereto on agricultural products and foodstuffs" includes Item 2. Section A(a), Subsection A.1 Food additives, which allows Code 160b Annatto, Bixin, Norbixin in the preparation of foodstuffs of animal origin, specifically in Red Leicester cheese, Double Gloucester cheese, Scottish cheddar and Mimolette cheese.

**7. Information regarding EPA, FDA, and State regulatory authority registrations, including registration numbers.**

Annatto Extract, Water Soluble is addressed under Code of Federal Regulations Title 21, revised April 1, 2005, Part 73 – Listing of Color Additives Exempt from Certification under §73.30:

TITLE 21--FOOD AND DRUGS

CHAPTER I--FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH AND HUMAN SERVICES
SUBCHAPTER A--GENERAL

PART 73 -- LISTING OF COLOR ADDITIVES EXEMPT FROM CERTIFICATION

Subpart A--Foods
Sec. 73.30 Annatto extract.
(a) Identity. (1) The color additive annatto extract is an extract prepared from annatto seed, Bixa orellana L., using any one or an appropriate combination of the food-grade extractants listed in paragraph (a)(1) (i) and (ii) of this section:

(i) Alkaline aqueous solution, alkaline propylene glycol, ethyl alcohol or alkaline solutions thereof, edible vegetable oils or fats, mono- and diglycerides from the glycerolysis of edible vegetable oils or fats. The alkaline alcohol or aqueous extracts may be treated with food-grade acids to precipitate annatto pigments, which are separated from the liquid and dried, with or without intermediate recrystallization, using the solvents listed under paragraph (a)(1)(ii) of this section. Food-grade alkalis or carbonates may be added to adjust alkalinity.

(ii) Acetone, ethylene dichloride, hexane, isopropyl alcohol, methyl alcohol, methylene chloride, trichloroethylene.

(2) Color additive mixtures for food use made with annatto extract may contain only diluents that are suitable and that are listed in this subpart as safe in color additive mixtures for coloring foods.

(b) Specifications. Annatto extract, including pigments precipitated therefrom, shall conform to the following specifications:

(1) Arsenic (as As), not more than 3 parts per million; lead as Pb, not more than 10 parts per million.

(2) When solvents listed under paragraph (a)(1)(ii) of this section are used, annatto extract shall contain no more solvent residue than is permitted of the corresponding solvents in spice oleoresins under applicable food additive regulations in parts 170 through 189 of this chapter.

(c) Uses and restrictions. Annatto extract may be safely used for coloring foods generally, in amounts consistent with good manufacturing practice, except that it may not be used to color foods for which standards of identity have been promulgated under section 401 of the act unless added color is authorized by such standards.

(d) Labeling. The label of the color additive and any mixtures prepared therefrom and intended solely or in part for coloring purposes shall conform to the requirements of 70.25 of this chapter. Labels shall bear information showing that the color is derived from annatto seed. The requirements of 70.25(a) of this chapter that all ingredients shall be listed by name shall not be construed as requiring the declaration of residues of solvents listed in paragraph (a)(1)(ii) of this section.

(e) Exemption from certification. Certification of this color additive is not necessary for the protection of the public health and therefore batches thereof are exempt from the certification requirements of section 721(c) of the act.

GRAS status is not available or necessary for Annatto Extract, Water Soluble as explained in FDA's **Guidance for Industry, Frequently Asked Questions About GRAS,** which notes the following question and answer:

"Is a substance that is used to impart color eligible for classification as GRAS?

The short answer is "No." Under section 201(s) of the Act, the GRAS provision applies to the definition of a food additive. There is no corresponding provision in the definition (in section 201(t) of the Act) of a color additive.

However, under section 201(t)(1) and 21 CFR 70.3(f), the term color additive means a material that is a dye, pigment, or other substance made by a process of synthesis or similar artifice, or extracted, isolated, or otherwise derived from a vegetable, animal, mineral, or other source, and that is capable (alone or through reaction with another substance) of imparting color when added or applied to a food; except that such term does not include any material which FDA, by regulation, determines is used (or intended to be used) solely for a purpose or purposes other than coloring. Under 21 CFR 70.3(g), a material that otherwise meets the definition of color additive can be exempt from that definition on the basis that it is used or intended to be used solely for a purpose or purposes other than coloring, as long as the material is used in a way that any color imparted is clearly unimportant insofar as the appearance, value, marketability, or consumer acceptability is concerned. Given the construct of section 201(t)(1) of the Act and 21 CFR 70.3(f) and (g), the use of a substance that is capable of imparting color may constitute use as both a color additive and as a food additive or GRAS substance. For example, beta-carotene is both approved for use as a color additive (21 CFR 73.95) and affirmed as GRAS for use as a nutrient (21 CFR 184.1245); in some food products, beta-carotene may be used for both purposes."

**8. <u>The Chemical Abstract Service (CAS) number or other product numbers of the substance and labels of products that contains the petitioned substance.</u>**

CAS numbers for Annatto Extract, Water Soluble is:

1393-63-1 Annatto Extract

These international code numbers may also identify Annatto:

CI #75120
EU #E160(b)
INS #160(b)

In the European Union, Annatto is known as E160(b) and is permitted in a variety of products.

Please see Attachment #5, the product label for Annatto Extract Water Soluble.

**9. <u>The substance's physical properties and chemical mode of action.</u>**

Please see Attachment #6, the Specification sheet for Annatto Liquid, Water Soluble.

<u>Annatto Extract Overview</u>

**History**

South, Central and North Americans have depended on Annatto for color since antiquity. The red of concentrated Annatto represented fire, the sun and blood - all images of majesty and power. The Mayans of what is now Mexico and Guatemala used Annatto, along with other colors, to identify points on the compass. Other indigenous civilizations painted their faces and bodies with Annatto for rituals and found it useful for coloring textiles, cosmetics and food. According to folklore, Annatto repels insects and is effective against colds and fevers. While it is commonly used today to color cheese, baked goods and snack foods, researchers are continuing to develop new applications.

Source

Annatto color originates from the evergreen shrub *Bixa Orellana*, named after Francisco de Orellana, historian and botanist for the conquistador Pizarro. The Annatto shrub - also known as the Lipstick Pod Tree - is found throughout Latin America, the Caribbean, India and some parts of Africa. Most of the world's supply comes from Peru, Ecuador, Brazil and Kenya. Other names for Annatto are Achiote, Rocou, Bija and Orlean.

Processing

At harvest, clusters of capsular fruit decorate the Annatto shrub's branches. The fruit, in the form of pods, contains between 25 and 50 seeds. Cultivators collect and dry the fruit, and then thresh the seeds from them. The seeds are coated with a paste-like oil containing Bixin pigment, a yellow-orange carotenoid. The classical methods for extracting this pigment use aqueous alkaline solutions and edible oils as solvents.

Vegetable oil extraction yields the Bixin pigment used for oil-based products such as butter, margarine and processed cheese.

Water based Annatto Extract occurs when annatto seeds are suspended and mechanically agitated in aqueous alkali (potassium or sodium hydroxide) to remove the pigment. Additional alkali is added to the resultant suspension, which is then heated to dissolve the pigment and cooled. This pigment provides the characteristic golden yellow color of cheddar cheese. In cheese production, Norbixin associates with the curd rather than the whey, making it the color product preferred by cheese producers.

**Chemical interactions with other substances, especially substances used in organic production**

The stability of Annatto varies, depending on the media, and should be tested for each application. Some factors to consider include:

Light - Annatto will fade upon prolonged exposure to strong, direct light.

Acid/Alkali - Annatto is susceptible to chemical change in acidic media where aqueous alkaline solutions of Norbixin will precipitate. As Norbixin precipitates, it can combine with proteins, starches and other ingredients and produce inconsistency of color in finished products. On the other hand, Annatto is very stable in alkaline systems.

Oxidation - Annatto colors are susceptible to degradation by oxidation.

Microbial Attack - Annatto colors are resistant to microbial attack.

Annatto Extract, Water Soluble will precipitate in acidic systems with a pH less than ~4.0. Sometimes this is used as an advantage. For example, if a customer wants to color fruit filling.

Annatto Extract Water Soluble will bind to free calcium to form an insoluble precipitant.

**Toxicity and environmental persistence**

There is no evidence of toxicity from the processing of Annatto Extract, Water Soluble. After harvest and extraction, the remaining Annatto seeds may be composted. In Canada, where seeds were extracted in the past, a certificate of approval from the Ministry of the Environment was obtained for use of the spent seeds in urban leaf composting programs. The evaluation prior to approval was done by a consultant, Compost Management, in Elora, Ontario, Paul Taylor, President (ph 519-846-8317), who knew what was required for ministry approval. Mr. Taylor in turn, had samples of seeds sent to Woods End Research Lab in Mt. Vernon, Maine (ph 207-293-2457) where the seed was analyzed for composting suitability.

In 1992 approval from the Canadian Ministry of the Environment was received and Annatto seeds have been approved for composting ever since. The seeds are blended with the leaf compost in large windrows turned with bulldozers. The Woods End Research Lab recommended a 1:1 mix of leaf to spent seed for compost efficacy.

**Environmental impacts from its use or manufacture**

There is no documented evidence of environmental impact from the use or manufacture of Annatto Extract, Water Soluble. There is although an environmental and agricultural benefit from the production of Annatto seed. The seeds are contained in the fruit of the wild grown and cultivated shrubs, depending on geographic location. Some farmers plant Annatto producing shrubs as wind barriers. The harvest does not damage the shrubs. The fruit is harvested and the seed removed. After harvest, the plant survives and produces another harvest each following year.

**Effects on human health**

A search of the Food Safety Risk Analysis Clearinghouse for risk and safety assessment of food and color additives did not produce any results directly related to Annatto, instead referred back to regulations in FDA 21 CFR for specific color additives.

Annatto as a color additive has been evaluated by USDA, FDA, CODEX and JECFA and is not considered to be classified as a known allergen.

**Effects on soil organisms, crops, or livestock**

Annatto seed, crop or processing waste is not fed to livestock without neutralizing its pH as its high alkalinity would degrade living animal tissue. It is acceptable as a livestock feed additive if its alkalinity is lowered.

**10. Safety information about the substance including a Material Safety Data Sheet (MSDS) and a substance report from the National Institute of Environmental Health Studies.**

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A Material Safety Data Sheet for Annatto Extract Liquid W.S. is Attachment #7.

**Regulatory information: EPA/NIEHS/Other Sources**

In 1998 The National Institute of Environmental Health Sciences National Toxicology Program (NTP) under EPA published a Request for Comments on Chemicals Nominated to the NTP for Toxicology Studies- Recommendations by the Interagency Committee for Chemical Evaluation and Coordination (ICCEC) for Study, No Studies, or Deferral to Obtain Additional Information. A total of 16 substances were nominated including Annatto. No information could be found as a result of this request.

**11. Research information about the substance, which includes comprehensive substance research reviews and research bibliographies, including reviews and bibliographies, which present contrasting positions to those presented by the petitioner in supporting the substance's inclusion on or removal from the National List.**

During the NOP public comment period prior to the April 2006 NOSB meeting, the Organic Materials Review Institute submitted a comment on the NOSB recommendation to renew Colors, non-synthetic sources only, during the National List sunset review. The comment opposed the categorical renewal.

OMRI also mentioned a process by which Annatto could be used as an ingredient in an organic product, which is exactly the process used to produce Annatto Extract, Water Soluble.

"Various organic products certified under the National Organic Program use organic annatto as an ingredient. When extracted with a 5% solution of potassium hydroxide, seeds from organically produced achiote would meet NOP standards to be labeled 'organic annatto,' which could in turn be used in a processed product labeled as 'organic.'"

Please see Attachment #8, the OMRI public comment.

As justification for this petition to place Annatto Extract, Water Soluble on National List §205.606, we have done considerable research into the commercial availability of organic forms of Annatto.

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Our general research included information found in the following resources:

The Handbook of U.S. Colorants, Third Edition, by Daniel M. Marmion, a Wiley-Interscience publication, published by John Wiley & Sons, Inc. As noted previously, relevant text is attached.

Natural Food Colorants, Science and Technology, edited by Gabriel J. Lauro, California State Polytechnic University, Pomona, California and Jack Francis,
University of Massachusetts, Amherst, Massachusetts, Chapter 6 – Annatto. Please see Attachment #11.

The Sensient Colors, Inc. corporate website, http://www.sensient-tech.com/food_sku_processed/annatto_extract_overview.htm also provides a thorough overview of the history, source, processing, regulatory status, solubility, and stability of Annatto Extract.

## 12. Petition Justification Statement

Natural colors used in food processing have a long history of use and consumer acceptance. Many of the qualities consumers take for granted in the food they buy everyday are expected and relied upon for consistency and comfort. Color is one of those qualities.

Organic consumers, too, expect the familiarity of the appearance of their favorites foods. Cheddar cheese has always been available in its recognizable yellow hue, even though the milk from which it is made is naturally white. Macaroni and cheese, while available in White Cheddar varieties, is best known for its bright cheddar color. Peach yogurt is expected to be a peachy orange color.

Food processing, organic and conventional, uses heat to bring everyday products to market. Heat, along with oxidation in food preparation, change the natural color of fresh food, and while there is an expected and reasonable loss of the original ingredients' natural colors, finished processed products lack the appeal of food as it is naturally.

The addition of small amounts of concentrated extracts of natural food colors restore the inherent vibrancy of processed food and fulfills the expectation consumers have in the quality and appearance of products that are most familiar to them.

While there is an ongoing effort to develop certified organic colors for organic processed food, some colors are more difficult than others to produce organically and provide to the organic industry, the quality, quantity and form organic processors require.

The production of Annatto seed has a lesser-known but important impact on global, cultural and societal sustainability. The Andean Trade Preference Act of 1991, as part of the U.S. Trade Partnership with the Andean Countries, administered by the U.S. Department of Commerce, was enacted to combat drug production and trafficking in the Andean countries: Bolivia, Columbia, Ecuador and Peru. The program offers trade benefits to help these countries develop and strengthen legitimate industries. Expanded under the Trade Act of 2002, now called the Andean Trade Promotion and Drug Eradication Act, it provides duty-free access to U.S. markets for approximately 5,600 products. Annatto seeds come from Peru and Ecuador and are provided duty-free access under this act, thus providing an alternative to the agricultural production of illegal drugs that end up being exported to the United States.

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Until such time as an organic form is commercially available, we respectfully submit this petition for Annatto Extract, Water Soluble, to provide an NOP compliant natural color to the organic handling sector, to fill the gap created by the impending loss of the allowance of Colors, non-synthetic. Organic handlers will then be able to continue their production schedules, continue with proven formulations and continue to use existing labeling without interruption or damage to their business. Any damage to their businesses is damage to the whole organic industry.

While the procedural circumstances that made the sunset of Colors, non- synthetic, unfortunately inevitable, the silver (colored) lining is the incentive for companies like Sensient Colors, Inc. to develop organic colors. With approval of this petition, Annatto Extract, Water Soluble will only be used until such time as a commercially available organic Annatto Extract is introduced. Until then, organic consumers will continue to buy organic cheddar cheese and organic peach yogurt because they are products that live up to their expectations. And their trust in the organic label, which has grown out of integrity and familiarity, will be maintained with the consistency they expect from the USDA organic seal.

**13. <u>Commercial Confidential Information Statement</u>:**

CBI

# CBI Deleted Version

## Petition to the National Organic Program and National Organic Standards Board for Annatto Extract, Water Soluble to be Added to National List Section 205.606

<u>List of Attachments</u>

1. **The Handbook of U.S. Colorants**, Third Edition, by Daniel M. Marmion, a Wiley-Interscience publication, published by John Wiley & Sons, Inc, Section on Annatto Extract, page 120-122.

2. **Overview of Food Color Additives** Prepared for the USDA National Organic Program and the National Organic Standards Board, October 14, 2005

3. **Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on food Additives,** January 8, 2005

4. **Annatto Extracts (Alkali-Processed Norbixin, Not Acid-Precipitated) Monograph 3,** Prepared at the 67th JECFA 2006

5. **Product Label** for Sensient Colors, Inc. Annatto Extract Liquid, W.S.

6. **Specification Sheet** for Annatto Extract Liquid W.S

7. **Material Safety Data Sheet** for Annatto Extract Liquid W.S.

8. **Organic Materials Review Institute April 2006 Public Comment** to the NOSB recommendation to renew Colors, non-synthetic during Sunset of the National List

9. **CBI Deleted**

10. **CBI Deleted**

11. **Natural Food Colorants, Science and Technology**, edited by Gabriel J. Lauro, California State Polytechnic University, Pomona, California and Jack Francis, University of Massachusetts, Amherst, Massachusetts, Chapter 6 – Annatto

12. **Evaluation Criteria** for Substances Added to the National List, NOSB Decision Sheets

Attachment 1



# HANDBOOK OF U.S. COLORANTS

## Foods, Drugs, Cosmetics, and Medical Devices

Third Edition

DANIEL M. MARMION

A WILEY-INTERSCIENCE PUBLICATION
JOHN WILEY & SONS, INC.
New York • Chichester • Brisbane • Toronto • Singapore

kingdoms. Some, like β-carotene and zinc oxide, are essentially pure factory-produced chemicals of definite and known composition. Others, including annatto extract, cochineal extract, caramel and beet powder are mixtures obtained from natural sources and have somewhat indefinite compositions. Many of the additives included in Tables 1.1–1.4 are relatively unimportant as colorants and are only classified as such because of the loose definition of a color additive given in the 1960 amendments. Only the more important of the colorants are considered in detail here.

In general, exempt colorants have less coloring power than certified colorants and thus have to be used at higher levels. Some—particularly those of plant origin—tend to be less stable, more variable in shade, and therefore more complicated to use than certified colorants, and are more likely to introduce undesirable flavors and odors into the products in which they are incorporated. Also, depending on their nature and origin, exempt colorants can vary substantially in composition from batch to batch, are more likely to be contaminated with undesirable trace metals, insecticides, herbicides, and bacteria such as *Salmonella,* and can be more difficult to obtain in steady supply compared with certified colorants.

