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A survey and measurement of residues of lindane (organochlorine pesticides) in four species of the most consumed fish in the Caspian Sea (Iran)

AG Ebadi[a] and M Shokrzadeh[b]

[a]*Department of Biology, Islamic Azad University of Sari, Sari Branch, Iran*
[b]*Department of Toxicology, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran*

In this study, samples of four species of the most consumed fish (sefid, koli, kilca and kafal fish) were analysed for concentrations of lindane (organochlorine pesticides). Fish were caught using electric fishing at four sites (Chalous and Babolsar cities, Khazar Abad and Miankaleh regions) in the Mazandaran provinces of Iran. Quantitative determination of the lindane was performed by gas chromatography electron-capture detection (GC-ECD). Samples contained detectable concentrations of lindane, but at concentrations below the maximum residue limit (MRL). No differences were found in the lindane concentrations between the types of fish at each site, but there were two groups of sites that were significantly different from one another in terms of lindane concentrations: sites II–IV < site I ($P < 0.05$). However, the concentrations of lindane residues in the muscle were found to be less than the FAO/WHO (1993) recommended permissible intake and should not be of public concern in Mazandaran province. *Toxicology and Industrial Health* 2006; **22:** 53–58.

Key words: *Caspian Sea; fish; Iran; lindane; organochlorine pesticides*

Introduction

The growing demand for food as a result of increasing human populations has led to a substantial increase in the production and use of agro-chemicals, such as pesticides and fertilizers (Agarwal, 1976; Albaiges *et al*., 1987; Berny *et al*., 2002). The increased use of various types of pesticides, particularly organochlorine pesticides (OCs), has led to concerns regarding the potential for contamination of environmental media (ie, water, sediment, and biota) and the associated effects on human health and wildlife. The hazards associated with the bio-accumulation of persistent and toxic pesticides are highlighted in Rachael Carson's book *Silent spring* (Carson, 1963).

Each year, parts of the northern provinces in Iran (Mazandaran and Golestan), including agricultural lands, are submerged under water during the wet season. It is not surprising, therefore, that pesticides find their way into ponds, streams, and rivers and residues of OCs in aquatic biota have been an environmental concern. Due to their toxicity, persistence, tendency to accumulate in biota, and adverse impacts on wildlife, the majority of OCs (including lindane) were banned in the US during the 1970s. Yet, more than 20 years later, residues of lindane, DDT and other OCs continue to be detected in water and aquatic biota throughout the world. Although these compounds are primarily industrial in origin, PCBs mimic the OCs with

Address all correspondence to: AG Ebadi, Department of Biology, Islamic Azad University of Sari, Sari Branch, Iran
E-mail: Dr-ebadi2000@yahoo.com

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regard to physical and chemical properties, environmental fate and the potential for accumulation in biota. Organochlorine compounds (such as OCs and PCBs) have the potential to affect the development, reproduction and behavior of fish and wildlife (Gudrun and Lillemark, 1998).

The organochlorine insecticide γ-hexachlorocyclohexane (γ-HCCH), commercially available as lindane, is used on a wide range of soil-dwelling and plant-eating (phytophagous) insects. It is commonly used on a wide variety of crops, in warehouses, in public health to control insect-borne diseases, and (with fungicides) as a seed treatment. Lindane is also presently used in lotions, creams, and shampoos for the control of lice and mites (scabies) in humans (Gudrun and Lillemark, 1998). Although the use of technical lindane in crop protection has been nominally banned worldwide, restricted permission in some countries has led to considerable amounts of residue in agricultural fields. Moreover, lindane is poorly hydrolyzed and biodegrades slowly in the environment (Padmaker and Karanth, 1994; Bevenue, 1976; Bindelman *et al*., 1990). This compound is persistent in food chains. It is thus readily accumulated by animals, and has been reported to bioaccumulate in fish at ratios of 500:1200 (La Rocca *et al*., 1991). Lindane is highly to very highly toxic to fish and aquatic invertebrate species. It is poorly hydrolyzed and biodegrades slowly in the environment. Therefore, this compound is persistent in food chains and is readily accumulated by animals. Fish absorb lindane directly from water or by ingesting contaminated food. Lindane is very lipophilic and is solubilized easily in lipids (Cossarini-Dunier *et al*., 1987); it is bioaccumulated in fish at ratios of 500:1200 (La Rocca *et al*., 1991). The maximum lindane residue limit for fish is 100 ng/g and 5 ng/g for humans, certified by FAO/WHO (1993).

