Saving Marine Life,
One Color at Time

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1. Acronyms/Abbreviations

The following list presents some acronyms and abbreviations used in this document.

CAS: Chemical Abstracts Service Number
EPA: Environmental Protection Agency
FDA: The Food and Drug Administration
GHS: Globally Harmonized System of Classification and Labelling of Chemicals
IARC: International Agency for the Research on Cancer
IUPAC: International Union of Pure and Applied Chemistry
Kow: Octanol-water coefficient
LD50: Lethal dose 50
NIOSH: National Institute of Occupational Safety and Health
OSHA: Occupational Safety and Health Administration
PEL: Permissible Exposure Limit
PHA: Poly-hydroxyalkanoate
REACH: Registration, Evaluation, Authorisation and Restriction of Chemicals (EU)
SDS: Safety Data Sheet
TWA: Time-weighted Average
UV: Ultraviolet light
T_g: Glass Transition Temperature
T_m: Melting Temperature
X_{cr}: Degree of Crystallinity
E: Young's modulus
σ: Tensile strength
$ε_f$: Elongation at break
WVTR: Water Vapor Transmission Rate
OTR: Oxygen Transmission Rate
BCF: Biological Concentration Factor

2.   The Challenge

Currently, there are over 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. Toxic chemicals leach from plastics and pose a threat to the marine environment (Eriksen et al., PLoS ONE, 2014). These plastics take many tens to hundreds of years to degrade (National Parks Service, n.d.). While they do undergo fragmentation by the mechanical forces of the ocean, they persist as micron sized pieces. These fragments concentrate and transplant microbial colonies away from their natural ecosystems, invading and disturbing others. Plastics are visually appealing to animals. Because of their bright colors, they are consumed by wildlife in large quantities. This poses a huge health hazard for marine life (Derraik, 2002). Our challenge was to identify several biodegradable, environmentally-benign and non-toxic colorants for PHB coatings, which maintain the durability, mechanics, and chemical properties of the plastic to be used in the application of marine buoys.

Our Client: Mango Materials

We partnered with Mango Materials, a new start-up that produces a naturally occurring biopolymer, poly-hydroxyalkanoate (PHA), from methane gas. Their mission is, *"To transform waste gas streams into affordable, biodegradable materials while creating a positive environmental impact"* (Mangomaterials.com). PHA is a biodegradable polymer produced naturally under conditions of excess carbon and limited nutrient availability (NSF, 2016). Mango Materials' process uses bacteria grown in fermenters to transform methane (supplied from Redwood City), oxygen and other additives into PHA. The PHA-rich bacteria are then removed from fermenters and the polymer is separated from the cell mass. The polymer is then rinsed, cleaned and dried and finally made into the desired product. At the end of the product's life, the polymer can be easily degraded anaerobically to produce methane gas (NSF, 2016). The use of methane gas would close the waste-cycle loop and provide a fresh feedstock for PHA production.

Image source: mangomaterials.com

Structure and properties of PHA

Polyhydroxyalkanoates (PHAs) are thermoplastic or elastomeric polyesters of R-hydroxyalkanoic acid (HA) monomers that are intracellularly deposited by bacteria as energy storage or reserves (Keshavarz, 2010). PHA is produced by microorganisms in response to conditions of physiological stress such as nutrient-limited conditions (Roy, 2014; Hankermeyer, 1999). PHAs are fully biodegradable and nontoxic polyesters with high melting temperatures. PHAs can have different physical and chemical characteristics owing to their varied monomer content. The type of microorganisms, media ingredients and fermentation conditions can influence monomer content.
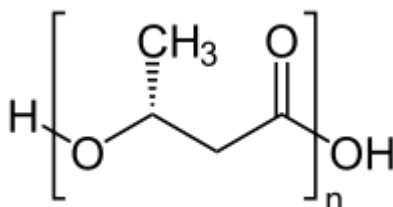
Figure 1: *The chemical structure of PHA*

Polyhydroxybutyrate (PHB) is a homopolymer that belongs to the PHA family. It exhibits an absolutely linear isotactic structure and is highly crystalline, meaning very stiff but brittle. However, PHB is thermally unstable during processing due to chain scission, which results in a rapid reduction of its viscosity and molar mass decrease (Tănase, 2015; Cyras 2000). The mechanical properties of PHB change over time due to recrystallization with aging (embrittlement) at room temperature (Sharma 1995; De Koning 1993). These previous studies have shown that ductility (i.e. percent elongation) of the polymer decreases from 40% to 10% after two weeks of storage. Some grades of PHB are similar in their mechanical properties to polypropylene (Doyle 1991). The average properties of PHAs are summarized in the table below.

Table 1: *Range of typical properties of PHA*

Property [units]	Values
T_g [^{o}C]	2
T_m [^{o}C]	160-175
X_{cr} [%]	40-60
E [GPa]	1-2
σ [MPa]	15-40
ε_f [%]	1-15
WVTR [g·mm/m^2·day]	2.36
OTR [cc·mm/m^2·day]	55.12

Adapted from Bugnicourt, 2014

**Manufacturing and Properties of PHA**

Polyhydroxyalkanoates are manufactured in anaerobic bacterial fermenters in nutrient-limited conditions. When sulfur or nitrogen, trace elements, or oxygen is lacking in the nutrient supply, this polymer is produced as granules by the bacteria, functioning as energy storage analogous to fat molecules in humans. This structure can be made into copolymers by tweaking

the starting materials available to the bacteria. The PHA granules can be collected by disrupting the cells and centrifuging and rinsing away the cell materials. As much as 80% of the dry weight of these cells can become PHA in the fermentation process (Jacquel, 2008).

In the manufacturing process of PHA, colorant will be added to the polymer mixture after the isolated and rinsed granules have been converted into pellets. Multiple formulations involving varied loading of colorants and the other additives (e.g., plasticizers and UV stabilizers) will need to be tested for longevity in response to the marine environmental conditions. This formulation will happen before the batch is molded into its final form.

PHA is similar to polypropylene (PP) in its structure and properties (see figure below). PHA is soluble in chloroform, dichloromethane or dichloroethane, while polypropylene is only soluble in p-xylene and tetrachloroethylene at high temperatures (140° to greater than 165° C) (Holten-Anderson, 1987; Drain, 1983). PHA differs from polypropylene in that it does not have the option for multiple types of tacticity in the stereochemical arrangement of its monomer functional groups. Polypropylene has a density between 0.895 and 0.92 g/cm³. Polypropylene's Young's modulus is between 1.3e9 and 1.8e9 GPa (Meier, 1998), much greater than that of PHA. This means that PHA is much more delicate in response to stress and strain than standard polypropylene. Polypropylene's melting temperature varies from 130° to 171°C depending on its tacticity (Kaiser, 2011). PHA has a very similar melting temperature range to the upper end of polypropylene, meaning it will likely need to undergo similar processing conditions.

Polypropylene degrades over time in response to the UV radiation found in sunlight. Usually, antioxidant materials, carbon black, and/or UV-stabilizer materials must be included in polypropylene used in external applications such as in the marine environment. The tertiary carbon atom of every repeat unit is the first to be subjected to radical degradation. Once the radical is formed at this site, oxygen can react with the site to break down the adjacent bonds into aldehydes and carboxylic acids. Fine cracks appear in over time in the polypropylene surface as a result of this degradation (Cacciari, 1993; Smillie, 1981).
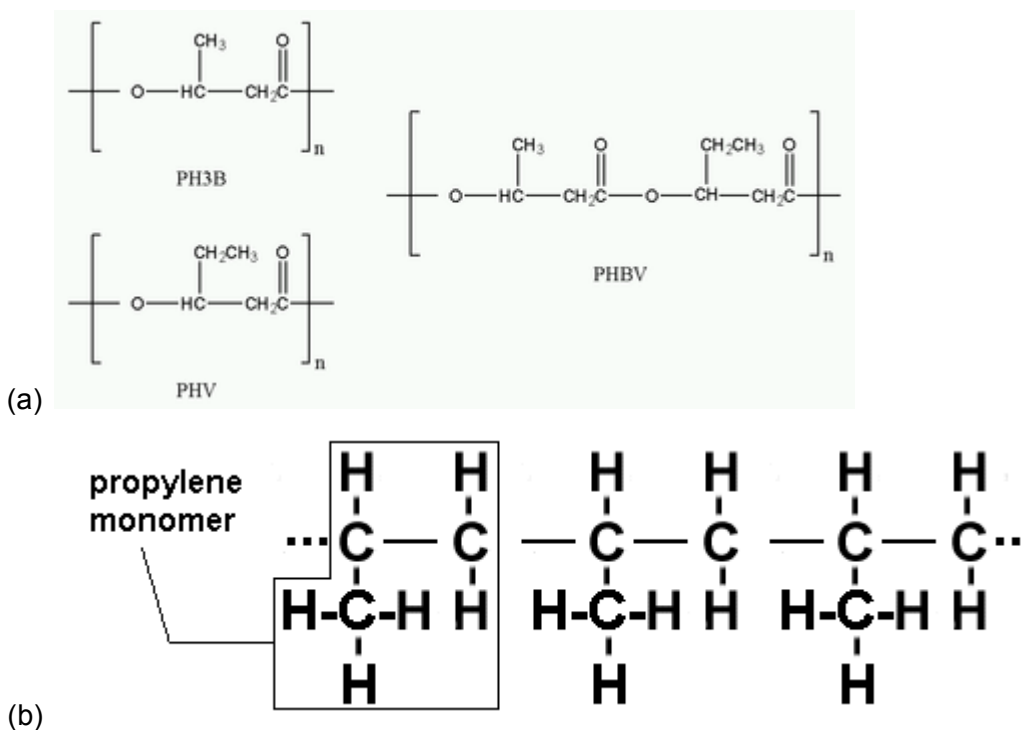
Figure 2: *(a) Three types of polyhydroxyalkanoates. Source: https://en.wikipedia.org/; (b) Polypropylene chemical structure. Source: www.advancedpolymersolutions.com.*

3.   Introduction to colorants

Various additives and reinforcements are added to polymers to assist processing and achieve the desired properties. Each polymer resin has its own color that might vary from grade to grade, or even from batch to batch. However, consumer desires dictate that the end-products have a specific, consistent color. To achieve this, colorants can be incorporated into the polymer coating along with other additives in a processing step such as extrusion or molding.

