Advance Analytical Course

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Lecture No. # 10

Recent developments in assessing the bioavailability of persistent organic pollutants in the environment – now, this is a very crucial and important area where analysis plays a very vital role.

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We know that there is lot of pollution all over and all around us, but how do we find out the levels of pollution? And for that, we need to bank on the analytical method and follow the protocol of analysis very carefully to be able to assess the bioavailability of persistent organic pollutants (POP), as we call it. POPs are very notorious because once they come to the biological system, they have no way to exit. And as a result, they go on bioaccumulating in the system and reach a toxic level, where they start showing their effects.

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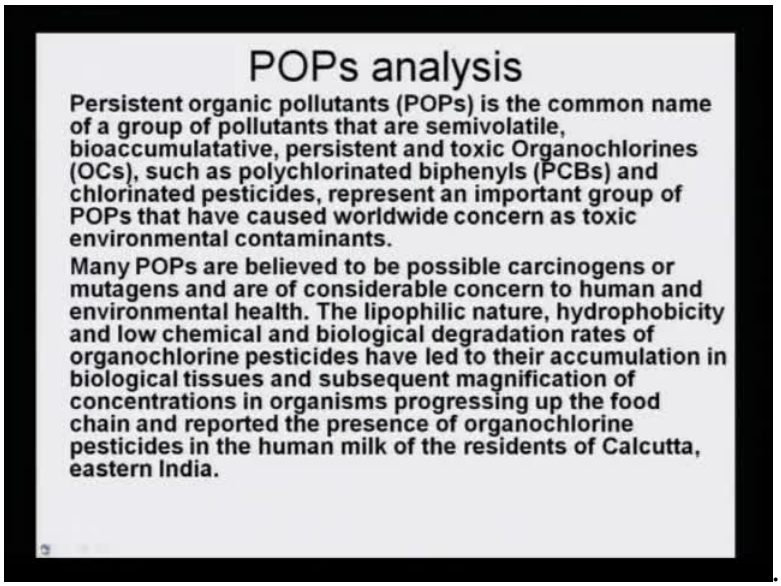

POPs then need to be analyzed. And so, what is the protocol for analysis of POPs? Persistent organic pollutants or POPs is the common name of a group of pollutants that are semivolatile, bioaccumulative, persistent and toxic organochlorines, such as polychlorinated biphenyls and chlorinated pesticides; and they represent an important group of POPs that have caused worldwide concern as toxic environmental contaminants. So, you see in the newspaper, everyday, that particular area or that particular industrial area has been found to be contaminated with x compound and that is, because proper diffusal of the reminants or the residues have not been taken care. And as a result, it has contaminated the soil; it has contaminated the underground water level and so on and so forth.

Many POPs are believed to be possible carcinogens or mutagens and are of considerable concern to human and environmental health. The word carcinogen means a compound, which is liable or which is causing cancer or is known to be causing cancer; and mutagens means that the genetic material by mutagenic effect have actually changed into another genetic material. And so, it is not acceptable to the normal or health condition. The lipophilic nature, hydrophobicity and low chemical and biological degradation rates of organochlorine pesticides have led to their accumulation in biological tissues, and subsequently, magnification of the concentrations in organisms progressing up the food chain and reported the presence of organchlorine pesticides in human milk of the residents of Calcutta and many other places. How did this happen? Because from the

food chain, from the lowest member of the food chain, it has risen and that the final food that was consumed by human beings, contained bioaccumulated organo pesticide, and that is how it has reached the human body. It is not that we are swallowing directly the organo pesticide; it is going through our food chain, because pesticides are used, the firms from the soil goes into the plant, the plant that we consume is then going into our body. So, the pesticide reaches our body in that sequential order.

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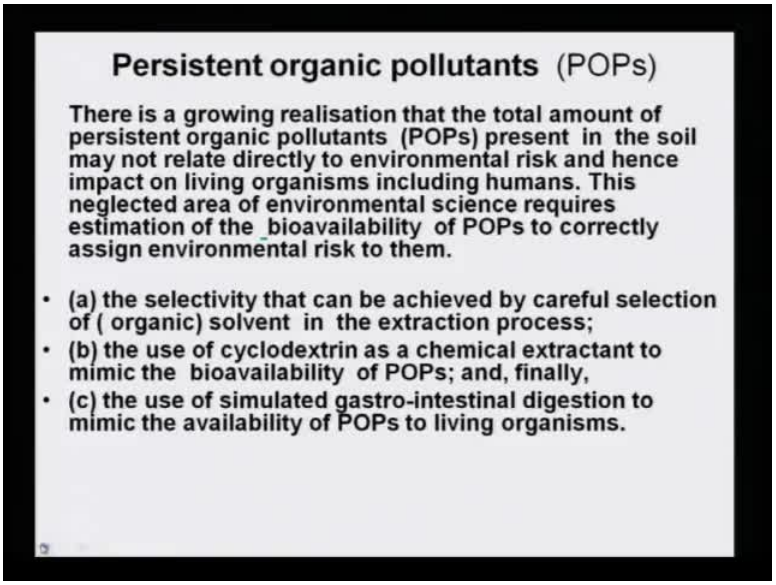

Pollutant organic pollutants or persistent organic pollutants – there is a growing realization that the total amount of persistent organic pollutants or the POPs present in the soil may not relate directly to environmental risk and hence, impact on living organisms including human beings. What does this statement mean? That directly if we try to find a correlation, there may not be a direct correlation between the persistent organic pollutants and the living beings and their health condition. But over a period of time, if this is neglected, and if it is not taken into consideration, if environmental science is not taking due recognition of the presence of POPs and the estimation is not done on the bioavailability of the POPs, it will not be able to ascertain what environmental risk it is posing.

