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# Measurement of Organophosphorus pesticide in fish from the Tajan River

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**Abstract:** In this study concentrations of Parathion (organophosphorous pesticides) was determined in four commercially valuable fish species, *Rutilus frisikutum*, *Clupeonella delicatula*, *Mugila auratus* and *Vimba vimba*, from four sites of Tajan River in July 2004. The concentration of parathion was measured by gas chromatography electron-capture detection (GC-ECD). Concentrations of the parathion in examined fish species ranged from 5.94 (site III) to 49.57 (site IV) lg $kg^{-1}$ (wet weight). No difference found between kind of fishes in each sites about parathion concentrations but there are two groups of sites that were significantly different from one another in terms of parathion concentrations: sites 2, 3 and 4 < site 1 ($p<0.05$). Parathion in the edible parts of the investigated fish were in the permissible safety levels for human uses and should not be public concern among peoples in Mazandaran province.

**Key words:** Organophosphorus pesticides, parathion, Tajan River, fish, Iran

## INTRODUCTION

For centuries pesticides have been used in agriculture to enhance food production by eradicating unwanted insects and controlling disease vectors[1]. Among pesticides, Organophosphorus compounds (OPs) are commonly used as insecticides[2].

Because of their rapid decomposition in water and their low environmental persistence, organophosphorus (OP) pesticides have largely replaced by the use of organochlorine pesticides in recent years. However, OP pesticides are themselves also a potential threat to aquatic ecosystems. Many organophosphorus pesticides, after being applied, remain in the environment and undergo chemical, physical and biological changes[3]. Parathion and malathion were reported to produce toxicological effects as a result of metabolism of their phosphothionate groups to the oxon form[3-5]. Repeated or prolonged exposure to organophosphates may result in the same effects as acute exposure including the delayed symptoms. Other effects reported in workers repeatedly exposed include impaired memory and concentration, disorientation, severe depressions, irritability, confusion, headache, speech difficulties, delayed reaction times, nightmares, sleepwalking and drowsiness or insomnia. An influenza-like condition with headache, nausea, weakness, loss of appetite and malaise has also been reported[6]. One study found that dietary doses of 50 ppm (about 2.5 mg/kg/day) produced toxic symptoms, growth retardation and death in rats. In another feeding study, dietary doses of 2.5 mg/kg/day for 2-years had no effect on rats, while doses of 5 mg/kg/day produced only slight signs of toxicity and growth retardation, but no deaths[2].

Parathion is an OP pesticide most commonly used in agriculture in China and its extensive use has caused high mortality of farmed shrimps[7]. It is a broad spectrum, organophosphate pesticide used to control many insects and mites[5, 8]. It has non-systemic, contact, stomach and fumigant actions[5,8]. It has a wide range of applications on many crops against numerous insect species[1]. Parathion is available in dust, emulsion concentrate, granular, UVL liquid and wettable powder formulations[3].

Its toxic effects have also been investigated *in vivo* in mammals[8,9] and insects[10] and in vitro in mammalian[6,11], fish[12] and insect cultured cells. However, thus far there have been few reports on the toxic effects of parathion on marine fish or cultured cells derived from marine species of fish.

Parathion is moderately toxic to fish and aquatic invertebrates (like crayfish, snails and worms)[5,8,13]. The 96 h LC50 for parathion in fish in general is 1.43 mg $L^{-1}$[3]. The 96 h LC50 in trout is 1.6 mg $L^{-1}$, 1.8 mg $L^{-1}$ in goldfish, 2.7 mg $L^{-1}$ in catfish, 0.3 mg $L^{-1}$ in mosquito fish and 0.02 mg $L^{-1}$ in bluegill but for more consumption of fishes in Mazandaran province ( 4-5 times per week) it is important to assay amount of parathion residues in fish.

The goal of this study was to survey levels of parathion (Organophosphorus pesticide) 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.

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## MATERIALS AND METHODS

**Monitoring sites:** The study area is located in Mazandaran province. A total of 4 sites were selected along the Tajan River in July 2004. The sites were selected according to the localization principal sources of pollution.