Two categories of commercially available colorants exist: dyes and pigments. Both dyes and pigments can be organic or inorganic. Dyes are colorants that are soluble in the substrate into which it is added. Pigments, on the other hand, are generally insoluble in water or in the substrate. Dyes are usually brighter and more transparent than pigments (Ebewele, 2000). Dyes are usually retained in the substrate by adsorption, solution and mechanical retention or by ionic or covalent chemical bonds, while organic pigments remain physically and chemically unaffected by the substrate (Board, 2003).

4.   Performance Criteria for Alternative Colorants

The end application for a plastic plays a crucial role in determining the type and amount of colorant to be used in the polymer. The desired colorant will be used for marine applications, specifically for the manufacturing and production of marine buoys. We focused on marine buoys as an application to define the performance criteria for the desired colorant. The desired colorant should be stable under processing and service conditions. The colorant should also be non-toxic, biodegradable, environmentally benign and relatively cheap to purchase or extract. The requirements for the safety and technical performance of the desired colorant are briefly explained in this section.

The desired colorant should not pose a serious threat to either the environment or human health. Acceptable chemicals would not be carcinogenic, mutagenic or cause any other serious health effects including acute or chronic toxicity, irritation or sensitization. The ideal colorant would also have low ecotoxicity, persistence (i.e. half life) and bioaccumulation.

The initial step in the selection of a technically feasible colorant is to determine whether the colorant is compatible with PHA and other additives. The compatibility of the colorant should be evaluated not only on the basis of the ease of its addition to the polymer but also on the requirement that it neither degrades or is degraded by the base resin and other additives (Ebewele, 2000). Incompatibility of the colorant with the polymer substrate can adversely influence the mechanical and chemical properties of the polymer. The desired colorant should not cause any adverse effects to the polymer mechanics while maintaining its own properties.

Migration fastness of the colorant indicates whether the colorant would easily leach out of the polymer. Migration fastness relates to colorant solubility in its substrate and in water. Bleeding of the colorant can occur if the colorant has a degree of solubility in the adjacent material (Brydson, 1999). In this case, the colorant can diffuse out of the current medium and impart its color in the adjacent material. Blooming can occur when the colorant is partially soluble in the polymer at ambient temperature even though it is fully dissolved at the processing temperature (Brydson, 1999). As a result, the colorant could be drawn out of solution upon cooling, leading to accumulation on the polymer surface. Blooming is undesired due to inhomogeneous mixture of the colorant in the polymer and can be prevented if the desired colorant is completely insoluble at processing temperature or if it is completely soluble at room temperature (Ciesielski, 1999). Given the specific application of the desired colorant, we need to evaluate the solubility of the colorants in PHA and water to determine whether the issue of bleeding or blooming would occur. For reference, the solubility of the colorants in water was determined based on the following data.

Table 2: *Water solubility of colorants*

Water Solubility (mg/L)	Classification
>10,000	Very soluble
>1,000 - 10,000	Soluble
>100 - 1,000	Moderately soluble
>0.1 - 100	Slightly soluble
<0.1	Insoluble

Adopted from the United States Environmental Protection Agency

U.S. Coast Guard personnel indicated buoys have a lifespan of approximately 8 years or longer if they are not weathered. Buoys will be directly exposed to sunlight, thermal gradients due to seasonal changes, and saline solution from the natural marine environment. The desired colorant should have good weather resistance and thermal stability to meet long lifespan requirements of marine buoys. The weather stability of the colorant indicates its ability to retain its color upon exposure to sunlight and/or atmospheric impacts. The colorant should neither be sensitive to sunlight nor rapidly degrade/decolorize upon exposure to UV light. The weather resistance of colorants has shown to change in combination of other factors including temperature, pH and salinity of the environment (Mitchell, 1996). Therefore, the weather stability of the colorant needs to be assessed in conditions that best mimic the service conditions. Thermal stability of the colorant indicates its resistance to thermal changes. The colorant should withstand not only the process temperature during the manufacturing process but also, the temperatures in the end-use conditions for prolonged periods. Colorant systems without the heat stability might show darkening, streaking or change of shade (Charvat, pp. 261, 2005). Heat stability of any colorant during processing is a function of both time and temperature. Both time and temperature dependency requires the specifics of the processing conditions. However, due to our limited information on processing conditions, we are unable to assess the thermal stability of the colorants during processing. On the other hand, the average temperature of ocean/sea surface waters is about 17 degrees Celsius, where maximum temperature can reach 35 degrees Celsius. On the basis of the end-use conditions of our colorant, the colorant should not degrade under ~40° C to be thermally stable.

The colorant should provide the desired coloristic properties (i.e. brightness, saturation, etc.) alone or in combination with other additives. The desired colorant for our specific application should have high saturation and opacity for buoys to be visible at long distances. The exact regulations are specified in a guide released by the United States Coast Guard (U.S. Aids to Navigation System). U.S. Aids to Navigation System guide Furthermore, buoys are generally colored green, yellow, red, orange or white.

Figure 3: A marine buoy.

Each buoy color provides a different navigation information. Therefore, the colorant should have one of the specified colors above.

5.   Methods
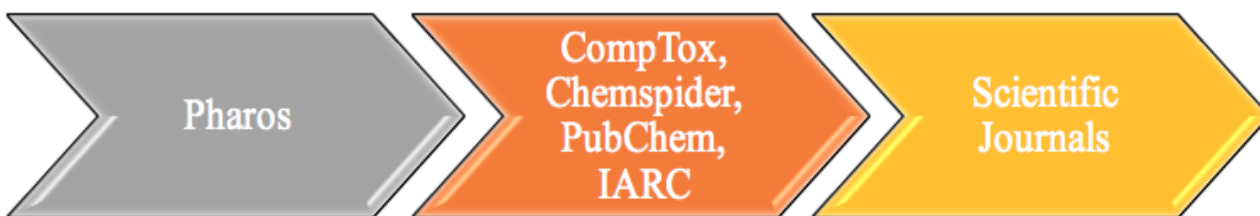
Figure 4: *Graphical summary of our approach to finding information for this project.*

Our initial research began with a simple Google search of industrially used colorants in the plastics industry. Similarly, we researched plant-based colorants that were commercially available. To narrow our long list of chemicals, we gathered information from safety data sheets (SDS). Next, we excluded chemicals that were patented, had limited hazard information, and were not used widely in industry. We then used Pharos (https://www.pharosproject.net) to determine if the colorants were on any authoritative lists. To further narrow the data gaps, we used toxicological databases like CompTox, ChemSpider, PubChem, and IARC. We also used scientific literature to assess the chemistry and mechanical properties of each colorant. Despite consulting multiple resources, we have several data gaps in our assessment.

We collected persistence and bioaccumulation data to assess the environmental fates of the colorants. We obtained the half-life of the chemicals from the databases to assess the persistence of the colorants. Half-life denotes the amount of time required for the chemical to diminish to half of its original amount. Similarly, we determined the bioaccumulation of each colorant by collecting information for K_{ow} values of colorants. Low K_{ow} values indicate low

potential for bioaccumulation. In the absence of experimental data for colorants' bioaccumulation and persistence, we used EPI Suite and PBT profiler to estimate these environmental fate parameters. The PBT profiler uses criteria set forth by the EPA in the Federal Register to evaluate the persistence and bioaccumulation of the chemicals (http://www.pbtprofiler.net/criteria.asp). Bioconcentration factor (BCF) is used as the indicator of a chemical's potential to be bioaccumulative. For reference, chemicals which have half-life less than 60 days in water, soil and sediment are not considered persistent. Similarly, chemicals with BCF factors 1,000 and 5,000 are considered bioaccumulative and very bioaccumulative, respectively.

6.   Current Industry Standards

We selected these colorants for review based on their known compatibility for use with polypropylene considering that polypropylene and PHA have similarities in their mechanical and chemical properties (Clariant, 2007). However, further testing is required to ensure that the colorants are compatible with PHA chemistry.

Industry Red Colorant: *Iron Oxide Red*
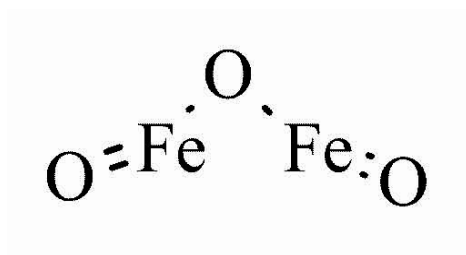
Figure 5: *Iron oxide red chemical structure.*

Iron(III) oxide red (Fe2O3), or ferric oxide, is an inorganic compound that is industrially used as a red colorant (Christie, pp.148-154, 2014). It can be produced naturally from iron compounds or synthetically from iron salts (Charvat, pp. 128-130, 2005). Both synthetic and natural iron oxide pigments are used commercially. Iron oxide red is used for paints, plastics and in building materials like cement and concrete (Revolvy, 2016). Synthetic iron compounds are used to color plastics because their natural form is inferior to their synthetic counterparts (Charvat, pp. 128-130, 2005).

Industry Yellow Colorant: *Iron Oxide Yellow*
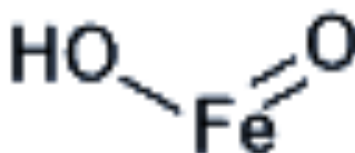
Figure 6: *Iron oxide yellow chemical structure.*

Iron Oxide Yellow ($Fe_2O_3.H_2O$), also known as C.I. Yellow Pigment 42, is a synthetically produced industrial colorant. It is a coloring agent in paints, lacquers, varnishes, and polymers. Additionally, it is used as a food colorant and permitted for use as an inert ingredient in non-food pesticide products (hazmap.nlm.nih.gov).

