行政院國家科學委員會專題研究計畫　成果報告

食物砷之攝取對職業性砷暴露評估之影響

計畫類別：個別型計畫
計畫編號：NSC91-2320-B-002-173-
執行期間：91 年 08 月 01 日至 92 年 07 月 31 日
執行單位：國立臺灣大學公共衛生學院職業醫學與工業衛生研究所

計畫主持人：黃耀輝

計畫參與人員：搖晚林、陳由瑄、許家晴

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一、中文摘要

本研究目的在於探討攝食不同海產食物及攝食後時間長短對於各種砷代謝產物量測之干擾。十種海產食物經分析後顯示有機砷含量最高的是花蟹 219.4 ug/g，最低的是海沙蝦 2.58 ug/g。十二名志願者分組參與後續的飲食控制實驗，使用的海產食物包括茶葉菜、蛤、牡蠣及海沙蝦。在一週實驗期間，由本計畫提供受試者每日三餐以避免受試者暴露於含海產食物之飲食。各組實驗指定之特定一種海產係在第四天午餐及晚餐提供。自進食海產前一天起至實驗結束前的另三天，受試者必須收取其所有尿液樣本，分別存放在 500 ml 塑膠瓶。尿中砷代謝物種，包括三價砷、五假砷、單甲基砷酸及雙甲基砷酸，係以高效率液相層析儀結合原子吸收光譜進行分析。結果顯示，雙甲基砷酸是最主要的尿中砷代謝物種，佔 80％ 左右。在進食茶葉菜、蛤、牡蠣等三組，其攝食後第一天早起第一泡尿液中的雙甲基砷酸濃度平均分別達到 36.4±7.8 ug/L, 48.6±15.1 ug/L, 26.6±5.2 ug/L。一般而言，在正常飲食攝取 150g 的海產就有可能讓尿中砷濃度上升幅度達 30 ug/L，顯示海產飲食會干擾對無機砷暴露後的尿中砷代謝產物偵測。本研究的發現也顯示不同人間對砷的代謝機制與解毒能力有很大的變異情形。需進一步針對個體易感受性對砷暴露的關係進行探討，以瞭解攝食海產對尿中砷物種分佈，甚至是砷相關疾病的綜合效應。

關鍵詞：海產食物、尿、砷、物種分離。

Abstract

The present study was conducted to explore the potential confounding effects resulting from dietary arsenic intake from seafoods with the aims to delineate the effects of seafood items and time since seafood ingestion on the elevation of speciated urinary arsenic metabolite levels. Among the ten seafoods determined for arsenic species, the most common arsenic species was arsenobetaine, with the highest total arsenic content found in flower crab, 219.42 ug/g and the lowest observed in seasand shrimp, 2.58 ug/g. Twelve volunteers were invited to participate in the next step seafood-restrain study. The seafoods used for this study included brown seaweed, clam, oyster, and shrimp from the ten aforementioned seafoods. During the one-week study period, study subjects were provided with all meals for dietary control and restrained from any aquatic and seafoods, except the designated study seafoods aforementioned provided in the lunch meal and dinner meal on the fourth day. All the urine excretions of the study subjects were separately collected in 500 ml bottles for the day prior to seafood ingestion, and the following 3 consecutive days. Urinary arsenic metabolites were determined with high performance liquid chromatography - hydride generation - atomic absorption spectrometry, including arsenite(As^{3+}), arsenate (As^{5+}), monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA). DMA dominated the urinary arsenic metabolites, constituting about 80 % of the total arsenicals excreted. Average DMA concentrations in the first morning void samples after ingestion of the brown seaweeds, clam, oyster were 36.4±7.8 *u*g/L, 48.6±15.1 *u*g/L, 26.6±5.2 *u*g/L, respectively. In general, an elevation of urinary arsenic levels by 30 ug/L is possible by regular meals composed of 150 g seafoods, implying a critical confounding effect of seafood ingestion on the biological marker for inorganic arsenic exposure monitoring. The findings of the present study also shed light on the significant individual variation in the arsenic metabolism and/or the capability of arsenic detoxification. Further studies emphasizing on the individual susceptibility to arsenic exposure are warranted in order to explore the comprehensive effects of seafood intake on the distribution of urinary arsenic species, and even more on the arseniasis.

