

Prevalence of Xenoestrogen in muscle tissues of edible fin and shell fish collected from local fish market in Mumbai, India

Hitesh U. Shingadia

SVKM's Mithibai College of Arts, Chauhan Institute of Science and Amrutben Jivanlal College of Commerce and Economics (Autonomous), Vile Parle, West Mumbai 400 056 M.S., India

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ABSTRACT

The uptake of noxious waste transpires directly from surrounding marine water across the absorptive body surface and from food in conjunction with the seawater to finally into the alimentary canal of the aquatic organisms. Xenoestrogen are often associated with sewage outfalls and industrial sites. Recent studies also suggest that the public may be exposed to BPA (Bisphenol A) by handling cash register receipts apart from plethora of sources. Phthalate esters are widely used plasticizers that are present in many daily used products. Fishes and various other invertebrates have been widely documented as bio-indicators of pollution and have been investigated to estimate health of the environment. The bioavailability of various xenoestrogen compounds is a key factor in of concern in tissues of aquatic biota. Researchers have raised concern as some laboratory animal studies report subtle developmental effects in fetuses and newborns exposed to low doses of BPA. The widespread use of phthalates makes them almost ubiquitous and thus, the issues about their long-term effects on human health are of great concern. The aim of this study was to survey the actual presence of xenoestrogens in muscle tissue of few of the edible variety of fishes of commercial significance. In the present study the actual presence of the suspected endocrine disrupter Monomethylphthalate in 12-51 ng/g & Bisphenol A (BPA) in 5 to 68 ng/g in the muscle tissue of finfish and monomethylphthalate in 18-26 ng/g & BPA in 8 to 36 ng/g in the muscle tissue of shell fish. Based on the present measured concentrations in the edible muscle tissues and on literature derived toxicity data it was concluded that neither eco-toxicological effects nor estrogenic effects are likely to occur in the present situation. The bottom dwelling organisms predominantly devour upon the benthic fauna which might have accumulated variety of xenoestrogens that was absorbed by the sediment since BPA, justify its occurrence. Safe disposal of industrial effluents, domestic sewage and navigational activities should be practiced in harmony with nature and enforcement of laws be enacted to protect our marine environment and/or, recycled to circumvent these contaminants from entering into the marine environment.

Key words: Monomethylphthalate, Bisphenol A, Edible Fin Fish, Shell Fish

Introduction

Human biomonitoring is a tool for evaluating the exposure to potentially critical chemicals found in the environment, contaminated foodstuffs, lifestyle, use of different products and also other sources of

pollutants. This approach allows depicting the body burden from all exposure sources and has been increasingly applied in environmental health surveys in the last decade all across the globe (Angerer *et al.*, 2007). The progress in industrial development has led to augmented emission of pollutants into envi-

ronment dilapidating ecosystems. As a consequence of anthropological activities, aquatic animals are exposed to elevated intensities of these environmental contaminants. BPA is a persistent environmental pollutant sourced from certain thermal paper products, including cash registers and ATM receipts, some dental sealants and composites, leached into food from the epoxy resin lining of cans and from consumer products such as polycarbonate tableware, food storage containers, water bottles and baby bottles (Biedermann *et al.*, 2010). Additional traces of BPA can leach out of these products when they are heated at high temperatures. Human exposure to BPA is widespread, for instance in 2003-2004 National Health and Nutrition Examination Survey (NHANES), conducted by the Centers for Disease Control and Prevention (CDC), found detectable levels of BPA in 93% of Americans six years and older. Another reason for concern, especially for parents, may be because some laboratory animal studies report subtle developmental effects in fetuses and newborns exposed to low doses of BPA (Calafat *et al.*, 2009). Exposure to bisphenol A and other phenols in neonatal intensive care unit premature infants. *Environmental Health Perspectives* 117(4):639-644.

The coastal zones of the maritime states are vastly inhabited and developed areas due to industrial set ups. Sea food, viz. fin fish as well as shell fish like shrimp, crab, oyster and mussel are delicacies and form an essential food commodity of the coastal populace. The bioaccumulation potent of pollutants in marine/estuarine organisms is of imperative concern to mankind. Bioconcentration is defined as 'the net result of the absorption, distribution and elimination of a substance in an organism, after an exposure via water'. Phthalates and their metabolites are classified as endocrine modulators. They affect the hormonal balance in both children and adult. Phthalates not only induce oxidative stress but also activate compensatory anti-oxidant systems (Kang *et al.*, 2010). Sea food forms the chief link for the probable relocation of these impurities into the human beings. Information on the level of heavy metal pollution in coastal environment is essential as they are the source for deleterious environmental health hazards. Based on data collected under COMAPS (Coastal Ocean Monitoring & Prediction System) since 1991 coastal waters along Mumbai are reported as some of the areas of concern that needs continuous intensive monitoring (Sakthivel *et al.*,

2000).