Industry Green Colorant: *Solvaperm Green*
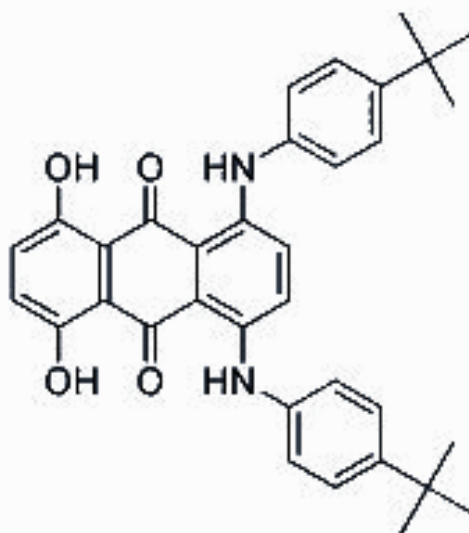
Figure 7: *Solvaperm Green chemical structure.*

Solvaperm Green G is currently available on the market. It is an anthraquinone-derivative dye. Solvaperm Green has an average density of 1.24 g/cm³ and a melting point of 245 °C. We were unable to find any information describing the particulars of its solubility in organic media, but we do know that it is basically insoluble in water (Clariant, 2007).

Industry White Colorants: *Titanium Dioxide and Zinc Oxide*

$$O = Ti = O$$

(a)

$$Zn = O$$

(b)

Figure 8: *(a) Titanium dioxide chemical structure; (b) zinc oxide chemical structure.*

Titanium dioxide (TiO_2) and zinc oxide (ZiO) are two colorants that are currently available on the market. Unlike colored pigments that primarily provide opacity through absorption of visible light, white colorants also provide opacity by scattering light (McKeen, 2009).

Titanium dioxide is an FDA-approved inorganic pigment naturally found in several kinds of rock and mineral sands. Due to its light scattering properties, it can impart whiteness, brightness, and opacity when incorporated into plastics. Varying particle-size fractions of titanium dioxide, including fine and ultrafine sizes are produced and used in the workplace (Dankovic, 2011). Two crystal structures of titanium dioxide, rutile (CAS Number 1317– 80–2) and anatase (CAS Number 1317–70–0), are currently available. Rutile titanium dioxide is the first choice for most applications since rutile titanium dioxide pigments can scatter light more efficiently. Rutile form is also not very reactive and is more structurally durable than anatase titanium dioxide pigments (IARC, 2010). Anatase titanium dioxide is not recommended for outdoor use due to its greater reactivity and lower durability (IARC, 2010).

Zinc oxide is another common white colorant used in plastics. Zinc oxide can be obtained by oxidation of zinc or zinc vapor with atmospheric oxygen or by calcination of different components such as zinc hydroxide, zinc carbonate or zinc nitrate (Kołodziejczak-Radzimska,

2014). Zinc oxide is mainly used as a control pigment in evaluating tint due to the highly bright white color they impart (Mitchell, 1996). Although zinc oxide has hides and opacifies material well, titanium dioxide performs better in both of these criteria. For applications that require high whiteness and opacity, titanium dioxide is mostly preferred as a colorant.

7.   Strategy: Bio-utilization

Our approach to alternatives was bio-utilization which entails harnessing naturally occurring organisms and materials in the design process. We believe this strategy would align with Mango Materials' goal to reduce waste and pollution in the environment. Bio-utilization can reduce waste because naturally occurring chemicals would biodegrade with the polymer-based coating, and thus would not contribute significantly to the overall pollution burden.

Alternative Red Colorant: *Paprika Oleoresin*
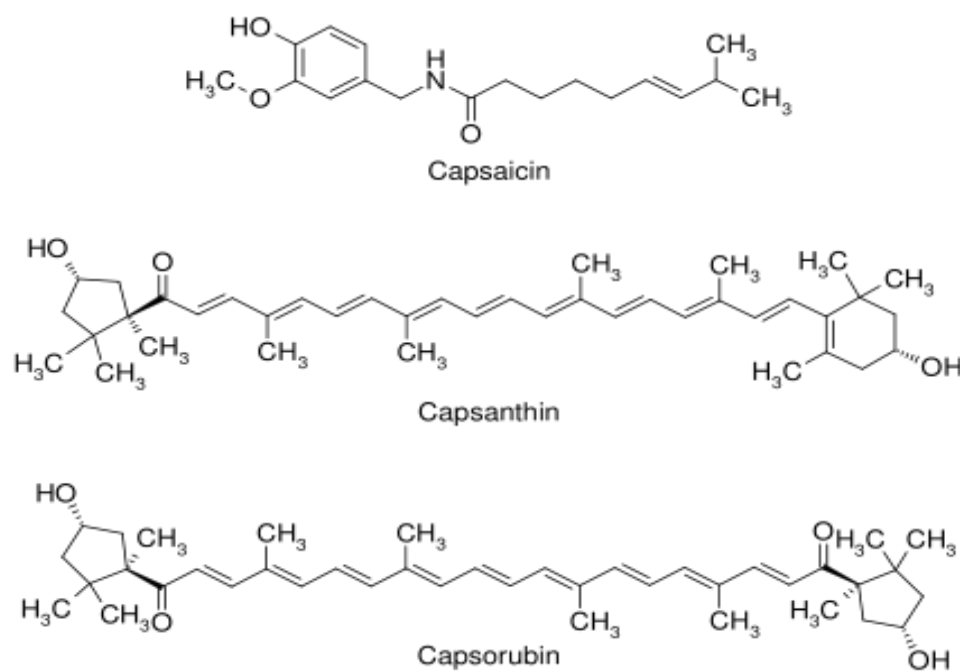
Figure 9: *Paprika oleoresin's three carotenoid components.Source: http://njhealthfederation.org*

An alternative chemical that can produce a red color is paprika. Paprika is a spice that is used widely as a flavoring and coloring agent (Matsufuji et al., 1998). It is made from the grinding of dried fruits of *Capsicum annuum* (Naz, K) and is a good source of carotenoid pigments. Paprika is manufactured exclusively in Hungary (Uragoda, 1967; Hunter, 1974). Paprika owes its vivid red color almost exclusively to the presence of carotenoids in its composition **(**Minguez-Mosquera, 1992**)**. Carotenoids are known to harvest light energy in photosynthesis and protects plants from photosensitized oxidative damage (Matsufuji et al., expected to1998). Some forms of carotenoids scavenge free radicals, making scientists and health professionals hopeful about their ability to prevent some forms of cancers and cardiovascular diseases (Matsufuji et al., 1998). The red carotenoids in paprika are comprised of capsanthin and capsorubin. The carotenoid pigments are present in the thylakoid membranes of chloroplasts (Matsufuji et al., 1998). Thin layer chromatography and column chromatography experiments have shown that capsanthin composes 30-60% of the total carotenoids (Matsufuji et al., 1998).

Paprika has a small dipole moment and is almost nonpolar. It is soluble in methanol, ethanol, 2-propanol, acetone, hexane, ethyl acetate and supercritical carbon dioxide. These solvents are used to extract the carotenoid molecules of paprika out of the oleoresin. Given its chemical structure, it is insoluble in water. The carotenoids of the paprika oleoresin each contain conjugated pi systems of electrons which allow the molecules to emit red light in the visible region. The maximal absorbance of paprika in the UV spectrum occurs at 462 nm in acetone and at 470 nm in hexane. Using atomic absorption hydride technique, the paprika's extract contains only 2 mg/kg of lead and 3 mg/kg of arsenic (fao.org).

Alternative Yellow Colorant: *Curcumin*
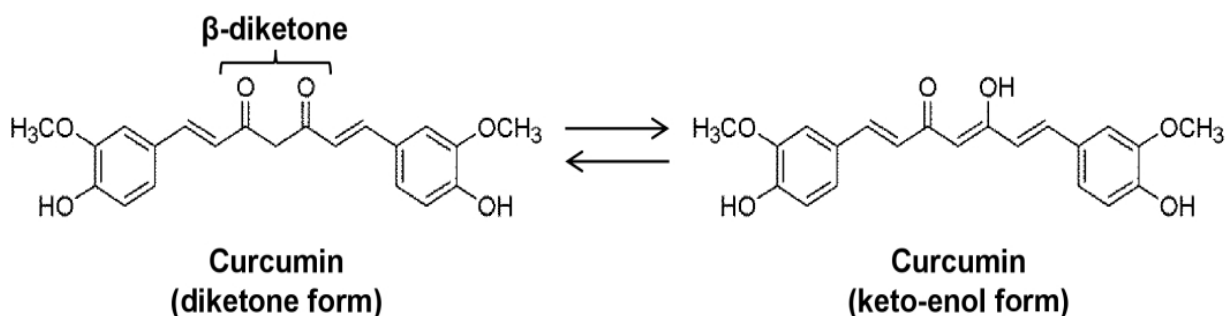
Figure 10: *Curcumin chemical structures. Source: http://www.intechopen.com*

Curcumin ($C_{21}H_{20}O_6$), a curcuminoid, is the principle component of the Indian spice turmeric. Curcuminoids are polyphenols. They are responsible for imparting the yellow color of turmeric (Priyadarsini, 2014). Curcumin is used as a yellow coloring agent in various food. Its radical-scavenging properties make it a great additive to protect food products from sunlight. Curcumin exists in its keto and enol forms; the enol form is less chemically reactive than the keto form (Priyadarsini, 2014).

Curcumin exhibits two strong bands in its absorbance profile, one in the visible light region (410-430 nm) and one in the UV light region (maximum of 265 nm) (Priyadarsini, 2014). Curcumin has a dipole moment of zero due to its molecular symmetry, making it a nonpolar molecule. The dipole moment of PHB is smaller than the dipole moment of water, making it more similar to that of curcumin than that of water. It is reasonable that curcumin will be more soluble in the polymer than in water. Alternatively, iron oxide yellow has polar covalent bonds, but its bond electronegativity differences resemble that of ionic bonds. Therefore, it is a polar covalent compound with ionic character and would not be expected to favor the polymer.

