

# Total mercury concentrations in edible tissues of two elasmobranch species from Crete (eastern Mediterranean Sea)

by

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**ABSTRACT.** - In 2003-2004, 47 spiny dogfish (*Squalus acanthias*) ranging from 430 to 729 mm in TL and 18 smooth-hound sharks (*Mustelus mustelus*) ranging from 428 to 720 mm in TL were collected from the eastern Mediterranean Sea, around the island of Crete. Total mercury levels in their edible tissues were determined with Cold Vapor Atomic Absorption Spectrometry. The highest concentration was found in the muscle tissue of a spiny dogfish (5.79 mg/kg w.w) and the lowest in the muscle tissue of a smooth-hound shark (0.22 mg/kg w.w). In all muscle samples of smooth-hound shark, except one, the concentrations of total mercury were below the maximum permitted level of 1 mg/kg w.w., whereas mercury concentrations were above this limit in 81% of the muscle samples of spiny dogfish.

**RÉSUMÉ.** - Concentrations en mercure total dans les tissus comestibles de deux espèces d'élasmobranches de Crète (Méditerranée orientale).

En 2003-2004, 47 aiguillats communs (*Squalus acanthias*) de 430 à 729 mm LT et 18 émissoles lisses (*Mustelus mustelus*) de 428 à 720 mm LT ont été pêchés en Méditerranée orientale autour de l'île de Crète. Les concentrations en mercure total dans leurs tissus comestibles ont été mesurées par spectrométrie par absorption atomique à vapeur froide. La concentration la plus élevée a été trouvée dans le muscle d'un aiguillat (5,79 mg/kg w.w) et la plus basse dans le muscle d'une émissole lisse (0,22 mg/kg w.w). Dans tous les échantillons musculaires d'émissoles lisses, sauf un, la concentration en mercure total était en dessous du niveau maximum autorisé, c'est-à-dire 1 mg/kg w.w., tandis que les concentrations en mercure étaient au-dessus de cette limite dans 81% des échantillons du tissu musculaire des aiguillats.

Key words. - *Squalus acanthias* - *Mustelus mustelus* - MED - Crete - Mercury concentration.

Fish is the most critical dietary source of mercury for human beings. Among them, species that belong to higher trophic levels tend to accumulate higher quantities of mercury (Miller *et al.*, 1972; Forrester *et al.*, 1972; Walker, 1976; Lyle, 1986; Vas, 1991; Hueter *et al.*, 1995; Adams and McMichael, 1999; Storelli and Marcotrigiano, 2001; Storelli *et al.*, 2002). Especially shark species tend to have higher mercury concentrations than other fish (Walker, 1976) because they belong to higher trophic levels exhibiting intense predatory behavior, higher longevity and lower metabolic rate. Consequently, sharks represent sites for biological accumulation of pollutants, and good indicators of mercury contamination in marine ecosystems (Vas, 1991).

In Europe, the limit value for total mercury, regulated by European Commission Decision 93/351 of 19 May 1993, is set at 0.5 mg/kg w.w. except for some species with a high trophic level for which it is raised up to 1 mg/kg w.w. (Official Journal of the European Communities, 1994). Although there is a wealth of information on the concentration of

mercury in marine organisms from the Mediterranean Sea, there are comparatively few data for mercury levels in elasmobranchs. The objectives of this study were 1) to provide data about total mercury concentrations in the muscle tissues of spiny dogfish (*Squalus acanthias*, Linnaeus 1758) and smooth-hound shark (*Mustelus mustelus*, Linnaeus 1758) from the eastern Mediterranean Sea, and 2) to ascertain whether the concentration of total mercury in these species exceeds the maximum level fixed by the European Commission.

