

ORIGINAL ARTICLE

Seafood Consumption and Blood Cadmium Level of Respondents along the Coastal Area of Melaka, Malaysia

Hamizah Md Zulkifli¹, Saliza Mohd Elias¹, Ahmad Zaharin Aris², Suhaili Abu Bakar³, Farrah Atiqah Mohd Noor¹

¹ Department of Environmental and Occupational Health, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400, UPM Serdang, Malaysia

² Dean Office, Faculty of Environmental Studies, Universiti Putra Malaysia, 43400, UPM Serdang, Malaysia

³ Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, , 43400, UPM Serdang, Malaysia

ABSTRACT

Introduction: Eating seafood has become a major health concern for many people due to the present of heavy metal especially cadmium (Cd). Cd can accumulate in the body and disrupt the normal cellular processes which will eventually lead to organ damage. This study aims to determine the seafood consumption pattern and blood cadmium (BCd) as well as the association between these two variables among respondents living along the coastal area of Melaka. **Methods:** Pretested questionnaires were used to collect background and food frequency intake from coastal villagers through convenient sampling method. Venous blood samples were analysed by using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for BCd determination. **Results:** A total of 63 respondents who 54% were female with median age of 34 years old provided complete data in this study. The most frequently consumed seafood and its product were shrimp paste (31.5%) followed by mackerel (13.6%), hardtail-scad (6.2%), flatfish (4.5%) and fish ball (4.0). All blood samples showed the present of Cd with median (IQR) = 0.076 (0.1) µg/L and ranged between 0.007 to 1.284 µg/L. The finding showed no association between frequently consumed seafood and low BCd of the respondents. On the other hand, gender was found to be significantly associated with the BCd. **Conclusion:** Seafood consumption pattern was not significantly associated with BCd which suggests that frequent seafood consumption may not contribute much to BCd level among the respondents as well as it may indicates safe consumption of these seafood available in the study location.

Keywords: Blood cadmium level, Seafood frequency intake, Coastal area, Malaysia, ICP-MS

Corresponding Author:

Saliza Mohd Elias, PhD

Email: saliza_me@upm.edu.my

Tel: + 603-97692402

INTRODUCTION

Cadmium (Cd) is listed as one of the most toxic metals that exist in the environment (1). Cd is released into the air through natural processes such as volcanic eruption and forest fires (2,3) It is also has been widely dispersed into the environment through its mining and smelting as well as man-made routes which includes the usage of the phosphate fertilizers and the presence in sewage sludge (4). Incineration of municipal waste such as plastics and nickel-Cd batteries is also one of the main sources of Cd (4).

Cd has a very long biological half-life which is about 10 to 30 years. They accumulate in the body with

age (5,6). Cd can enter and leave human body via inhalation, ingestion and dermal contact. For the non-occupationally exposed population, tobacco smoking, consuming contaminated food and drinking water are the major source of Cd intoxication in human body (7). In general population, smoking is the main route of Cd exposure to human (8). For the non-smoking population, food supply is the main route of exposure to Cd (4). Crops such as lettuce, spinach, potatoes, grains, peanuts and soybeans might contain high level of Cd from the phosphate fertilizers that is use to grow them. Animal offal which includes kidney and liver can also exhibit high level of Cd as these are the organs in animals where Cd concentrates (9). Cd also can be found in the sea because of the industrial waste, illegal littering and also illegal dumps of oils into the sea. These factors can affect the marine organisms as the sea is their habitat.

Today, seafood such as fish and seashells has become the main supply of protein besides meat and poultry.

Heavy metals contamination found in this type of food raised the interest of researchers on its safety and how it affects the human health. Sea life especially those lives in the sediments which includes cockles, crabs and prawn are greatly exposed to Cd because of the immobile Cd that is absorb into the sediment. Solubility of Cd in water determines its wide distribution in aquatic systems compared to the other metals which make it a metal of major concern with respect to aquatic animal exposure as well as human food chain (10). Norimah and friends in 2008 found that marine fish was consumed everyday by Malaysian with the prevalence of 40.78% of the respondents consumed it in their daily diet (11). This might explained why the research on the level of heavy metal in local seafood has gained interest among the researcher.

