Risk assessment of mercury and methyl mercury intake via sardine and swordfish consumption in Algeria

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ABSTRACT. Total mercury (Hg) and methylmercury (MeHg) concentrations in the flesh of sardine *(Sardina pilchardus)* and swordfish *(Xiphias gladius)* fished in three Algerian coasts were determined by a direct mercury analyzer (DMA). We also assessed the risk to which the consumer was exposed to by calculating the estimated daily intakes (EDIs), target hazard quotient (THQ) and hazard index (HI). The average concentrations of Hg and MeHg in the flesh of sardine were similar (0.04 mg/kg wet weight) and in swordfish were 0.61 mg/kg wet weight; 0.57 mg/kg wet weight, respectively. These concentrations have not surpassed the thresholds set by the Algerian and European regulations. The estimated daily intakes for Hg and MeHg were similar in sardine (0.0064 µg/kg/day) and were 0.098 µg/kg/day and 0.092 µg/kg/day for Hg and MeHg, respectively, in swordfish. These values did not exceed the provisional tolerable weekly intake (PTWI) established by the European Food Safety Authority (EFSA) and by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The target hazard quotient (THQ) and the hazard index (HI) calculated were < 1. Consequently, consumption of these fishes does not pose any risk for the adult groups of the Algerian population regarding mercury, and methylmercury studied.

Keywords: Mercury, Methylmercury, Sardine, Swordfish, Risk assessment.

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INTRODUCTION

Seafood is a source of energy and protein with high biological value, and contributes to the intake of essential nutrients, such as iodine, selenium, calcium, and vitamins A and D, with well-established health benefits. Seafood also provides n-3 (also called omega-3) long-chain polyunsaturated fatty acids (LC-PUFA) and is a component of dietary patterns associated with good health. Most European Food-Based Dietary Guidelines recommend a minimum of two servings of fish per week for older children, adolescents, and adults to ensure the provision of key nutrients (EFSA, 2014). However, there has been heightened concern about the presence of toxic metals such as mercury (Ricketts et al., 2016), which is released in the environment from both natural sources and human activities. It exists mainly in different forms of elemental mercury (HgO), inorganic mercury (Hg^+, Hg^{2+}) and organic mercury ($MeHg^+$, $EtHg^+$,$PhHg^+$, etc.) (Zhu et al., 2017). Mercury, an element also known as quicksilver, causes different toxic effects on the nervous, digestive and immune systems, as well as the lungs, kidneys, skin, and eyes. Exposure to it can result in severe illness and death (WHO, 2018). Consumption of fish is the main path for human exposure to mercury especially for coastal populations (Ricketts et al., 2016). Inorganic mercury is converted into the organic form (methylmercury) through methylation and the enzymatic process performed by bacteria and other aquatic microorganisms (Manavia et al., 2018). It is the predominant chemical form since the majority of total mercury that accumulates in the muscle tissue of fish is in this form methylmercury (95%-99%) (Carroll and Warwick, 2017) and has the ability to biomagnify through the aquatic food chain (Henry et al., 2017). It is the most toxic organic form of mercury which is considered by the International Agency for Research on Cancer (IARC) to be possibly carcinogenic to humans (Group 2B) (Torres-Escribano et al., 2010; Ricketts et al., 2016). Methylmercury is at the origin of neurologic damage such as mental retardation, seizures, vision and hearing loss, delayed development, language disorders, and memory loss or renal damage and has a long biological half-life. It can also cross the blood-brain and placental barriers (Torres-Escribano et al., 2010; Kral et al., 2017; Guérin et al., 2018). The first and most serious case illustrating the potential hazard of chemical contamination of food was encountered in Japan in the 1950s following industrial releases of mercury salts in a closed bay (Minamata Bay) producing fish for the consumer after concentration through the food chain and methylation, a mysterious neurological disease that reached alarming epidemic proportions (more than two thousand cases of poisoning and almost a hundred deaths were observed between 1953 and 1960, known as "Minamata Disease" (Roger and Guéry, 1991). Since then, the competent authorities have become aware of the hazard that these substances may represent and put in place standards to protect the health of consumers (JECFA, 1972). For this reason, it is necessary to monitor mercury and methylmercury levels in fishes and assess the risk they pose to the consumer. This study aimed to determine the levels of total mercury and methylmercury in the flesh of sardine (*Sardina pilchardus)* and swordfish *(Xiphias gladius)* collected in three Algerian coasts (Béjaia, Algiers, and Oran) and also assessed the risk related to consumption of these fishes for adult consumer.

