

Special Article - Endocrinology

Determination of Heavy Metals (Pb, Cd and Hg) in Crude and Refined Edible Salt at Production Level in Cameroon – A Risk Assessment

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Abstract

Edible salt is the most commonly used for iodization worldwide. Therefore, any contamination of processed iodized salt could be a health hazard. The present study aimed to determine the levels of heavy metals in the crude salt as well as after processing it, to assess the risk involved in consuming food grade salt that has passed through this process in order to propose amendments. Eighty-two crude salt samples and the same number of refined salt samples were collected from three factories that are producing iodized to serve the CEMAC Region in 2006, 2010 and 2018. The levels of lead (Pb), cadmium (Cd), mercury (Hg), were determined using atomic absorption spectroscopy method. The study reveals that imported crude salt from Namibia, Egypt and Djibouti does contain high levels of these heavy metals contaminants. The levels expressed as average (range) in µg/g of Pb, Cd, Hg, in refined salt samples were 3.7 (0.8 – 8.0), 0.23 (0.0 – 0.6) and 0.45 (0.01 – 0.9) in 2006; 2.5 (0.2 – 9.0), 0.32 (0.0 – 0.8) and 0.37 (0.0 – 0.9) in 2010; 2.6 (0.1 – 8.0), 0.2 (0.0 – 0.8) and 0.35 (0.0 – 1.0) in 2018, respectively. The results obtained in the present study were compared with Codex standards. Except for Cd that posed no problem, all values for these metals in refined salts were higher than the permitted consumption level defined by Codex (2 µg/g of Pb, 0.5 µg/g of Cd and 0.1 µg/g of Hg).

Keywords: Pb; Cd; Hg; Heavy metals; Quality assurance; Risk Assessment

Introduction

Heavy metals present in foods and the environment constitute a source of danger for human health [1,2]. Over the past decades there is increased industrial use of heavy metals leading to their accumulation in the environment with consequence being increase food contamination [3]. The continuous consumption of foods or salt contaminated by these minerals would normally result in their accumulation in body tissues and organs and with an eventual consequent effect on health. For example, increasing concentration of lead (Pb) under chronic exposure, will result to irreversible functional disorders that may even cause death. A similar scenario has been reported in the case of Cadmium, mercury etc [4]. Mindful of the deleterious effects on health because of the accumulation of heavy metals in the body, the acquisition of knowledge of their level of concentrations in crude and especially processed food like salt that is consumed by man and animal is of paramount importance. Biologically, edible salt cannot be avoided totally, as it provides sodium and chlorine which are two essential macronutrients for the human body. Edible salt improves food taste and can also be used for food preservation. In addition, it is the most widely used additive in food industry [5].

Meanwhile, a joint expert meeting of the Food and Agricultural Organization and World Health Organization [6] on the evaluation of certain food additives and contaminants had reported that lead, cadmium and mercury were the most pollutant heavy metals.

Furthermore, lead (Pb) is amongst the chemical goitrogens that affect thyroid function, promoting thyroid growth or goiter [7,8] or thyroid hormonal malfunctioning [9]. It may act in isolation or association with iodine insufficiency to produce a spectrum of clinical conditions called Iodine Deficiency Disorders (IDD) such as cretinism, physical or mental retardation, deafness or muteness and spastic etc [10,11].

Based on the foregoing, the present researchers were curious to know the levels of heavy metals in the crude salt as well as after processing it. In other words, they were curious to know how successful the Quality Assurance was in reducing the heavy metal contaminants which are a known health risk. The concerned heavy metals were Lead, Cadmium and Mercury.

The hypotheses for this research were:

- Imported crude salt contains undesirably high levels of toxic heavy metal (Pb, Cd, and Hg) constituents.
- The Quality Assurance practiced by the Iodized salt Refineries does not completely eliminate these heavy metal constituents.

Objectives

The General Objective was to determine the heavy metal content of crude salt by source of origin as well their residue after processing and iodizing the salt ready for commercialization and the risk involved after consumption.

Specific objectives

- To chemically quantify the undesirable heavy metals, particularly Lead, Cadmium and Mercury in crude and processed salt.

- To assess whether or not the levels of these metals in processed salt constituted a health risk to the consuming population.