Muscle tissue of fish is the most frequently used for analysis because it is a major target tissue for metal storage and is the main edible part of the fish for the consumers. Fish is a vital source of food for hundreds of millions of people across the globe. In 2008, about 81% (115 million tons) of estimated world fish production was used as human food with an average per capita of 17 kg (FAO, 2010a). Consumption of fish provide an essential source of protein, polyunsaturated fatty acids (PUFA, Omega-3-fatty acid), lipid soluble vitamins and essential minerals that are concomitant with health benefits and normal growth. According to FAO (2010b) statistics, fish accounted for about 16% of the global population's intake of animal protein and 6% of all protein consumed. Evaluation of heavy metals along the food chain may throw light on the heavy metal input to the human body from sea food. Marine organisms exposed to heavy metals have been consumed as sea foods and thus are linking pathways for the relocation of toxic heavy metals in human beings. Therefore, it often becomes mandatory to check chemical contaminants in foods from aquatic environment to comprehend their hazardous intensities.

Xenoestrogen refers to 'foreign estrogens', concentrated endocrine disrupting chemicals, which are found in food, soil and air (Dussault *et al.*, 2008). Xenoestrogen are capable of mimicking body's estrogen hormone and attaching to estrogen receptors. Estrogen mimickers interfere with body's natural circulating estrogens; disrupt hormonal balance and normal physiological functions. Among these high-volume chemicals are the alkylphenolpolyethoxylates (APEOs) including their degradation products, the alkylphenols and bisphenol A. Both the alkylphenols and bisphenol A have been observed to possess estrogenic activity in in-vitro and in-vivo screening systems. The APEOs belong to one of the largest groups of non-ionic surfactants and are used both as detergents and in many formulated products like herbicides, pesticides and paints. Bisphenol A is used in the production of polycarbonate, epoxy resins, flame retardants and various other products. Phthalate esters are widely used plasticizers that are present in many daily used products. Although some of their reproductive effects have been reported, pubertal development effects from prenatal exposure to phthalates awaits further investigations. Pen *et al.* (2015) re-

ported no evidence of an association between phthalate exposure and ovarian volume or testicle size investigated a birth cohort with prenatal phthalate ester exposures assessed in mothers' urine samples at pregnancy in relation to puberty outcomes, focusing on bone age for both genders, uterus size and ovarian volume for girls and testicle size for boys at age eight and 11 years old in central Taiwan. This analysis suggests phthalate exposure may affect specific pubertal development characteristics in human beings. Chemicals like phthalates are almost ubiquitous in our living environment because of our modern life style. Research interest in their biological and clinical effects to the health of reproductive system or other related organs in human beings or animals is increasing. Newborns admitted into neonatal intensive care unit are potentially exposed to a higher dosage of plasticizers from medical devices (Su *et al.*, 2012b).

Aim of the present study was to assess the concentrations of xenoestrogen in edible muscle tissues of commercially valuable fishes from marine/estuarine fishes sold in the local fish market in Mumbai Coast of India. Further, their hazardous levels were compared with available certified safety guidelines proposed by World Health Organization (WHO) and Food and Agricultural Organization (FAO) for human consumption.

Materials and Method

The study was aimed to investigate metal concentration in muscle tissue of eleven commercially importance marine/estuarine water fin fishes, four crustaceans (prawns and crabs) and one molluscan (green lip mussel) shell fishes collected from the local fish market in Mumbai city. The following species of fin fishes were selected: Shark (*Scoliodon sorrakowah*), Sting ray (*Dasyatis uarnak*), Mackerel (*Rastrelliger kanagurta*), Oil Sardine (*Sardinella longiceps*), Seer fish (*Scomberomeros guttatus*), Silver pomfret (*Stromateus cinereus*), Indian Salmon (*Elutheronema teradactylum*), Bombay duck (*Harpodon nehereus* Ham-Buch), common Sole (*Cynoglossus elongatus* Linnaeus, 1758), Ribbon fish (*Leptutacanthus savala*) and Black pomfret (*Parastromateus niger*). The crustacean shell fishes like prawns (*Penaeus indicus* and *Solenocera crassicornis*), crabs (*Scylla serrata*, *Neptunus pelagicus* and *Charybdis cruciatus*) and molluscan shell fish like green lip mussel (*Perna viridis*) were analyzed.