MATERIALS AND METHODS

Sampling

A total of 87 samples of sardine (*Sardina pilchardus*) (n = 43) and swordfish (*Xiphias gladius*) (n = 44) were collected from April to September 2017 from three fishing major ports of Algeria, 15 samples of sardine and 20 samples of swordfish were collected from the coast of Algiers (North centre, NC), 12 and 13 from the coast of Bejaia (North East, NE), 15 and 11 from coast of Oran (North West, NW). The sampling procedure was carried out according to the EU (2016/582) regulation. The number of elemental samples that make up each aliquot varies according to the weight of the lot: elemental samples of 100 g in a number of 3, 5 and 10 were used if the weight of the lot was < 50 kg, 50 kg < weight < 500 kg or > 500 kg, respectively. The samples were placed immediately in blank polyethylene bags and transported to the laboratory in icebox for preparation.

Sample preparation

The preparation of the samples for the analysis was carried out, according to the requirements of the European standard EN 13804 (2013), in the laboratory of the National Center for Toxicology (CNT) in Algiers for the samples of the center (Algiers) and east (Bejaia) and at Federal Laboratory for the Safety of Food Chain Gentbrugge (FLSFC-G) of the Federal Agency for the Safety of the Food Chain (FASFC) in Gentbrugge (Belgium) for those of the west (Oran). The test samples were quickly prepared after the arrival of the fishes in the laboratory. The fishes were rinsed with potable wa-

ter before cutting to prevent leaching of the cut surfaces, then rinsed with distilled water. In the sardine samples, all inedible parts are removed. Swordfish samples were rinsed and peeled. Only the flesh was used for subsequent tests. From each sample, 100 g of matrix obtained was homogenized using a grinder (Retsch Grindomix 200), identified and placed in small closed black plastic bags and stored at -18°C.

Analysis of total mercury

The analysis was performed using a direct mercury analyzer (AMA-254), without any prior chemical treatment or digestion. A total of 50 to 100 mg of homogenate flesh was directly weighted in nickel boats. The nickel boats were rinsed with distilled water and cleaned by a thermal program by the apparatus to avoid contamination. Before the commencement of the analysis, a list was prepared in advance on the computer that was directly linked to the device where the date, the number of the samples, the weight and the position of nickel boats on the carousel are recorded. The nickel sample boats were automatically inserted into the combustion/ catalyst tube by the autoloader. The samples were firstly dried and then thermally degraded at 750°C. To determine the amount of mercury that was caught on the amalgamator, the amalgamator was briefly heated up to 900°C where by the mercury was released in the form of a cloud. The mercury cloud was transferred by the oxygen flow to the measurement cells. The amount of mercury was measured in each cell at 254 nm. Once finished, the detector was linked to a computer that gives the concentrations in ppm. The duration of the analysis for each sample was about 10min. The limit of detection and the limit of quantification of Hg and MeHg were 0.005 and 0.010mg/kg w.w.

Quality control of the analysis

Reference materials were used to control the quality of the analysis; canned fish (Fapas) with an internal reference number (12130869) and the tissue of lyophilized mussels ERM-CE278k with a known concentrations of Hg (0.359; 0.071mg/kg w.w, respectively). The results were in good agreement with the certified values located in this interval (0.404-0.674 mg/kg w.w; 0.053-0.089 mg/kg w.w) for both reference materials, respectively.

Methylmercury analysis

Analysis of MeHg was carried out according to the European document TC 275 WI0275321 (2017).

