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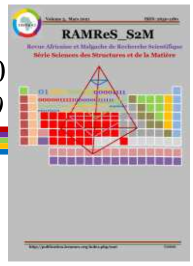

Evaluation of potential risks of farmed fish consumption on human health

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***Abstract*:**

The objective of this study is to evaluate the levels of metallic trace elements in the muscle of tilapia *Oreochromis niloticus* fish reared in a floating cage in order to assess the potential risks of the consumption of farmed fish on human health in Bingerville, a town situated in the southeast of Côte d'Ivoire. Fish samples were monthly taken from February to July 2017. The levels of metallic trace elements were determined in the muscle of the fish using an atomic absorption spectrophotometer and the potential health risk was determined by the calculation of the estimated daily intake (EDI), the estimated weekly intake (EWI), the hazard quotient (HQ) and the hazard index (HI). The arsenic levels in the samples were higher than the ones of lead, mercury and cadmium. Concentrations of arsenic (1.12 ± 0.04 - 1.4 ± 0.3) mg/kg in muscle were above the maximum authorized concentration (0.1 mg/kg) for human consumption. However, the daily and weekly intakes of all metals were below the tolerable values recommended by the joint FAO/WHO committee. Moreover, the hazard quotients of the different metals were less than 1 (HQ < 1). In addition, the hazard index of all metallic trace elements was less than 1 (HI < 1). Therefore, the result of this assessment is that the consumption of tilapia reared in a floating cage is not harmful to human health.

***Keywords*:** Fish farm; Floating cage; Farmed fish; Metallic trace elements; Risk assessment; Côte d'Ivoire.

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1. Introduction

Fish is an important part of human diets. Moreover, fish is an important source of protein, but it is also rich in essential minerals, vitamins and unsaturated fatty acids [1]. With the increase in fish consumption and the slowdown in fishing catches in recent years, aquaculture has become the driving force behind the production of fish for consumption [2]. Farmed fish are therefore called upon to play an important role in the satisfaction of food security. However, fish farms like all aquatic environments are threatened by pollutants such as metallic trace elements.

Metal trace elements are toxic pollutants that are harmful to the aquatic environment [3]. The toxicity of trace metal elements can affect fish growth, their physiology as well as fish production [4]. Once in fish farms, these trace metals from human activities, atmospheric transport and erosion from rain contaminate water, sediment and farmed fish. Fish contaminated with trace metal elements are now a worldwide concern because trace metal elements cause health problems to humans [5]. Indeed, the consumption of aquatic resources containing toxic metals can cause serious health risks due to their bioaccumulation in the food chain [6]. Besides, metallic trace elements are not biodegradable, they are carcinogenic and accumulate in living organisms to cause a wide range of diseases and disorders [7, 8]. Arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb) are metals known to harm, even at low concentrations, fish [4] and humans [9].

In the present study, the levels of the metallic trace elements in the muscle (edible part) of the tilapia *Oreochromis niloticus* fish reared in a floating cage were determined in order to assess how dangerous the consumption of farmed fish in the Aghien lagoon can be for human health.

2. Materials and methods

2.1. Sampling and preservation of samples

Sampling was carried out from February to April 2017 (dry season) and from May to July 2017 (rainy season) in a floating cage fish farm located on the Aghien lagoon in Bingerville between longitude 3°53'10" W and latitude 5°24'14" N (Figure 1). During the samplings, reared tilapia *Oreochromis niloticus* (Figure 2) were captured alive using dip nets. The fish were carefully placed in new plastic bags immediately and stored in a cooler at 4 °C in order to be transported to the laboratory. Once in the laboratory, the fish samples were stripped of inedible parts using a stainless steel knife. Subsequently, 5 g of fish muscle were taken, kept in pill organizers and stored at -20 °C for further evaluation in order to check the amounts of trace metal elements contained in the fish muscle.