Alternative Green Colorant: *Chlorophyll*

(a)

(b)

(c)

(d)
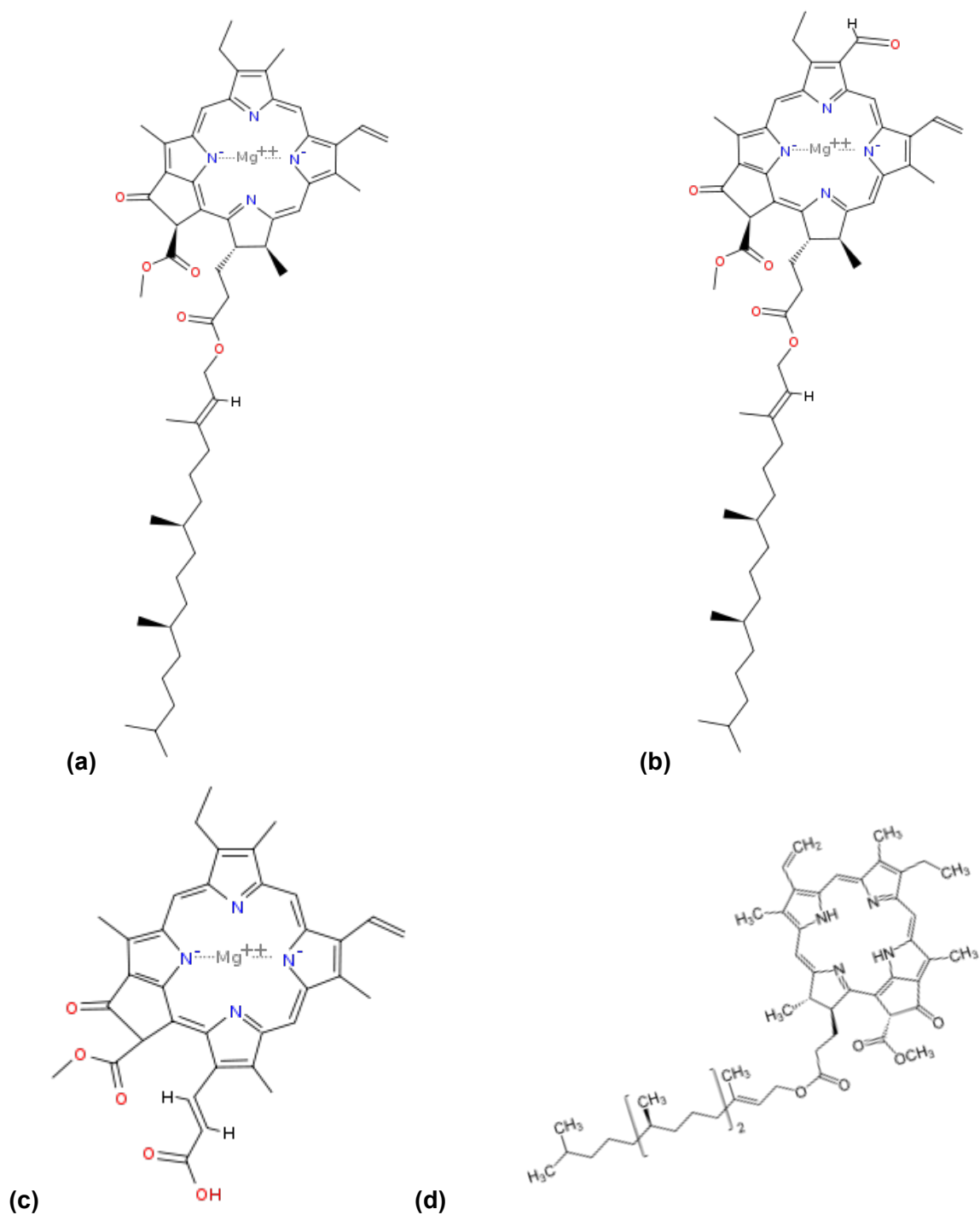
Figure 11: *Sample of chlorophyll chemical structures. (a) Chlorophyll a, (b) Chlorophyll b (note the additional aldehyde functional group at the top right of the structure), (c) Chlorophyll c1*

(note the lack of a long aliphatic tail), and (d) phaeophytin a (notice the lack of a metal ion in the center, with added protons instead).   Source: Wikipedia.

Chlorophyll is commonly found in the chloroplasts within plant cells. It can be easily extracted from plant leaves and vegetables. Chlorophyll is green in color and is involved in the process of harvesting light energy for plants to make their own food. Chlorophyll exists in many forms, including chlorophylls a, b, and c1. These molecules each contain a porphyrin structure that is metalated with one magnesium dication. Unlike chlorophylls a and b, chlorophyll c1 does not contain a long aliphatic chain extending from the porphyrin structure.   In the presence of acidic conditions, chlorophyll can lose its metal, becoming a protonated form of the molecule known as phaeophytin. While chlorophyll is green in metalated form, it turns brown in demetalated form (http://www.people.oregonstate.edu/; Lowe, 1955).

Chlorophyll is redox active, meaning that it participates in donating and accepting electrons from other chemicals in its immediate chemical environment. Depending on these local environmental conditions, chlorophyll a can be hydroxylated or deprotonated, or even have another type of functional group attached (Orzel, 2015).

Active in the visible region, chlorophyll demonstrates a known fluorescence profile (Kotz et al). This fluorescence profile has been used in many studies to identify chemical and other changes to chlorophyll content in various media - from plants to materials (Borowitzka, 2010).
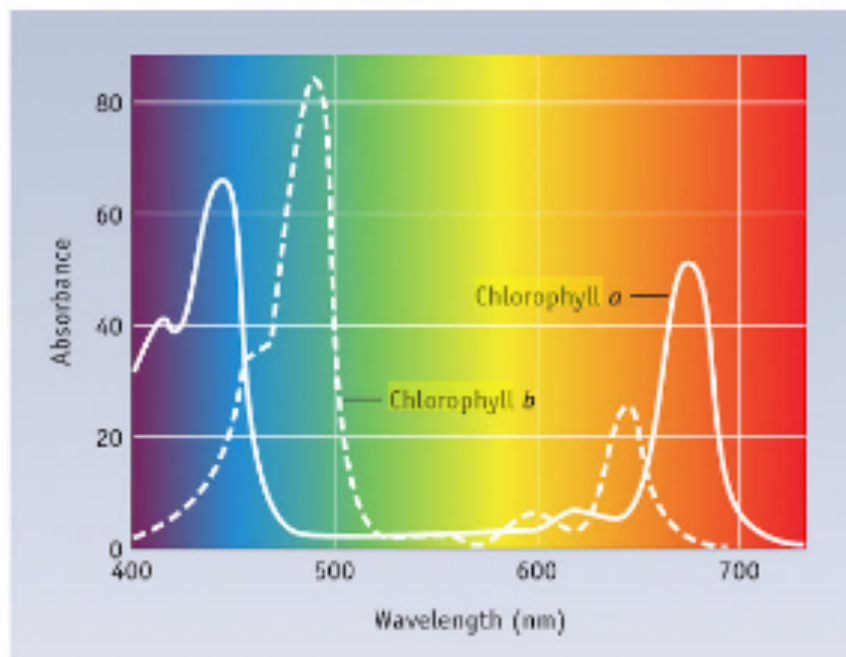
Figure 12: *Absorbance profile of chlorophyll a and b. Source: Kotz et al*

Chlorophyll and its aggregate forms

Chlorophyll's thermal, mechanical, and chemical stability can be improved by keeping it intact with its native proteins. However, its strong fluorescence profile is quenched in the presence of these proteins, when arranged as oligomers or large aggregates (http://www.esrf.eu). Chlorophyll in combination with several surrounding native proteins form an aggregate called the light harvesting complex. Two types of light harvesting complexes exist in plants: I and II. The crystal structure of the light harvesting complex II is known, but the light harvesting complex I has not been resolved. Light harvesting complex II is the main complex responsible for harvesting energy from light (Kühlbrandt, 1994).

Chlorophyll is insoluble in water. It is soluble in ethanol, diethyl ether, chloroalkanes, hydrocarbons and nonvolatile ("fixed") oils. The molecular structure does not contain any highly reactive functional groups which would suggest bioactivity or other unfavorable reactivity. Chlorophyll also does not contain any halogens that would suggest high persistence. (fao.org)

Acetone, dichloromethane, methanol, ethanol, propan-2-ol and hexane have all been used to successfully extract chlorophyll from plant matter. Analysis of gas chromatography

headspace has shown that extracted chlorophyll samples retain no more than 50 mg/kg, either singly or in combination of acetone, methanol, ethanol, propan-2-ol, and/or hexane. The samples will also retain <10 mg/kg of dichloromethane. Atomic absorption analysis has shown that extracted chlorophyll samples contain no more than 5 mg/kg of lead and 3 mg/kg of arsenic. (fao.org)

The extraction procedure has been optimized in the literature. Optimal conditions for extraction use acetone as solvent. Chlorophyll extraction per unit plant matter has been optimized using *Conyza triloba*, a member of the sunflower family, with methanol: water extraction solvents (El-Sayed, 2013). It has also been optimized to yield 659 µg/g of chlorophyll a and 261 µg/g of chlorophyll b using *A. sessilis* and a 80% *v/v* aqueous acetone buffer. Once extracted, chlorophyll can be stored at 15 °C for 3 days without significant content loss (Jinasena, 2016).

Alternative White Colorant: *Calcium Carbonate*
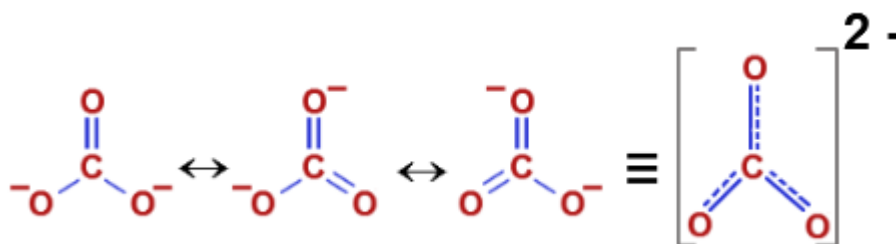
Figure 12: *Calcium carbonate chemical structure with resonance forms (NCS Pearson TutorVista).*

Calcium carbonate ($CaCO_3$) is commonly found in nature, including rocks, pearls, the shells of some organisms, eggs, and even in our bones. It makes up about 4% of the earth's outer crust (Gao, 2012). Pure calcium carbonate can be extracted by mining or quarrying, or produced from a pure quarried source (e.g. marble). The two natural forms of calcium carbonate that are commercially available are aragonite and calcite (Iwasawa, 2009).

Calcium carbonate is highly stable since it has three different resonance forms. It does not contain any heavy metals which would disrupt organism metabolism. It reacts with water that is saturated with carbon dioxide to form calcium bicarbonate. In plastics it is relatively inert due to its ionic nature.