Exempt colorants are inherently neither more nor less safe than their certified cousins. However, some see them as having been obtained from nature ("natural") and, as such, imagine them as less of a health hazard than certified colorants. In fact, they, like all color additives, are fabricated products.

In the descriptions of exempt colorants which follow, Colour Index (CI), Chemical Abstract Service (CAS), and European Economic Community (EEC) identification numbers are included where known.

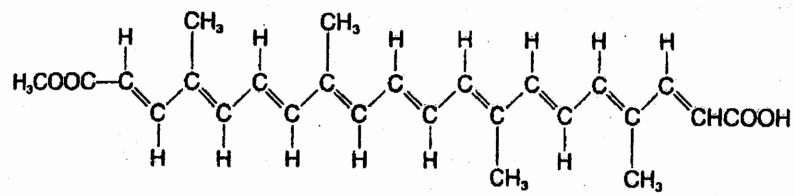
## ANNATTO EXTRACT

The annatto tree (*Bixa orellana)* is a large, fast-growing shrub cultivated in tropical climates, including parts of South America, India, East Africa, and the Caribbean. The tree produces large clusters of brown or crimson capsular fruit containing seeds coated with a thin, highly colored resinous coating or mark that serves as the raw material for preparation of the colorant known an annatto extract (CAS Reg. No. 8015-67-6).



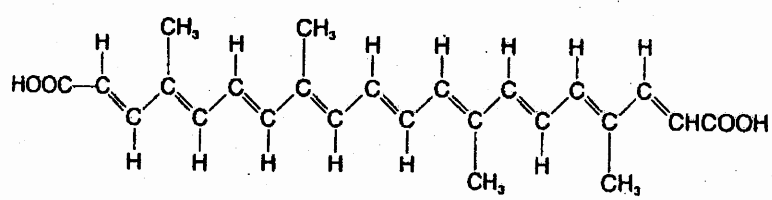
The colorant is prepared by leaching the annatto seeds with an extractant prepared from one or more approved, food-grade materials taken from a list that includes various solvents, edible vegetable oils and fats, and alkaline aqueous and alcoholic solutions. Depending on the use intended, the alkaline extracts are often treated with food-grade acids to precipitate the annatto pigments, which in turn may or may not be further purified by recrystallization from an approved solvent. Annatto extract is one of the oldest known dyes, used since antiquity for the coloring of food, textiles, and cosmetics. It has been used in the United States and Europe for over 100 years as a color additive for butter and cheese.

The chief coloring principle found in the oil or fat extracts of annatto seeds is the carotenoid bixin (CI Natural Orange 4, CI No. 75120, EEC No. E 160b), which is the monomethylester of the dicarboxylic acid norbixin:




BIXIN: $C_{25}H_{30}O_4$ (mw 394.51)

The major colorant in alkaline aqueous extracts is norbixin:




NORBIXIN: $C_{24}H_{28}O_4$ (mw 380.48)

Annatto extract is sold in several physical forms, including dry powders, propylene glycol/monoglyceride emulsions, oil solutions and suspensions, and alkaline aqueous solutions containing anywhere from 0.1–30% active colorant calculated as bixin or norbixin, as appropriate. It is used in products at levels of 0.5–10 ppm as pure color, resulting in hues ranging from butter-yellow to peach, depending on the type of color preparations employed and the product colored. Annatto extract's chief use is in foods such as butter, margarine, processed cheeses, non-dairy creamers, cooking oils, salad dressings, cereals, ice cream, ice cream cones, sausage casings, bakery goods, snack foods, and spices. It is often used in combination with turmeric.

The chemistry and performance of annato extract is essentially that of bixin, a brownish-red crystalline material that melts at 198°C. It is moderately stable toward light and has good stability toward oxidation, change in pH, and microbiological attack. Bixin is very stable toward heat up to 100°C, fairly stable at 100–125°C, and unstable above 125°C, where it tends to form 13-carbomethoxy-4,8-dimethyltridecahexane-oic acid.

Attachment 2

# OVERVIEW OF FOOD COLOR ADDITIVES
**Prepared for the USDA National Organic Program and the National Organic Standards Board**
**October 14, 2005**

This paper provides a general overview of color additives and how they are regulated in the United States. Use of colors in organic food production and potential adverse effects from the consumption of some specific colorants also are discussed.

## I. EXECUTIVE SUMMARY

Colors are defined as any dye, pigment, or other substance that can impart color to a food, drug, or cosmetic or to the human body. Colors are regulated in the United States by the U.S. Food and Drug Administration (FDA) and are categorized either as "certifiable" (those derived primarily from petroleum and known as coal-tar dyes) or "exempt from certification" (those obtained largely from mineral, plant, or animal sources). Currently, there are no GRAS ("generally recognized as safe") exemptions for color additives. Consequently, all color additives are subject to premarket approval requirements. To obtain approval from FDA for a new color additive, the manufacturer must submit a petition demonstrating the safety and suitability of the new color additive or new use. FDA is then responsible for evaluating the petition and determining whether the color additive is safe for human consumption. Additionally, the decision regarding batch certification is made during FDA's review of the petition. If required, a sample from each manufactured batch must be submitted to FDA for analysis and certification. With this regulatory process, color additives generally have a good safety record; however, some adverse reactions have been noted. Specifically, allergic effects to Yellow No. 5 and carmine and cochineal extract have been observed. Additionally, possible carcinogenic effects have led FDA to ban uses of FD&C Red No. 3 and FD&C Red No. 2.

## II. CHARACTERIZATION

Color additives are defined as any dye, pigment, or other substance that can impart color to a food, drug, or cosmetic or to the human body. Color additives include those that are white, black, and gray (Barrows et al., 2003). They also may include any chemical that reacts with another substance and causes formation of a color. In the United States, FDA is responsible for regulating color additives. For regulation purposes, FDA categorizes colors as "certifiable" (those derived primarily from petroleum and known as coal-tar dyes) and "exempt from certification" (those obtained largely from mineral, plant, or animal sources).

Certifiable colors can be further categorized into straight colors, mixtures, and dyes and lakes. Straight colors are those color additives that have not been mixed or chemically reacted with any other substance. Mixtures are the resulting color additives that are formed by mixing one color additive with one or more color additives or non-colored diluents, without a chemical reaction. Dyes are defined as those that "...dissolve in water and are manufactured as powders, granules, liquids or other special purpose forms. They can be used in beverages, dry mixes, baked goods, confections, dairy products, pet foods and a variety of other products" (FDA, 1993). Lakes are the water insoluble form of the dye. Lakes tend to be more stable than dyes and ideal for coloring products containing fats and oils or items lacking sufficient moisture to dissolve dyes. Some examples where lakes are used include coated tablets, cake and donut mixes, hard candies, and chewing gums. Additionally, certifiable colors that are added to food are chemically classified as azo, xanthene, triphenylmethane, and indigoid dyes.

## III. REGULATION

### A. History

Color additives were initially regulated in the United States under the U.S. Department of Agriculture's (USDA) Bureau of Chemistry. In 1906, the Food and Drugs Act was passed by Congress, which prohibited the use of poisonous or deleterious colors in confectionery and the coloring or staining of food to conceal damage or inferiority. In 1927, responsibility of the Food and Drugs Act was transferred to FDA. Increasing government oversight, the Federal Food, Drug, and Cosmetic Act (FFDCA) was passed in 1938 and established the three following categories for colors:

- **FD&C:** colors used in foods, drugs and cosmetics;
- **D&C:** colors used in drugs and cosmetics when in contact with mucous membranes or ingested; and
- **Ext. D&C:** colors used in products applied externally.

The FFDCA mandated a listing of those coal-tar colors that were determined to be "harmless and suitable" for use in foods, drugs, and cosmetics. FDA interpreted "harmless" to mean harmless at any level (Francis, 2000). Additionally, the FFDCA required the listing of new colors, mandated the previously voluntary certification program for batches of listed color with associated fees, and contained adulteration and misbranding provision for the use of coal-tar colors in food, drugs, and cosmetics (Barrows et al., 2003).

The Color Additive Amendments to the FFDCA were established in 1960 because FDA's interpretation of "harmless" was not workable. Under the Color Additive Amendments, "color additives" were defined and a requirement was established that only color additives (except coal-tar hair dyes) listed as "suitable and safe" for a given use could be used in foods, drugs, cosmetics, and medical devices. A current listing of FDA approved colorants, including those that do and do not require certification, is provided in Table 1 (Barrows et al., 2003). As illustrated in Table 1, all of these colorants are straight colors.

**Table 1. FDA Approved Food Color Additives**

| 21 CFR Section | Straight Color | Use and Restrictions |
|---|---|---|
| **Color Additives Subject To Certification** | | |
| 74.101 | FD&C Blue No. 1 | Foods generally |
| 74.102 | FD&C Blue No. 2 | Foods generally |
| 74.203 | FD&C Green No. 3 | Foods generally |
| 74.250 | Orange B | Casings or surfaces of frankfurters and sausages, NTE 150 ppm |
| 74.302 | Citrus Red No. 2 | Skins of oranges not intended or used for processing, NTE 2.0 ppm (by weight) |
| 74.303 | FD&C Red No. 3 | Foods generally |
| 74.340 | FD&C Red No. 40 | Foods generally |
| 74.705 | FD&C Yellow No. 5 | Foods generally |
| 74.706 | FD&C Yellow No. 6 | Foods generally |
| **Color Additives Exempt From Certification** | | |
| 73.30 | Annatto extract | Foods generally |
| 73.35 | Astaxanthin | Salmonid fish feed |
| 73.40 | Dehydrated beets (beet powder) | Foods generally |
| 73.50 | Ultramarine blue | Salt for animal feed |
| 73.75 | Canthaxanthin | Foods generally, NTE 30 mg/lb of solid or semisolid food or per pint of liquid food; broiler chicken feed; salmonid fish feed |
| 73.85 | Caramel | Foods generally |
| 73.90 | ß-Apo-8'-carotenal | Foods generally, NTE 15 mg/lb solid, 15 mg/pt liquid |
| 73.95 | ß-Carotene | Foods generally |
| 73.100 | Conchineal extract; carmine | Foods generally |
| 73.125 | Sodium copper chlorophyllin | Citrus-based dry beverage mixes, NET 0.2% dry mix |
| 73.140 | Toasted partially defatted cook cottonseed flour | Foods generally |
| 73.160 | Ferrous gluconate | Ripe olives |
| 73.165 | Ferrous lactate | Ripe olives |
| 73.169 | Grape color extract | Nonbeverage food |
| 73.170 | Grape skin extract (enocianina) | Still and carbonated drinks and ades; beverage bases; alcoholic beverages |
| 73.185 | Haematococcus algae meal | Salmonid fish feed |
| 73.200 | Synthetic iron oxide | Sausage casings, NTE 0.1% |

| 21 CFR Section | Straight Color | Use and Restrictions |
|---|---|---|
| | | (by weight); dog and cat food, NTE 0.25% (by weight) |
| 73.250 | Fruit juice | Foods generally |
| 73.260 | Vegetable juice | Foods generally |
| 73.275 | Dried algae meal | Chicken feed |
| 73.295 | Tagetes (Aztec marigold mean and extract) | Chicken feed |
| 73.300 | Carrot oil | Foods generally |
| 73.315 | Corn endosperm oil | Chicken feed |
| 73.340 | Paprika | Foods generally |
| 73.345 | Paprika oleoresin | Foods generally |
| 73.355 | Phaffia yeast | Salmonid fish feed |
| 73.450 | Riboflavin | Foods generally |
| 73.500 | Saffron | Foods generally |
| 73.575 | Titanium dioxide | Foods generally, NTE 1% (by weight) |
| 73.600 | Turmeric | Foods generally |
| 73.615 | Turmeric oleoresin | Foods generally |

The Color Additive Amendments also established the "Delaney Clause" that prohibited the listing of a color additive shown to be carcinogenic.

## B. Petition Process

Under the current regulatory system, FDA is responsible for ensuring the safety of new food additives, including colors. However, food additive petitions are not required for food additives that are identified as "generally recognized as safe" (GRAS) substances. Currently, there are no GRAS ("generally recognized as safe") exemptions for color additives. Consequently, all color additives are subject to premarket approval requirements. These requirements are listed in Title 21 of the Code of Federal Regulations (CFR), Part 71. In filing a color additive petition, the manufacturer is responsible for providing FDA with information including, but not limited to the following:

- Identification of the food additive;
- Physical, chemical, and biological properties;
- Chemical specifications;
- Manufacturing process description;
- Stability data;
- Intended uses and restrictions;
- Labeling[1];

[1] Any labeling that will be required by applicable provisions of the FFDCA on the finished food by reason of the use of the food additive.

- Tolerances and limitations[2];
- Analytical methods for enforcing chemical specifications;
- Safety studies; and
- Estimate of probable exposure.

## C. Safety Assessment

A color additive petition must demonstrate the safety and suitability of the new color additive or new use. FDA is responsible for evaluating petitions and determining whether the additive is safe for human consumption. Generally, this determination is made by examining the following parameters:

- History of use or natural occurrence;
- Consumption ratio, if applicable;
- Exposure levels;
- Inherent toxicity of the substance;
- Toxicological data on the substance or on structurally-related compounds; and
- Metabolism of the substance (either know of forecasted on the basis of data for structurally-related compounds).

FDA's safety assessment includes a review toxicity data such as the results of controlled animal studies. Ideally, a complete range of data, including short- and long-term toxicity studies, as well as studies that examine possible reproductive, carcinogenic, mutagenic, and sensitization characteristics of the color additive would be available for review. Sometimes a complete set of toxicology data is not available. One method of gaining additional insight on a color lacking a complete set of data is to evaluate the toxicity of structurally related substances. By evaluating structurally related substances, scientists can try to determine how the compound is absorbed, distributed, and metabolized within the body, and how it may act on target organs in the body. Based on these data and various safety factors, FDA determines a safe exposure level for the color additive.

FDA then compares the safe exposure level to the amount likely to be consumed in food taking into consideration the composition and properties of the substance and the proposed conditions of use. Because the absolute safety of any substance can never be proven, FDA must determine if the additive is safe under the proposed conditions of use, based on the best scientific knowledge available. For more information, see http://vm.cfsan.fda.gov/~dms/opa-cg8e.html.

[2] According to 21 CFR Part 571, "If the food additive is one for which a tolerance limitation is required to assure its safety, the level of use proposed should be no higher than the amount reasonably required to accomplish the intended physical or other technical effect, even though the safety data may support a higher tolerance."

## D. Batch Certification

As described in Section II, FDA requires certification of every manufactured batch of some color additives. Color additives requiring and exempt from batch certification are listed in Table 1.

Batch certification is required when the composition of the color needs to be controlled in order to protect public health. Procedures for color additive batch certification are available in 21 CFR Part 80. Under these procedures, a sample from each manufactured batch of certifiable color additive, as well as a "Request for Certification," must be submitted to FDA's Color Certification Branch. The "Request for Certification" should provide information regarding the batch weight, storage conditions, and the use for which it is being certified. FDA is then responsible for evaluating the batch's physical appearance and performing chemical analyses including, but not limited to the following:

- Purity (total color content);
- Moisture;
- Residual salts;
- Unreacted intermediates;
- Colored impurities other than the main color;
- Any other specified impurities; and
- Heavy metals (lead, arsenic, and mercury).

If the sample meets FDA's requirements, FDA will issue a certificate for the batch that identifies the color additive, batch weight, uses for which the color additive is certified, the name and address of the owner, as well as other information. The batch also is assigned a unique lot number.

Colors that are exempt from certification are usually derived from plant or mineral sources and must comply with the identity and purity specification and use limitation described in their listing regulations. According to 21 CFR 71.1(c)G, "If exemption from batch certification is requested, the reasons why it is believed such certification is not necessary (including supporting data to establish the safety of the intended use)." Consequently, a petition for exemption from certification must show why such certification is not necessary for the protection of public health (21 CFR 71.18). Color additives that are exempt from batch certification for one use may be subject to batch certification for other uses. Because natural colorants are exempt from a lengthy certification process, there has been a strong trend over the past 50 years toward the use of these color additives as compared to synthetic coal-tar dyes (Francis, 2000).

# IV. ADVERSE EFFECTS

Although food colors generally have a good safety record, some adverse reactions have been noted. For example, Yellow No. 5 (listed as tartrazine on medicine labels; a color found widely in beverages, desserts, processed vegetables, drugs, makeup, and many other products) causes itching or hives in a small population sub-group (FDA, 2001).

Another color that causes allergic reactions is carmine and cochineal extract. Carmine and cochineal extract are scarlet red pigments that come from the female coccid insect *Dactylopius coccus* var. Costa (family Dactylopiidae, superfamily Coccoidea), which is parasitic on several species of cacti, particularly the cochineal figs produced by prickly pear (Opuntia) cactus *Nopalea cochenillifera*. There have been several case reports of anaphylaxis and urticaria resulting from ingestion of food or drink containing carmine (Beaudouin et al., 1995; Baldwin et al., 1997; DiCello et al., 199a,b; Chung et al., 2001).

In 1960, FDA banned uses of FD&C Red No. 3 including cosmetics and externally applied drugs because large amounts of the color caused thyroid tumors in male rats (FDA, 2001). In 1976, FDA issued a ban on FD&C Red No. 2 because there appeared to be a statistically significant increase in malignant tumors when fed high doses of the color (FDA, 2001).

## V. USE OF COLORS IN ORGANIC FOODS

Colors are currently on the National List of Allowed and Prohibited Substances for use in organic foods. Colors were not added to the National List as the result of a petition. Instead, they were included among substances initially placed on the National List when USDA promulgated regulations pursuant to the Organic Food Production Act of 1990. According to 21 CFR Part 205.605, nonagricultural (nonorganic) colors are allowed as ingredients in or on processed food products labeled as "organic" or "made with organic." Only nonsynthetic colors (as a group) are allowed.

**References:**


Baldwin J.L., Chou A.H., and Solomon W.R. 1997. Popsicle-induced anaphylaxis due to carmine dye allergy. Annals of Allergy, Asthma & Immunology 79:415-419.

