Endocrine Disruptors Research in Malaysia

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EDC research in Malaysia is very new and began to be carried out intensively only since 1999. Since we just started the research a few years ago, the team is not very big. EDC research in Malaysia is being conducted by a few institutions including universities. The main activities in the country were conducted at our center. That is the center which is co-organized by Shimadzu Corporation and University of Malaya, the Shimadzu-UMMC Center for Xenobiotic Studies (SUCXeS). This center is considered to be the most active EDC research centre in South east Asia.

In Malaysia, there are, of course, some other institutions involvement, for example, the Ministry of Health has already started to note the existence of EDCs and also granted some funding for EDC research in the country. The Ministry of Agriculture has begun to look into the EDC aspect.

The United Nations University, together with Shimadzu Corporation, has initiated and successfully conducted a project for the past five years. During the last three years, the research was on EDC. This research had international involvement by several countries in this region: China, Japan, Vietnam, Phillipines, Korea, Thailand, Indonesia, Malaysia and Singapore.

In the year 2000, the research in EDC was given a big boost by the mandate given by the United Nations University. Our centre were given a task to host an international symposium on EDC in Kuala Lumpur. We also conducted a workshop on analysis of EDC in water. This symposium and workshop has triggered aggressive work on EDC in our laboratory.

Our main aim of research activities in the country is, of course, to assess the extent of contamination of these hazardous chemicals in our country. We wanted to know how serious it is or otherwise in the context of Malaysia. We also needed to assess the potential effects of these chemicals on the population. We have disseminated information by publications to the public; not to alarm them, but to inform them of the existence of these chemicals and that research is being carried out to assess these chemicals in detail.

These studies that we planned here, and that we conducted in Malaysia, were necessary because we were aware that studies conducted elsewhere may not be the same. There may be some differences, especially due to the differences in our way of life, economics status, the temperature, weather conditions, and so on. We have some data to show that there are some differences. So our own study is also relevant to compare with what has been assessed elsewhere. More research needs to be done in the lower part of the globe on EDC distribution and the fate of these chemicals.

Current research activities that were conducted includes some analytical and monitoring work. Most of our work were also based on method development for analytical purposes, and for these we worked closely with the NIES, Japan, to use methods that are available and to adapt the method or modify the method for our work in respective areas.

One important study that we conducted in Malaysia is the monitoring of phytoestrogens in blood of the Malaysian population. We wanted to look at the distribution of phytoestrogens in our population, to find out how much is there. This study was partly funded by Japan Chemical Industries Association, JCIA. It is going on now and we have managed to collect the blood and analysis is underway.

We have finished analysis of phthalates in our rivers. This project was sponsored by Shimadzu Corporation and the UNU. We also assessed the distribution of phytoestrogens in our local plants and vegetables because we believe that not much data has been worked out in local plants. We have some data from this study. We also analyzed pesticides in fish, water, and blood of an agricultural community. That means we do a detailed study of a community living in a specific place where agricultural is the main activity for the population. We look at all the aspects: from water, the fish that they take, and the blood levels. This is a thorough study for a small population.

We also looked at the distribution of DINP, bisphenol A, and phenols in domestic plastic materials manufactured locally: baby feeding bottles, canned drinks, and mineral water. This study involves the division of food quality control, Ministry of Health, Malaysia. They have assigned us to look at these chemicals in domestic utensils and also food. This involvement of the Ministry shows how concern the government of Malaysia is on EDC and the involvement of government institutions in this studies. We have already started these studies, and part of it is still going on in our laboratory.

Another monitoring study that we did was to look at the environmental distribution of bisphenol A in water. A few rivers in Malaysia were selected for this study and the concentration of pesticides, bisphenol A and phthalates were studied. A study on leaching of bisphenol A from baby feeding bottles were also carried out. This study includes the leaching of diisononyl phthalate from feeding bottles, plastic containers, and also toys manufactured locally. A nationwide study was conducted to look at the contamination of pesticides in blood of schoolchildren where we collected blood from the whole country in schoolchildren and we assessed the levels of certain selected pesticides.

The first study was to look at the distribution of bisphenol A in river water. We used a method developed by the NIES, Japan, using GCMS. If we look at the results of this study, we found that the distribution and the concentration of this chemical varies with time and month. This is due to the volume of water in the river because we have a very fluctuating season: rainy and dry seasons are not consistent and do not change very much except for the river volume. It was found that it varies with time and sites. This type of variations may be very peculiar to our climate and environment.

The next study is to assess the pesticide residues in the blood of school children. A total of 645 samples of blood were sampled from school children in selected schools all over the country. The children were aged between 13 to 15 from rural and urban areas schools.

Some of the pesticides that we monitored are currently in use and some are already banned. The analysis were carried out using whole blood and analysed by GCMS, and the sample preparation carried out by liquid-liquid extraction.

