Natural Dyes

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Lecture No. # 05

Today we will try to take a look at a newer aspect of dyestuff, and that is the toxicity factor. You see all chemicals are not our friends; and those chemicals, which are not our friends, could become a toxin at a particular concentration. So, let us try to understand why and how these dyestuffs can become our friend, and how they can become a toxin? So trying to understand the toxicity factor of dyestuff.

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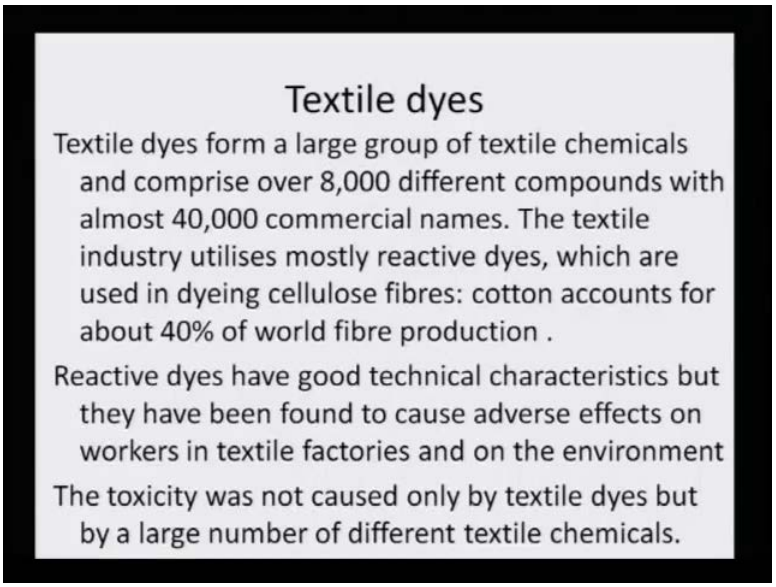

Let us take an overview of the textile dyes. Textile dyes form a large group of textile chemicals and comprise over 8,000 different compounds with almost 40,000 commercial names. The textile industry utilises mostly reactive dyes, which are used in dyeing cellulose fibers, such as cotton and that accounts for about 40 percent of the world fiber production.

So, that is the kind of volume of chemical dyes, that are being used particularly the reactive dyes, and then the cotton itself is utilizing about 40 percent. Reactive dyes have

good technical characteristics, but they have been found to cause adverse effects on workers in textile factories and into the environment. The toxicity was not caused only by the textile dyes, but a by a large number of different textile chemicals.

So, it is not just the dyes that are toxic, but even the textile processing chemicals are also sometimes not so good for our health as well as for the environment, but nevertheless let us even try to understand, what is it that makes a dye molecule toxic, and which has safer to deal with and so on and so forth. As we go along we must understand that this is the very important aspect, because a lot of textile affluent carries these dyes when they run out of factory.

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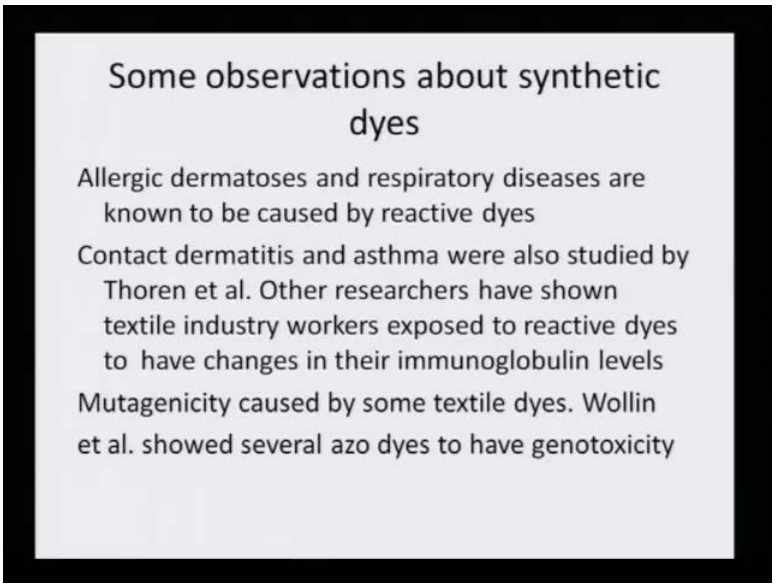

Some observations about synthetic dyes, allergic dermatoses and respiratory diseases are known to be caused by reactive dyes. So, now we at least know for conclusion that reactive dyes are little dangerous they should be used optimum and that these dyes when let in the affluent should be a processed properly. Contact dermatitis and asthma were also studied by Thoren et al. Other researchers have shown textile industry workers exposed to reactive dyes to have changes in their immunoglobulin levels.

Mutagenicity caused by some textile dyes was also noticed. Wollin et al. showed several azo dyes to have genotoxicity. So, it not only can create a mutagenesis in the DNA material, but it can also alter the gene content in the DNA and RNA. That is the nucleic acid of a cytoplasm or a nucleus and therefore, these chemicals are more toxic than other

chemicals, which do not interfere with the cell body. So, that is why it needs a special reference.