The selectivity that can be achieved by careful selection of organic solvent in the extraction process; secondly, the use of cyclodextrin as a chemical extractant to mimic the bioavailability of POPs; and finally, the use of simulated gastro-intestinal digestion to

mimic the availabilities of POPs in living organisms. So, these are the three areas where one needs to focus and the analysis should be done in such a manner that the POPs are extracted most efficiently.

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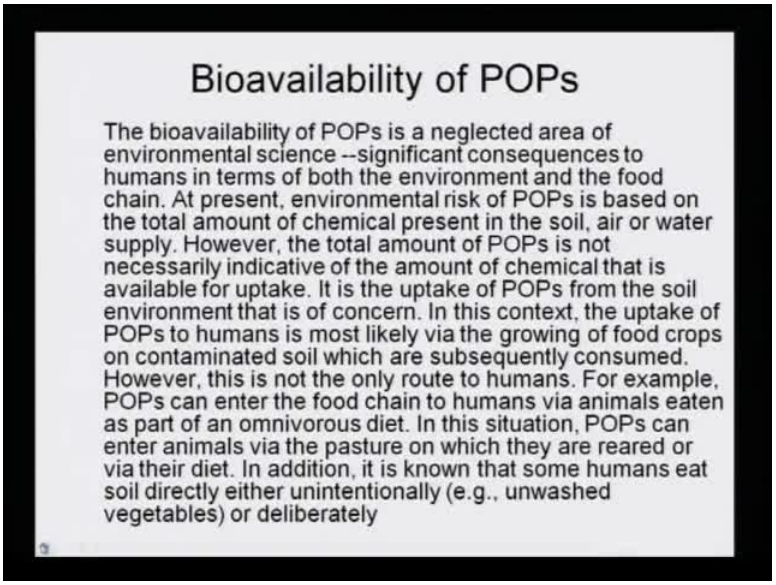

Bioavailability of POPs – the bioavailability of POPs is a neglected area of environmental science, because people are not seeing these chemicals; they feel that anything, which is not been seen is not known, but that is not true; significant consequences to humans in terms of both the environment and the food chain. At present, environmental risk of POPs is based on the total amount of chemical present in the soil, air or water supply that we consume. However, the total amount of POPs is not necessarily indicative of the amount of the chemical that is available for uptake. There may be kilos of pesticides lying on the soil, but it is not that all 1 kg will go into the human body, but a part of it will go slowly if that soil is being used for agricultural purpose.

It is the uptake of the POPs from the soil environment that is of concern. In this context, the uptake of POPs to humans is most likely via the growing of food crops on contaminated soil, which are subsequently consumed. So, that is what I told you, that it is not that human beings are swallowing the pesticide; it is going from soil to plant and from plant to the human being. However, this is not the only route to humans. For example, POPs can enter the food chain to humans via animals eaten as a part of an

omnivorous diet. Suppose those who are non-vegetarian, even they have another source of this contaminate reaching, because the animal must be gracing and that if it has graced on the fields, which were already contaminated with pesticide, then that pesticide will go into the body of the animal. And if that animal – sheep or goat, is then consumed by human being, then that would subsequently come to the human body as well. In this situation, POPs can enter animal via the pasture, on which they are reared or via their diet. In addition, it is known that some humans eat soil directly either unintentionally or unwashed vegetables or deliberately. So, of course, there are other reasons how it can reach?

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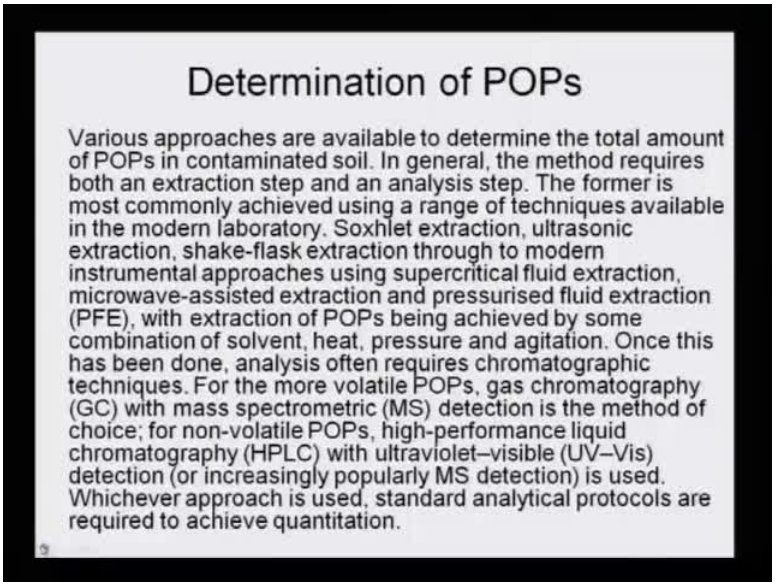