Comparison of Health Hazards

We must disclose that we did not conduct a full GreenScreen hazard assessment. While we did search the literature and consult multiples types of sources thoroughly, our findings do not fulfill all the requirements of a GreenScreen benchmark assessment. We followed the GHS standards to assess the biohazards of our colorants by ranking their hazards from low, indicated by green color to very high, indicated by dark red color. We assessed the following hazard endpoints:

Table 3: Hazard Endpoint Criteria; Adapted from Clean Production Action

Hazard Endpoint	Definition
Carcinogenicity	Capable of increasing the incidence of malignant neoplasms, reducing their latency, or increasing their severity or multiplicity.
Mutagenicity and Genotoxicity	The more general terms genotoxic and genotoxicity apply to agents or processes which alter the structure, information content, or segregation of DNA, including those which cause DNA damage by interfering with normal replication.
Reproductive Toxicity	The occurrence of biologically adverse effects on the reproductive systems of females or males that may result from exposure to environmental agents.
Developmental Toxicity	Adverse effects in the developing organism that may result from exposure prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation.
Endocrine Activity	a substance having the inherent ability to interact or interfere with one or more components of the endocrine system resulting in a biological effect, but need not necessarily cause adverse effects.
Acute Mammalian Toxicity	Refers to those adverse effects occurring following oral or dermal administration of a single dose of a substance, or

	multiple doses given within 24 hours, or an inhalation exposure of 4 hours.
Systemic Toxicity	Includes all significant non-lethal effects in a single organ that can impair function, both reversible and irreversible, immediate and/or delayed, not otherwise covered by any other endpoint; or generalized changes of a less severe nature involving several organs.
Neurotoxicity	An adverse change in the structure or function of the central and/or peripheral nervous system following exposure to a chemical, or a physical or biological agent.
Skin Sensitization	A skin sensitizer is a substance that will lead to an allergic response following skin contact.
Respiratory Sensitization	Hypersensitivity of the airways following inhalation of the substance
Skin Irritation	The production of reversible damage to the skin following the application of a test substance for up to 4 hours
Eye Irritation	Eye irritation is the production of changes in the eye following the application of a test substance to the anterior surface of the eye, which are fully reversible within 21 days of application.
Acute Aquatic Toxicity	The intrinsic property of a substance to be injurious to an organism in a short-term, aquatic exposure to that substance.
Persistence	The length of time the chemical can exist in the environment before being destroyed (i.e., transformed) by natural processes.
Bioaccumulation	A process in which a chemical substance is absorbed in an organism by all routes of exposure as occurs in the natural environment (e.g., dietary and ambient environment sources).

Red Colorants

Iron Oxide Red

Iron oxide red is classified as a Group 3B Carcinogen, which means it has shown evidence of carcinogenic effects, but sufficient data is not available for classification (Pharosproject.net, 2016). However, it has low mutagenic, reproductive, and developmental toxicity. In addition, evidence suggests that exposure leads to systemic toxicity. This is especially problematic because iron oxide red is a highly persistent molecule (Pharosproject.net, 2016). We found that the colorant has a predictive octanol-water partition coefficient (K_{ow}) of -5.88, meaning it may bioaccumulate in an organism.

In a study by Zhu et al., rats were exposed to iron oxide red particles via inhalation exposure. In the the rats iron oxide particles were shown to be significantly high in lung tissues. Histopathological examination showed that exposure caused severe damage in lung tissues. This may be due to the oxidative stress caused by the induction of reactive oxygen species (Zhu, 2008). Exposure to iron oxide red also showed evidence of skin and eye irritation. While it had low aquatic toxicity, its carcinogenic properties create concern for both human and marine life.

Paprika Oleoresin

Exposure to paprika has had inconsistent results in human exposure studies. It has low carcinogenic properties and mutagenicity, reproductive toxicity, and developmental toxicity. Additionally, its impact on endocrine activity has not been thoroughly investigated.Paprika itself poses no irritation to workers after the pepper fruit has been separated from its ribs. However, in order to separate the fruit from its ribs, workers are often exposed to fungus that often grows within the ribs of the fruit. Workers who inhale the fungus during the course of work show symptoms such as loss of weight, poor appetite, poor sleep, and even haemoptysis, and even bronchitis. Chronic exposure may lead to the development of pulmonary fibrosis and bronchiectasis (Uragoda, 1967; Hunter, 1974). It has some toxicity to aquatic life, which is a concern given our application. Safety data sheets also indicate there may be high skin and eye irritation. Other endpoints such as ecotoxicity, environmental fate, and physical hazards of paprika are uncertain (Pharosproject.net, 2016). We believe this compound may be favorable to iron oxide red because it does not have carcinogenic properties. Complete hazard information is not available for paprika but it still appears to be worth investigating further as an alternative colorant.

Yellow Colorants

Iron Oxide Yellow

Exposure to iron oxide yellow has not been well studied. Limited literature available on iron oxide yellow states that it is unlikely to be carcinogenic. Given that it is a metal compound, it is highly persistent in the environment and has low acute mammalian toxicity. This is favorable because it appears to have very little toxicity to animals and humans. However, it is important to note that acute exposure may induce coughing, sneezing, and other respiratory issues. Aquatic toxicity has not been studied for this compound (Pharosproject.net, 2016). We recommend further testing and evaluation of this chemical in marine environments. Overall, there are large data gaps of safety and hazard information on this compound.

Curcumin

Research on curcumin does not show carcinogenic, mutagenic, reproductive, and developmental toxicities. In addition, research suggests that curcumin has low acute mammalian toxicity, physical hazards, and environmental fate. Curcumin causes some skin sensitization and eye irritation, but this is not life-threatening. No aquatic toxicity data are available for this compound. Further research on aquatic toxicity and other missing hazard data is needed for both yellow coloring agents to have comprehensive hazard profiles.

Green Colorants

Solvaperm Green

We were unable to find any studies curating the effects of Solvaperm Green on any animal or cell model. We were able to find, however, that anthraquinone (of which Solvaperm Green is a derivative) can be used to induce oxidation of adenine in DNA (Abou-Elkhair, 2008). This lack of information in combination with the possible correlation we found to DNA-altering is poignant considering Solvaperm Green's widespread use in the plastics industry.

Chlorophyll

A study by Jubert et al. revealed that ingestion of chlorophyll has been found to reduce absorption of known carcinogens in the human body (2009). Chlorophyll has also been implicated in two studies to absorb superoxide radicals and other free radicals, leading to increased resilience against oxidative stress in the body. Zhang et al. observed this effect in experiments by applying chlorophyllin, a semi-synthetic derivative that contains sodium copper salts, on human umbilical vein endothelial cell (2012). El-Sayed et al. also observed anti-carcinogenic effects on multiple cell lines treated with chlorophyll. Five cell lines showed actual

inhibition of tumor growth, while every cell line tested in the study showed noticeable abatement of tumor growth (2013).

Subramoniam et al also observed that both chlorophyll a and pheophytin a suppress inflammation in rats subjected to paw edema (2012). They also found that chlorophyll a suppressed the expression of tumor necrosis factor α (TNF-α), a gene commonly implicated in the development of cancer and other inflammatory and neurodegenerative diseases (Gahring, 1996).

White Colorants
Titanium Dioxide

Occupational exposure to titanium dioxide is regulated by OSHA under the permissible exposure limit (PEL) of 15 mg/m^3 for titanium dioxide as total dust (8-hr time-weighted average [TWA] concentration) (NIOSH, 2011). In 1988, NIOSH has evaluated ultrafine titanium dioxide (particle diameter <100 nm) as a potential carcinogen despite the lack of sufficient data to classify fine titanium dioxide (particle diameter >100 nm) as a potential occupational carcinogen (NIOSH, 2011). Epidemiological studies involving workers in the titanium dioxide manufacturing industry indicate no association of titanium dioxide with an increased risk of cancer or with any other adverse lung effects (Boffetta, 2001; Boffetta 2004; Chen 1988; Fryzek 2003; Ramanakumar 2008). In 2006, IARC evaluated titanium dioxide as "possibly carcinogenic to humans" (Group 2B) based on the results of previous chronic inhalation studies on rats (NIOSH, 2011). Inhalation exposures to titanium dioxide in rats were observed to result in lung effects and lung tumors (Lee, 1985). However, it is generally recognized that rat is uniquely sensitive to the effects of "lung overload" although the same results were not observed in other species including humans. (IARC, 2010). Upon a review of all the relevant data from animal inhalation studies and epidemiologic studies, NIOSH has concluded that titanium dioxide is not a direct-acting carcinogen, but acts through secondary genotoxicity mechanism that is not specific to TiO2 but is primarily related to particle size and surface area (NIOSH, 2011). The evidence suggests that inhalation of lower surface area TiO2 is not likely to result in carcinogenicity. The potency of ultrafine particles compared to fine particles is due to greater surface area of ultrafine fine particles for a given mass (NIOSH, 2011; Sagar, 2009).

Titanium dioxide has medium hazards to mutagenic and reproductive toxicity. The health hazards through potential exposures to titanium dioxide by inhalation, oral intake and skin contact are classified to be low. Titanium dioxide is approved as a food colorant and has not

previously shown any health hazards through oral exposure. Previous in vivo and in vitro dermal penetration studies have also indicated that titanium dioxide particles do not penetrate either intact or damaged skin (Gamer, 2006; Mavon 2007)

Titanium dioxide has low acute aquatic toxicity, medium chronic toxicity, very high persistence and low bioaccumulation. A previous study by López-Serrano Oliver et al. analyzed the experimental bioaccumulation capability of ionic titanium dioxide and titanium dioxide nanoparticles by zebrafish through BCFs after 48 or 72 hours of exposure (López-Serrano Oliver, 2015). The BCF values found in this study were <100, which classifies ionic titanium and titanium dioxide particles as non-bioaccumulative substances, under the REACH regulations.

