

Structures of some typical carotenoid compounds biology essay

[Science](#), [Biology](#)



Part III Specific additives

Colorants

F. J. Francis, University of Massachusetts, Amherst⁸. 1 Introduction The appreciation of color and the use of colorants dates back to antiquity. The art of making colored candy is shown in paintings in Egyptian tombs as far back as 1500 bc. Pliny the Elder described the use of artificial colorants in wine in 1500 bc. Spices and condiments were colored at least 500 years ago. The use of colorants in cosmetics is better documented than colorants in foods. Archaeologists have pointed out that Egyptian women used green copper ores as eye shadow as early as 5000 bc. Henna was used to redden hair and feet, carmine to redden lips, faces were colored yellow with saffron and kohl, an arsenic compound, was used to darken eyebrows. More recently, in Britain, in the twelfth century, sugar was colored red with kermes and madder and purple with Tyrian purple. Until the middle of the nineteenth century, the colorants used in cosmetics, drugs and foods were of natural origin from animals, plants and minerals. That changed with the discovery of the first synthetic dyestuff, mauve, by Sir William Henry Perkin in 1856. The German dyestuff industry rapidly developed a large number of 'coal tar' colorants and they rapidly found applications in the food and cosmetic industries. At the turn of the century, over 700 synthetic colorants were available for use in foods in the US. The potential for fraud and personal harm was obvious and horror stories abounded. For example, Marmion¹ described a situation where a druggist gave a caterer copper arsenite to make a green pudding for a public dinner and two people died. History is rife with anecdotes about adulteration of food, from the recipe for bogus claret

wine² in 1805 to the attacks on synthetic colorants today. Two centuries ago, adulteration had become a very sophisticated operation. Accum³ commented, 'To such perfection of ingenuity has the system of counterfeiting and adulterating various commodities of life arrived in this country, that spurious articles are everywhere to be found in the market, made up so skilfully, as to elude the discrimination of the most experienced judges - the magnitude of an evil, which in many cases, prevails to an extent so alarming, that we may exclaim - There is death in the pot'. Many of the adulterants involved color and flavor. Elderberries and bilberries were added to wine. Copper acetate was used to color artificial tea leaves. Red lead was a colorant for cheese. Obviously government regulation was essential and this led to a long series of publications on the safety of colorants for food, drugs and cosmetics. This chapter is devoted to a description of the chemistry, applications, and safety of the wide variety of natural and synthetic colorants available today. But another aspect has entered into consideration. Food safety of colorants has usually been considered to be a negative if we ignore the many benefits of making food more attractive in appearance. The recent meteoric rise of the nutraceutical industries has made it possible to claim health benefits for many categories of food including the colorants. Where appropriate, the health claims will be included in this chapter.

8. 2 Food, drug and cosmetic colorants

8. 2. 1

IntroductionThe chaotic situation existing in the synthetic colorant industry was evident in the 80 colorants available in 1907 to the paint, plastic, textile, and food industries. Obviously very few of them had been tested for safety. Dr Bernard Hesse, a German dye expert employed by the US Department of

Agriculture, was asked to study the situation and he concluded that, of the 80 colorants available, only 16 were more or less harmless and he recommended only seven for use in food. This led to the US Food and Drug Act of 1906 which set up a certification procedure which ensured the identity of the colorant and the levels of impurities specifications for each food, drug and cosmetic (FD&C) color permitted for each colorant. 4The Federal Food, Drug, and Cosmetic Act of 1938 set up three categories: FD&C colors for use in foods, drugs and cosmetics D&C colors for use in drugs and cosmetics when in contact with mucous membranes or ingested Ext. D&C colors for use in products applied externally. The 1938 law required colorants on the permitted list to be 'harmless and suitable for food' but the FDA interpreted 'harmless' to mean harmless at any level and this proved to be unworkable. The Color Additive Amendment of 1960 eliminated the 'harmless per se' interpretation and resulted in a list of nine permitted colorants. The list was reduced to eight with the delisting of FD&C Fig. 8. 1 Structures of eight permitted food, drug and cosmetic colorants. Red No. 3 in 1998 (Fig. 8. 1). Colorants in the three categories above were termed 'certified' colorants but the Color Additive Amendment also set up a category of 'exempt' colorants which were not subject to the rigorous requirements of the certified colorants. There are 26 colorants in this category (Table 8. 1) and they comprise most of the preparations which would be called 'natural' in other countries. The US does not officially recognize the term 'natural' but it is often used in the popular press. Table 8. 1 Regulatory and safety status of colorants exempt from certification in the USA Color additive US food use limit EU status JECFA ADI (mg/kg/bw) Algal meal, dried GMPb for chicken

feedNLcNEdAnnatto extractGMPeE160b0-0. 065Dehydrated
beetsGMPE162NEUltramarine blueSalt for animal feed up toNLNone0. 5//5 by
weightCanthaxanthinNot to exceed 30 mg/lb ofE161gNonesolid/semisolid
food or pintof liquid food or 4. 41 mg/kgof chicken feedCaramelGMPE1500-
200Beta-apo-8-carotenalNot to exceed 15 mg/lb ofE160a0-5solid or
semisolid food or 15mg/pint of liquid foodBeta caroteneGMPE1500-5Carrot
oilGMPNLNECochineal extract orGMPE1200-5carmineCorn endosperm oilGMP
for chicken feedNLNECottonseed flour, GMPNLNEToasted partiallydefatted,
cookedFerrous gluconateGMP for ripe olives onlyNLNEFruit
juiceGMPNLNEGrape color extractGMP for non-beverage foodsE1630-2.
5Grape skin extractGMP for beveragesE1630-2. 5(Enocianina)Iron oxide,
syntheticPet food up to 0. 25%E1720-2. 5PaprikaGMPE160cnonePaprika
oleoresinGMPE160cSelf-limiting asa spiceRiboflavinGMPE1010-0.
5SaffronGMPNLFood ingredientTagetes meal andGMPNLNEextract
(AztecMarigold)Titanium dioxideNot to exceed 1% by weightE171noneof
foodTurmericGMPE100Temporary ADITurmeric oleoresinGMPE100Temporary
ADIVegetable juiceGMPNLNEaAdapted from Francis, F. J., 1999. Chap. 4,
Regulation of Colorants in Colorants, Eagan Press. St. Paul, MN. pp. 223-32. b
Good Manufacturing Practice. c Not listed. d Not evaluated. e Calculated as
bixin. 8. 2. 2 Chemistry and usageThe certified and exempt colorants is the
most important group of colorants in the US both from a poundage and
diversity of applications point of view. The chemical structures of the
certified group are shown in Fig. 8. 1. This group also includes the lakes
which are prepared by reacting the pure FD&C colorants with alumina and
washing and drying the resultant precipitate. 5 FD&C colorants are available