Extraction of the organic phase of mercury

Homogenate samples of 0.7 g to 0.8 g were weighed accurately (or 0.2 g in the case of lyophilised reference material) in a 50 ml centrifuge tube. 10 ml of hydrobromic acid was added and shaken manually for at least 2 min. Then, 20 ml of toluene was added and shaken vigorously for at least 20 min using an agitator (Stuart), centrifuged using a centrifuge (SIGMAᴿ) for 10 min at 2300 g according to the TC 275 WI0275321 (2017). 15 ml of the organic supernatant was transferred into a 50 ml centrifuge tube containing already 6 ml of L-cysteine solution. 15 ml of toluene was added to the initial centrifuge tube (containing the hydrobromic acid phase) and repeated the second extraction with the organic phase. After centrifugation, the remaining upper organic phase was transferred into the 50 ml centrifuge tube with the L-cysteine solution. It was then shaken vigorously using an agitator (Stuart) for at least 20 min and centrifuged in a centrifuge (SIGMAᴿ) for 10 min at 2300 g. An aliquot of 2 ml to 3 ml from the lower phase with the L-cysteine (and the extracted organic mercury) was taken. Ensured that the sample to be analysed is toluene free. Test samples were analysed as soon as possible to minimize instability issues.

Determination of concentration

The analysis was performed using direct mercury analyzer (AMA-254), where 500 μl to 200 μl of extract was put in a nickel boat cleaned by the apparatus and introduced in the DMA. Drying time, decomposition and waiting time were 350 s, 150s, and 55s respectively for samples and were150s, 150s and 55s respectively for reference materials, cleaning, and blanks. The results were given after a few minutes in μg/l Hg in the extract. The MeHg concentration was calculated using the formula:

$$MeHg\,(mg/kg, expressed\,as\,Hg) = C \times 6 \times D \div 1000 \times m$$

where *6* is the volume of L-cysteine solution (6 ml); C is the concentration in the extract expressed in μg/l Hg; D is the dilution factor if needed; m is the mass of the test portion, in g.

Results of MeHg expressed in mg/kg.

The limits of detection and the limit of quantification of MeHg were 0.010 and 0.020mg/k w.w.

Quality control

To ensure the trueness of the method a (certified) reference material (RM) with a known content of

MeHg was used. Fish lyophilized TORT-2 with internal reference number (EU-RL-HM-15/IMP-115) and a known concentration (0.152 mg/kg w.w), the results were in agreement with the certified values located in this interval (0.0914-0.2126 mg/kg w.w).

Statistical analysis

Microsoft Excel ® (2007) software was used for calculating averages, standard deviation, minimum and maximum values. Statistical analyses were carried out using software R version (3.0.2). The Chapiro-Wallik normality test was used. The nonparametric Mann-Whitney test was used to compare the differences in the metal content studied in the two species (sardine and swordfish) (significant difference at a probability threshold of less than 5%). The Kruskal-Wallis test was conducted to compare the difference in the Hg and MeHg content of both species in the study areas.

Risk assessment for sardine and swordfish consumption

The risk assessment was performed using estimated dietary intakes (EDI/EWI), target hazard quotient (THQ) and hazard index (HI).

Determination of the estimated daily intake (EDI) for Hg and MeHg

The average concentrations of the metals analyzed were used to determinate the estimated daily intake (EDI; µg/kg/day) for an Algerian adult weighting an average of 60 kg and consuming 9.7 g per day (MFRR, 2018). The EDI was calculated using the following equation (Ju et al., 2017):

$$EDI = C \times DC \div BW$$

Where C: the mean concentration of heavy metals in fish flesh (µg/g), DC: the daily fish consumption (g/day), BW: the mean body weight of population (kg).

Determination of the target hazard quotient (THQ)

The target hazard quotient (THQ) is a complex parameter introduced by the United States Environmental Protection Agency and is commonly used to assess the potential of non-carcinogenic risks associated with long-term exposure to contaminants, such as heavy metals from foods such as fish and water. THQ represents the ratio of chronic daily intake of metals studied (EDI) in mg/kg/day to the oral reference dose (RfD) also expressed in mg/kg/day. In addition, THQ parameter does not estimate the risk; it only indicates

a level of risk associated with exposure to pollutants; if the value of THQ is < 1, it means that there are no adverse effects for the exposed population; when THQ > 1, there is a potential risk related to the metal studied in the exposed population (Al-Mahaqeri and Ahmad, 2015). The THQ can be calculated using the formula (Orosun et al., 2016):

$$THQ = (EFr \times EDtot \times FIR \times C) \div (RfDo \times BW \times ATn) \times (10\text{-}3)$$

where EFris is the exposure frequency (365 days/year), EDtot the exposure duration (70 years, average lifetime), FIR the food-intake rate (g/day), C the mean of Hg and MeHg concentrations in sardine and swordfish muscular tissue (mg kg^{-1}), RfDo the oral reference dose of Hg and MeHg fixed by US EPA are 3×10^{-4} and 1×10^{-4} mg/kg/day, respectively (USEPA, 2017). BW is the average body weight (60 kg of body weight refers to adult people) and ATn the period of average exposure for non-carcinogens (365 days/year × number of exposure years, 70 years).