There were varied levels of heavy metals in fish samples were reported from various collection sites in Malaysia (12,13,14). Nor Hasyimah and friends (15) found that the amount of Cd in fish samples fall well between the permissible limit for human consumption when compared to the Malaysian Food Regulation, 1985. Crustaceans which includes cockles, crabs and prawn are greatly exposed to heavy metals especially Cd because of their habitat which is sediment where various kind of hazardous and toxic substances are accumulated (14). Although the amount of Cd were found higher in cockles, the concentration was below the permissible value stated by the Malaysian Food Act 1983 and are safe to be consumed by human (16).

Cd was also found in trace concentration in shellfish (17). Seaweeds are also one of the marine organisms that are susceptible to be contaminated with Cd (18). Based on the research done by Hwang and friends (18) in South Korea, they traced the presence of Cd in all 426 seaweed samples that they analysed. These findings proved that besides animals and human, Cd also contaminated plant in the sea. This should be a great concern since seafood and seafood products has become the main supply of protein to humankind besides meat and poultry. Even though the findings of above studies creates a health concern particularly in community, due to less visible effects, the problem is neglected. Cd are toxic to human being. When they accumulate in the body, they will disrupt the normal cellular processes which will lead to the organ damage and later can cause variety of health effects. The exposure and the toxicity of the Cd to human body have led researchers to conduct this study.

This study aimed to determine the seafood consumption pattern to understand the possible risk of cadmium exposure through seafood consumption as well as the concentration of Cd in blood sample as the indicator of Cd exposure in the respondent's body. This research provides information about blood Cd level in the community and thus helps in the planning to reduce the risk of the health effect that might rise in the later age.

MATERIALS AND METHODS

Subjects

This cross-sectional study was carried out between September 2014 to February 2016. Healthy non-occupationally exposed (to heavy metals) Malay adults who fulfilled the inclusive criteria of age between 18-60 years old, and consume seafood and seafood products in their daily diet were conveniently approached from coastal areas which located in three Dewan Undangan Negeri (DUN) of Malacca namely Alor Gajah, Melaka Tengah and Jasin. Those who resided in the study area less than 3 months and those who were pregnant and just delivered the baby (within 60 days of delivering) were excluded. They were given a set of questionnaire which include background information and a semi quantitative Food Frequency Questionnaire (FFQ) which was adapted from Malaysian Adult Nutrition Survey (11) and was modified following the feasibility of the respondents to gather information about seafood frequency intake and background information of potential exposure to Cd. The conversion of food frequency to the amount of food intake was carried out using the formula below; Amount of food (g) per day = frequency of intake (conversion factor) X serving size X total number of serving X weight of food in one serving (11). Based on the questionnaire, the food were classified under frequently consume and not frequently consumes seafood. Fig. 1 summarised the data collection flow.

Sample Size Calculation

The sample size of the survey part of this study was calculated by using the formula proposed by Aday and Cornelius (17) as below:

$$n = \frac{Z^2 \cdot 1 - \alpha / 2 \cdot P(1 - P)}{d^2}$$

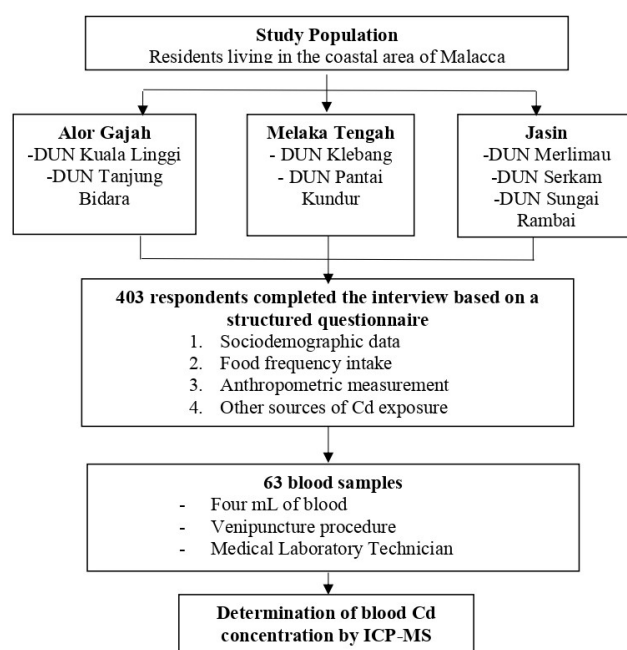


Figure 1: Flowchart of the simplified methodology of the study

Where,

- n = Total number of respondents
- $(Z_{1-\alpha/2})$ = Confidence interval = 1.96
- P = Estimated proportion
- d = Absolute precision required = 0.05