Zinc Oxide

Compared to titanium dioxide, zinc oxide shows lower hazard of carcinogenicity and reproductive toxicity. However, zinc oxide exhibits high ecotoxicity, making it dangerous for the organisms in the marine environment. Since there is a limited number of studies which quantified the concentration of zinc oxide nanoparticles in the marine environment, the environmental concentrations were usually estimated by mathematical modeling methods (Yunga, 2014). An experimental study assessing bioaccumulation and sub-acute toxicity of nano zinc oxide particles indicated that after a 30-day exposure, 50 mg/L of nano-ZnO and fine particles of ZnO could be significantly accumulated and distributed in various tissues of fish, causing severe histopathological changes (Hao, 2013). The same study also reported that nano-ZnO exhibited more bioaccumulation than fine particles. Moreover, zinc oxide nanoparticles can adsorb onto pollutants and dissolved organic matter (e.g., fulvic acids from algae) in the marine environment. Dissolved organic matter is derived from algae or higher plants in the marine environment, inhibiting zinc oxide nanoparticles' aggregation/agglomeration. The inhibition of their aggregation and agglomeration would enhance their mobility in the marine environment and increase the ecotoxicological hazards of zinc oxide (Yunga 2014).

Calcium Carbonate

Calcium carbonate has low hazards for human toxicity and ecotoxicity, but its persistence in the marine environment is similar those of the current industrial colorants. Routes of exposure to calcium carbonate include inhalation and eye or skin contact (Mackison, 1981). Previous animal studies on rats showed that calcium carbonate can cause severe eye irritation (Mackison, 1981) and moderate skin irritation upon exposure for 24 hours (NIOSH, 1991). The acute toxicity of this chemical (oral dosage, LD50) in rats was measured to be 6,450 mg/kg (NIOSH 1991), which is classified as very low according to GreenScreen Hazard criteria.

This chemical is highly hazardous for skin and eye, and may cause respiratory irritation. Overall, this chemical has lower human health and environmental hazards compared to the current industrial colorants.

9.   Technical performance of Current colorants
Red Colorant: Iron Oxide Red

Oxides of iron are major constituents of some of the most abundant minerals in the Earth's crust (Revolvy, 2016). Iron oxide red is soluble in water in the presence of tartaric acid. We believe that iron oxide red may leach into the water when combined with the polymer.

Iron oxide can be found and made in several polymorphs. It can be found most commonly in its alpha and gamma forms, with iron adopting octahedral coordination geometry (Revolvy, 2016). This means each Fe center is bound to six oxygen ligands (Revolvy, 2016). The Alpha phase occurs naturally as the mineral hematite and is mined as the main ore of iron (Revolvy, 2016). The Gamma phase is metastable and readily converts from the alpha phase at high temperatures (Revolvy, 2016). Iron (III) oxide is a product of iron oxidation (losing electrons to oxygen atoms). It can be produced in a laboratory by electrolyzing a solution of sodium bicarbonate with an iron anode. The resulting hydrated iron(III) oxide can be dehydrated at approximately 200   C (Revolvy, 2016). It can also be prepared by thermal decomposition of iron(III) hydroxide at temperatures above 200   C (Christie, pp.148-154, 2014).

Synthetic iron oxides are favorable for coloring plastics than their natural form due to their chemical purity and improved control of physical form (Christie, pp. 148-154, 2014). Natural iron oxides are less chromatic and less uniform in particle size and distribution and are not as widely used in plastics as synthetic iron oxides (Charvat, pp. 128-130, 2005). Given that iron oxide red is widely used to color plastics, we believe that when mixed with the PHA polymer matrix, it would not alter polymer mechanics.

The various forms of iron oxide can yield a range of different physical properties. Density can range from 4.5-5.2 g/cm^3 and particle size ranges 0.3 to 4um (Charvat, pp. 128-130, 2005). Red iron oxide, similar to yellow iron oxide, has excellent weather stability and absorbs ultraviolet radiation (Charvat, pp. 128-130, 2005). Iron hydroxide is dehydrated to iron oxide at 200 degrees Celsius; furthermore, iron oxide has excellent heat stability and begins degrading at 525 degrees Celsius (Christie, pp. 148-154, 2014). it is insoluble in water and would possibly be soluble in the PHA polymer matrix. It has excellent heat stability and can be used in almost all engineered plastics. In combination with organic red toners, iron oxide red, in comparison to

other industrially used red colorants such as cadmium- or molybdate-containing formulations, lowers the cost of formulation, produces an opaque color, and enhances UV stability of the formulation (Charvat, pp. 128-130, 2005).

Yellow Colorant: Iron Oxide Yellow

Iron Oxide Yellow ($Fe_2O_{3.}H_2O$) use is limited to low-temperature processes because it begins to lose its water of hydration above ~175 °C, which converts to Fe_2O_3 and changes to a red color (Charvat, 2005). It is stable outdoors and is able to absorb ultraviolet radiation. In regards to its ability to impart color, iron oxide yellow has excellent opacity but limited saturation and chroma (Charvat, 2005). There was limited information available on the mechanical properties of iron oxide yellow. Further testing of its compatibility with the polymer matrix is recommended.

Green Colorant: Solvaperm Green

Solvaperm green is a colorant that is currently used to impart green color to plastics. Its chemical class is anthraquinone, which is a building block of many dyes (Murphy, pp. 129, 2001). Due to its poor solubility in water, this colorant is not expected to leach out of the polymer. There is not much information available specifically for Solvaperm Green.

White Colorants: Titanium Dioxide and Zinc Oxide

Previous studies were considered to evaluate the photoreactivity of titanium dioxide pigments on the photodegradation of polymeric coatings for outdoor uses. There was conflicting data between earlier studies regarding the photoreactivity of titanium dioxide. Some investigators claimed that titanium dioxide provided good UV protection by reflecting and/or scattering most of the UV-rays through its high refractive index whereas some believed that it absorbed UV radiation because of its semiconductive properties (Mossotti, 2010; Bohringer 2010; Gupta; 2002; Thiry, 2002). A recent study showed that titanium dioxide absorbs UV light and works as a UV-blocking additive mainly through UV absorption (Yang, 2004). Titanium dioxide pigments show strong absorption of UV for wavelengths below c. 400 nm and below c. 370 nm for rutile and anatase pigments, respectively (Day, 1990). However, absorption of UV radiation can release electrons and positive holes in the titanium dioxide crystals, some of which can diffuse to the surface and result in the production of free radicals such as hydroxy, peroxy, single oxygen etc. These free radicals can cause photo-oxidation and photocatalytic degradation of the polymer in the presence of moisture and oxygen (Day, 1990). Considering

the exposure to humidity in marine environment and the lability of the oxygen in titanium dioxide, there might be a risk of the free radicals that can increase the photodegradation of the polymer.

The influence of the addition of titanium dioxide on the degradation and mechanical properties of the polymer depends on its amount. When titanium dioxide was added to the polypropylene, maximum degradation temperature increased while degradation rate decreased (Esthappan, 2012). The same study reported an increase in thermal stability of polypropylene matrix when the concentration of titanium dioxide was increased. Titanium dioxide was proven to act as a flame retardant, antioxidant and help to improve the thermal stability of the polymer (Supaphol, 2007; Allen 1998; Turton, 2001). The incorporation of 1 wt% titanium dioxide in the polypropylene was observed to result in an increase in the tensile strength and modulus (Esthappan, 2012). However, higher content of titanium dioxide can result in aggregation, and in turn, a decrease in the contact area and weaker interfacial interaction, which can reduce the strength of the polymer coating.

Zinc oxide has high heat capacity and high heat conductivity (Porter, 1991). It has been used in plastics not only to provide color, but also add durability and abrasion resistance. Its excellent UV blocking ability makes it a good ingredient for sunscreens (Girigoswami, 2015). It doesn't generate free radicals upon long exposure to sunlight. This colorant is also compatible with most of the polymers and doesn't exhibit any adverse effects to the polymer matrix. The incorporation of zinc oxide has shown to increase the thermal stability of polypropylene (Silvestre, 2013).

Due to their poor solubility in water, titanium dioxide and zinc oxide are expected not to pose any risk of bleeding. In addition, both titanium dioxide and zinc oxide have shown to enhance mechanical properties of polypropylene. In a study by Altan et al., the addition of titanium dioxide and zinc oxide into polypropylene did not cause any significant change in the melt and crystallization temperature of the polymer (Altan, 2011). The mechanical properties including tensile strength, elastic modulus and failure stress under tension also increased with the addition of metal oxides. The increase in failure stress implies that the sample can endure higher stresses before failure occurs. Based on the thermal analysis of the polymer, zinc oxide particles were not dispersed as fine as titanium dioxide particles. Due to higher stiffness of the titanium dioxide particles as well as their improved adhesion to the polymer matrix, the addition of titanium dioxide into polypropylene improved its tensile mechanics more than that of zinc oxide.

Table 3: *Summary of Technical Performance of Industrial Colorants*

Chemical	Soluble in Polymer	Soluble in Water	No Bleeding	Opacity	Thermal Stability	Weather Resistance	Saturation	Mechanics
Iron Oxide Red	✓	X	✓	✓	✓	✓	X	✓
Iron Oxide Yellow	X	X	✓	✓	✓	✓	X	✓
Solvaperm Green	?	X	✓	✓	✓	✓	?	?
Titanium Dioxide	✓	X	✓	✓	✓	✓	✓	✓
Zinc Oxide	✓	X	✓	✓	✓	✓	✓	✓

✓ = favorable   ? =data gap

X = not favorable

10.   Technical Performance of Alternative Colorants
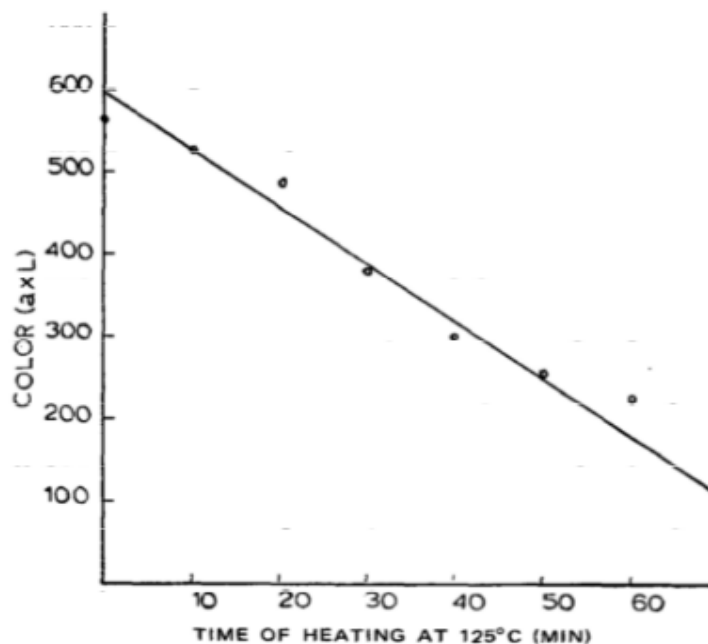***Red Colorant: Paprika***

Paprika naturally has a deep, saturated red color which can be altered with heated processes. The redness of paprika is related to the carotenoid content. The degradation of carotenoids is dependent on the quality and quantity of fat and this varies with the paprika powder itself [4]. The very light and orange paprika powders owe their color to the smaller particle size rather than chemical composition (Ramakrishnan, 1973).

