Dioxin: Persistent Organic Pollutant and a member of "Dirty Dozen"

*Ashish Kumar Jha, Remya S and G.K.Sivaraman
Veraval research centre of Central Institute of Fisheries Technology
Corresponding Author : ashisjha@gmail.com

Introduction

Dioxins and PCBs (polychlorinated biphenyls) are highly persistent organic chemical contaminants. They are polychlorinated aromatic compounds with similar structures, chemical and physical properties.The term 'dioxins' refers to a group of 75 polychlorinated dibenzo-p-dioxins (PCDDs) and 135 polychlorinated dibenzofurans (PCDFs) congeners.The dioxins are produced in small amounts as unwanted by-products during the manufacture of certain industrial chemicals and during combustion and incineration processes. Of the 210 different dioxin compounds, only 17 are of toxicological concern. The most widely studied and most toxic form of dioxin is 2,3,7,8-tetrachlorodibenzo-p-dioxin, abbreviated as 2,3,7,8- TCDD. It is measured in parts per trillion (ppt). PCBs or polychlorinated biphenyls are chlorinated aromatic hydrocarbons which are synthesised by direct chlorination of biphenyls. Technical PCB mixtures are still widespread and are present in various forms which are used in transformers, building materials, lubricants, coatings, plasticisers and inks. Some of the PCB compounds have toxicological properties that are similar to dioxins and are therefore often termed "dioxin-like" PCBs.

Dioxins and PCB's have the dubious distinction of belonging to the "dirty dozen" - a group of dangerous chemicals known as persistent organic pollutants (POPs). POPs are a set of toxic chemicals that are persistent in the environment and able to last for several years before breaking down. According to United Nations Environment Programme "Dirty Dozen" is a group of 12 highly persistent and toxic chemicals which includes aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzen, mirex, polychlorinated biphenyls, polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, and toxaphen. Many of the pesticides in this group are no longer used for agricultural purposes but a few continue to be used in developing countries.

Major Source of Dioxin and Dioxin like compounds

Dioxin is ubiquitous in the environment. The most common source of dioxin currently results from incineration. The major environmental sources of dioxin and dioxin-like compounds are emissions from combustion, incineration, any industrial processes using chlorine, paper mills, fireplaces, grass fires, etc. Dioxins have been found in soil, surface water, sediment, plants, and animal tissue throughout the earth. The aerial transport of these emissions is the primary pathway dioxins enter the terrestrial environment and food chain.

Dioxins are lipophilic compounds which accumulate in the fat of animals. The types of foods which tend to have the highest dioxin concentrations are dairy products, meat and poultry, eggs, fish, and animal fats. Green vegetables, fruits and grains are the types of foods with the lowest dioxin concentrations. A person's intake of dioxins through the diet therefore, depends on the relative intake of foods with high or low levels of contamination and the quantity consumed. More than 90% of human dioxin and dioxin-like PCB exposure is from food stuffs with those from animal origin contributing approximately 80% of the overall exposure. Deposition of airborne dioxins onto plant and soil surfaces, and subsequent ingestion of this contaminated vegetation and soil by food animals, is considered to be the primary pathway by which dioxins enter the food chain. The levels produced in this way are considered background levels. Fish become contaminated due to airborne dioxin deposition into the water and also from contaminated soil or industrial waste washed into rivers and lakes, leading to high sediment concentrations. Inhalation and water pathways are not considered significant sources of exposure for terrestrial animals. Contamination above background levels can occur if food animals are fed products which are adulterated, either by natural or unnatural means, with high concentrations of dioxins.

Environmental Fate of PCDDs and PCDFs

The widespread presence of PCDDs and PCDFs is quite expected considering the innumerable sources that emit these compounds into the atmosphere and the overall resistance of these chemicals to abiotic and biotic transformation. Moreover, PCDDs/PCB emitted to the atmosphere can be transported long distances in the atmosphere before they are deposited on vegetation, soil and water via dry and wet deposition.

Deposition onto vegetation and subsequent ingestion of that plant material by animals is hypothesised to be the primary mechanism by which CDD/CDFs enter the terrestrial/agriculture food chain. Deposition onto the soil and its subsequent erosion and runoff into the water bodies with subsequent bioaccumulation by aquatic biota is believed to be the major pathway by which CDD/CDFs enter the aquatic food chain in most fresh water bodies. The ultimate environmental sink of PCDD/PCDFs is believed to be the aquatic sediments.

