The Use of a Chlorophyll Degradation Product as a Marker for Locating Fecal Contamination

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Abstract

The Digestion of green plants in the gastrointestinal tract of warm-blooded mammals produces a specific product of degraded chlorophyll that appears to be emitting a strong fluorescent spectrum. This fluorescent signal can be used as a marker for fecal matter contamination for any mammal that ingests green plants as a part of its diet. Through the use of this fluorescence spectroscopy this signal can be easily located and identify. This fluorescence property from the degradation of chlorophyll in the intestinal track of mammals is very useful as a marker for possible fecal matter contamination of food and possibly water resources. Fecal contamination can be located easily and cheaply in real-time making it possible to test large quantities without much difficulty. This process was originally designed with food safety as a priority, and inspection of the meat industry, but has other useful application in most other areas where fecal contamination is of concern. This process has already been applied for the protection of the public with improved food safety procedures, and research has already begun for its implementation in other areas.

Key Words: Fecal Contamination; Fluorescence Spectroscopy; Real-Time Detection; Chlorophyll Degradation; Pheophorbide

Introduction

Every year in the United States approximately 76 million people experience some sort food or water born illness (CDC 1999). A large portion of these illnesses can be traced back to microorganisms found in food and water contaminated with fecal matter. Even with the regulations, in this country, concerning food and water safety we still have been unable to eliminate the majority of these pathogenic bacteria. The safety of these extremely important resources increases the importance of locating fecal contamination before infected food and water reaches the public at large.

There exists many markers for the location of fecal contamination, but most of these are slow and some can be costly. The use of a specific chlorophyll degradation product as a marker for fecal contamination is a new process developed with food safety in mind. This process uses fluorescence spectroscopy to locate a specific degraded product of chlorophyll that is a major product of the digestion of green plants in the gastrointestinal tract of mammals (Ashby et al, 2003).

This is a real-time detection process meaning there is no wait time for results. This has many advantages with the main one being you can test large quantities quickly and easily. There are disadvantages to this including the possibility of false positives, and the inability to distinguish concentration.

There is always room for more research and this is no different. A lot is already known about fluorescence spectroscopy, but more work can be done with this specific marker. Looking for other compounds the may present and give false positives are possible and should be looked at. It has also been found during testing that transmissible spongiform encephalopathy (TSE) infected animals can be located easily and before they have been sent for processing. This could be invaluable in locating Bovine Spongiform Encephalopathy (BSE) in animals that are still

alive (Casey, 2005). The new idea of phototherapy has also been looked at and may be of great help in this area.

This new technology is a process of improved detection of fecal contamination in real-time. It has been shown that this specific chlorophyll product has a fluorescence signal that can act as a marker in contamination in food and water. The usefulness in the area of food safety is documented and begin used today. Location of fecal contamination in water has yet to be studied in depth, but will have uses if not as great an impact as in food safety.

Fecal Contamination

Fecal contamination is the presents of mammal feces in large or small quantities in human food or water supply. This is dangerous for several reasons including the presents of pathogenic bacterial, and protozoa, which is often found in this type of contamination.

There are several different pathogens that can be attributed to fecal contamination. These include Campylobacter, Salmonella, and Escherichia coli (E. Coli). Campylobacter is a bacterium that lives in the intestines of birds and is one of the most common diarrhea causing bacteria in the world. E. Coli is a pathonogenic bacteria found in the feces of cows and other cattle. Consumption of food or water contaminated with cow feces causes variety illnesses including bloody diarrhea and painful abdominal cramps. The presents of virus and helminthes in fecal contamination are also likely. These other pathogens can cause a series of sever health problems and although are not a large problem in the U.S. they do cause lot of food poisoning each year. They are also a huge problem in other parts of the world where food and water safety are not as strictly enforced as it is in the more industrialized nations (CDC, 2000).