Now, how do we determine these POPs? Various approaches are available to determine the total amount of POPs in contaminated soil. In general, the method requires both an extraction step and an analysis step. As what I have repeated time and again, that sample preparation, sample extraction is of equal importance as the analysis step. The former is the most commonly achieved using a range of techniques available in the modern laboratory. Soxhlet extraction, ultrasonic extraction, shake-flask extraction through to the modern instrumental approach using supercritical fluid extraction, microwave-assisted extraction and pressurised fluid extractions, with extractions of POPs being achieved by some combination of solvent, heat pressure and agitation. So, either we can use these modern techniques or we can even take care by using the older techniques. But the whole purpose is to be able to extract the POPs most efficiently by using the right kind of

solvent, by using the proper heat temperature and not very high temperature or not very low temperature, using a pressure, which can help in solubilizing these POPs, and of course, agitation, because we know that when we have to stir sugar or salt in a tumbler of water, we stir it; by stirring it, it dissolves in a more efficient manner. Similarly, by agitation of the flask, the solubility of POP will get enhanced.

Once this has been done, analysis often requires chromatographic techniques. For the more volatile POPs, gas chromatography is an ideal machine, which must be hyphenated with mass spectrometry or we can say that GC/MS is the most ideal machine. Detection by this method is the best choice. For nonvolatile POPs, high-performance liquid chromatography, that is, HPLC with or without mass, is also suitable, but it must have a UV-visible detector and increasingly, the more popularly, the mass detector is also being used. Whichever approach is used, standard analytical protocols are required to be achieved for quantitation.

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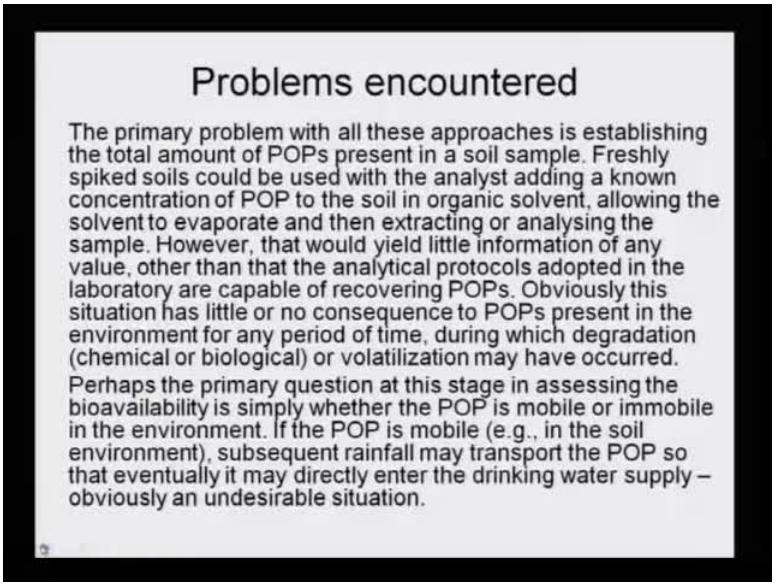

There are of course, obvious problems that are encountered during the analysis of POPs. The primary problem with all these approaches is establishing the total amount of POPs present in a soil sample. Freshly spiked soil could be used with the analyst adding a known concentration of POP to the soil in organic solvent, allowing the solvent to evaporate and then extracting or analysing the sample. However, that would yield little information of any value, other than that the analytical protocols adopted in the

laboratory are capable of recovering POPs. Obviously, this situation has little or no consequence to POPs present in the environment for any period of time, during which degradation – either chemical or biological, or volatilization may have occurred.

Now, there are methods where the analyst can directly take soil, inject some POP and analyze. But, that will not give a very holistic approach of how much of the POP would have originally been present in the soil, because this is a simulated experiment. But, simulated experiments are not the same as what the real time experiments or real experiments are. POPs undergo certain kind of chemical and biological degradation, although the process may be very slow. So, they may be generating many metabolites during the process. And so, if a soil sample is collected, it may have a metabolite of the same POP and the concentration of these metabolites would be much different. So, that is why, it is important to understand that a simulated experiment does not give a very correct picture. Perhaps, the primary question at this stage is assessing the bioavailability in simply whether the POP is mobile or immobile in the environment. If the POP is mobile, that is, in the soil of the environment, subsequently, rainfall may transport the POP, so that eventually, it may directly enter the ground water or the drinking water supply. Obviously, this is an undesirable situation. So, one has to understand the chemical nature of these POPs and then try to understand the extraction methods that need to be adapted.