Materials and Methods

Between 2006 and 2018, the researcher accompanied the factory inspection team of the International Council for the Control of Iodine Deficiency Disorders (known today as Global Iodine Forum) which, apart from promoting Universal Salt Iodization for sustainable elimination of iodine deficiency in the CEMAC Region also seeks to encourage Quality Assurance and Quality Control at the level of Refineries. During these inspection visits, the production managers were interrogated about the source of origin of the crude salt as well as the methods of Quality Assurance and Quality Control. During each inspection visit (that took place in 2006, 2010 and 2018), 8 -12 samples of crude salt and refined salt at sacking point of production were collected from three factories and carefully labeled for eventual analysis at the Centre for Research in Food and Nutrition (CRAN) of the Institute of Medical Research and Medicinal Plants Studies (IMPM) – Yaounde. In all, 82 crude salt samples and 82 refined salt samples were collected.

Standard solutions of Lead (Pb), Cadmium (Cd), and Mercury (Hg) were prepared from stock solutions of $1000\mu\text{g l}^{-1}$ (Fisher chemicals) by following appropriate dilutions using 10% nitric acid. Glassware was cleaned by overnight soaking in HNO_3 ; H_2O (1:1) followed by repeated rinsing with water. Only de-ionized water was used throughout this work and acids were all of analytical grade.

Samples were weighed after putting them in a desiccator overnight to eliminate traces of water. Mineralization of samples was carried in a humid medium using 65% nitric acid. About 1.0g of each sample was weighed in quadruples into teflon capsules, and 6ml of nitric acid added. The capsules were sealed in the bombe and carried to the fumes cupboard for digestion overnight at 240°C for about 9hours (using a timer). After cooling, the samples were transferred into 25ml round bottom flasks with the help of sterile transfer pipettes. The samples were thus ready for reading at the atomic absorption spectrometer.

Atomic Absorption Spectrophotometer analysis of samples

The heavy metal analysis was done using Perkin 311 model Atomic Absorption Spectrophotometer, as described by Burtis and Ashwood [12]. With the standard curve the unknown concentration of the particular cation in the sample was obtained. Air-acetylene gas was used as fuel, while the following wavelengths were used for the cationic estimations- Lead 283.3nm, cadmium 228.8nm and mercury 253.65nm (with special attention for Hg; using nasal masks). The absorption signals were evaluated by subtracting the value of blank (made of 10% nitric acid) from the signal of the sample.

Estimate of heavy metal intake through salt consumption

Based on Estimated Average Requirement (EAR) for food grade salt (= 10g) per day [13], the daily intake of heavy metals per individual through the consumption of edible salt was estimated

using the formula:

$$DI = A \times B \text{ where}$$

DI = the daily intake of heavy metal

A = average requirement for food grade salt per day

B = Average quantity of heavy metal per gram of salt consumed.

Through this method intake of heavy metal contaminating edible salt was calculated

Potential toxicity index of heavy metal

Using the results of Heavy Metal concentration found in salt samples the values were compared to Maximum Permissible Level (MPL) for each metal.

Given that Heavy Metal can be toxic to human health, Codex has defined MPL in food grade salt: $2.0\mu\text{g/g}$, $0.5\mu\text{g/g}$ and $0.1\mu\text{g/g}$ for Pb, Cd and Hg respectively [14]. Concentrations higher than these limits in any food grade salt render it potentially toxic.

In this respect Potential Toxicity Index (PTI) in food grade salt may be defined as;

$$PTI = \frac{OCf}{PCf} \text{ Where } OCf \text{ is observed concentration in food grade salt and } PCf \text{ is permissible concentration in edible salt.}$$

Based on this relationship food samples with PTI lower than 1 are considered as potentially not toxic to the body while PTI higher than 1 are considered to be potentially toxic.

Health risks and hazards of exposure [15]

Risk of intake of heavy metal-contaminated food grade salt to human health characterized by Health Risk Index (HRI) was calculated:

C_{metal} : heavy metal (Pb) concentrations in food grade salt sample ($\mu\text{g/g}$), considering the average requirement for food grade salt per day.

$D_{salt \text{ intake}}$: daily intake of edible salt (= 10g).

C_f : Conversion factor (average fraction of dry matter contained in edible salt from the factory: approximated to be 1).

B (average weight): average body weight (70kg) [15].