Table 1: Incidence of infection with nine pathogens and one syndrome under surveillance in the food borne diseases active Surveillance Network in United States 2001

Pathogen/syndrome	CA	CO	CT	GA	MD	MN	NY	OR	TN	Overall incidence	National health objective for 2010
Campylobacter	31.7	15.9	14.5	7.4	7.0	19.4	11.7	17.4	7.5	13.8	12.3
E. coli O157	1.1	1.9	1.1	0.6	0.4	4.8	1.5	2.3	1.4	1.6	1.0
Listeria	0.5	0.2	0.4	0.2	0.3	0.1	0.3	0.4	0.2	0.3	0.25
Salmonella	14.3	14.7	13.3	20.6	14.7	14.1	12.8	8.2	15.4	15.1	6.8
Shigella	13.2	7.1	1.8	8.6	3.3	10.0	1.3	3.2	3.5	6.4	NA†
Vibrio	0.6	0.2	0.3	0.3	0.4	0.1	0.1	0.1	0.1	0.2	NA
Yersinia	0.5	0.4	0.3	0.6	0.3	0.4	0.3	0.4	0.4	0.4	NA
Cryptosporidium	0.9	0.7	0.5	1.9	0.7	3.9	0.7	1.7	1.0	1.5	NA
Cyclospora	NR§	NR	0.1	0.3	NR	NR	NR	NR	NR	0.1	NA
HUS¶	1.0	1.3	0.3	0.3	1.0	1.8	0.5	1.7	0.8	0.9	NA

* Per 100,000 persons.
† Not applicable.
§ None reported.
¶ Hemolytic uremic syndrome. Incidence per 100,000 children aged <15 years.

(CDC, 2001)

Chlorophyll Degradation

When chlorophyll breaks down it can create several different end products. The major product for the degradation of chlorophyll during the dietary process of mammals is known as pheophorbide. Pheophorbide is the end product after several intermediate reactions of chlorophyll within the intestinal tract. These reactions are show in figure 2. This pathway shows how the phytyl tail is removed by Chorophyllase, to create Chlorophyllide. Mg Dechelatase then removes the center Mg ion from the chlorophyll leaving the final product Pheophorbide. Both of these processes are enzymatic in nature and occur as chlorophyll proceeds through the intestinal track. The two main types of chlorophyll a, and b are unimportant and will both be treated the same for all purposes related to this process (Hortensteiner, 1999). Chlorophyll c, and d form separate degraded products, which will be, talked about later.

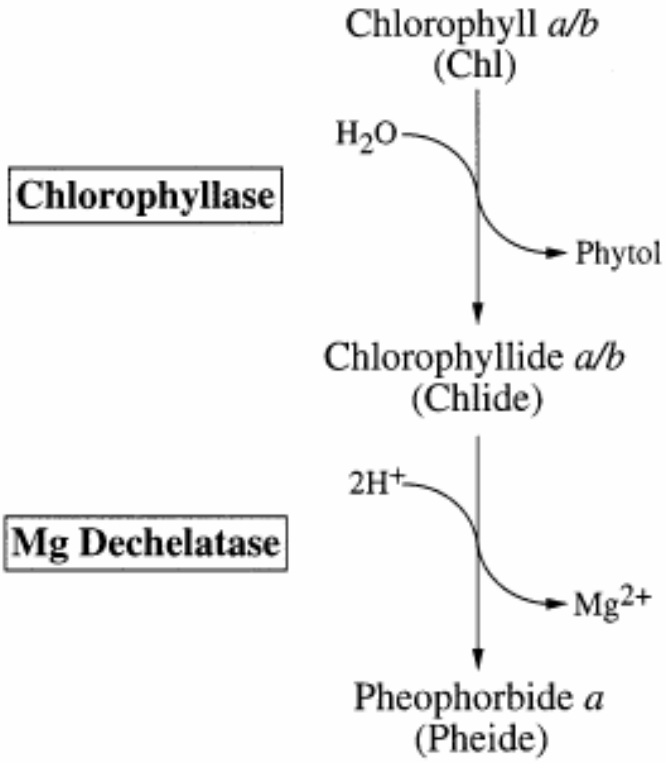

Figure 2: Degradation of chlorophyll to Pheophorbide
(Hortensteiner, 1999)

This figure shows how chlorophyll is broken down into pheophorbide. First figure 3 shows the intact chlorophyll molecule with both the phytyl tail and the Mg ion center. After these are removed using the aforementioned process you obtain Pheophorbide molecule shown in figure 4 (Ashby et al, 2003).