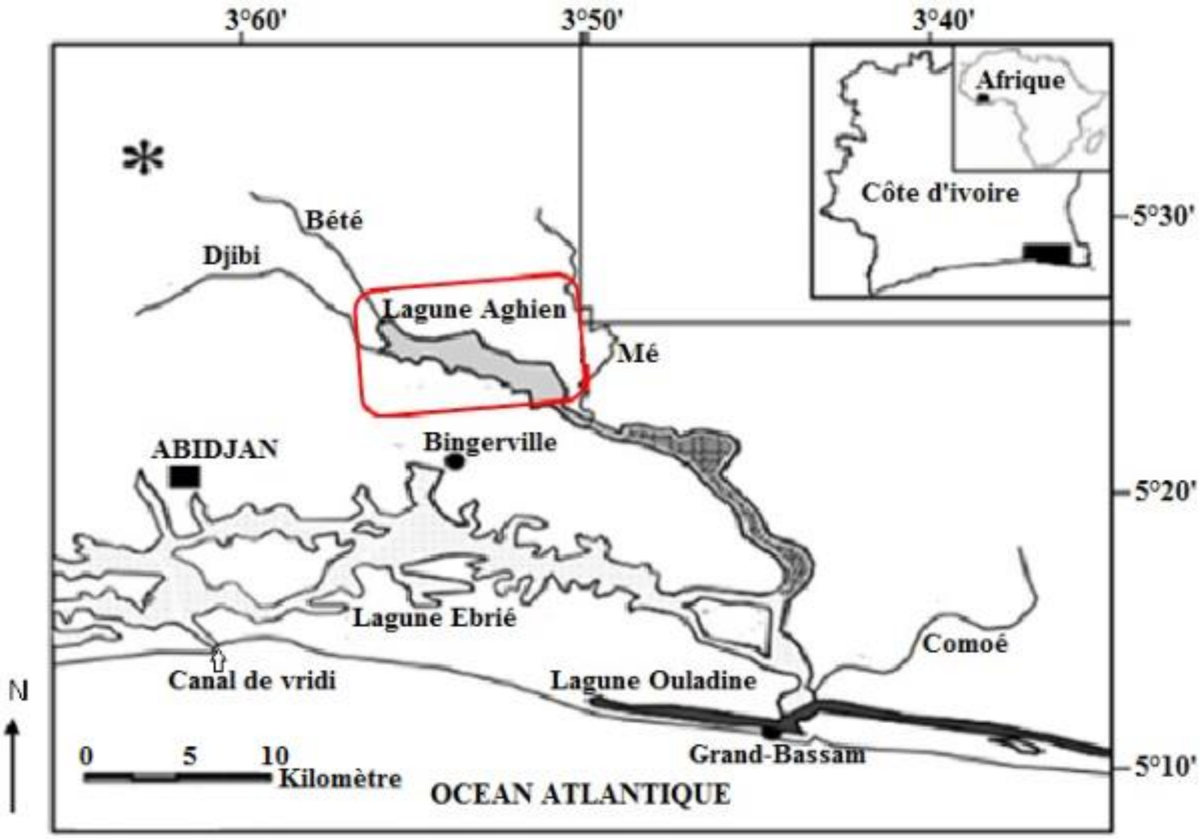

Fig. 1. Location map of the fish farm on the Aghien lagoon in Bingerville [10].

Fig. 2. Photograph of the fish (tilapia *Oreochromis niloticus*) collected on the farm.

2.2. Determination of the concentration of metallic trace elements

Metal trace elements were determined using a Shimadzu brand atomic absorption spectrophotometer (Shimadzu AA 660). The assays were performed at wavelengths of 253.7 nm for mercury (Hg); 228.8 nm for cadmium (Cd); 283.3 nm for lead (Pb) and 193.7 nm for arsenic (As).

Prior to any atomic absorption spectrometry analysis, fish samples were dried in an oven at 70 °C for 12 hours to improve the efficiency of acid mineralization.

For the determination of cadmium, lead and arsenic, samples are mineralized through heating in microwaves. In each Teflon bomb adapted to the microwave digestion apparatus, 200 mg of sample homogenate are weighed. 8 mL of nitric acid are added to the homogenate. The bombs are closed and positioned in the mineralizer, and then the temperature is gradually increased to 180 °C. After cooling down, the solutions are transferred to polyethylene tubes. The metallic element content is measured by atomic absorption in the sample of mineralisates. The concentration of mineralisates is corrected for the content of the blanks, and then divided by the dilution factor P/V [11]:

$$C_M = (C - C_B) \times \frac{V}{P} \quad (1)$$

With:

- C_M: the concentration of the metallic trace element (mg/kg);
- C: the concentration of metallic element measured in the mineral (µg/L);
- C_B: the concentration of metallic element measured in the blank (µg/L);
- V: the final volume of the mineral (L);
- P: the weight of the starting sample (mg).