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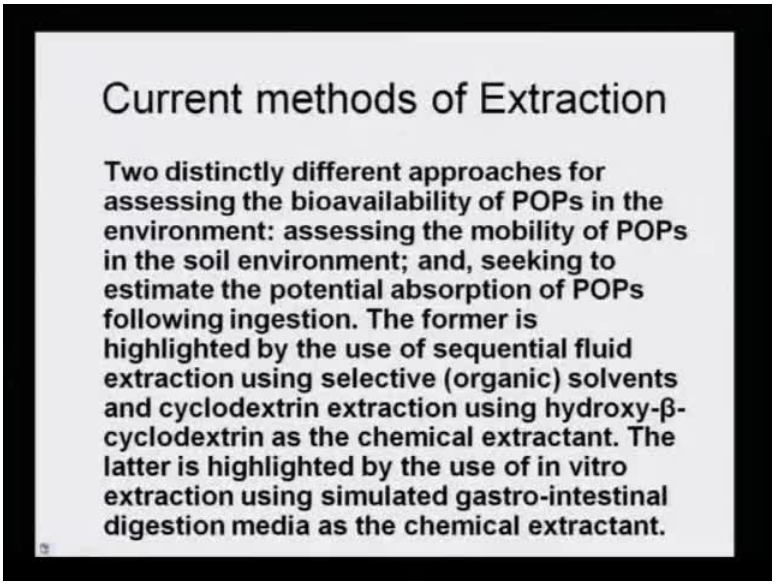

Current methods of extraction of POPs are two distinctly different approaches for assessing the bioavailability of POPs in the environment: assessing the mobility of POPs in the soil environment; and, seeking to estimate the potential absorption of POPs following ingestion. The former is highlighted by the use of sequential fluid extraction using selective organic solvents and cyclodextrin extraction using hydroxyl-beta-cyclodextrin as the chemical extractant. The latter is highlighted by the use of in vitro extraction using simulated gastro-intestinal digestion media as the chemical extractant. So, it is very important to be able to design a method, to be able to extract the quantitative amount of the POP that is present.

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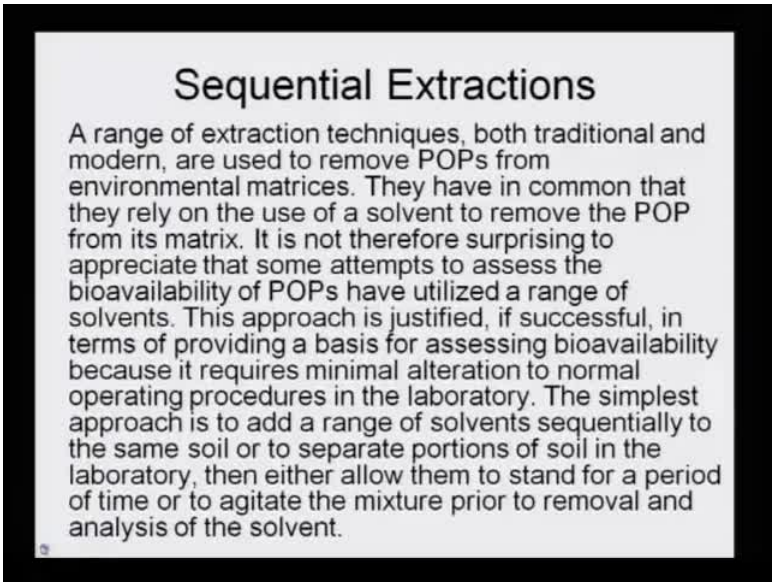

Sequential extraction definitely has an edge over the other methods that I just mentioned. A range of extraction techniques, both traditional and modern, are used to remove POPs from the environmental matrices. They have in common that they rely on the use of a solvent to remove the POP from its matrix. It is not therefore surprising to appreciate that some attempts to assess the bioavailability of POPs have utilized a range of solvents. This approach is justified, if successful, in terms of providing a basis for assessing bioavailability, because it requires minimal alteration to normal operating procedures in the laboratory. The simplest approach is to add a range of solvents subsequently to the same soil or to separate portions of soil in the laboratory, then either allow them to stand for a period of time or to agitate the mixture prior to removal and analysis of the sample.

Now, the basic fundamental about this whole extraction and assessment of bioavailability of POPs, relies on how we can take this POP out of the matrix – soil matrix. Now, once we can do that, then analysis is very simple. And for doing that, the best method is that some of them are organic solvent soluble; some of them are water soluble. So, a variety of solvents must be used and with the same sample of the soil we can start, and subsequently use the solvent in higher polarity. And, when we use slowly, gradually higher polarity, then these solvents will have a selective effect on the extraction. And, when it is extracted effectively, the analysis will be done properly.

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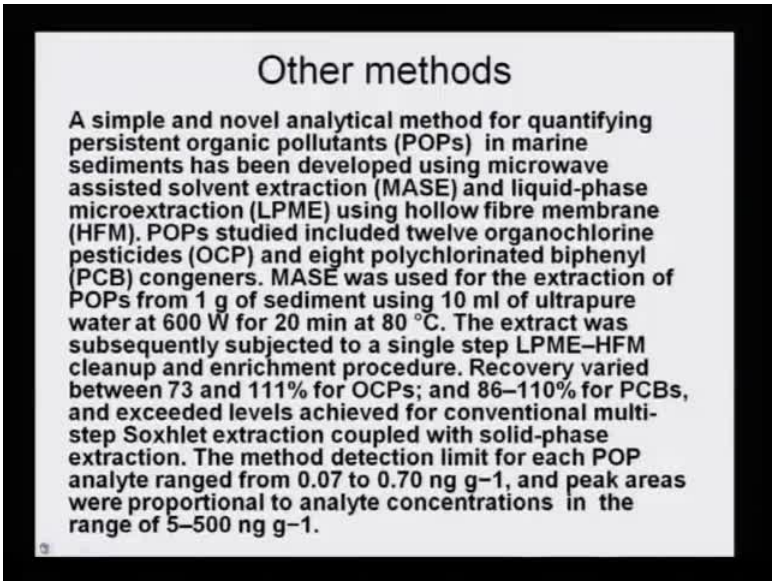