in many formulations: dry powders, solutions in a variety of solvents, blends, formulations with a variety of carriers, and lakes. The colorant preparations are used in a wide variety of foods available in the food markets. Lakes are used in food formulations where it is desirable to minimize the 'bleeding' of color from one ingredient to another. Stability and solubility of FD&C colorants under a variety of conditions was published by Marmion⁴ and Francis. 58. 2. 3 Safety The history of the safety of the synthetic colorants has been replete with controversies and contradictions. The decisions by Bernard Hesse at the turn of the century were based primarily on the concept that the colorants should be harmless when consumed in everyday food. The authors of the 1938 Act were primarily concerned with developing a list of colorants which were allowed and would be harmless under the conditions of toxicological testing accepted in the 1930s. These included consideration of the anticipated levels of consumption with the levels of usage likely to be found across the range of expected products (the Acceptable Daily Intake, ADI). It became evident that this concept could be abused when, in 1950, some children became ill after eating popcorn with excessive levels of colorants. The FDA then launched a new round of toxicological investigations. The original list of seven colorants, which had grown to sixteen, was reduced to seven, and it included only two of the original seven. The toxicological protocols were based on weight changes, tumor production, biochemical and physical changes, etc. and teratological, immunological, multi-generation changes, allergenicity, carcinogenicity, and other changes were added. With the possible exception of saccharin, the FD&C colorants became the most tested group of additives in foods. The

case of FD&C Red No. 2 (Amaranth) is an example. Amaranth was approved for use in foods in 1907 and survived the batteries of tests until a Russian study in 1972 concluded that it was carcinogenic. It was difficult to repeat the study because the Russian material was of textile grade and contained about 9% impurities, but a flurry of activity resulted. Two studies indicated a problem with carcinogenicity and 16 had negative effects. Regardless, FD&C Red No. 2 was delisted in 1976 in spite of the controversy over the interpretations. For example, the Canadian authorities allowed FD&C Red No. 2 to be used in foods and banned FD&C Red No. 40 (Allura Red) which largely replaced Red No. 2. The American authorities banned FD&C Red No. 2 and allowed FD&C Red No. 40. Similar discrepancies are found in the approved colorants for many countries. A detailed discussion of the safety of each FD&C colorant⁶ and the exempt colorants⁷ was published but space does not allow a discussion of the safety considerations here. The general protocols for toxicological testing may be found in the FDA publication Toxicological Principles for the Safety Assessment of Direct Food and Color Additives Used in Food. Information on specific additives may be found in the US Code of Federal Regulations - CFR 21. Listings of the available colorants are also available. 8'98. 2. 4 Future prospects Synthetic colorants were severely criticized in the US in the 1960s by consumer groups critical of the 'junk food' concept. Activists had difficulty criticizing the foods themselves but the colorants which were essential to the formulation of convenience foods were vulnerable. The belief that natural additives were superior to synthetic compounds from a health point of view is a little naive since 5, 000 years is too short a time for humans to develop genetic resistance.

Regardless, the popularity of natural formulations increased worldwide and shows no signs of decreasing. The last synthetic food colorant to be approved by the FDA was FD&C Red No. 40 (Allura Red) in 1971 and there are not likely to be any more in the near future. A case can be made that there is no need for any more since, from a tristimulus approach with three primary colors such as red, blue and green, any color can be matched. This is not quite true since the current primary colors available do not cover the desired spectrum. Industry would like a wider choice and several new colorants are being considered. 5 Conventional wisdom would suggest that approvals for new colorants are likely to be in the natural group. 8. 3

Carotenoid extractsIntroductionCarotenoids are probably the best known of the colorants and certainly the largest group of pigments produced in nature with an annual production estimated at 100, 000, 000 tons. Most of this is fucoxanthin produced by algae in the ocean and the three main pigments, lutein, violaxanthin and neoxanthin in green leaves. 10 Over 600 carotenoid compounds have been reported. Chemistry and usageThe chemical structure of some typical carotenoids is shown in Fig. 8. 2. Beta- carotene occurs in nature usually associated with a number of chemically closely related pigments and extracts have been used as food colorants for many years. For example, palm oil has a high concentration of carotenoid pigments, primarily beta-carotene and about 20 others. Crude palm oil has been used extensively as a cooking oil because of its desirable flavor and as a generalO OCH₃ BixinFig. 8. 2 Structures of some typical carotenoid compounds. edible oil after purification. Both the crude oil and the semi-purified oil are effective colorants. Xanthophyll pastes, well known in Europe, consist of extracts of

alfalfa (lucerne), nettles, broccoli and other plants. Unless saponified, they are green because of the chlorophyll content. Many xanthophyll pastes contain as much as 30% carotenes with the major pigments being those of green leaves, lutein, beta-carotene, neoxanthin, and violaxanthin. Extracts of carrots contain about 80% beta-carotene and up to 20% alpha carotene with traces of other compounds including lycopene. Extracts of citrus peels have been suggested for coloring orange juice since the more highly colored juices command a higher price. Astaxanthin is a desirable colorant for trout and salmon in aquaculture and the usual source is byproducts of the shrimp and lobster industries, but demand has led to the cultivation of the red yeast *Phaffia rhodozyma* to produce astaxanthin. 11 Extracts of marigold, *Tagetes erecta*, are well known commodities used primarily as colorants in poultry feed. Extracts are available in three main forms, dried ground petals, crude oleoresins and purified oleoresins in a wide variety of formulations.

Preparations from tomatoes have been used to provide flavorful and colorful food ingredients for many years. Recent increases in the demand for natural beta-carotene has led to the production of beta-carotene extracts from microalgae, and some species of *Dunaliella* can accumulate up to 10% d. w. of beta-carotene. 11 All carotenoid extracts are effective yellow to orange colorants and can be used in a variety of foods depending on government regulations. These include vegetable oils, margarine, salad dressings, pastas, baked goods, dairy products, yoghurt, ice cream, confectionery, juices, and mustard. Toxicology There is little toxicological data available for extracts of carrots, alfalfa, corn oil, palm oil, tomatoes, etc. The JECFA had no objections to their use as food colorants provided that the levels of use did not exceed

that normally present in vegetables. A number of toxicity experiments were conducted on *Dunaliella* algae in view of its increasing importance in the health food area. Twelve studies on *D. salina* indicated no problems. Cis beta-carotene was absorbed to a lesser extent than trans beta-carotene. Furahashi suggested a no-observed-effect level (NOEL) of 2.5 g/kg/day for extracts from *D. Hardawil*.¹² The Joint Expert Committee on Food Additives of the World Health Organization/United Nations (JECFA) did not establish an NOEL or an ADI because of the variation in the composition of the products.

Health aspects Carotenoids are of physiological interest because some of them are precursors of vitamin A. They have been in the news recently because many exhibit radical or single oxygen trapping ability and as such have potential antioxidant activity *in vivo*. They may reduce the risk of cardiovascular disease, lung cancer, cervical dysplasia, age-related macular degeneration, and cortical cataract.¹³ The beneficial effects of beta-carotene are thought to occur through one of several modes: singlet oxygen quenching (photoprotection), antioxidant protection, and enhancement of the immune response. Evidence suggests that a diet rich in carotenoids reduces the risk of coronary heart disease but supplementing the diet with synthetic beta-carotene did not produce the same benefit. Possibly other carotenoids are important in the diet and this has led to increased interest in carotenoids such as lutein. Interest in lycopene has increased dramatically in recent years due to epidemiological studies implicating lycopene in the prevention of cardiovascular disease, and cancers of the prostate and gastrointestinal tract.

s. 3.5 **Future prospects** The dramatic increase in the health aspects of the carotenoids has spurred a great deal of interest in

these compounds as colorants. The prospect of having both a health and a colorant aspect is very appealing to merchandisers so we can expect an increase in the number of carotenoid extracts available. But with over 600 carotenoids existing in nature, it will be difficult to determine which compounds exhibit health effects.