Dioxins and dioxin-like PCBs are poorly soluble in water, but are adsorbed to the mineral or organic particles suspended in water. When dioxin emissions are transported by air to the surface of oceans and seas they end up being concentrated in the aquatic food chain.

Problems associated with Dioxin and Dioxin like compounds

Dioxins are not soluble in water and are highly soluble in fat. This means that they bind to sediment and organic matter in the environment accumulate in animal and human fatty tissue. In addition they are not biodegradable so they are persistent and bio-accumulate in the food chain. This indicates that once released into the environment, via air or via water, they pile up in the fat tissue of animals and humans.Human exposure to dioxin can occur through working in industries where dioxin is a
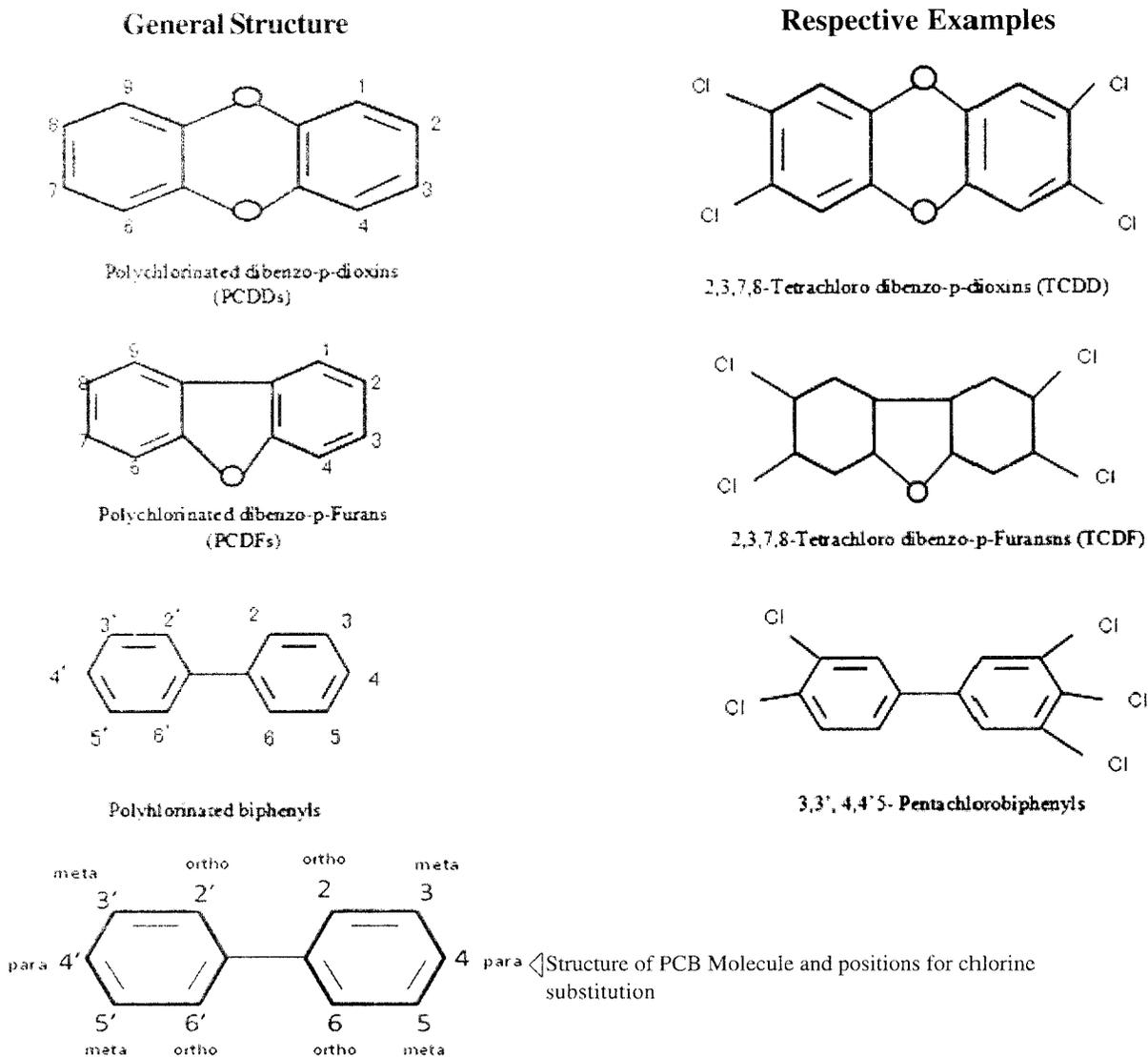
General Structure