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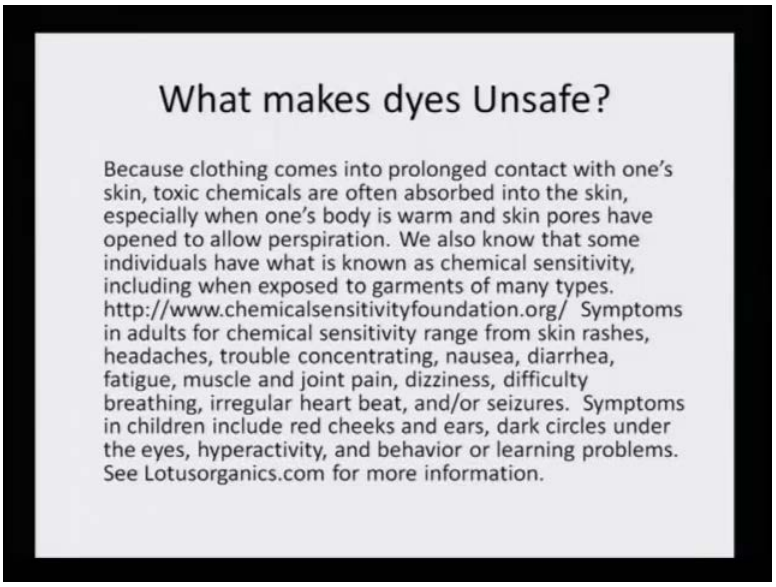

What makes these dyes unsafe? Because clothing comes into prolonged contact with one's skin, toxic chemicals are often absorbed into the skin. Especially when one's body is warm and skin pores have opened to allow perspiration. We also know that some individuals have what is known as chemical sensitivity, including when exposed to garments of many types. And this has been written extensively in the website mentioned here.

Symptoms in adults for chemical sensitivity range from skin rashes, headaches, trouble concentrating, nausea, diarrhea, fatigue, muscle and joint pain, dizziness, difficulty in breathing, irregular heartbeat, and or seizures. Symptoms in children include red cheeks and ears, dark circles under the eyes, hyperactivity, and behavior or learning problems. So, it is important to understand what is that makes these dyes. So, unsafe and they can create such serious a health hazards for us. So, therefore, it is important to understand the structure and the toxic nature.

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Synthetic dyes, dyes are so problematic because the families of chemical compounds that make good dyes are also toxic to humans. Each new synthetic dye developed is a brand new compound, and because it is new no-one knows it is risks to humans and the environment. Actually that is the problem the problem does not lie in the dye itself, but because the dye to human relationship have not been worked out the behavioral pattern of the dye has not been understood properly.

That is why a new compound when it is launched in the market has a lot of these a unknown facts, which need to be actually worked out just like medicines. Medicines there is a lot of study that goes on for animal studies and once the animal studies are proven to be correct then only it is allowed for human consumption and even on human consumption, a few a tester human beings are taken and these medicines are administer.

Only then when they get positive responses, and they feel that there are no negative a toxic effects visual a visually a known to the person who is actually observing then, it is passed. So, nothing like this actually happens with the dyes and therefore, there is a problem, because these dyes are completely new compounds, and their behavior to human being is not understood and is not known by them.

Many dyes like Amaranth have entered the market, then have subsequently been discover to be carcinogenic and withdrawn later. The European Union in particular has been pro-active in banning dangerous dyes and dyes formulated from toxic chemicals.

Although the European Union as well as many other developed countries like US are taking a very pro-active role. On trying to ban any chemical which shows any kind of toxicity including the dyes, but even then sometimes it is already maded syntax into the society.

But it is drawback to create a dye, see if it is a hazardous then ban it if so. It is you know a very tedious process, and by that time the harm is already done. Especially since so many dyes are known to be dangerous and carcinogenic. So, how much testing can be done and who which dye is being used, and how many these dyes are being used on one particular garment, and then this sensitivity of the person to person also matter. So, therefore, it is a very tedious task.

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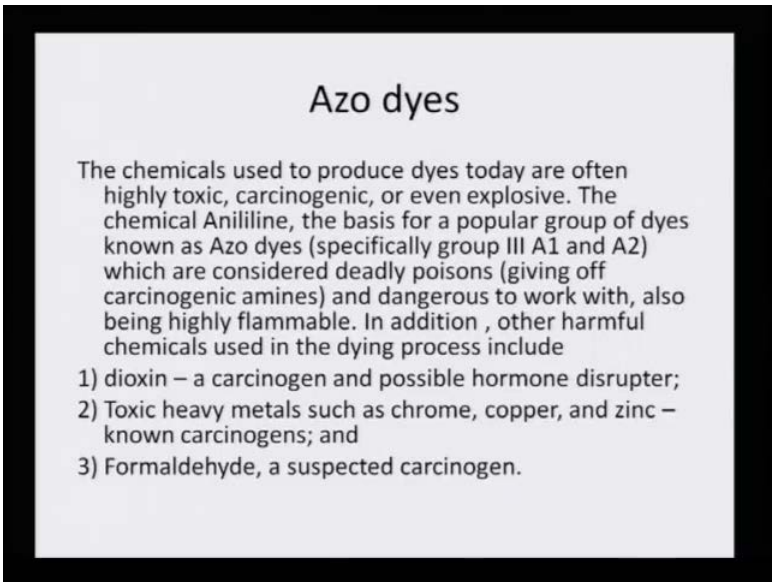

Azo dyes are definitely one of the most notorious dyes, and they have been banned. And that is the reason why they have been studied extensively and some information about their structural detail, but what actually makes the azo dye dangerous or toxic will be revealed to you in a while. The chemicals used to produce dyes today are often highly toxic, carcinogenic, and even sometimes explosive.

The chemical Anililine, the basis for a popular group of a dyes known as Azo dyes specially group three A1 and A2, which are considered deadly poison giving a carcinogenic amines and dangerous to work with also being highly flammable. In addition, other harmful chemical use in the dyeing process include dioxin - a carcinogen

and possible hormone disrupter; Toxic heavy metals such as chrome, copper, zinc - known carcinogens; and Formaldehyde, a suspected carcinogen.

So, it is not only azo dyes, but azo dyes related and processing chemicals are equally bad. Like for example, the dioxin mordant that is sometimes use these dyes like the metal mordant made out of chromium copper and zinc are also known carcinogens. And then there is fabric processing chemical formaldehyde which is also a suspected carcinogen.