Barrows J.N., Lipman A.L., Bailey C.J. 2003. Color Additives: FDA's Regulatory Process and Historical Perspectives. Available at: http://www.cfsan.fda.gov/~dms/col-regu.html.

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Chung K., Baker J.R., Baldwin J.L., and Chou A. 2001. Identification of carmine allergens among three carmine allergy patients. Allergy 56(1):73-77.

DiCello M.C., Baldwin J.L., Myc A., and Baker J.R. 1999a. Anaphylaxis after ingestion of yogurt colored with carmine. Annals of Allergy, Asthma, & Immunology 82:73 (Abstract).

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Francis F.J. (2000) Safety assessment of flavor ingredients. In: Watson D.H. (ed.). Food Chemical Safety. Volume 2. Woodhead Publishing Limited: Cambridge, England. Pp. 173-206.

Attachment 3

# Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives

## *ANNATTO EXTRACT (ALKALI-PROCESSED NORBIXIN, NOT ACID-PRECIPITATED)*

| | |
|---|---|
| ***See:*** | ANNATTO EXTRACTS |
| ***INS:*** | 160b |
| ***Chemical names:*** | 9'-cis-6,6'-DIAPOCAROTENE-6,6'-DIOIC ACID; MONO- OR DISODIUM SALT, MONO- OR DIPOTASSIUM SALT |
| ***Synonyms:*** | **ANNATTO** G; L. ORANGE |
| ***Functional class:*** | COLOUR |
| ***Colour code:*** | CI (1975) 75120 (NATURAL ORANGE 4) |
| ***Latest evaluation:*** | 2003 |
| ***ADI:*** | No ADI allocated |
| ***Comments:*** | No ADI was allocated because no data on toxicity were available |
| ***Report:*** | TRS 922-JECFA 61/11 |
| ***Specifications:*** | COMPENDIUM ADDENDUM 11/FNP 52 Add. 11/11 |
| ***Tox monograph:*** | FAS 52-JECFA 61/13 |
| ***Previous status:*** | See "ANNATTO EXTRACTS". THE PREVIOUS SPECIFICATIONS FOR ANNATTO EXTRACTS (OIL- AND ALKALI-EXTRACTED) PREPARED AT JECFA 46 (1996) HAVE BEEN REPLACED BY THESE AND SEPARATE SPECIFICATIONS FOR "ANNATTO EXTRACT (AQUEOUS-PROCESSED BIXIN)", "ANNATTO EXTRACT (ALKALI-PROCESSED NORBIXIN)", AND "ANNATTO EXTRACT (OIL-PROCESSED BIXIN)". |

8 Jan 05

Attachment 4

# ANNATTO EXTRACTS (ALKALI-PROCESSED NORBIXIN, NOT ACID-PRECIPITATED)

*Prepared at the 67th JECFA (2006) and published in FAO JECFA Monographs 3 (2006), superseding specifications prepared at the 61st JECFA (2003) and published in FNP 52 Add 11 (2003) and in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). An ADI for bixin of 0 – 12 mg/kg bw and a group ADI for norbixin and its disodium and dipotassium salts of 0 – 0.6 mg/kg bw expressed as norbixin were established at the 67th JECFA (2006). The colouring matters bixin and norbixin derived from annatto extracts (solvent-extracted bixin; solvent-extracted norbixin; aqueous-processed bixin; alkali-processed norbixin, acid-precipitated; and alkali-processed norbixin, not acid-precipitated) are included in the ADIs for bixin and norbixin. All previous ADIs for annatto extracts were withdrawn.*

**SYNONYMS**

Annatto G, Orlean, Terre orellana, L. Orange, CI (1975) 75120 (Natural Orange 4), INS 160b

**DEFINITION**

Alkali-processed norbixin (not acid-precipitated) is prepared by removal of the outer coating of the seeds of the annatto tree (*Bixa orellana* L) with aqueous alkali. The bixin is hydrolysed to norbixin in hot alkaline solution. The precipitate is filtered, dried and milled to give a granular powder. Extracts contain mainly the potassium or sodium salt of norbixin as the major colouring matter.

Alkali-processed norbixin (not acid-precipitated) contains several coloured components; the major colouring principle is *cis*-norbixin, a minor colouring principle is *trans*-norbixin; thermal degradation products of norbixin may also be present as a result of processing.

Products supplied to the food industry may be formulated with appropriate carriers of food grade quality.

Chemical name

*cis*-Norbixin: 6,6'-Diapo-Ψ,Ψ-carotenedioic acid
*cis*-Norbixin dipotassium salt: Dipotassium 6,6'-diapo-Ψ,Ψ-carotenedioate
*cis*-Norbixin disodium salt: Disodium 6,6'-diapo-Ψ,Ψ-carotenedioate

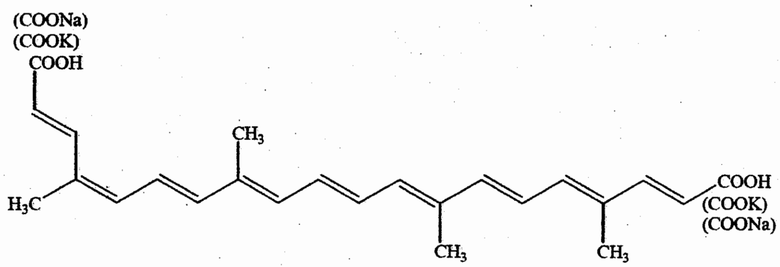
C.A.S. number

*cis*-Norbixin: 542-40-5
*cis*-Norbixin dipotassium salt: 33261-80-2
*cis*-Norbixin disodium salt: 33261-81-3

Chemical formula

$C_{24}H_{28}O_4$, $C_{24}H_{26}K_2O_4$ , $C_{24}H_{26}Na_2O_4$

Structural formula




*cis*-Norbixin

Formula weight — 380.5 (acid), 456.7 (dipotassium salt), 425 (disodium salt)

Assay — Not less than 15% colouring matter (expressed as norbixin)

## DESCRIPTION

Dark red-brown to red-purple powder

## FUNCTIONAL USES

Colour

## CHARACTERISTICS

### IDENTIFICATION

Solubility (Vol. 4) — Soluble in alkaline water, slightly soluble in ethanol

UV/VIS absorption (Vol. 4 ) — The sample in 0.5% potassium hydroxide solution shows absorbance maxima at about 453 nm and 482 nm.

Thin Layer Chromatography — Activate a TLC plate (e.g. LK6D SILICA GEL 60 A (layer thickness: 250 µm, size: 5 x 20 cm)) for 1 h at 110°. Prepare a 5% solution of the sample in 95% ethanol and apply 10 µl to the plate. Allow to dry and develop using a mixture of n-butanol, methyl ethyl ketone and 10% aqueous ammonia (3:2:2 by volume) until the solvent front has ascended about 10 cm. Allow to dry. Bixin and norbixin appear as yellow spots with $R_f$ values of about 0.50 to 0.45, respectively. Spray with 5% sodium nitrite solution and then with 0.5 mol/l sulfuric acid and the spots immediately decolourise.

### PURITY

Arsenic (Vol. 4) — Not more than 3 mg/kg
Determine using an ICP-AES/AAS-Hydride technique. Alternatively, determine arsenic using Method II of the Arsenic Limit Test. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4.

Lead (Vol. 4) — Not more than 2 mg/kg
Determine using an AAS ICP-AES technique appropriate to the specified level. The selection of the sample size and method of sample preparation may be based on the principles of the method described in Volume 4.

Mercury (Vol. 4) — Not more than 1 mg/kg
Determine using cold vapour atomic absorption technique. Select sample size appropriate to the specified level.

## METHOD OF ASSAY

Proceed as directed in Food Colours, Colouring Matters Content by Spectrophotometry (Vol. 4), procedure 1, using 0.5 % potassium hydroxide as solvent. Measure the absorbance at the $A_{max}$ of about 482 nm. The specific absorbance ($A^{1\%}_{1\,cm}$) is 2870.

Attachment 5

SENSIENT

# ANNATTO EXTRACT LIQUID W.S.
## ORGANIC UNDER SEC. 205.606

BT/LOT NO. **SAMPLE**

RESOURCE # [illegible]

**CUST SPEC**

MANUFACTURING DATE **06/14/06**

KOSHER SYMBOL **OVK**

BT/LOT # SAMPLE

CONTAINS: WATER, ANNATTO EXTRACT, AND POTASSIUM HYDROXIDE.

THE COLORANTS USED IN THIS PRODUCT HAVE BEEN CERTIFIED BY THE U.S. FOOD & DRUG ADMINISTRATION OR ARE EXEMPT FROM CERTIFICATION. NOT FOR USE IN THE AREA OF THE EYE, COLORING DRUGS FOR INJECTION OR SURGICAL SUTURES.

CAS # 7732-18-5 WATER
CAS # N/A ANNATTO EXTRACT
CAS # 1310-58-3 POTASSIUM HYDROXIDE
CAS #
CAS #

**PROTECT FROM FREEZING* *STORE REFRIGERATED**
**SHAKE WELL BEFORE USE* *SHAKE WELL BEFORE USE**

GROSS WT. **42.70** TARE WT. **2.70** NET WT. **40.00** **LB**

WEIGHT [illegible]

Sensient Colors Inc. 2515 N. Jefferson, St Louis, Missouri 63106 U.S.A. 1-800-325-8110 SEN-501

A Hachment 6

Specification

SENSIENT®

**Sensient Colors Inc.**
2526 Baldwin Street
St. Louis, MO 63106-1903
Tel 314 658-7443 Fax 314 658-7314
www.sensient-tech.com

# ANNATTO EXTRACT LIQUID W.S.

DESCRIPTION: A dark, reddish-brown, aqueous alkaline solution of the norbixin pigment extracted from the annatto seed *Bixa orellana* L.

IDENTIFICATION: A 1:20,000 dilution in purified water compares favorably with the accepted standard diluted comparably using any spectrophoto-meter, colorimeter or visual method. This dilution should be adjusted to pH 9 or higher with potassium hydroxide.

SPECIFIC GRAVITY: 1.04 - 1.08 at 60°F.

pH: (5% solution): Not less than 10.0.

RECOMMENDED SHIPPING CONDITIONS: Product is packed for HAZMAT ground transportation at ambient temperatures.

RECOMMENDED STORAGE CONDITIONS: Upon receipt, product should be kept in closed container at 40°F.

SHELF LIFE: Nine months if kept under recommended storage conditions. Once opened, product should be used as quickly as possible to prevent oxidation.

PACKAGING: The standard packaging for this product is a five-gallon plastic containers. One gallon (packaged four per case), thirty and fifty-five gallon plastic containers are also available.

Four trailing digits are added to the five-digit product number shown above. The additional four digits are used to identify package size and type.

KOSHER STATUS: This material is Kosher certified.

APPLICATIONS: This product is designed for use in water based systems. It produces a yellowish-orange color depending on product and quantity used. Applications include baked goods, cheese, cereal, ice cream cones, ice cream and sausage casings. Keep out of contact with calcium salts.

STATUS: This product is approved by the FDA and meets the specifications outlined in the Code of Federal Regulations, Title 21, Parts 73.30, 73.1030 and 73.2030. This product meets organic description under Section 205.606.



CSL/PM
11/06

Attachment 7

**Sensient Colors Inc.**
2526 Baldwin Street
St. Louis, MO 63106
800-325-8110, ext. 7475

# **MATERIAL SAFETY DATA SHEET**

CHEMTREC 24 HOUR EMERGENCY TELEPHONE NUMBER - 800/424-9300
CHEMTREC TELEPHONE NUMBER - 800/262-8200
CHEMTREC INTERNATIONAL EMERGENCY NUMBER – 01-703 527-3887

DATE PREPARED: 11/06 REVISION DATE: Original CHEMIST INITIALS: PM

## SECTION I - PRODUCT IDENTIFICATION

PRODUCT CODE & DESCRIPTION: Annatto Extract Liquid W.S.

TECHNICAL NAME: Corrosive Liquid, Basic, Inorganic, n.o.s. (contains potassium hydroxide) UN3266.

CLASSIFICATION CODE: Class 8, PG II.

SYNONYMS: Color Additive; Cheese Color.

PRODUCT CAS NOS. (NEW JERSEY COMPLIANCE): 1393-63-1 Annatto, 1310-58-3 Potassium Hydroxide, 7732-18-5 Purified Water.

## SECTION II - HAZARDOUS INGREDIENT(S)

| COMPONENT CAS # | % WEIGHT | TLV (ppm) | STATUTE |
|---|---|---|---|
| Potassium Hydroxide 1310-58-3 | NMT 4% | Not Found | OSHA/CERCLA |

## SECTION III - PHYSICAL DATA

BOILING POINT (□C): Not determined.

MELTING POINT (□C): Not applicable.

SPECIFIC GRAVITY ($H_2O$=1): 1.04 - 1.08 at 60°F.

SOLUBILITY IN WATER: Soluble.

FLASH POINT (METHOD): Not applicable.

% VOLATILE BY VOLUME: Not applicable.

APPEARANCE AND ODOR: A dark, reddish-brown liquid with faint characteristic odor.



## SECTION IV - FIRE AND EXPLOSION HAZARD DATA

EXTINGUISHING MEDIA: For small fires: dry chemical, $CO_2$, water spray or foam. For large fires: water spray, fog or foam. Move container from fire area if you can do it without risk.

SPECIAL FIRE FIGHTING PROCEDURES: Some of these materials may burn but none of them ignite readily. Some of these materials may ignite combustibles (wood, paper, oil, etc.). Some of these materials may react violently with water.

UNUSUAL FIRE AND EXPLOSION HAZARD: None.

## SECTION V - REACTIVITY DATA

STABILITY: This is stable material in closed containers at room temperature under normal storage and handling conditions.

MATERIALS/CONDITIONS TO AVOID: May react with acid and amphoteric metals, such as aluminum.

HAZARDOUS DECOMPOSITION PRODUCTS: None.

HAZARDOUS POLYMERIZATION: None.

## SECTION VI - HEALTH HAZARD DATA

INGESTION: Swallowing may result in corrosion of tissues.

INHALATION: Vapors may cause irritation of mucous membranes.

SKIN CONTACT: Contact may burn skin.

EYE CONTACT: Contact may cause severe burn to eyes.

OTHER EFFECTS: Fire may produce irritating or poisonous gases. Runoff from fire control or dilution water may cause pollution.

## SECTION VII - EMERGENCY FIRST AID PROCEDURES

SKIN: Immediately wash contaminated skin with soap or mild detergent and water. If clothing is soaked, remove clothing and wash skin with soap and water. Get medical attention as soon as possible.

INGESTION: Do not induce vomiting. Seek medical attention immediately.

INHALATION: If person breaths in large amounts of this chemical, move the exposed person to fresh air at once. Get medical attention as soon as possible.

EYES: Immediately wash the eyes with large amounts of water, occasionally lifting the lower and upper lids. Continue for 15 minutes. Get medical attention immediately.

## SECTION VIII - PERSONAL PROTECTION

EYES: Wear face shield or mono goggles.

VENTILATION: Provide general and local exhaust ventilation to meet TLV requirements.

RESPIRATORY: Use approved NIOSH dust/mist respirator in absence of proper ventilation and dependent upon the extent and severity of likely exposure.

OTHER PROTECTIVE EQUIPMENT: Wear gloves when handling material. Wear impervious clothing.

WORK/HYGIENIC PRACTICES: Use good personal hygiene practices; limit exposure to product whenever possible. Wash any contaminated clothing or shoes before reuse.

## SECTION IX - SPILL OR LEAK PROCEDURES

STEPS TO BE TAKEN IN CASE MATERIAL IS RELEASED OR SPILLED: Do not touch spilled material; stop leak if you can do it without risk.

SMALL SPILLS: Take up with sand or other noncombustible absorbent material and place into containers for later disposal.

SMALL DRY SPILLS: With clean shovel, place material into clean, dry container and cover. Move containers from spill area.

LARGE SPILLS: Dike far ahead of spill for later disposal.

WASTE DISPOSAL METHOD: Sanitary landfill in accordance with Local, State and Federal regulations.

## SECTION X - STORAGE AND HANDLING

Prevent eye and skin contact. Do not breathe dusts or mists. Avoid storing next to strong acids.

MATERIAL SAFETY DATA SHEET PREPARED BY SENSIENT COLORS INC.

For additional information on this product, call 800-325-8110, ext. 7443 during normal business hours.

Attachment 8

Organic
Materials
Review
Institute

Katherine Benham
Director of Program Administration
National Organic Program
USDA-AMS-TMP-NOP
Room 4008 South Bldg.
1400 Independence Ave., SW,
Ag Stop 0268
Washington, D.C. 20250

RE: Sunset Recommendations - Colors
**Sent Via E-Mail to:** Katherine.Benham@usda.gov.

Sent Via Fax to: 202.205.7808

Dear Ms. Benham:

OMRI appreciates the opportunity to comment on the proposed renewal of sunset items on the USDA National List of Allowed and Prohibited Substances (National List), 7 CFR 205.601 – 205.606. As stated in previous comments, OMRI sees the Sunset process as an important and necessary step in maintaining the National List and keeping it up to date.

As a general rule, however, OMRI does not take position regarding the renewal or renewal or removal of any substances on the list. Only in cases where our work has identified confusion or a lack of clarity with resulting inconsistencies do we comment.

## *Executive Summary*

'Colors–Nonsynthetic sources only' should not be renewed on the National List of allowed non-organic nonagricultural ingredients used in or on organic processed foods. As currently listed, the item does not have a clear standard of identity. The annotation is not enforceable and subject to varying interpretation. There is no petition for non-synthetic colors as a category, no TAP review, and no NOSB recommendation prior to its appearance on the National List. The review conducted for sunset fails to address the criteria in the Organic Foods Production Act. Individual colors may be synthetic or non-synthetic depending on the origins and processes used to manufacture them. Most natural colors are agricultural products. OMRI recommends that colors be removed from 7 CFR 205.605(a), that colors in organic food from agricultural sources be treated as any other agricultural ingredient, and that non-agricultural colors be considered on an individual basis through the petition process.