Polychlorinated dibenzo-p-dioxins
(PCDDs)

Polychlorinated dibenzo-p-Furans
(PCDFs)

Polychlorinated biphenyls

Respective Examples

2,3,7,8-Tetrachloro dibenzo-p-dioxins (TCDD)

2,3,7,8-Tetrachloro dibenzo-p-Furansns (TCDF)

3,3',4,4'5- Pentachlorobiphenyls

para ◁ Structure of PCB Molecule and positions for chlorine substitution

Fig 1: Basic Strucutre and numbering scheme

by-product, industrial accidents, through food, human breast milk and drinking water. Overall, skin contact or breathing represents very small sources of dioxin exposure. Dioxins and related compounds are suspected of causing neurological disruption. Epidemiological studies indicated that exposure to these compounds caused neurodevelopmental disturbances such as learning disability and attention deficit hyperactivity disorder, which are thought to be closely related to dopaminergic dysfunction. Its accumulation also causes inability to maintain pregnancy, decreased fertility, reduced sperm count, endometriosis, diabetes, immune system suppression, lung problems, skin disorders, lowered testosterone levels. The International Agency for Research on Cancer reported Dioxin, 2,3,7,8-TCDD as Group 1 Carcinogen which means it causes cancer to human beings.

Chemical structures

Both compound classes have a triple-ring structure that consists of two benzene rings connected by a third oxygenated ring. For CDDs, the benzene rings are connected by a pair of oxygen atoms. CDFs are connected via a single oxygen atom.

The PCBs assume a dioxin-like structure when the substituent chlorines occupy: (a) usually no more than one of the ortho positions; (b) both para positions; and (c) at least two meta positions; and (d) the structure is not hindered from assuming the preferred planar configuration. The basic structure and numbering scheme for PCBs is shown in Figure 1 (in pre-page)

Toxicity equivalents

There are many different terms used to express the strength or potency of different PCDDs, PCDFs, and PCBs. Generally the potency of different PCDDs, PCDFs, and PCBs represented in relative term of 2,3,7,8-TCDD.Some commonly used terminologies are Relative Potency (ReP), Relative Potency Factor (RPF),and Toxic Equivalent Factors (TEFs).

Relative Potency (ReP): It is the potency of an individual chemical to cause AhR-mediated toxic or biological effect in an individual organism, cellular, or biochemical assay relative to 2,3,7,8-TCDD. The relative potency of a chemical must be derived from a single *in vitro* or *in vivo* study/experiment, that is a study in which a potencies of a PCDD, PCDF, or PCB congener and a reference chemical (2,3,7,8-TCDD or PCB 126) to cause a particular effect are measured in single experiment or by the same author/s using the same study design in both experiments.

Relative Potency Factor (RPF): It is the potency estimate of PCDD, PCDF, or PCB congener relative to 2, 3, 7, 8-TCDD based on one or more studies to cause AhR mediated toxicity or biological effects, determined using careful scientific judgement after considering all available relative potency data. The ReP database used to derive an RPF for a chemical may include multiple end points, species, and /or *in vitro or in vivo* studies. RPF may be used as alternatives to TEFs when more specific data for the species, end point, and/or site conditions are judged to improve the accuracy of the risk assessment. RPF and ReP are equivalent if RPF is based on single ReP study.
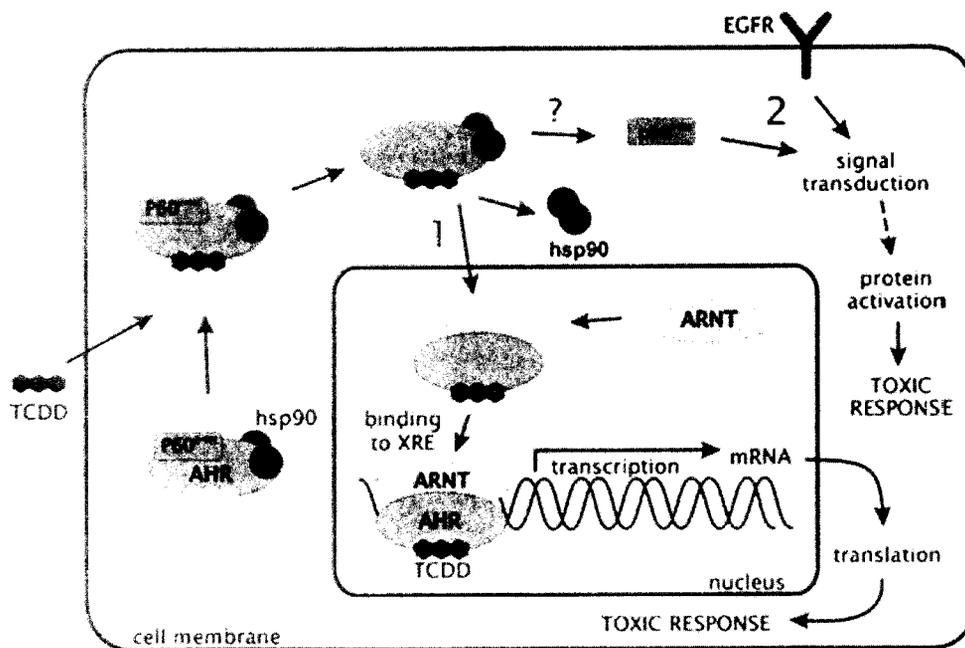
Toxicity equivalent Factors (TEFs): It is the potency estimate of individual PCDD, PCDF, or PCB congeners relative to 2,3,7,8-TCDD based on all available relative potency data obtained from scientific studies. Environmental protection agency (of USA) presently applies to TEFs derived through an international scientific consensus building process supported by the World Health Organisation.