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Not only dyes other processing chemicals are also harmful. So, we took a look that even other chemicals are also very harmful. In addition to the dyes themselves, the garment finishes are often equally as harmful. We will save this discussion on garment finishes for another time, but just briefly, they are used for creating wrinkle-free, stain resistant, flame retardant, anti-static, anti-fungal, anti-bacterial, odor-resistant, permanent-press, and non-shrink fabrics. So, these are very special processing chemicals which are applied to the fabric.

They can also be used as softening agents, and for creating other easy-care treatments. In fact it is often the dye fixative, used to bond the dye color to the fabric that causes the most of the problems. All of these can be particularly challenging for people with chemical sensitivities. You see for it is from individual to individual, that these dyes can have very different effect it may affect me, but it may not affect you. It may effect of a, it

may not effect person b, but some of these processing chemicals also have highly toxic nature.

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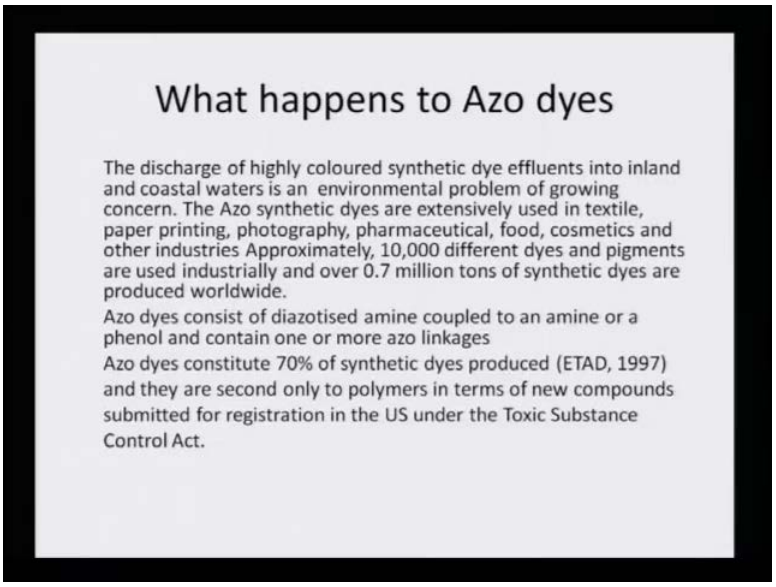

What happens to Azo dyes, why is it that it turns from a good you know friendly a dye to foam. That is because these azo dyes liberate I mean on hydrolyses, and because of the cleavage of the N double bond N they the problem begins. The discharge of highly colored synthetic dye effluents into inland and coastal waters is an environmental problem of growing concern. The Azo synthetic dyes are extensively used in textile, paper printing, photography, pharmaceutical, food cosmetics and other industries. Approximately, 10,000 different dyes and pigments are used industrially and over 0.7 million tons of synthetic dyes are produced worldwide.

So, you imagine that that is the kind of magnitude in which these dyes are produced and used. Azo dyes consist of diazotised amine coupled to an amine or a phenol and contain one or more azo linkages. Azo linkages mean N double bond N containing chromophore, azo dyes constitute 70 percent of the synthetic dyes produced and this is a detailed that was a issued by ETAD in 1997 and things must have gone up by now. It is an old data, and they are only by polymers in terms of new compounds submitted for a registration in the US under the Toxic Substance Control Act.

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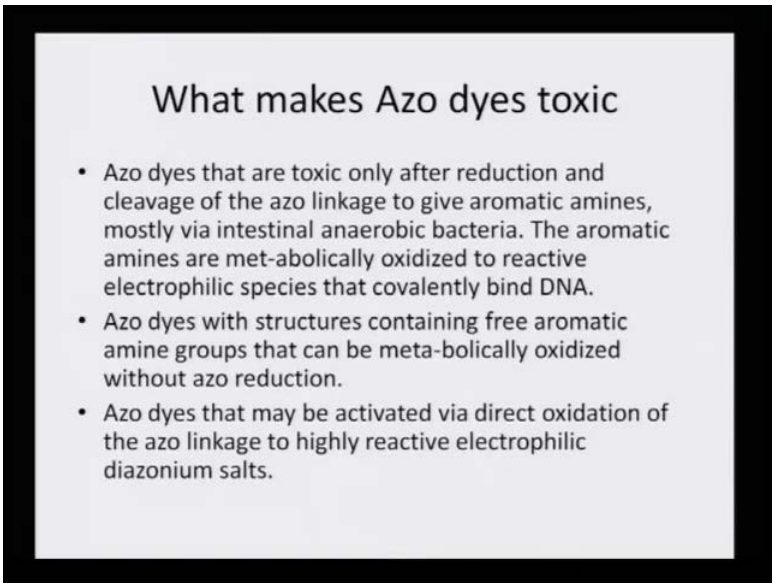

So, you see that they are already registered as toxic chemicals. What makes Azo dyes toxic? The Azo dyes that are toxic only after reduction and cleavage of the azo linkage to give aromatic amines, mostly via intestinal anaerobic bacteria. The aromatic amines are meta-bolically oxidized to reactive electrophilic substitute species that covalently bind the DNA.

So, you see that how kind of notoriously goes and attacks the DNA that is the genetic material of any cell of the body. Azo dyes with structures containing free aromatic amine groups that can be meta-bolically oxidized without azo reduction. Azo dyes that may be activated via direct oxidation the azo linkage to highly reactive electrophilic diazonium salts.

So, either it can go through a reductive cleavage of the N double bond N or it can undergo some kind of an oxidation without reducing the azo bond, and the third thing is that it may get activated by direct oxidation into a reactive electrophilic diazonium salt. So, these three things can happen under the cell condition and if these three things happen although three of them are very reactive species and they tend to attack the DNA.