Mazandaran province has a vast fishery potential within its coasts. Chalous and Babolsar cities and Khazar Abad and Miankaleh regions are important fishing centers of Mazandaran province of Iran. These areas also have abundant potential for supporting aquacultural development, including the propagation of numerous marine and brackish water fish species. Considering the importance of these areas as a food source for Mazandaran, it is essential to characterize contaminant levels in the fish and other environmental compartments. Although it would be desirable to generate information on levels of OC pesticides on a variety of fish and wildlife species, resource limitations preclude this option. Fish has also been selected for bio-monitoring because:

- Fish concentrate pollutants in their tissues directly from water and through their diet, thus enabling the assessment of the transfer of pollutants through the food chain.
- Fish generally metabolize and excrete organochlorines and consequently should reflect the levels of pollution in the aquatic environment (Muir *et al*., 1990).
- Fish occupy different habitats in the ecosystem and have different feeding behaviors, thus offering the potential to study the influence of environmental and biological factors on the bioaccumulation of pollutants (Porte and Albaiges, 1993).
- Data on chlorinated compounds in edible fish are important from a human health perspective (Pastor *et al*., 1996; Ferraro *et al*., 1991; Fon *et al*., 2001).

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. Among the pesticides used in Iran, organochlorine pesticides are particularly problematic because they are persistent, broad-spectrum toxicants that tend to accumulate in the food chain and have the potential to adversely affect the ecosystem and human health.

In the present study, four species of the most consumed fish, locally called 'sefid, koli, kilca and kafal', have been selected as bio-monitoring species. These fish are carnivorous species with relatively high lipid levels in muscle tissues and, hence, tend to concentrate contaminants to a higher degree than other species. In addition, they have a high commercial value and are widely consumed by the people of this region. These characteristics make these species appropriate models for assessing the concentrations of persistent OCs, such as lindane in fish.

Material and methods

Monitoring sites

The study area was located in Mazandaran province. Four sites were selected along the Mazandaran province – Chalous and Babolsar cities and Khazar Abad and Miankaleh regions. The sites were selected according to the localization of principal sources of pollution.

Fish sampling

The fish were caught using electric fishing (powered by a 220 V electric generator) in July 2004. Collected fish (four species: sefid = *Rutilus frisikutum*, koli = *Clupeonella delicatula*, kafal = *Mugila auratus* and kilka = *Vimba vimba*) were weighed, measured for total length and classified by species and size. Each batch (same species, same size) was ground and kept frozen (−20°C) until further analysis.

Preparation, extraction and clean-up

The dorsal muscle tissue from each fish was homogenized separately in a high-speed blender until a paste-like consistency was formed (ie, following the procedure of the US Food and Drug Administration; Albaiges *et al*., 1987). After preparing the homogenate, 20 g of the sample was taken and mixed with 20 g of anhydrous sodium sulfate (Na_2SO_4). This mixture was then ground in a mortar until it reached the consistency of a free-flowing powder. The powder was transferred to a paper thimble and extracted in a Sohulet apparatus using 250 mL of solvent (double distilled (DD) hexane:DD dichloromethane, 1:1) for 6 h at 25°C. The extract was then evaporated to dryness to calculate the fat content. Then, 0.3 g of fat was taken and cleaned up through glass column chromatography with deactivated florisil (mesh: 60–100 μ) and the extract was eluted through a glass column with 100 mL eluting mixture (DD hexane:DD dichloromethane, 1:1). The extract was then evaporated to dryness and made to a final volume (5 mL) with DD hexane for gas-chromatographic (GC) analysis.

Gas-chromatographic analysis

Final volume (1 μL) was injected into the GC and the analysis was carried out using a PU 4500 equipped with electron capture detector (ECD) for determination of the level of lindane residues. Lindane residues were identified based on comparison of the measured retention times to those of known standards and also by co-injecting the samples with standards. Lindane was quantified by comparing the areas under each peak with the area under the standard peak. The GC result was verified by using thin layer chromatography (TLC). The results of internal laboratory quality assurance analysis indicated the lindane determinations were within accepted levels of accuracy (89–90% recovery).