4 Lycopene

4. 1 Introduction

Lycopene is the major pigment in tomatoes and is one of the major carotenoids in the human diet. It also accounts for 5% of the carotenoids in human serum. Tomato products are widespread in diets around the world and are highly prized for their flavor and color contributions.

4. 2 Chemistry and usage

The major source of lycopene is tomato products but it also occurs in water melons, guavas, pink grapefruit, and in small quantities in at least 40 plants. The structure of lycopene is shown in Fig. 8. 2. It is a long chain conjugated hydrocarbon and its structure suggests that it would be easily oxidized in the presence of oxygen and isomerized to cis compounds by heat. Both of these reactions occur in purified solutions of lycopene but in the presence of other compounds normally present in tomatoes, lycopene is more stable. Actually the absorption of lycopene in the human gut is increased by heat treatment probably because the breakdown of the plant cells makes the pigment more accessible. Preparations from tomatoes are widely used in pizza, pasta, soups, drinks and any product compatible with the flavor and color of tomatoes.

4. 3 Toxicology

There is little safety data available for tomato products probably because they have been a major food for so long.

4. 4 Health aspects

A recent review of 72 independent epidemiology studies revealed that intake of tomatoes and tomato products was inversely related to the risk of developing cancers at several sites

including the prostate gland, stomach and lung. The data were also suggestive of a reduced risk for breast, cervical, colorectal, esophageal, oral cavity and pancreas. 13 Obviously, the role of lycopene is going to get more research attention in the future. s. 5 Lutein s. 5. 1 Introduction Lutein is a major component of many plants. It is a component of most of the carotenoid extracts suggested as food colorants. s. 5. 2 Chemistry and usage Lutein has a structure similar to beta-carotene with a hydroxyl group on the ionone ring at each end of the molecule. It is somewhat less sensitive to oxidation and heat degradation than beta-carotene. It contributes a yellow color. s. 5. 3 Toxicology Little data is available but it would be expected to be non-toxic by comparison to similar carotenoids. 8. 5. 4 Health effects Several studies have linked lutein to a lower risk for eye, skin and other health disorders, probably through its antioxidant activity. Lutein is apparently metabolized to zeaxanthin, an isomer, and several other compounds which protect the macula from ultraviolet radiation. The suggestion is that lutein may play a positive role in reducing macular degeneration. Other reports have linked lutein to a reduction of risk of cancer. 13 Regardless, lutein is currently being promoted as an important dietary supplement. 8. 6 Annatto and saffron Introduction Annatto is one of the oldest colorants, dating back to antiquity for coloring food, cosmetics and textiles. Annatto is produced from the seeds of the tropical shrub *Bixa orellana*. Saffron is also a very old colorant dating back to the 23rd century BC. It is produced from the dried stigmas of the flowers of the crocus bulb, *Crocus sativa*. Saffron is known as the gourmet spice because it produces a desirable flavor and color. Its high price is assured because it takes about 150, 000 flowers to produce one

kilogram of saffron. Chemistry and usage The main pigments in annatto are bixin (Fig. 8. 2) and norbixin. Bixin is the monomethyl ester of a dicarboxyl carotenoid. Norbixin is the saponified form, a dicarboxyl acid of the same carotenoid. The carboxylic acid portion of the molecule contributes to water solubility and the ester form contributes to oil solubility. Annatto is available in both water soluble and oil soluble liquids and powders. Annatto is somewhat unstable to light and oxygen but, technically, it is a good colorant. The principal use of annatto is as colorant for dairy products due to its water solubility but it is also used to impart a yellow to red color in a wide variety of products. The main pigments in saffron are crocin (Fig. 8. 2) and crocetin. Crocin is the digentiobioside of the dicarboxylic carotenoid crocetin. The carboxylic and the sugar portion of the molecule contribute to water solubility. It is more stable to light and oxygen than annatto but, technically, it is a good colorant and is used in a variety of gourmet foods. 15 Toxicology A series of studies have shown annatto to be non-genotoxic¹¹¹⁵ but others have suggested some mitotic aberrations¹⁶ and some genotoxicity. 17 The acute oral toxicity is very low. The oral LD₅₀ for rats is greater than 50 g/kg for the oil soluble form and 35 g/kg for the water soluble form. Lifetime toxicity studies in rats at the level of 26mg/kg/day showed no toxic effects. Rats showed no reproductive problems when fed at 500 mg/kg/day for three generations. It was concluded that annatto was not carcinogenic. The JECFA established an ADI of 0-0. 065 mg/kg/day for annatto based on studies nearly 40 years ago. Current research is under way to increase the ADI. Little data is available for saffron but the chemical similarity to the pigments in annatto, and other carotenoids, would suggest that saffron would pose no problems in

the food supply. Health effects Annatto seeds have long been used by the South American Indians as a traditional medicine for healing of wounds, skin eruptions, healing of burns, and given internally for diarrhea, asthma and as an antipyretic. 15 Annatto is claimed to have strong antioxidative potency, as shown by inhibition of lipid peroxidation and lipoxidase activity. 8. 6. 5 Future projects Annatto is well established in the market and its use is increasing in poundage probably due to its superior technological properties. If some of the health claims prove to be true, annatto will enjoy increased interest. Saffron is well established in the gourmet markets but its use will be restrained because of its high price.

8. 7 Paprika

Introduction Paprika is a very old colorant and spice. It is a deep red, pungent powder prepared from the dried pods of the sweet pepper, *Capsicum annum*. Chemistry and usage Paprika contains capsorubin and capsanthin (Fig. 8. 3) which occur mainly as the lauric acid esters, and about 20 other carotenoid pigments. Paprika is produced in many countries which have developed their own specialties. Cayenne or cayenne pepper, produced from a different cultivar of *C. annum*, is usually more pungent. *C. frutescens* is the source of the very pungent Tabasco sauce. Paprika oleoresin is produced by solvent extraction of the ground powder. Obviously paprika supplies both flavor and color and its use is limited to those products compatible with the flavor. The recent rise in demand for tomato products in the form of pizza, salsa, etc., has increased the demand for paprika. Paprika is used in meat products, soups, sauces, salad dressings, processed cheese, snacks, confectionery and

baked goods. 1018ToxicologyThe acute oral toxicity of paprika is very low with an LD50 for mice of 11 g/kg. Several studies have indicated that paprika is not genotoxic. The JECFA did not establish an ADI because they considered that the levels of paprika and its oleoresins in foods would be self-limiting.