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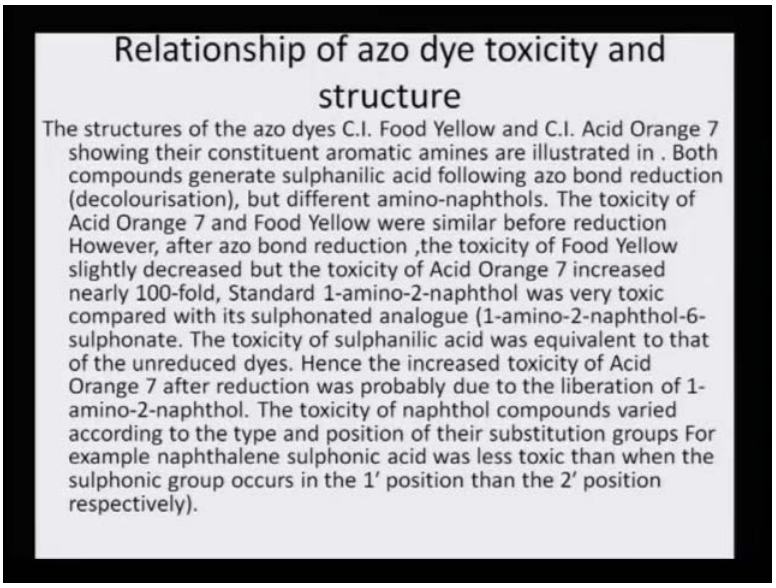

So, that is what makes azo dyes very toxic Relationship of azo dyes toxicity and structure. Let us try to understand how the structure and toxicity are related. The structures of azo dyes that is the C.I. number Food Yellow and C.I Acid Orange 7 showing their constituent aromatic amines are illustrated. Both compounds generate sulphanilic acid following azo bond reduction that is the decolourisation, but different amino-naphthols.

The toxicity of Acid Orange 7 and Food Yellow were similar before reduction. However, after azo bond reduction, the toxicity of Food Yellow slightly decreased, but the but the toxicity of Acid Orange 7 increased nearly 100-fold, Standard 1-amino-2- naphthol was very toxic compared with it is sulphonated analogue 1-amino-2-naphthol-6-sulphonate. The toxicity of sulphanilic acid was equivalent to that of unreduced dyes.

Hence the increased toxicity of Acid Orange 7 after reduction was probably due to the liberation of 1-amino-2-naphthol. The toxicity of naphthol compounds varied according to the type and position of their substitution groups for example naphthalene sulphonic acid was less toxic than one with the sulphonic acid when it occurs in the one position other than if it is at the two positions. So, the toxicity also varies if it is one substituted sulphonic acid group is at the one position, and the same sulphonic acid if it is at the two positions the toxicity will vary.

So, you see the main culprit in azo dye and the reduction of these two compounds. That is the Food Yellow and the Acid Orange 7 has been because they both liberate different quantities of 1-amino-naphthol-2-naphthol, but at the same time in the Acid Orange reduction there is another compound that is liberated, and that is 1-amino-2-naphthol-6-sulphonic. And that is what makes the orange Acid Orange both toxic than the Food Yellow.

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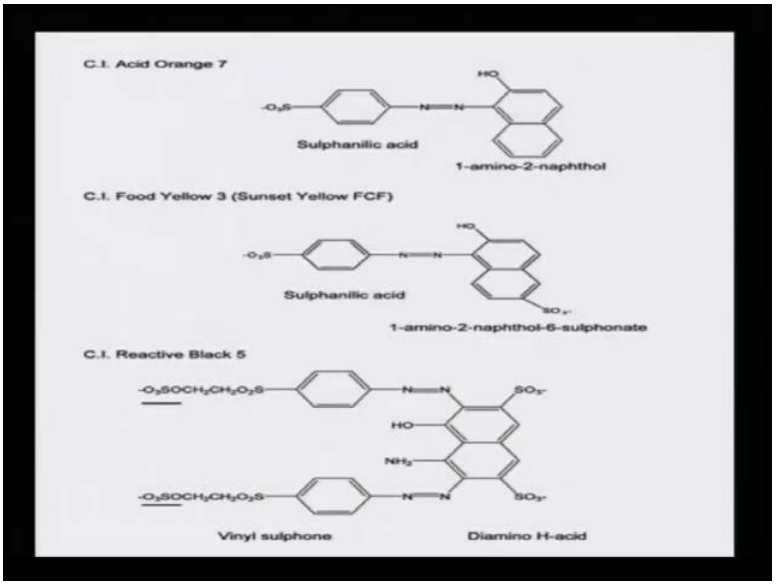

Now, if try to look at these structures if you see sulpholinic acid is a simple structure and it has 1-amino-2-naphthol, but Food Yellow has substitution of sulphonic acid at the 6 position. So therefore, it liberates these kind of compounds and the and the so, it is less toxic as compared to 2 Acids Orange. So, these are the structural details and Reactive Black 5. If you see, it has more sulphonic acid, and it has vinyl sulphones. So, makes it more and more toxic because the complexity and the electro you know the electrophilic edition of these kinds of cleaved moieties becomes more feasile.

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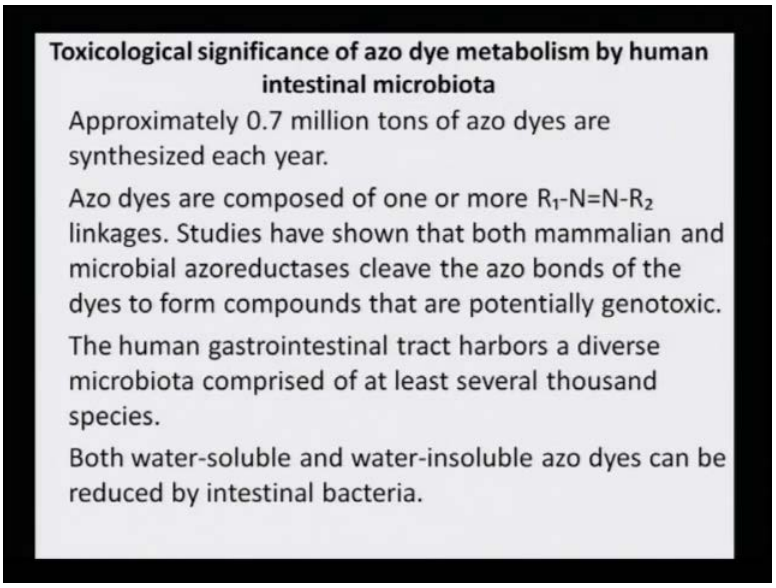

Toxicological significance of azo dyes metabolism by human intestinal micro microbiota, approximately 0.7 million tons of azo dyes are synthesized every year. Azo dyes are suppose are composed one or more R 1-N double bond N-R 2 linkages. Studies have shown that both mammalian and microbial azoreductases cleave the azo bonds of the dyes to form compounds that are potentially genotoxic.