Keywords: seafood, urine, arsenic, speciation.

二、緣由與目的

Food arsenic species, usually in organic forms and used to be thought non-toxic (1), got great attention in past years since this concept of non-toxic has been challenged recently. Usually, the summation of urinary inorganic arsenic metabolites, i.e., As^{3+}, As^{5+}, MMA, DMA, could be used as the biological marker for external inorganic arsenic exposure. However, recent studies reported that the urinary DMA might be confounded by arsenosugar in seafood, such as seaweed and mussels (2-5) or by arsenobetaine in seafood after cooking, such as manufactured seafood products (6). The present study was conducted to explore the potential confounding effects resulting from dietary arsenic intake from seafoods with the aims to delineate the effects of seafood items and time since ingestion on the elevation of speciated urinary arsenic metabolite levels, i.e., As^{3+}, As^{5+}, MMA, DMA.

三、研究方法

Ten different seafood species were obtained from a local wharf to determine the contents of various arsenic species, including As^{3+}, As^{5+}, MMA, DMA, arsenobetaine, arsenocholine, trimethylarsineoxide, teramethyarsoniumiodide, and total arsenic species with high performance liquid chromatography (HPLC, Agilent 1100 series) linked with inductive couple plasma – mass spectrometer (ICP-MS, Agilent 7500c). Meanwhile, the NIST SRM 1566b for oyster was used in the present study for total arsenic level reference.

Four items of seafood were further used in the following seafood ingestion study, i.e., brown seaweed, clam, oyster, and shrimp. Twelve volunteers were invited to participate in the present study. At the first stage, these 12 subjects were randomly assigned into 3 groups for the one-week food intake study with focus on brown seaweed, clam, and oyster. About one month after the first stage, 4 out of the 12 volunteers took part in the second stage study with seafood of shrimp. During the one-week seafood exposure study, study subjects had three meals per day provided by the project and were restrained them from any food containing seafoods. On the third day, two aliquots of designated seafood, according to the study group assigned, were supplied along with lunch and dinner, respectively, for each study subject.

For urine sampling, study subjects were

asked to provide with all their urine samples after the noon of the second day until the end of the present study, characterized as a 24-hour urine sample and a first morning void (FMV) sample, respectively. Urine samples were analyzed for levels of As^{3+}, As^{5+}, MMA, and DMA, respectively by high performance liquid chromatography (HPLC, Waters 600 along with Waters 717 Autosampler) on-line linked to hydride generation atomic absorption spectrometry (HGAAS, Perkin Elmer AAnalyst 100 equipped with Perkin Elmer FIAS 400 for hydride generation) (7).

四、結果

Table 1 presents the arsenic contents in ten various seafood. Among them, the most common arsenic species was arsenobetaine, followed by dimethylarsonic acid, and arsenocholine was found in some sorts of seafood. The highest total arsenic content was found in flower crab, 219.42 ug/g with the lowest observed in seasand shrimp, 2.58 ug/g. As to the arsenic not speciated in this study, most of them was believed the arsenosugar, of which commercial reference is not currently available for speciation and quantification.

All the study subjects were master students except one faculty member of the authors' Institute. The amount of seafood ingested by the study subjects from the two designated meals were 169.3±1.3 g, 92.9±8.1 g, 166.7±30.1 g, 115.7±9.0 g for the subgroups of 'Brown Seaweed', 'Clam', 'Oyster', and 'Shrimp', respectively.

Figure 1 indicated high correlations between 24-hour urine sample and first morning urine sample for total urinary arsenic levels ($r=0.740$, $p<0.0001$) and dimethylarsinic acid (DMA) ($r=0.737$, $p<0.0001$). Figure 1 shows DMA concentrations in the FMV samples of the 'Brown Seaweed,' 'Clam,' and 'Oyster' subgroups reaching their peaks during the 0~24 hours stage of the study period, while only the DMA level of the 24-hour urine sample for the 'Clam' subgroup showed peak in the 0~24 hour stage.