## MATERIALS AND METHODS

During 2003 and 2004, a total of 65 samples of spiny dogfish and smooth-hound shark were obtained from the incidental catches of the Greek commercial fishing vessels, around the island of Crete in depths from 350 to 420 m (Fig. 1). All samples were initially preserved in ice (on

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Figure 1. - Sampling sites (black dots) of the spiny dogfish (*Squalus acanthias*) and smooth-hound shark (*Mustelus mustelus*) specimens caught in the eastern Mediterranean Sea during the period 2003-2004. [Sites d'échantillonnage (points noirs) des spécimens d'aiguillat commun (*Squalus acanthias*) et d'émissole lisse (*Mustelus mustelus*) capturés en Méditerranée orientale au cours de la période 2003-2004.]

board) and later frozen at  $-20^{\circ}\text{C}$  until dissection. Total length (TL) of the specimens was measured to the nearest centimetre, and total weight (TW) to the nearest gram. Sex was determined macroscopically by direct observation of the claspers in males and sexual maturity by examination of the internal and external reproductive organs (Chatzisprou and Megalofonou, 2005). Out of 47 specimens of spiny dogfish, ranging from 430 to 729 mm in TL and from 354 to 1955 g in TW, 36 were females and 11 males. Moreover, out of 18 smooth-hound shark specimens, ranging from 428 to 720 mm in TL and from 350 to 1165 g in TW, 9 were females and 9 males. All the specimens of smooth-hound shark were classified as immature whereas most of spiny dogfish specimens were mature.

A stainless-steel knife was used to remove samples of axial muscle tissue from the left dorsal area above the lateral line and anterior to the origin of the first dorsal fin. White

Table I. - Range and mean value  $\pm$  S.D. of total mercury concentrations (T-Hg, mg/kg w.w.) in muscle tissue of females, males and the total of specimens of spiny dogfish (*Squalus acanthias*) and smooth-hound shark (*Mustelus mustelus*) caught in the Eastern Mediterranean Sea during the period 2003-2004. [Gamme de variation et moyenne  $\pm$  E.T. de la concentration en mercure total (T-Hg, mg/kg w.w.) dans les tissus musculaires des femelles, mâles et l'ensemble des spécimens d'aiguillat commun (*Squalus acanthias*) et d'émissole lisse (*Mustelus mustelus*) capturés en Méditerranée orientale au cours de la période 2003-2004.]

Species		T-Hg		
		Females	Males	All
<i>S. acanthias</i> (spiny dogfish)	range	0 - 3.78	0 - 5.79	0 - 5.79
	mean $\pm$ S.D.	2.01 $\pm$ 0.94	2.34 $\pm$ 1.94	2.07 $\pm$ 1.17
<i>M. mustelus</i> (smooth-hound shark)	range	0.22 - 0.53	0.26 - 1.83	0.22 - 1.83
	mean $\pm$ S.D.	0.31 $\pm$ 0.1	0.48 $\pm$ 0.51	0.39 $\pm$ 0.37

muscle tissue taken from this part of the body is representative of the portion of shark used for human consumption. During the extraction process, care was taken to assure that the samples made no contact with epidermal or dermal layers, scales, or other surrounding surfaces. Tissue samples were immediately placed in sterile polypropylene vials, sealed, and frozen at  $-20^{\circ}\text{C}$ . Then tissue samples were freeze-dried, powdered and homogenized. A 50 ml calibrated borosilicate glass vial was used as the digestion vessel. Samples were weighted ( $\sim 0.3$  g) directly into the digestion vessels and they were acid-digested (10 ml  $\text{HNO}_3$ ) on a pre-heated hotplate at  $80^{\circ}\text{C}$  for two hours using standard procedures in accordance with the US Environmental Protection Agency: method 245.6 (EPA, 1991). Before bringing the digest to the 50 ml mark,  $\text{K}_2\text{Cr}_2\text{O}_7$  5% w/v was added as preservative. Cold Vapour Atomic Absorption Spectrometry (CVAAS) was used for the final determination of mercury concentration with stannous chloride as reducing agent. The detection limit of this method is  $0.1 \mu\text{g/l}$ . Mercury concentration was calculated on a wet weight (w.w.) base in all samples. A Kruskal-Wallis test was used to examine for statistically significant differences between total mercury concentrations in the two species.