For the determination of mercury, the method used is a semi-automatic method, which allows the determination of the mercury present in the samples after dry mineralization. About 10 to 30 mg of the dried sample are weighed into a cuvette. Placed on its rack, this cuvette is automatically introduced into the oven of the device where the temperature is gradually raised to 550 °C. After calcination, the mercury present in the sample is volatilized. The formed elemental mercury is carried in the form of vapor by a stream of oxygen and amalgamated on a golden sand trap. After heating the trap to separate the formed gold-mercury amalgam, the mercury vapor is sent to the cell of the atomic absorption spectrophotometer. During analyzes, the computer calculates the amounts of mercury present in the sample from the absorbance by referring to the calibration curve [12].

2.3. Assessment of the health risks linked to the consumption of tilapia farmed in floating cage in the Aghien lagoon

2.3.1. Estimated Daily Intake (EDI)

The estimated daily intake is the estimate of the amount of a toxic substance in a food, based on body weight, which is daily ingested by the consumer. The estimated daily intake of trace metals elements linked to the consumption of fish is determined by the following equation [13]:

$$EDI = \frac{C_{metal} \times D_C}{Bw} \quad (2)$$

In which EDI is the estimated daily intake for trace metals (mg/kg/day); C_{metal} (mg/kg) is the maximum concentration of metal in the muscle of the fish; Dc (kg/day) represents the daily average consumption of fish in Côte d'Ivoire and Bw (kg) is the body weight of an adult.

The average quantity of fish consumed by an Ivorian is 15 kg/year, i.e. 0.041 kg/day [14, 15]. The average body weight of an adult is conventionally equal to 70 kg according to the United States Environmental Protection Agency (US EPA) [16].

2.3.2. Estimated Weekly Intake (EWI)

The estimated weekly intake permits to evaluate the amount of a toxic substance in a food, based on the body weight, which is ingested per week by the consumer. The estimated weekly intake of trace metal elements linked to the consumption of fish is determined by the following relationship [17]:

$$EWI = \frac{C_{metal} \times W_C}{Bw} \quad (3)$$

In which EWI is the estimated weekly intake for trace metal elements (mg/kg/week); C_{metal} (mg/kg) is the maximum concentration of metal in the muscle of the fish; W_C (kg/week) represents the weekly average fish consumption in Côte d'Ivoire and Bw (kg) is the body weight of an adult.

The weekly average quantity of fish consumed by an Ivorian is 0.288 kg/week [14, 15].

2.3.3. Hazard quotient (HQ)

Although it is necessary to determine the daily and weekly intakes to understand the level of exposure to metallic trace elements, the assessment of risks is a rapid and essential method for measuring the effect of trace metals on human health [18]. The risk characterization for threshold effects is expressed by the hazard quotient. It is a term commonly used in assessing the risk of consuming a food. The HQ is the simplest tool to assess a risk.

It makes it possible to evaluate the risk associated with a single metal [18]. The HQ can be evaluated by Eq. (3) [19, 20]:

$$HQ = \frac{EDI}{ADI} \quad (4)$$

Where HQ is the hazard quotient, EDI (mg/kg/day) is the estimated daily intake and ADI (mg/kg/day) is the acceptable daily intake.

For HQ values < 1, the presence of a toxic effect is very unlikely, whereas for HQ > 1, the appearance of a toxic effect is evident [19].

2.3.4. Hazard index (HI)

The hazard index is a simple measure, which allows bringing together hazards linked to several metals. This is the sum of the hazard quotients [21]:

$$HI = \sum_1^n HQ_i \quad (5)$$

In which HI is the hazard index, HQ is the hazard quotient of metal i, and n is the total number of metals.

The HI value < 1 indicates the absence of harmful effects and the HI value > 1 indicates harmful effects [21].