Figure 3 presents the profile of urinary arsenic metabolites of first morning void (FMV) urine sample. There were significant increases in total urinary arsenic level for all study seafood subgroups after ingestion of designated seafood except the subgroup of shrimp. For all these 4 subgroups, DMA was found the absolute dominant among the four various arsenic metabolites. As to the highest increment in excreted urinary arsenic, it was found for the 'Brown Seaweed' subgroup of 52.0±27.2 *u*g, for the 'Oyster' and 'Clam' subgroups of 32.5±18.9 *u*g and 29.8±20.1 *u*g, respectively.

Figure 4 presents the fluctuations of study subject's individual urinary DMA levels in the first morning void (FMV) during the study period. For the "Brown Seaweed" and "Shrimp" subgroups, most of the four study subjects showed sharp elevation in DMA level during the stage right after brown seaweed ingestion. On the other hand, for the "Oyster" subgroup, the DMA levels did not get down back to baselines through the end of this one-week study period.

五、討論

From the findings in the present study, it is evidently demonstrated that the total urinary arsenic level, composed of As^{3+}, As^{5+}, MMA and DMA, and the urinary DMA level could be significantly affected by the organic arsenic source from seafood. Even just having total 100~200 g seafood in two consecutive meals might result in an elevation of urinary DMA level and total urinary arsenic level by averagely about 7~29 *u*g/L, to some extent, consistent with previous reports (4,6,8). And, such phenomenon might explain why the islanders usually have higher background of total urinary arsenic levels.

Besides, this study provided strong evidence to indicate the effects of seafood ingestion on the increment of urinary total arsenic metabolites and the latency of such elevation, the latter further reflecting different metabolism efficiency for these study seafoods. Further study is warranted to explore how much the time-dependent urinary arsenic excretion was affected by the organic arsenic species in seafood and their metabolism process.

In the present study, it was noted that the baseline urinary arsenic levels and the urinary arsenic levels after ingesting designated seafood varied significantly among the subjects within the same exposure group, implying that there must be significant variation in the pathway and/or efficiency for the seafood organoarsenic metabolism inside human body, and so is the variation in the individual's capability in arsenic detoxification. Great concern has also been imposed on the susceptibility to arseniasis. Further studies are needed to better understand the individual's capability in metabolizing organic and inorganic arsenic, and the susceptibility to arsenic exposure for a more confident risk assessment.

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Table 1. Total and speciated arsenic contents in study seafoods.

Seafood	Speciated Arsenic, ug/g				Total As, ug/g	Arsenic not speciated, %
	DMA	AsB	AsC	Sub Total		
Oyster(蚵)	2.00	2.69	-	4.69	8.53	45.1
Cod(鱈魚)	-	4.98	-	4.98	7.10	29.9
Variegate venus(海瓜子)	0.93	5.24	-	6.17	6.27	1.6
(脆捲)	-	8.20	-	8.20	10.81	24.1
Seasand Shrimp(海沙蝦)	-	1.62	-	1.62	2.58	37.2
Flower Crab(花蟹)	0.02	191.52	-	191.54	219.42	12.7
Clam	0.57	5.89	0.95	7.41	13.52	45.2
Seaweed	0.15	0.59	-	0.74	2.67	72.3
Brown Seaweed(茶葉菜)	6.24	0.79	0.10	7.12	8.43	15.5
Haliotis diversicolor(九孔)	0.26	1.80	0.35	2.41	4.03	40.2

