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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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Investigating and Measurement of Residues of Chlorobenzilate (Organochlorine Pesticides) in Four Species of the Most Consumed Fishes in Caspian Sea (Iran)

M. Shokrzadeh[1] and A.G. Ebadi[2]
[1]Department of Toxicology, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran
[2]Department of Biology, Islamic Azad University of Sari, Sari Branch, 48164-194, Iran

Abstract: In this study samples of four species of the most consumed fishes (Sefid, koli, kilca and kafal fish) were analyzed for concentrations of Chlorobenzilate (organochlorine pesticides). Fish were captured using electric fishing on four sites (Chalous and Babolsar city and Khazar Abad and Miankaleh regions) in Mazandaran provinces of Iran. Quantitative determination of the Chlorobenzilate was performed by gas chromatography electron-capture detection (GC–ECD). Samples contained detectable concentrations of Chlorobenzilate but at concentrations below the maximum residue limit (MRL). No different found between kinds of fishes in each sites about Chlorobenzilate concentrations but there are two groups of sites that were significantly different from one another in terms of Chlorobenzilate concentrations. According to insecticides analyzed in four kinds of fishes the Kafal in the hunting region of Khazar abad had greatest amount of 0.038 PPM. Kilca from Babolsar had the highest amount of Chlorobenzilate, 0.035 PPM ($P<0.05$). In the case of Sefid and Koli fishes in regions, significant difference was not seen. It is necessary to mention that no research has so far been done to be a criterion for comparison in this area sites. However, the concentrations of Chlorobenzilate residues in the muscle were found to be lower than the FAO/WHO recommended permissible and should not be public concern among peoples in Mazandaran province.

Key words: Chlorobenzilate, organochlorine pesticides, caspian sea, fish, Iran

Introduction

The term organochlorine refers to a wide range of organic chemicals, which contain chlorine and sometimes several other elements. A range of organochlorine compounds have been used in Iran, including herbicides, insecticides, fungicides and industrial chemicals such as polychlorinated biphenyls (PCBs). The compounds are characteristically very stable (Ware, 1986; Shereif and Mancy, 1995; Ingrid and Joan, 2002).

This characteristic is widely recognized as being a problem in some uses such as pesticides and transformer oils, because the chemicals can be distributed in the environment especially river and sea where they persist long after their original use. They degrade slowly and being fat-soluble, accumulate in the food chain, eventually ending up in the fat of our bodies (Smith, 1991; Kidd and James, 1991).

Key properties of organochlorines, which cause concerns, are persistence and toxicity. While organochlorine pesticides were manufactured for their toxicity, the fact that they were also persistent had advantages in that they remained effective against target pests for prolonged periods. Therefore the chlorinated organic compounds held an important position in pest control in agriculture for a long time, being versatile and, against some pests, very effective (Ware ,1986; Shereif and Mancy, 1995; U.S., 1995).

Chlorobenzilate was introduced in 1952. It is used for mite control on citrus crops and in beehives (U.S., 1990; Vidar and Anuschka, 1998). It is nonsystemic, meaning that it is not absorbed or transported throughout a plant. It has little insecticidal action, killing only ticks and mites (Vidar *et al*., 1998).

Chlorobenzilate is available as emulsifiable concentrate and as wettable powder formulations (Vidar *et al*., 1998; Hall, 1999). Chlorobenzilate is slightly to moderately toxic to humans. Symptoms of acute poisoning from ingestion of chlorobenzilate include incoordination, nausea, vomiting, fever, apprehension, confusion, muscle weakness or pain, disturbed sense of balance, dizziness, weight loss, wheezing and coma. Symptoms may occur within several hours after exposure. Death may result from discontinued breathing or irregular heartbeats (Hall ,1999).Chlorobenzilate is a mild skin and eye irritant (Berg, 1986). The oral LD50 for chlorobenzilate in rats and hamsters is 700 mg/kg, and 729 mg/kg in mice (Hayes and Laws, 1990).

Prolonged or repeated exposure to chlorobenzilate may cause the same effects as acute exposure (Gosselin, 1984). After continuous exposure to chlorobenzilate, 16 out of 73 workmen tested had abnormal electroencephalograms, or EEGs. EEGs are recordings of electrical activity of the brain. The most severe brain activity changes were seen in those persons exposed to the herbicide for one to two years (Griffeth and Duncan, 1985). Chronic skin exposure to chlorobenzilate may cause inflamed skin or rashes, also known as dermatitis. Chronic eye exposure to this material may cause conjunctivitis (Gosselin, 1984; Griffeth and Duncan, 1985).

The use of chlorobenzilate has been restricted in the U.S. because the compound is tumor-forming (oncogenic) in rats and mice. Atrophy of the testes was observed in a two-year study of male rats. Symptoms of poisoning in test animals included depression, salivation, tearing, diarrhea and deep, rapid breathing (Griffeth and Duncan, 1985; Lyman, 1983).

Chlorobenzilate has a slight tendency to accumulate in fatty tissues. Intense activity or starvation may mobilize the stored pesticide and cause the reappearance of toxic symptoms. In a study funded by the National Pesticide Hazard Assessment Program of EPA, detectable traces of chlorobenzilate were found in urine collected from Texas and Florida citrus-grove growers and workers. The collected information showed a range of exposure: from low levels in harvest season pickers, exposed to little or no chlorobenzilate exposure, to higher levels among permanent or semi- permanent workers employed during the spraying season. Among all workers, urinary values ranged from zero to 63.6 ppm. This acaricide has not been found in human milk in the United States (Meister, 1992). Chlorobenzilate is not expected to bioconcentrate in aquatic organisms. Its LC50 is 0.7 mg/l for 95 hours in rainbow trout .

