INDIAN INSTITUTE OF TECHNOLOGY ROORKEE

NPTEL

NPTEL ONLINE CERTIFICATION COURSE

Biomedical Nanotechnology

Lec – 20
Nanotoxicology

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Hello everyone I welcome you all to the 20th lecture of this course this 20th lecture is on nanotoxicology so this is the last lecture of this course okay so till now we have learnt various biomedical applications of this nano materials in this course and today we are going to see the other side of nano materials that is toxicity of nano materials.

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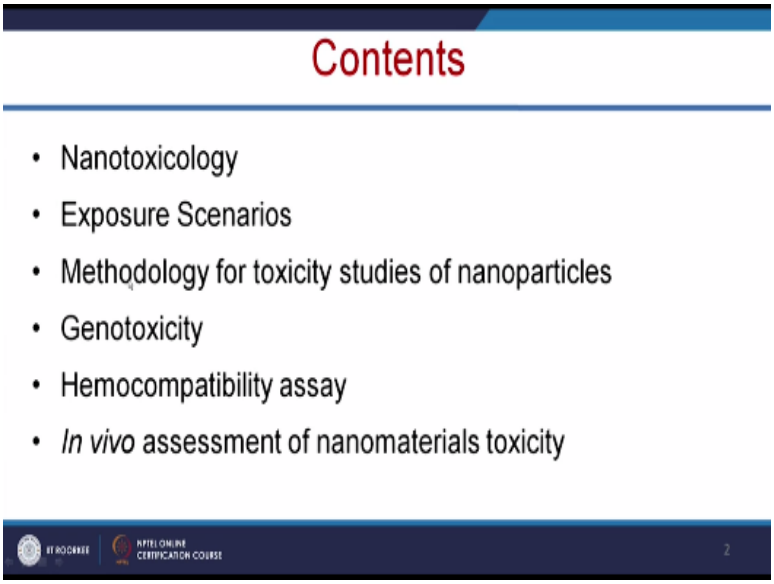

So in this lecture we are going to learn what is nano toxicology hat are the various exposure scenarios and what are the various mythology available to study the toxicity nano particles and we are also going to learn wheat is genotoxicity and how to study the hemocompatibility of your nanomaterials okay and also how to study the in vivo assessment of your nano materials toxicity.

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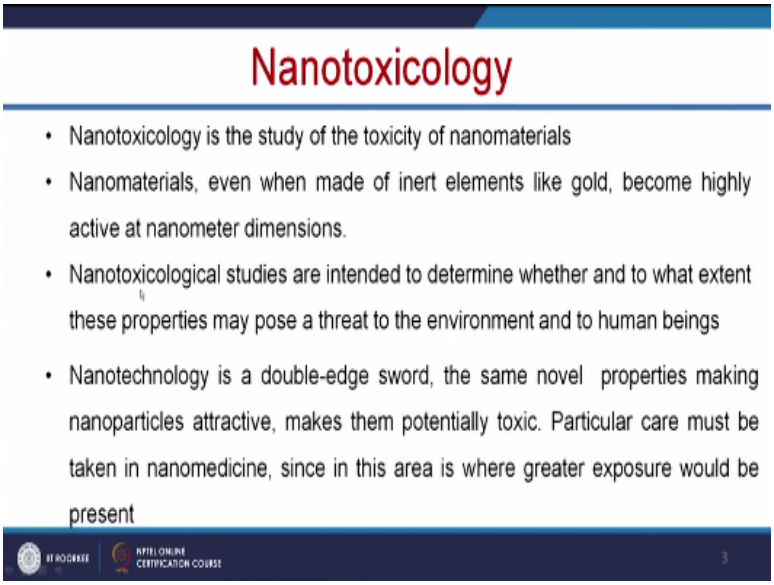

So let us see what is nanotoxicology so this nanotoxicology is a study of toxicity of nano materials okay and nanomaterials even when made of inert elements like gold become highly active at nanometer dimension as we know that gold is a inert material we will free use it for making ornaments or jewels and when it goes to nano scale it will show you a different kind of properties.

So here this nano toxicology studies are intended to determine whether and what extent these properties may pose a threat to the environment and to the human beings okay so here this nano technology is said double edge sword the same novel properties making nano particles attractive makes then poetically toxic to okay so the particular care must be taken in nano medicines since in this area where greater exposure would be present.

So most of the feel have his own advantage as well as disadvantages similarly this nano technology also like a double edge sword so it has it is own advantages as well as disadvantages so we have to select the nano materials wisely for the various biomedical applications.

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So why we have to study the nano toxicology you can see here these are the various consumer products where they are using this nano scale materials for examples so this loyal companies using this nano particles based cosmetics and motorolize using the CNT based nano image displace okay and also we have a nano care fabrics shirts and carbon nano fiber racks okay and also in India also we are having several washing machine okay which is coated with the silver nano particle and also in the now a days in the TV advertisement we are seeing that some of the soap.

There we are using silver nano particles okay so what happens this silver nano particles and everything goes into the water bodies okay so when it goes into the water body how it is going to affect the living or concern in the water bodies and again it will contaminate the ground water and how it is going to cause toxicity so those things we do not know okay so in this lecture we try to learn what are the nano toxicity methodology available to study the toxicity of nano materials.

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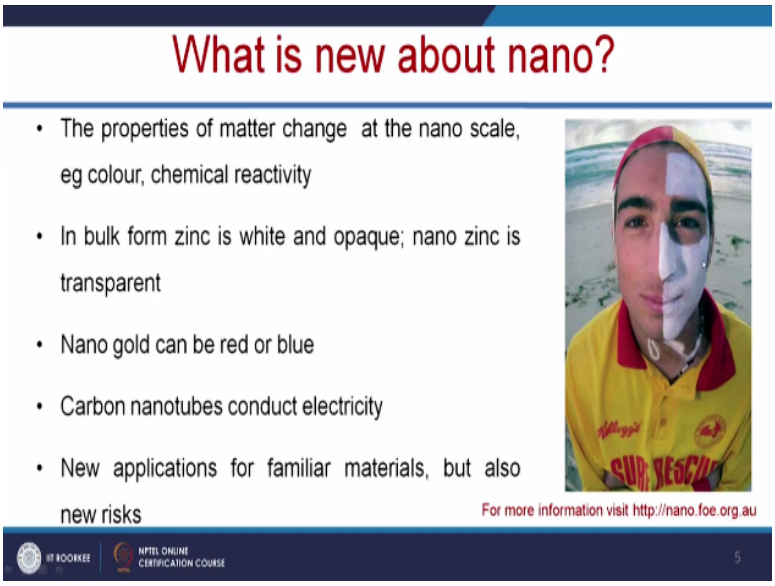

So this you might of seen in cricket okay so in the Australian players mostly they uses to apply this kind of sun screen okay so this is a bulk form zinc which is white and opaque okay and this side he applied the same sun screen but it is in the nano zinc so the nano zinc is transparent and this bulk zinc that is your sun screen it is a white and opaque okay so when the material goes to nano scale the properties of the material get changed okay.

And the nano gold controller be red and blue and copper nano tubes can connect electricity so applications for familiar materials okay and again but also new risks so the inert material which we are using day to day to okay when it goes nano scale it is going have some different kind of properties and different kind of properties it may have advantage at the same time it may have new risk also.

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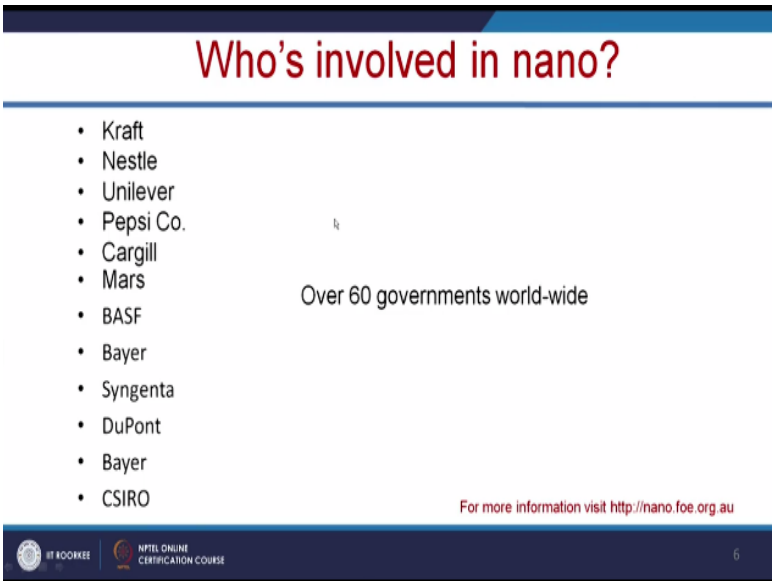

So these are the various companies are involved in this nano for example nestle, Pepsi and some of the other companies are involved in this oaky so most of the companies they product the product in the name of trade secrete or in the name of pattern produced loss okay so we are not aware about what are the nano materials in the food products.

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So these are the other products nano products nano based products okay for example sunscreen cosmetic and even the baby diaper and baby bottle feeding bottle so everything they started using this nano materials and nano coatings so but we do not know what will be the long term effects okay.

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So this in a early 2008 approximately 104 foods and food additives contain nano particles okay which is on sale internationally and some of the earliest suggest that almost 500 nanofoods are available in the world wide okay so almost 500 nanofoods are already in the market so we are not aware about what are those and there is no labeling for example if you are using the GM of food so there should be GM or label similarly if you are using nano food or nano coating there should be some label to indicate this these are coated with nano particles are this food raped with nano package okay.

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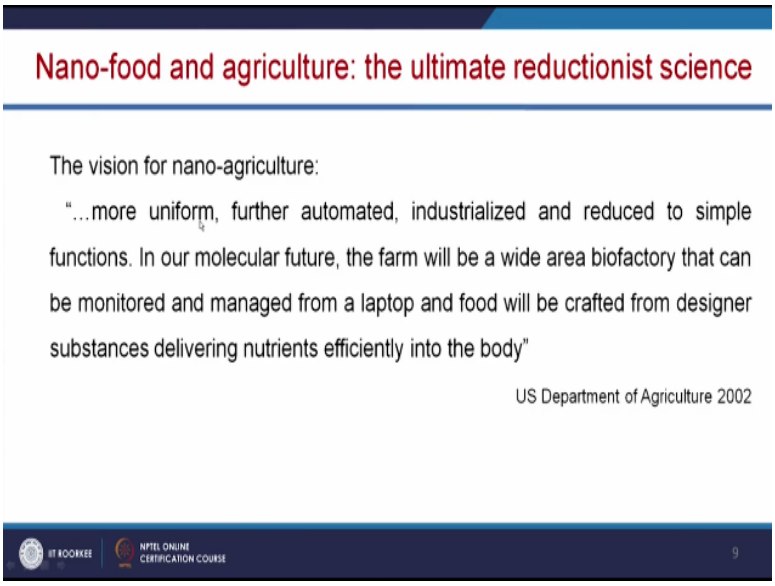

So there is no such labeling then again this nano can play a major role in achrticulte also so It can make the agriculture uniform and it can make further automated and industrialized okay and so due to which what will happen this the farms may lose the farming knowledge but at the same time but it can have the designers substance which can deliver the nutrients efficiently at the body okay.

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And these are some of the nano foods and sale include cooling oils teas and also we can have the antibacterial kitchen where okay and also food processing and food packaging material so this nano based food packing materials will interact with the food, and it will prevent the microbial growth but this nano materials also interacting with their foods substance so when it interact with the food how much amount of nano particles realizing into a food material and when you take those food what will happen to you. So these things are not understood thoroughly.