Other methods a simple and novel analytical method for quantifying persistent organic pollutants in marine sediments has been developed using microwave-assisted solvent extraction and liquid-phase microextraction using hollow fibre membrane. POPs studied included 12 organochlorine pesticides and 18 polychlorinated biphenyls congeners. The microwave assisted solvent extraction was used for the extraction of POPs from 1 gram of sediment using 10 ml of ultrapure water at 600 watt for 20 minutes at 80 degrees. The extract was then subsequently subjected to a single step LPME cleanup and enrichment procedure. Recovery varied between 71 to 111 percent for POPs; and 86 to 110 percent for PCBs, and exceeded levels achieved for conventional multistep Soxhlet extraction coupled with solid-phase extraction. The method of detection limit for these POPs analytes ranged from 0.07 to 0.7 nanogram per gram, and peak areas were proportional to analyte concentrations in the range of 5 to 500 nanogram per gram. So, you see that

very small quantities also can be analyzed very effectively if a proper extraction method is used.

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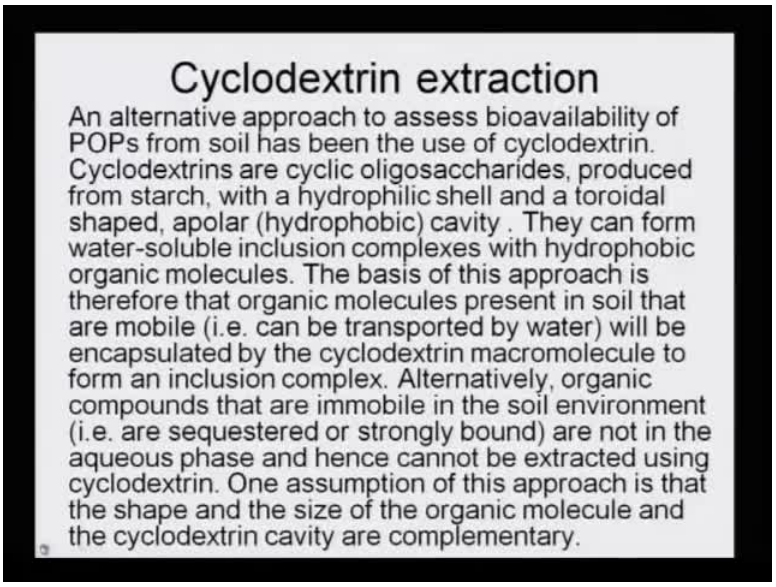

Cyclodextrin extraction – I have been talking about this chemical extractant for a while, but I thought that I should dedicate one slide to this, so that you are able to understand what cyclodextrin is, because it is not a common compound that you would have probably come across. An alternative approach to assess bioavailability of POPs from soil has been the use of cyclodextrin. Cyclodextrins are cyclic oligosaccharides, produced from starch. So, cyclodextrins are basically derivative from starch, with a hydrophilic shell and a torodial shaped, apolar hydrophobic cavity. They can form water-soluble inclusion complexes with hydrophobic organic molecules. The basis of this approach is therefore that organic molecules present in soil that are mobile can be transported by water, will be encapsulated by the cyclodextrin macromolecule to form an inclusion complex.

Alternatively, the organic compounds that are immobile in the soil environment are sequestered or strongly bound, are not in the aqueous phase and hence, cannot be extracted using cyclodextrin. One assumption of this approach is that the shape and size of the organic molecule and the cyclodextrin cavity are actually complementary. And that is the reason why cyclodextrin was chosen, because it could discriminate and take the organic molecule, because the sizes of these pesticides or POPs were matching

exactly into the cavity. And that is how it would encapsulate it and transport it or separate it.

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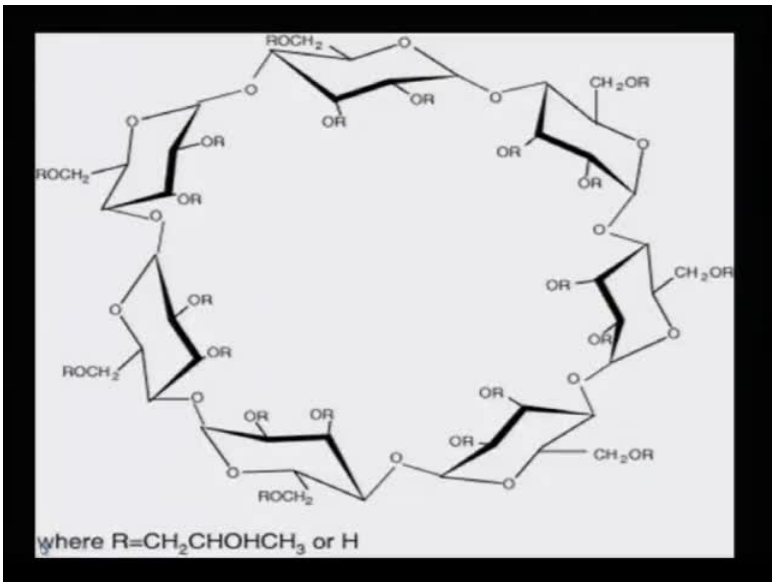

It is something like this; it has a ring structured and there is a hollow cavity in between.