**Fish sampling:** The fishes were captured using electric fishing (powered by a 220V electric generator). Collected fishes (4 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.

**Chemical analysis:** A 1.0 g sample was taken from each batch and 30 mL of hexane/acetone 75/25 mix was added. Each sample was blended with an Ultraturrax® (Ika, Werke, Germany).

The supernatant was removed and filtered through a phase separator membrane. This extraction procedure was performed twice. The extract was evaporated at 60°C in a rotary evaporator. The dry extract was dissolved in 10 mL hexane. Two ml of fuming sulphuric acid ($SO_3$ 7%) were added and the test tube was shaken immediately. After centrifugation at 3000 rpm for 10 min, a part of supernatant was used for OP chromatography[14] and 1 mL of the supernatant was added to 1 mL of 2% potassium hydroxide in ethanol for parathion analyses. The tubes were placed in a water bath at 50°C for 30 min. At the end of this period, 2 mL ultrapure water was added, the samples were vortexed and centrifuged once again for 10 min. Samples underwent another acid hydrolysis with 1 mL sulphuric acid. After a final centrifugation, the final supernatant was removed and kept frozen until further analysis. A gas chromatograph Hewlett-Packard HP5890 series 2 equipped with an electron-capture detector was used. Total duration of analysis was 110 min. Injection was performed automatically with an automatic injector (HP 6890).

**Statistical analysis:** Data were analyzed using the one-tailed variance test.

The results are expressed as mean±standard error mean. The data were tested for homogeneity of variances at significant level of $p<0.05$. Statistical data analysis was performed with SPSS program for Windows software.

## RESULTS AND DISCUSSION

The concentrations of parathion 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 $kg^{-1}$ (Site 1), However this concentration does not represent any risk for fishes.

Among the sites, there were significant differences in total parathion concentration between site 1 and other sites ($p<0.0001$). Fish were increasingly contaminated in site 4 samples. The mean parathion concentration ranged from 5.94 (site III) to 49.57 (site IV) l g $kg^{-1}$ (wet weight). There are two groups of sites that were significantly different from one another in terms of parathion concentrations: sites II, III and IV < site I ($p<0.05$) and there is not significantly different amount Sites 2, 3 and 4 (Table 1) No difference found between kinds of fishes in each sites. We did not highlight a point source of parathion but there was a progressive increase along the Tajan River, compatible with the increase in urbanization and impacts of human activities.

The bioconcentration of parathion in aquatic organisms correlates with the degree of chlorination, the stereochemistry and lipophilicity[14]. 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 France. Therefore, it is extremely difficult to demonstrate either temporal or geographical trends of environmental contamination in the country. The concentration is higher in the Site 4 could be associated with the localization agricultural lands and application of

Table 1: Total concentrations of parathion (in µg $kg^{-1}$ wet weight) in four species collected from four monitoring sites

| Region | Kind of fish | Parathion mean±SEM |
|---|---|---|
| (Site IV) | Sefid n = 15 | 38.34±1.08 |
| | Koli n = 16 | 41.56±0.40 |
| | Kafal n = 17 | 43.45±0.47 |
| | Kilca n = 16 | 49.57±0.64 |
| (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 |
| (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 |
| (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 |

n = number of individuals analyzed

parathion by local farmers and industrial plants. Caution is required in making a direct comparison because of the differences between studies (year, species, methods of analysis, congeners)[15].

In all sites, parathion concentrations do not exceed the French Food Standards (2 mg $kg^{-1}$) authorized for OPs in fish muscle since 16/02/1988 (French Food Safety Agency). In our study, almost all the sites (site 1-4) have values lower than 50 lg $kg^{-1}$.

It is assumed, therefore based on MRL limits and our data, that the current concentrations of pollutants in the Tajan River should not pose a serious threat to the fishes and that chemical contamination should not be a limiting factor.

The Organophosphorus 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 Organophosphorus pesticide residues in fishes as well as its environment.

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