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*E-mail info@omri.org • www.omri.org*

## Colors

OMRI is responding to the NOSB Processing Committee's recommendation on both colors and flavors. While most of our comments focus on colors, we acknowledge that many of the comments apply also to flavors. There are, however, significant technical differences. The NOSB should consider the colors and flavors separately.

The inclusion of colors on the National List is problematic for five reasons that we have identified:

1) Standard of Identity
2) Procedural Irregularities
3) Process Verification
4) Agricultural Origins
5) Health concerns

### Standard of Identity

Even if so-called 'colors, nonsynthetic sources only' remain on the National List, there is ambiguity over what colors comply with the annotation. It is difficult to comment on 'colors, nonsynthetic' because it is not clear what falls within the category. Some colors are commonly referred to as 'natural colors,' but even that term has no accepted industry standard of identity. The Color Additives Amendment of 1960 removed any prior differentiation between synthetic and non-synthetic colors (Zuckerman, 1964). The Technical Advisory Panel (TAP) Overview provides a description of the batch certification process and exemption from batch certification, so there is no need to repeat it here (TAP, 2005). Natural occurrence is a factor in exempting a color from batch certification. However, the exemption from batch certification does not ensure that a given color is non-synthetic, and not all non-synthetic colors are exempt from batch certification.

One source considers all color additives to be 'fabricated' products (Marmion, 1991). The FDA Policy Guidance on food labeling indicates an objection to the reference to colors as either 'food' or 'natural.'

> The use of the words "food color added," "natural color," or similar words containing the term "food" or "natural" may be erroneously interpreted to mean the color is a naturally occurring constituent in the food. Since all added colors result in an artificially colored food, we would object to the declaration of any added color as "food" or "natural." (US FDA, 1976).

Most items that appear on the National List are single substances rather than categories of substances. There are a few broad categories of substances, such as enzymes and flavors, but both of these categories have a petition from the industry and a record of what the NOSB considered to fall within those categories prior to making their recommendation. A major reason for the lack of clarity regarding non-synthetic colors arises from the lack of a petition and TAP review.

## Procedural Irregularities

As noted in the Overview conducted by the TAP, colors were not added to the National List as the result of a petition (TAP, 2005). Unlike every other substance on the National List, there is no record of a TAP review, position paper, or NOSB recommendation prior to natural colors appearing on the National List. In 2001, OMRI requested that colors be removed from the National List as a technical correction to an apparent drafting error (Brown Rosen and Baker, 2001).

Because natural colors were never petitioned and because colors are not a single, well defined substance, it is unclear what substances are covered by the listing. In response to the 1997 proposed rule, the USDA received considerable public comment against placing any item on the National List that had not gone through all the steps in the petition process.

Colors are distinctly different from other items scheduled for sunset. Various references acknowledge that the use of color additives have long been controversial and often considered a deceptive practice (Saltmarsh, 2000). A survey of organic food processors found that colors were considered one of the additives least compatible with organic processing (Raj, 1990). The lack of a petition indicates that there was no solid support for colors so there was no point to debate or challenge them. The procedural issues are a concern for public health, because the food safety literature has identified that specific colors such as annatto and carmine are capable of eliciting allergic sensitization (Taylor and Hefle, 2001).

While the overview provided to the NOSB for sunset addresses some of the criteria in the OFPA, they are not all addressed. In fact, the Overview identifies a number of concerns for specific colors that should be studied in greater depth, suggesting that a case-by-case review is more appropriate. Like all other items on the National List, individual coloring substances should be petitioned, that petition should be reviewed by the Technical Advisory Panel, and the NOSB should make a recommendation based on that TAP review and petition. Without a petition, it is not possible to understand what the industry requested. Without a TAP review it is not possible to assess the claims made regarding the health, safety, and environmental impacts of permitting using the criteria in the Organic Foods Production Act.

The NOP standards allowance of non-organic colors poses several issues for international trade. Non-organic colors are not categorically permitted in the European Union Regulations on organic food products [EC 2092/91], the Japanese Agricultural Standards for organic processed food products [JAS 60], the Codex Alimentarius guidelines on organically produced foods [CAC GL32-1999 rev. 1-2001], or the IFOAM Basic Standards (IFOAM, 2005). Conversely, the FDA identifies color additives that are not permitted in the US, but appear in imported food to be a problem area (FDA, 2001b). Therefore, the assumption that FDA regulations are sufficient to address safety concerns related to colors may not be valid when dealing with imported organic food products.

## Process Verification

With the NOP's evaluation of the NOSB's recommendation on the definition of synthetic (Frances, 2006a), and recommended framework to further clarify the definition of synthetic (Frances, 2006b), the need for a case-by-case review of colors becomes even more apparent. Certifiers often do not check the origin, sources, and manufacturing processes of non-agricultural substances that appear on the National List.

With single, well-identified items that are on the National List, verification that a given ingredient meets the established standard of identity is relatively simple. The presumption is that because a substance was petitioned, received a TAP review, and was recommended by the NOSB, no further verification is needed. With flavors, the NOSB came up with clear, verifiable guidelines consistent with organic production and handling, even though it did not require a full TAP review or case-by-case evaluation of flavors.

The premise that certifiers do not need to look at the manufacturing process of a non-organic ingredient because it was evaluated during the National List process is obviously false in this case. The NOSB did not recommend that colors extracted using volatile synthetic solvents or in synthetic carriers appear on the National List. Such solvents are synthetic incidental ingredients that would need to be reviewed. It is unclear what meets standard of identity for non-synthetic colors, particularly when petroleum derived solvents are used in the extraction process. For example, turmeric oleoresin may be extracted by one or a combination of the following solvents: acetone, ethyl alcohol, ethylene dichloride, hexane, isopropyl alcohol, methyl alcohol, methylene chloride, and trichloroethylene (Marmion, 1991). While this was considered and discussed with flavors, there are significant technical differences with colors that need to be considered.

More important to the case of colors are the aniline, azo, and other coal tar derivatives used to make dyes, and the metals that are used to make lakes. These are obviously synthetic substances, but their coloring agents often come from plant or animal sources. It is OMRI's opinion that such colors are synthetic. The industry needs greater clarity what given colors are 'non-synthetic.' Every component needs to be non-synthetic, including substances used to extract, stabilize and disperse the coloring agent.

Colors that are exempt from batch certification are not necessarily natural. Some familiar 'natural' colors may be wholly synthesized. While some may assume that all ß-carotene (INS 160; CAS 7235-40-7) is naturally derived from carrots or other orange vegetables, in more recent years commercial sources have been factory produced by synthesis from acetone (Marmion, 1991). These are acknowledged to be synthetic, and are marketed as 'nature identical' as opposed to 'natural' (Downham and Collins, 2000). Also, a growing number of coloring agents are potentially produced from genetically modified organisms or by 'excluded methods' prohibited under 7 CFR 205.105(e) (Downham and Collins, 2000).

Because of international trade, it is not safe to assume that all organic handlers and processors are operating under the FDA's jurisdiction or subject to FDA regulations. A number of colors that have been banned in the United States could be considered to be non-synthetic, thus rendering the safety of a blanket category of 'colors–non-synthetic' suspect.

Various synthetic dyes are given natural sounding names. For example, amaranth is a plant that is cultivated and could potentially be a source for a non-synthetic color. However, amaranth is also known as FD&C Red Dye #2. While the amaranth plant was once used in the process to make FD&C Red Dye #2, it is more accurately described as a monoazo or azoaniline coal tar derivative. FD&C Red Dye #2 was banned by the FDA (2001a).

Some ingredients used for coloring may be of agricultural origin, but also are produced synthetically and commonly contain synthetic ingredients used as preservatives or carriers. FD&C Blue No. 2 can be made from indigo, and is one of the oldest known and most extensively utilized natural pigments (von Elbe and Schwartz, 1996). FD&C Blue No. 2 can also be synthesized (Merck, 2001). However, indigo is not exempt from the requirement of batch certification, even when it is extracted from an indigo plant [21 CFR 74.102].

## Agricultural Origins

Agricultural products are often used to make colors. Corn, cottonseed, beets, carrots, grapes, marigolds, turmeric, and paprika all are clearly agricultural and therefore do not qualify to be included in a category that appears on a list of 'nonagricultural' substances.' Agricultural ingredients used as ingredients in organic food products are supposed to be organically produced [7 CFR 205.270; 7 CFR 205.301]. Colors of agricultural origin should be subject to the same rules as any other agricultural commodity. The growing market for organic food is seen as an obstacle as well as an opportunity for the makers of food colorings in those markets—such as the European Union—that do not permit non-organic colors (Downham and Collins, 2000).

## Health Concerns

Annatto, caramel, and carmine can be used as models to illustrate the various origins of colors, and discuss possible health concerns related to specific colors.

### Annatto

Annatto (INS 160b; CAS 1393-63-1) is a carotenoid pigment produced from the bean-like fruit of achiote, *Bixa orellana,* a tropical bush that originated in South America and is cultivated in various tropical regions. Annatto is extracted from the thin layer of intense pigmentation on the pericarp on the seed. The principle components of annatto that provide color are bixin, an oil-soluble orange-red and its water-soluble analogue norbixin. One method of extraction uses alkaline hydrolysis that involves a 5% solution of potassium hydroxide at a pH of 12-14. Annatto can also be obtained through oil processing or solvent extraction. The FDA also allows acetone, ethylene dichloride, hexane, isopropyl alcohol, methyl alcohol, methylene chloride, and trichloroethylene all to be used as solvents to extract annatto. Solvent extracted forms are arguably synthetic, or at least inconsistent with the parallel standard applied to natural flavors.

Products sold as annatto may contain diluents, pigment enhancers, and other substances that may be synthetic. The Food Chemicals Codex has established residual tolerances of solvents of not more than 0.003% for acetone; not more than 0.0025% for hexanes; not more than 0.005% for isopropyl alcohol; not more than 0.005% for methyl alcohol; and not more than 0.003% for trichloroethylene and dichloromethane;

There are also food safety concerns about the use of bixin and norbixin. The UN's Joint Evaluation Committee for Food Additives (JECFA) recommends that countries place restrictions on the Average Daily Intake of Annatto and Bixin, and the extraction process used drives these limits. Annatto was historically prepared in an alcohol solution (MacPhail, 1864). More recently, propylene glycol (Kocher, 1958) and other synthetic carriers are used. These carriers would make the color synthetic.

Various organic products certified under the National Organic Program use organic annatto as an ingredient. When extracted with a 5% solution of potassium hydroxide, seeds from organically produced achiote would meet NOP standards to be labeled 'organic annatto,' which could in turn be used in a processed product labeled as 'organic.'

**Caramel**

Caramel (INS 150; CAS 8028-89-5) is made most simply from the heating of sugar (Merck, 2001). Sugar is an agricultural commodity and organic sugar is commercially available from a number of sources. Such a caramel color would be known as Class I (INS 150a), For example, caramel color can be produced by the browning of sugars without the use of synthetic acids or alkalis. Some sources consider caramel to be an 'artifact' as distinct from a natural or artificial color (MacKinney and Little, 1962).

It is unclear if caramel would be considered synthetic or non-synthetic. The answer would depend on the manufacturing process. Four distinct classes of caramel can be distinguished by the reactants used in their manufacture (Food Chemicals Codex, 2003).

Class I caramel (INS 150a) is known as 'plain caramel' and is prepared by the heating of carbohydrates, often simple refined sugar or sucrose. Class I caramel's standard of identity permits the use of synthetic acids and bases in their processing. 'Caustic caramel' is a form of Class I caramel that has been alkali treated. Some of the acids and bases used to make Class I caramel are on the National List—such as citric acid, sodium hydroxide, and potassium hydroxide.

Caramel made in the presence of sulfite compounds such as sulfur dioxide but without the use of ammonium compounds is considered Class II caramel (INS 150b). Class II caramel may or may not involve the use of acids or alkalis. Food Chemicals Codex permits a maximum of 0.2% sulfur dioxide and caramel color (Food Chemicals Codex, 2003).

Class III caramel is prepared by heating carbohydrates in the presence of ammonium compounds. Class III caramel also may or may not be treated with acids or alkalis. The NOSB determined that ammonia is synthetic, and it follows that the reaction products of ammonia would be as well (NOSB, 2001).

Class IV caramel is prepared using both ammonium and sulfite compounds, either with or without acids or alkalis.

It is therefore possible to make caramel entirely from sugar alone or sugar and substances on the National List. If sugar is an agricultural product that is commercially available in organic form, it is therefore possible to make an organic caramel. All caramel acceptable for use in organic production would be Class I, but not all Class I caramel would meet organic standards even if the main ingredient was organic sugar. Caramel made from organic sugar and that is reacted with an acid or base on the National List that does not exceed 5% by weight net of the water would qualify as 'organic' caramel.

It is OMRI's opinion that if colors remain on the National List with their current annotation, natural caramel would be limited only to plain caramel. Caustic caramel would not meet the annotation, even if it is Class I. Class II, Class III, and Class IV are all synthetic as defined by the NOP rule, and the presence of sulfites in Class II and Class IV caramel raises serious concerns regarding the general prohibition of added sulfites in organic food and associated adverse health effects on a segment of organic food buyers.

**Carmine**

Carmine and carminic acid (INS 120; CAS 1390-65-4) are obtained from cochineal *Coccus cacti (Dactylopius coccus costa)*–a scale insect that lives on cactus plants endemic to Mexico. The process by which the coloring agent is extracted from the insect would need to be reviewed by the NOSB in order to determine if the coloring agent is acceptable for use under the current annotation. Carmine used in food coloring is usually not the naturally extracted acid but rather an aluminum lake or aluminum-calcium lake of carminic acid produced by reaction of aluminum hydroxide (Merck, 2001). Aluminum hydroxide is a synthetic substance not on the National List and is therefore prohibited in organic handling [7 CFR 205.105(c)] Sodium benzoate is also commonly added as a preservative (Marmion, 1991).

Carmine is cited as an example of a 'natural' color exempted from safety requirements and has never been fully reviewed for its safety to the same degree as other, more recently developed colors (Barrows, Lipman, and Bailey, 2003). While the FDA has withdrawn approval for some uses, it is reported as 'permanently listed' and is considered by some to be non-synthetic (Winter, 1989). The TAP overview notes that carmine is associated with adverse health effects, in particular allergic reactions (TAP, 2005). Carmine has also been associated with salmonella poisoning (Komarmy, Oxley, and Brecher, 1967; Lang et al., 1971).

As with beeswax and shellac, carmine is the product of domesticated insects that can be managed consistently with organic livestock standards. While there is no known source of organic carmine at the present time, the development of apiculture standards can be extended to the production of cochineal bugs.

## Recommendations

Once again, OMRI urges the NOSB and NOP work together to correct the technical errors in the National List.

1) The NOSB should address colors separately from flavors when considering their renewal.
2) The NOSB should *not* renew natural colors on the National List of nonagricultural ingredients used to process or handle organic agricultural products because:
   (a) there is no clear standard of identity,
   (b) the NOSB has not been presented with a petition or TAP review that addresses the OFPA criteria and did not make a recommendation prior to the appearance of colors on the National List.
   (c) Verification that sources are nonsynthetic is inadequate.
   (d) Most non-synthetic colors can be obtained from agricultural sources.
   (e) There are health concerns that need to be addressed by a more comprehensive review.
3) Sources and manufacturing processes need to be disclosed to determine whether a specific color is synthetic or non-synthetic.
4) Colors currently used in organic processing are agricultural in origin and should be treated as all other agricultural ingredients in organic food products.
5) Colors from non-agricultural sources should be petitioned on a case-by-case basis subject to the criteria in 7 CFR 205.600.

Thank you for your consideration,

David DeCou
Executive Director

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Attachment 11

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# Natural Food Colorants

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# 6
# Annatto

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## INTRODUCTION

The food color annatto is the extract of the outer layer of the seeds of *Bixa orellana*, the tropical tree named after the Spanish conquistador Francisco de Orellana, who discovered the Amazon River in 1541 starting his expedition from Quito, Ecuador. Patiño (1967) provides a complete description of the aboriginal uses of annatto seed. For centuries it was a traditional ingredient for food preparation and for cosmetic purposes in Central and South America. The natives prepared it by rubbing the seed with oils pressed from other plants. The Aztecs, in what is now Mexico, mixed it with cocoa to give a special taste and a more pleasing color to chocolate. A ceremonial drink with annatto resembled human blood, and its consumption became an important ritual for the Indians. This was probably also the basis for the cosmetic uses of annatto in pre-Spanish Latin America. Painting the human body with annatto was usually done by the women on the bodies of male warriors as a symbolic act representing the color of blood. During the Spanish colonization of America this ceremony was banned at times as a pagan ritual. However, the Spaniards also became intensely interested in this color.

The Dutch and British traders who arrived at ports of South America in the seventeenth century also became enchanted with annatto. They traded European products for annatto seed, which they took back to Europe. Annatto was the first vegetable color brought to Europe in large quantities after the discovery of America. Just from the Mexican port of Veracruz more than 35,000 pounds of annatto seed were shipped to Europe in the year 1644 (Patiño, 1967).

Annatto seeds and extracts have been used for over 100 years in Europe and North America to impart a yellow to red color to foods, especially dairy products such as cheddar and colby cheeses. According to Parish (1994), "The English started their own gold rush in the 18th century with the discovery that annatto was a useful coloring for cheese. The warm-toned cheeses of the Cheddar variety, Cheshire, Double Gloucester and Leicester, all get their blush from annatto. Before that, the English dairy maids wrung out (the juice of) scrapings of carrots, marigold leaves or saffron to get the light orange color." In the United States, the first modern annatto food color was exhibited at the Philadelphia World's Fair in 1876 by the Christian Hansen Laboratory.