It is also important to look and compare the differences with other degradation products of chlorophyll. Some other major products are shown in figure 5 and will be compared with their fluorescent spectrum later.

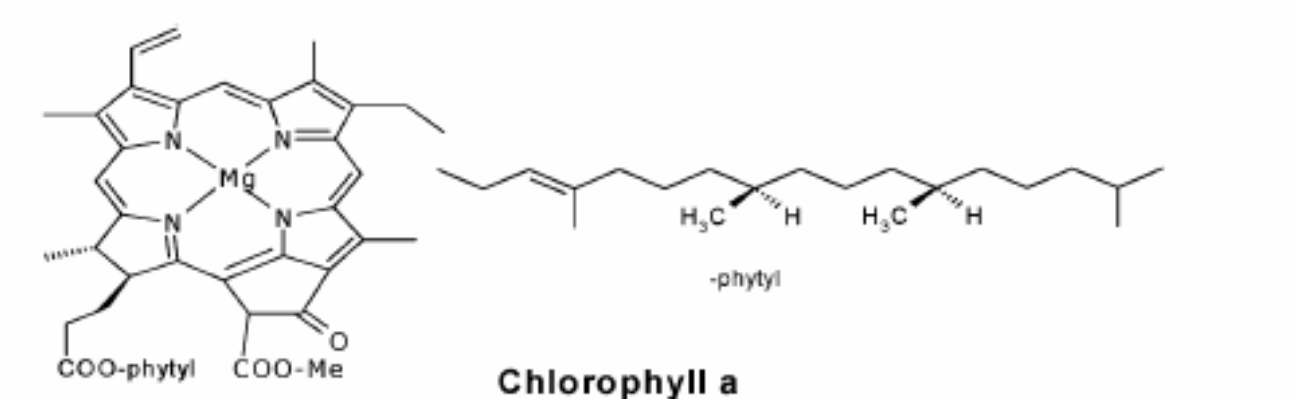

Figure 3: Chemical Make up of Chlorophyll
(Ashby et al, 2003)

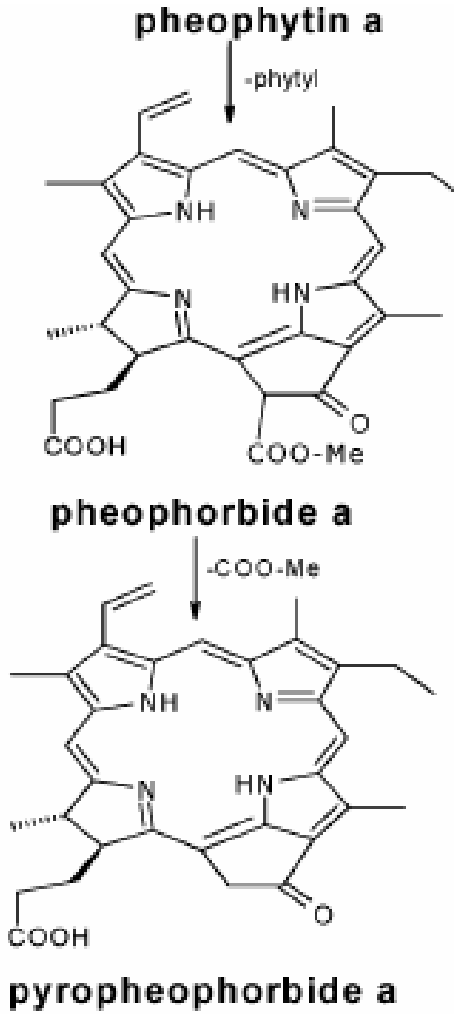

Figure 4: Chemical makeup of Pheophorbide, and Pyropheophorbid
(Ashby et al, 2003)