Note: DMA - dimehtylarsonic acid, AsB - Arsenobetaine, AsC – Arsenocholine

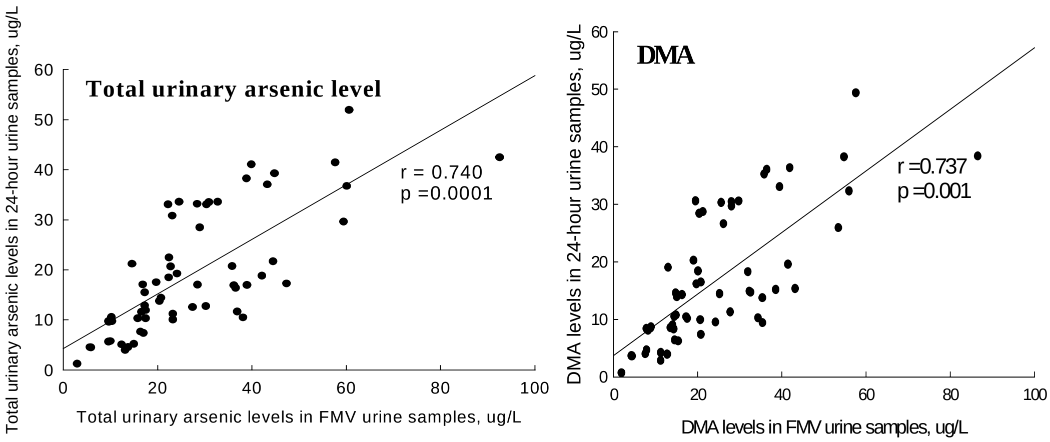

Figure 1. Correlation between 24-hour urine samples and FMV urine samples for total urinary arsenic level, and DMA.

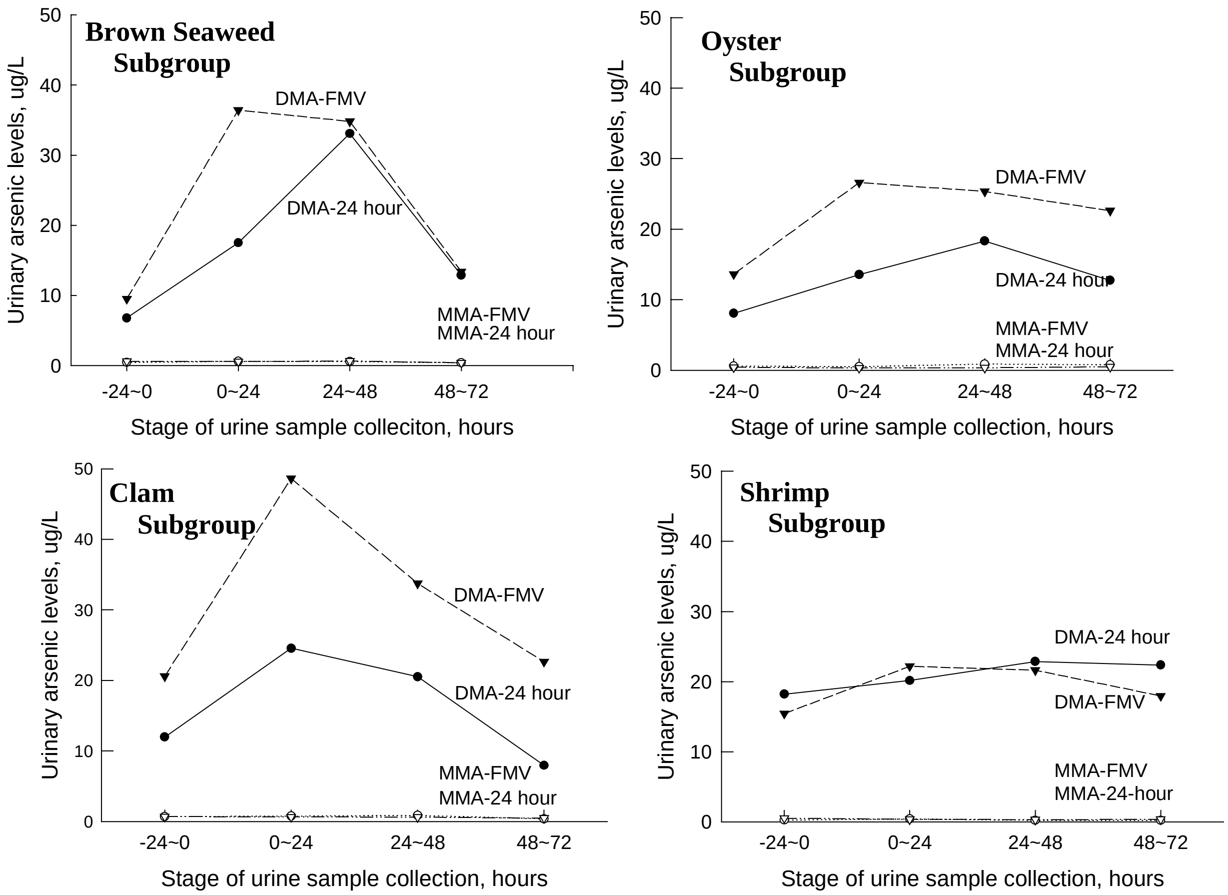

Figure 2. Fluctuation comparison for urinary DMA and MMA levels between 24-hour urine samples and FMV urine samples.