Chlorobenzilate adsorbs to sediment and suspended particulate material in water. It is practically insoluble in water. It is not expected to volatilize or to bioconcentrate in aquatic organisms, but may be subject to biodegradation .

The Caspian Sea, the largest inland sea in the world, is bordered by five countries: Iran, Azerbaijan, Turkmenistan, Kazakhstan and Russia. It has no outlets and acts as a reservoir for water in the region. Environmental pollutants found in the sea probably arrive via the Mazandaran and Gillan rivers. Industrial complexes along the coast particularly in mazandaran and Gillan provinces, in Iran, also discharge waste directly into the Caspian sea.

It is important to note that the use of almost all the chemicals mentioned above is now banned in Iran, and that a nationwide plan is being developed for their overall management (Hayes and Laws, 1990).

The goal of this study was to survey levels of organochlorines (Chlorobenzilate) in the Four species of the most consumed fishes that have been hunted from four central fishery locations in order to estimate the potential of human exposure.

Materials and Methods

Four commonly consumed fish (Sefid = Rutilus frisikutum, Koli = Clupeonella delicatula, Kafal = Mugila auratus and Kilka = Vimba vimba) were selected to analysis.

Procedure: All samples were collected from Caspian Sea in July and August 2004. 100 individuals of each fish were collected from four sites (*Chalous and Babolsar city and Khazar Abad and Miankaleh region*). Dorsal muscle of the samples were removed and frozen at –20°C and shipped to central laboratory (Sari city) for analysis and finally concentration of residues of Chlorobenzilate, were determined.

Sample preparation and analysis: The sample preparation and analysis protocols are similar to those described in Vidar and Anuschka, 1998. Briefly, approximately 5 g of dorsal muscle from Samples fish was thawed and homogenized with 60 g of anhydrous sodium sulphate in a mortar until a free-flowing powder was obtained. The sample was extracted with 225 ml of 1: 1 methylene chloride/hexane. Extracted sample was injected to Gas chromatography in electron capture detector (ECD). OC levels (Chlorobenzilate) were measured using the internal standard method in conjunction with the corresponding external standards using selected ion monitoring mode (FAO/WHO ,1993).

Results and Discussion

The Amounts of Chlorobenzilate Contents in all Samples of four Examined fishes (Sefid, Kafal, Kilca and Koli) of Caspian Sea Were Measured and Represented in Table 1.

According to Table 1, Residues of Chlorobenzilate in Kafal fish Samples had maximum amounts (0.038 ppm) in Khazar abad Region. In Babolsar Region, Kilca fish presented the greatest quantity of Chlorobenzilate (0.035 ppm). In the case of Sefid and Koli fishes significant difference was not seen.

Statistical analysis (One–way ANOVA), indicatad a significant difference regarding Chlorobenzilate ($P< 0.05$, Sig 0.082) among fishery sites.

The results presented that difficulty of poisonous residues is very serious and important and Since researches in the case of Caspian sea fishes is Very Little, to compare with this Study, then, there is a requirement to complete Survey in Caspian sea (Mazandaran Province) and Southern Coasts of Caspian sea .

Other Study in North Atlantic indicated that means of DDT (0.002 ppm), lindane (0.002 ppm), Dieldrin (0.006 ppm) and Endosulphan (0.007 ppm) in Liver Samples (in Shirbit fish), that these Levels ware lower than quantities proposed by WHO (0.05 ppm) (FAO/WHO, 1993).

Quantities of Chlorobenzilate in Caspian sea (Table 1) was lower than WHO Standard Levels (0.05ppm) But in comparison to all regions and other poisons , Presented higher quantities for great use by farmers in Northern Province in Iran (Southern coasts of Caspian sea) and great distribution by agriculture center in Mazandaran among Farmers.

Table 1: The average quantities of Chlorobenzilate Contents (ppm) in four Speices of fishes under study in the Caspian Sea

Region	Kind of fish	Mean of Chlorobenzilate (ppm)
Chalus	Sefid	0.025
Site IV	Koli	0.028
	Kafal	0.026
	Kilca	0.019
Babolsar	Sefid	0.032
Site III	Koli	0.017
	Kafal	0.018
	Kilca	0.035
Khazar abad	Sefid	0.031
Site II	Koli	0.025
	Kafal	0.038
	Kilca	0.023
MianKaleh	Sefid	0.028
Site I	Koli	0.024
	Kafal	0.023
	Kilca	0.027

There is evidence that the population of seals in the Caspian Sea is declining and fertility rates are decreasing. Further studies on contaminants in live animals and biomarker responses that may indicate reproductive interference are needed before we can conclude that the high levels of Chlorobenzilate insecticides in this population are lexicologically important (FAO/WHO, 1993; Shereif and Mancy, 1995; U.S., 1995).

Additionally, chemical analysis has demonstrated the presence of highly toxic contaminants such as the Chlorobenzilate. No long-term monitoring data exist for these compounds, which may affect fish and wildlife at extremely low concentrations (Occupational Health Services, 1986). New approaches and technologies, capable of detecting chemical exposure and its effects at all levels of biological organization, will be required to monitor and assess highly toxic chemicals and those that do not accumulate in fish and wildlife before concentrations reach harmful levels (Shereif and Mancy, 1995; Ingrid and Joan, 2002).

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