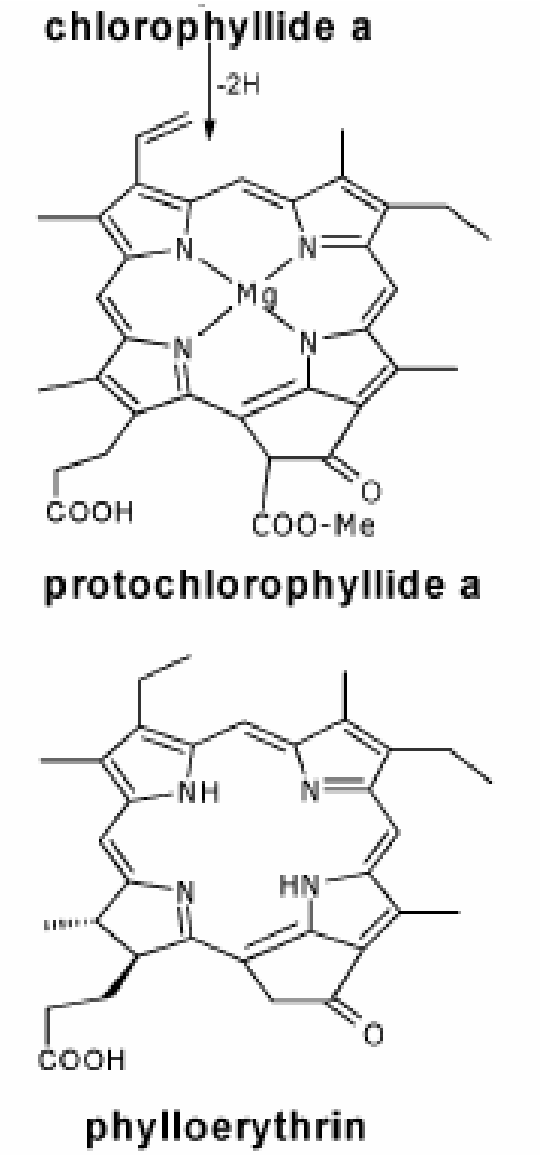

Figure 5: Chemical make up of Protochlorophyllide, and Phlloerythrin
(Ashby et al, 2003)

Fluorescence Spectroscopy

"Fluorescence spectroscopy or fluorometry is a type of electromagnetic spectroscopy used for analyzing fluorescent spectra. It involves using a beam of light, usually ultraviolet light, that excites the electrons in molecules of certain compounds and causes them to emit light of a lower energy, typically, but not necessarily, visible light" (Wikipedia, 2005). Fluorescence spectroscopy is a long known about, and much used process that has many applications in both the areas of chemistry and biology. Some work has been done using electronic imaging devices to detect bioluminescence and fluorescents in many different areas. It has been found that some bioluminescent bacteria and green fluorescent protein, such as Salmonella, can be successfully detected in water, vegetables and meat (Kocak et al, 2002).

When light photons interact with matter they can either collide or become absorbed. Collision of photons with matter is not important for this process. When light energy is absorbed into matter the amount of free energy is increased, and the molecule is considered to be in an excited state. There are several ways for excited molecules to return to their ground state. Molecules that are very efficient absorbers of this kind of energy may release light photons of lower energy as a way to return to the ground state. This release of light photons is called fluorescence.

Pheophorbide as a Marker for Fecal Contamination

It has been previously discussed how chlorophyll degrades and forms many different degradation products. Pheophorbide is not the only chlorophyll degradation product that has a fluorescent spectrum. It is important to distinguish the difference between these products and the wavelength absorbed and wavelength released. Pheophorbide is the specific dietary porphyrin to ensure that the chlorophyll was degraded in animals and not by some other method. Refer to figure 6 for the wavelength absorbed and released by four major chlorophyll products.