The result of the analysis showed that that some pesticides that were screened were present, while some other were not present. Some pesticides were detected in a large number of samples, while some were detected n small number of samples. It was found that only 79 out of 645 samples or only 12% of the samples show the presence of pesticides.

Currently used OCs were detected. Aldrin and p,p'-DDT were also detected in blood, despite their banned status. This is attributed to their persistent characteristic, and we also suspected that this contamination may be through air; it may not be from our agricultural activity *per se*, because these pesticides, when we analysed from the water, were also detected in the water from far upstream where there is no agricultural activity. Diazinon and chlorpyrifos were detected and particularly chlorpyrifos, was found to be more abundant in the urban areas, This could be attributed to the anti-termite spray used in residential areas and so on.

The next study was on the leaching of bisphenol A from babies feeding bottles. Used baby bottles were collected from all over the country. To date, we have studied about 1,000 bottles. Methods used in this study were adapted from NIES, Japan, and it uses GCMS.

The results showed that most of the samples leached out some detectable level, except for a few samples which leached out significantly high amount of bisphenol A. Most of these bottles are manufactured from different factories, and they are of different ages. They are all used bottles. When hot water is used, the leaching is much greater. This means that the bottle can leach more bisphenol A when it is used with hot water. Most of the bottles showed about the same phenomenon, except for a few which did not increase in the leaching when used with hot water. We compared the amount of leaching between used bottles and new bottles. We found that used bottles give a much higher amount, about ten times more, of leaching of bisphenol A compared to the new bottles.

The next study is on the *in vivo* effect to cytochrome enzymes after exposure to extracts of local plants and fruit juices. The aim of this study is to find out the effect of these juices on the cytochrome enzymes. This study is important because these enzymes are involved in the metabolism of several steroids, hormones, their precursors and their metabolites.

Some Malaysians take these plants as a tonic, called *JAMU,* and when they take these plants every day, we expect the concentration will be persistent in the blood. It is important to know whether the presence of this plant materials in the blood might be detrimental or maybe advantageous to the system. It is also important to know whether the presence of this enzyme leads to any increase or reduction in cytochrome enzyme.

In the study, the juice or the plant extract were fed to the animals for four days, and on the fourth day, the animals were injected with phenobarbitone to induce sleep. This phenobarbitone will be metabolized by the enzyme. If the enzyme production is inhibited, the concentration of the enzyme is lower, the sleeping time will be longer because the drug concentration will be high; if the enzyme production is induced, the concentration of the enzyme is higher, and the sleeping time will be shorter because most of the drugs will be metabolized rapidly.

In this study, it was found that some plant materials inhibited the enzyme while some induced the enzyme significantly. A few of the plants had significant effects, but the rest were not so significant.

One interesting thing that can be highlighted here is that the inhibition of one of the plant extract is antagonistic: that means, as the concentration is reduced, the effect is stronger. This may be related to the inverted log dose response. This needs to be looked into more seriously to see whether there is any indication or not to explain the inverted log dose response.

Some animal studies were also conducted by feeding the anmals with several suspected EDCs such as sorbic acid, glyphoshate, paraquat, sodium benzoate and phthlates. Several parameters of the fed animals were observed after two and six weeks. The parameters includes body weight, liver weight, the testis weight and the testosterone levels. There are some important results here. Glyphosate, paraquat, lindane, caused a slight reduction in the testis weight after six weeks. The testosterone levels showed some effects after six weeks while lindane fd animals showed some effects after two weeks.

Phthalate exposure leads to an increase in liver weight, decrease in testis weight, and a decrease in testosterone level. The morphology of testes were also compared. We find that the testis has differentiated quite significantly.

Studies were conducted on toxicological effects of endosulfan. Animals were exposed to endosulfan the levels of endosulfan in the blood were monitored at different hours. The body weight, liver weight, and kidney weight were also monitored. It was found that the body weight gain is not really much different and there is no significant changes in body weight. The tissue distribution of α- and β-isomers of endosulfan and their effect on the serum testosterone, T3, insulin levels were studied. The animals were divided into two groups and one group were analyzed after thirty days and the other group were analysed after fifty days. The testosterone levels of those animals treated with endosulfan were much reduced. Some pharmacological studies were conducted, and the preliminary results show that there is some significant difference in the testis tubular diameter at a dosing of 10mg/kg on day 16.

In conclusion, we would like to conclude that Malaysia has started monitoring of EDC in the local environment. Some basic research on local sources of potential EDCs were carried out. Basic research facilities, especially for monitoring and analysis of EDCs were available and some collaborative work with Japanese groups and UNU has progressed significantly .

The government has noted the need to intensify the research in EDCs; there is already government involvement in Malaysia for monitoring of EDCs, and the Malaysian scientists are willing to collaborate further with overseas institutions and agencies.

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