Therefore;

$$\text{Daily intake of Pb (DIM)} = \frac{C_{metal} \times C_f \times D_{salt \text{ intake}}}{B(\text{average})}$$

Health Risk Index (used to assess hazard exposure) was then calculated as follows;

Calculation of HRI for lead

$$\text{Health Risk index for Pb (HRI)} = \frac{DIM}{RfD}$$

$$HRI (Pb) = \frac{DIM}{RfD \frac{\mu\text{g}}{\text{g}}} \text{ RfD} = \text{Reference dose or heavy metal MPL}$$

Statistical analysis

All determinations were done in duplicate and Statgraphic centurion XVI version 16.1.18 (StatPoint Technologies, Inc., USA) used for statistical analysis. The Duncan test was used to compare the different dependent variables.

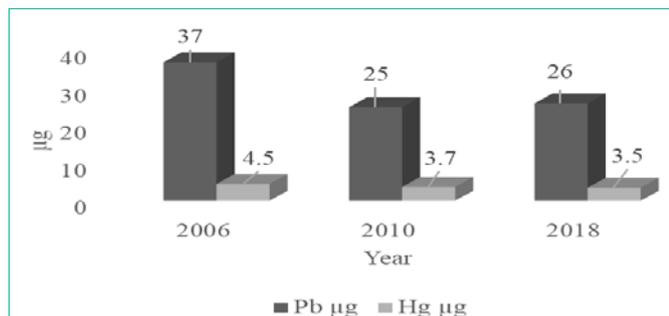


Figure 1: Estimate of Pb and Hg consumed daily in refined salt per individual per year of inspection.

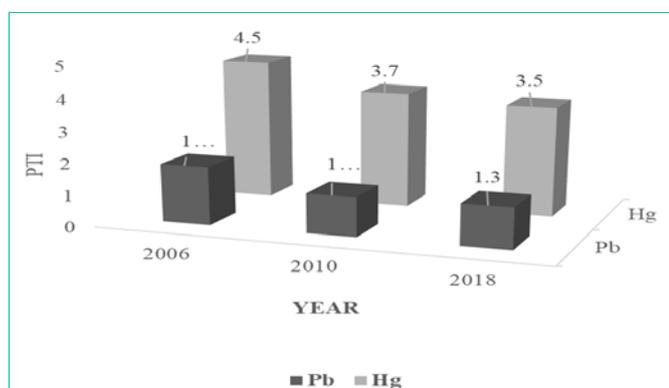


Figure 2: Potential toxicity of Pb and Hg in refined salt per year of inspection.

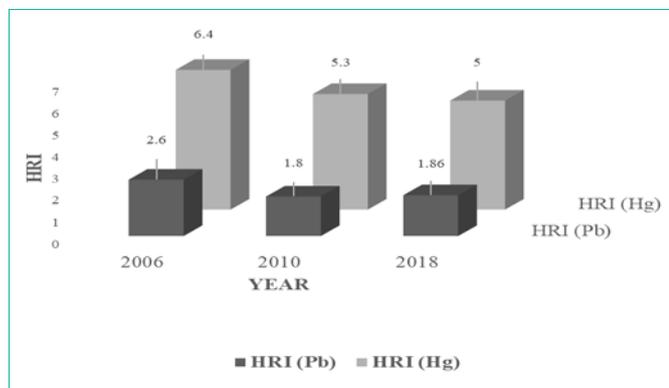


Figure 3: Health risk index (HRI) of Pb and Hg in refined salt per year of inspection.

Discussion

Cameroon does not mine or produce salt by solar system. She imports crude salt from other countries and processes it to produce refined iodized salt for marketing in the Central African Sub-Region (CEMAC-Communauté Economique et Monetaire de l’Afrique Centrale). The countries from which Cameroon imports crude salt in tons include NAMIBIA, EGYPT, DJIBUTI.

The crude salt imported often carries a supplier’s quality certificate which indicates that the sodium chloride content is adequate and that the heavy metal contaminants are within certain standards of safety. To convert this to food grade salt, the crude salt is subjected to a long process of Quality Assurance consisting of: crude sieving, crushing, washing with brine, removal of metallic iron by magnets, drying,

Table 1: Pb, Cd and Hg content of crude salt collected at 2006, 2010 and 2018.