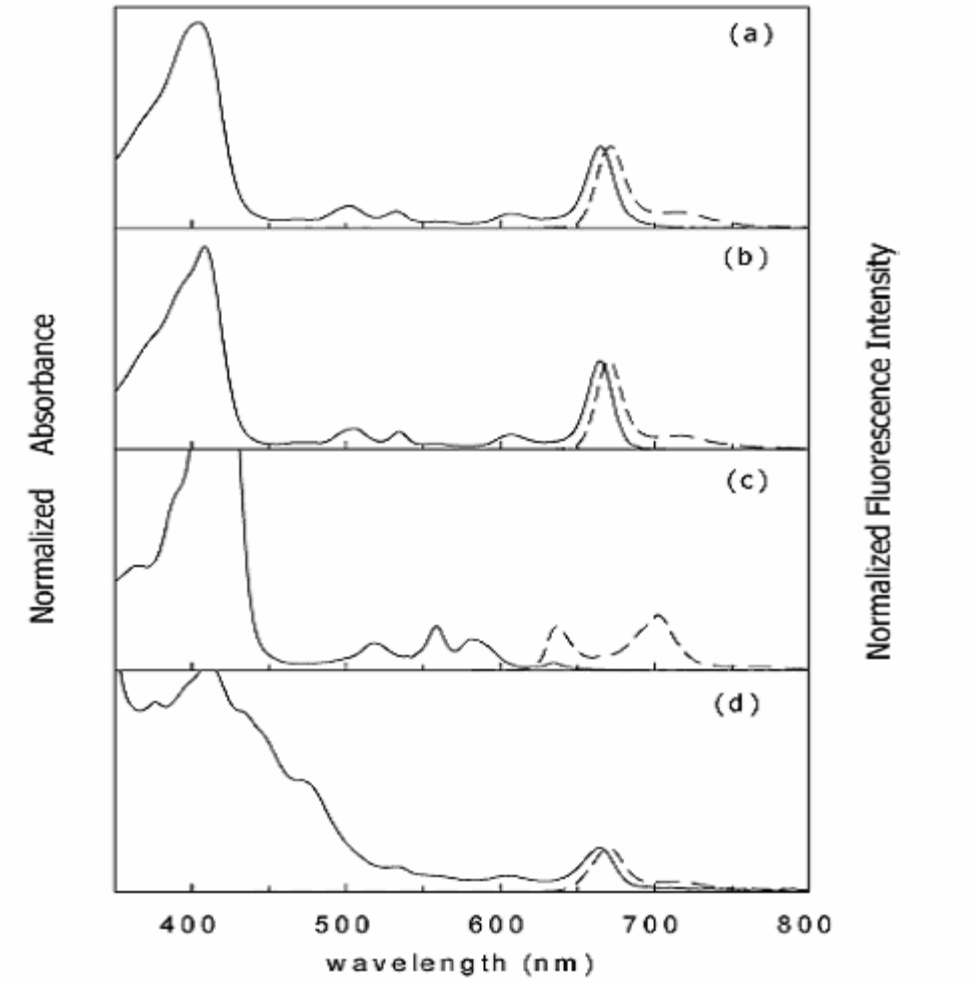

Figure 6: Absorption (Solid line) and Fluorescence emission (Dashed line) of (a) Pheophorbide a; (b) Phyropheophorbide-a methyl ester; (c) Phylloerythrin dihydrochloride; and (d) feces in acetone. (Ashby et al, 2003)

It becomes obvious from figure six that the fluorescent spectrum for pheophorbide and phyropheophorbide matches the spectrum from feces. Feces require more light energy to be absorbed before a release of smaller amounts of light. This is attributed to the fact that even if the animal in question lives entirely off green plants fecal matter is still not completely made up of pheophorbide. Feces contain smaller amounts of pheophorbide so to obtain the same levels of light emission it requires more absorption.

Some information about the fluorescent signal in general can be found in table 2. The fluorescence wavelength is in the red area. The area of contamination needed to give a possible is shown a 1 mm. The total amount of time needed before the contamination will fluoresce is 2 seconds (Kocak et al, 2002).

Table 2: Fecal Contamination Parameters

Parameter	Value
Fluorescence Wavelength	675 nanometers
Resolvable Contamination Spot Size	1mm
View Area	26 mm
Observation Time	2 sec maximum

(Table information from Kocak, 2002)

Advantages and Disadvantages

There are many distinct advantages and disadvantages to detection of fecal contamination thought Fluorescence Spectroscopy. These can be examined in many different ways. In many cases one aspect can be considered both an advantage and a disadvantage depending on the situations. So it becomes important to look at them in different ways so we can make and objective discussion.