11Future prospectsPaprika is well established worldwide and will probably increase in volume due to the popularity of tomato products and possibly by analogy to the health effects being attributed to the carotenoids. R = N, i R = C 7 H15Monascin AnkaflaviriOHCapsorubinFig. 8. 3 Top. Pigments in Monascus. Middle. Phycobilins in algae. Bottom. Pigments in paprika. HOCaps an thinnRubropunctatin R = C5H,, Monascorubtn R = C;

H15Rubropunctamine R = C5 H,, Monascorubramine R = C7Hi6HOOC

COOHHOOC COOHPhycocyanobilinHHPhycoerythrobilin8. 8 Synthetic

carotenoidsIntroductionThe success of the carotenoid extracts led to the commercialization of synthetic carotenoids, some with the same chemical structure as those in the plant extracts and others with modifications to improve their technological properties. The yellow beta-carotene was synthesized in 1950, followed by the orange beta-8- carotenal in 1962 and the red canthaxanthin in 1964. A number of others soon followed, methyl and ethyl esters of carotenoic acid, citraxanthin, zeaxanthin, astaxanthin, and recently lutein. Chemistry and usageThe structures of four of the synthetic carotenoids (beta-carotene, canthaxanthin, beta-apo-8'-carotenol, beta-apo-8'-carotenoic acid) are shown in Fig. 8. 2. By virtue of their conjugated double bond structure, they are susceptible to oxidation but formulations with antioxidants were developed to minimize oxidation.

Carotenoids are classified as oil soluble but most foods require water soluble

colorants; thus three approaches were used to provide water dispersible preparations. These included formulation of colloidal suspensions, emulsification of oily solutions, and dispersion in suitable colloids. The Hoffman-LaRoche firm pioneered the development of synthetic carotenoid colorants and they obviously chose candidates with better technological properties. For example, the red canthaxanthin is similar in color to lycopene but much more stable. Carotenoid colorants are appropriate for a wide variety of foods.

10 Regulations differ in other countries but the only synthetic carotenoids allowed in foods in the US are beta-carotene, canthaxanthin, and beta-8-carotenol.

8. 8. 3 Toxicology

When the first synthetic carotenoid colorant, beta-carotene, was suggested as a food colorant, it was subjected to an extensive series of tests¹¹ despite the belief that carotenoid extracts containing beta carotene were non-toxic. It was thought that synthetic preparations might contain a different profile of minor contaminants than those in plant extracts. Beta-carotene has a very low acute oral toxicity. The LD₅₀ for dogs is greater than 1000mg/kg and a single intramuscular injection of 1000 mg/kg in rats had no significant effect. Lifetime dietary administration showed no carcinogenicity with a NOEL of 100 mg/kg/ day for rats and 1000 mg/kg/day for mice. No teratogenic or reproductive toxicity was shown when four generations of rats were fed up to 100 mg/kg/day. No cytogenic or teratogenic effects were seen in the offspring of rabbits given 400 mg/kg/day by stomach tube. Similar experiments with rats given up to 1000 mg/kg/day by intubation showed no embryo toxicity, teratogenicity or reproductive effects. Apparently rodents can tolerate large amounts of beta-carotene but extrapolation to humans is difficult because rats and humans

metabolize beta-carotene in different ways. With humans, the absorbed beta-carotene is largely converted to vitamin A, esterified, and transported in the lymph. With rats, the beta-carotene is largely converted to non-saponifiable compounds. Regardless, there is ample evidence that beta-carotene in reasonable quantities is harmless to humans. There is, however, evidence that humans with a high intake of beta-carotene may develop hypercarotenemia leading to an orange skin coloration. The JECFA established an ADI of 0-5 mg/kg/day for the sum of all carotenoids used as colorants. Canthaxanthin was also subjected to an extensive series of toxicological tests¹¹ which indicated that it was essentially non-toxic. The acute oral toxicity was very low with an oral LD₅₀ for mice greater than 10g/kg. No effect was seen in dogs fed 500 mg/kg/day for 15 weeks. Canthaxanthin was not carcinogenic at feeding levels of 1000 mg/kg/day for 104 weeks for rats and 98 weeks for mice and it may have been anti-carcinogenic. No reproductive or teratogenic effects were seen when rats were fed up to 1000 mg/kg/day for three generations. Ingestion of large amounts of canthaxanthin caused deposition of canthaxanthin crystals, producing retinal impairment, in the eyes of humans, cats and rabbits, but not in rats, mice and dogs. In humans the visual impairment was reversible in a few months. Effects of high doses are available because canthaxanthin is used as a tanning aid. Other adverse effects were hepatitis and urticaria. The JECFA was unable to assign an ADI because of the problem with retinal crystal deposition in humans. Canthaxanthin, in the amounts required for appropriate coloration, is believed to be completely safe. Beta-8-carotenal and the methyl and ethyl esters of carotenoic acid were also tested for toxic

effects with results similar to beta-carotene. 8. 8. 4 Health effects There is considerable current research under way to determine if the synthetic carotenoids have the same physiological effects, and consequent health benefits, as the naturally occurring compounds. If this proves to be true, we can expect increased interest in this group of colorants. 8. 9 Anthocyanins 8. 9. 1 Introduction Anthocyanins are ubiquitous in the plant kingdom. They are responsible for many of the orange, red, blue, violet, and magenta colors. They have been the object of intensive research from a taxonomic point of view and this has resulted in about 275 known structures and about 5, 000 of the chemically closely related flavonoid compounds. Their use as colorants dates back to antiquity since the Romans used highly colored berries to augment the color of wine. 188 Food chemical safety 8. 9. 2 Chemistry and usage Anthocyanins consist of an aglycone combined with one or more sugars. Twenty-two aglycones are known but only six are important as colorants (Fig. 8. 4). In view of the ubiquity and high tinctorial power it is not surprising that many sources have been suggested as colorants. Francis listed pigment profiles and methods of extraction for over 40 plants^{19'20} and also 49 patents on anthocyanin sources as potential colorants. 21 However, despite the large number of sources, only one dominated the supply for many years. Colorants from grape skins as a by-product of the wine industry is the major source. Grapes are the largest fruit crop for processing and since 80% of the estimated 60, 000, 000 Pelargonidin (4' = OH) Cyanidin (3', 4' = OH) Delphinidin (3', 4', 5' = OH) Peonidin (4' = OH, 3' = OMe) Petunidin (4', 5' = OH, 3' = OMe) Malvidin (4' = OH, 3', 5' = OMe) (All other substitution positions = H) HOOC; OH Betanine HR. $\hat{R}^{\wedge}OCH_j R, =$

H R2= OCH3 P P^—H2CurcuminDe methoxycu rcu m i n

BisdemethoxycurcuminFig. 8. 4 Top. Structures of the major anthocyanidins in foods. Middle. Structure of betanine. Bottom. Pigments in turmeric. metric tons is used annually for wine production, this situation is not likely to change. Recently anthocyanins from red cabbage have enjoyed some success. The sugars attached to the anthocyanin molecule are in order of relative abundance glucose, rhamnose, galactose, xylose, arabinose, and glucuronic acid. The molecule may also contain one or more of the acyl acids p-coumaric, caffeic, and ferulic or the aliphatic acids malonic and acetic esterified to the sugar molecules. Extracts of anthocyanins invariably contain flavonoids, phenolic acids, catechins and polyphenols. The net result is that it is impossible to express the chemical composition accurately. Specifications usually present tinctorial power, acidity, per cent solids, per cent ash and other physical properties. The major market for the colorants from grapes (generic term enocyanin) is in fruit drinks. Anthocyanins are pH sensitive and show the greatest tinctorial power around pH 3-3.5 and most fruit drinks are in this range. Anthocyanin colorants have been used in a wide variety of food products such as beverages, jams, jellies, ice cream, yoghurt, gelatin desserts, canned fruits, toppings, confections, and many others.