Source	Count	Specifications	Pb (µg/g)	Cd (µg/g)	Hg (µg/g)
Namibia	26	Mean	3.0 ± 1.9 ^a	1.2 ± 0.71 ^a	2.0 ± 0.54 ^a
Egypt	28	Mean	4.2 ± 2.3 ^b	1.4 ± 0.69 ^a	1.8 ± 0.53 ^a
Djibouti	28	Mean	3.8 ± 2.5 ^b	1.4 ± 0.92 ^a	1.7 ± 0.51 ^a
Total	82	Average (range)	3.7 (0.2 – 9.1)	1.3 (0.1 – 3.4)	1.84 (0.5 – 3.4)
-		F-ratio	7.21	0.36	1.56
-		p value	0.0013	0.6971	0.2166

Values in the same column having the same superscripts are not significantly different ($p > 0.05$).

iodization, crushing and filtration to obtain the desired grain size, sacking into standard bags, sewing and storing in a magazine ready for commercialization.

Crude salt values (µg/g) of lead (Pb), cadmium (Cd) and mercury (Hg) are presented in Table 1. The difference between Pb content of crude salt from Namibia compared to that from Egypt and Djibuti was statistically significant ($p < 0.05$), with great variation between the crude sources (Namibia, Egypt and Djibuti) (F-ratio = 7.21). On the contrary, no significant difference was observed for crude Cd and Hg ($p = 0.6971$ and $p = 0.2166$ respectively). The F-ratio of 0.36 for Cd and 1.56 for Hg show respectively that there was no variation in the values of Cd, but slight variation in Hg values amongst the sources of crude under study.

Similarly, refined salt values (µg/g) of lead (Pb), cadmium (Cd) and mercury (Hg) are presented in Table 2. Apart from 2010 and 2018 where there were statistical differences ($p = 0.0436$ and $p = 0.049$ respectively) between the Pb values of the refineries under study, no other refinery showed any statistical differences for any of the heavy metals (Pb, Cd and Hg) under study ($p > 0.05$ in all cases). According to the Codex standards, the Cd values are below the Maximum Permissible Limit (MPL) of 0.5µg/g. With the exception of one refinery that produced salt with average Pb values of 0.5µg/g and 1.0µg/g for 2010 and 2018 respectively, all other Pb and Hg values are above the MPL of 2µg/g and 0.1µg/g respectively. There are great variations for Pb along the years of inspection as observed with F-ratio values (2.53, 3.65 and 3.18), but little variation for Hg (with F-ratio 1.67) in 2010, showing no variations in 2006 and 2018 as observed with F-ratio 0.09 and 0.13 respectively.

Also, Table 2 shows that Pb and Hg are dropping both in refined and crude across the years of refinery visit for reasons yet to be understood as concern crude salt. Though the drops for refined salt didn’t reach acceptable standard for many of the refineries, they were remarkable and could be due to improved quality assurance at the level of the factories as an impact of the inspection visits.

The findings of this study suggest that the Cameroonian and CEMAC sub-region population could be suffering from toxic effects of heavy metal consumption particularly lead and mercury. Lantum [16] demonstrated the potential of a similar endemic toxicity of lead poisoning in the city of Rochester, New York State, but this was attributed to decaying lead pipes which have been used as water conductors for a long time – about 30years.

The presence of Pb in iodized processed salt is evidence that consumers run the potential risk of spontaneous abortion (for pregnant women) and increased blood pressure that affect a

Table 2: Pb, Cd and Hg content of refined edible salt collected at 2006, 2010 and 2018.