Quality control measurements included analysis of laboratory blanks, duplicate or triplicate tissue samples, and standard fish tissue reference materials: DORM-2 (*Squalus acanthias*) from the National Research Council of Canada and IAEA-350 (*Thunnus thynnus*) from the International Atomic Energy Agency.

## RESULTS

The accuracy of the analytical method that was followed for the determination of total mercury concentration in different sample tissues of spiny dogfish and smooth-hound shark was high with recovery percentages  $100 \pm 1.19$  and  $100 \pm 3.18$  for the certified reference materials, DORM-2 and IAEA-350 respectively.

The highest value of total mercury concentration (5.79 mg/kg w.w.) was found in the muscle tissue of a male spiny dogfish with total length 575 mm and total weight 785 g and the lowest (0.22 mg/kg w.w.) in the muscle tissue of a female smooth-hound shark with total length 630 mm and total weight 925 g. The range and mean mercury concentration in muscle tissue, separately for females, males and all of the specimens of each species examined, are shown in table I. At the 95% confidence level there was not found a statistically significant difference in mercury concentration (medians) between males and females of spiny dogfish ( $P = 0.78 > 0.05$ ) and smooth-hound shark ( $P = 50.29 > 0.05$ ).

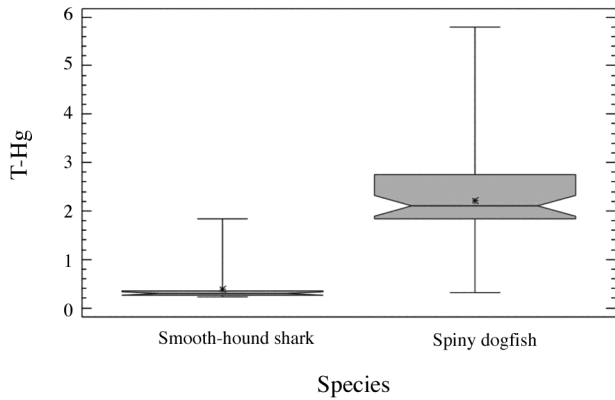


Figure 2. - Total mercury concentration (T-Hg in mg/kg w.w.) in muscle tissue of 47 spiny dogfish (*Squalus acanthias*) and 18 smooth-hound shark (*Mustelus mustelus*), caught in the Eastern Mediterranean Sea during the period 2003-2004; (\*): mean; horizontal line: median; grey area: 50% of the values; notch: 95% confidence level for median; vertical line: minimum, maximum. [Concentration en mercure total (T-Hg en mg/kg w.w.) dans les tissus musculaires de 47 aiguillats communs (*Squalus acanthias*) et 18 émissoles lisses (*Mustelus mustelus*), capturés en Méditerranée orientale au cours de la période 2003-2004; (\*) : moyenne ; ligne horizontale : médiane ; zone grisée : 50% de la valeur ; indentation : niveau de confiance de 95% pour la médiane ; ligne verticale : minimum, maximum.]

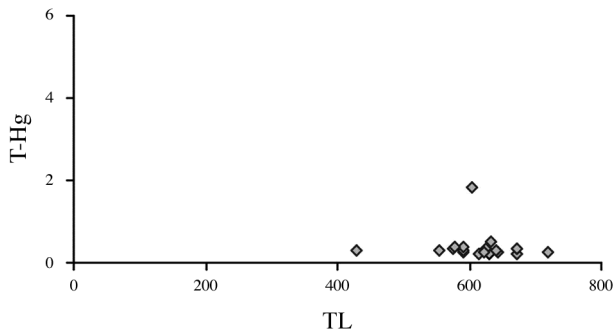


Figure 3. - Total length (TL in mm) against total mercury concentration (T-Hg in mg/kg w.w.) in muscle tissues of 18 smooth-hound shark (*Mustelus mustelus*) caught in the eastern Mediterranean Sea during the period 2003-2004. [Longueur totale (TL en mm) en fonction de la concentration en mercure total (T-Hg en mg/kg w.w.) dans les tissus musculaires de 18 émissoles lisses (*Mustelus mustelus*) capturés en Méditerranée orientale au cours de la période 2003-2004.]