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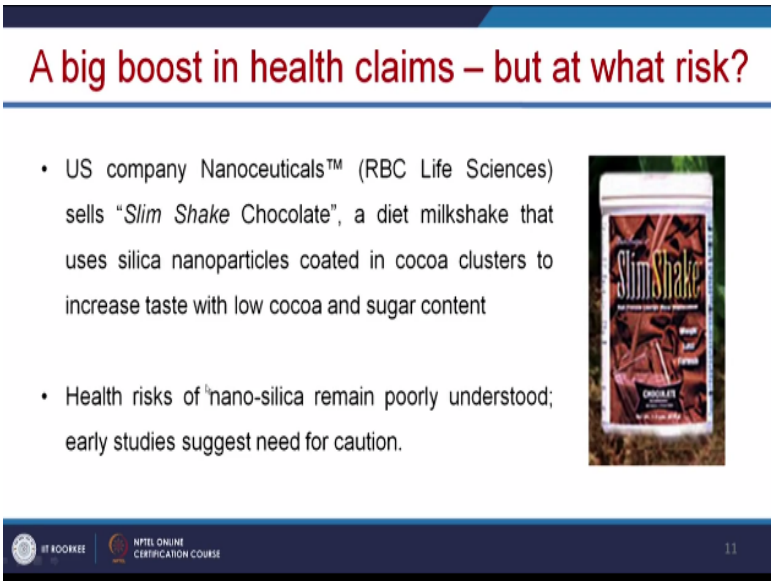

So recently a US based company nanoceuticals okay so they made us slim shake chocolate okay so this a diet milk shake that uses silica nano particles coated in coco clusters to increase the taste with low coco and sugar content okay but this health risk of nano silica remain fully understood and early studies suggest need for causation. So this slim shake is made up of silica nano particles so what is silica nano particles are basically a sand particles okay.

So in your childhood if eat a sand your mom will be hit you but the same sand particles your drinking in the form silica slim shake, so but we do not know the what will be the long term of effect of this silica based chocolate drinks okay.

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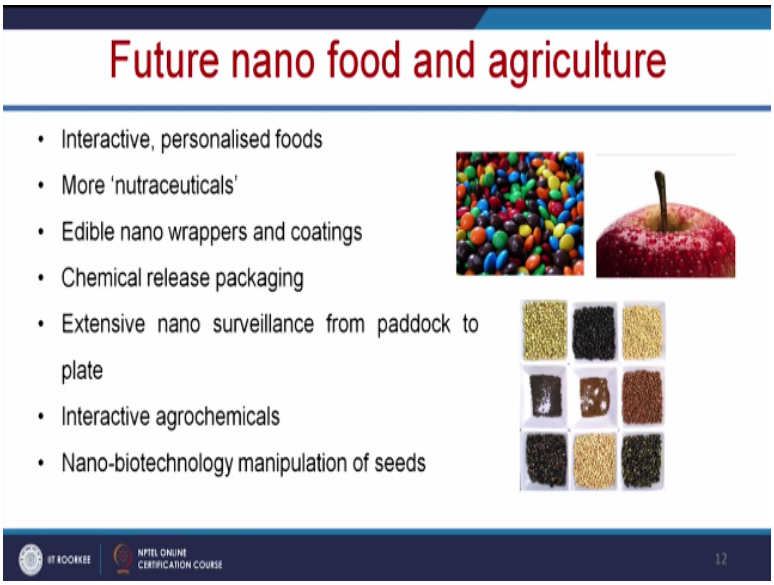

And these are the some of the other further nano food and agriculture like you can have the edible nano rappers and coatings okay and also we can use the nano biotechnology to manipulation of seeds so we can sue these technologies to manipulate the seeds to increase the growth okay and also we can increase the productivity but again what will be the drawback long term effects still unknown.

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And again this nano technology could enable junk food to be fat free sugar free and also it can reduce the carbohydrate and it can increase the vitamin and protein and it can be fiber enhanced

okay so then it can be marketed as a healthy alternative so this junk food can been enriched with protein and fiber and it can be solved in the name of like healthy alternative but the problem is our relationship with the real food will be rolled.

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And again this nano could displace the workers and erode the forming knowledge because everything will be automated so automated nano surveillance and management system okay so that could reduce the need for form workers and this nano could commodity farming knowledge and the nano will play a major role and it will take up the technology like a propriety technologies okay.

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So only the some of the multinational companies they can control the food and agriculture.

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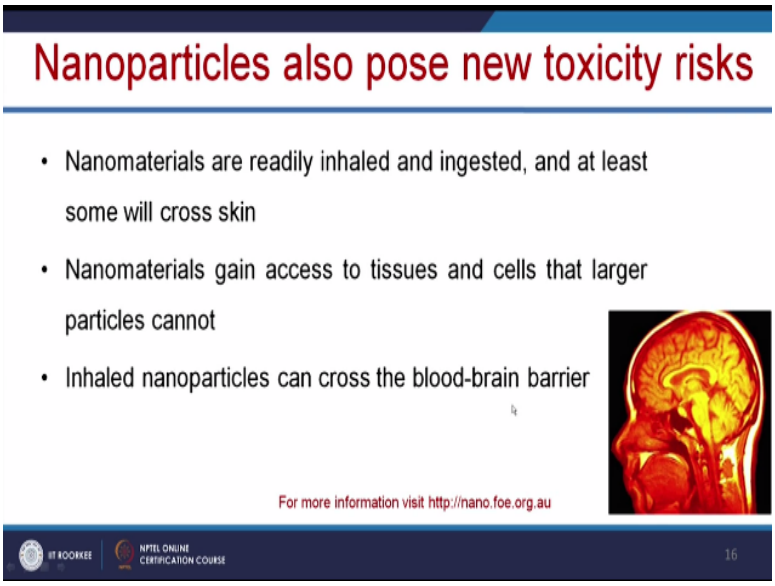

So let us see some of the examples of how this nano particles pose some new toxicity risks so here the nano materials are readily inhale and ingested and at least some will cross the skin and nano materials gain access to tissues and cells that in larger particles okay and inhale nano particles can also cross the blood brain barrier okay so that is a important property of this nano particles. So most of the other drugs cannot enter the blood brain barrier but this nano particles it can cross the blood brain barrier.

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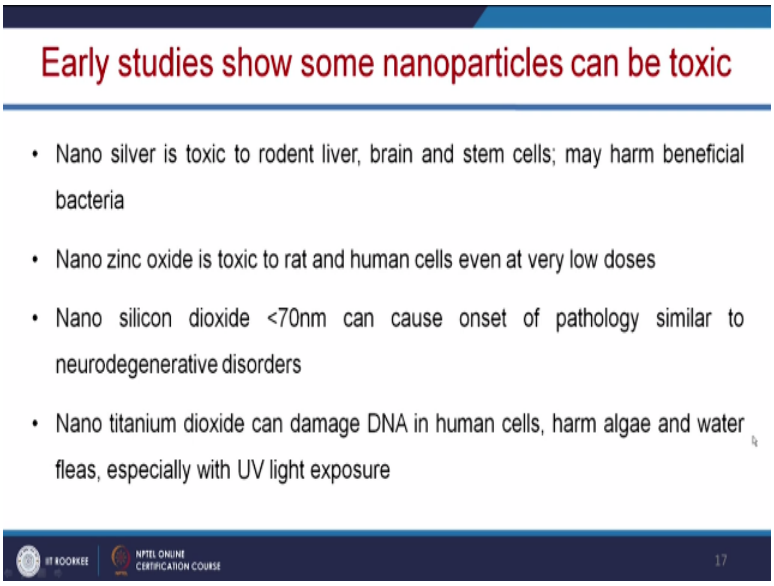

So let us see some more example so this nano sliver is toxic to rodent liver brain and stem cells and may harm beneficial bacteria also and this nano zinc oxide is toxic to rat and human cells even at low doses and nano Silicon dioxide <70nm can cause onset pathology similar to your neurodegenerative disorders and this nano titanium dioxide can damage DNA in human cells harm algae and water fleas especially with help of UV light exposure.

So this titanium dioxide is everywhere in your familiar tooth paste and your talcum powder and ever where this titanium dioxide is a and this nano titanium dioxide when it go into the water bodies and it is a very good photo catalyzed presence of sunlight so it will act like a very good photo so it can harm the agile and other living organs in the water bodies.

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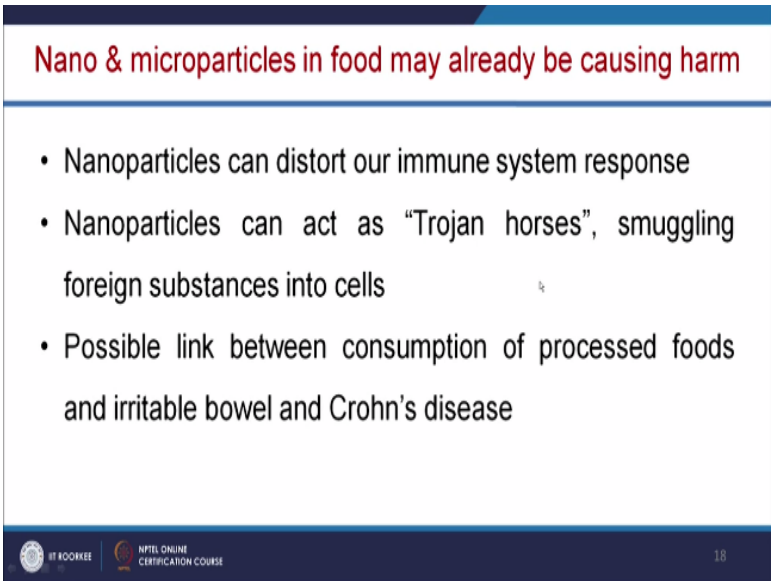

So here this nano particles can distort our immune system response and here nano particles can also cat like a Trojan horses and the Trojan horses means like this nano particle will be in your body for more time so it can hit as more circulation time and suddenly it may cause some new toxic effects, so that affect is called as Trojan horse effects and this possible link between the consumption of processed foods and irritable bowl and crown disease. So there is nano materials can also induce this kind of diseases, okay.

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So the UK royal society recommended in 2004 they made these are some of the rules for the nano safety so that is the full safety assessment of all products that contain nano prior to market release and all nano ingredients to be labeled and environmental release of nano materials to be avoided as far as possible and factories and research laboratories to treat nano materials as if they were hazardous. So let us see these properties of nano scale materials.

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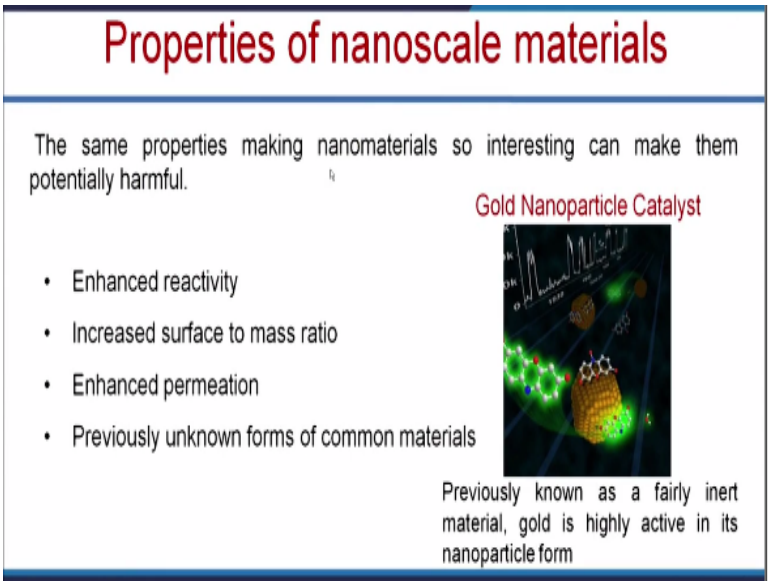

The same properties making nano material so interesting can make them potentially harmful as I told you earlier so this enhanced reactivity and increase surface to mass ratio and enhanced permeation okay and also previously unknown forms of common materials so these are the properties which has a wide application biomedical field and also the same properties will also have some toxic effects, okay.