Year	Refinery (count)	Specifications	Pb ($\mu\text{g/g}$)	Cd ($\mu\text{g/g}$)	Hg ($\mu\text{g/g}$)
		Codex standards	2.0	0.5	0.1
2006	1 (8)	Mean	2.5 \pm 1.6 ^a	0.2 \pm 0.21 ^{ab}	0.4 \pm 0.24 ^a
	2 (8)	Mean	4.1 \pm 1.2 ^a	0.36 \pm 0.19 ^b	0.48 \pm 0.1 ^a
	3 (8)	Mean	4.4 \pm 2.4 ^a	0.14 \pm 0.16 ^a	0.45 \pm 0.3 ^a
	(24)	Average (range)	3.7 (0.8 – 8.0)	0.23 (0.0 – 0.6)	0.45 (0.01 – 0.9)
	-	F-ratio P value	2.53 0.1040	3.09 0.067	0.09 0.9137
2010	1 (8)	Mean	0.5 \pm 0.3 ^a	0.3 \pm 0.25 ^{ab}	0.38 \pm 0.24 ^a
	2 (8)	Mean	2.9 \pm 3.6 ^{ab}	0.46 \pm 0.22 ^b	0.49 \pm 0.1 ^a
	3 (8)	Mean	4.0 \pm 2.7 ^b	0.20 \pm 0.19 ^a	0.25 \pm 0.3 ^a
	(24)	Average (range)	2.5 (0.2 – 9.0)	0.32 (0.0 – 0.8)	0.37 (0.0 – 0.9)
	-	F-ratio P value	3.65 0.0436	2.82 0.0820	1.67 0.2217
2018	1 (10)	Mean	1.0 \pm 1.2 ^a	0.26 \pm 0.2 ^a	0.33 \pm 0.32 ^a
	2 (12)	Mean	3.5 \pm 2.9 ^b	0.18 \pm 0.23 ^a	0.38 \pm 0.34 ^a
	3 (12)	Mean	3.0 \pm 2.7 ^{ab}	0.17 \pm 0.15 ^a	0.32 \pm 0.25 ^a
	(34)	Average (range)	2.6 (0.1 – 8.0)	0.2 (0.0 – 0.8)	0.35 (0.0 – 1.0)
	-	F-ratio P value	3.18 0.049	0.48 0.6213	0.13 0.8779

Values in the same column having the same superscripts are not significantly different ($p > 0.05$). NB: 1, 2 and 3 correspond to salt refineries, identity withdrawn to avoid advertisement.

community under Pb exposure as reported by [17,18], together with the classical signs of lead poisoning. Its effects on women include infertility, miscarriage, premature membrane rupture and premature delivery [19,20]. Huang *et al.*, [19] and [20] revealed that lead may be toxic at levels previously thought to have no effect. The U.S. Public Health Services stated that there is no safe level for Pb and as a practical measure recommended reduction of blood Pb levels to less than 10 $\mu\text{g}/100\text{g}$ in women of childbearing age. At higher levels individuals affected are susceptible to comma, seizures or death [16]. When industrial workers are exposed to low doses of Pb for several years, they can develop “plumbism” or chronic lead Intoxication [21].

In addition, Pb is amongst the chemicals that are known to alter the mechanisms of iodine transport [22]. Studies of populations with high exposure to Pb (blood Pb levels higher than 20 $\mu\text{g}/\text{dL}$) have reported negative associations with circulating T_4 , or T_3 [23-26]. In fact, Pb can cause serious hematological, neurological, gastrointestinal, renal, rheumatological and endocrine manifestations in man even at levels previously considered safe [27-29]. Contaminating iodized processed salt with Pb might render iodine contained in it inaccessible to the thyroid hormone synthesis mechanism stagnating IDD and jeopardizing the efforts of iodine fortification of salt put in place by the Cameroon Government.

Cadmium is a wide-spread heavy metal element in the environment, but as it did not pose a risk in this study there is no call for discussion.

FAO/WHO, [6] also described various forms of toxic manifestations of mercury in the environment including tremors, gingivitis and/or minor psychological changes. Spontaneous abortions and congenital malformation have been reported in literature. Mono-ethyl mercury causes damage to the brain and central nervous system, and when the foetus has been exposed, the

results have been abortion and other development changes in young children. The main pathway for mercury to humans is through the food chain and not by inhalation [30]. In the present study the risk of mercury intoxication through inadequate Quality Assurance of Iodized salt production has been suggested, and can be argued based on Codex standards [14].

Estimate of heavy metal intake through salt consumption

Heavy metals above the MPL are Pb and Hg and estimates of their daily intake are presented on Figures 1. These values estimate the quantity of Pb, and Hg ingested by Cameroonian people and the central Africa sub-region inhabitants served by these salt refineries. Daily intake of Pb starts from 37 μg dropping to 25 μg in 2010 increasing slightly to 26 μg in 2018. Similarly, daily intake of Hg starts from 4.5 μg dropping to 3.7 μg in 2010 then to 3.5 μg in 2018. These drops could be due to improved quality assurance at the level of the factories as an impact of the inspection visits, as stated above.