Xenoestrogen estimation was carried out using

Gas Chromatography Mass Spectrometry (APHA, 2005; Hatcher *et al.*, 1988). The weighed fish muscle tissues were digested overnight in a mixture of solvent, Dichloromethane:Methanol (2:1, v/v), filtered with Whatman 42 filter paper. 4 mL Potassium chloride solution was added to the filtrate and centrifuged at 1000 rpm for 10 min. Organic layer was collected and evaporated to dryness and redissolved in Methanol:Hexane (1:20, v/v) mixture for estimation of xenoestrogen.

Statistical analysis of data was carried out using Statistical package program (Zar, 1984). Inter-heavy metal correlations in the fish muscle were investigated. The Pearson correlation coefficient was used to measure the strength of the association between heavy metal concentrations in muscle tissue and presented in correlation matrices (Pentecost, 1999). The p-values of less than 0.05 and 0.01 were considered to indicate statistical significance.

Results and Discussion

In the present study the actual presence of the suspected endocrine disrupter monomethylphthalate in 12-51 ng/g and bisphenol A (BPA) in 5 to 68 ng/g in the muscle tissue of finfish and monomethylphthalate in 18-26 ng/g and 8 to 36 ng/g in the muscle tissue of shell fish respectively (Table 1 and 2; Fig. 1 & 2). Highest concentration of both the xenoestrogens was detected in *S. longiceps* and *S. serrata*. However, BPA was only present in detectable level in *S. serrata* among the shell fishes, while in green lip mussel (*P. viridis*) both were not detected. The cartilaginous fishes like *S. sorrakowah*, *D. uarnak* and demersal bony fish, *C. elongatus* only BPA was observed to be present. In *H. nehereus* & *L. savala* only monomethylphthalate was detected. Lindholm *et al.* (2000) also detected BPA in the muscle of rainbow trout, but at much lower concentrations. This observation is in correspondence with the present investigation. The risks of estrogenic effects of the present BPA levels in the environment can be based on the observed estrogenic response in rainbow trout (Lindholm *et al.*, 2000). At a selected number of location, the presence of BPA in fish was studied, which showed that BPA varied from 2 to 75 ng/g in the liver and 1 to 11 ng/g in the muscle. Based on present measured concentrations in surface water and on literature derived toxicity data Belfroid *et al.* (2002) concluded that eco-toxicological effects nor estrogenic effects are likely to occur in the field situ-

Table 1. Concentration of Xenoestrogens in muscle tissue of fin fishes

Fin Fish Species	Xenoestrogens	
	Monomethylphthalate (ng/g)	Bisphenol A(ng/g)
<i>Scoliodon sorrakowah</i>	ND	27.97±0.146**
<i>Dasyatus uarnak</i>	ND	22.61±0.228*
<i>Harpodon nehereus</i>	13.42±0.165**	ND
<i>Rastrelliger kanagurta</i>	24.79±1.098**	47.54±2.194
<i>Sardinella longiceps</i>	50.92±2.416	68.01±1.936**
<i>Stromateus cinereus</i>	46.237±0.923*	67.43±1.295*
<i>Elutheronema tetradactylum</i>	26.75±3.142	41.72±0.094*
<i>Scomberomorus guttatus</i>	36.07±2.074*	66.9±1.641**
<i>Cyanoglossus elongatus</i>	ND	23.37±3.284
<i>Lepturacanthus savala</i>	42.18±3.231	ND
<i>Parastromateus niger</i>	46.85±2.653	63.71±1.782*

All values are mean ± SE. * & ** indicate P < 0.05, P < 0.01

ation. Yejikim *et al.*, (2020) found BPA concentration to be higher in men who frequently consumed large fish and tuna, shellfish and other seafood.

Only mono Bisphenol A (BPA) is a widely used compound in the production of epoxy resins and

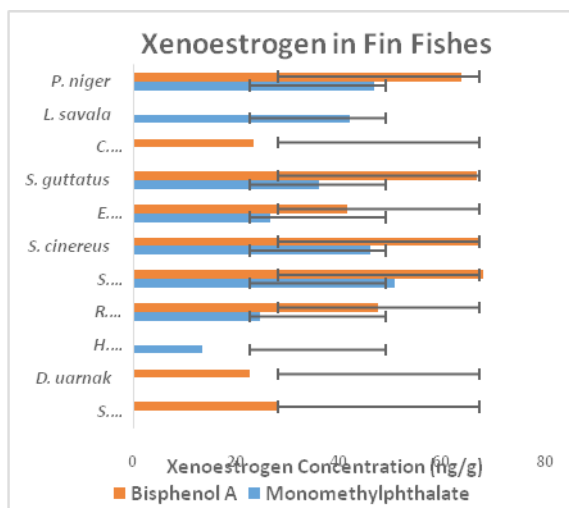


Fig. 1. Concentration of Xenoestrogen in muscle tissue of fin fishes

polycarbonate plastics. Recent research showed that BPA also has estrogenic potency and is therefore generally mentioned as one of the suspected endocrine disrupter (Toppari *et al.*, 1995). However, based on measured and calculated environmental concentrations (the calculated values derived from discharge levels) and the predicted no effect concentration (PNEC, set at 64l g/L), no potential adverse effect is expected for aquatic organisms (Staples *et al.*, 2000) as observed in the present study. The risks