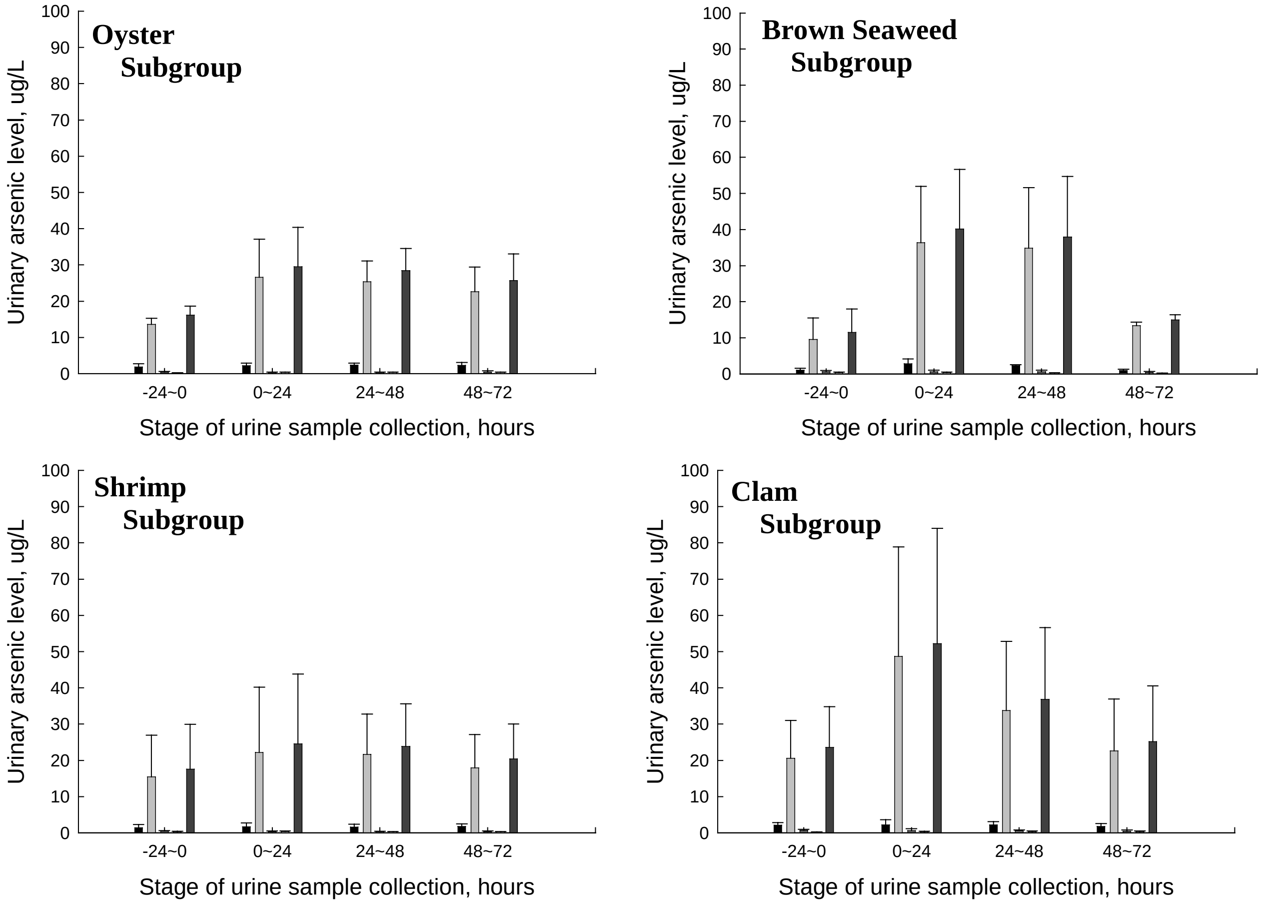

Figure 3. Speciated urinary arsenic levels by ingested seafood subgroup and stage of study phase.