2.3.5. Metal pollution index (MPI)

The Metal Pollution Index allows the comparison of the total content of metallic trace elements between study sites, periods and organisms. So to compare the total trace metal elements content in fish muscle over the two sampling seasons, the MPI was calculated with Eq. (5) [22, 23]:

$$MPI = (C_1 \times C_2 \times \ldots \times C_n)^{1/n} \quad (6)$$

In which C is the concentration of metal in the sample and n is the total number of trace metal elements measured in the sample.

2.4. Statistical analysis

The STATISTICA software (Version 7.1) allowed us to perform the Bravais-Pearson correlation test to establish a relationship between the concentrations of metallic trace elements in muscle.

3. Results and discussion

3.1. Concentration of metallic trace elements in the muscle of tilapia farmed in a cage in the Aghien lagoon

The study revealed that there are the metals in the muscle of the sampled fish. The concentrations of cadmium (Cd), arsenic (As), mercury (Hg) and lead (Pb) in the muscle of raised tilapia are shown in Table 1. The mean concentrations of Cd were 0.03 mg/kg in both the dry season and the rainy season. The concentrations of Hg ranged from 0.05 ± 0.03 to 0.08 ± 0.01 mg/kg, then Pb from 0.09 ± 0.01 to 0.10 ± 0.01 mg/kg and As from 1.12 ± 0.04 to 1.4 ± 0.3 mg/kg. As, Pb and Hg concentrations are higher in the rainy season than in the dry season. This seasonal variation observed in the accumulation of metallic trace elements would be due to local and seasonal pollution [24] in the living environment (water and sediments) of fish [25]. Over both seasons, the accumulation of metals in muscle was in the following decreasing order: As > Pb > Hg > Cd. This order of accumulation of metallic trace elements in the soft tissue of fish could be due to the difference in the accumulation process of different metals in fish [23]. Cadmium has the lowest content (0.03 mg/kg) in the muscle of fish (Table 1). This could be because farmed tilapia *Orechromis niloticus* weakly accumulates cadmium in its flesh. Indeed, Sanou *et al*. [26], in a study conducted on a fish farm in Taabo, showed that cultured tilapia had a cadmium bioconcentration factor of less than 1 as well as a bioaccumulation factor of less than 1; thus translating an almost zero accumulation of this metal in the fish. In contrast, arsenic is the most accumulated metal (1.12 ± 0.04 - 1.4 ± 0.3 mg/kg) in tilapia muscle (Table 1). This could be due on the one hand to the excessive use of arsenic-based pesticides in the plantations bordering the fish farm and on the other hand to the use of contaminated amendments and fertilizers with a significant concentration of arsenic. Indeed, many fertilizers contain traces of arsenic [27].

The toxic metal content in tilapia muscle was compared to the maximum authorized limit to assess the potential risk of farmed fish for human consumption.

The results show that the concentrations of Cd, Hg and Pb in the muscle of the fish did not exceed the upper limits authorized by the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) [28, 29]. These results tell that for Cd, Hg and Pb the consumption of farmed fish has no toxic effect on human health. However, the arsenic content exceeded the maximum authorized level of 0.1 mg/kg (Table 1), indicating that it could be a risk for human consumption. Indeed, the accumulation of metallic trace elements in the soft tissue of fish can present serious health risks [30] because the concentration of toxic metals in the muscles of fish can exceed the limits authorized for the human consumption [31].

Furthermore, the overall content of trace metal elements in the muscle of reared tilapia during the two sampling seasons was compared using the Metal Pollution Index (MPI). The recorded values indicate a slightly elevated overall contamination in the rainy season (Table 1). This could be attributed to the high concentration of arsenic recorded during the rainy season. However, these seasonal values are less than 1 (MPI < 1). Therefore, this shows that all the metals contained in the muscle of farmed fish are safe for human consumption both in the dry season and in the rainy season [23]. However, these levels of trace metal elements in fish muscle do not provide information on the level of exposure to metals. It is therefore necessary to evaluate the weekly and daily intakes of exposure to trace metal elements from the consumption of fish.