Based on the calculation, at least 384 respondents should be recruited in this study. In this study, 403 potential respondents were approached and briefed about the study and were asked to participate based on the voluntarily basis. However, only 63 agreed to participate and provide all the information required especially the blood sample for Cd determination. The written consent was signed by the respondents before the commencement of data and blood collection. All respondents were given a choice to continue participating in the study or to pull out at any time they chose to do so. The information about respondents involved in this research remained confidential. This poor participation could be due to the fear of the procedure of blood sample preparation. Respondents might also have the feeling that this study was not important for them.

Blood Sample Collection

Sixty three blood samples were collected from the respondents. Four millilitre (ml) of the blood sample was collected via the venepuncture procedure in 4 ml monovette containing ethylene diamine tetra acetic acid (EDTA). The blood collection procedure was performed by certified medical laboratory technician (MLT). All the blood samples were stored at -20°C and were analysed within 2 days.

Blood Sample Analysis

Pre-treatment of the apparatus and glassware

All glassware and sample containers were thoroughly washed by using detergent solution to ensure all dirt were removed. The apparatus were then rinsed with tap water until free of detergent followed by rinsed with distilled water. The glassware was soaked in 10% of nitric acid, HNO_3 for 24 hours and finally rinsed with distilled water. They were dried in 60°C oven for 24 hours prior to analysis.

Wet Acid Digestion

Blood samples were digested by using the conventional wet acid digestion method adopted from Memon et al. (20). A fresh mixture of concentrated nitric acid, HNO_3 and hydrogen peroxide, H_2O_2 was prepared by adding the reagent in the ratio of 2:1 respectively. In this study 69% of HNO_3 and 30% of H_2O_2 were used to prepare the mixture. The blood samples were thawed for 30 minutes. Accurately 0.5 ml of each of the blood samples was taken into conical flasks separately. Three ml of a mixture of HNO_3 and H_2O_2 was added into blood samples. The flasks were then stood for 10 minutes. Watch glasses were used to cover the conical flasks and heated on the hot plate at 70°C for 2 hours. Two ml of HNO_3 was added into the flask while heating continued

on the hot plate at a temperature of 80°C . A few drops of H_2O_2 were added into the flask until clear solution obtained. Excess acid mixture was evaporated on the filter paper. It was then cooled and diluted with 0.1 ml of HNO_3 . The sample mixture was transferred into 100 ml volumetric flask and diluted to mark by using distilled water. The procedures were repeated for the preparation of blank sample without the use of blood sample. The sample mixtures were stored in polyethylene containers in a refrigerator at 4°C prior to inductively coupled plasma mass spectrometry (ICP-MS) analysis.

Sample Introduction into the ICP-MS

Cd level in blood were determine by ICP-MS 7500c; Agilent Technologies, USA. Briefly, sample aerosol generated by a nebuliser was carried to an argon plasma of about 8000 Kelvin for the production of the elemental ions. The samples were then introduced into a mass spectrometer for the ion identification and quantification of the ion.

Ethical Approval

The data collection methods and procedures to conduct this research have been approved by Ethical Committee of UPM for Research Involving Human Subject (Reference Number: UPM/TNCPI/RMC/1.4.18.1 (JKEUPM)/F2

Statistical analysis

All data was analysed using the Statistical Package for Social Science (SPSS) Software (version 22). Descriptive analysis was used to describe the background information, seafood frequency intake, frequency of consumed foods and also Cd concentration in blood. Mann-Whitney U and Kruskal-Wallis test analyses were used to determine the association between background information and seafood frequency intake with Cd concentration in blood. The value of $p < 0.05$ was considered as statistically significant.

RESULTS

Socio demographic characteristic of the respondents

A total of 63 respondents, whom 54% female completed in providing all the required data for this study. About 64% of them were married, 81% educated up to secondary school level, and majority of them employed either with government or private agencies. The median age was 38 years old and median income was RM2500 monthly. About 35% respondents were smoker. Table I summarised the socio demographic characteristics of the respondents.