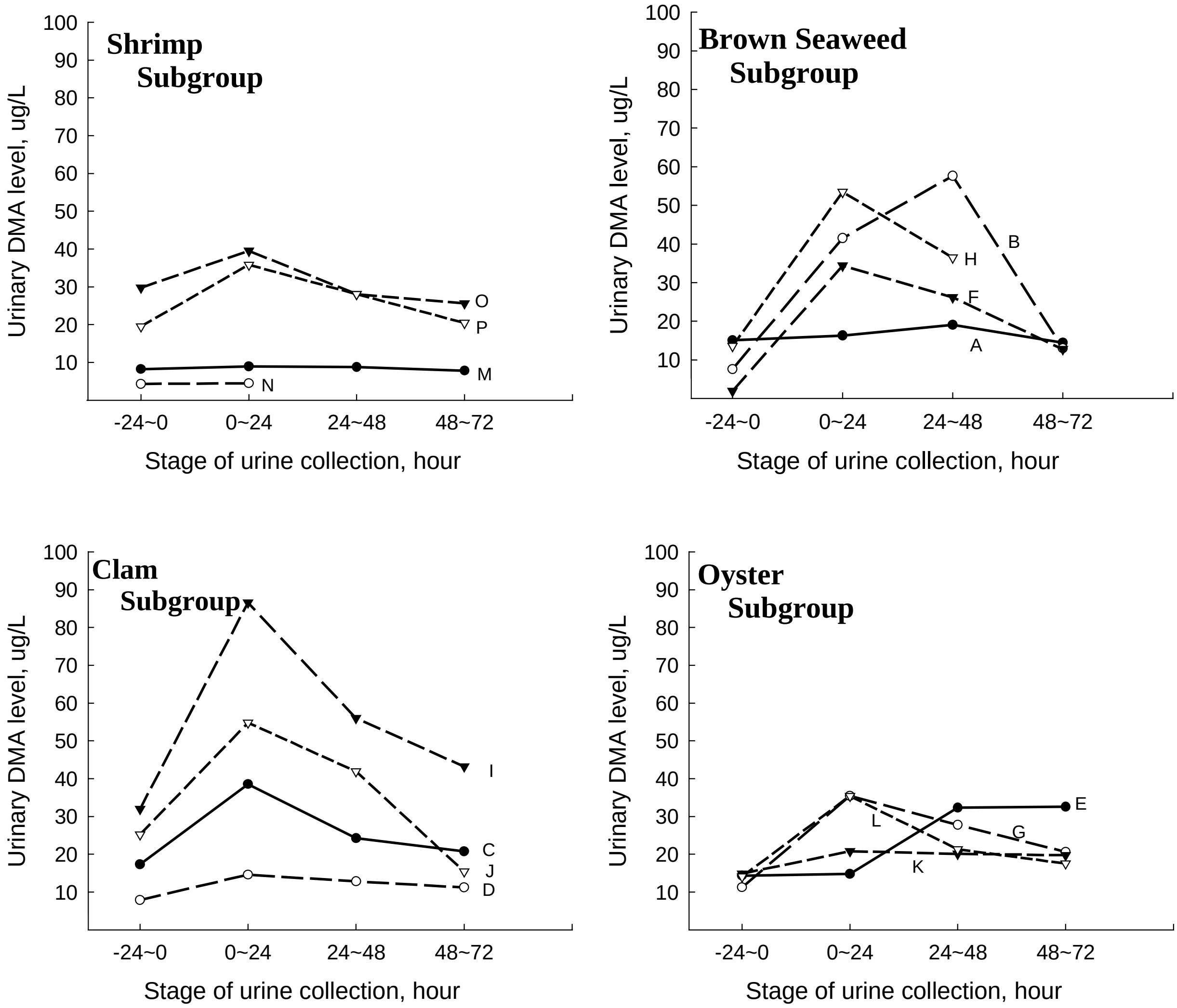

Figure 4. Fluctuation of individuals` urinary DMA levels by designated seafood subgroup.