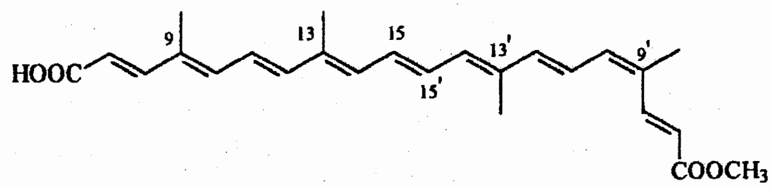
In modern times, annatto ranks second in economic importance worldwide among all natural colors (Lauro, 1991) and it is the most frequently used natural colorant of the food industry in the United Kingdom (Scotter et al., 1998).

9′-*cis*-Bixin (Fig. 6.1) is the major color component of annatto seed, accounting for over 80% of the annatto pigment (Preston and Rickard, 1980; Lauro, 1991). Water-soluble annatto extracts are prepared by alkaline hydrolysis whereby bixin is converted to norbixin (Fig. 6.1). Reviews on the extraction, chemistry, and applications of annatto have been published by Dinesen (1974), Preston and Rickard (1980), Collins (1992), and Green (1995).

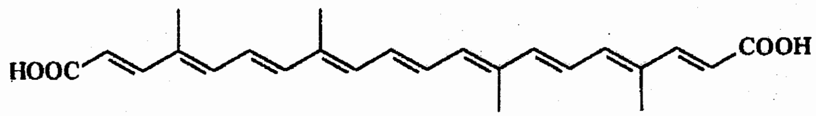
## ANNATTO SEEDS

Commercial annatto extracts are obtained from the red oily layer covering the seeds of the tree *Bixa orellana* (Annatto), native of northern South America and introduced into Central America, India, and Africa (Heiser, 1965). It is one of the four species of the family Bixaceae, order Violales, subclass Archichlamydeae, division Angiosperms/Dicotyledons (Evans, 1996). The plant thrives from sea level to about 4000 feet altitude in a moist climate and prefers a deep, loamy, well-drained soil (Dendy, 1966). When large clusters of capsular fruits have formed at the end of the branches they are harvested. The capsules, about one inch in diameter, are cut off the stems. They contain 50–60 small seeds with a bright crimson oily covering, weighing 10–20 mg each. The capsules are allowed to dry in the sun, are

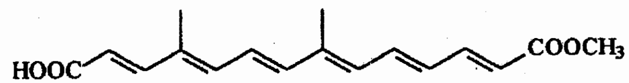





**9′-*cis* – BIXIN**




***trans*-NORBIXIN**




**C-17 COMPOUND**

**FIG. 6.1** Molecular formulas of bixin, norbixin, and C-17 compound.

cracked open, and the small seeds are separated by hand from the husk. Dried annatto seeds and their extract are traded worldwide. The cultivation of annatto has been reviewed by Ingram and Francis (1969), Arce (1983), and Aparnathi et al. (1990).

### Wild Annatto and Home-Grown Annatto—Their Social Significance

The major proportion of the world production of annatto seed comes from the collection of seed from wild trees or trees planted on family farms, especially in northern South America. Each mature tree may yield between 0.5 and 4 kg of seed. The collection and sale of seeds from wild annatto trees of the tropical jungle areas provide a subsistence income for thousands of families in the most economically depressed areas. The family income is frequently supplemented by the harvest of a few dozen annatto trees they have planted on the land around their home. The dried seeds are taken to an intermediary. After the intermediary has accumulated a few hundred pounds, they are transported to the next major city for sale to a wholesaler, who supplies the local extraction industry, or to an exporter, who waits to complete the 10–20 metric tons of seed necessary to fill a container for ocean shipment.

More than 2000 individual seeds have to be harvested to get just one gram of bixin. For the food technologist using annatto color, it may be a sobering thought to consider that 240,000 seeds in 4000 seed pods harvested by hand are required for just one gallon of cheese color—a very labor-intensive operation.

Thousands of the poorest peasants of the tropics derive their subsistence income from the collection and sale of annatto seed, which gives it enormous social and economic importance in the tropical areas of many countries. In the case of Peru, the collection of annatto seed is one of the few practical alternatives available to the production of illegal drugs, which are grown in the same areas. Major efforts of the so-called "war on drugs" are directed towards providing such alternative sources of income to the population in order to direct the financial temptation (or, indeed, their vital needs of survival) away from the illegal drug harvest and trade and replace that source of income by a legitimate occupation. Annatto plays an important role in this effort.

## Plantation-Grown Annatto

A significant proportion of annatto seed is now produced in Ecuador, Central America, and West Africa on plantations or semi-plantations, with yields of 900–1500 kg of seed per hectare per year (Green, 1995). Possibly the first commercial annatto plantation based on modern agricultural techniques was established in Ecuador in 1986 by the firm INEXA, based on selected annatto varieties of high color content (Evans, 1996). The seeds are extracted in the INEXA industrial plant in Quito within 72 hours of harvest for maximum extraction yield and highest purity of the extract.

## Stability of Annatto Seed

Prompt extraction after harvest is only possible when extraction facilities are located near the cultivation areas. If this is not the case, the seeds must be shipped overseas. Several months elapse before extraction. Cold storage is advisable to prevent the degradation of bixin through air oxidation. Annatto seeds that have been stored for more than 6 months in the tropics frequently contain peroxides, which can cause autocombustion. At times the



peroxides can even carry over to the extracted bixin. A case of spontaneous combustion has been observed with bixin powder of low purity that had been extracted from one-year old annatto seed.

## World Production and Commerce

The quantity of annatto seed harvested in the producing countries has been estimated at 6,500 metric tons (dry weight) per year (Wood et al., 1991) and 10,000 metric tons (Green, 1995). A more realistic estimate may be 14,500 metric tons because most statistics refer only to exports from the producing countries and do not include their local consumption. Such regional trade is considerable in some producer countries but is difficult to assess because these quantities do not go into the official statistics and because thousands of small producers are involved. An example is Brazil, where informal estimates put the harvest at the impressive figure of 5000 metric tons/year that escape the official statistics. They are for domestic use by the local population, mainly as the condiment called "Coloral," which is ground annatto seed and is as popular in Brazil for cooking as is pepper in the rest of the world. In Peru and Ecuador about 1000 metric tons of annatto seed may be consumed by the local population. Thus, the annatto export statistics, which are the only easily obtainable figures, seriously underestimate the total annatto production of the world. Table 6.1 shows estimated production figures of the main producing countries matched by internal consumption

**TABLE 6.1** Estimated World Production and Commerce

| Producing Countries | Metric tons dried seed | Importer | Metric tons dried seed[a] |
|---|---|---|---|
| Brazil | 5,000 | North America | 3,000 |
| Peru, Ecuador, Colombia, Bolivia | 3,000 | Europe | 2,500 |
| Kenya, Tanzania | 2,500 | Japan | 1,500 |
| Guatemala, Mexico, Caribbean | 2,000 | Other | 500 |
| Ivory Coast, Ghana | 1,500 | | |
| | | Total | 7,500 |
| India, Asia | 500 | | |
| Total | 14,500 | | |
| Of which: | | | |
| Domestic consumption | 7,000 | | |
| Available for export | 7,500 | | |

[a]Or its equivalent in extract.

*Source*: Adapted from UNCTAD/GATT, 1990; Wood, 1991; Green, 1995; N. Dinesen, private communication.

and export. For the equivalence in terms of bixin, a good approximation is 20 kg of pure bixin per ton of seed.

The price history of annatto seed shows the typical roller coaster fluctuations for most agricultural commodities of the third world. The historical average price over the past 30 years is around $1,100 per metric ton, but with fluctuations from highs of $1,500–$2,500 per metric ton (1973, 1978, 1987) to lows of $500 per metric ton (1969, 1974, 1982, and 1999).

## PRODUCTION OF ANNATTO EXTRACT

Extraction methods may either aim at the production of the native bixin from the annatto seed or may involve aqueous hydrolysis and simultaneous extraction of norbixin.

### By Mechanical Means

The seventeenth-century Spanish conquerors of Ecuador and Peru discovered for Europe not only the annatto plant but also the first rudimentary extraction method, which to this day is practiced by the Colorado Indians of western Ecuador. For over 600 years the natives have been rubbing the annatto seed by hand to dislodge the red seed coating and obtain a powder or paste, which they apply to their skin and hair to dye them red (*colorado* in old Spanish) and as a dye or spice for cooking.

The same dry-extraction method in modern times uses equipment in which the annatto seeds are forced by an air current through a draft tube into a spouted bed extractor (Guimaraes et al., 1989; Massarani et al., 1992). The particle impact loosens the surface layer of the seed, yielding an average of 114 g of powder containing about 15% bixin (Passos et al., 1998).

The earliest "wet" industrial extraction method involved the same principle, but mechanical abrasion was provided by stirring the annatto seed in water to dislodge and separate the pigment layer, which was then filtered off and dried to give a paste with a bixin content of about 20%. For use as a food color the paste was then dissolved in vegetable oil to prepare dilute bixin solutions or mixed with hot aqueous alkali to hydrolyze the bixin and obtain dilute solutions of norbixin.

### Directly Extracted Food Colors

In the original and traditional food recipes of Latin America, annatto seeds are heated with cooking oil, separated, and the colored oil used for preparing rice, soups, and tortillas. A similar direct-extraction system is applied industrially by immersion of the seed in vegetable oil to produce a bixin slurry, which is heated and filtered. The filtrate is marketed as a color for high-fat foods (Barnett and Espoy, 1957; Murthi et al., 1989). The use of alkaline propylene glycol instead of vegetable oil extended its application



to both high-fat and low-fat foods and gave solutions of greater purity (Kocher, 1958). In all these cases, the extraction liquids, which contain up to 1.5% bixin, or in some cases oil suspensions with up to 8% bixin are marketed directly as oil-soluble annatto food color after standardization of color content.

For aqueous applications (e.g., cheese color) the extraction of the annatto seed is done with aqueous potassium hydroxide or sodium hydroxide, which hydrolyzes the bixin on the seed to the water-soluble norbixin salt that goes into aqueous solution. These solutions of 1–3% norbixin content are marketed directly as finished food colors after standardization of color content. Thus they are called directly extracted food colors.

### Acid-Precipitated Norbixin Concentrates

Norbixin concentrates of between 25 and 50% purity can be prepared by acid precipitation of norbixin from the aqueous alkaline extraction liquid (Marcus, 1963; Dendy, 1966; Tadamasa, 1974) or by direct spray-drying (Park et al., 1990).

### Solvent Extraction

Microcrystalline bixin products of 80–97% purity have been developed as a response to the modern need for more concentrated annatto extracts by extraction of annatto seed with volatile organic solvents and subsequent production of a solvent-free product, which is then processed by the manufacturers of food colors according to specific applications.

Numerous patents and research reports cover a variety of organic solvents for producing concentrates, such as chlorinated hydrocarbons (Marcus, 1956), mixtures of ethanol and chloroform (Alvarez-Smith, 1957), acetone (Todd, 1964), ethanol (San-Ei, 1975; DaSilva et al., 1994), ethyl acetate (Bahl et al., 1971), alcoholic sodium hydroxide (Tadamasa and Yasuda, 1985), or ethyl acetate (Srinivasulu and Mahapatra, 1989). It should be noted that chlorinated solvents are no longer suitable for food products. Several authors reported between 1991 and 1997 on the successful use of supercritical carbon dioxide fluid extraction (Jay et al., 1991; Chao et al., 1991; Degnan et al., 1991; El-Sharkawi et al., 1995; Anderson et al., 1997), but it is not known if this has been applied commercially so far.

The market trend is towards annatto extracts of the highest concentration and purity. The production processes now involve additional purification steps with different solvents.

## MOLECULAR STRUCTURE OF BIXIN AND NORBIXIN

The groundbreaking work by McKeown and associates at the Department of National Health and Welfare of Canada provided much of the current basic knowledge about the composition of oil-soluble annatto food colors (McKeown 1961, 1963, 1965; McKeown and Mark, 1962). More recently, Scotter (1995) gave valuable insight into the mechanism of formation of a 17-carbon colored thermal degradation product of bixin, which is a common component of oil-soluble annatto food colors first reported by McKeown.

Bixin was used as annatto color long before its molecular structure and the intricacies of its isomeric composition were known. First isolated by Boussingault in 1825, its molecular formula ($C_{25}H_{30}O_4$) was established in 1917 by Heiduschka and Panzer. Much of the basic chemistry of bixin was developed by Karrer and associates (1929), who used such words as "magnificent" and "one of the most beautiful compounds." And yet, Karrer debated the molecular formula proposed by Heiduschka, erroneously claiming that it should be $C_{26}H_{30}O_4$.

Bixin is a half-ester carotenoid and more precisely a diapo-carotenoid, which means that for the purpose of IUPAC nomenclature it is considered a central part of a carotene molecule, i.e., without the terminal rings. The central carbons of the bixin molecule are thus numbered 15 and 15′ towards the free carboxylic acid side and the methyl ester side, respectively. The carboxylic carbon atoms at each end of the molecule are assigned the numbers 6 and 6′ and not the numbered 1 and 20, as would otherwise be the case (see Appendix). The two end groups of the bixin molecule are both designated with the Greek letter Ψ. (For further explanation of the IUPAC nomenclature of carotenoids, see Britton, 1998.)

Historically bixin was the first carotenoid in which geometrical isomerism was encountered (Karrer et al., 1929). Bixin, and even more so norbixin, are special among the carotenoids because their molecule contains two strongly polar groups. As with all the carotenoid molecules, the numerous conjugated double bonds may give rise to several geometrical isomers. Most carotenoids in nature have the all-*trans* configuration. Bixin is the exception; it is a *cis*-carotenoid in nature. But just exactly which of the nine double bonds is in the *cis* configuration was the subject of long and sometimes bitter controversy for almost 25 years (Zechmeister and Escue, 1944; Barber et al., 1961). The unambiguous and stereo-chemically controlled total synthesis of methylbixin and the comparison of its nuclear magnetic resonance spectrum with methylbixin derived from natural bixin (Pattenden et al., 1970) finally confirmed that natural bixin has the 9′-*cis* structure (at the time still called the *cis*-4 configuration because the carbon atom of the free carboxylic acid group was still assigned the number 1). Valuable insight into the electronic and molecular structure of bixin was obtained by NMR and X-ray crystallography (Kelly et al., 1996) and by resonance Raman spectroscopy (Oliveira et al., 1997). The first total synthesis of bixin has recently been accomplished (Häberli and Pfander, 1999)

Bixin is unique among the naturally occurring carotenoids not only because of its *cis* configuration, but also because the molecule has two carboxylic groups, one of which is a methyl ester. Bixin is thus a half-ester. This gives it some liposolubility. By alkaline hydrolysis of this methyl ester group the water-soluble salt of the dicarboxylic acid norbixin ($C_{24}H_{28}O_4$) can be prepared (see Appendix). It is an important cheese color.



## COLOR CHANGES CAUSED BY HEAT

The oil-solubility of the natural *cis*-bixin is improved by heating the suspension in vegetable oil to around 100°C, which causes an isomeric change to *trans*-bixin, which is more lipid-soluble. The commercial use of this isomerization was described by Preston and Rickard (1980). However, it had long been observed that the heating of bixin in oil also changes the color to a more yellow tone, which is desirable for many applications. Iversen and Lamm (1953) suggested that this change involves a partial decomposition of the bixin molecule into a yellow decomposition product of (at the time) unknown molecular structure. McKeown (1963, 1965) later identified this product as the monomethyl ester of 4,8-dimethyltetradeca-hexanedioic acid. It is commonly called the C-17 compound (Fig. 6.1). It is formed by the loss of a xylene molecule from the bixin molecule and the molecular mechanism leading to its formation has recently been elucidated by Scotter (1995, 2000).

The formation of the yellow product upon heating bixin in vegetable oil has commercial importance because it allows the production of different color shades from orange-red to yellow to suit product requirements. The desired final color can be adjusted by controlling the degree of degradation (Barnett, 1957; Perret, 1958). Heating time is a critical factor in this thermal degradation (Prentice-Hernandez, 1993).

The change of color tone upon heating of solutions of bixin allows the food technologist great versatility to adjust the "hue" of the food product from reddish to orange to yellow as desired. For a more objective measurement of the red-to-yellow balance of annatto extracts, we have developed a hue index defined as the ratio of optical absorbances at 404 nm and 470 nm when the extract is dissolved in chloroform. Pure bixin has a hue index of 0.22. Depending on heating temperature and time, this value can go up to 1.0, as in oil-soluble annatto food colors used for processed cheese.

The proportion of yellow to red components in annatto food color can also be established by comparing reverse-phase HPLC chromatograms obtained at two wavelength settings of the detector. By this method we have found two groups of yellow components in annatto food colors. An early-eluting group of peaks are the $C_{17}$-type yellow compounds formed by the action of heat, whereas a group of late-eluting peaks, appearing about 9 minutes after the *cis*-bixin peak, are natural, less polar yellow components from the annatto seed. The latter were first found in a sample of annatto food color by Scotter (1998) and marked as unknowns.

## BIXIN DIESTERS

Esterification of the free carboxylic acid group of bixin yields the diester methylbixin. It is prepared from bixin by reaction with dimethyl sulfate (Zechmeister and Escue, 1944; Buchta and Andree, 1959; Jondiko and Pattenden, 1989). The use of ethylbixin has also been described (Geminder and McDonough, 1957). The improved liposolubility shown by the diesters has long been thought to increase their usefulness as food colors (Bahl et al., 1971), but the fact that in the strict sense they can no longer be considered natural compounds has limited their application.

It should be noted that traces of methylbixin were found in annatto extract for the first time by Mercadante et al. (1997b). These authors were in fact the first ones to find methylbixin in annatto, although they acknowledged the erroneous priority of Jondiko and Pattenden (1989). The latter, however, had prepared methylbixin from bixin. A useful distinction between methylbixin artificially prepared from natural bixin and natural methylbixin was given by Barber et al. (1961), who proposed the name "methyl-natural bixin" for the former and "natural methylbixin" for the latter, although at that time no natural methylbixin had yet been found.