The human gastrointestinal tract harbors a diverse microbiota comprised of at least several thousands of search species. Both water-soluble and water-insoluble dyes can be reduced by intestinal bacteria. So, you see that they have such a wide spectrum of different types of intestinal microbiota. That they are able to react with these azo dyes, whether they are in the solubilize form or they are in a insoluble form.

In either of the cases they are able to cleave this N double bond N, and release 1-amino-2-naphthol and that is the reason that this particular azo dye is so susceptible for oxidated or reductive cleavages, and this can be brought about in the human intestine by the microbial azo reductase as the name suggest suggest. The enzyme that place role is azo reductases. It is a class of enzymes which is present in the microbiota of the human intestine.

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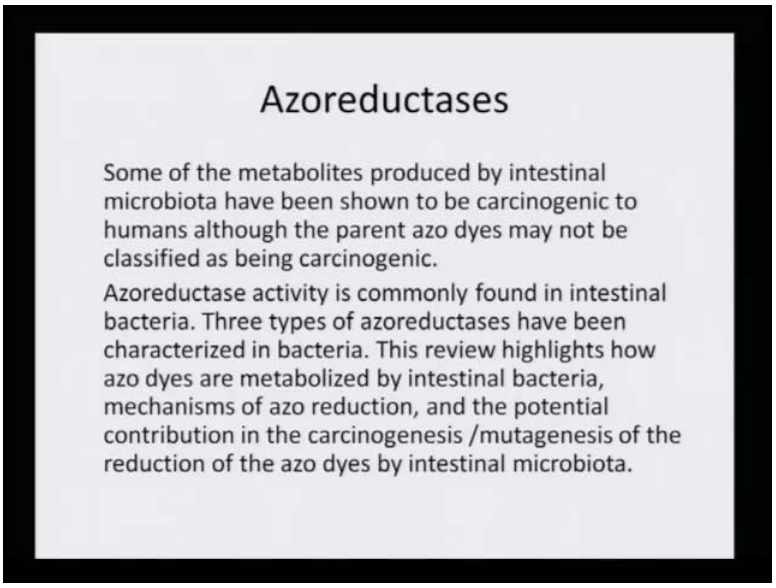

Azoreductases: Some of the metabolites produced by intestinal microbiota have been shown to be carcinogenic to humans although the parent azo dyes may not be classified as being carcinogenic. So, you see that only after the cleavage of that azo linkage. That the primary amine that is released is what is the culprit. It is not the dye that is the culprit. Azoreductase activity is commonly found in intestinal bacteria. Three types of azoreductases have been characterized in bacteria.

This particular lecture highlights how azo dyes are metabolize by intestinal bacteria, mechanisms of azo reduction, and the potential contribution in the carcinogenesis mutagenesis of the reduction of the azo dyes by intestinal microbiota. So, you see that we have at least understood. So, for that what makes the reactive dyes and particularly the azo dyes toxic? If we try to correlate with the structure of these azo dyes, there is one very common feature in all the azo dyes and that is the N double bond N linkage.

And it will have a aromatic ring on this side and it will have a another aromatic ring on this side and these rings will be further function carrying many functional group or in other words now we can said they will be carrying auxochromes, and they will contribute to the electronic effect into the rings, but these rings when they are sitting like this with the azo linkage are not so bad or not so dangerous or not so toxic, but once this N double bond N bond breaks or is reduced by the intestinal azoreductases.

That is the time when they become dangerous compound. So, if the dye itself is not dangerous, but when it is metabolized in the human body that is the intestinal. Then it starts becoming more and more dangerous and so much so that that it actually culminates in a carcinogenicity or mutagenicity. So, that is what is to be understood very clearly.

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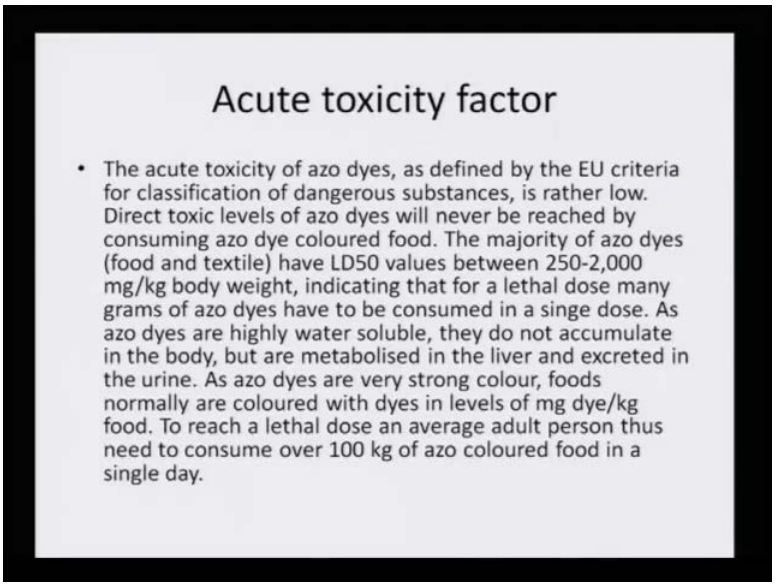

Acute toxicity factor: The acute toxicity of azo dyes, as defined by the European criteria for classification of dangerous substances, is rather low. Direct toxic levels of azo dyes will never be reached by consuming azo dye colored food. The majority of azo dyes food as well as textile have LD50 values between 250-2,000 milligram per kilogram body weight, indicating that for a lethal dose many grams of azo dyes have to be consumed in a single dose, as azo dyes are highly water soluble, they do not accumulate in the body, but are metabolize in the liver and excreted in the urine.