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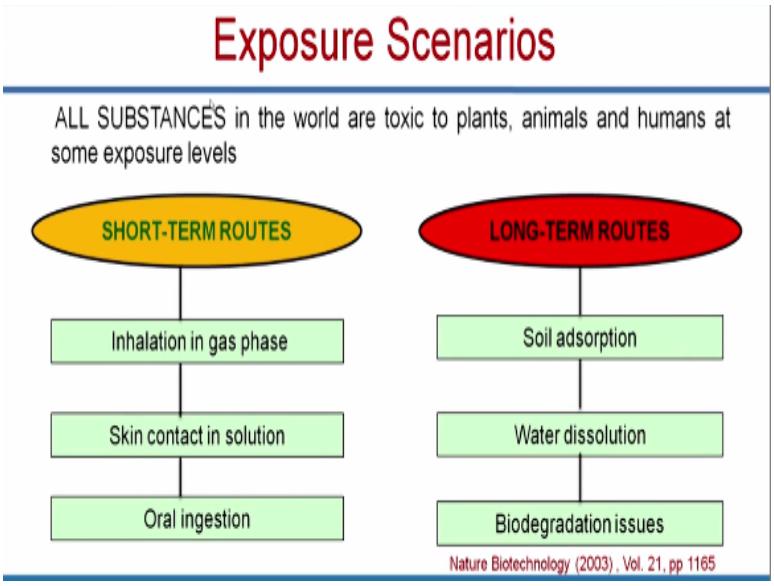

So let us see what are the various exposure scenarios, so all substance in the world or toxic to plants animals and humans at some exposure levels, so there are two roots short term routes and long term route so in the short term routes it is mainly due to inhalation gas phase and skin contact in solution and also oral ingestion and in the long term routes mainly due to the soil absorption and water reservation and also biodegradation issues. So the risk evolution for exposure to nano technology product is.

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Hindered by the law protected secrecy of product formulation and also there is a lack of specific regulations on nano technology and non-mandatory reports on toxicity of products okay and we are still using the old criteria and methods becoming obsolete, and here these researches from national institute for occupational safety and health so determines the concentration of nano particles in the air while unloading a reactor for producing the metal oxide nano particles.

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And these are the various international initiatives okay, so recently some agencies have taken some actions to establish regulations to nano technology so international standard organization and US national nano technology initiative and British standard institute okay and also the environmental protection agency okay. So all these agencies have published reports and guidelines related to the handling of nano materials. And research approaches to nano toxicology, so but all them are voluntary to follow there is no mandatory rules and regulation.

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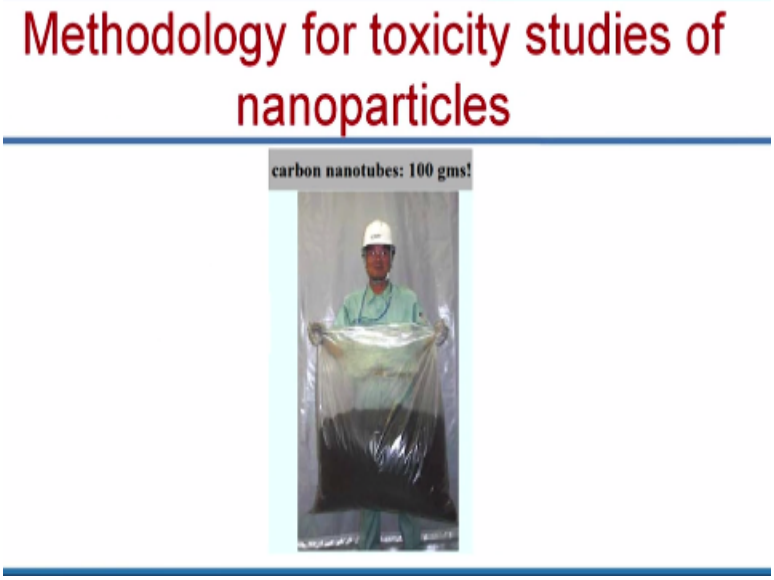

So let us see the methodologies for studying the toxicity of nano particles, so if we see this is your carbon nano tubes 100 grams of carbon nano tubes but it look like a 100 kg, okay.

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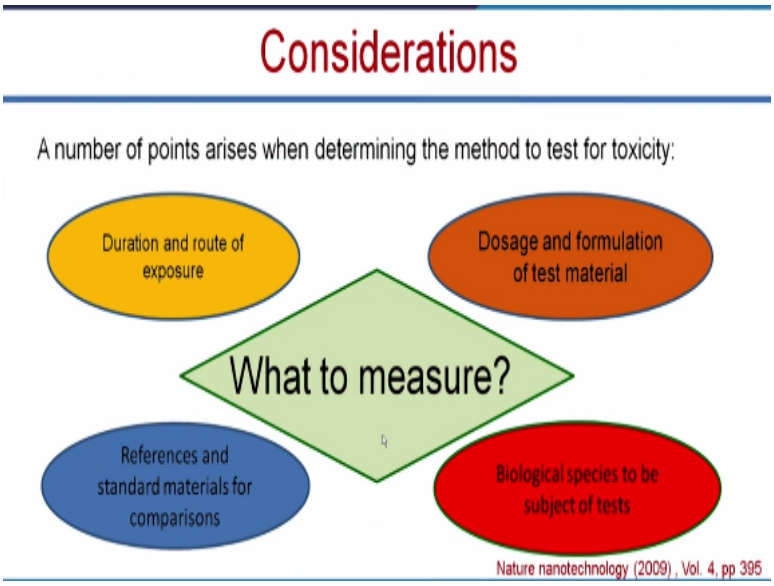

So there are several points we have to understand to study the toxicity of a nano materials so what to measure like how much duration and what is the root of exposure and what is the dosage and formulation of test material okay and also what kind of biological species you are going to use to study the toxicity of nano materials and whether do you have standard references and standard materials for comparisons, so these are the questions to be consider before we start the nano toxicity studies.

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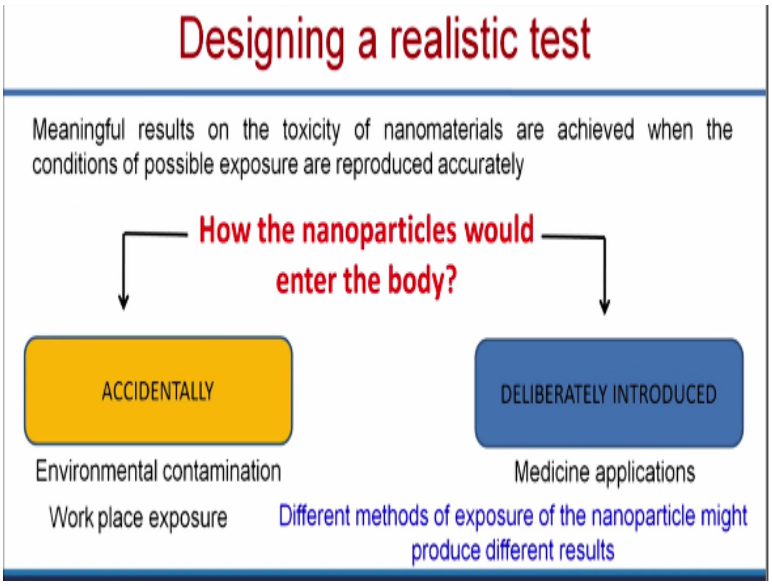

And also the main important thing is how to design a realistic test, okay. So the meaningful results on the toxicity of nano materials are achieved when the conditions of possible exposure are reproduced accurately. So let us see how the nano particles would enter the body, the first one is accidently so it may be due to environmental contamination and work place exposure, the next one is deliberately introduced. That is your nano material and applications so different methods of exposure of the nano particle might produce different results, so let us see this with some examples.

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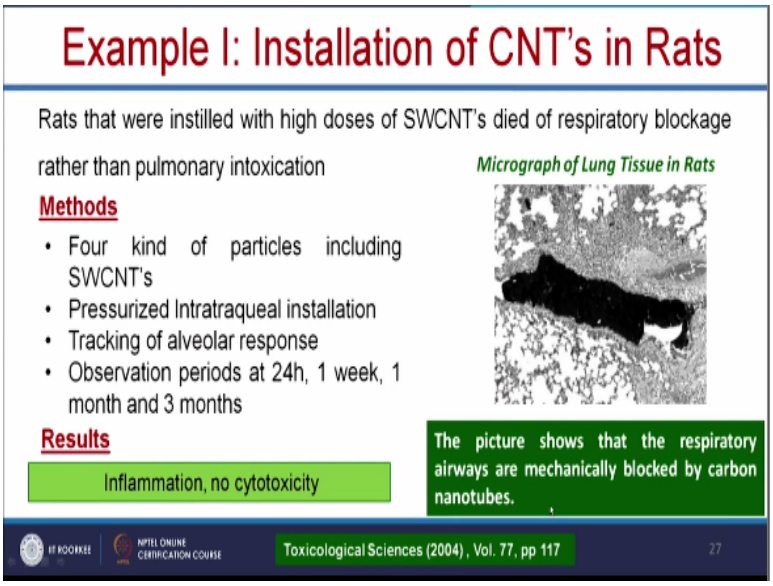

So the example 1 is insulation of CNT in the rats so here the rats that where in still with high doses of single wall carbon nano tubes it is died due to respiratory blockage rather than pulmonary intoxication. So here they use four kinds of particle including single wall carbon nano tubes by pressurized intratraqueal installation SWCNT's was given to this rats , okay. And it was the absorbed at 24 hours one week, one months and 3 months.

And here the results are like there is inflammation but no cytotoxicity and this picture is showing that the respiratory airways are mechanically blocked by carbon nano tubes sue to which the rat was died, okay.

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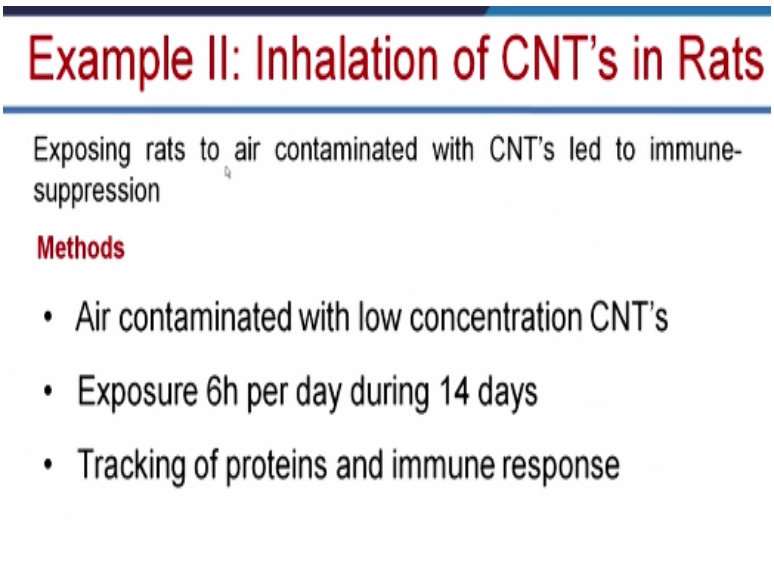

The next example is inhalation of carbon nano tubes in rats, so exposing the rats to air contaminated with this carbon nano tubes led to the immune suppression, okay. So in the previous example we have seen that when you eject this carbon nano tubes through intra tech inhalation what happens is, that rat is died, okay. So in this case so we are passing this air contaminated with low concentration of carbon nano tubes and exposure of 6 hour per day during 14 days, okay and the tracking of proteins and immune response in the rats.