## ANALYSIS

### Annatto Seeds

The usual solvents for analytical extraction of annatto seeds are chloroform (McKeown and Mark, 1962), chloroform/acetone (Ramamurty and Bhalerao, 1964), or chloroform/ethanol (Bahakar and Dubash, 1973). Supercritical carbon dioxide with soybean oil as entrainer has also been suggested (Degnan et al., 1991). Extraction at ambient temperature is preferred to prevent isomerization and other molecular changes of the *cis*-bixin.

The main problem of the analysis of annatto seed is the long time it takes to achieve complete extraction at ambient temperature, even with chloroform, which is the best solvent for bixin. Thus, for routine analytical work on numerous samples, many laboratories have resorted to hot extraction as a compromise between speed and stability.

Prior grinding of the seed sample has not been considered necessary because the pigment is located on the surface of the seed. However, the speed and completeness of extraction at ambient temperature is greatly improved by grinding the seed in a tissue homogenizer equipped with a porous glass filter (Avila et al., 1982). In our laboratory we use a high-frequency dispersing Polytron (model PT 10/35, Brinkman Instruments, Westbury, NY) positioned with the seed sample in an extraction thimble for easy filtration of the extraction liquid. We have found that the best extracting solvent is the 79:21 azeotropic mixture of chloroform and methanol (E. Regalado, unpublished), which has the added advantage of being recyclable by distillation without change in solvent composition.



Although chloroform or chloroform/methanol still appear to be the most efficient solvents for quantitative extraction of bixin from annatto seed, there have been repeated warnings against the use of chloroform as a solvent for carotenoids, due to the difficulty of ensuring the removal of all traces of HCl, which may cause molecular changes in carotenoids (Schiedt and Liaaen-Jensen, 1995). Severe degradation of carotenoids in chloroform have been reported (Scott, 1996), and dichloromethane has been suggested as an alternative (Furr, 1997). For the analytical extraction of bixin from annatto seed, however, no entirely satisfactory substitute for chloroform has been found.

An alternative procedure for routine color measurement of annatto seed is the determination of bixin as its norbixin derivative after hydrolytic extraction. The seed sample is boiled in aqueous potassium hydroxide (N. Dinesen and P. Collins, private communications). The spectrophotometric result must be multiplied by 1.037 to correct for the ratio of molecular weights of bixin and norbixin.

Optical absorbance for the determination of carotenoids is generally measured at one of the wavelengths of maximum absorption (see below). A suggestion has been made to replace optical spectrophotometry for the analysis of annatto seed by photoacoustic spectrophotometry (Haas and Vinha, 1995), but this system does not appear to be widely used.

### Annatto Extracts and Formulations

For all practical purposes, the measurement of the color intensity of annatto food colors is the most important and at times the only criterion applied. Color intensity is the spectrophotometric absorbance of a 1 g/L solution of food color measured at 470 nm in chloroform/1% glacial acetic acid for oil-soluble annatto extracts and in 0.1 M sodium hydroxide at 453 nm for water-soluble extracts (Food Chemicals Codex, 1986) or at 454 nm in acetone/1% glacial acetic acid for oil-soluble extracts (Food Chemicals Codex, 1992). The absorbance is used as the final result. No extinction coefficient (specific absorbance) is used in the calculation, and thus the result does not reflect the actual bixin or norbixin content.

However, more precise methods are necessary for stating the bixin and norbixin content of annatto extracts, of food colors, and of foods containing them as more stringent specifications for these products are imposed and a better understanding for their chemistry and biochemistry is warranted (Mercadante et al., 1996).

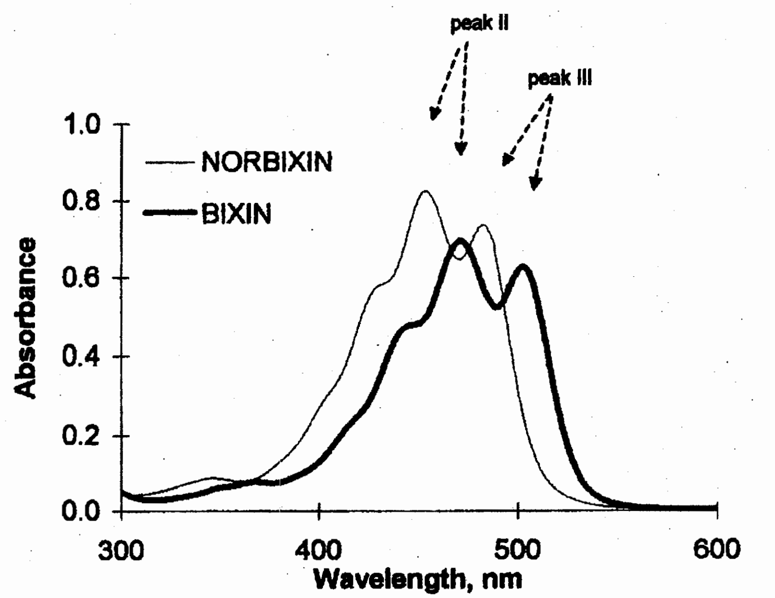



**FIG. 6.2** Spectrometric curve of 2.5 mg 87.5% bixin/L, before and after hydrolysis.

**Spectrophotometry.** A precise examination of the entire visible spectrum provides important information on the qualitative composition of annatto extracts and food colors. Carotenoids usually show three peaks of absorption maxima. These peaks are numbered I, II, and III, going from the lower wavelength to the higher.

Chloroform has been used historically as the solvent for spectra of bixin despite its problems (see above). In the case of bixin in chloroform, peak I is actually only an inflection point at about 445 nm of the spectral curve. The main peak of *cis*-bixin is at 471 nm. It would normally be considered the first peak, but it is, by convention, designated as peak II. A lower but well-defined second peak is at about 503 nm and is designated by convention as peak III. For norbixin in 0.1 N NaOH, peak II is at 453 nm and peak III at 482 nm (Fig. 6.2).

Absorbance measurements at either peak II or peak III are used for quantitive determination of bixin and norbixin, although peak III is generally preferred because at its wavelength there is less interference from a possible presence of yellow decomposition products with overlapping spectral curves, which may require the application of a correction through an additional absorbance measurement at 404 nm (McGeown and Mark, 1962).

As in most carotenoids (Englert, 1995), the position of the wavelengths



**TABLE 6.2** 1% Extinction Coefficients[a]

| Peak II (453nm) | Peak III (482nm) | Ref. |
|---|---|---|
| | | **Norbixin[b]** |
| 2850 | 2550 | Rieth and Gielen (1971), FAO/WHO (1976), Smith (1983) |
| 2818 | 2503 | Scotter (1994) |
| 3473 | — | FAO/WHO (1981), Hirata (1989) |
| 2620[c] | 2290[c] | Smith (1983), Scotter (1998) |
| 3208 | — | Collins (private communication,1990) |
| — | 2870 | EEC (1995) for E160b, FAO/WHO (1996) |

| Peak II (470nm) | Peak III (501nm) | Ref. |
|---|---|---|
| | | **Bixin in chloroform[b]** |
| — | 2826 | McKeown (1962), Dendy (1966) |
| 3230 | 2880 | Reith and Gielen (1971), FAO/WHO (1976), Smith (1983) |
| 3130[d] | 2790[d] | Reith and Gielen (1971), Smith (1983) |
| 2826 | — | FAO/WHO (1981), Hirata (1989) |
| 3092 | 2773 | Scotter (1994) |
| — | 2870 | EEC (1995) for E160b, FAO/WHO (1996) |

[a]In chronological order of original research report.
[b]In 0.1 N KOH unless otherwise indicated.
[c]In chloroform + 0.5% acetic acid, peak II at 473 nm and peak III at 503 nm.
[d]In chloroform + 3.7% acetic acid, peak II at 474 nm and peak III at 505 nm.

of maximum absorption of the all-*trans* isomer shows a bathochromic shift of about 4 nm in respect to the *cis* isomer.

*Conflicting Extinction Coefficients*: Published 1% extinction coefficients (also called specific absorbance) or molar extinction coefficients are used for the calculation of results (McKeown and Mark, 1962; Dendy, 1966; Reith and Gielen, 1971; Avila et al., 1982; Smith et al., 1983; Hirata et al., 1989; Scotter 1994, 1998). Surprisingly we find a wide and disturbing variation among the published 1% extinction coefficients of bixin and norbixin (Table 6.2).

Depending on which extinction coefficient is chosen, the results of the determination of bixin or norbixin are obviously quite different. At least one of the erroneous values can be traced back to the extinction coefficient of norbixin published by Reith and Gielen (1971), which has been quoted and used extensively in most later publications. Their 1% extinction coefficients for norbixin of 2850 (at 453 nm) and 2550 (at 482 nm) in aqueous alkaline solution are in serious doubt as is the value of 3473 at 453 nm used in the earlier FAO/WHO specification (1981).

In 1990 P. Collins (private communication) was the first to question the extinction coefficient of 2850 for norbixin at peak II (453 nm). He purified a sample of norbixin by repeated recrystallization to a purity of 99.6% (HPLC, photodiode array detection) and found a 1% extinction coefficient of 3208 for peak II in aqueous 0.1 M potassium hydroxide, quite different from the published value of 2850. We have used the absorbance ratio of 0.894 between the norbixin peaks II (453 nm) and III (482 nm) to calculate from Collins's findings that the extinction coefficient of norbixin at peak III must be 2867 and not 2550 as published by Reith and Gielen. As was pointed out in 1990 by N. Dinesen (private communication), it must be a curious coincidence that Reith and Gielen's figure for peak II is actually rather close to the extinction for peak III as calculated on the basis of Collins's data.

The following practical conversion factors may be useful to correlate optical absorbances at peaks II and III. To convert absorbance at peak II in chloroform (472 nm) to absorbance at peak III in the same solvent (504 nm), multiply by 0.900. To convert norbixin absorbance at peak II in aqueous alkali to absorbance at peak III in the same medium, multiply by 0.894.

*Correlation of Optical Absorbances of Norbixin and Bixin*: When a sample of bixin is hydrolyzed, the optical absorbance increases. Figure 6.2 shows the spectrum of a 2.5 mg/L solution of bixin in chloroform before hydrolysis and after hydrolysis of the same sample of bixin (norbixin in aqueous alkali) without change in concentration. The norbixin curve is higher than was the curve of the same sample of bixin before hydrolysis, which confirms that the 1% extinction coefficient of norbixin is higher than that of bixin.

In an attempt to calculate the 1% extinction coefficient of bixin in chloroform on the basis of Collins's value for norbixin, we have made a statistical study with optical absorbance data recorded during 9 years for more than 1000 different samples of bixin before and after hydrolysis. This study shows that the difference between the two extinction coefficients is of the order of 6%. If the value of 3208 for norbixin at peak II (P. Collins, private communication) is adopted, then the 1% extinction coefficient of bixin in chloroform must be 3016 at peak II and 2714 at peak III. This is in acceptable agreement with the findings by Scotter (1994) of 3029 and 2773 at peaks II and III, respectively, for bixin in chloroform.

However, these values are in conflict with the extinction coefficient of 2870 at peak III for both bixin and norbixin as established in Europe (European Economic Communities, 1995; FAO/WHO, 1996). Their assumption of equal extinction coefficient for both bixin and norbixin may have been reasonable in theory because both molecules have the same chromophore. However, from our results it is clear that the ionization of the carboxylic groups of norbixin in aqueous alkali exerts a hyperchromic effect (as defined by Britton, 1995) and thus a revision of the European specification E160b may be in order.



Our findings also provide an explanation for the mention by Smith et al. (1983) that "unexplainably" a sample of oil-soluble annatto food color gave them a higher bixin assay result after saponification than before. These authors thought that perhaps some other component of the food color was responsible for this increase, but they did not at the time consider the possibility of a hyperchromic effect due to ionization.

It is obvious that further research is needed to finally establish the correct extinction coefficient of bixin. This requires a systematic purification of bixin by the methods of classical organic chemistry until achieving 100% purity as proven by a molecular weight determination and compliance of C, H, and O analyses with the molecular formula.

*Never Use a Quartz Cuvette*: An aspect often overlooked in the spectrophotometric analysis of bixin in chloroform solution is the type of cuvette used in the spectrophotometer. We have found that when using a quartz cuvette, photodegradation occurs at a surprisingly fast rate after placing the sample in the spectrophotometer solely by the action of the light energy of the instrument on the sample. The addition of antioxidants does not prevent this degradation. In as little as 10 seconds the absorbance of the sample is reduced by almost 5%, and the decrease continues thereafter. Figure 6.3 shows the spectral curves of a solution of 0.25 mg bixin in 100 mL of chloroform measured at 40-second intervals while in a quartz cuvette in the spectrophotometer.

As stated, this degradation occurs only when the sample is in a quartz cuvette, which transmits ultraviolet radiation below 300 nm. It does not occur when using glass cuvettes that do not transmit light in the UV area of the spectrum. It is therefore important to use only glass cuvettes for bixin analysis, a recommendation already made by Dendy (1966), which has been largely ignored. It is possible that some of the discrepancies in the extinction coefficients published by different authors may thus be explained.

**Reverse-Phase HPLC.** Methods are available for the determination of the colored components of annatto extracts including the geometrical isomers of bixin and norbixin (Smith et al., 1983; Nishizawa et al., 1983; Amakawa et al., 1984; Rouseff, 1988; Luf and Brandl, 1988; Chatani and Adachi, 1988; Wood et al., 1991). The method of Scotter et al. (1994) is practical and very reliable. A reverse-phase HiRPB column is used with an isocratic (65%:35%) mobile phase of acetonitrile:2% aqueous acetic acid. We have found that a mobile phase with a 70%:30% ratio of the two components improves peak separations in some cases, especially when using a Spherisorb S50DS1 column.

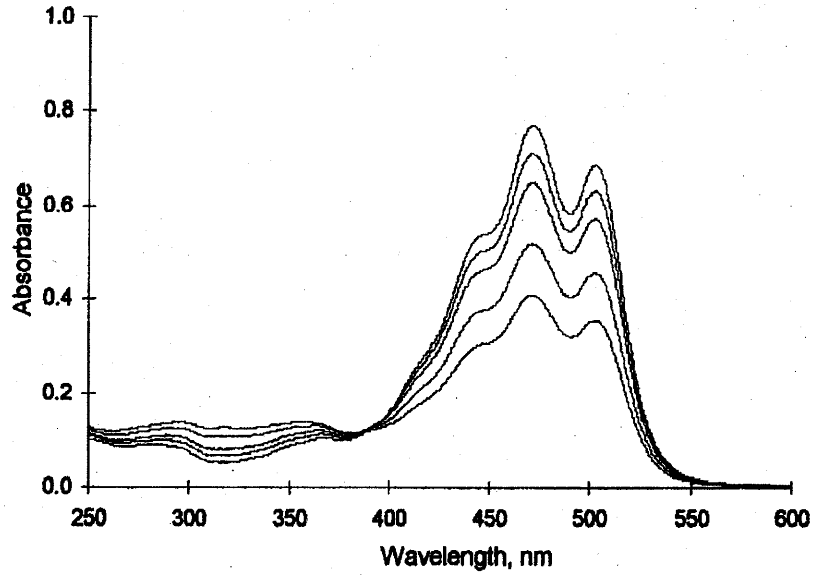



**FIG. 6.3** Reduction of optical absorbance of bixin in chloroform in quartz cuvettes placed in spectrophotometer (40 sec interval between measurements).

An interesting feature of HPLC of annatto colors is the possibility to distinguish which of the peaks refer to the red and which to the yellow components if two detector settings are used. An aliquot of the sample is first injected with the detector set at 460 nm. Then an aliquot of the same sample if again injected but with the detector set at 404 nm. The two chromatograms are compared. Peaks that decrease in area between the first and the second chromatogram are the red components; peaks that increase in the second are the yellow components.

A method for the determination of bixin and norbixin in human plasma was recently published (Levy et al., 1997).

### Methods for Specific Products

For mixtures of bixin/norbixin with other colors such as β-carotene or turmeric, open column chromatography or thin-layer separation must be used prior to spectrophotometric measurement because of overlapping spectral curves. An interesting method for direct measurement of annatto colors in the presence of β-carotene involves the use of derivative spectrophotometry (Luf, and Brandl, 1988). Photo-acoustic spectrometry has been proposed (Haas and Vinha, 1995).



Of special interest for the food technologist are specific methods for the determination of annatto in margarine, cheddar cheese, and boiled sweets (Smith et al., 1983) and in high-fat dairy products, margarine, and hard candy (Lancaster and Lawrence, 1995), the detection of bixin as adulterant in products derived from red pepper (Minguez-Mosquera et al., 1995), the determination of bixin in the presence of carminic acid (Lancaster and Lawrence, 1996), the group analysis of natural coloring matters in food products (Yamada, 1997), and the analysis of food coloring formulations (Scotter, 1998).

## CONTAMINANTS

Tolerance levels in the United States for annatto extracts (U.S. Code of Federal Regulations, 21CFR73.30) are 3 mg/kg for arsenic and 10 mg/kg for lead. Maximum residual solvent levels shown in the Food Chemicals Codex (1996) are 0.003% for acetone, 0.0025% for hexane, 0.005% for methanol or isopropanol, and 0.003% for trichloroethylene or dichloromethane individually or in combination. The same limits are shown in the European specifications (1995) for "annatto, bixin, norbixin" (food color E160b) with additional limits for heavy metals (40 mg/kg expressed as lead), mercury (1 mg/kg) and cadmium (1 mg/kg) (European Economic Community, 1995).

There is a certain incongruence in the tolerance levels of contaminants in annatto food colors. In the European specification the same maximum levels of contaminants are shown for annatto extracts of the whole range of color concentration, i.e., for oil-extracted annatto of 0.1% bixin, for alkali extracted annatto of 0.1% norbixin, as well as for solvent-extracted annatto of 75% bixin, even though in practical use the dilutions at which these are incorporated into the food product vary from 1: 70 (for the 0.1% bixin extract) to 1: 40,000 (for the 75% bixin extract). The aim of establishing tolerances for contaminants is obviously to safeguard the health of the consumer of the ready-to-eat food product containing the annatto color. Such contaminants will appear in the final food product at different levels with each of the various annatto extracts, which nevertheless are used at widely differing dosages because of the range of bixin/norbixin concentrations available. The U.S. limits do not refer to any specific concentration of annatto color either. They refer simply to "annatto extract, including pigments precipitated therefrom" (21CFR73.30) and thus show the same incongruence.