As azo dyes are very strong colour, foods normally are colored with dyes in levels of milligram dye per kilogram of the food. To reach a lethal dose an average adult person thus need to consume 100 kilogram of azo coloured food in a single day. So, is now understand the at least the magnitude, but it is not that if you consume azo dye you will die. It is this slow release of these and over a period of time these coloured food which carry the azo dye or metabolize in the body.

And it is the metabolism which creates the toxic substance. So, if you consume the large quantity as large as 100 grams 100 kilo grams. Only then there is a possibility that

somebody may die, but because they have a slow effect, because they start attracting attacking the cytoplasm and the nucleus of every cell in the abdomen. And therefore, if the you know, toxicity is not visible immediately or the effect of the toxicity is not available or seen or observed immediately, because the LD50 values are fairly low.

It is only between 250-2,000 milligram per kilogram of the body weights so, you imagine that such low as is the LD value therefore, it it has to be noticed that on long run it will create a hey walk, which is not that it is as lethal as a poison that if you take this you will die. So, it has a slow impact on the health.

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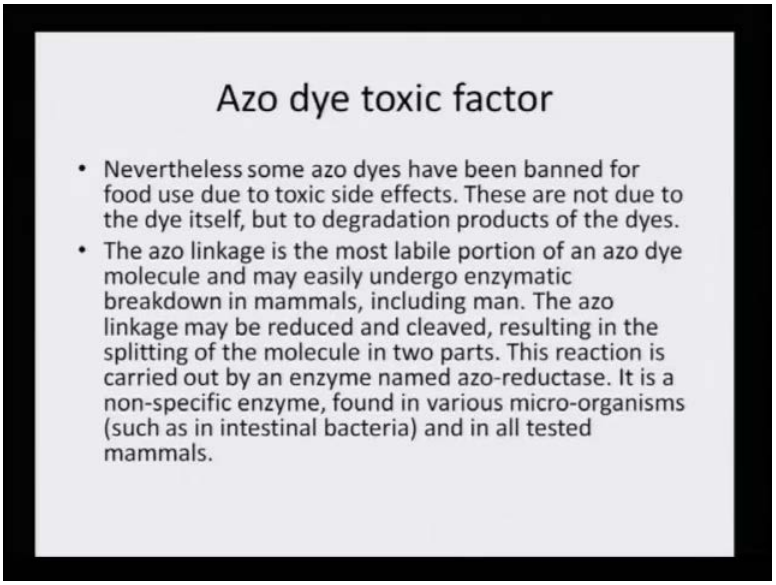

Azo dye toxic factor: Nevertheless some azo dyes have been banned for food use due to toxic side effects. These are not due to dye itself, but due to the degradation product of the dyes. Azo dye emphasize a while ago the dye itself for say is not toxic, is not poisonous, but it is the breakdown of the metabolite or the metabol or the you know, hydrolyze product or reductive product; that is what makes it a toxic substance. The azo linkage is the most liable portion of an azo dye molecule and may easily undergo enzymatic breakdown in mammals, including man.

The azo linkage may be reduced and cleaved, resulting in the splitting of the molecule into two parts. This reaction is carried out by an enzyme known as the azo-reductase. It is a non-specific enzyme, found in various micro-organisms which thrives in the

intestinal portion and they are called as intestinal bacteria and in all it is present in all the tested mammals.

So, that is where if colored food and the food that has been colored with azo dye. Although it is a food grade azo dye, when it is consumed by the mammals or man it actually goes through the intestinal column and in the intestinal column resides these microbiota, which contain a azo reductives, and that is what creates the awake or that creates the degraded product of the azo dye and subsequently the releases the primary amine.

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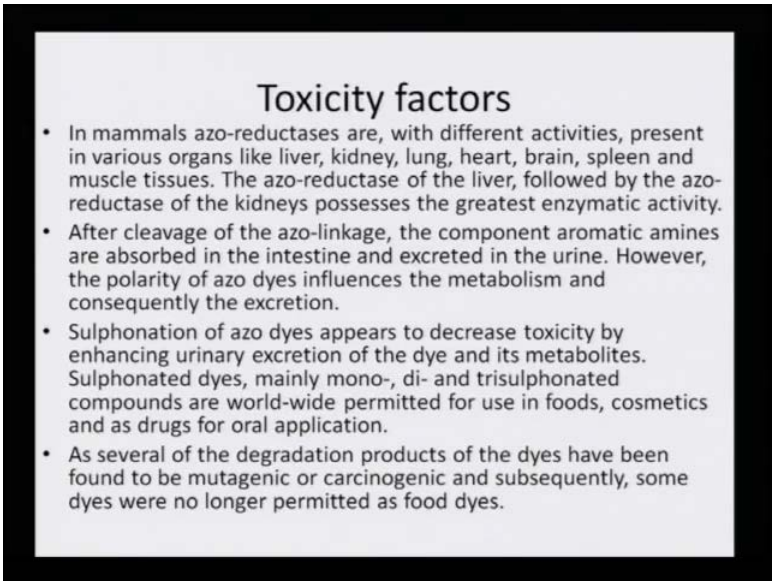

Toxicity factors: In mammals azo-reductases are, with different activities, present in various organs like liver, kidney, lung, heart, brain, spleen and muscle tissues. The azo-reductases of the liver, followed by the azo-reductase of the kidneys possesses the greatest enzymatic activity. After cleavage of the azo-linkage, the component aromatic amines are absorbed in the intestine and excreted in the urine. However, the polarity of the azo dyes influences the metabolism and subsequently the excretion.