Mode of action of Dioxins

One of the most important pathways of dioxin toxicity is mediated through aryl hydrocarbon receptor (AhR). Aryl hydrocarbon receptor is also known as dioxin receptor. It is ligand activated transcription factor belonging to basic helix loop helix family. The cytosolic form of AhR is dormant or inactive and remains in complex with two units of heat shock protein 90, i.e HSP 90.Upon ligand binding, AhR gets activated. The activation of AhR is associated with conformational change and expose of NLS (Nuclear Localisation Signal). With the activation HSP 90 gets dissociated from the complex and the receptor translocates to the nucleus, where it forms heterodimer with a related protein, aryl hydrocarbon nuclear translocator (ARNT). This heterodimer complex binds to the xenobiotic response element (XRE) and alters expression of genes controlled by enhancer XREs. XREs are found in the promoter regions of many genes involved in the metabolism of xenobiotics, including CPY1A1, CYP1A2, CYP1B1 and NAD(P)H-*Quinone oxidoreductase*. The AhR activates several drug metabolising enzymes and proteins involved in growth and differentiation and is also known to modulate number of other nuclear transcription factors such as receptors of the steroid hormone receptor superfamily and of cell surface receptors such as EGF (Epidermal Growth Factor). Alteration of growth and differentiation of epithelial tissues may be the reason behind most of the toxic response. Overall it may be concluded that persistent activation of AhR through ligand binding is responsible for toxic responses.

Dioxin's action as a tumor promoter and developmental toxicant presumably reflects its ability to alter cell proliferation and differentiation processes. There are several plausible mechanisms by which this could occur. First, TCDD might activate a gene (or genes) that is directly involved in tissue proliferation. Second, TCDD-induced changes in hormone metabolism may lead to tissue proliferation (or lack thereof) and altered differentiation secondary to altered secretion of a trophic hormone. Third, TCDD-induced changes in the expression of growth factor or hormone receptors may alter the

EGFR

? 2

signal
transduction

1

hsp90

protein
activation

ARNT

TOXIC
RESPONSE

TCDD

hsp90 binding
to XRE

ARNT
AHR

transcription mRNA

ARNT
AHR
TCDD

translation

nucleus

TOXIC RESPONSE

cell membrane

Fig 2: Schematic diagram of AhR mediated mechanism of action of Dioxins

sensitivity of a tissue to proliferative stimuli. Fourth, TCDD-induced toxicity may lead to cell death, followed by regenerative proliferation. These mechanisms likely differ among tissues and period of development, and they may be modulated by different genetic and environmental factors.

Determination of dioxins

Determinations of dioxins are quite different from routinely analysed chemicals, the reason being its ultra low presence in technical and environmental samples. Dioxins are generally measured in terms of parts per trillion (ppt) or parts per quadrillion (pqd) whereas other organics are measured in parts per million (ppm) and parts per billion (ppb). The trace analysis of dioxins requires a high sample enrichment which can be obtained by efficient extraction methods and highly selective purification and fractionation techniques. The analytical methods for dioxin and dioxin like compounds must also be highly sensitive and specific such as high resolution gas chromatography along with mass detection system which can separate and quantify the trace amount of the individual PCDD, PCDF and selected PCB congeners. The most common procedure for dioxin determination in various sample types are those approved by the Environmental Protection Agency of United states (US EPA).