In a study by Ramakrishan et al., a series of temperatures were tested on paprika to assess the degree of color change in paprika. At 150 degrees celsius, the color changed to an extremely dark color in 1.5 hours (Ramakrishnan, 1973). At 100 degrees celsius, the color changed very slowly (Ramakrishnan, 1973). The various temperatures were associated with a loss in carotenoid compounds. Similarly, heating paprika for 1 hour at 125 degrees Celsius changed the color drastically (Ramakrishnan, 1973).

In an inert atmosphere such as nitrogen, heating of paprika powder did not change the compound significantly (Ramakrishnan, 1973). After 30 minutes at 125 degrees Celsius, carotenoid losses were 12.2% in air and 9.8% in nitrogen (Ramakrishnan, 1973). It appears that in nitrogen sufficient atmospheres, there is a slowing of carotenoid compound degradation.

As Ramkrishan found, the Hunter "a" and "L" values both decrease when paprika is heated. The "a" value is related to the carotenoid content and the "L" function is the percent increase in browning agents (Ramakrishnan, 1973). Therefore, *a x L* function can be used to indicate various shades of red color [see figure below]. Samples with an *a x L* value above 500 would be visually rated as red; samples between 300-500 would be medium red, and values below 300 would be rated as light red (Ramakrishnan, 1973). For samples with fine particle size, values of *a x L* above 900 were brilliant-red (Ramakrishnan, 1973).

Figure 13: *A typical calibration curve for time of heating vs. a x L values for a sample of paprika*
Image source: Color and carotenoid changes in heated paprika (Ramakrishan et al).

Given that the colorant will be used in marine applications, we believe the red colorant would not degrade in marine environments. Safety data sheets indicated that paprika oleoresin would be insoluble in water; therefore we would not have an issue of the colorant bleeding into the adjacent material. Given the limited data found on its mechanical properties, we are uncertain if paprika oleoresin would alter PHA's mechanical properties.

Yellow Colorant: Curcumin

Thermal stability of the colorant is important to consider since color changes can occur due to thermal decomposition during or after processing. The degradation of curcumin would take place if the pigment is processed above its decomposition temperature. Curcumin is very sensitive to temperature changes. Heating enhances the solubility of curcumin but provokes its degradation (Siddiqui, 2015). The solubility of curcumin in water can increase by twelve fold by applying heat (Kurien, 2007). Partial degradation of curcumin was found to start at around 50 degrees Celsius. This temperature threshold is not of concern given our application because buoys are unlikely to reach such high temperatures in the ocean.

The weather stability of the colorant indicates its ability to retain its color upon exposure to sunlight and/or atmospheric impacts. Curcumin undergoes chemical degradation in aqueous-organic solutions and its degradation increased as the pH is increased. It has shown to be sensitive to light and rapidly decolorized upon exposure to UV light (Lee, 2014). Lee et al. found that curcumin degradation in water or phosphate buffered saline solution was accelerated under 24 hours of UV radiation, where the residual levels of curcumin following were measured to be 36.9 and 16.8%, respectively (Lee, 2014). In an alternative study, in dilute solutions (i.e. micromolar solutions), 90% of the curcumin in the medium degraded in 30 minutes (Priyadarsini, 2014). We have found contrasting degradation rates for curcumin but can ultimately determine that curcumin undergoes faster degradation when exposed to sunlight. The results of these studies are applicable to our research considering that the buoys will be exposed to an environment with some degree of salinity and sunlight (Lee, 2014).

To counteract the adverse effects of fast UV degradation, UV stabilizers can be added to PHB intended for outdoor exposure. However, additional care must be taken while incorporating UV stabilizers because one finding observed the mechanical properties of UV stabilizers were altered by the presence of other additives and pigments in polypropylene (Maier, 1998). Other approaches have been developed to tune the weather stability of curcumin. An example is the addition of zinc sulfate to curcumin can reduce the UV degradation rate by providing links between the curcumin and the zinc sulfate through divalent ions (Zebib, 2010). The curcumin and zinc sulfate mixture exhibited a much slower degradation rate than curcumin alone, and resulted with degradation of only 50% of the curcumin after 30 hours of exposure to UV light in 0.1 M phosphate buffer solution (Zebib, 2010). However, due to their positive charges, most of metal-curcumin complexes can bind to DNA, and thereby induce DNA damage and pro-oxidant behavior (Ali, 2013). Therefore, metal-curcumin complexes should be avoided due to their mutagenic effects to biological systems, including humans and aquatic life. Degradation of curcumin is significantly reduced when it is attached to some macromolecular and

microheterogeneous systems like lipids, liposomes, albumins, cyclodextrin, cucurbituril, and surfactants (Priyadarsini, 2009).

Green Colorant: Chlorophyll

Mechanical properties of chlorophyll

Significant improvements of the mechanical properties, high ductility, impact strength, ultimate strength, and modulus of elasticity in polypropylene were demonstrated by adding a 0.5% loading of chlorophyll to the polymer as a plasticizer (Sultan, 2016). When used as surfactants, chlorophyll a was able to be formed into a close-packed monolayer. Chlorophyll a monolayers were observed to have properties similar to rubber, with a Poisson's ratio of ~0.55 (Periasamy, 2012).

UV stability of chlorophyll

Studies conducted on the effect of UV light on chlorophyll only examine the effect of the UV on chlorophyll *in vivo* or *in vitro*. These responses could be noticeably different from the actual way in which chlorophyll would respond to UV light alone or once integrated into the polymer matrix. The *in vitro* study showed that chlorophyll extract dissolved in n-hexanes would reduce its absorbance values from 1.2 to 0.4 absorbance value in 30 min of exposure to UV-B light (Zvezdanović,2008). *In vivo* studies showed that in Chinese kale and in Tahitian limes, exposure to UV-C light and UV-B light, respectively, actually delayed the decrease of chlorophyll content that normally happens when vegetables and fruits are stored (Chairat, 2013; Srilaong, 2011).

Chemical stability can be modified

Improvement of chemical stability of chlorophyll was made by binding a copper (II) ion in place of magnesium ion in the porphyrin structure. A less significant improvement in stability over magnesium was also observed by replacing the magnesium ion with a zinc (II) ion. Adding the zinc ion also significantly reduced the pKa of chlorophyll, increasing its ability to dissociate protons. Copper-metalated chlorophyll also showed higher solubility in organic phase over the magnesium-metalated analog (Gerola, 2011). Increased preference for organic phase would eliminate concerns for bleeding and blooming of chlorophyll out of the polymer. Metalation with

copper could also potentially improve mechanical and UV stability by strengthening the bonding interactions between the metal ion and the organic ligands of the porphyrin structure.

Thermal stability of chlorophyll

Chlorophyll will degrade completely at over a span of 45 min at 50 °C (Jinasena, 2016).

White Colorant: Calcium Carbonate

Calcium carbonate is poorly soluble in water and thereby pose no risk of bleeding from PHA coatings. It has good thermal stability and weather resistance. Calcium carbonate decomposition starts at ~825 °C, a temperature that is much higher than the aquatic temperatures and temperatures experienced during the manufacturing of PHA coatings (Hills, 1968). This chemical is expected to be durable both during processing and its service time. Although calcium carbonate has good opacity, it has low saturation, meaning that the chemical can't impart brightness in the polymer.

Despite the lack of saturation, calcium carbonate has low cost, easy processing and favorable mechanical and chemical properties as a functional additive in the polymer (Kulshreshtha, 2002). When the particle size of calcium carbonate is carefully controlled, it can result in an increase in stiffness of the finished plastic product. The use of calcium carbonate with titanium dioxide can not only increase whiteness but also facilitate the dispersion of the white pigments in the polymer matrix contributing to the polymer's opacity. Calcium carbonate can be incorporated into PHA coatings to reduce the amount of the colorant needed. The addition of this chemical in polymer matrix would also reduce the risk of human health effects

and ecotoxicity due to lower potential risks of calcium carbonate compared to titanium dioxide or zinc oxide.

Table 4: *Summary of Technical Performance of Alternative Colorants*

Chemical	Soluble in Polymer	Soluble in Water	No Bleeding	Opacity	Thermal Stability	Weather Resistance	Saturation	Mechanics
Paprika Oleoresin	X	X	✓	?	✓	✓	✓	?
Curcumin	✓	X	✓	?	✓	X	✓	✓
Chlorophyll	✓	X	✓	?	✓	?	✓	✓
Calcium Carbonate	X	X	✓	✓	✓	✓	X	✓

✓ = favorable   ? =data gap

X = not favorable

11.   Data gaps

From our research on colorants we experienced difficulty finding information regarding the health and environmental impacts of both alternative colorants and industry standards (see Hazard Summary table for a visual summary of what we could and could not find from the literature).

Red Color

Iron oxide red has been shown to be a moderate carcinogenic hazard. It is a low health hazard for mutagenicity, reproductive toxicity, developmental toxicity, skin sensitization, and respiratory sensitization. It is a moderate hazard for both single and repeat systemic exposure, as well as for skin and eye irritation. It presents low hazard for both acute and chronic aquatic toxicity. Finally, iron oxide red is known to have high persistence, moderate bioaccumulation, and low reactivity and flammability. Iron oxide red has no data detailing its hazard levels as an endocrine disruptor or as a neurotoxin.

Paprika has a low health hazard for carcinogenicity. However, we could not find any information about the status of paprika as a mutagen, reproductive toxicant, developmental toxicant, or endocrine toxicant. Paprika is known to be a moderate hazard for both acute toxicity and repeat systemic toxicity, while presenting as a high health hazard for single exposure systemic toxicity. Paprika also has some known issues with skin and eye sensitization (high hazards). However, we weren't able to find any information about its ecotoxicity in the aquatic environment or its fate or physical hazards. We do know that iron oxide red is highly persistent in the environment. We don't know what the long-term impact of this persistence and bioaccumulation could be, so we recommend fully testing paprika to acquire data for a fair comparison.