This first and foremost advantage would have to be its speed. The results are received in real-time. This means there is little to no waiting for results; they are obtained as you perform the test. This is important because you can test large quantities very quickly and easily. Figure 7 shows the speed at which the fluorescence is absorbed and released. With the reaction speed show in nano-seconds (10^-9 sec) this reaction is occurring at extremely fast speeds. This is most important for inspection in the areas of food safety. Formally, in food safety, fecal contamination inspections were done by sight with a zero tolerance level. This means that if any contamination was visible to whole product had to be disposed of. Things are safer now, because smaller quantities of contamination can be located with ease and the infected area can be removed without destroying the entire product. This has the possibility to save the food producers in general large amounts of money and provide a safer product. When applied to contamination in our water supply it does not hold as much value. As has already been said the fluorescence product acting as our marker is a specific degradation product that comes form digestion in the intestinal tract of mammals. So the prescreens of plant materials present in the water do not exactly cause a large problem, but the dilution of this marker does cause a problem. As the maker becomes to dilute it will require far more light energy that can easily be used. This is also a disadvantage because fluorescence spectroscopy will not give you any information on the concentration of the contamination (Ashby, 2001).

This test is very easy to perform. As has already been stated it is as simply as shining a light on your sample and looking for the fluorescent single to be returned. Anyone can perform this test they with very little training on how the equipment works. Fluoresce spectroscopy equipment is

not cheap, but is easily maintained and may be more cost effective in the long run. All the light used in this method is in the visible light spectrum.