Determination of the hazard index (HI)

This is the sum of the hazard quotients for substances that affect the same organ or target organ systems. Ideally, hazard quotients should be combined for pollutants that cause adverse effects through the same toxic mechanism (USEPA, 2017). As with the hazard quotient, overall exposures below 1 calculated using hazard quotients are unlikely to result in any chronic systemic risk adverse health effects during a lifetime of exposure and would normally be considered as acceptable. The hazard index (HI) from THQs is expressed as the sum of the target hazard quotients (Núñez et al., 2018):

$$HI = THQ (Hg) + THQ (MeHg)$$

RESULTS

Concentrations of Hg and MeHg

Concentrations of Hg and MeHg in the flesh of sardine (*Sardina pilchardus*) and swordfish (*Xiphias gladius*)

The results in (Table 1) showed that the concentrations of Hg and MeHg were higher in swordfish (0.61 ± 0.47; 0.57 ± 0.45 mg/kg w.w, respectively) than in the sardine (0.04 ± 0.03; 0.04 ± 0.028mg/kg w.w, respectively).

The nonparametric Mann-Whitney test showed a significant difference for both Hg and MeHg concen-

trations between the two species (sardine p-value = $9.99 \times 10^{-16} < 0.05$; swordfish p-value = $7.536 \times 10^{-10} < 0.05$).

Hg and MeHg concentrations in the three study areas for both species

The results in (Table2) showed that the concentrations of Hg and MeHg were higher in the swordfish flesh than in sardine in all study areas. The highest concentrations of Hg and MeHg were found in the swordfish of NC (0.77 ± 0.41 mg/kg w.w; 0.64 ± 0.38 mg/kg w.w, respectively). The Kruskal-Wallis test showed no difference between the three study areas for MeHg concentrations, while a difference was recorded for the Hg concentrations between the NW area and the others.

In sardine, the statistical test showed a difference between NE and other areas for Hg and between NC and NE for MeHg.

The lower case letters showed the presence of difference or not according to the Kruskal-Wallis test.

The average concentrations of Hg and MeHg obtained in the flesh of sardine and swordfish in the three study areas were compared with the national and European regulatory thresholds (JORDPN°25/2011; EU N° 1881/2006). The results showed that the average concentrations of this metal and its organic form are lower than the set thresholds (0.5 mg/kg w.w in sardine and 1 mg/kg w.w in swordfish).The difference in threshold between these two species is due to the different concentrations of metals in fish flesh. Swordfish is a predatory fish found at the top of the marine food chain, which allows it to accumulate more mercury, particularly the organic form methylmercury than the sardine.

Risk assessment

Estimated dietary Intake (µg/kg/ body weight/day/week) to Hg and MeHg in sardine and swordfish

The estimated weekly intakes (EWI) of Hg and MeHg due to swordfish consumption (0.7; 0.64µg/kg/bw/week, respectively) were higher than the EWI's recorded for consumption of sardine (0.5/kg bw/week) for both Hg and MeHg. These values were lower than the provisional tolerable weekly intake (PTWI) established by the European Food Safety Authority (EFSA) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (Table 3).