ToxicologyAnthocyanins are not genotoxic as shown by a number of studies. 19 The oral toxicity of mixed anthocyanins was greater than 2G mg/kg/day for rats. Dogs fed a diet containing 15% anthocyanins from Concord grapes for 9G days showed no significant toxic effects. Another multigeneration study on rats fed 15% powder from Concord grapes showed no effects on reproduction. A study on Roselle, a popular drink made from the calyces of

Hibiscus sabdariffa, showed no toxic effects. 22 The lack of toxic effects is not surprising in view of the long history of wine consumption. Health aspects Anthocyanins and the related flavonoids have been very much in the news lately for a wide variety of health claims. These include anticarcinogenic, anti-inflammatory, antihepatotoxic, antibacterial, antiviral, antiallergenic, anti-thrombotic, and antioxidant effects. 13 Antioxidation is believed to be one of the most important mechanisms for preventing or delaying the onset of major diseases of aging including cancer, heart disease, cataracts and cognitive dysfunction. The antioxidants are believed to block oxidative processes and free radicals that contribute to the causation of these chronic diseases. Anthocyanins are non-competitive inhibitors of lipid peroxidation comparable in potency to the classical antioxidants such as butylated hydroxy anisole (BHA), butylated hydroxytoluene (BHT), and alpha tocopherol. Anthocyanins were reported to have anti-inflammatory properties comparable to commercial pharmaceuticals. The property of anthocyanins to decrease the fragility and permeability of blood capillaries is common to many flavonoids and was the basis for the original definition of Vitamin P by Szent Gyorgi in 1936. This claim has been commercialized by the marketing of extracts of bilberry (*Vaccinium myrtillus*). Interest in the health effects of anthocyanins was piqued by the 'French paradox' in which the mortality from cardiovascular disease was lower than that predicted from the intake of dietary saturated fatty acids. The beneficial effects were greater in association with alcohol taken in the form of wine suggesting that there may be a protective effect of other components of wine. Needless to say the wine industry was pleased with this research. 8. 9. 5 Future

prospects The potential health effects of anthocyanins and flavonols has stimulated much research in this area but, in view of the chemical complexity of the plant extracts, we are a long way from determining the chemical compounds responsible for the wide variety of claims. Regardless, a colorant with associated health benefits is a very desirable situation from an industry point of view. This is a very active research area. 8. 10

Betalains Introduction The betalains are confined to ten families of the order Caryophyllales. 20 The only foods containing betalains are red beet (*Beta vulgaris*), chard (*B. vulgaris*), cactus fruit (*Opuntia ficus-indica*) and pokeberries (*Phytolacca americana*). They also occur in the poisonous mushroom *Amanita muscaria* but this is not a normal food source. The importance of the betalains as colorants is confined to preparations from red beet. Chemistry and usage The betalains have two main groups; the red betacyanins and the yellow betaxanthins. The main pigment of red beets is betanine and its structure is shown in Fig. 8. 4. Beets usually contain both betacyanins and betaxanthins and the ratio depends on the cultivar. Some cultivars contain only the yellow betaxanthins and this makes it possible to formulate a range of colorants from yellow to red. Betanine is relatively stable to changes in pH as contrasted with the anthocyanins and this makes it preferable for foods in the pH range 5-6. Both the red and yellow betalains are susceptible to degradation by heat, light and the presence of metal ions. Within these limitations, betalains are ideally used to color products that have a short shelf life, are packaged to reduce exposure to light, oxygen and high humidity, do not receive extended or high heat treatment, and are marketed in the dry state. Despite these limitations, betalains have been

suggested for coloring ice cream, yoghurt, cake mixes, gelatin desserts, meat substitutes, gravies, frostings and many others. 20'23 8. 10. 3

Toxicology Betalain pigments have been tested on rodents by feeding 50mg/kg pure betanin, 2000 ppm betanine in the diet and several other conditions. 11 No carcinogenic or other toxic effects were observed and the authors concluded that red beet extracts were safe as food colorants. 8. 10.

4 Future prospects Betalain colorants are well established in the food chain and will probably continue in a limited capacity. 8. 11

Chlorophylls Introduction The chlorophylls are a group of naturally occurring pigments produced in all photosynthetic plants including algae and some bacteria. Hendry²⁴ estimated annual production at about 1, 100, 000, 000 tons with about 75% being produced in aquatic, primarily marine, environments. Obviously as a source of raw material for food colorants, chlorophylls present no problem with supply. Chemistry and usage Five chlorophylls and five bacteriochlorophylls are known but only two, chlorophylls a and b are important as food colorants. Chlorophyll a has a complex structure with a magnesium ion in the molecule which is easily removed in acid media to form pheophytin a. Removal of the phytyl portion of the molecule produces chlorophyllide a whereas removal of both magnesium and phytyl produces pheophorbide a. Chlorophyll b reacts in the same manner. Chlorophyll and chlorophyllide are both bright green in color but pheophytin is olive green and pheophorbide is brown. Attempts to produce a food colorant from chlorophyll are centered around trying to stabilize the molecule by retaining or replacing the magnesium ion.

Treatment with copper and zinc salts substitutes copper or zinc for the

magnesium and the derivatives are bright green in color. Commercial colorants are usually made from lucerne (alfalfa), *Medicago sativa*, or nettles, *Urtica dioica*, and a series of pasture grasses. The plants are dried, extracted with a solvent and dried resulting in a mixture of chlorophyll, pheophytin and other degraded compounds. The dry residue can be purified to obtain an oil-soluble preparation or treated with an acidified copper solution to prepare a more stable water-soluble copper chlorophyllin. It is not commercially feasible to prepare a colorant containing pure chlorophyll because of the instability of the molecule. The major portion of the chlorophyll colorants are in the water soluble forms and are used in dairy products, soups, oils, sugar confections, drinks and cosmetics.

25ToxicologyThe JECFA classified chlorophyll under List A which means that the colorant has been fully cleared and its use is not limited toxicologically since when used with 'good manufacturing practice' does not represent a hazard to health. A subchronic oral toxicity study showed no adverse effects.