Mean mercury concentration of muscle tissue was found higher in spiny dogfish (mean =  $2.07 \pm 1.17$  mg/kg w.w) than in smooth-hound shark (mean =  $0.39 \pm 0.37$  mg/kg w.w) (Fig. 2). At the 95% confidence level a statistically significant difference was found in mean mercury concentrations between the two shark species examined ( $P = 5.12 \cdot 10^{-7} < 0.05$ ). Moreover, mercury concentration and size in each species were not highly correlated (Figs 3, 4). In smooth-hound shark total mercury concentration was below the maximum permitted level of 1 mg/kg w.w., in all specimens apart from one male of 604 mm and 730 g (1.83 mg/kg w.w.)

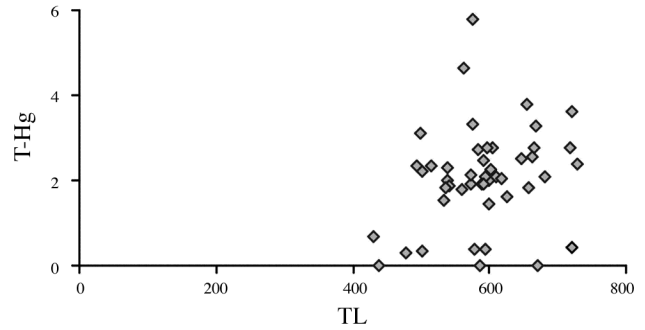


Figure 4. - Total length (TL in mm) against total mercury concentration (T-Hg in mg/kg w.w.) in muscle tissues of 47 spiny dogfish (*Squalus acanthias*) caught in the Eastern Mediterranean Sea during the period 2003-2004. [Longueur totale (TL en mm) en fonction de la concentration en mercure total (T-Hg en mg/kg w.w.) dans les tissus musculaires de 47 aiguillats communs (*Squalus acanthias*) capturés en Méditerranée orientale au cours de la période 2003-2004.]

(Fig. 3). In spiny dogfish total mercury concentration was above this limit in 38 out of 47 specimens (mean =  $2.49 \pm 0.86$ ;  $n = 38$ ) (Fig. 4).

**DISCUSSION**

The present study reports for the first time total mercury levels in muscle tissue of the spiny dogfish and smooth-hound shark from two areas around the island of Crete in Greece. Our results revealed that mercury concentrations measured in the spiny dogfish samples were significantly higher than those in the smooth-hound shark, although the specimens from both species examined had similar length. Differences in mercury concentration levels among species of similar length are probably associated with differences in growth rates and age (Walker, 1976). According to unpublished data (project Pythagoras II), the ages of smooth-hound shark specimens ranging in total length from 428-720 mm were estimated from 3 to 9 years while the ages of the spiny dogfish ranging in total length from 430-729 mm were from 6 to 21 years. Similar results on length at age estimations for the two species were obtained in previous studies (Goosen and Smale, 1997; Ketchen, 1975). It is obvious that in the same length the spiny dogfish specimens are older than the smooth-hound sharks and therefore they have more time to bioaccumulate mercury and other heavy metals from their environment.