Table 1. Concentrations of Hg and MeHg in the flesh of sardine *(Sardina pilchardus)* and swordfish *(Xiphias gladius)* (mg/kg w.w). n: number of samples; SE: standard deviation; Min: Minimum; Max: Maximum, ND: No detected concentration

	Sardine*(Sardina pilchardus)* (n= 43)		Swordfish *(Xiphias gladius)* (n= 44)	
	Mean ± SD	Min.-Max. Value	Mean ± SD	Min.-Max. Value
Hg	0.04±0.03	0.01-0.12	0.61±0.47	0.16-2.18
MeHg	0.04±0.02	ND-0.07	0.57±0.45	0.12-1.92

Table 2. Variations in concentrations of Hg and MeHg in sardine and swordfish flesh depending on the study areas

Species	Study areas	N	Mean±SE (Max-Min)	
			Hg	MeHg
Sardine (*Sardina pilchardus*)	**Algiers**	15	0.02[d]±0.01(0.01-0.06)	0.02[c]±- (ND-0.04)
	Bejaia	13	0.08[c]±0.01(0.07-0.12)	0.06[b] ± 0.006 (0.04-0.07)
	Oran	15	0.02[d]±0.04(0.01-0.03)	ND
Swordfish *(Xiphias gladius)*	**Algiers**	20	0.77[a]±0.41(0.25-1.25)	0.64[a]±0.38 (0.12-1.92)
	Bejaia	13	0.69[a]±0.59 (0.16-2.18)	0.59[a]±0.52 (ND-0.01)
	Oran	11	0.23[b]±0.02 (0.19-0.27)	0.20[a] ±0.02 (0.13-0.23)

Table 3. Estimated Dietary Intake (µg/kg/ body weight/ day/week) for the intake of Hg and MeHg in sardine and swordfish.

Heavy Metals		EDI	EWI	Established PTWI by EFSA	Established PTWI by JECFA
Hg	Sardine	0.0064	0.05	-	**5 (JECFA, 2010)**
	Swordfish	0.098	0.7		
MeHg	Sardine	0.0064	0.05	1.3	1.6
	Swordfish	0.092	0.64	**(EFSA, 2012)**	**(JECFA, 2010)**

Table 4. Estimated Dietary Intake (µg/kg/ body weight/day/week) for the intake of Hg and MeHg in sardine and swordfish by region of study.

Heavy metals	Species		EDI	EWI	Established PTWI by EFSA	Established PTWI by JECFA
Hg	Algiers	Sardine	0.0032	0.0224	-	5 (JECFA, 2010)
		Swordfish	0.124	0.90		
	Bejaia	Sardine	0.013	0.91		
		Swordfish	0.111	0.78		
	Oran	Sardine	0.0032	0.0224		
		Swordfish	0.037	0.26		
MeHg	Algiers	Sardine	0.0032	0.0224	1.3 (EFSA, 2012)	1.6 (JECFA, 2010)
		Swordfish	0.103	0.721		
	Bejaia	Sardine	0.010	0.07		
		Swordfish	0.095	0.67		
	Oran	Sardine	-	-		
		Swordfish	0.032	0.224		

Estimated dietary Intake (µg/kg/ body weight/day/week) to Hg and MeHg by region of study

These results (Table 4) showed that the values of estimated daily/weekly intakes (EDI's/EWI's) were different among the studied regions for the same species. The estimated EDI's/EWI's of Hg and MeHg in the swordfish of NC gave the highest values (0.124; 0.103 µg/kg/bw/day; 0.90; 0.721 *µg/kg/bw/week*, respectively). All the estimated values in the three studied regions were lower than the fixed values (PTWI's) of EFSA and JECFA.

Target hazard quotient (THQ) and hazard index (HI)

We have estimated the THQ for Hg and MeHg and the HI due to the consumption of the two fish species. All the registered values have been < 1 (Table 5).

Table 5. Estimated of target hazard quotient (THQ) and hazard index (HI)

	THQ (Hg)	THQ (MeHg)	HI
Sardine	2.15×10^{-5}	6.46×10^{-5}	8.61×10^{-5}
Swordfish	3.28×10^{-4}	9.21×10^{-4}	1.24×10^{-3}

DISCUSSION

Hg and MeHg concentrations in the flesh of sardine and swordfish

The recorded average concentration of Hg in sardine flesh in the three studied regions (Table 1) was higher than that reported by Yabanli (2013) in Izmir (Turkey). However, it was much lower than reported in our previous study (2016) than that reported by Chahid (2016) in five areas (Agadir, Essouira, Dakhla, Sidi Ifni and Laayoune) of Morocco.

The MeHg concentration recorded (Table 1) was higher than that reported by Cano-Sancho et al. (2015) and Carbonell et al. (2009) in Spain.