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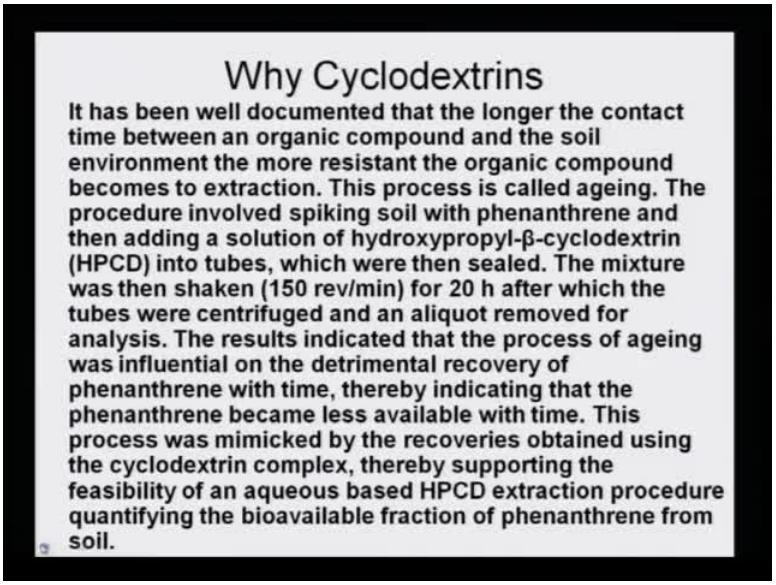

Why cyclodextrins? It has been well documented that the longer the contact time between the organic compound and the soil environment, the more resistant the organic compound becomes for extraction. This process is called ageing. The procedure involved spiking soil with phenanthrene and then adding a solution of hydroxypropyl-beta-

cyclodextrin into tubes, which were then sealed. The mixture was then shaken 150 revolves per minute for 20 hours, after which the tubes were centrifuged and an aliquot removed for analysis. The results indicated that the process of ageing was influential on the detrimental recovery of phenanthrene with the time; thereby indicating that the phenanthrene became less available with time. This process was mimicked by the recoveries obtained using the cyclodextrin complex; thereby supporting the feasibility of an aqueous based HPCD extraction procedure quantifying the bioavailable fraction of phenanthrene from the soil. So, you see, so many experimentations need to be carried out in order to come to a conclusion which method should be adapted for these various different types of chemically different compounds.

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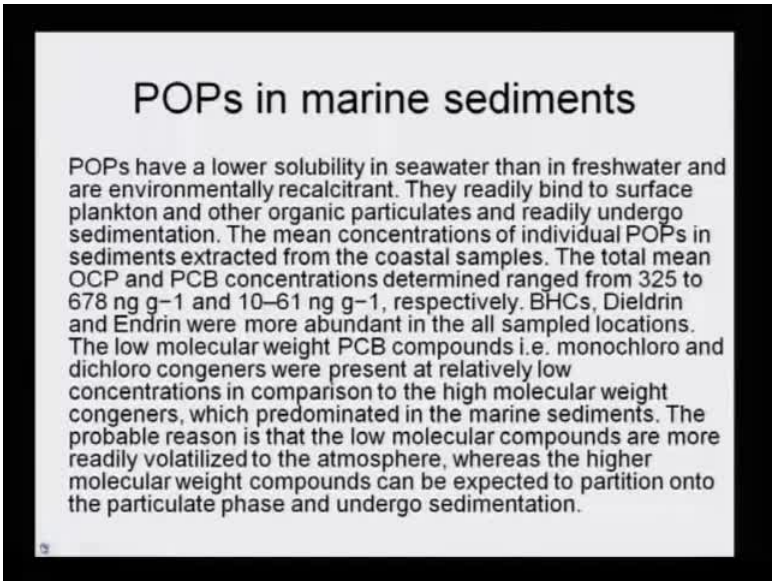

POPs in marine sediments – POPs have a lower solubility in seawater than in freshwater and are environmentally recalcitrant. They readily bind to surface plankton and other organic particulates and readily undergo sedimentation. The mean concentrations of individual POPs in sediments extracted from the coastal samples are given in the following example: the total mean OCP and PCB, that is, organochlorine pesticide and polychlorinated biphenyl concentrations determined ranged from 325 to 678 nanogram per gram and 10 to 61 nanogram per gram, respectively. The BHCs, Dieldrin, Endrin were more abundant in all the sampled locations. The low molecular weight PCB compounds, that is, the monochloro and the dichloro congeners were present at relatively low concentrations in comparison to the high molecular weight congeners, which

predominated in the marine sediments. The probable reason is that the low molecular compounds are more readily volatized to the atmosphere, whereas the higher molecular weight compounds can be expected to partition onto the particulate phase and undergo sedimentation. So, that means they are bioavailable; they have already interacted with some biological system and they are not freely available for extraction.