Food habits can contribute as well to differences between mercury concentrations among different species. In a previous study, De Pinho *et al.* (2002) showed that mercury levels for all cartilaginous species analyzed from the Brazilian offshore waters were mainly affected by their eating habits. It is known that spiny dogfish is a primarily piscivorous species which feeds also on mollusks, crustaceans and other inverte-

brates (Compagno, 1984), while smooth-hound shark is an omnivorous species, that feeds mainly on crustaceans secondly on cephalopods and thirdly on bony fishes (Costantini *et al.*, 2000). Probably, the higher mercury concentrations in spiny dogfish may be due to its piscivorous diet. Mercury concentration was found higher, at least one order of magnitude, in fish than in mollusks which can represent the prey of carnivorous fishes (Juras, 1988).

In a previous study (Storelli *et al.*, 2002) on shark species from the Mediterranean Sea, significant differences were observed in total mercury concentrations between samples from different areas. In blackmouth catshark, *Galeus melastomus*, mean mercury concentration in muscle tissue was 2.66 mg/kg w.w. in the Adriatic Sea (Italy); 1.01 mg/kg w.w. in the Adriatic Sea (Albania); 0.82 mg/kg in the Ionian Sea; and 2.14 mg/kg w.w. in the Aegean Sea. The mean value of total mercury in muscle tissue of *Mustelus mustelus* (0.39 mg/kg w.w.) found in the present study was similar to the mean value of muscle tissue of the same species (0.31 mg/kg w.w.) from Adriatic Sea (Storelli *et al.*, 2002). However, the mean concentration of mercury in muscle tissue of *Squalus acanthias* (2.07 mg/kg w.w.) was smaller than the mean value that was found in muscle tissue of *Squalus blainvillei* (4.53 mg/kg w.w.) from Adriatic Sea (Storelli *et al.*, 2002). In the eastern Mediterranean Sea higher mean concentrations of mercury in muscle tissue were found in *Centrophorus granulosus* (9.66 mg/kg w.w.) from the Adriatic Sea and in *Dalatias licha* (4.38 mg/kg w.w.) from the Ionian Sea (Storelli *et al.*, 2002). Low mean mercury levels muscle tissues were found in *Etmopterus spinax* (0.63 mg/kg w.w.) from the Ionian Sea, as well as in *Scyliorhinus canicula* (1.01 mg/kg) and *Mustelus mustelus* (0.31 mg/kg w.w.) from the Adriatic Sea (Storelli *et al.*, 2002; Storelli *et al.*, 2005). Likewise, low concentrations of mercury were also found in species of the genus *Mustelus* from the Atlantic Ocean. In *Mustelus canis* the mean value found was  $0.41 \pm 0.35$  and in *Mustelus norrisi*  $0.36 \pm 0.28$  mg/kg w.w. (De Pinho *et al.*, 2002).

Apart from the results of the present study, where in one specimen of smooth-hound shark and in 81% of spiny dogfish mercury concentration was above the maximum level fixed by the European Commission, mercury concentrations exceeding this limit were observed also in the following shark species: *Carcharhinus signatus* (mean =  $1.77 \pm 0.56$ ), *Squalus megalops* (mean =  $1.90 \pm 0.58$ ) and *Squalus mitsukurii* (mean =  $2.22 \pm 0.72$ ) from the Atlantic Ocean (De Pinho *et al.*, 2002); *Squalus blainvillei* (mean =  $4.53 \pm 1.18$ ) and *Scyliorhinus canicula* (mean =  $1.49 \pm 0.61$ ) from the Adriatic Sea (Storelli *et al.*, 2002). In *Squalus acanthias* from Pacific Ocean mean mercury value was found above 0.5 (Harbo and Birtwell, 1975; Hall *et al.*, 1977) and 1.01 (Harbo *et al.*, 1983).

Muscle tissue of many shark species gets used to being edible and has increasing commercial interest in several countries all over the world. Both spiny dogfish and smooth-hound shark are marketable species in several European and Mediterranean countries. The fact that in 81% of spiny dogfish muscle samples examined, the concentration of total mercury exceeded the maximum permitted level of 1 mg/kg w.w., should draw our attention for control and monitoring.

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