Table 1
Seasonal values of metallic trace elements (mg/kg) in muscle of *Oreochromis niloticus*

	Metallic trace elements				**MPI**
	Cadmium (Cd)	Mercure (Hg)	Plomb (Pb)	Arsenic (As)	
Dry season	0.03	0.05±0.03	0.09±0.01	1.12±0.04	0.1086
Rainy season	0.03	0.08±0.01	0.10±0.01	1.4±0.3	0.1347
MPL*	**0.05**	**0.5**	**0.2**	**0.1**	

***MPL**: Maximum permissible limits authorized by the joint committee FAO/WHO [28, 29]

3.2. Interdependence of metallic trace elements in muscle of farmed tilapia in a cage in the Aghien lagoon

A Bravais - Pearson correlation matrix was performed to make clear the relationships between assayed metallic trace elements in the muscle of the cage-reared tilapia in the Aghien Lagoon. The correlation coefficients are shown in Table 2. Analysis of the correlation matrix points out the existence of positive and significant correlations between Hg-Pb (r = 0.89), Hg-As (r = 0.87), Cd-As (r = 0.83) and Pb-As (r = 0.55) in the dry season. Moreover, in the rainy season, positive and significant correlations were observed between Hg-Pb (r = 0.96) and Cd-As (r = 0.85).

These strong positive correlations observed between metallic trace elements show a mutual dependence of metals, common sources of contamination and similar behavior during transport [32]. However, in the rainy season, significantly negative correlations were recorded between Cd-Hg (r = - 0.90) and Cd-Pb (r = - 0.75). These results point out the increase in cadmium in the muscle of fish, which brings about the decrease in lead and mercury. In addition, a negative correlation is observed between Hg-As (r = - 0.54). This indicates that the concentration of arsenic in the muscle of tilapia decreases with increasing mercury content.

Table 2

Bravais - Pearson linear correlation matrix

Dry season				
	Cd	Hg	Pb	As
Cd	1			
Hg	0.45	1		
Pb	0.00	0.89	1	
As	0.83	0.87	0.55	1
Rainy season				
Cd	1			
Hg	-0.90	1		
Pb	-0.75	0.96	1	
As	0.85	-0.54	-0.29	1

3.3. Assessment of the health risks linked to the consumption of farmed fish in a cage in the Aghien lagoon

Chronic ingestion of trace metal elements, beyond the tolerance threshold in humans, has harmful effects and can cause neurological damage, headaches, liver disease [33], renal, hematopoietic and gastrointestinal systems [34].

3.3.1. Evaluation of weekly and daily estimated intakes

The estimated weekly intake (EWI) was used to assess the level of exposure to each metallic trace element per week. The results of the EWI values over the two seasons are given in Table 3. The EWI, for all metals, are significantly higher in the rainy season than in the dry season. However, As has the highest EWI followed by those of Pb, Hg and Cd. However, the obtained EWI for Cd (1.11×10^{-4} – 1.56×10^{-4} mg /kg/week), Hg (3.91×10^{-4} – 3.99×10^{-4} mg/kg/week), Pb (4.11×10^{-4} – 4.61×10^{-4} mg/kg/week) and As (4.81×10^{-3} – 6.62×10^{-3} mg/kg/week) are very much below the tolerable intakes recommended by the joint committee of FAO and WHO. Indeed, according to the joint FAO/WHO committee [35], the provisional tolerable weekly intakes (PTWI) for Cd, Hg, Pb and As are respectively 0.007; 0.0016; 0.025 and 0.015 mg/kg of body weight/week which correspond to 0.49; 0.112; 1.75 and 1.05 (mg/kg/week) for a 70 kg adult [36], respectively.

Table 3
Estimated Weekly Intake (EWI) to metallic trace elements

		Metallic trace elements			
		Cd	Hg	Pb	As
EWI (mg/kg/week)	**Dry season**	1.11×10^{-4}	3.91×10^{-4}	4.11×10^{-4}	4.81×10^{-3}
	Rainy season	1.56×10^{-4}	3.99×10^{-4}	4.61×10^{-4}	6.62×10^{-3}
PTWI*(mg/kg bw/week)		**7×10^{-3}**	**1.6×10^{-3}**	**25×10^{-3}**	**15×10^{-3}**
PTWI**(mg/kg/week)		**0.49**	**0.112**	**1.75**	**1.05**

* Provisional tolerable weekly intake [35]; ** For an adult weighing 70 kg [36]