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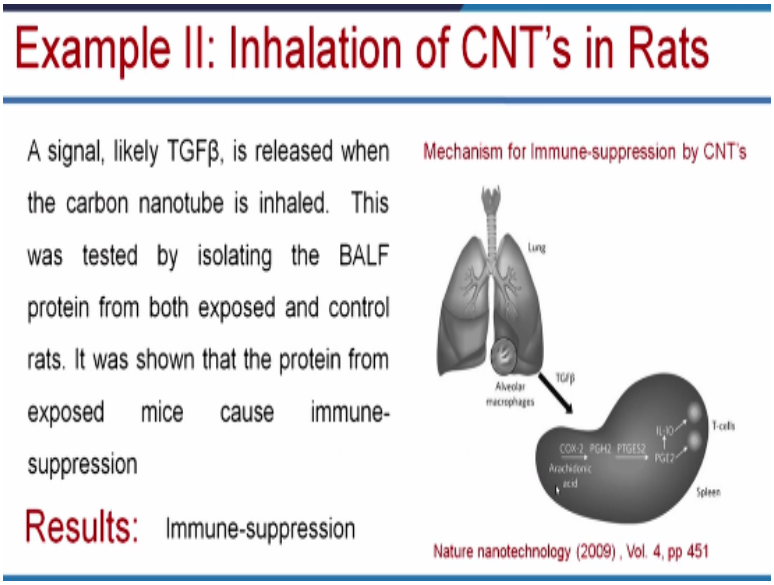

So here the researches absorbed that signal like TGFß is released when the carbon nano tube is inhaled, so this was tested by isolating the Bronchoalveolar Lavage fluid protein from both exposure and control rats and it was shown that the protein from exposed mice cause immune suppression, okay. So here there is no death of rat and here only the immune suppression, so from this example we can understand.

So based on the method by you are giving the carbon nano tubes dosage to this animals the results also varying, okay. So when you install this carbon nano tubes the rats are died so when the carbon nano tubes are inhaled by the rats only the immune suppression happened.

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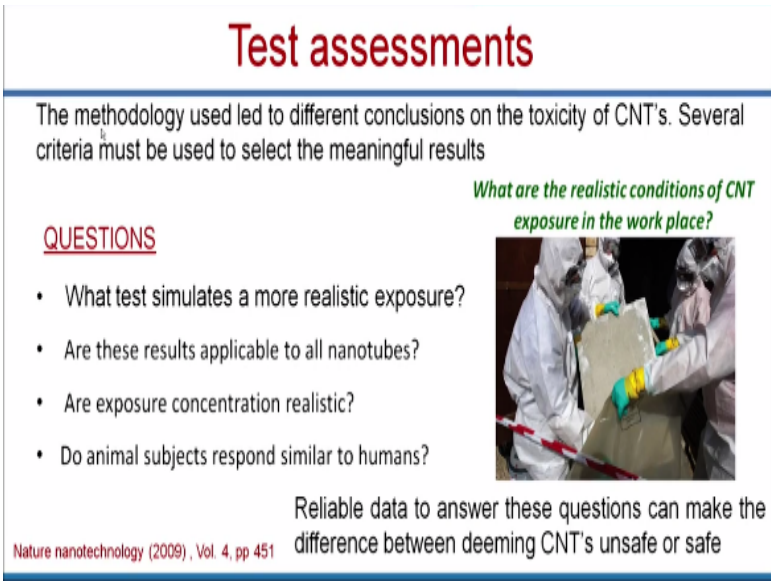

So what are the various test assessments the methodology used led to different conclusions on the toxicity of carbon nano tubes, okay. So several criteria must be used to select the meaningful results like a what test simulate the more realistic exposure and also these results applicable to all nano tubes or exposure concentration realistic and do animal subject response similar to humans, okay. So this reliable data to answer these questions can make different between deeming this carbon nano tubes unsafe or safe.

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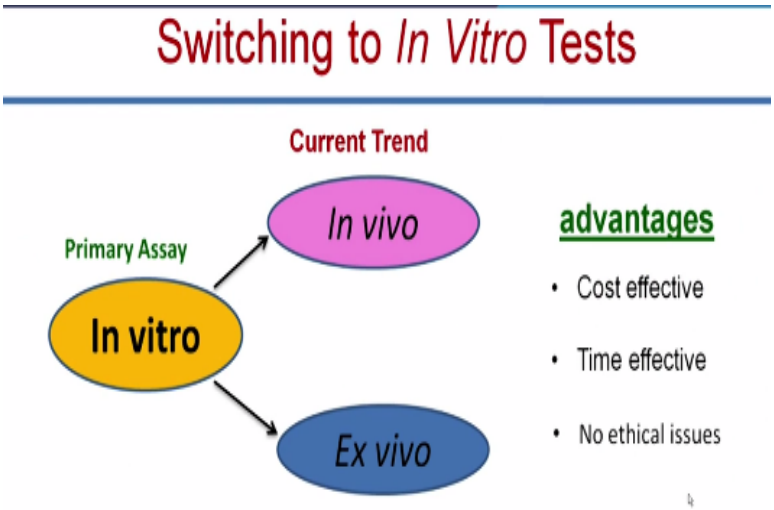

So what are the various methods available like we can use this In vitro methods so In vitro methods means you can do it in the laboratory condition using the bacteria or cell lines to understand the toxicity and environments inside their living system like living animal models like mouse or the zebra fish or some other animal models and Ex vivo is a combination of In vitro and Ex vivo so we can study the toxicity of nano materials using the bacteria and circulatory system In vitro, okay.

And we can also study the toxicity of nano materials using the animal model like mouse ort rat or zebra fish, okay. So that is your In vivo so in the twins are in the living system, so this Ex vivo is a combination of In vitro and In vivo, okay. So we can take out the particular organ and we can grow in the lab and we can study the toxicity of nano materials, so here the advantages are like this cost effective, time effective and no ethical issues when you use the In vitro condition.

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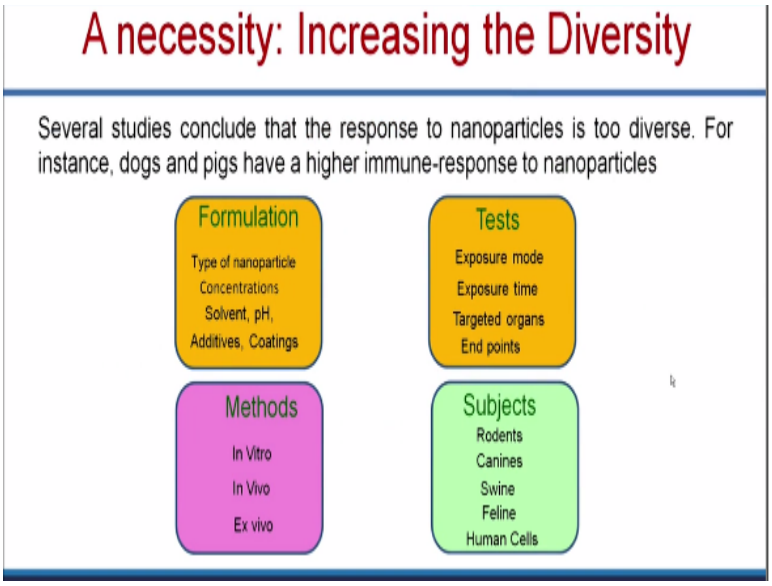

And several studies conclude that response nano particles is too diverse for instance dogs and pigs have a higher immune response to nano particles okay. And again this nano particles talks the will differ depends on the formulation, so depends on what kind of nano particle you are using what was the concentration and also what is the PH and what are the coatings of the nano materials and also what are the exposure mode exposure time and which size the targeted organ, okay.

And what kind of animal model you are using are you going to use the rat or human cell line or fish model which model you are going to use and what are the methods you are going to incorporate to understand toxicity for example In vitro or In vivo or Ex vivo methods. So depends on this four parameters okay formulation test and subjection methods so the nano particles toxicity will also vary.

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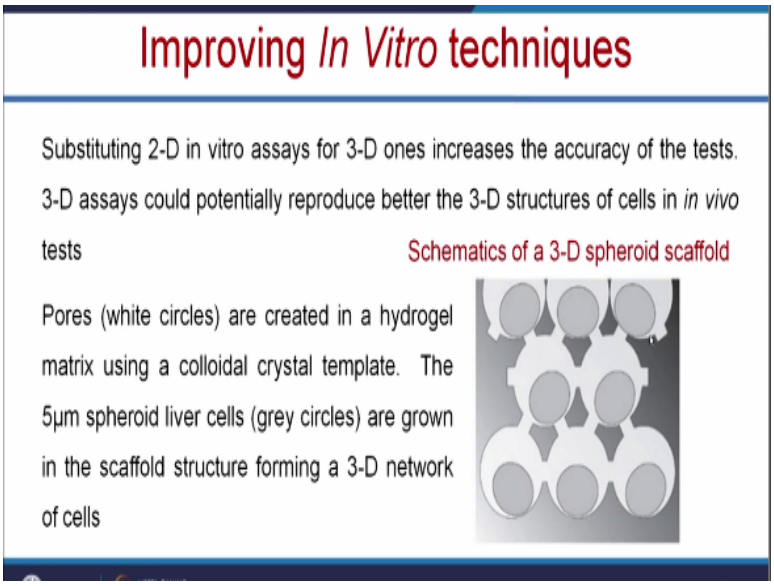

And we can also improve the In vitro techniques by substituting that 2D with 3 dimensional scare fold okay so here this poles are the white circles, okay. So these are created in a hydrogel matrix and this is a liver cells are like a grey color circles, okay.

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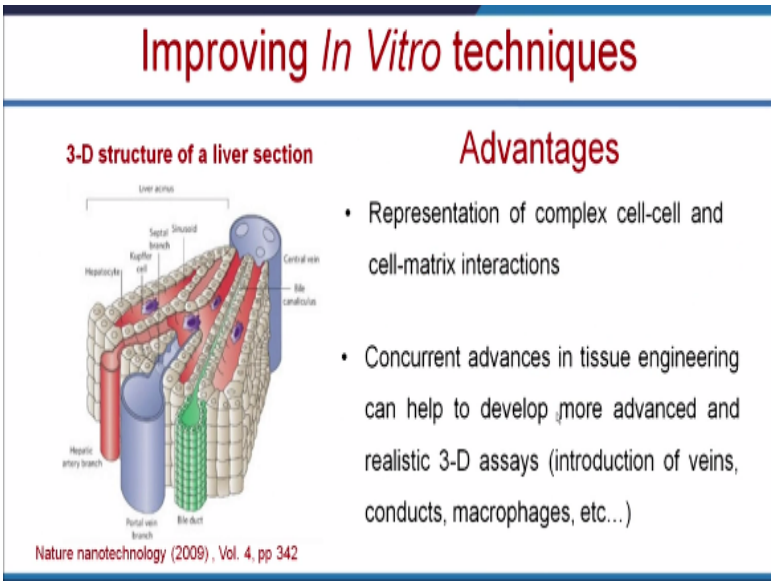

So the liver can be useful to study the toxicity of nano materials and in a 3D dimensional scare fall so here the advantages are like it will represent the complex cell-cell and cell-matrix interaction an also so with a help of tissue engineering we can develop this artificial liver and we can easily understand the toxicity of nano materials.