Statistical analysis

Data were analysed using the one-tailed variance test.

Result and discussion

The concentrations of lindane were measured from each site. Most of the concentrations were below the limit of detection of chemical analyses performed. The highest concentration was 49.57 ± 0.64 g/kg (site I). However, this concentration does not represent any risk to the fish. The whole body total concentrations of lindane (l g/kg wet weight) are presented in Table 1.

There were significant differences in total lindane concentration between site I and the other sites ($P < 0.0001$). Fish were increasingly contaminated in Chalus samples. The mean lindane concentration ranged from 7.8 (site I) to 43.23 (site IV) g/kg (wet weight). There were two groups (sites II–IV versus I) of sites that were significantly different from one another in terms of lindane concentrations: sites II–IV < site I ($P < 0.05$) and there was no signifi-

Table 1. Total concentrations of lindane (in μg/kg wet weight) in four species of the most consumed fish collected from four monitoring sites along the Mazandaran province (Iran) in July 2004.

Region	Species	Lindane (mean ± SEM)
Chalus (site IV)	Sefid (N=15)	38.34 ± 1.08[a]
	Koli (N=16)	41.56 ± 0.40[a]
	Kafal (N=17)	43.45 ± 0.47[a]
	Kilca n = 16	49.57 ± 0.64[a]
Babolsar (site III)	Sefid (N=16)	6.62 ± 0.42
	Koli (N=18)	7.12 ± 0.54
	Kafal (N=18)	7.42 ± 0.68
	Kilca (N=16)	5.94 ± 0.41
Khazar Abad (site II)	Sefid (N=18)	5.51 ± 0.99
	Koli (N=19)	6.22 ± 0.35
	Kafal (N=19)	7.82 ± 0.55
	Kilca (N=19)	6.12 ± 0.29
Miankaleh (site I)	Sefid (N=18)	6.93 ± 0.33
	Koli (N=17)	8.23 ± 0.58
	Kafal (N=14)	7.57 ± 0.52
	Kilca (N=17)	8.69 ± 0.10

All values (median); N, number of fish analysed.
[a]$P < 0.001$.

cant difference among sites II–IV. No difference was observed between fish species based upon site location. We did not highlight a point source of lindane, but there was a progressive increase along the Mazandaran province, compatible with the increase in urbanization and human activity.

Due to their known persistence, liposolubility and bioaccumulation in species at higher trophic levels, OCPs should be monitored in the environment with special attention given to animal species which may be part of the human food chain. Several fish species representative of human consumption of fish in the Mazandaran province region were selected for investigation of their contamination with OCPs.

Relatively low concentrations of γ-HCH were found in the fish.The levels of γ-HCH isomer in the fish species from the sampled area were lower than values found by Barlas (1999) in the Sakarya basin, suggesting different pollutant factors in the two aquatic environments. γ-HCH isomer was not predominant in the investigated fish species. Although the use of HCB has been severely restricted in Iran since 1980, all analysed fish samples contained low but detectable residues of HCB. This low level contamination can have its origin in the various industrial processes from which HCB can be emitted as a by-product in high-temperature processes. HCB may also be present as an impurity in other chlorinated pesticides and, most probably, it is related to long-range air transport from more contaminated areas of Europe (Jaward *et al*., 2004; Nhan *et al*., 1998; Nowell *et al*., 1998). Interestingly, concentration of γ-HCH in the muscle of fish species from the sampled area in Caspian Sea is in accordance with and similar to γ-HCH isomers in the muscle of fish samples from the Danube Delta (Covaci *et al*., 2003). In addition, amounts of γ-HCH have also been measured in trout muscle from Lake Michigan (Wong *et al*., 2002) and whole cod from the Baltic Sea (Wiberg *et al*., 2000), Polynya Northwater (Moisey *et al*., 2001) and the Arctic Ocean (Hoekstra *et al*., 2003), where it was also the most predominant HCH isomer.

The bioconcentration of lindane in aquatic organisms correlates with the degree of chlorination, the stereochemistry, and lipophilicity (Bordajandi *et al*., 2003) In spite of the severity of the toxicity and the persistence of organic and metallic micropollutants, data concerning contamination levels in aquatic continental ecosystems, especially in fish, are limited in many countries. Therefore, it is extremely difficult to demonstrate either temporal or geographical trends of environmental contamination in the country. The concentration in Chalus city could be associated with the localization of agricultural lands and application of lindane by local farmers and industrial plants.