Sulphonation of azo dyes appears to decrease toxicity by enhancing urinary excretion of the dye, and it is metabolize. We also took a notice that you saw that in Acid Orange 7. There was no sulphonic acid group, whereas in food yellow there was a sulphonic acid group. So, as and when sulphonation is added to the aromatic rings of the either side of

the azo-linkages, that is where it reduces the toxicity and that has been observed very carefully.

And now how it has been observed that it also you know a lot dyes is run out in the urine and therefore, it is not assimilated in the body sulphonated dyes, mainly mono-, di-trisulphonated compounds are world-wide permitted for use in foods, cosmetic as well as drugs for oral application. And therefore, it has been found that such dyes, which have more obvious azo three groups, can be used for safely for food and cosmetic and drugs.

As several of the degradation products of these dyes have been found to be mutagenic and carcinogenic and subsequently, some dyes were no longer permitted as food dyes. So, it was found that over period of time all the dyes food dye varieties that were available in the market. They were kind of properly investigated and those which were found to be more carcinogenic or mutagenic on animal's studies were then banned from the food dye category.

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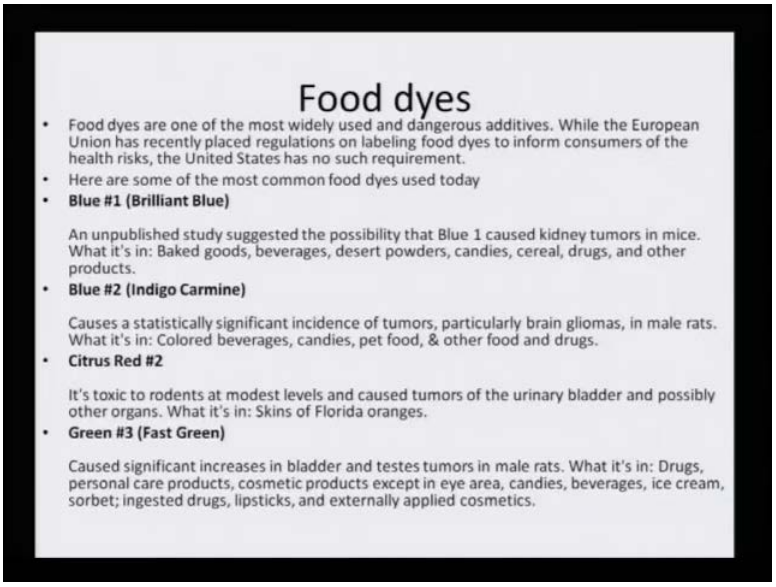

Food dyes is one of the most widely used dangerous additives; while the European Union has recently placed regulations on labeling food dyes to inform consumers of the health risk, the united state has not has no such requirement, but nevertheless they are important and therefore, we should take a very serious look at it. Blue Brilliant Blue indigo carmine citrus red green fast green that is they have been used extensively in baked food beverages desert can these and therefore, it is important that these dyes which

have been labeled as dangerous dyes by European union need to be taken into account very seriously.

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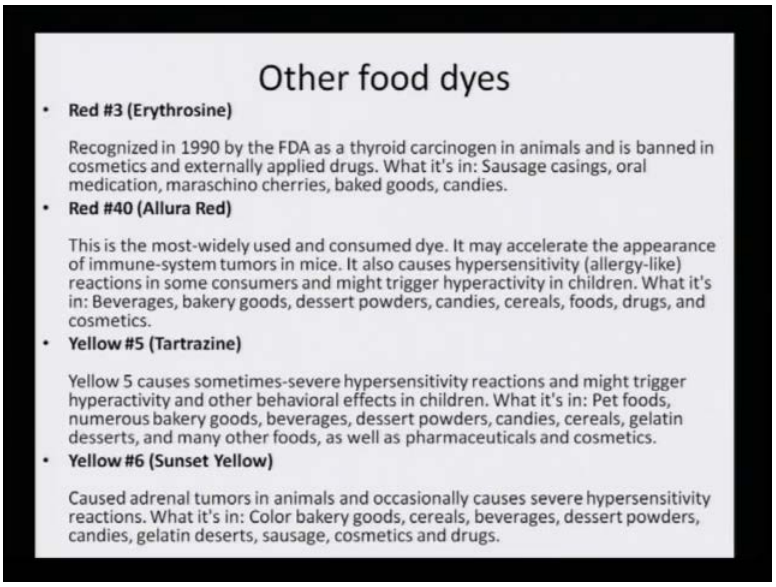

Other food dyes are Erythrosine, Allura Red, Tartrazine, Sunset Yellow. These also have been used it was recognized in 1990 by FDA that is the food development authority as a thyroid carcinogen in animals and is banned in cosmetics and externally applied drugs. So, erythrosine is no longer used because it was identified, then allure red is most widely used and consumed dye.

And therefore, it was found that you know it actually attacks the immune system of the mikes studies. Were carried out and it creates hypersensitivity allergy. And therefore, it was also found to be dangerous similarly tartrazine, and sunset yellow they have also been found to be toxic at a particular you know threshold level therefore, because the studies we carried out only when these products were brought into the market.