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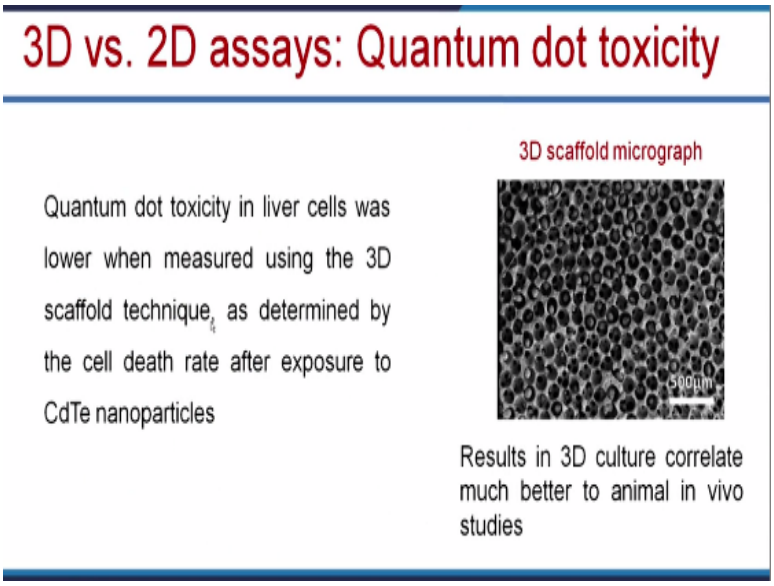

So in this the researchers have study the toxicity of quantum dot using this 3 Dimensional as well as 2 dimensional scaffold.

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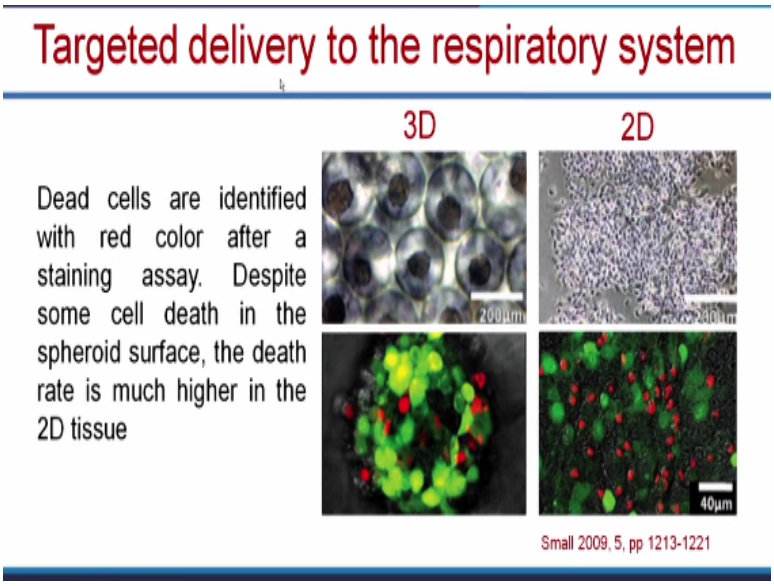

So and they concluded that the 3 dimensional scaffold it is exactly mimicking the In vivo condition, okay so you can see here this red colors are dead cells so as I explained in your previous lecture so this red cells are dead cells in the 2D with the same concentration you are seeing more amount of dead cells and in the 3D culture you are seeing only few cell's are dead cells, okay. But the concentration nano particle is same, okay. So when you use this 3 dimensional scaffold and that will exactly mimic like you are In vivo condition.

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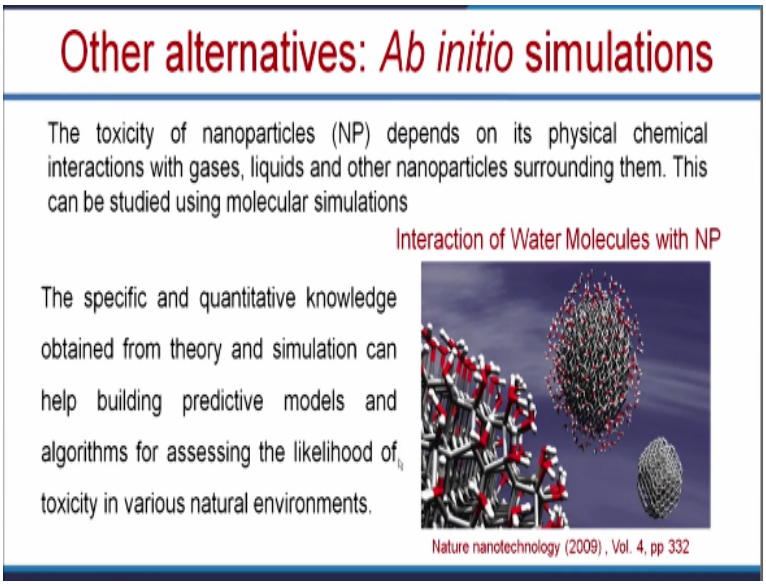

And other alternate is like we can also do these modeling in simulation the toxicity of nano particles depends on a physical and chemical interaction with the gases, liquids and other nano particles surrounding them, so this can be studied using these molecular simulation the toxicity of nanoparticles depends on its physical chemical interactions with gases liquids and other nanoparticles surrounding them.

So this can be studied using molecular simulations. So the specific and quantitive knowledge obtained from theory and simulation can help building the predictive models and algorithms for assessing the likelihood of toxicity in various natural environments so if you know that toxicity of nano materials we can do the molecular simulation and we can accelerate the nano toxicity field okay.

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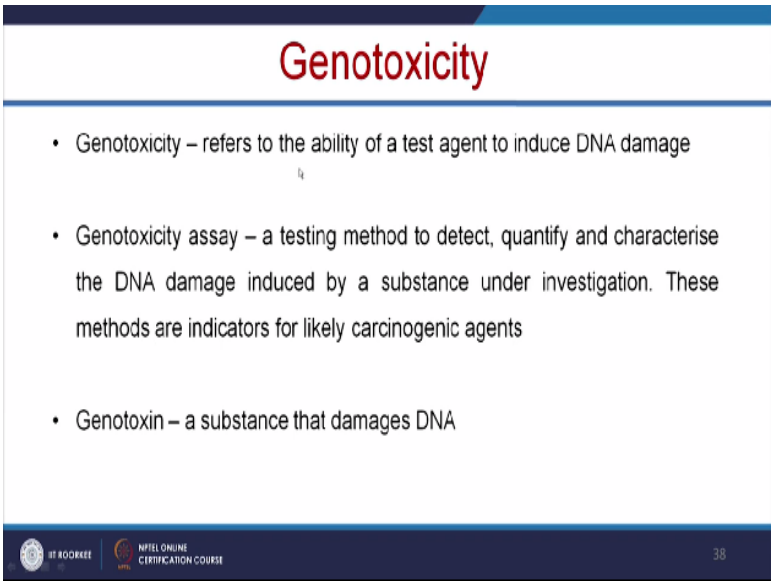

So let us see what is genotoxicity so genotoxicity refers to the ability of a test agent to induce DNA damage okay so the psycho toxicity means if it cause toxic to the cells okay and genotoxicity is if it damage your genetic material okay so here genotoxicity assay like a testing method which can detect quantify and characterize the DNA damage induced by a substance under investigation and these methods are indicators for likely carcinogenic agents and this genotoxin is a substance that damages DNA you know the toxin.

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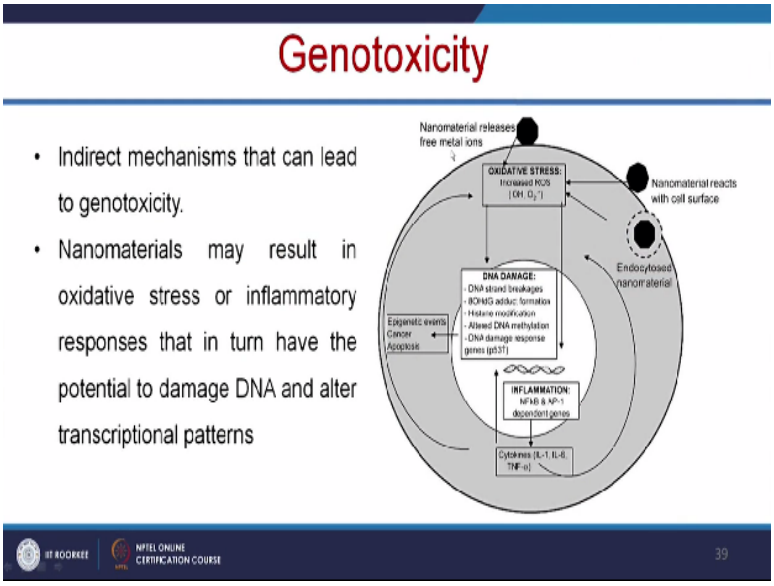

So this nano material release free metailions so that will induce the oxidative stress and this oxidative stress and this oxidative stress will damage your DNA and it will induce the upper toxins or it will induce the inflammation okay so we are to understand whether this nano material inducing any damage to DNA or not.

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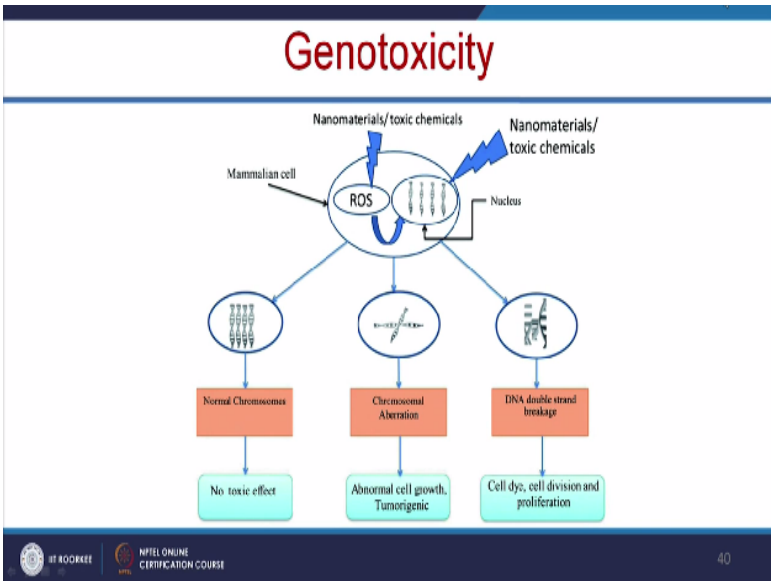

Secondly you have know expose the self to this nano materials what happens is like a there may be chances for chromosome abbrievation so if it is a normal chromosome there is no toxicate and if you are identifying the there is achromosome abbrievation that means it due to the it can leads to the abnormal self growth and it may be compound may be tumeriogeneic and we can understand the geno toxicity by studying the DNA double stand breakage and by using this various nucleous stained and cell division and proliferation.