Table 2 summarizes reported contamination levels in fish in Europe in the early 1990s and 2000s. Caution is required in making a direct comparison because of the differences between studies (year, species, methods of analysis, congeners).

In all sites, lindane concentrations do not exceed the French Food Standards (2 mg/kg) authorized

Table 2. PCB content (g/kg of wet weight and dry weight) in the whole body of fish from European waters.

	Species	OCPs (wet weight) (min–max)
Mazet *et al*. (2004); Ardeche (France)	10	135 (35/524)
Lopez-Martin *et al*. (1995); Network Catalonia (Spain)	12	181 (9/2212)
Bordajandi *et al*. (2003); Turia (Spain)	14	5.14–126
Bressa *et al*. (1997); Po Delta (Italy)	12	11–265
Binelli and Provini (2003); Lake Iseo (Italy)	7	105.9–786.4

for PCB in fish muscle since 16 February, 1988 (French Food Safety Agency). In our study, almost all the sites (sites 1–4) had values <50 g/kg.

Therefore, it is assumed, based on MRL limits and our data, that the current concentrations of pollutants in the Mazandaran province should not pose a serious threat to fish, and that chemical contamination should not be a limiting factor.

The organochlorine pesticide residues accumulated in lipid portion are mobilized into the eggs and transferred to the fingerling after spawning. A year-round monitoring program should be undertaken to acquire adequate information regarding the level of organochlorine pesticide residues in fishes as well as its environment.

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References

Agarwal, H.C. 1976: Residues of DDT and its metabolites in human blood sample in Delhi, India. *Bulletin of the World Health Organisation* 54, 349.

Albaiges, J., Farran, A., Soler, M. *et al*. 1987: Accumulation and distribution of biogenic and pollutant hydrocarbons, PCBs and DDT in tissues of western fishes. *Marine Environmental Research* 22, 1–18.

Barlas, N.E. 1999: Determination of organochlorine pesticide residues in aquatic systems and organisms in Upper Sakarya basin. Turkey. *Bulletin of Environmental Contamination and Toxicology* 62, 278–85.

Berny, P.J., Lachaux, O., Buronfosse, T. *et al*. 2002: Zebra mussels (*Dreissena polymorpha*) as indicators of freshwater contamination with lindane. *Environmental Research* 90, 142–51.

Bevenue, A. 1976: The bioconcentration aspects of DDT in the environment. *Residue Review* 61, 37–112.

Bindelman, T.F., Patton, G.W., Hinckley, D.A. *et al*. 1990: Chlorinated pesticides and polychlorinated biphenyls in the atmosphere of the Canadian arctic. In Kurtz, D.A., editor. *Long range transport of pesticides*. Lewis Publishers, 237–372.

Binelli, A. and Provini, A. 2003: The PCB pollution of Lake Iseo (N. Italy) and the role of biomagnification in the pelagic food web. *Chemosphere* 53, 143–51.

Bordajandi, L.R., Gomez, G., Fernandez, M.A. *et al*. 2003: Study on PCBs, PCDD/Fs, organochlorine pesticides, heavy metals and arsenic content in freshwater fish species from the river Turia (Spain). *Chemosphere* 53, 163–71.

Bressa, G., Sisti, E. and Cima, F. 1997: PCBs and organochlorinated pesticides in eel (*Anguilla anguilla* L.) from the Po Delta. *Marine Chemistry* 58, 261–66.

Carson, R. 1963: *Silent spring*, Hamish Hamilton, Ltd.

Economic and Social Commission for Asia and the Pacific (ESCAP), 1988: *Coastal environmental management plan for Bangladesh*, Volume 2 (Final Report).

Cossarini-Dunier, M., Monod, G., Demael, A. and Lepot, D. 1987: Effect of γ-hexachlorocyclohexane (lindane) on carp (*Cyprinus carpio*). Effect of chronic intoxication on humoral immunity in relation to tissue pollutants levels. *Ecotoxicology and Environmental Safety* 13, 339–45.