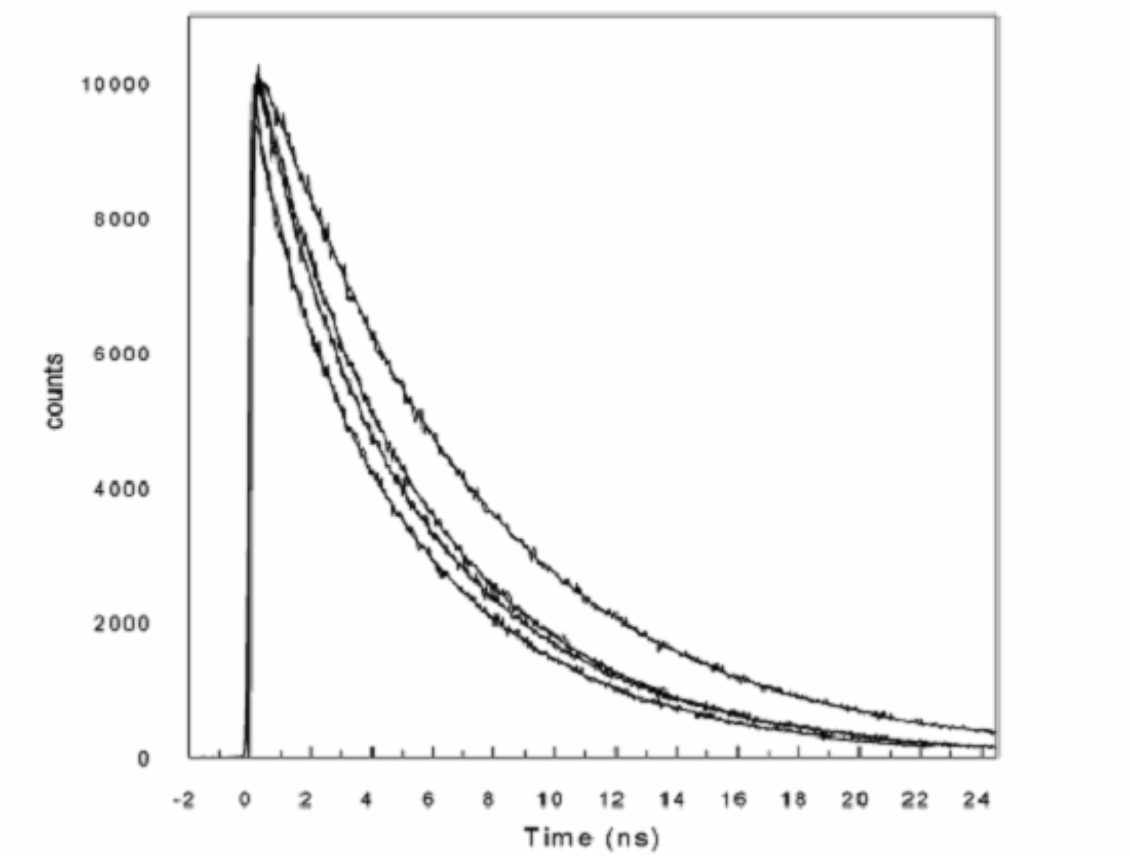

Figure 7:Fluorescence lifetime decays, from right to left; Pyropheophorbide-a Chylloerythrin dihydrochloride, Pheonophorbide.

More Research

There are always more things that need to be researched. In this case it would be important to look at other things that may give off a fluorescent signal close to the one being uses for fecal contamination location. This would be important to low the chances of getting false positives during testing and to be able to better control what is contaminated and what may not be contaminated. It is also interesting to look at other things that give off a fluorescent signal and how they may be used to improve the safety of our food and water supply.

It would also be important to understand the process that occurs in the intestinal tract. The production of this specific chlorophyll degradation production may lead to important insights into how to better design a system to location the contaminants. Currently those responsible for this breakthrough are not entirely sure which chlorophyll product is giving off the fluorescent signal used, thought they have a very good idea. If this information can be found more insight into the fluorescent signal used would be nice to have.

One area where more research could be done is how this may work in water contaminated by fecal matter. With the amount of things in the environment it is hard to say that nothing present in environmental water will give off the same signal as the one we are looking at. That is why at this

time water samples should be taken to a lab to be tested. This is done mostly for the reasons of removal of larger living organisms that may inhabit that water.

It has also been found in recent research that it may be possible to detect Bovine Spongiform Encephalopathy (BSE) (Mad Cow Disease) in animals that are still alive. It was found during testing of animal carcasses that nerve cells infected by TSE, the disease found in other animals that resembles BSE, give off a fluorescent signal much like the used in to locate fecal contamination. It was found that a large accumulation of lipofuscin &/or prion fluorescence build up was found in animals that are affected by TSE. This build up can be located in the optical tissue with the use of shining a light into the eye and seeing if a signal is given off. This may not be a foolproof way of locating mad cow disease, but it could use useful in locating possible infection quickly and easily. This could be invaluable in for food safety and helping to reduce the already extremely small chance of humans being infected by this horrible degenerative neurological disease. It could also be useful in reducing the economic reproductions of finding a BSE infected animal (Casey, 2005).

These areas of research could greatly improve the usefulness of fluorescent spectroscopy in locating fecal matter contamination in both the environment and in controlled laboratory situations. If these problems can be addressed along with any more that may arise while more research is being done then it would be possible to make our food and water supply even safer then they already happen to be.

Conclusion

Aside from the research that still needs to be done in a few areas the use of chlorofloral degradation as a marker for fecal contamination has a lot of promise in the area of food and possibly water safety. When chlorophyll is digested in the digestive system of a mammal it produces a specific degradation product, known as Pheophorbide that gives off a fluorescent signal under the right conditions in the red spectrum wavelength. This fluorescent signal acts as a marker for fecal contamination. This test can be done in real-time and is very easy to perform. Simply fast and effective, it can greatly improve our safety from bacteria that a prevalent in fecal matter.

Once more research has been done it may also be useful in locating contamination in water and the ability to easily diagnose Mad Cow Disease or BSE which is currently of major concern in the food production industry. It can also be used to locate contaminated water though currently is not as usefully as it is in locating contamination in food products.

This process is already being used in the food production to increase safety (Kocak et al, 2002). It has the possible use in any other areas where fecal matter is one of the possible sources of contamination. Anywhere you find contaminated fecal matter you have the possibility of bacterial infection. With this process we can greatly reduce the risk of bacterial infection from fecally contaminated food and water.

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