Seafood Consumption Pattern

A total of 23 types of seafood and seafood-based product were listed in the questionnaire. The lists of seafood and seafood based product were modified according to a study conducted by Alina et al. (14) where the location of the study was similar to this

Table I : The socio demographic characteristics of respondents

Variables	Frequency (%)	Median (25 th – 75 th percentile)
Gender		
Male	29 (46.0)	
Female	34 (54.0)	
Marital Status		
Married	40 (63.5)	
Others	23 (36.5)	
Education Background		
Secondary education and lower	51 (81.0)	
Tertiary education and higher	12 (19.0)	
Type of occupation		
Government	10 (15.9)	
Private	28 (44.4)	
Others	25 (39.7)	
Smoking		
Yes	22 (34.9)	
No	41 (65.1)	
Age (years)		38(27-48)
Household Income (RM)		2500 (1500-3500)

N=63

research which was along the coastal area of Malacca. The final intake was expressed in two distinct categories which were frequently consumed (≥ 3 times a week) and not frequently consumed (<3 times a week) (21). Table II showed the prevalence and mean frequency of top 10 frequently consumed seafood and seafood based product among the respondents daily.

Blood Cd Level of the Respondents

Based on the ICP-MS result, all 63 blood samples traced the present of Cd with the median (IQR) = 0.076 (0.1) and ranged from 0.007 $\mu\text{g/L}$ to 1.284 $\mu\text{g/L}$. According to Agency for Toxic Substances and Disease Registry, ATSDR (2012), the permitted Cd level in human blood for general population (≥ 1 year old) is 0.315 $\mu\text{g/L}$. Four (6.35%) of this study respondents exceeded the blood Cd level permitted by ATSDR. Table III illustrated the level of Cd in blood sample of the respondents.

Table II : Prevalence and mean frequency of top 10 most consumed seafood and seafood based product daily

Seafood/ Seafood based product	Prevalence who answered daily consumption (%)	Mean frequency per day \pm SD
Shrimp paste	31.5	0.33 \pm 0.517
Mackerel	13.6	0.19 \pm 0.514
Hardtail scad	6.2	0.08 \pm 0.318
Flatfish	4.5	0.08 \pm 0.351
Fish ball	4.0	0.05 \pm 0.245
Fish crackers	2.2	0.03 \pm 0.221
Shrimp ball	2.2	0.02 \pm 0.148
Squid	2.2	0.02 \pm 0.148
Crab ball	2.2	0.02 \pm 0.171
Wolf herring	2.0	0.02 \pm 0.140

Table III : Cadmium Level in Blood

Variable	Frequency (%)	Median (25 th – 75 th percentile)	Min-Max Value
Blood Cd Level ($\mu\text{g/L}$)	-	0.076 (0.038-0.138)	0.007-1.284
< 0.315	59 (93.65)	-	-
≥ 0.315	4 (6.35)	-	-

N = 63

Association between Background Information and Blood Cd Level of the Respondents

Mann-Whitney U and Kruskal-Wallis tests were used to analyse the association between Cd level in blood and the background information of the respondents. The results revealed that, gender was associated with the blood Cd level of the respondents, where the female had significantly higher Cd level in blood as compared to male ($p < 0.05$). The other factors such as marital status, educational status, type of occupation, and household income showed no significant association. Table IV summarised the results of those analysis.

Association between top 10 Frequently Consumed Seafood and Blood Cadmium Level of the Respondents

All type of seafood and seafood based product that were frequently consumed by the respondents showed no association with the blood Cd level ($p > 0.05$). Table V

Table IV : Association between Socio Demographic data and Blood Cadmium Level of the Respondents

Variables	Frequency (N)	Median (25 th – 75 th percentile)	Statistical Analysis	P-value
Gender				
Male	29	0.055 (0.032-0.094)	308.5 ^a	0.011*
Female	34	0.096 (0.057-0.179)		
Marital status				
Yes (married)	40	0.073 (0.039-0.131)	423.0 ^a	0.597
No (single or divorce)	23	0.092 (0.036-0.176)		
Education status				
Secondary education and below	51	0.074 (0.037-0.133)	243.5 ^a	0.274
Tertiary education and higher	12	0.114 (0.049-0.187)		
Type of Occupation				
Government	10	0.066 (0.040-0.210)	3.117 ^b	0.538
Private	28	0.073 (0.035-0.146)		
Self employed	10	0.061 (0.025-0.156)		
Retired/not working	10	0.095 (0.050-0.134)		
Others	5	0.149 (0.070-0.205)		
Smoking				
Yes	22	0.057 (0.031-0.110)	350.0 ^a	0.145
No	41	0.078 (0.049-0.153)		