Covaci, A., Gheorghe, A. and Schepens, P. 2003: Levels and enantiomeric signatures of γ-HCH in an aquatic food chain from the Danube Delta, Romania. *Organohalogen Compounds* 62, 305–308.

FAO/WHO, 1993: Food standards programme. *Codex Alimentarius Commission* 9, 149–58.

Ferraro, S.P., Lee, H., Smith, L.M. *et al*. 1991: Accumulation factors for eleven polychlorinated biphenyl congeners. *Bulletin of Environmental Contamination and Toxicology* 46, 276–83.

Fox, W.M., Connor, L., Copplestone, D. *et al*. 2001: The organochlorine contamination history of the Mersey estuary, UK, revealed by analysis of sediment cores from salt marshes. *Marine Environmental Research* 51, 213–27.

Gudrun, H. and Lillemark, L. 1998: Reduction of organochlorine contaminants from fish oil during refining. *Chemosphere* 37, 1241–52.

Hoekstra, P.F., O'Hara, T.M., Karlsson, H. *et al*. 2003: Enantiomer-specific biomagnification of alpha-hexachlorocyclohexane and selected chiral chlordane-related compounds within an arctic marine food web. *Environmental Toxicology and Chemistry* 22, 2482–92.

Jaward, F.M., Farrar, N.J., Harner, T. *et al.* 2004: Passive air sampling of PCBs, PBDEs, and organochlorine pesticides across Europe. *Environmental Science Technology* 38, 34–41.

La Rocca, C., Di Domenico, A. and Vittozzi, L. 1991: Chemobiokinetic study in freshwater fish exposed to lindane: uptake and excretion phase rate constants and bioconcentration factors. *International Journal of Health Research* 1, 103–16.

Lopez-Martin, J.M., Ruiz-Olmo, J. and Borrell, A. 1995: Levels of organochlorine compounds in freshwater fish from Catalonia, N.E. Spain. *Chemosphere* 31, 523–35.

Mazet, A., Keck, G. and Berny, P. 2004: PCBs in fish of the Ardeche river: potential implications for the survival of the otter (*Lutra lutra*). *Bulletin of Environmental Contamination and Toxicology* 72, 784–90.

Moisey, J., Fisk, A.T., Hobson, K.A. and Norstrom, R.J. 2001: Hexachlorocyclohexane (HCH) isomers and chiral signatures of alpha-HCH in the arctic marine food web of the

Northwater Polynya. *Environmental Science and Technology* 35, 1920–27.

Muir, D.C.G., Norstrom, R.J. and Simon, M. 1990: Organochlorine in Arctic marine food chains: accumulation of specific polychlorinated biphenyls and chlordane related compounds. *Environmental Science and Technology* 22, 1071–79.

Nhan, D.D., Am, N.M., Hoi, N.C. *et al*. 1998: Organochlorine pesticides and PCBs in the Red River delta, North Vietnam. *Marine Pollution Bulletin* 36, 742–49.

Nowell, L.H., Capel, P.D. and Dileanis, P.D. 1999: Pesticides in stream sediment and aquatic biota: distribution, trends, and governing factors. Lewis Publishers.

Padmaker, G.D. and Karanth, N.G. 1994: Biodegradation of hexachlorocyclohexane isomers in soil and food environment. *Critical Reviews in Microbiology* 20, 57–78.

Pastor, D., Boix, J. and Albaiges, J. 1996: Bioaccumulation of organochlorinated contaminants in three estuarine fish species (*Mullus barbatus*, *Mugil cephalus* and *Dicentrarcus labrax*). *Marine Pollution Bulletin* 32, 257–62.

Porte, C. and Albaiges, J. 1993: Bioaccumulation pattern of hydrocarbons and polychlorobiphenyls in bivalves, crustaceans and fish. *Archives of Environmental Contamination and Toxicology* 26, 273–81.

Wiberg, K., Letcher, R.J., Sandau, C.D. *et al*. 2000: The enantioselective bioaccumulation of chiral chlordane and HCH contaminants in the polar bear food chain. *Environmental Science and Technology* 34, 2668–74.

Wong, C.S., Hoekstra, P.F., Karlsson, H. *et al*. 2002: Enantiomer fractions of chiral organochlorine pesticides and polychlorinated biphenyls in standard and certified reference materials. *Chemosphere* 9, 1339–47.