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*Research Note*

# Measurement of Residues of Chlorobenzilate (Organochlorine Pesticides) in Fishes from Caspian Sea (Iran)

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**Abstract:** Pesticides are compounds used by human beings especially farmers for controlling biotical factors for a long time and can bring a lot of environmental problems. Among these compounds organochlorine insecticide sprays have high half-life, the property of maintaining for a long time and high lipophilicity characteristic due to having chronic consequences that result from consuming of this compound. It is necessary to evaluate and analyse in environment especially in foods as well in fishes which have high fat and can carry this kind of insecticide. So we have investigated the remains of chlorideorgano insecticide sprays such as chlorobenzilate in the four most-consumed kinds of fish in the Caspian sea. In this investigation we have chosen four different kinds of fish, which arc highly used and available in the Caspian sea (sefid, koli, kilca and kafal). We selected 100 samples of fish from four different hunting regions (Noushahr port and three cities: Babolsar, Mahmoud Abad and Ramsar) in 2006. After cleaning, we prepared Hun muscle of the samples and after the process of extraction, we distilled them in vacuum by means of organic solvent. At last we evaluated the remains and reported based on PPM by Gas Chromatography (GC) with ECD detector. The results of insecticides analyzed in four kinds of fish showed that kafal in the hunting region of Mahmoud Abad had highest amount of 0.032 PPM. Kilca from Noushahr port had the highest amount of chlorobenzilate, 0.028 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, but, fortunately the amount of the insecticide sprays has not reached to standard level in fishes, and it raises an alarm bell.

**Key words:** Chlorobenzilate, organochlorine pesticides, Caspian sea, fish.

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## 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 (Agarwal, 1976; Albaiges et al., 1987; Barlas, 1999).

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 (Berny et al., 2002; Bevenue, 1976).

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 (Bindelman et al., 1990).

Chlorobenzilate was introduced in 1952. It is used for mite control on citrus crops and in beehives. It is non-systemic, meaning that it is not absorbed or transported throughout a plant. It has little insecticidal action, killing only ticks and mites. Chlorobenzilate is available as emulsifiable concentrate and as wettable powder formulations (Binelli and Provini, 2003).

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. Chlorobenzilate is a mild skin and eye irritant. The oral LD50 for chlorobenzilate in rats and hamsters is 700 mg/kg, and 729 mg/kg in mice (Bressa et al., 1997).

Prolonged or repeated exposure to chlorobenzilate may cause the same effects as acute exposure. 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. Chronic skin exposure to chlorobenzilate may cause inflamed skin or rashes, also known as dermatitis. Chronic eye exposure to this material may cause conjunctivitis (Carson, 1963; Cossarini-Dunier et al., 1987).

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 (FAO/WHO, 1993).

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. 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 (Ferraro et al., 1991).

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. 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.

## Material 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 2006. Hundred individuals of each fish were collected from four sites (Noushahr port, Babolsar city, Mahmoud Abad city and Ramsar city). 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 sample fish was thawed and homogenised 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 (Fox et al., 2001).

## Result 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 Figure 1.

According to Figure 1, residues of chlorobenzilate in kafal fish samples had maximum amount (0.032 ppm) in Mahmoud Abad city. In Noushahr port, kilca fish presented the greatest quantity of chlorobenzilate (0.028 ppm). In the case of sefid and koli fishes significant difference was not seen. Statistical analysis (One-way ANOVA) indicated a significant difference regarding chlorobenzilate ($P< 0.05$, Sig 0.078) among fishery sites.

The results showed that problem of poisonous residues is very serious and important and since research 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 mean values of DDT (0.002 ppm), lindane (0.002 ppm), dieldrin (0.006 ppm) and endosulphan (0.007 ppm) in liver samples (in Shirbit fish), were lower than quantities proposed by WHO (0.05 ppm). Quantities of Chlorobenzilate in Caspian sea (Figure 1) were lower than WHO standard levels (0.05 ppm) but, in comparison to all regions and other poisons, presented higher quantities for large use by farmers in Northern Province in Iran (southern coasts of Caspian sea) and great distribution by agriculture centre in Mazandaran among farmers (Gudrun and Lillemark, 1998; Hoekstra et al., 2003; Jaward et al., 2004; La Rocca et al., 1991).

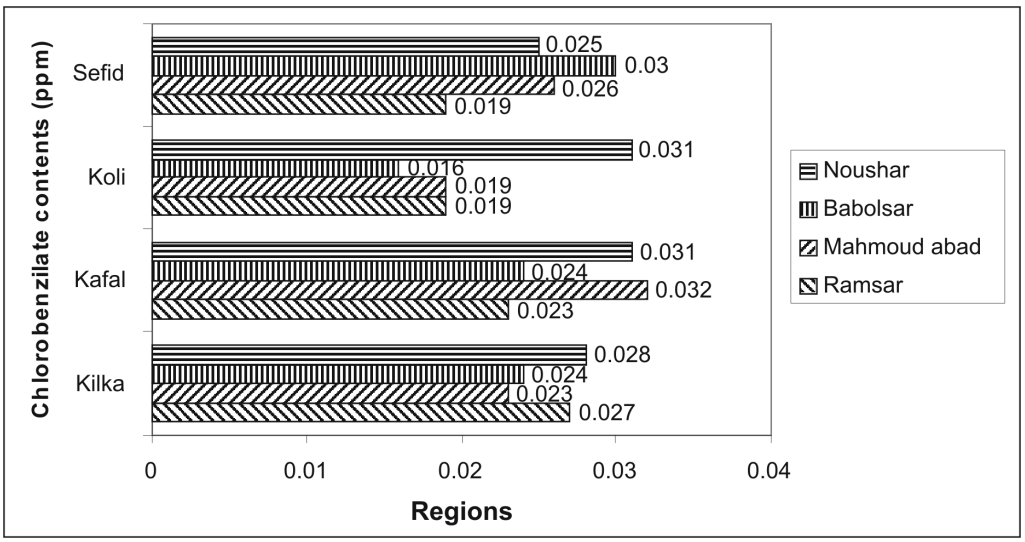


**Figure 1: The average quantities of chlorobenzilate contents (ppm) in four species of fishes under study in the Caspian Sea.**

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 (Jaward et al., 2004; La Rocca et al., 1991; Lopez-Martin et al., 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. 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 (Mazet et al., 2004).

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