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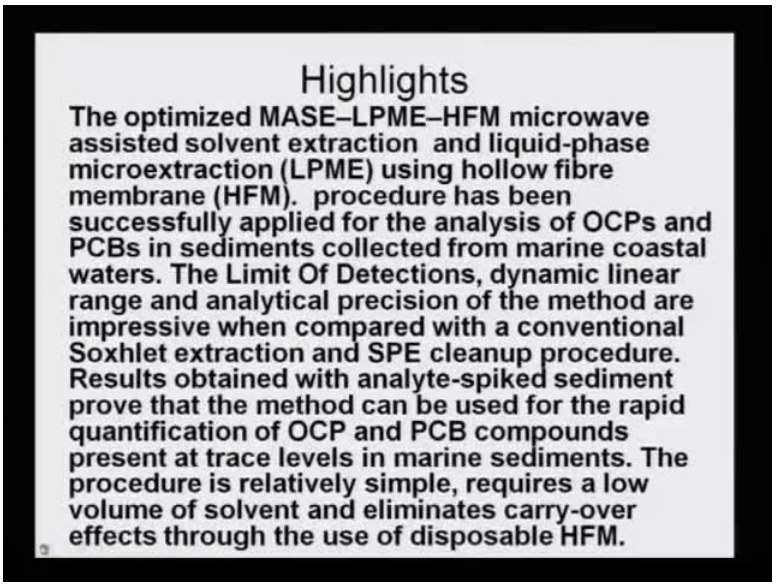

Highlights of this particular method is that an optimized MASE-LPME microwave-assisted solvent extraction and liquid-phase microextraction using hollow fibre membrane, was the ideal method for extraction of POPs. Procedures have been successfully applied to the analysis of organochlorine pesticides and polychloro biphenyls in sediments collected from marine coastal waters. The limits of detections, dynamic linear range and analytical precision of the method are impressive when compared with a conventional Soxhlet extraction and SPE cleanup procedure. Results obtained with analyte-spiked sediment prove that method can be used for the rapid quantification of OCPs and PCBs compounds that are present at trace levels in marine sediments. The procedure is then relatively very simple, requires a low volume of solvent and eliminates any carry-over effects through the use of disposable hollow fibre membranes. And, that is what the beauty of this procedure is.

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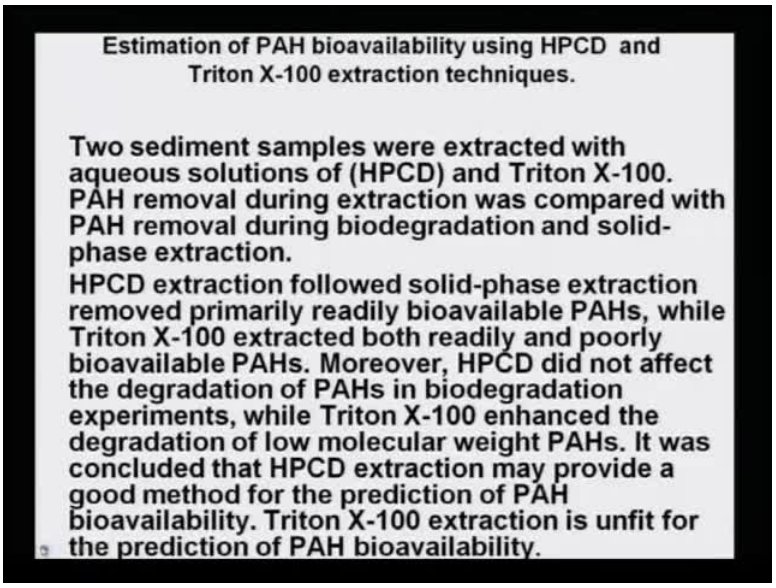

Estimation of another very notorious POP, which is called polyaromatic hydrocarbon – their availability using HPCD and triton X-100 extraction techniques – now, you will see that every time I am giving a new method for extraction. Why is it so? It is so because new methods have to be derived for certain new compounds and for their effective and efficient extraction. Two sediment samples were extracted with aqueous solutions of HPCD and triton X-100. The polyaromatic hydrocarbon removal during extraction was compared with PAH removal during the biodegradation and solid-phase extraction.

The HPCD extraction followed solid-phase extraction removed primarily and readily bioavailable polyaromatic hydrocarbons, while triton X-100 extracted both readily and poorly available polyaromatic hydrocarbons. Moreover, HPCD did not affect the degradation of polyaromatic hydrocarbons in biodegradation experiments, while triton X-100 enhanced the degradation of low molecular weight PAHs. It was concluded that HPCD extraction may provide a good method for the prediction of polyaromatic hydrocarbon and their bioavailability. Triton X-100 extraction is unfit and it is predicted that it will not give good results. So, by making these kind of research and by using different materials for extraction, one can conclude which is a superior method and which is not fit for the extraction.