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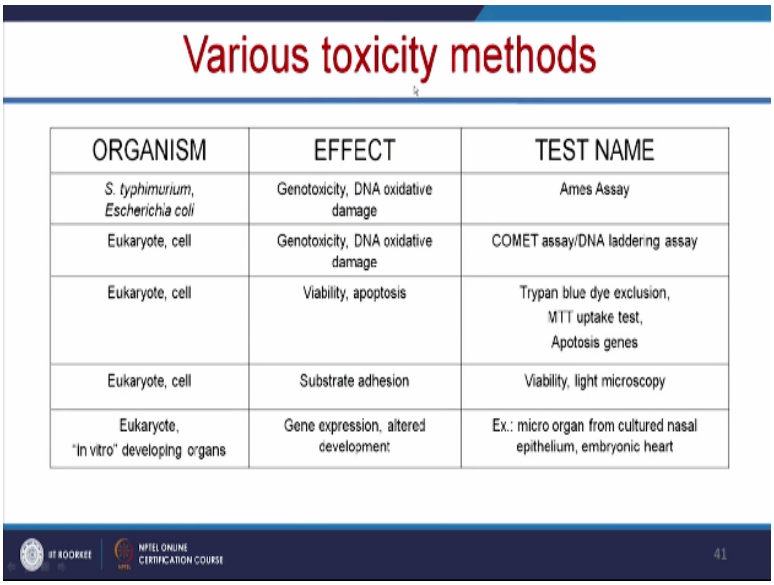

So this are the various toxic methods available okay so we can use this typimurium to understand whether this nano material this inducing any mutation in your genetic material okay so as I called as ames assay okay and also we can use the cell lines memaline cell lines to understand the whether it is inducing their DNA laddering and we can also understand the apoptosis andv everything so using this MTT assay and other apotosis genes okay.

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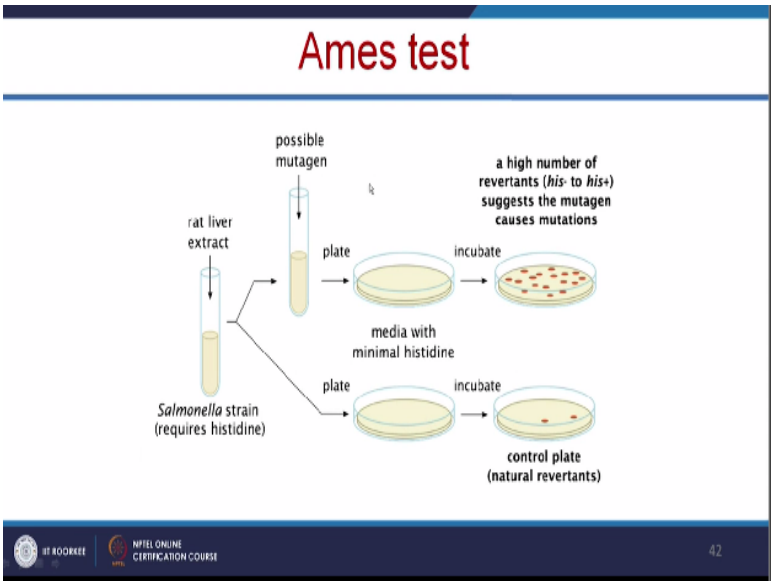

So these I already explained in the previous lecture so let us see what is the ames test okay os here we can use the bacteria called salmonella okay and which requires histidine for his growth and to that we can add a possible mutagen in this case we are using this nano particles so this bacteria will all be grown in their media with minimal histidine okay.

And in presence of this possible mutagen if it cause some mutation okay sob this bacteria will grow more on this plate so if bacteria growing more on this plate that means the nano particle may be induced some mutation in the genetic material so we can see here some compare to control plate where few particles are there okay.

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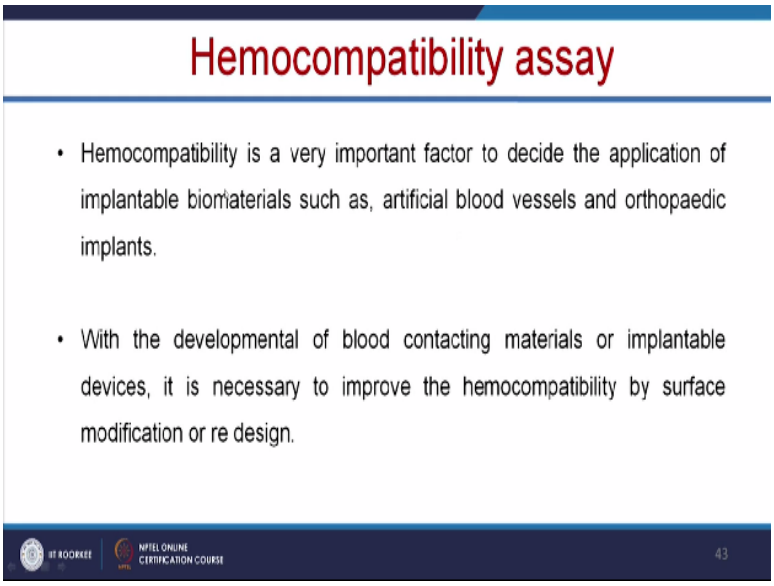

So let us see what is hemocompatibility assay so because hemocomptibility is a very important factor to decide the application of implantable biomaterials such as artificial blood vessels and orthopaedic implants and with the developmental of blood contacting materials or implantable devices it is necessary to improve the hemocomptability by surface modification or redesign.

So whenever you make the nano materials or nano particles just coatings we are to undewrstand whether it is comfortable with the system and also we have to understand whether it is compatable with the blood. So by using this hemocomaptability test we can understand the compatible of the material with the blood.

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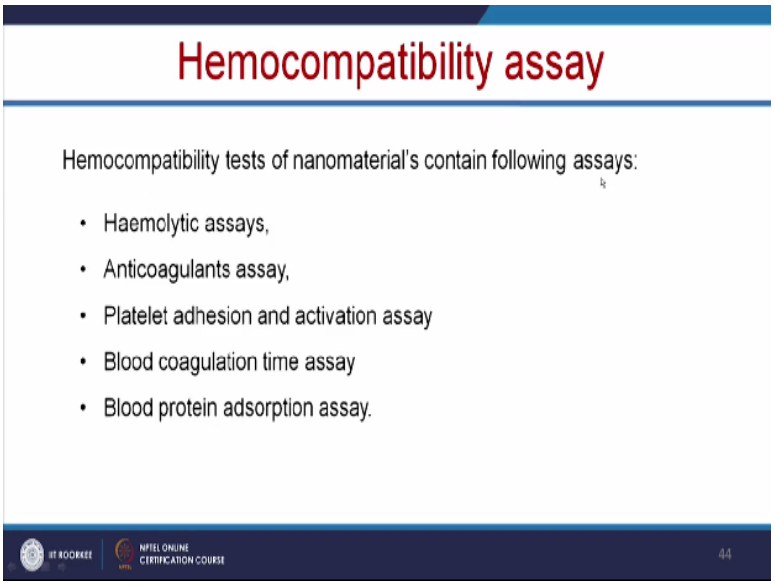

So the hemocompatibility test of nano material contains the following assays so it is a haemolytic assay or anticoagulants assay and platelet adhesion and activation assay blood coagulation time assay and blood protein adsorption assay.

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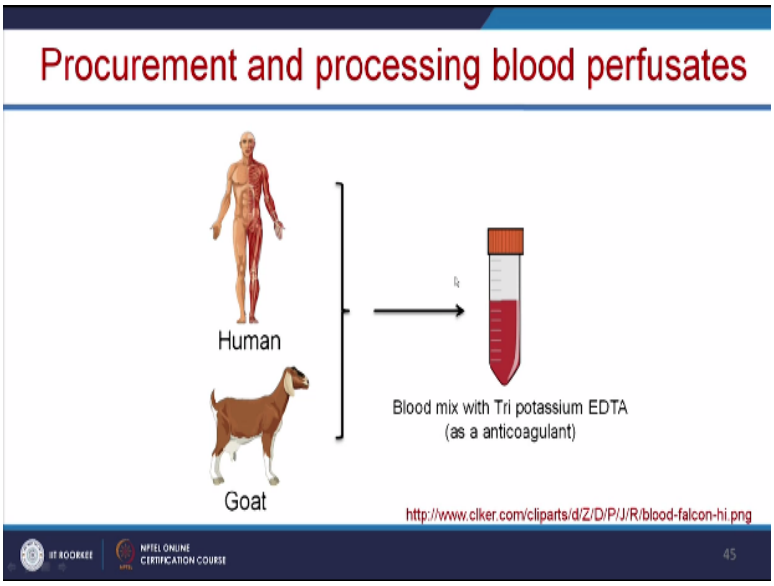

So how to collect the blood we can take the blood from human okay you take a 2ml of blood okay or you can collect the blood from goat also but in case of human when you collect your own blood also so you have to take the ethical committee human ethical committee permission so it is easy to take the blood from the goat so you can collect the blood from the nearby slutter house and you can use it for hemocomptbility studies.

So here when you collect the blood you have to mix the blood immediately with the dry production EDTA that is your anticoagulant okay so this blood can be the goat blood can be characterized from the slutter house and it present of the anti coagulant okay.

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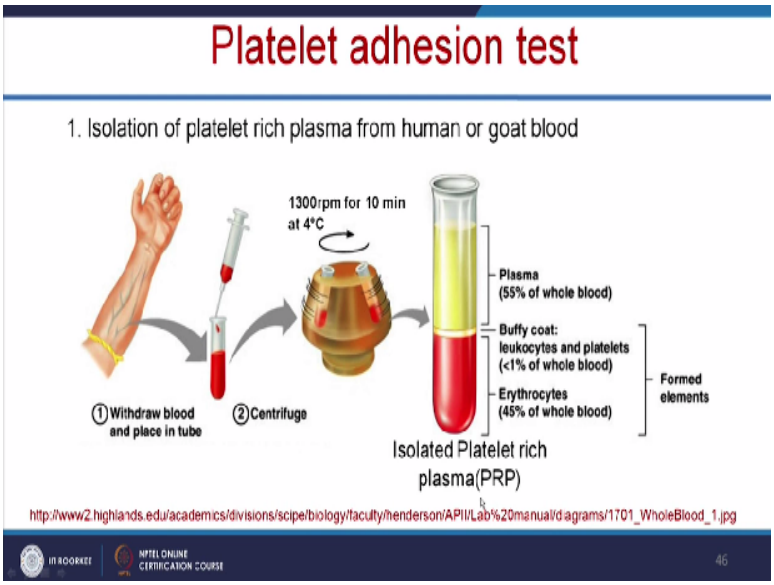

So then you can centrifudge it at 13000 rpm for the 10 minutes 4degree Celsius so when you centrifudge you will get this kind of less and you can use this isolated plated rich plasma that is PRP for your further studies.