This is beyond the scope of this article to explain the entire methodology of sampling, extraction, purification and analysis of dioxins.

Maximum allowable limits of intake

The European Union Scientific Commission for Food (EU SCF) has assessed the risks for public health arising from the presence of dioxins and dioxin-like PCBs in food. Considering the persistent nature of these compounds the SCF has established a tolerable weekly intake (TWI) of 14 picogram toxic equivalents (TEQ) per kilogram body weight for dioxins and dioxin-like PCBs. This TWI is in accordance with the Provisional Tolerable Monthly Intake (PMTI) of 70 pg/kg bodyweight/ per month established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) at its fifty seventh meeting (Rome,5-14 June 2001) and occurs with the lower end of the range Tolerable Daily Intake (TDI) of 1-4 pg WHO-TEQ/Kg body weight, established by the World Health Organization (WHO) Consultation in 1998. (Table 1)

Maximum permitted levels of dioxins and PCBs in Sea food: There are three different ways to denote the maximum levels of dioxins and PCBs in sea food, they are as follows:

1) The sum of polychlorinated-dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDFs), expressed as world Health Organization (WHO) toxic equivalent using WHO-TEFs (Toxic Equivalent Factors)

2) The sum of dioxins, furans and dioxin like polychlorinated biphenyls(PCBs), defined as the sum of PCDDs, PCDFs and PCBs, expressed as WHO toxic equivalent using the WHO-TEFs and

3) The sum of the 6 PCB congeners (PCB 28, PCB52, PCB101, PCB138, PCB153 and PCB180).

Table 1: Maximum permitted levels of dioxins and PCBs in Sea food

Seafood Products	Maximum Permitted level		
	Sum of dioxins (WHO-PCDD/F-TEQ)	Sum of dioxins and dioxin like PCBs (WHO-PCDD/F-PCB-TEQ)	Sum of PCB28, PCB52,PCB101, PCB138, PCB153 and PCB180
Muscle meat of fish and fishery products thereof, with exemption of: - Wild caught eel, - Wild caught fresh water fish, with exception of diadromous fish species caught in fresh water. - Fish liver and derived products - Marine oils - Where fish are intended to be eaten whole, the maximum level applies to the whole fish - The maximum level for crustaceans applies to muscle meat from appendages and abdomen. - In case of crabs and crab like crustaceans (Brachyura and Anomura) it applies to muscle meat from appendages.	3.5pg/g wet weight	6.5 pg/g wet weight	75 ng/g Wet weight
Muscle meat of wild caught fresh water fish, with the exception of - Diadromous fish species caught in fresh water, and products thereof - Where fish are intended to be eaten whole, the maximum level shall apply to the whole fish.	3.5pg/g wet weight	6.5 pg/g wet weight	125 ng/g Wet weight
Muscle meat of wild caught eel (Anguilla anguilla) and products thereof	3.5pg/g wet weight	10pg/g wet weight	300 ng/g Wet weight
Fish liver and derived products thereof with the exception of - Marine oils referred to below. in the case of liver, the maximum applies to the whole of the edible content.	-	20pg/g wet weight	200 ng/g Wet weight
Marine oil(fish body oil, fish liver oil and oils of other marine organisms intended for human consumption)	1.75 pg / g fat	6.0 pg / g fat	200 ng/g Wet weight

References

BAILAR, J.C., III. How dangerous is dioxin? N.Engl. J. Med. (1991) 324: 260-262.

Commission Regulation (EU) No 1259/2011. Amending Regulation (EC)No 1881/2006 as regards maximum levels for dioxins, dioxins-like PCBs and non dioxin-like PCBs in food stuffs, Official Journal of European Union 2011, L320/18-L320/23

EDULJEE GH, GAIR A]. Validation of methodology for modelling PCDD and PCDF intake via the food chain. Science of the Total Environment 1996, 187 (3), 211-229

FRIES GF. A review of the significance of animal food products as potential pathways of human exposures to dioxins. Journal of Animal Science 1995, 73 (6), 1629-1650

FUMIO MATSUMURA. Mechanism of action of dioxin-like chemicals, pesticides and other xenobiotics effecting nutritional

indexes, Am J Clin Nutr.(1995); 61

HYUNSUNG P, KO, STENVEN T OKINO, QIANGMA and JAMES P, WHITLOCK JR. Dioxin-Induced CYP1A1 transcription In Vivo: the Aromatic Hydrocarbon Meceptor Mediates Transaction, Enhancer-Promoter Communication, and Changes in Chromatin Structure, (1996), Molecular and Cellular Biology, 430-436.