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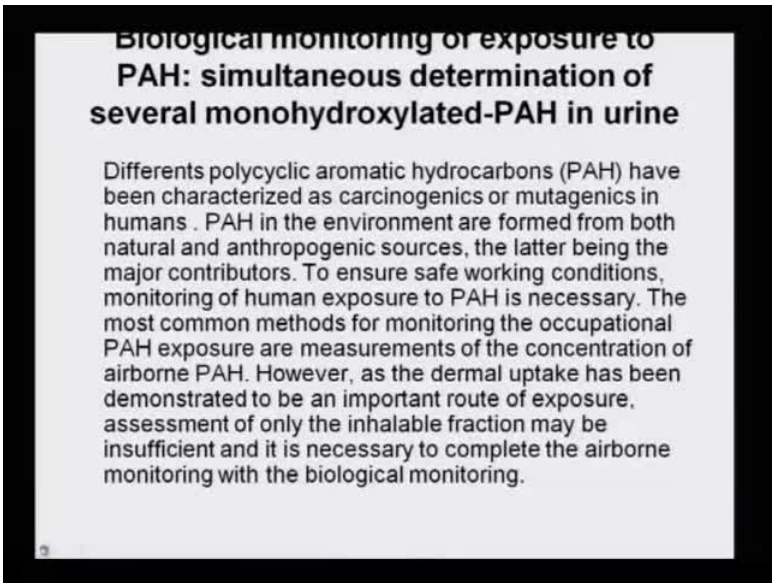

Biological monitoring of exposure to PAH: simultaneous determination of several monohydroxylated-PAH in urine. Different polycyclic aromatic hydrocarbons, that is, the PAH have been characterized as carcinogenics or mutagenics in humans. PAH in environment are formed from both natural and anthropogenic sources, the latter being the major contributors. To ensure safe working condition, monitoring of human exposure to PAH is very necessary. The most common methods for monitoring the occupational PAH exposure are measurements of the concentrations of airborne PAH. That means the polycyclic aromatic hydrocarbons that are present in the air. However, as the dermal uptake has been demonstrated to be an important route of exposure, assessment of only the inhalable fraction may be insufficient and it is necessary to complete the airborne monitoring with the biological monitoring also.

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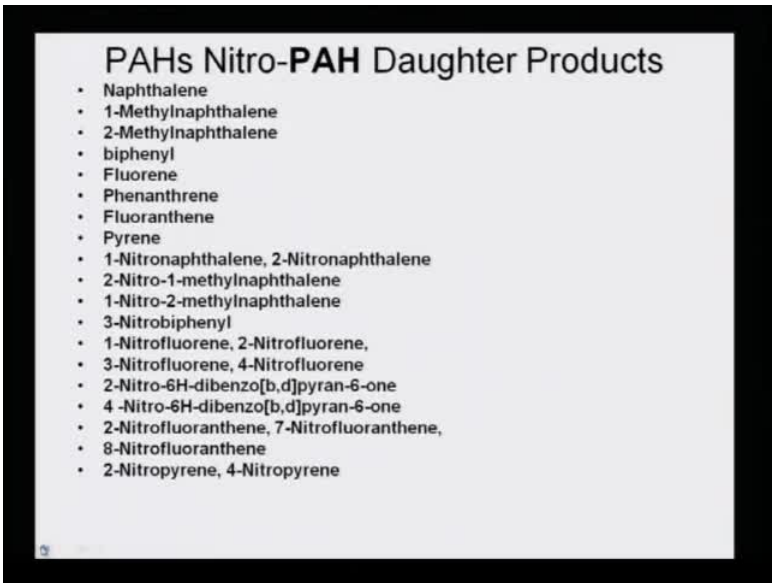

I have listed different types of PAH. Some of them are nitro-PAH. And these then, subsequently produce different metabolites or daughter products, as we call it.

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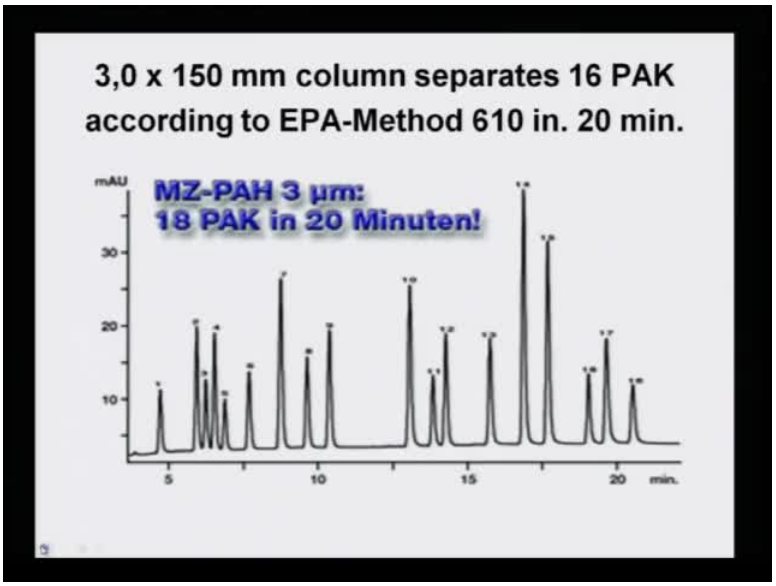

This particular chromatogram shows the presence of these many components. And, this is our EPA method that is a very scientific protocol of analysis. So, with this, we have come to an end of analysis of very specified products.