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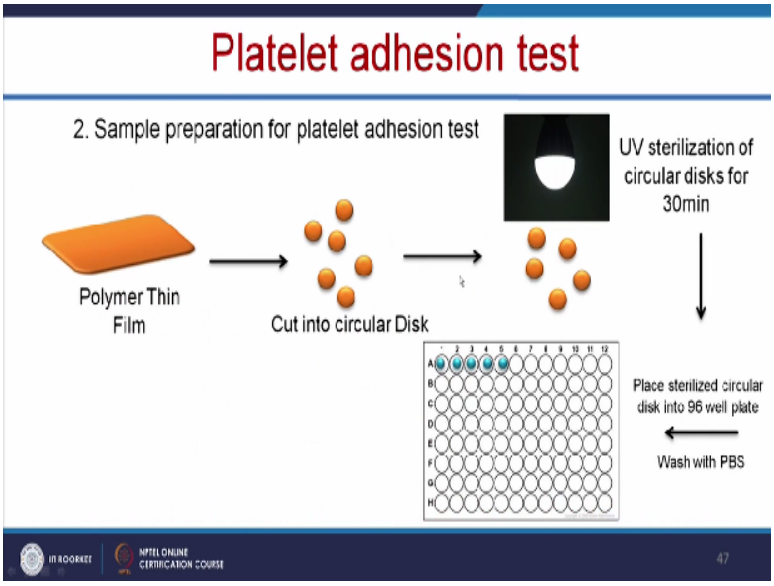

And here this is your polymer film and you want to understand whether this polymer film is haemocomapatable or not okay so you cut into circular disk using this even a simple punch machine okay so it can cut the uniform size and this can be sterlised by keeping it under the UV light for 30 minutes and thi9s can be transferred to 96 well plate okay and wash with PBS

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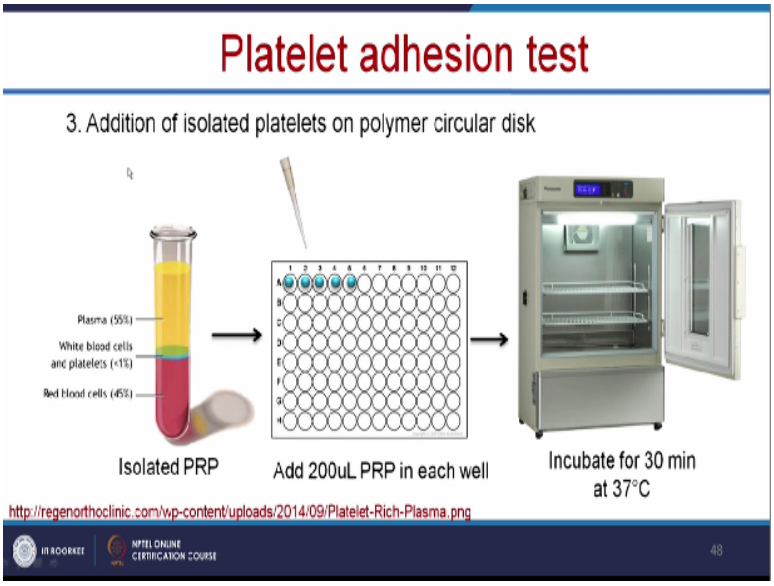

So then you can add the isolated platelets on the polymer circular disk so you can add 200 PRP in each well and you can incubate in the incubator for 30 min at 37 degree Celsius okay.

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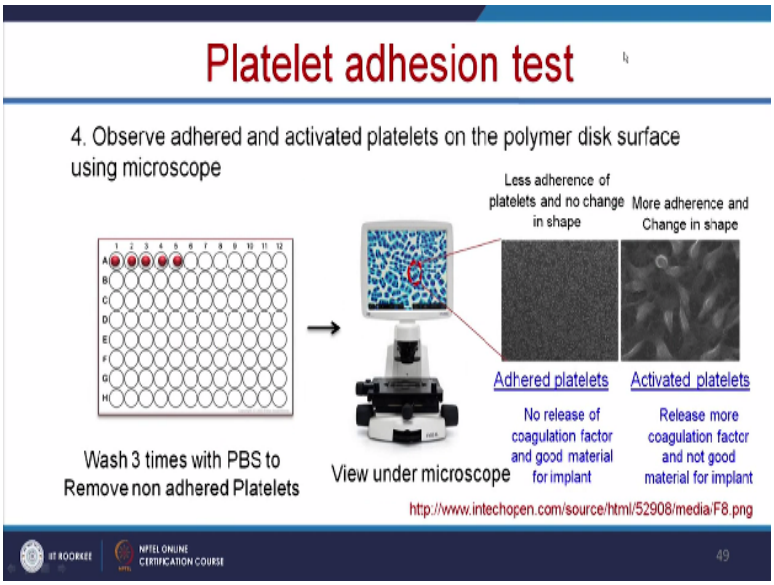

And you can observe the adhered and activated platelets on the polymer disk surface using the microscope and you can wash this 3 times with the PBS to remove the non adhered platelets okay so when you view under the microsocope you will get a this kind of results if you are getting this kind of results that means a less adherence of platelets okay.

And there is no change in the shape that means there is no release of coagulant factor and it is a good material for implants so under the microscope if I seeing this kind of shapes that means more adherence and the change in shapes so it is mainly due to the activated platelets it is releasing more coagulation factor and it is not good material for implants okay.

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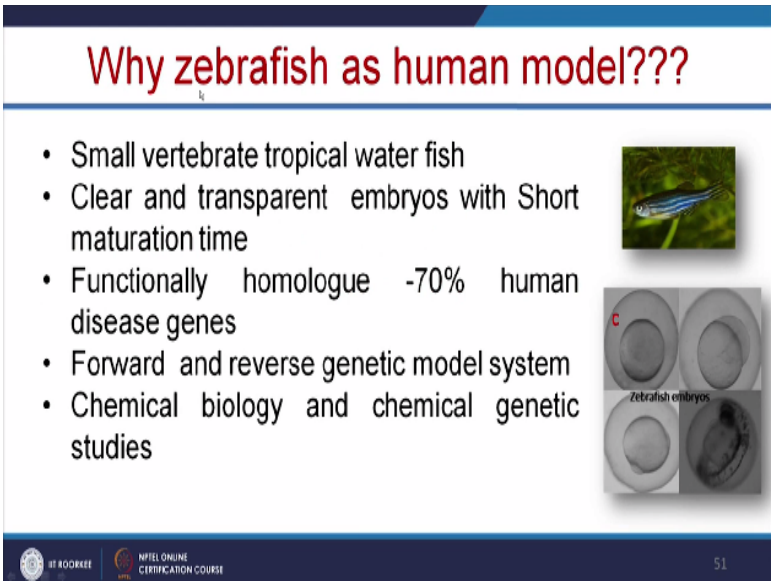

So next one is in vivo assessment of nanomaterials toxicity so why we are used zebrafish human model it is a small vertebrate tropical water fish okay so it has a clear and transparent embryos with short maturation time and functionally homologue with 70% of human disease genes and forward and reverse genetic model system is possible here and we can also do the chemical biology and chemical genetics studies using zebrafish.

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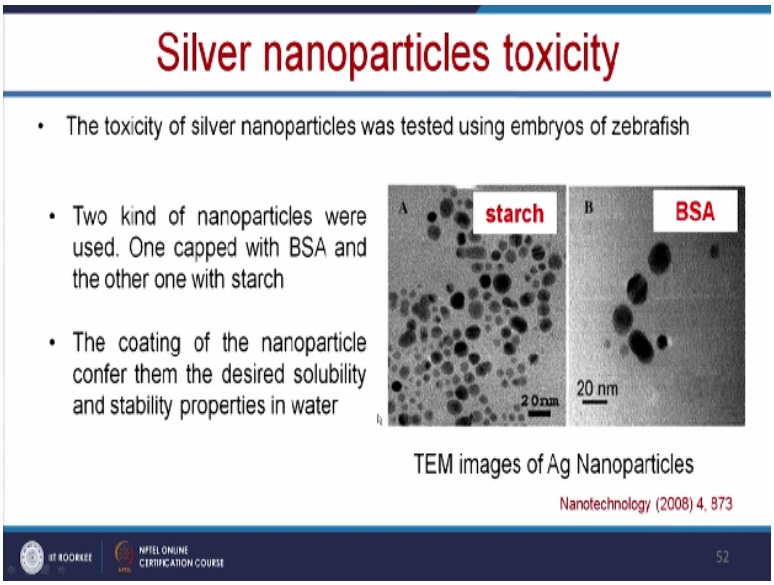

So let us see some of the examples how we can study the toxicity sliver particles using this zebrafish embryos okay so here they in this paper they were use a two different kind of nanoparticles okay one is starch coated sliver nanoparticles and one is BSA coated sliver nanoparticles.

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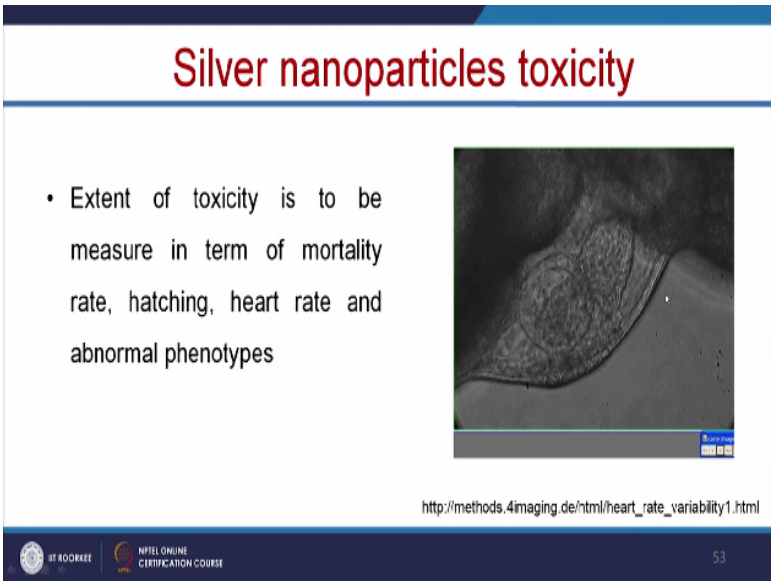

And we can study the toxicity of the nano materials in term of mortality rate of zebrafish are hatching as well as the heart rate and abnormal phenotypes okay so this is the zebrafish heart beating okay so you can measure the heart rate also compare to the control what is the heart rate in treated in zebrafish embryos.

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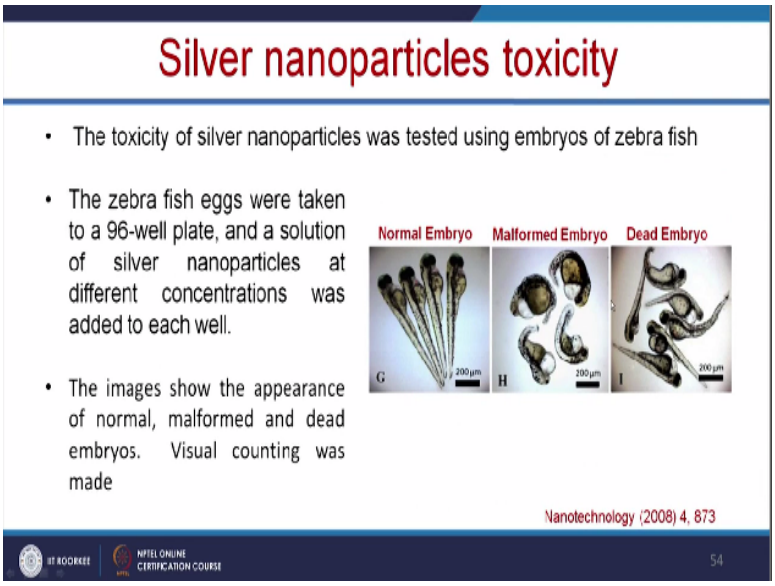

So here you can see here so they have use the zebrafish embryo and related with thedifferent concentration of sliver nanoparticles so this is our normal embryo and this is the malformed embryo and these are the dead embryo and presence of sliver nanoparticles so based on that you can count the number of a embryo which got the malformed embryo as well as the dead embryo and based on you can calculate the toxicity of nanomaterials.