Yellow Color

Investigations of the literature surrounding iron oxide yellow only revealed that iron oxide is known to have low acute toxicity, low flammability hazard, and very high persistence. It is unclear what the impact of this very high persistence would be for marine life, considering that iron ions are important in marine organism metabolism. Every other category is presented as a data gap (see Hazard Summary table).

Curcumin is known to present as a low hazard for carcinogenicity, mutagenicity, reproductive toxicity, and developmental toxicity. It also presents as a low health hazard for acute toxicity, while being a moderate hazard for single exposure systemic toxicity, skin sensitization, respiratory sensitization, skin irritation, and eye irritation. Of the colorants presented in this project, curcumin is the colorant about which we know the most fate and physical information. Curcumin degrades quickly, exhibiting low persistence in the environment. It also is low for bioaccumulation. It is not easily flammable or reactive. However, we do not have information to qualify curcumin's effect on aquatic life. We also could not find information about its neurological effects or endocrine effects. Finally, we don't know what the effect of

repeat exposure would be to the organs. These are all data gaps that should be investigated through testing before curcumin could be wisely chosen as a substitute to iron oxide yellow.

Green Color

Although Solvaperm Green has known low and moderate hazard impacts for human health, it is very problematic as a very highly acute aquatic toxicant. It also presents as a very high hazard for bioaccumulation. Solvaperm Green is known to have low hazards for carcinogenicity, mutagenicity, reproductive toxicity, and it has low persistence in the environment. It is not very flammable or reactive. We were not able to find data regarding the endocrine toxicity, developmental toxicity repeat exposure systemic toxicity, neurotoxicity, respiratory sensitization, and chronic aquatic toxicity categories.

No information was available in the literature regarding the health and environmental impacts of chlorophyll, but we do know that it is a highly persistent molecule. While chlorophyll is frequently ingested through foods like spinach and blue-green algae, we recommend that Mango Materials would perform a comprehensive set of tests to determine these data prior to making a decision about which green to use.

White Color

Titanium dioxide presents high carcinogenicity and developmental toxicity, as well as high repeat systemic organ toxicity. It is a moderate hazard for mutagenicity, reproductive toxicity, acute toxicity, respiratory sensitization, eye irritation, and chronic aquatic toxicity. Titanium dioxide is a low hazard acute aquatic toxicant. It also shows low hazard for endocrine disruption, single exposure systemic toxicity, and skin sensitization. It has low tendency to bioaccumulate and does not pose much hazard for reactivity and flammability. Titanium dioxide However, its great technical performance may outweigh its hazards.

 The ecotoxicological hazards of zinc oxide are very high, due to the presence of zinc ions in structural and catalytic proteins in organisms. Zinc oxide might not be the best option considering that our colorant will be used for marine applications.

Calcium carbonate has high persistence and shows low hazard levels for carcinogenicity, mutagenicity, reproductive toxicity, and endocrine disruption. Calcium carbonate also has moderate hazard for single exposure systemic toxicity, but low acute toxicity and repeat exposure systemic toxicity categories. It is known to be a low hazard for neurotoxicity, skin sensitization, skin irritation, and eye irritation. Notably, it poses low toxicity to aquatic life. Developmental toxicity is not known for calcium carbonate. This chemical can be used as a filler to improve the mechanical properties of the polymer and increase its surface finish and saturation.

12.   Conclusions/Future directions

UV stabilizers and fillers

Colorant and the base polymer might degrade upon direct or indirect impact of heat and UV light. Photodegradation takes place when UV radiation breaks down the chemical bonds in a polymer, ultimately causing adverse effects on polymer mechanics. The degradation of the colorant or the polymer would cause loss of strength, stiffness or flexibility as well as discoloration and loss of gloss. To prevent fast degradation of the colorant, UV stabilizers can be incorporated into polymer matrix along with other additives to improve and preserve mechanical properties of the polymer. UV stabilizers bond with the base polymer and prevent the polymer chains from breaking down due to UV light. However, the effectiveness of the stabilizers against weathering depends on various factors including their solubility, distribution in matrix and evaporation loss during processing and service use. UV stabilizers must be water-repellant, and would not precipitate out of the polymer. Otherwise, they could be easily extracted from the polymer coatings.

There are various types of UV stabilizers that are currently used. These include UV absorbers (e.g. benzophenone), nickel organic complexes and hindered amine light stabilizers (HALS). UV absorbers are not effective in thin cross sections such as coatings and films and may not provide the surface with sufficient protection (Harper, 2003). Benzophenone UV absorbers have been previously used in plastic coatings. HALS stabilizers do not act by absorbing UV radiation but by scavenging radicals (Hussain, 2002). Weathering of HALS causes the formation of nitroxyl radicals which combine with free radicals in polymers. Although HALS are observed to be effective in some polymers including polyethylene and polyurethane, they are shown to be ineffective in PVC. It is thought that the ability of HALS to form nitroxyl radicals in PVC is disrupted. Oligomeric HALS have excellent resistance to migration unlike other UV stabilizers and very low volatility (Schaller, 2009). On the contrary, HALS can be partially absorbed by the inorganic fillers in the polymer (e.g. talc and calcium carbonate), causing considerable loss of the UV stability of the color/polymer matrix (Gray, 1998)

The adverse effects of colorants might be mitigated by the introduction of modifiers or fillers which are nontoxic, biodegradable and environmentally friendly. The content of non-biodegradable fillers is limited by DIN EN 13432 (*German Institute for Standardisation*) to a maximum of 5 % in total and 1 % per individual filler (Priyadarsini, 2009). PHA has similar mechanical properties to polypropylene. Talc and calcium carbonate are the most commonly used fillers for polypropylene and improves its mechanical properties such as: stiffness, impact

strength, ductility and dimensional stability (Maier, 1998). The filler needs to be chosen based on the properties that need to be improved. Compared to talc, polypropylene modified with calcium carbonate exhibits better impact strength, ductility, and surface quality whereas its tensile strength and stiffness are reduced (Maier, 1998). Mango Materials currently uses Ecoflex to enhance processing and properties of PHA. Ecoflex is a commercially available, biodegradable material which has poly(butylene adipate/terephthalate) chemical structure (Müller, 2001). However, we are not providing any specific suggestions for UV stabilizers or fillers due to the scope of our challenge question. Further research is needed to find fillers and UV stabilizers that are compatible with the polymer.

Alternative approaches to tune properties

The innovation of biodegradable plastics has created new windows of opportunities to reduce future plastic debris in the ocean. We partnered with Mango Materials and proposed several plant-based alternative colorants to be used in the application of marine buoys. The colorants would biodegrade with the polymer and would not pose a threat to marine life.

We chose red, yellow, green and white plant-based colorants and current industry colorants were used as a baseline to compare toxicities. We estimated the compatibility of the colorant and polymer based on chemistry and physical properties. We acknowledge the large data gaps present in our analyses and recommend further testing of the colorants in the polymer matrix.

Paprika oleoresin is a possible alternative to iron oxide red because it has low toxicity profile in comparison to iron oxide red, which is carcinogenic and very persistent in the environment. We recommend testing paprika oleoresin with PHA to further assess its compatibility in the polymer matrix.

Curcumin is a possible alternative to iron oxide yellow because more hazard information is available on this plant-based yellow colorant. We acknowledge there is limited data available on iron oxide yellow and recommend further testing of iron oxide yellow to fully assess its toxicity. Given that iron oxide yellow can be dehydrated and converted to iron oxide red, we believe it would not be favorable to use over curcmin as a yellow colorant. In our literature search to assess curcumin's ability to impart color, we discovered this colorant degrades quickly upon UV light exposure. Curcumin's degradation rate can be altered by adding several additives, such as UV stabilizers. However, some UV stabilizers may alter the mechanical properties of the polymer. We recommend testing curcmin with several UV stabilizers in the polymer matrix to assess their compatibilities.

We propose chlorophyll as a viable alternative to solvaperm green as a green colorant. Chlorophyll is a naturally occurring compound which can be extracted easily from plants. Solvaperm green poses a threat to aquatic life and would not be a suitable candidate for our application. We acknowledge chlorophyll has an incomplete hazard profile and recommend conducting an exposure assessment of this plant-based green colorant.

We propose calcium carbonate as an alternative white colorant. It is naturally occurring in rocks, pearls and shells of some organisms. This colorant is currently used as a filler in plastics due to its low cost, easy processing, and its ability to enhance polymer mechanics. We recommend using calcium carbonate, in addition to another white colorant, in the polymer matrix. Calcium carbonate is not able to impart a white color solely on its own and would need an additional colorant, such as titanium dioxide and zinc oxide, to produce a white color. Despite its low color profile, using calcium carbonate can reduce the amount of other harmful white colorants in the matrix. Therefore, we believe calcium carbonate would be a strong candidate for a white colorant.

In this report, we assessed colorants to be used for PHA-based coatings. While we encountered numerous data gaps in our research and were unable to perform a full GreenScreen Hazard Assessment, we identified several promising plant-based alternatives. We hope our research has served as a foundation for Mango Materials' search for biodegradable colorants.

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15.   Meet the Authors

Semih Bezci (bezsem11@berkeley.edu) is a Mechanical Engineering PhD student with over three years of research experience in mechanical characterization of engineering materials and biological tissues. He will be in charge of investigating the effect of different colorants on the polymer mechanics.

Tashnia Hossain (thoss1001@berkeley.edu) is an Industrial Hygiene MPH candidate with a background in environmental health research and occupational safety. She has an interest in green chemistry and providing safer workplaces for people working with hazardous chemicals. She will contribute her knowledge of toxicology and environmental health research to this project.

Hannah Stringfellow (hstringf@berkeley.edu) is a Chemistry Master's Student with experience in advanced coursework in inorganic chemistry and in chemical lab techniques. She has a year of experience teaching general chemistry, with green chemistry principles, to non-majors. Hannah will provide expertise relating to evaluating the chemical compatibility of colorants with PHB chemistry. She can also elucidate the chemical terms in a way that requires limited chemical background to understand.