In swordfish, the Hg recorded average concentration (Table 1) was higher than that reported by Zaza et al. (2015) in the central Atlantic Ocean. While it was lower than that recorded in our previous study in Algiers (2016) and that reported by Liu et al. (2018) in the United States and Torres-Escribano et al. (2010) in Spain. It was observed that the values reported in our study in 2016 are different from those reported in the current study, which can be attributed to the changes of heavy metals concentrations with time, and also as a result of the different assay methods.

The average concentration of MeHg reported was higher than that reported by Cano-Sancho et al. (2015) in Spain.

Many studies on Hg and MeHg concentrations in sardine and swordfish have shown different results with a high or low concentration. This difference could be due to variations in the study area, the size, age, sex and the physiological status of the fish (Manavia and Mazumder, 2018). Other factors should be considered such as fishing seasons, microbial activity and mercury content in sediment, water chemistry characteristics (dissolved organic content, salinity, pH, and redox potential) (Ramos, 2012; Rajeshkumar and Li, 2018).

The concentrations of metals differ from one fish species to another; the highest concentrations of Hg

and MeHg were recorded in swordfish, which could be attributed to its high marine trophic position. This trophic position has variable effects on the bioaccumulation of metallic elements in pelagic fish depending on the element considered (Bodin et al., 2017). Hg levels in fish showed significant interspecific differences reflecting the trophic level and biomagnifications. Hg tends to bioaccumulate in organisms with higher trophic levels. Large predatory fish reaches high concentrations of Hg; usually larger individuals have higher concentrations than the smaller ones, due to age, diet and the time of exposure to pollutants. Casadevall et al. (2017) studied the contamination of swordfish muscle samples from Madrid markets and showed that approximatively 35% of the samples exceeded the maximum limit of Hg, some specimens reached levels up to 1900 ng/g, which agree with our results.

The lowest concentrations of total Hg and MeHg found in the sardine could be a result of their diet, which consists mainly of water plants and plankton (Vieira et al., 2011).

The literature on heavy metal concentrations in fish shows that the fishing areas are an important factor of variation of metal accumulations, which was observed in this study (Table 2).

The main results obtained in this study confirm what several researchers have reported previously. The majority of accumulated total mercury is in its organic form (methylmercury), due to its high lipophilicity and low solubility in water compared to other forms (Ramos, 2012; Carroll and Warwick, 2017).

Risk assessment

The risk assessment conducted for adult Algerian consuming sardine showed that the EWI for Hg in sardine (Table 3) was similar to that reported by Vieira et al. (2011) in Portugal but lower than that reported by Falcoä et al. (2006) in Spain in adults and Chahid (2016) for a Moroccan adult of 60 kg. The MeHg EWI (0.05 ug/kg/body weight/week) was higher than that reported by Cantoral et al. (2017) in Mexico.

In the swordfish, the Hg EDI and EWI (Table 3) were lower than that reported by Falcoä et al. (2006), Cano-Sancho et al. (2015) and Aranda et al. (2017) in Spain for adult men and women.

The values of estimated dietary intakes for both sardine and swordfish we recorded did not exceed the provisional tolerable weekly intake (PTWI) established by the European Food Safety Authority (EFSA) and Joint FAO/WHO Expert Committee on Food Additives (JECFA) (Table 3, 4).

From the results of this study, the consumption of sardine and swordfish, wherever they are caught, does not expose the consumer to toxic risk.

Since all calculated target hazard quotient and risk index are below 1 (Table 5), sardine and swordfish do not pose chronic systemic risk to the Algerian population.

CONCLUSIONS

The average concentrations of Hg and MeHg in swordfish were higher than those obtained in sardine fished in the three Algerian coasts; these values were lower than the national and European regulatory thresholds (OJPDRAN°25/2011; EU N°1881/2006).

The species and the fishing area are two important factors that influence the bioaccumulation of Hg and MeHg.

In the risk assessment performed, estimated dietary intake (EDI) was lower than the provisional tolerable weekly intake (PTWI) established by the European Food Safety Authority (EFSA) and Joint FAO/WHO Expert Committee on Food Additives (JECFA), the target hazard quotient (THQ) and the index hazard (IH) were below 1. As a result, the consumption of sardine and swordfish do not pose any risk to the adult Algerian population.

CONFLICT OF INTEREST

None declared by the authors.

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