26Health aspectsChlorophyllin is an effective antimutagenic agent and has been used as a dietary supplement to diminish the intensity of the uncomfortable side effects of cyclophosphamide treatments. 13

Cyclophosphamide is a potent antitumor agent and is used against many forms of cancer and other diseases. Chlorophyllin protects against radiation induced DNA single strand breaks possibly by its ability to scavenge OH^+ and ROO^+ groups. 8. 11. 5 Future prospectsChlorophyllins are approved for use in Europe and Asia but only for dentifrices in the US. Since there are no other commercially available natural green colors, these colorants are attracting interest. 8. 12 Turmeric 8. 12. 1 IntroductionTurmeric is a very old

colorant produced from the rhizomes of several species of *Cucuma longa*, a perennial shrub grown in many tropical areas around the world. 8. 12. 2

Chemistry and usage Turmeric is the ground dried rhizomes and contains three main pigments, curcumin, demethoxycurcumin, and bisdemethoxycurcumin (Fig. 8. 4) together with about four potent flavor compounds. 27 28 Turmeric oleoresins are prepared by extracting the dried ground rhizomes with a variety of chemical solvents and concentrating the resins perhaps with the addition of oils and other carriers. Turmeric and turmeric oleoresins are unstable to light and alkaline conditions and a number of substances are sometimes added to stabilize the molecule. Curcumin is insoluble in water but a water-soluble form can be made by complexing the compound with tin or zinc to form an intensely orange colorant but it is not allowed in most countries. The major applications of turmeric are to color cauliflower, pickles and mustard but it is also used in combination with annatto in ice cream, yoghurt, baked goods, oils, salad dressings and confectionery. Toxicology Turmeric has been subjected to a number of safety studies because its consumption in Europe, India and the Middle East is very high. Consumption in India was estimated at up to 3. 8 g/day. 11 The acute oral toxicity is very low. The oral LD50 for the oleoresin in rats and mice is greater than 10 g/kg and for curcumin in mice is greater than 2 g/kg. Rats fed turmeric for 52 weeks at 500 mg/kg/day showed no significant toxicity. Similar experiments with dogs and monkeys showed similar results. There was no evidence of carcinogenicity, reproductive toxicity or teratology. The JECFA did not allocate an ADI for turmeric because they considered it to be a food. A temporary ADI of 0. 01 mg/kg for turmeric

oleoresin and 0-0. 1 mg/kg for curcumin was established in 1990. 12 In 1995, the temporary ADI for curcumin was increased to 1 mg/kg. Health effectsIt has been known since the early 1950s that turmeric had strong antioxidant effects with curcumin being the major compound responsible followed by demethoxycurcumin and bisdemethoxycurcumin. All three inhibit lipid peroxidation and have a positive anti-oxidant effect for hemolysis and lipid peroxidation in mouse erythrocytes. 11 Curry pills containing turmeric are being marketed as a prevention for colon cancer. 29Future prospectsTurmeric is well established in the food supply and if it is proven to have a health effect as well as a colorant and flavor component, its future would seem assured. 8. 13 Cochineal and carmineIntroductionCochineal is a very old colorant. References go back as far as 5000 BC when Egyptian women used it to color their lips. It was introduced to Europe by Cortez who found it in Mexico. Production peaked around 1870 and then declined due to the introduction of synthetic colorants, but it is still a major commodity for Peru, Mexico, and the Canary Islands. 25 30Chemistry and usageCochineal extract is obtained from the bodies of the female cochineal insects, particularly *Dactylopius coccus* Costa, by treating the dried bodies with ethanol. After removal of the solvent, the dried residue contains about 2-4% carminic acid, the main colored component. The cochineal insects grow on cactus and, since it takes about 50, 000-70, 000 insects to produce one pound of the colorant, production will always be labor intensive. Solutions of carminic acid, at pH4 show a range of colors from yellow to orange depending on the concentration. When complexed with aluminum, a series of stable brilliant red hues ranging from 'strawberry' to 'blackcurrant' can be

produced depending on the ratio of aluminum to carminic acid. Purified extracts of cochineal have been termed 'carmine' but the term usually refers to a lake of carminic acid with aluminum, calcium or magnesium. Carmine usually contains about 50% carminic acid. Carmine is considered to be technologically a very good food colorant. It is ideally suited to foods with a pH above 3.5 such as comminuted meat and poultry products, surimi, and red marinades. It is also used in a wide variety of other products such as jams, gelatin desserts, baked goods, confections, toppings, dairy products, non-carbonated drinks, and many others.

8. 13. 3 Toxicology A number of studies have shown that cochineal extract and carmine are not carcinogenic. 11 Rats fed carmine up to 100mg/kg/day showed only reduced growth at the higher levels. Other studies show no reproductive problems in a single generation study or reproductive/teratology problems in a multigeneration study. The JECFA assigned a combined ADI of 0-5 mg/kg/day for cochineal and carmine.

8. 13. 4 Future prospects Carmine is well entrenched in the food industry and probably will remain there because of its superior technological properties. It is, however, a very labor intensive industry because of the hand harvesting of the insects with a consequent high price.

8. 13. 5 Related compounds Carmine belongs to the anthraquinone class of compounds and several other chemically closely related compounds are also used as colorants. 25 Kermes is a well known colorant in Europe. It is obtained from the insects, *Kermes ilicis* or *Kermococcus vermilis*, which grow on oak trees. It contains kermisic acid, the aglycone of carminic acid, and its isomer ceroalbolinic acid. Its properties are very similar to carmine. Lac is a red colorant obtained from the insect *Laccifera lacca* which is found on several

families of trees in India and Malaysia. The lac insects are better known for their production of shellac. They contain a complex mixture of anthraquinones. Alkanet is a red pigment from the roots of *Alkanna tinctoria* Taush and *Alchusa tinctoria* Lom. All three have been cleared for food use in Europe but not in the US. 8. 14 *Monascus* 8. 14. 1 Introduction *Monascus* colorants are well known in Asia and in Chinese medicine date as far back as 1590. The colorants are produced by several fungal species of the genus *Monascus* which grow readily on a number of carbohydrates, especially rice, but also on wheat, soybeans, corn and other grains. The Koji process involves inoculating the solid grain mass with the fungus, primarily *Monascus purpureus*, and drying the substrate to produce 'red rice' which is used as a colorant for many foods. 3132 Chemistry and usage *Monascus* species produce six pigments (Fig. 8. 3). Monascin and ankaflavin are yellow, rubropunctatin and monascorubin are red, and rubropunctamine and monascorubramine are purple. The pigments are very reactive and have been reacted with a variety of compounds such as polyamino acids, amino alcohols, chitin amines or hexamines, proteins, sugar amines, aminobenzoic acid and many others^{21, 28} to produce compounds with greater water solubility, thermostability and photostability than the parent compounds. The ability to grow on a solid substrate has led to a large body of data on optimization of pigment production in solid state fermentation and later in submerged fermentation. 31 The conditions of growth can be modified to optimize the ratio of the different pigments and also for several other compounds with health implications. *Monascus* pigments are soluble in ethanol and the derivatives are soluble in water. A range of colors from