In practice, a disturbing aspect of the analytical data of trace contaminants is the highly variable performance of different laboratories as shown in an assessment of 136 laboratories in 21 countries carried out by the World Health Organization. As many as 40% of the laboratories assessed showed unsatisfactory accuracy in the analytical trace element results they reported (Weigert et al., 1997). In the specific case of the determination of mercury at the 1 ppm level, we have found wide variations of results received from various internationally recognized independent laboratories to whom we sent aliquots of the same bixin sample. They reported results varying from 0.6 ("sample complies with international specifications") to 1.6 ppm ("sample does not comply"), all for the same sample.

## STABILITY: LIGHT, OXYGEN, HEAT

Like all highly colored and highly unsaturated compounds, bixin and norbixin are unstable to light (Najar et al., 1988). This means, of course, that certain wavelengths of light are absorbed by the molecule, as we know from the spectrophotometric absorption curve, and it has led to the use of bixin or norbixin in sunscreens (Grollier et al., 1989). In practice, however, bixin and norbixin show better light stability than many other carotenoid colors.

Like all the other antioxidant carotenoids, bixin and norbixin are also unstable in the presence of atmospheric oxygen. Stabilization of bixin and norbixin can be achieved by the addition of antioxidants with higher affinity for oxygen (Hettiarachy et al., 1986; Ford and Mellor, 1987; Najar et al., 1988; Ono, 1988).

When bixin is formulated in a powder base, certain levels of moisture enhance color stability (Glória et al., 1995).

Normally no color fading is observed in practice in food products colored with annatto because bixin and norbixin are more stable than many other air-susceptible colors (Berset and Marty, 1986) and thus the products containing them have an excellent shelf life. In the few cases when fading has been observed, the cause was traced to quality defects of the food product such as rancidity of an ingredient, bacterial action, or defects in packaging materials (N. Dinesen, private communication). In fact, annatto colors tend to extend the shelf life of products because of their antioxidant action.

Bixin has even been used to stabilize vitamin D preparations (Yamada, 1988).

Relative to other carotenoids, bixin and norbixin have good heat stability during food processing. By heating bixin in oil, a partial thermal decomposition can be achieved intentionally to provide a purer yellow tone to the product.

## USES AND APPLICATION RATES

The applications of oil-soluble and water-soluble annatto colors have been reviewed by Collins (1992). Between 0.1 and 50 parts per million of bixin (as pure color) provide pleasing "butter to egg-yolk shades" when applied to otherwise colorless food products. Bixin is completely soluble in fats and



oils up to 0.75% by weight, whereas norbixin is soluble in water as the sodium or potassium salt up to 7% (Dinesen, 1974). Norbixin reacts with protein with a slight shift to a delicate peach-red color. This is seen in some cheeses colored with annatto. This binding property is useful in coloring products that must hold the color fast and not bleed into the surrounding medium (Lauro, 1991).

Commercial annatto food colors are available as water-soluble extracts with norbixin contents of 1.4% ("single-strength cheese color"), 2.8% ("double-strength"), or 3.8% ("triple strength") and as a 15% norbixin powder. Oil-soluble annatto colors contain between 0.2 and 5% bixin. Higher concentrations are available as emulsion or suspension colors. "Acid-proof" annatto colors are also available.

Annatto is especially suited for coloring cheese, meat, and fish because of its ability to combine with protein, thus creating a very stable color. The categories of foods in which annatto is used are (in alphabetical order): biscuit fillings, breakfast cereals, cakes, cheeses, dairy drinks, decorations and toppings, desserts, edible ices, fruit curds, imitation crab legs, jams, maraschino cherries, margarine, marmelade, marzipan, pickles, pie fillings, sauces and seasonings, sausage casing, smoked fish, snacks, soft drinks, and sugar confectionary (U.K. Food and Drink Federation, 1992). The use of annatto in surimi paste and in new surimi-based foods has been reviewed by Lauro (1999).

Numerous patents have been issued involving the preparation of annatto food colors and their applications (Kocher, 1958: annatto extract in propylene glycol; Todd, 1964: combination with turmeric; Marcus, 1963: margarine and cheese color; Sato, 1966: Vienna sausage; Unilever, 1981: margarine; San-Ei, 1983: acid-stable food color; Schmidt, 1985: acid-soluble annatto powder; Berset and Marty, 1986: extrusion cooking; Ford, 1987: stabilization with ascorbic acid for beverages; Ono, 1988: stabilization with quercitin; Maeda, 1988: bread manufacture, Winning and Isager, 1997: water-dispersible compositions). A drawback in the use of norbixin for coloring beverages is that it is water-soluble only at a high pH. To color products of lower pH, "acid-proof" forms been developed by the combination with gum arabic (Hettiarachy et al., 1986), modified food starch, milk proteins, or cyclodextrins and polysorbate-80, all of which produce stable emulsions in acidic media (Todd, 1991). A recent Japanese patent (Nakajima et al., 1999) describes the addition of unsaturated carboxylic acid esters to stabilize norbixin solutions at pH 3.

The ever-increasing reporting requirements to the governmental regulatory agencies have opened up the former industrial secrecy surrounding the application and usage level of many food ingredients. Thus the varied applications of bixin and norbixin and the allowed rates of application can now be known in greater detail than before. Table 6.3 has been compiled from such sources.

For cheese, the most important food product in which annatto is used,

**TABLE 6.3** Maximum Application Levels for Annatto Colors in Various Food Products

| Food product | mg/kg (as 100% bixin or norbixin) |
|---|---|
| Red Leicester cheese | 50 |
| Cake decorations, marzipan toppings | 40 |
| Mimolette cheese | 35 |
| Extruded or puffed breakfast cereals | 25 |
| Sauces, seasonings, pickles | 20 |
| Snacks, extruded snacks | 20 |
| Decorations and coatings, ice cream | 20 |
| Edible cheese rind | 20 |
| Process cheese | 15 |
| Margarine | 10 |
| Fine bakery wares, confectionary | 10 |
| Cakes, biscuit fillings, deserts, liqueurs | 10 |
| Nonextruded snacks | 10 |
| Sausage casings, smoked fish | 10 |
| Marmelade | 3 |
| Dairy drinks | 2 |
| Soft drinks | 0.5 |

*Source*: Adapted from U.K. Food and Drink Federation, 1992, and European Economic Community, 1994.

the maximum application rate in Europe is 600 mg "annatto color" (probably based on 2–4% norbixin, thus equivalent to 12–24 mg pure pigment) per kg of cheese (Luf and Brandl, 1988), followed by baked goods, cake decorations, and breakfast cereals. Other applications for annatto are the coloring of sausage casings (Sato and Susuki, 1966), sausages and ham at up to 95 mg/kg (Chatani and Adachi, 1988), and the quality improvement of bread dough (Maeda, 1988). Lancaster and Lawrence (1995) reported on the norbixin and bixin content of commercial samples of various types of cheeses sold in Canada. The bixin content per kg was 5.9 mg in process cheese spread and 5.1 mg in Canadian wine cheese. The norbixin content in mg pure pigment per kg was 1.1 in process cheese spread, 2.5 in process cheese slices, 15.6 in skim milk cheese, 16.8 in Canadian wine cheese, 18.2 in medium Cheddar cheese, 21.2 in Canadian Colby, and 68.8 in sharp Cheddar cheese. Samples of commercial butter sold in Canada were found to contain 0.017–0.199 mg/kg of bixin and 0.033–0.914 mg/kg of norbixin.

Of interest to the food technologist is an abnormal color change that occurs occasionally in annatto-colored processed cheese commonly referred



to as "pinking." Its relation to the processing techniques and to the types of annatto color used has recently been studied (Shumaker and Wendorff, 1998). It was found that the anomaly occurs more often with aqueous emulsions of annatto than with bixin-in-oil suspensions.

Annatto acts as an antioxidant in foods improving the stability of the product. Haila et al. (1996) have shown that the autoxidation of fats is significantly reduced with 30–60 mg bixin per kg. Unlike other carotenoids, such as β-carotene, lutein, or lycopene, no pro-oxidant action has been found for bixin.

The rather unusual application of *cis*-bixin as the rigid hydrophobic core of a bolaform amphiphile surface monolayer was reported by Fuhrhop et al. (1990), who also was able to integrate the gluconamide derivative of bixin into helical micellar fibers.

## DAILY HUMAN INTAKE

This is an important consideration from the regulatory standpoint. Based on the application levels, many calculations have been attempted to establish the estimated daily human intake of bixin/norbixin through foods containing them. The estimates vary from 0.032 mg/kg body weight/day for bixin and 0.051 mg/kg body weight/day in the United States (N. Dinesen, private communication) to 0.065 mg in the United Kingdom (Ministry of Agriculture, Fisheries and Food, 1993). Calculated for a person of 60 kg average body weight, these figures represent an average daily bixin/norbixin intake of 1.9 mg for bixin and 3.1 mg for norbixin in the United States and of 3.9 mg in the United Kingdom. Another British study (UK Food and Drink Federation, 1992) suggests an 8 mg/day average intake, which appears to be greatly exaggerated. A recent French study (Verger et al., 1998) shows a theoretical maximum daily intake of 0.16 mg/day/kg body weight (2.5 mg/day for a 60 kg person) and a mean daily intake of 0.005 mg/day/kg body weight (0.3 mg/day/person). The study is based on the additive list on the labels of foods, and thus the figures most probably are based on annatto color E160b as such and not on pure bixin or norbixin. If so, the results shown should at least be halved to reflect bixin or norbixin intakes.

The maximum acceptable daily intake (ADI) established by the Joint FAO/WHO Committee on Food Additives (JECFA, 1982) is 0.065 mg/day/kg body weight expressed as bixin, which for the average human weight of 60 kg works out at 3.9 mg/day.

These calculations have achieved more significance since it has been shown that bixin is rapidly absorbed into the blood stream after ingestion (Levy et al., 1997). The experimental human ingestion of 16 mg bixin in a single dose gave rise to a maximum bixin/norbixin level in the plasma of 144 μg/L, which is comparable to the ranges of other carotenoids normally found in human plasma (e.g., β-carotene, 40–530; lutein, 90–140;

lycopene, 70–460 μg/L). Complete plasma clearance occurred for bixin by 8 hours after ingestion and for norbixin by 24 hours. This confirmed an anonymous report mentioned earlier by Preston and Rickard (1980).

## SAFETY

Annatto is considered essentially nontoxic. In the United States it is a natural colorant "exempt from certification" (U.S. Code of Federal Regulations as of 1994). Its safety is based on its traditional use for many centuries as a food color by millions of people in South America and on the early work of Zbinden and Studer (1958) and van Esch et al. (1959), who conducted chronic feeding tests with aqueous alkaline annatto extracts on rats and found no adverse effects. A safety assessment of annatto extracts was made by the International Life Sciences Institute (1985). Based on prior work by Lück and Rickerl (Ghorpade et al., 1995) with rats and one-year chronic oral toxicity studies with dogs using solvent-extracted annatto and aqueous annatto extracts (J. H. Kay and J. C. Calandra, unpublished), as well as the genotoxicity tests of Haveland-Smith (1981), it was concluded that high levels of bixin or norbixin in the diet of experimental animals do not cause any toxicological or carcinogenic effects and that 0.065 mg/kg body weight (expressed as bixin) is an acceptable daily intake for humans.

Some care must be exercised when reviewing some of the toxicological information mentioned in the literature. Ghorpade et al. (1995), for example, erroneously quote Dunham and Allard (1960), saying that "annatto extracts" are antispasmodic and hypotensive, but they do not warn the reader that Dunham and Allard's work is irrelevant to annatto food color because it was done with extracts of the roots of the annatto plant, which contain no bixin.

More recently it was reported that petroleum ether extracts of annatto seed probably containing mainly nonbixin extractives have shown genotoxicity when applied to onion roots, as have total extracts with chloroform containing all the seed components soluble in this solvent (Aranez and Rubio, 1996). Also, a petroleum ether extract made from the chloroform extract given to male mice before mating appeared to produce more dead offspring in the females (Aranez and Bayot, 1997). A critical evaluation of this work is necessary. It would be important to find out if the observed result could be explained by the presence of residual chloroform in the sample.

Certainly the petroleum ether extract of annatto seeds contains only negligible amounts of bixin. In any event, these reports lack any practical importance because none of these solvents are used commercially for the extraction of annatto seed.

Occasional individual cases of allergic reactions to foods containing annatto color have been reported (Mikkelsen et al., 1977; Nish et al., 1991),



but they are attributed to a trace of an annatto seed protein, which may have been in the annatto color as an impurity, rather than to bixin or norbixin.

The increased use of annatto colors means that the ADI needs to be raised. The international authorities have requested additional studies because all earlier studies had used addition rates for annatto now considered too low. The scientific and manufacturing groups involved in annatto are currently funding a major animal study at a well-known research laboratory in England.

## NONBIXIN COMPOUNDS IN THE ANNATTO PLANT

Although bixin is the only component of *Bixa orellana* that currently has commercial importance, the plant is a treasure chest of exotic compounds, some found in no other plant, most of which were discovered during the past 10 years. Prominent among them are geranylgeraniol in the annatto seed coat (Jondiko and Pattenden, 1989) and ishwarane in annatto leaf oil (Lawrence and Hogg, 1973). The presence of these two compounds converts annatto seed into their richest known natural source. Recent work of the group of Prof. Pfander at the University of Bern (Switzerland) has enormously expanded our knowledge of the minor carotenoid components of annatto seed extract (Mercadante et al., 1996, 1997a, 1997b, 1999). Most of the compounds are found only in annatto and have not been reported previously. It has been postulated that the presence of geranylgeraniol is responsible for some of the "unusual properties" of annatto colors (Craveiro et al., 1989). Recently Mercadante et al. (1999) discovered geranylgeraniol esters of bixin in annatto seed. Many of these minor constituents of annatto may give new insight into the biosynthesis and metabolism of bixin in the annatto plant.

Compounds other than bixin that have been discovered in the annatto plant since 1965 are shown in chronological order of their discovery in Table 6.4.

## PHARMACOLOGY

This is an entirely new field that is wide open for new research. Annatto seeds have long been used in traditional medicine of the South American Indians to promote the healing of wounds, against skin eruptions, and in the healing of burns "without a scar" and have been given internally to subdue diarrhea and asthma (Morton, 1989) and as an antipyretic (Terashima et al., 1991). It is not clear if these effects are attributable to bixin or to some other compounds in the annatto seed. Other botanical parts of the annatto

**TABLE 6.4** Compounds Other than Bixin and Norbixin Identified in *Bixa orellana*[a,b]

| Compound | Ref. |
|---|---|
| Tomentosic acid (in roots) | Schneider, 1965 |
| Ishwarane (in leaf oil) | Lawrence and Hogg, 1973 |
| β,β-Carotene, cryptoxanthin, lutein zeaxanthin, methylbixin (all in traces) | Tirimanna, 1981 |
| Geranylgeraniol | Jondiko and Pattenden, 1989<br>Craveiro et al., 1989 |
| Geranylgeraniol formate, geranylgeraniol octadecanoate farnesylacetone, δ-tocotrienol | Jondiko and Pattenden, 1989 |
| Methyl-9′-*cis*-apo-1-bixinal ester or Methyl-8-oxo-9′*cis*-8,6′-diapo-caroten-6′-oate[c] | Jondiko and Pattenden, 1989 |
| α- and β-Pinene (in essential oil) | Rath, 1990 |
| Iso-scutellarein, gallic acid, Pyrogallol (aqueous leaf extract) | Terashima et al., 1991 |
| Methyl-9′-*cis*-apo-6′-lycopenoate[c] | Mercadante et al., 1996 |
| Methyl-*trans*-8′-apo-á-caroten-8′-oate[d] | Mercadante et al., 1997a |
| Methyl-7,9,9′tri-*cis*-apo-6′-lycopenoate[c] | Mercadante et al., 1997a |
| Methyl-9-*cis*-apo-8′-lycopenoate[c] | Mercadante et al., 1997a |
| Methyl-*trans*—apo-8′-lycopenoate[c] | Mercadante et al., 1997a |
| Methyl-*trans*—apo-6′-lycopenoate[e] | Mercadante et al., 1997a |
| Methyl-9-*cis*-bixin | Mercadante et al., 1997b |
| Methyl-9,9′di-*cis* bixin[c] | Mercadante et al., 1997b |
| Methyl-9-*cis*-6′-oxo-6,5′-diapocaroten6-oate[c] | Mercadante et al., 1997b |
| Methyl-4-*cis*-4,8-dimethyl-12-oxo-dodecyl-2,4,6,8,10-penta-enoate[c,f] | Mercadante et al., 1997b |
| 6-Geranylgeranyl-8′-methyl-6,8′-diapo6,8′dioate | Mercadante et al., 1999 |
| Geranylgeraniol-*cis*-bixinate | Mercadante et al., 1999 |
| Geranylgeraniol-*trans*-bixinate | Mercadante et al., 1999 |

[a]In chronological order of discovery.
[b]In seed extracts unless otherwise indicated.
[c]New carotenoid, found only in *B. orellana*.
[d]Previously found only in *Staphylococcus aureus* bacteria.
[e]Previously found only in *Sepherdia canadensis*.
[f]Two central methyl groups missing, thus no longer a carotenoid, possibly a metabolic product.



plant also contain physiologically active compounds. Annatto root extracts, for example, have been shown to be antisecretory, antispasmodic, and hypotensive (Dunham and Allard, 1960), and the aldose reductase inhibitor iso-scutellarein (a flavone) has been found in annatto leaf extract (Terashima et al., 1991). Annatto seed extract given to dogs showed the presence of a hyperglycaemic principle (Morrison et al., 1991).