JOHN S. GRAY. Biomagnification in marine systems: the perspective of an ecologist. Marine Pollution Bulletin 45 (2002) 46-52

Office of the Science Advisor, risk Assessment Forum, United States Environmental protection Agency. Framework Application of the toxicity equivalence methodology for polychlorinated dioxins, furans and biphenyls in ecological risk assessment, (2008), EPA 100/R-08/004

SCHECTER A, CRAMER P, BOGGERS K, et al. Levels of dioxins, dibenzofurans, PCB and DDE congeners in pooled food samples collected in 1995 at supermarkets across the United States.Chemosphere.1997, 34(5), 1437-1447

SYLWIA OLESZEK-KUDLAK, ETSURO SHIBATA, TAKASHI NAKAMURA, XIAN-WEI LI, YONG-MEI YU. Review of the sampling and pretreatment methods for dioxins determination in solids, liquids and gases Journal of the Chinese Chemical Society, (2007), 54, 245-262

United Nations Environment Programme; Dioxin and Furan Inventories. National and regional Emissions of PCDD, Geneva, Switzerland, (1999)

UNEP/GPA, 2006a, The State of the Marine Environment: Trends and processes, The Hague

WARD, C.T. and F.MATSUMURA. Fate of 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) in a model aquatic environment. Arch. Environment Contam.Toxicol. (1978) 7:349-357.

www.helsinki.fi/science/dentenv/dioxin.html

Training on integrated brackishwater fish and crustacean culture

A ten day hands-on training course on the integrated fish and crustacean culture was organised by ICAR-Central Institute of Brackishwater Aquaculture (CIBA), Chennai during 20-30 July 2015 for the benefit of first time fish and shrimp farming entrepreneurs. Totally fifteen candidates from Gujarat, Maharashtra and Tamil Nadu participated in the training programme.

Dr. K. K. Vijayan, Director, CIBA welcomed the trainees and briefed the importance of sustainable brackishwater aquaculture practices as an economical development activity. He highlighted the farmer-friendly technologies developed by the institute for the benefit of the farmers in the brackishwater sector. He cautioned the farmers about the burgeoning of non-scientific aquaculture practices along the coastal states and emphasised the farmers to take up aquaculture in a scientific way with proper guidance. Dr. C. Gopal, Course Director and Head of the Crustacean Culture Division, CIBA, mentioned that there is a drastic upsurge in shrimp farms in India during the past three years due to better return on investment in Pacific white leg shrimp Litopenaeus vannamei culture compared to black tiger shrimp, Penaeus monodon. He emphasised the importance of diversification of shrimp culture with other economically viable candidate shrimp and finfishes and suggested the trainees to take up culture in scientific and eco-friendly manner.

The training programme covered various aspects of management practices to be adopted for successful scientific fish, shrimp and crab culture. The scientific faculty from CIBA explained in detail on various aspects of scientific management of fish and shrimp culture with respect to pond, feed, health management, evolving farming techniques like polyculture, integrated multitrophic aquaculture, biofloc based culture, periphyton based shrimp culture, etc. The trainees were also explained about site selection, design and construction of ponds and also guidelines of Coastal Aquaculture Authority for shrimp farming in India.

During the ten days training programme, the sessions were arranged in such a way that that the trainees get comprehensive information on various practical aspects of water and soil quality management, health management in brackish water aquaculture, and importance of disease diagnostics in shrimp culture. The trainees got hands-on experience in hatchery seed production and breeding of commercially important finfishes like Asian seabass, Chanos chanos, ornamental fishes and shrimp at the experimental research station of CIBA, Muttukadu. In addition to this they were trained in shrimp feed formulation and preparation of various types of feeds like sinking, floating and larval feeds, shrimp and crab nursery rearing in cages and their grow out management techniques, importance of live feed in fish and shellfish seed production. A field visit to mud crab grow out ponds at Chinna Cuddalore, Kalpakkam and automatic feeder demonstration farm site (L.vannamei shrimp farm) was also arranged as part of the training programme.

The training course came to an end with the valedictory programme, where in, an interaction session was arranged followed by certificate distribution to the trainee by the Director. A consolidated report about the training course was presented by Dr.Shyne Anand Scientist, CIBA. The vote of thanks was proposed by Dr. P. Nila Rekha, Principal Scientist CIBA, Chennai. ■