yellow to purple can be produced by manipulating the ratio of pigments and the pH. Their stability in neutral media is a real advantage. The colorants are suitable for processed meats, marine products, ice cream, jam, toppings, and tomato ketchup, and traditional oriental products such as koji, soy sauce, and kambuki. Their solubility in alcohol makes them appropriate for alcoholic beverages such as saki. Toxicology Monascus colorants have been consumed for hundreds of years and are believed to be safe for human consumption. Tests with a series of microorganisms have demonstrated no mutagenicity. No toxicity was observed in rodents or in fertile chicken eggs. The yellow pigments have an LD50 for mice of 132mg/20g. No ADI is available. 11 There has been some concern that some of the strains produced antibiotics which is obviously undesirable for a food colorant, but it is possible to choose strains of Monascus which do not produce antibiotics. Health effects A large number of reports describe the use of Monascus preparations in herbal medicines and food supplements. These include the suppression of tumor production, regulation of immunoglobulin production, lowering of lipids in hyperlipidemia, and reduction of aminoacetaphen-induced liver toxicity by antioxidase action. One example would be Cholestin distributed by the Pharmanex company. It is obtained from red rice imported from China as a nutraceutical and contains a natural inhibitor of the rate-limiting synthesis of cholesterol. It is claimed to reduce total cholesterol and low-density lipoprotein and triglyceride levels while increasing high-density lipoprotein levels. A nutraceutical with a beneficial effect for heart disease is a very desirable product so Cholestin was soon followed by several other products which may have been produced to maximize the content of the active

ingredient. One problem was that the active ingredient was identical to that in Mevacor, a prescription drug patented by Merck. The FDA ruled that Cholestin was a drug, not a food supplement, and banned it. The Pharmanex company sued the FDA and the courts made a temporary decision in favor of the company and reversed the ban. 31 This situation seems to permit an end run around the strict rules governing approval of prescription drugs by simply finding a natural source, manipulating the growing conditions to maximize the content of the drug, and calling the formulation a food supplement. 8. 14. 5 Future prospects Monascin colorants are well entrenched in Asia, particularly China, Japan, and Taiwan and probably will continue to be an important product in view of their long history. They are not allowed in the US and there seems to be little interest in them. Certainly, the situation illustrated by the Cholestin debate will have to be settled, probably by new legislation, before a commercial firm petitions to have Monascin colorants permitted in the US.

8. 15 Iridoids

Introduction The colorants from saffron have enjoyed good technological success as colorants and spices but their high price has led to searches for other sources of the same pigments. The pigments, but not the flavor, can be obtained in much larger quantities from the fruits of the gardenia or Cape jasmine plant. 33 **Chemistry and usage** The fruits of gardenia, *Gardenia jasminoides*, contain three groups of pigments, crocins, iridoids and flavonoids. Structures of six of the nine iridoid pigments are shown in Fig. 8. 5. The formulas for five flavonoids from *G. fosbergii* are also shown. This is a

different species but botanically closely-related species tend to have similar pigment profiles. The crocins are orange and the flavonoids are pale yellow. The iridoids are interesting because they can be reacted with amino acids or proteins to produce a range of colors from green to yellow, red, R'OH o Flavoroid compounds Fig. 8. 5 Top. Some flavonoid pigments in gardenia. Bottom. Six of the nine iridoid pigments in gardenia. and blue. A number of patents have described the manipulation of the reaction conditions such as time, temperature, pH, oxygen content, degree of polymerization, reaction with selected microorganisms, etc. The compounds can be hydrolyzed to produce genipin which reacts readily with taurine to produce an attractive blue color. Four greens, two blues and one red colorant have been commercialized in Japan. They have been suggested for use with candies, condiments, ices, noodles, imitation crab, fish eggs, chestnuts, beans, dried fish substitutes, liqueurs, baked goods, etc. Toxicology The geniposides from gardenia were found to have some hepatotoxicity due to the aglycone genipin produced by hydrolysis of the geniposides. 34 The yellow, green, red, and blue colorants were studied extensively and were found to be safe for human consumption as food colorants. 35 36 Future prospects The yellow crocins from gardenia have received some success for the same colorant applications as saffron, 37 but the iridoid derivatives have not received the same promotion. The range of colorants available from the same source would seem to make them attractive possibilities. 8. 16

Phycobilins Introduction The phycobilins belong to the heme group of pigments which include the green chlorophylls in plants and the red hemoglobins in animals. The phycobilins are major biochemical components

of the blue-green, red, and cryptomonad algae. 38

Chemistry and usage Phycobilins are colored, fluorescent, water-soluble pigment-protein complexes. They can be classified into three groups according to color: phycoerythrins are red with a bright orange fluorescence; phycocyanins and allophycocyanins are both blue and fluoresce red. The structures of two are shown in Fig. 8. 3. Phycocyanins and allophycocyanins share the same chromophore but differ in the protein portion. The attachment of the bilin chromophore to the protein is very stable and this makes them desirable from a colorant point of view. Phycobilin preparations can be made by simply freeze drying algal cell suspensions which can be grown in ponds or sophisticated tubular reactors. Suggested applications involve chewing gums, frozen confections, dairy products, soft drinks, and ice cream.

Toxicology One study on toxicity of phycocyanin from *Spiroplina platensis* reported no adverse effects. 39 There is little other data available on toxicology but no toxic effects would be expected in view of the long history of algal consumption. 8. 16. 4

Future prospects The future of the phycobilins looks promising for two reasons. First, there are no other blue natural colorants available and, admittedly, blue is not a favorite food color but niche markets are evolving. Second, *spiroplina* is becoming an attractive product in the health food area, and the same facilities used to produce *spiroplina* products could be used to produce phycobilins. 8. 17 Caramel 8. 17.

1 Introduction Caramel is a brown colorant obtained by heating sugars. 33 The official FDA definition is as follows: 'The color additive caramel is the dark brown liquid or solid resulting from carefully controlled heat treatment of the following food grade carbohydrates: dextrose, invert sugar, lactose,

malt syrup, molasses, starch hydrolysates and fractions thereof, or sucrose.' Heating sugar preparations to produce brown flavorful and pleasant-smelling products has been practised in home cooking for centuries but the first commercial caramel colorants appeared in Europe about 1850. Chemistry and usage Commercial caramel is a very complex mixture of heat degraded carbohydrates. In 1980, the JECFA recommended that further information on the chemical properties be obtained in order to establish a suitable classification and specification system. The International Technical Caramel Committee attempted to provide this information and undertook an extensive research program. The complexity of the mixtures made it impossible to define the chemical composition so the commercial preparations were divided into four groups (Table 8. 2) on the basis of a series of sophisticated chemical assay procedures. Caramel colorants must be compatible with the food products in which they are used, which usually means the absence of flocculation and precipitation in the food. These undesirable effects result from charged macromolecular components of caramel which react with the food. Hence the net ionic charge of the caramel macromolecules at the pH of the intended food product is the prime determinant of compatibility. Caramel colorants are used in a variety of foods (Table 8. 2) but over 80% of the caramel produced in the US is used to color soft drinks particularly colas and root beers. Toxicology One of the major considerations of the research requested by JECFA was the safety aspects which was not surprising in view of the chemical complexity of the caramels. The program resulted in the publication of 11 papers in the same issue of the journal Food and Chemical Toxicology 1992 (Vol. 30) and seven

of them were on toxicology. Caramel colorants were given a clean bill of health and JECFA assigned an ADI of 0-200 mg/kg/day. Table 8. 2 Caramel formulations