N = 63

* : significant at $p < 0.05$
a : Mann-Whitney U Test
b : Kruskal-Wallis Test

Table V : Association between top 10 Frequently Consumed Seafood and Blood Cadmium Level of the Respondents

Type of seafood/ seafood product	Frequency	Median (25 th -75 th percentile) (µg/L)	Mann- Whitney U test	P – value
Shrimp Paste				
Frequent	24	0.066 (0.035-0.121)	414.5	0.449
Not frequent	39	0.078 (0.042-0.158)		
Mackerel				
Frequent	29	0.074 (0.041-0.123)	470.0	0.751
Not frequent	34	0.079 (0.037-0.179)		
Hardtail scad				
Frequent	16	0.069 (0.033- 0.198)	367.5	0.893
Not frequent	47	0.076 (0.042- 0.138)		
Flatfish				
Frequent	8	0.075 (0.041-0.078)	184.0	0.457
Not frequent	55	0.076 (0.038-0.158)		
Fish ball				
Frequent	1	0.042 (0)	16.0	0.409
Not frequent	62	0.076 (0.037-0.140)		
Squid				
Frequent	15	0.065 (0.033-0.136)	329.5	0.623
Not frequent	48	0.077 (0.043-0.146)		
Wolf Herring				
Frequent	15	0.065 (0.033-0.136)	329.5	0.623
Not frequent	48	0.077 (0.043-0.146)		

N = 63

displayed the results of those analysis.

DISCUSSION

This study was carried out to provide a baseline data on the background, seafood consumption pattern, and Cd concentration in blood samples among residents of coastal area in Malacca. The most frequently consumed seafood in this study turned out to be shrimp paste with 31.5% of respondents consumed it daily. This is in agreement with Alina et al. (14). Shrimp paste is a finely ground fermented shrimp in sea salt. This particular food was the mostly consumed seafood might be due to the respondent in this study were from the Malay ethnic whose famously known to used shrimp paste as their food enhancer (22). Malay ethnic usually used shrimp paste as dipping sauce (commonly known as sambal belacan) for raw or lightly blanched vegetable called ulam.

The second mostly consumed seafood and seafood based product in this study was mackerel with 13.6% of the respondents consumed it in their daily meal. This is in line with the study done by Ahmad and friends in their research on fish consumption pattern among adults of different ethnics in Peninsular Malaysia where they also found mackerel to be in their top 10 mostly consume fish by their respondents (23). Besides that, squid was also placed on their top 10 mostly consumed

seafood. Fish ball, crab ball and shrimp ball were among the top 10 mostly consumed seafood because these types of food were considered as the Malaysian street food and they were easily spotted at the night and morning markets. This is the reason why these kind of seafood based product were mostly consumed by the respondents in their daily meal.

Consumption of seafood can be related to Cd exposure and accumulation in the human body. A study on the heavy metal level in seafood and seafood product done by Alina et al. (14) in the Strait of Malacca traced Cd in every seafood samples that have been collected. Although the concentration of Cd in these marine organisms is in low concentration, the effect of the seafood consumption by human might appeared in a long term duration. The accumulation of Cd in human body might affect cell proliferation, differentiation and cell apoptosis (24). Cd interrupt the DNA repair mechanism and trigger the generation of reactive oxygen species (ROS) and eventually induced the cell apoptosis. These activities may result to organ failure in human body

All 63 blood samples of this study were detected with some amount of Cd, However, the blood Cd level of this study was lower than findings by Higashikawa et al. (25) where they did a research on the correlation of the blood cadmium level and urine Cd level among Asian women. In their study, women living in Kuala Lumpur which representing Malaysia had the mean level of 0.74 µg/L of Cd in blood, which was higher than this study finding of median = 0.096 µg/L in women blood. Eum et al. also found a higher mean level of Cd in blood of their respondents compared to this study which was 1.67 µg/L (26). A research done in Thailand by Sirivarasai et al. also found a higher level of Cd in blood sample of Thai men which was 2.93 µg/L (27). These previous findings were higher than the ATSDR standard. Acute toxicity will be observed when the blood level exceeds 50 µg/L. Since this current study found lower Cd level, it indicates that the respondents in this study might have lower risk to be adversely affected by the recent Cd environmental exposure.