of estrogenic effects of the present BPA levels in the environment can be based on the observed estrogenic response in rainbow trout (Lindholm *et al.*, 2000). Belfroid *et al.* (2002) studied the distribution of the occurrence of BPA in surface water throughout the Netherlands. They reported the presence of BPA in sessile blue mussel (*Mytilus edulis*) and migratory fish like estuarine ounder (*P. esus*) from waters in Netherlands that contained BPA in the concentration of 10 ng/g wet tissue weight of mussel and 250 ng/g in the liver tissue of the flounder. The liver tissue has the additional advantage that it is an important organ for contaminants uptake and biotransformation and therefore generally contains high concentrations. Explanation for the observed presence of BPA is that the bottom dwelling species feed primarily on benthic fauna which might have accumulated BPA that was absorbed by the sediment since BPA is not a highly lipophilic compound (Staples *et al.*, 1998) and thus that its tendency to

Table 2. Concentration of Xenoestrogen in muscle tissue of shell fishes

Shell Fish Species	Xenoestrogens	
	Monomethylphthalate (ng/g)	Bisphenol A(ng/g)
<i>Penaeus indicus</i>	19.11±2.348	ND
<i>Solenocera crasicornis</i>	18.04±2.116	ND
<i>Scylla serrata</i>	25.07±0.793**	35.21±0.967**
<i>Neptunus pelagicus</i>	23.69±2.694	ND
<i>Charybdis cruciatus</i>	21.48±0.681*	ND
<i>Perna viridis</i>	ND	ND

All values are mean ± SE. * & ** indicate P < 0.05, P < 0.01

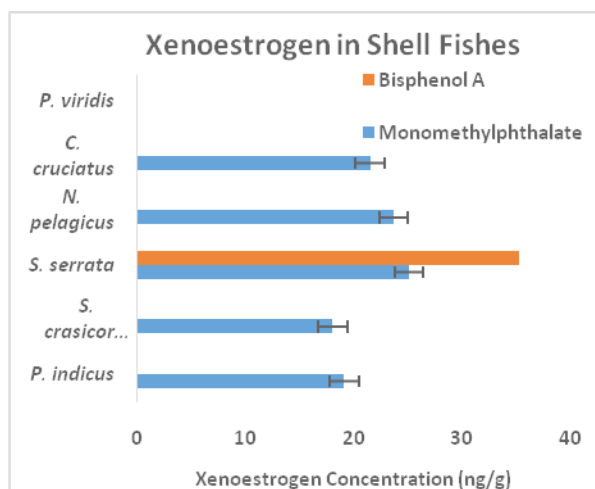


Fig. 2. Concentration of Xenoestrogen in muscle tissue of shell fishes

sorb to sediment is not considerably large.

Recommendations

- Avoid microwave polycarbonate plastic food containers. Polycarbonate is strong and durable, but over time it may break down from repeated use at high temperatures.
- Avoid plastic containers with the #7 on the bottom.
- Avoid washing polycarbonate plastic containers in the dishwasher with harsh detergents.
- Minimize consumption of canned foods, rather eat fresh or frozen foods.
- Whenever possible, choose for glass, porcelain or stainless steel containers, mainly for hot food or liquids.
- Promote usage of infant feeding bottles, tethers', etc. that are BPA free and also look for toys that are labeled BPA free.

Conclusion

In view of the prominence of finfish and shell fish that form an integral part of protein rich diet of human, it is necessary that biological monitoring of the fish meant for consumption should be carried out recurrently to warrant safety of the seafood. Fish is an important food resource for human consumption and a major component of the marine ecosystem with tremendous export potential, thus assessment of the metal effects is particularly important. Therefore, studies on the presence of xenoestrogen in marine fish will contribute to the accrual of new

data on their levels in species of marine organisms with commercial significance, thus to make a more valid conclusion further experimentation would be required so as to tap the source of these pollutants and bioaccumulation at various trophic structures of the marine ecosystem. From this analysis, status of coastal waters of the Mumbai Coast will be apparently predicted for their pollution by xenoestrogen. Elaborate studies with bigger sample sizes across the biota along with their environment over longer follow-up periods are obligatory.

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