Table 4 shows the results of the assessment of the estimated daily intake (EDI) in Cd, Hg, Pb and As linked to the consumption of tilapia reared in cages in the Aghien lagoon. The EDI values for trace metals are (1.58×10^{-5} – 2.23×10^{-5} mg/kg/day), (5.56×10^{-5} – 5.68×10^{-5} mg/kg/day), (5.86×10^{-5} – 6.56×10^{-5} mg/kg/day) and (6.85×10^{-4} – 9.43×10^{-4} mg/kg/day) for cadmium, mercury, lead and arsenic, respectively. The EDI values for all metals are lower than the intakes recommended by the joint FAO/WHO committee [35] both in the dry season and in the rainy season which are 1×10^{-3}; 2.29×10^{-4}; 3.57×10^{-3} and 2.14×10^{-3} (mg/kg bw/day) for Cd, Hg, Pb and As (Table 4), respectively. These results tell that the consumption of tilapia reared in cages in the Aghien lagoon is not harmful to the consumers' health because the weekly and daily exposure intakes are well below the recommended safety values.

Table 4
Estimated Daily Intake (EDI) to metallic trace elements

		Metallic trace elements			
		Cd	Hg	Pb	As
EDI (mg/kg/day)	**Dry season**	1.58×10^{-5}	5.56×10^{-5}	5.86×10^{-5}	6.85×10^{-4}
	Rainy season	2.23×10^{-5}	5.68×10^{-5}	6.56×10^{-5}	9.43×10^{-4}
ADI* (mg/kg bw/day)		**1×10^{-3}**	**2.29×10^{-4}**	**3.57×10^{-3}**	**2.14×10^{-3}**
ADI** (mg/kg/day)		**0.07**	**0.016**	**0.25**	**0.15**

* Acceptable Daily Intake of PTWI [35]; ** For an adult weighing 70 kg [36]

3.3.2. Evaluation of hazard quotients and indices

Table 5 shows the results of the Hazard Quotients (HQ) and the corresponding Hazard Index (HI). The hazard quotient (HQ) points out an indication of the level of risk of a single metal when we eat fish. It was therefore used to assess the toxic effects of each metal. The seasonal HQ values of cadmium (0.0002 – 0.0003), mercury (0.0035 – 0.0036), lead (0.0002 –0.0003) and arsenic (0.0046 – 0.0063) are less than 1 (HQ < 1). These results indicate that toxic effects from these metals linked to consumption of farmed fish is very unlikely for consumers. Indeed, HQ values < 1 show the absence of toxic effects for consumers' health [16, 19].

The Hazard Index (HI) provides information on the level of risk associated with all trace metals ingested by eating contaminated fish. The hazard index is 0.0085 in the dry season and 0.0105 in the rainy season. These values are less than 1 (ID < 1). This shows that all the trace metal elements cadmium, mercury, lead and arsenic contained in the muscle of farmed tilapia in a cage in the Aghien lagoon is not toxic. It is also not harmful to human health [18, 21].

Arsenic and mercury are the main causes of the risk with respectively 54.12% and 41.18% of the total HI in the dry season. This risk is 60.00% for As and 34.29% for Hg in the rainy season. This shows the need to develop strategies to reduce arsenic and mercury pollution in this fish farm.

Table 5
Hazard Quotients (HQ) and Indices (HI)

		Metallic trace elements				**HI**
		Cd	Hg	Pb	As	
HQ	Dry season	0.0002	0.0035	0.0002	0.0046	0.0085
	Rainy season	0.0003	0.0036	0.0003	0.0063	0.0105

4. Conclusion

The aim of this study was to determine the levels of contamination of Cd, Hg, Pb and As in the muscle of tilapia *Oreochromis niloticus* farmed in the Aghien lagoon and to assess the risk linked to the consumption of these fish. All the samples recorded higher concentrations of arsenic with values above the maximum limit authorized by the FAO/WHO. This therefore shows that this metal could have toxic effects on human health. However, the recorded values of Metal Pollution Indices (MPI) were less than 1, indicating that all metals are harmless to health. In addition, the evaluation of the estimated daily and weekly intakes as well as the hazard quotients and indices showed that the contributions of Cd, Hg, Pb and As through the consumption of farmed tilapia in a cage in the Aghien lagoon is harmless to consumers' health.

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