Exposure to Cd can be divided into two distinct categories which are acute and chronic. Acute Cd exposure or acute Cd toxicity is the adverse effect of cadmium that results from a single exposure or multiple exposures in a short period of time (28). The effect of acute exposure of Cd is mostly related to gastrointestinal disturbances such as nausea, vomiting, abdominal cramp and diarrhea. Adverse health effect of acute Cd exposure by inhalation includes bronchitis, chemical pneumonitis and pulmonary edema (28). Chronic Cd exposure or chronic cadmium toxicity is the continuous or repeated exposure of Cd over a long period of time. Due to their long biological half-life and accumulation properties, long term contact to Cd may cause chronic

adverse health effect (29). Chronic exposure to Cd may affect heavy smokers, industrial workers and people living in areas of contaminated with environmental Cd (28)

It has been proven that prolonged exposure to heavy metals might cause serious health effects in humans (22). Consumption of Cd via seafood over a long period of time will result in chronic organ damages which includes kidney, liver, lung, central nervous system, ovaries, testes and pancreas. Adverse toxicity of Cd is due to its bioaccumulation in the kidney. Kidney dysfunction may result in proteinuria, glycosuria, renal hypertension and other metabolic disorder.

In this study, convectional wet acid digestion was used to prepare the blood sample instead of the microwave induced acid digestion method before the introduction to ICP-MS. Even though this method is time consuming, it is more efficient as it gave higher percentage recoveries of Cd (30). In Malaysia, there was no unified diagnostic Cd criterion for the general population. Therefore, the standard by ATSDR (4) was used as the reference limit in this study.

The statistical analyses done to associate the BCd with sociodemographic background of the respondents revealed that gender was significantly associated with the BCd level. The higher blood Cd level among female might be explained by the differences in behaviours and dietary intake related to Cd exposure. The accumulation of Cd was higher in female due to the facts that women are more susceptible to Cd toxicity because of the increased Cd intestinal uptakes when level of the iron was lower in the body (31,32,33). Low iron stores in women body was due to the loss of iron during menstruation and pregnancy (34). Furthermore this higher percentage of Cd level in female also could be due to the possibility of them being the second hand smoker (35). Therefore, it was fair to conclude that female accumulated Cd more efficiently than male.

There was no association found between blood Cd level and smoking behaviour in this study although it was proven as the major source of exposure to Cd and its accumulation in human body (8). This could be due to most of the smokers were not consider as heavy smokers, which defines as those who are having 20 or more cigarettes per day over 10 to 20 years (9). The other factors of exposure to Cd also showed no association with the Cd level in human blood. Furthermore, respondents in this study consumed salad (Ulam) and other types of foods that contained glutathione which is believed to neutralize free radicals and help their body to detoxify Cd efficiently. Foods that rich in glutathione includes whey protein, sulphur foods which includes broccoli, cabbage, cauliflowers, kale and other fresh vegetables, vitamin C and E, beef liver and vitamin B6, B9 and B12 (36,37,38)

This study found lack of association between blood Cd level and the most frequently consumed seafood and seafood based products. The trends showed that those who frequently consumed the seafood (more than 3 times in a week) had lower blood Cd level. This could indicates that seafood and seafood based product from Malacca may be safe to be consumed in terms of cadmium concentration since the level was low and do not constitute a risk for human health (14). This is supported by a study done by Rosli and friends who found that the level of Cd in fish samples at Malaysia were generally low compared to the worldwide RDA (recommended dietary allowance) (39).

CONCLUSION

This study found no association between frequent consumption of seafood and seafood products with blood Cd level of respondents. This result suggest that seafood and seafood based product from Malacca may be safe to be consumed and they may not contributed much to the accumulation of Cd in blood since the level was below the standard of 0.315 µg/L.

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