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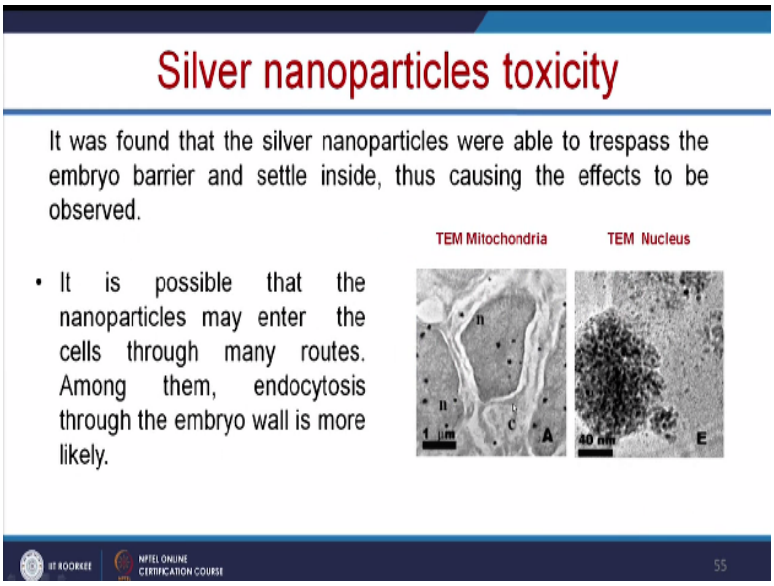

And in this case they were observed that sliver nanoparticles is most of the mitochondria as well as the nucleus that means it is possible that nanoparticles enter the cells to many routes among them endocytosis through the embryo wall is more likely okay and entering the mitochondria as well as the nucleus and it may induce some damage to the DNA also.

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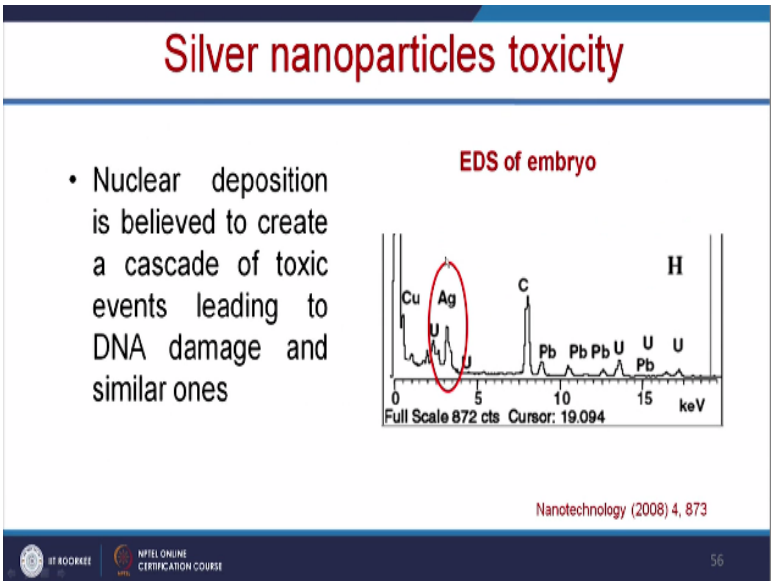

So by elemental analysis of embryo they have observed that sliver nanoparticles are more in the nucleus so they believe that it make create a cascade of toxic events leading to damage the DNA okay which will leads to various toxic effects.

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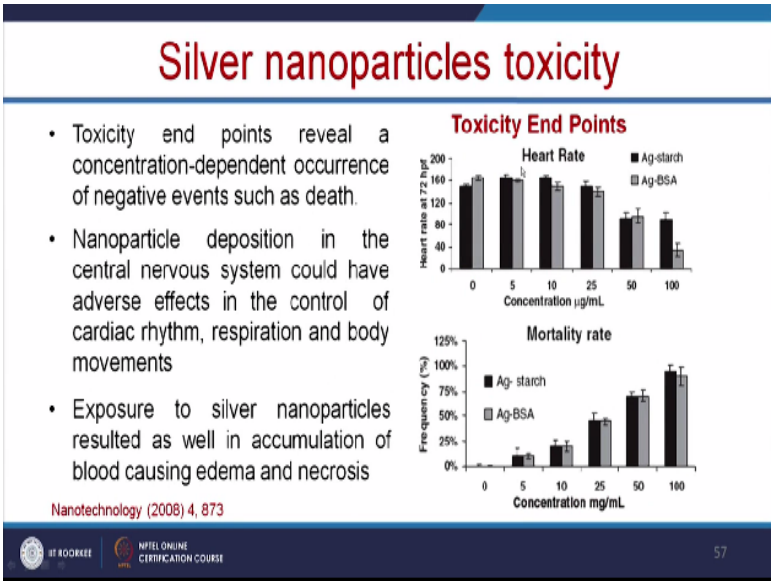

So the zebrafish embryo with the different concentration on the sliver nanoparticles so you can see here heart rate is going down and again this mortality rate is increasing okay so this nanoparticles deposition in central nerve system could have adverse effects in the control of the cardiac rhythm respiration and body movements so you can see here the heart rate is going down on the effective concentration.

And again this exposure to sliver nanoparticles resulted in accumulation of blood causing edema and necrosis so that is why there is some alternatives is increasing.

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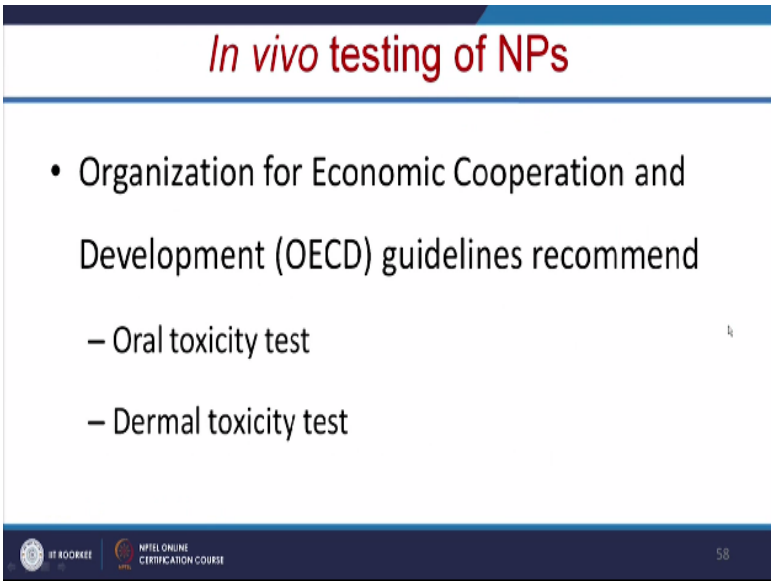

And we can also use that mouse or rat model for studying the toxicity of nano particles so organization for the economic cooperation and development guidelines recommend oral toxicity and dermal toxicity test to understand the toxicity of nano particle.

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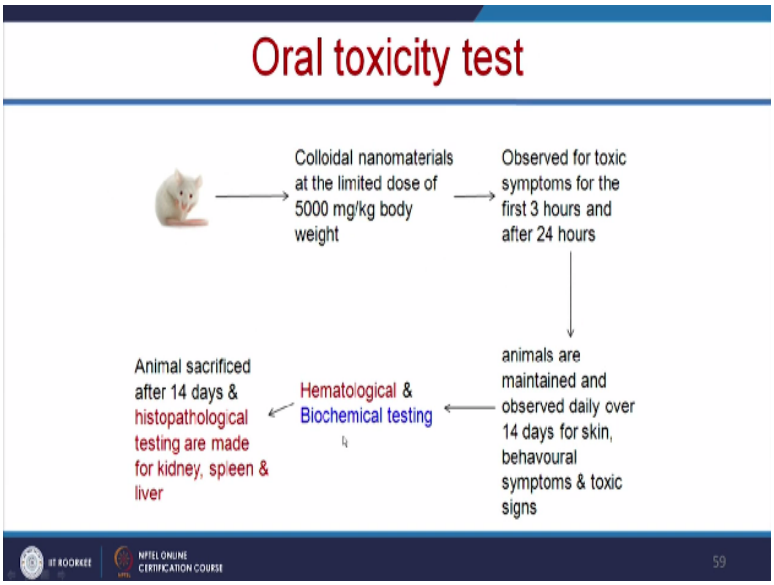

So for the oral toxicity test colodial nanomaterials at the limited dose of 5000 milligram/kilogram body weight could be given to the mouse okay and it can be observed for the toxic symptoms for the first three hours and after 24 hours so this animals could be a maintain and observe daily over 14 days for the speen and also behavioural symptoms and toxic signs then we can do the haemotological and biochemical testing so this animal cells be annual sacrificed after 14 days and histopathological testing could be made for understanding the how much is nanoparticles deposition in kidneys speen and liver.

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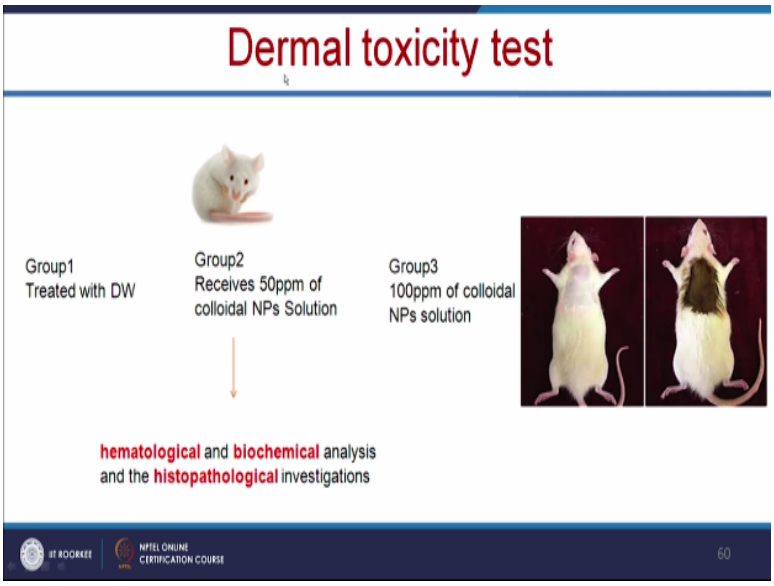

So next one is dermal toxicity test okay we can apply this nanomaterials on the surface of the animal models okay so group 1 is treated with the DW and group 2 receives 50ppm of colodial nanoparticles solution and group 3 we can use a 100ppm of colodial nanoparticles solution. So we can increase the concentration depends on the study so when you treat with the different kind of nanoparticlesthen you can study the hemothological and biochemical analysis and also the histopathelogical investigations to understand the toxicity of nano materials okay

As a summary of this lecture in this lecture we are learned the what is the nanotoxicology and what are the various exposure scenarios and also we learned what are the various methodologies available to the study the toxicity of nanoparticles and we also learned genotoxicity and how to study hemocomaptability of nonmaterial. So I end my lecture here I thank you all for listening this lecture and all the best for your exams.

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