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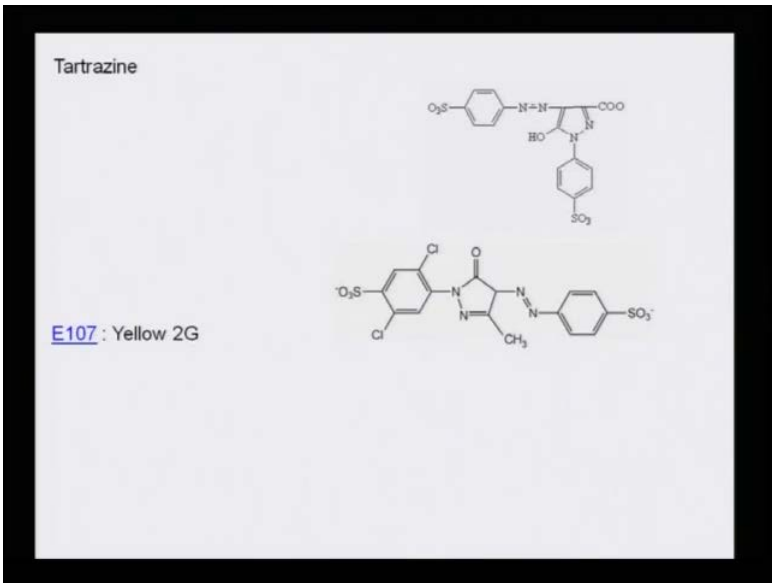

If you try to look at the structure of Tartrazine although it has sulphonic acid groups, they even it is somewhat dangerous, because when this bond cleaves, this particular bond cleaves. That is the time when it actually is you know releasing the primary amine. And all the time the point of breaking is the azo-linkage.

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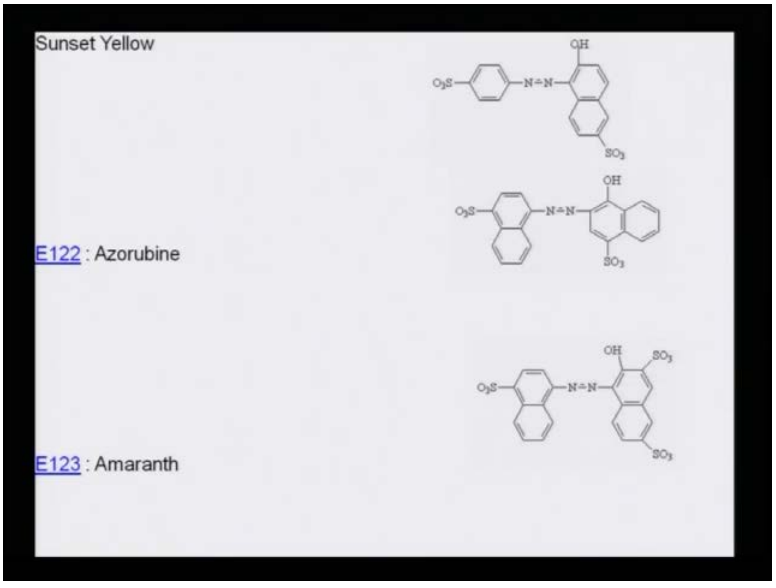

Similarly, the these are the structures of sunset yellow Azorubine and Amaranth. I mentioned all these because these are some of the very popular dyes that are used in the food in the stream, and they belong to the azo dye category and therefore, one should

know that what is the kind of structure and what it will liberate, after it it a hydrolizes the N double bond N. And therefore, it is important to understand the structural details of these dyes, so that one can truly understand what is you know dangerous for us.

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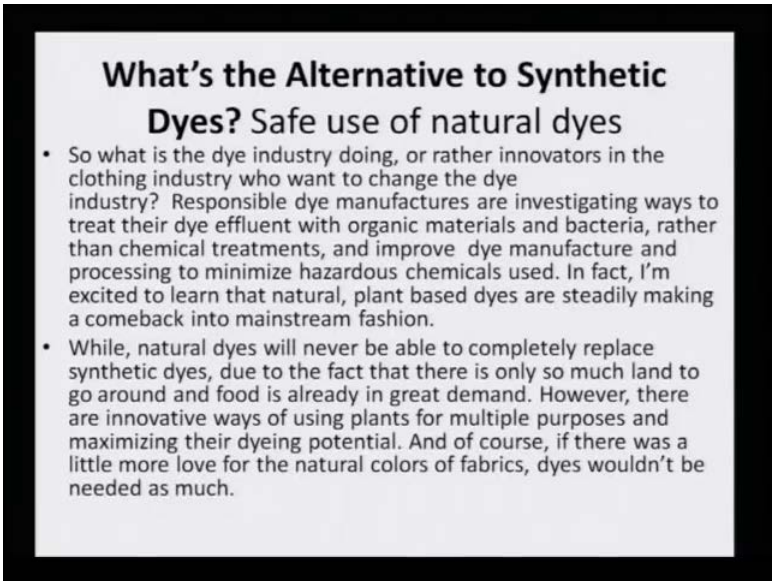

Now, what is the alternative to synthetic dyes? Safe use of natural dye: So what is the dye industry doing, or rather innovators in the clothing industry who want to change the dye industry? Responsible dye manufactures are investigating ways to treat their dye effluent with organic materials in bacteria, rather than chemical treatments, and improve dye manufacture and processing to minimize hazardous chemicals used.

In fact, I am excited to learn that natural, plant based dyes are steadily making a comeback into the mainstream fashion, because I personally work in that area. While, natural dyes will never be able to completely replace synthetic dyes, due to the fact that there is only so much land to go around and food is already in great demand. However, there are innovative ways of using plants for multiple purposes and maximizing the dyeing potential.

And of course, if there was little more love for the natural colors of fabrics, dyes would not be needed at all. So, you see that although there is a huge production of synthetic dyes, but people are now turning towards natural dyes and all precise reason is that they natural dyes are bio derivative, and they have at least a large number of them have not been found to be invasive of the DNA, RNA material.

And therefore, they are safe in turns of their toxicity factor, but the kind of demand that the food industry or the clothing industry has in today's present population. Globally it can never match the demand, but people are progressing towards the use of natural dyes, because it is safer and because it is safer it can be safely used not only for clothing, but also in in any food industry also. So, a lot of industrial are now looking for natural alternatives for food dyeing.