Bixin has strong physical quenching activity of singlet molecular oxygen and thus may excert a protéctive action against some types of cancer (DiMascio et al., 1990). However, bixin did not prevent the formation of cancer cells in experimental carcinogenesis with methylcholanthrene (Bertram et al., 1991). As a protectant against biological membrane oxidation, bixin is a potent inhibitor of lipid peroxidation at the same level of lutein and canthaxanthin and is only surpassed by α-tocopherol (Zhang et al., 1991). Bixin acts as a lipoxydase inhibitor and modulates lipid hydroperoxide formation (Canfield and Valenzuela, 1993). Oral administration of bixin significantly reduced the otherwise increased level of lipid peroxides in serum and liver of rats caused by gamma radiation and can thus be considered a candidate drug for protection against the side effects in cancer patients undergoing therapeutic irradiation (Thresiamma et al., 1995, 1998). Bixin does not upregulate Connexin-43 gene expression as some other carotenoids do, but it is active in membrane protection (Zhang et al., 1992). Bixin increases the activity of xenobiotic metabolizing enzymes (Jewell and O'Brien, 1999). Food colors in general enhance immunoglobulin production by rat spleen lymphocytes, although bixin was not specifically included in the study (Kuramoto et al., 1996). Some interesting spectral changes have been observed during the interaction of bixin with respiring rat liver mitocondria (Inada et al., 1971; Hirose et al., 1972).

Compared to the extensive current research effort on the health-promoting effects of the other natural carotenoids (Krinsky, 1994), bixin has not yet received much attention. It may well be that many beneficial actions of bixin are yet to be discovered (Levy et al., 1997).

## SPECULATIONS: ARE NATURAL COLORS GOOD FOR YOUR HEALTH?

The purpose for the use of colors in the food industry has been to make the product more pleasant to look at and, since "we eat through our eyes," to make it more palatable. Because most natural colors are also potent antioxidants, they also have the function of preservation of many nutritionally valuable food ingredients, increasing the shelf life of the product. Over the last few years more and more evidence has been accumulated that many natural antioxidants that are useful for the preservation of vitamins and lipids in foods also produce antioxidant activities in the human body after consumption of the foods (Lölinger, 1997). There is mounting evidence of the importance of the antioxidant carotenoids in human health. It is interesting to speculate that these colors not only make the food products look better and help to preserve it, but they may actually improve the health of the consumer.

## Action of Natural Colors Against Free Radicals

There is increasing awareness of the importance of controlling the metabolic production of free radicals in our bodies to maintain good health. Ever since animals and humans appeared on this planet, this has been accomplished by their eating foods from the plant kingdom that are rich in antioxidants, such as fruits and vegetables. The statistical increase of human diseases such as cancer and atherosclerosis in this century has been related to the increase of stress, atmospheric pollution, pesticide contamination of foods, and ultraviolet radiation caused by the thinning of the protecting ozone layer in the stratosphere. All of these are facts of life for modern humans. All of these promote the formation of more free radicals in our bodies than may have been the case in former periods of history. Thus the current strong medical recommendation for all of us to increase our consumption of fruits and green vegetables containing carotenoids, flavonoids, and other natural antioxidants. It is believed that within the biological defense system against free radicals, unpaired electrons are taken up by vitamin E (tocopherol), carotenoids, and vitamin C (ascorbic acid) in a cascade-like fashion, where all of these act in combination (Truscott et al., 1996). Vitamin E, after taking up the electrons, becomes a transient tocopheryl free radical. Free electrons are then transferred to one after the other of the carotenoids through their conjugated double bonds. The first carotenoid of this cycle regenerates the tocopherol to its original non–free radical state, the next carotenoid regenerates the former carotenoid, and so on. In this process the excess energy is slowly dissipated during the trip of the electron through the various conjugated double-bond systems as thermal energy, and the electrons are finally transferred at the lipid/water interphase into the aqueous phase, where ascorbic acid takes final care of them.

## Molecular Basis for Color and Free-Radical Scavenging

The color sensation of the human eye originates in the conjugated double-bond system of the carotenoids. A minimum of seven conjugated double bonds is necessary for a carotenoid molecule to absorb light of the visible spectrum (Britton, 1998). All carotenoid molecules have more than this number of double bonds. Free radical–scavenging activity is equally related to the number of conjugated double bonds in the carotenoid molecule.



Thus it can be postulated that both the color and the defense against free radicals originate on the same molecular basis.

## Bioavailability of Carotenoid Colors

The bioavailability for humans of the carotenoids of foods is rather low. This means that only a small proportion of the carotenoids of fruits, vegetables, and spices reach the blood stream where they are most needed. Spinach, for example, is a rich source of the carotenoids β-carotene and lutein, but normally only a fraction of these is actually absorbed into the blood stream (Castenmiller et al., 1999). This is due to the "matrix" in which the carotenoid is locked in the spinach (i.e., the cell walls surrounding the carotenoid, which are broken down only partially by the act of chewing). The same is the case for lycopene in tomatoes (Gaertner et al., 1997) and other vegetables and fruits. Recommendations have been made to prepare the vegetables in a food blender in order to break down the cells mechanically prior to eating. Also, against all common wisdom, it was found that cooked foods actually provide more antioxidants than fresh, uncooked foods. The reason is the same: by softening the cell walls the antioxidants become more "available" for absorption into the blood stream during digestion. All this means that nutritional supplementation of carotenoids is an ever-increasing necessity for modern humanity. Such supplementation may come, in part, from carotenoid extracts added to food products, which then become fortified foods.

## Artificial Dyes Versus Natural Colors in Relation to Cancer

The color effect of coal tar–derived artificial dyes is generally due to the electronic structure of the carbon-nitrogen or carbon-sulfur bond in their molecules, not by a system of conjugated double bonds as is the case with the carotenoids, which contain only carbon, hydrogen, and sometimes oxygen atoms. This fundamental molecular difference explains the apparent paradox that two substances having the same color—one an artificial dye and the other a carotenoid—have such radically different biological actions, such as one being a carcinogen and the other an anticarcinogen. Artificial dyes tend to produce free radicals, whereas the natural colors eliminate free radicals. For this reason artificial colors have been progressively banned for use in foods and are being replaced by natural colors.

## How Does Bixin Fit in?

Bixin is one of the most powerful carotenoid antioxidants. Its conjugated double-bond system is ideally suited for scavenging free radicals. After consumption it is well absorbed into the blood stream. Its possible benefit for human health awaits further research. In summary, for all the natural colors, including bixin, it is entirely possible that the food technologist may soon watch in fascination as new scientific findings demonstrate that the natural food colors of today will be the vitamins of tomorrow.

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## APPENDIX

### Annatto

Synonyms: Achiote (South America), Onoto (Venezuela, Mexico, West Indies), Urucum (Brazil), Atzuete (Philippines), Rocou, Terre orellana, Orlean, Orange-3, CI Natural Orange 4

Chemical Abstract number: 1393-63-1

EINECS number: 215-735-4 (annatto), 289-561-2 (annatto extract)

Color Index (1982) number: 75120

European Community Identification number: Food color E-160b

Identity (USA): 21 CFR 73.30. Food Chemicals Codex, 4th ed., 1996

*Bixin*

$C_{25}H_{30}O_4$, 6,6′-diapo-Ψ, Ψ-carotenedioic acid monomethyl ester, or 6′-methylhydrogen 9′-*cis*-6,6′ diapocarotene 6,6′-dioate (or *trans*), class: carotenoid, mol. wt. 394.51

New (2000) Chemical Abstracts nomenclature: 2,4,6,8,10,12,14,16,18 eicosanonaene dioic acid; 4,8,13,17 tetramethyl-monomethyl ester.

Chemical Abstract number 6983-79-5 and 39937-23-0 (the trans isomer

EINECS number: 230-248-7

Formerly called α-bixin or "unstable bixin" (the *cis* isomer) and β-bixin or "stable bixin" (the *trans* isomer)

*Norbixin*

$C_{24}H_{28}O_4$, 6,6′-diapo-Ψ, Ψ-carotenedioic acid, or 9′-*cis*-6,6′-diapocarotene-6,6′-dioic acid (or *trans*-), mol. wt. 380.48, class: carotenoid

New (2000) Chemical Abstracts nomenclature: 2,4,6,8,10,12,14,16,18 eicosanonaene dioic acid; 4,8,13,17 tetramethyl.



Chemical Abstract number 542-40-5 and 626-76-6 (the trans isomer)

Formerly called α-norbixin and β-norbixin (*cis*- and *trans*-isomers, respectively)

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Attachment 12

EVALUATION CRITERIA FOR SUBSTANCES ADDED TO THE NATIONAL LIST

**Category 1. Adverse impacts on humans or the environment?** Substance: Annatto Extract, Water Soluble

| Question | Yes | No | N/A[1] | Documentation (TAP; petition; regulatory agency; other) |
|---|---|---|---|---|
| 1. Are there adverse effects on environment from manufacture, use, or disposal? [§205.600 b.2] | | √ | | |
| 2. Is there environmental contamination during manufacture, use, misuse, or disposal? [§6518 m.3] | | √ | | |
| 3. Is the substance harmful to the environment? [§6517c(1)(A)(i);6517(c)(2)(A)i] | | √ | | |
| 4. Does the substance contain List 1, 2, or 3 inerts? [§6517 c (1)(B)(ii); 205.601(m)2] | | | √ | |
| 5. Is there potential for detrimental chemical interaction with other materials used? [§6518 m.1] | | √ | | |
| 6. Are there adverse biological and chemical interactions in agro-ecosystem? [§6518 m.5] | | √ | | |
| 7. Are there detrimental physiological effects on soil organisms, crops, or livestock? [§6518 m.5] | √ | | | Should not be fed to livestock due to high alkalinity unless neutralized to lower pH. There are also positive effects of the residual seed after extraction as beneficial components of compost. |
| 8. Is there a toxic or other adverse action of the material or its breakdown products? [§6518 m.2] | | √ | | |
| 9. Is there undesirable persistence or concentration of the material or breakdown products in environment?[§6518 m.2] | | √ | | |
| 10. Is there any harmful effect on human health? [§6517 c (1)(A)(i) ; 6517 c(2)(A)i; §6518 m.4] | | √ | | |
| 11. Is there an adverse effect on human health as defined by applicable Federal regulations? [205.600 b.3] | | √ | | |
| 12. Is the substance GRAS when used according to FDA's good manufacturing practices? [§205.600 b.5] | | | √ | |
| 13. Does the substance contain residues of heavy metals or other contaminants in excess of FDA tolerances? [§205.600 b.5] | | √ | | |



[1]If the substance under review is for crops or livestock production, all of the questions from 205.600 (b) are N/A—not applicable.

**Category 2. Is the Substance Essential for Organic Production?** Substance: Annatto Extract, Water Soluble

| Question | Yes | No | N/A[1] | Documentation (TAP; petition; regulatory agency; other) |
|---|---|---|---|---|
| 1. Is the substance formulated or manufactured by a chemical process? [6502 (21)] | | √ | | |
| 2. Is the substance formulated or manufactured by a process that chemically changes a substance extracted from naturally occurring plant, animal, or mineral, sources? [6502 (21)] | | √ | | |
| 3. Is the substance created by naturally occurring biological processes? [6502 (21)] | √ | | | Bixin and norbixin are naturally occurring as an oily film on the coating of the Annatto seed. Bixin is converted to norbixin during processing. Small amounts of bixin remain in the finished liquid. Norbixin is the water soluble form of the natural colorant. Processing is described in the petition text. |
| 4. Is there a natural source of the substance? [§205.600 b.1] | √ | | | Annatto Extract, Water Soluble is derived from a natural source, Annatto seed. |
| 5. Is there an organic substitute? [§205.600 b.1] | | √ | | |
| 6. Is the substance essential for handling of organically produced agricultural products? [§205.600 b.6] | | √ | | |
| 7. Is there a wholly natural substitute product? [§6517 c (1)(A)(ii)] | | | √ | Annatto Extract, Water Soluble is a wholly natural product. |
| 8. Is the substance used in handling, not synthetic, but not organically produced? [§6517 c (1)(B)(iii)] | √ | | | |
| 9. Is there any alternative substances? [§6518 m.6] | | √ | | |
| 10. Is there another practice that would make the substance unnecessary? [§6518 m.6] | | √ | | |

[1]If the substance under review is for crops or livestock production, all of the questions from 205.600 (b)are N/A—not applicable.

**Category 3. Is the substance compatible with organic production practices?**

Substance: Annatto Extract, Water Soluble

| Question | Yes | No | N/A[1] | Documentation (TAP; petition; regulatory agency; other) |
|---|---|---|---|---|
| 1. Is the substance compatible with organic handling? [§205.600 b.2] | √ | | | |
| 2. Is the substance consistent with organic farming and handling? [§6517 c (1)(A)(iii); 6517 c (2)(A)(ii)] | √ | | | |
| 3. Is the substance compatible with a system of sustainable agriculture? [§6518 m.7] | √ | | | |
| 4. Is the nutritional quality of the food maintained with the substance? [§205.600 b.3] | √ | | | At typical usage levels (ppm) Annatto Extract Water Soluble will have negligible nutritional effect on the finished food product. |
| 5. Is the primary use as a preservative? [§205.600 b.4] | | √ | | |
| 6. Is the primary use to recreate or improve flavors, colors, textures, or nutritive values lost in processing (except when required by law, e.g., vitamin D in milk)? [205.600 b.4] | √ | | | Section 205.600 b.4 references the use of synthetic substances used to recreate or improve flavors, colors, textures, or nutritive values lost in processing. Annatto Extract Water Soluble is not a synthetic substance. |
| 7. Is the substance used in production, and does it contain an active synthetic ingredient in the following categories: a. copper and sulfur compounds; | | | √ | |
| b. toxins derived from bacteria; | | | √ | |
| c. pheromones, soaps, horticultural oils, fish emulsions, treated seed, vitamins and minerals? | | | √ | |
| d. livestock parasiticides and medicines? | | | √ | |
| e. production aids including netting, tree wraps and seals, insect traps, sticky barriers, row covers, and equipment cleaners? | | | √ | |

[1]If the substance under review is for crops or livestock production, all of the questions from 205.600 (b) are N/A—not applicable.

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Company Sensient Colors Inc.

Address 2515 North Jefferson Ave Dept./Floor/Suite/Room

City St Louis State MO ZIP 63106

**2 Your Internal Billing Reference**

**3 To**

Recipient's Name NOSB - Robert Pooler Phone 202 720-3252

Company USDA/AMS/TM/NOP

Recipient's Address 1400 Independence Ave S.W.

We cannot deliver to P.O. boxes or P.O. ZIP codes. Dept./Floor/Suite/Room

Address Room 4008-50 Ag Stop 0268

To request a package be held at a specific FedEx location, print FedEx address here.

City Washington State DC ZIP 20250

8598 0354 8507

**4a Express Package Service** *Packages up to 150 lbs.*

1 [X] FedEx Priority Overnight — Next business morning.* Friday shipments will be delivered on Monday unless SATURDAY Delivery is selected.

5 [ ] FedEx Standard Overnight — Next business afternoon.* Saturday Delivery NOT available.

6 [ ] FedEx First Overnight — Earliest next business morning delivery to select locations.* Saturday Delivery NOT available.

3 [ ] FedEx 2Day — Second business day.* Thursday shipments will be delivered on Monday unless SATURDAY Delivery is selected.

20 [ ] FedEx Express Saver — Third business day.* Saturday Delivery NOT available.

FedEx Envelope rate not available. Minimum charge: One-pound rate.

* To most locations.

**4b Express Freight Service** *Packages over 150 lbs.*

7 [ ] FedEx 1Day Freight* — Next business day.** Friday shipments will be delivered on Monday unless SATURDAY Delivery is selected.

8 [ ] FedEx 2Day Freight — Second business day.** Thursday shipments will be delivered on Monday unless SATURDAY Delivery is selected.

83 [ ] FedEx 3Day Freight — Third business day.** Saturday Delivery NOT available.

* Call for Confirmation:

** To most locations.

**5 Packaging**

6 [ ] FedEx Envelope* 2 [ ] FedEx Pak* Includes FedEx Small Pak, FedEx Large Pak, and FedEx Sturdy Pak. 3 [X] FedEx Box 4 [ ] FedEx Tube 1 [ ] Other

* Declared value limit $500.

**6 Special Handling**

Include FedEx address in Section 3.

3 [ ] SATURDAY Delivery — Not available for FedEx Standard Overnight, FedEx First Overnight, FedEx Express Saver, or FedEx 3Day Freight.

1 [ ] HOLD Weekday at FedEx Location — Not available for FedEx First Overnight.

31 [ ] HOLD Saturday at FedEx Location — Available ONLY for FedEx Priority Overnight and FedEx 2Day to select locations.

Does this shipment contain dangerous goods? One box must be checked.

[ ] No 4 [ ] Yes As per attached Shipper's Declaration. [ ] Yes Shipper's Declaration not required. 6 [ ] Dry Ice Dry Ice, 9, UN 1845 ____ x ____ kg

Dangerous goods (including dry ice) cannot be shipped in FedEx packaging. [ ] Cargo Aircraft Only

**7 Payment** *Bill to:* Enter FedEx Acct. No. or Credit Card No. below. Obtain Recip. Acct. No. [ ]

1 [X] Sender Acct. No. in Section 1 will be billed. 2 [ ] Recipient 3 [ ] Third Party 4 [ ] Credit Card 5 [ ] Cash/Check

[redacted]

| Total Packages | Total Weight | | Total Charges |
|---|---|---|---|
| 1 | 2.5 lbs | [redacted] | Credit Card Auth. |

†Our liability is limited to $100 unless you declare a higher value. See the current FedEx Service Guide for details.

**8** ***NEW*** **Residential Delivery Signature Options** If you require a signature, check Direct or Indirect.

[ ] No Signature Required — Package may be left without obtaining a signature for delivery.

10 [ ] Direct Signature — Anyone at recipient's address may sign for delivery. *Fee applies.*

34 [ ] Indirect Signature — If no one is available at recipient's address, anyone at a neighboring address may sign for delivery. *Fee applies.*

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