Class	Charge	Reactants	Usage
1— No ammonium or sulphite	Distilled spirits, desserts, compound	spice blends	2— Sulphite compounds
Liqueurs	3+ Ammonium compounds	Baked goods, beer, gravies	4+ Both sulphite and ammonium

Soft drinks, pet foods, soups

compounds

8. 17. 4 Future prospects

Caramel colorants are well established in food formulations and probably will remain that way in the foreseeable future. 8. 18 Brown polyphenols

Introduction

There are two important sources of brown polyphenols, cocoa and tea, used as colorants for foods. Both are very old and date back to antiquity. Chemistry and usage

The cacao plant, *Theobroma cacao*, is the source of chocolate which is well known and highly prized in international commerce. The cacao pods contain beans which are fermented and pressed to provide a brown liquid which is the raw material for chocolate. The press cake is ground and sold as cocoa and it also provides a brown colorant. The pods, beans, shells, husks and stems have also been suggested as colorants. They contain a very complex mixture of acyl acids, leucoanthocyanins, flavonoid polymers, tannins, and catechin-type polymers.

33

The tea plant, *Thea sinensis*, has provided a desirable beverage for centuries but it is also used as a colorant. Extracts of tea contain a very complex mixture of glycosides of myricetin, quercetin and kaempferol, epicatechin, epigallocatechins, acyl acids and many other polyphenol compounds. 33 In black tea, the above compounds may act as precursors to the poorly defined compounds thearubin and theaflavin. Both cocoa and tea are used in a variety of food products, including beverages, bakery products,

confections, toppings, dry mixes, etc. Toxicology Tarka⁴⁰ wrote an extensive review of the toxicology of cocoa and the methylxanthenes, theobromine, caffeine and theophyllin. The review involved biological and behavioral effects, metabolism, species variation, pregnancy, lactation, reproduction, teratology, mutagenicity, fibrocystic breast diseases, and drug and dietary factors. No implications for human use as a beverage or a colorant were discussed. The toxicology of tea is similar to cocoa and no adverse effects for humans have been implied. 8. 18. 4 Future prospects Both cocoa and tea are well entrenched and this is not likely to change. Neither group is permitted as a food colorant in the US but they are permitted as food ingredients and this accomplishes the same end. 8. 19 Titanium dioxide Introduction Titanium dioxide is a large industrial commodity with world production over 4, 000, 000 tons but only a very small proportion is used as a food colorant. Commercial TiO₂ is produced from the mineral ilmenite, which occurs in three crystalline forms, but the only one approved for food use is synthetic anatase. Anatase occurs in nature but only the synthetic version is approved because it contains fewer impurities. 41 Chemistry and usage Titanium dioxide is a very stable compound with excellent stability towards light, oxidation, pH changes, and microbiological attack. It is virtually insoluble in all common solvents. It is available in oil-dispersible and water-dispersible forms with a wide variety of carriers. Titanium dioxide is a very effective whitener for confectionery, baked goods, cheeses, icings, toppings, and numerous pharmaceuticals and cosmetics. Toxicology Titanium dioxide has been subjected to a number of safety tests¹¹ and found to be non-genotoxic, non-carcinogenic and exhibited no adverse effects in rats, mice, dogs, cats,

guinea pigs, and rabbits. The LD50 values are greater than 25 g/ kg/day for rats and 10 g for mice. Apparently titanium dioxide is poorly absorbed and non-toxic. JECFA has not established an ADI since they consider titanium dioxide to be self regulating under GMP. In the US, it is allowed up to 1% by weight in food. 8. 19. 4 Future prospects Titanium dioxide is well established and will probably remain that way. 8. 20 Carbon black 8. 20. 1

Introduction Carbon black is a large volume industrial commodity but its food use is very small. 40 Chemistry and usage Carbon black is derived from vegetable material, usually peat, by complete combustion to residual carbon. The particle size is very small, usually less than 5 , um, and consequently is very difficult to handle. It is usually sold to the food industry in the form of a viscous paste in a glucose syrup. Carbon black is very stable and technologically a very effective colorant. It is widely used in Europe and other countries in confectionery. Toxicology In the US in the 1970s when the GRAS list was being reviewed, safety data were requested on carbon black in view of the possibility that it might contain heterocyclic amines. Apparently, the cost of obtaining the data was higher than the entire annual sales of food grade carbon black so the tests were never done. Carbon black is not permitted in the US. 8. 21 Miscellaneous colorants A number of preparations are used in small volume or with minimal effect on color. 41 Ultramarine blue, a synthetic aluminosulphosilicate blue colorant, is widely used in cosmetics and in salt intended for animal consumption. A variety of brown iron oxides are used in cosmetics, drugs and pet foods. Talc is a large industrial commodity with many uses. It is used as a release agent in the pharmaceutical and baking industries as well as a coating for rice grains.

Zinc oxide is an effective whitener for food and food wrappers. It is also added as a nutrient. Riboflavin provides an attractive yellow green color to foods as well as a nutrient benefit. Corn endosperm oil is added to chicken feed to enhance the yellow color of the skin and eggs. Dried algal meal is produced from *Spongiococcum* spp. and may be added to chicken feed to enhance the color of skin and eggs. Extracts from other algae are permitted in other countries. Four products from cottonseeds may be added to food but they are usually considered ingredients and impart only a slight yellow color. Shellac, obtained from the insect, *Laccifer lacca*, is added to foods as a surface coating or glaze and it does not affect the appearance. Octopus and squid ink contain mixtures of melanoidin polymers and are effective black colorants for pasta for special occasions for some ethnic groups. They are not permitted in the US. All of the above preparations are considered harmless for human consumption by virtue of a long history of use and sometimes because their use is in such small amounts or concentrations as to be considered of no concern even though the toxicological data may be a little hazy. 8. 22 Outlook

Colorants present a wide variety of preparations added to foods to increase their visual appeal. Depending on their classification, they may be subjected to extensive or minimal toxicity testing. For example, FD&C Red No. 2 is probably the most tested additive in our food supply, second only to saccharin. Others have been grandfathered in by virtue of a long history of consumption. But our society is moving towards a more formulated food supply with more attention being paid to ingredients available in large quantities but not necessarily in the most appealing form. Colorants and other additives, such as flavorants and texturants, are vital to

making our food supply appealing. Interest in colorants, in particular, is increasing as judged by the research activity. Over the last 50 years, there has been a distinct trend towards the use of natural colorants as compared to synthetic 'coal tar dyes'. In one study, 21 the five-year period from 1979-1984 yielded the same number of colorant patents as the previous ten-year span from 1969-1978. In the fifteen-year span, there were 356 patents on natural colorants and 71 on synthetics. This trend has continued and shows no sign of changing. 42-47 The confidence in natural colorants over synthetics may be a little naive but it is realistic.