Similar high but weekly intake of approximately 220 μg of methyl Hg was estimated in 80% of the fish from Naboc village in the Philippines [31]. Like in this case, 200 – 400 μg of Pb was estimated to be ingested in food daily in industrial societies [32]. Similarly, daily intake of Cd (0.018 $\mu\text{g}/\text{g}$) from food cultivated in an abandoned municipal waste dumpsite has been reported [33], indicative of the fact that intake of heavy metals can be estimated from contaminated food or substances ingested.

Potential toxicity index (PTI) of heavy metal

The PTI of the different metals analyzed in each sample are shown in the histograms (Figure 2). Potential Toxicity Index (PTI) for Pb starts from 1.85 dropping to 1.25 μg in 2010 increasing slightly to 1.3 μg in 2018. Similarly, PTI of Hg starts from 4.5 μg dropping to 3.7 μg in 2010 then to 3.5 μg in 2018.

The Potential Toxicity Index (PTI) used in this study to rank and compare the relative toxicity of edible salt due to heavy metal contamination is an adaptation from the method used for identifying levels of toxicity due to pesticides in surface water [34] and heavy metal contamination of kaolin [15]. In the later study potential toxicity makes use of the level of the heavy metal concentration in the samples and the reference dose [35], like in this study. This tool summarizes complex information into simple numerical ratings for application by public health experts or resource managers.

Health risks and hazards of exposure

$$\text{Daily intake of Pb (DIM 2006)} = \frac{37.1 \times 10}{70} = 5.29$$

DIM (2006) for Pb = 5.29

$$\text{HRI pb (low)} = \frac{5.29}{2} (\text{RfD} = 2\mu\text{g for Pb})$$

HRI (2006) for Pb = 2.6

Considering the RfD of 0.1µg/g for mercury, the same calculation was carried out for this metal.

Detailed results are presented on Figure 3. The population will have no risk if the index is less than or equal to 1 (obvious case for cadmium) and if the index is greater than 1 then, the population will experience health risk. Therefore, the values of HRI for Pb of 2.6, 1.8 and 1.89 for salt consumers, indicate a factor that is multiplying risk at each inspection year.

The calculated HRI values of 6.5, 5.3 and 5.0 for Hg are also presented in Figure 3. These HRI value are higher when compared to those of Pb, indicating that this heavy metal is affecting the population more than Pb.

This HRI risk assessment method has been used by researchers [15,36-38] and is useful illustration of the level at which the targeted population is exposed to a hazard. Salt consumers are prone to develop diseases that come about as a result of exposure to these heavy metals. The situation is more disturbing because these heavy metals are also getting into human food chain through eating contaminated edible salt by cows, goats, sheep etc.

Conclusion

There is evidence that imported crude salt imported from NAMIBIA, EGYPT and DJIBUTI does contain high levels of heavy metal contaminants, particularly Pb, Cd and Hg. Further, that after production/processing with technology available in local refineries, the risk of Pb and mercury intoxications were not eliminated as salt still contained these heavy metal above the maximum permissible limits.

Recommandation

1. That the Industrialist/salt producers (SOCAPURSEL, SOTRASEL and AIGLE) should be made aware of the presence of heavy metal contaminants in the imported Crude salt so that they can invite their plant manufacturers to redesign and add steps in the processing chain to eliminate most of these heavy metals to within tolerable ranges.

2. That internal quality laboratories attached to these salt production plants should also do quality control for heavy metal contaminants in refined salt.

3. That this investigation should continue and cover all the other metals listed in codex alimentarius, namely, Calcium, Magnesium, Sulphate, Arsenic, Copper and Iron.

4. That importers of crude salt should ensure that it gets into the market for consumption only after processing.

5. That from this present study it appears necessary for scientists to study the potential risk of other heavy metals contaminants in salt produced at the Douala port [14]. These include:

i) Calcium content (maximum permissible level, MPL 3g/kg)

ii) Magnesium content (MPL = 3g/kg)

iii) Sulphate content MPL = 5g/kg)

iv) Other chemical contaminants

- Arsenic content (MPL= 5g/kg)
- Copper content (MPL= 2mg/kg)
- Iron content (MPL = 50mg/kg)

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