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Intake of different chemical species of dietary arsenic by the Japanese, and their blood and urinary arsenic levels

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We calculated the intake of each chemical species of dietary arsenic by typical Japanese, and determined urinary and blood levels of each chemical species of arsenic. The mean total arsenic intake by 35 volunteers was 195±235 (15.8–1039) μg As day^{-1}, composed of 76% trimethylated arsenic (TMA), 17.3% inorganic arsenic (As_i), 5.8% dimethylated arsenic (DMA), and 0.8% monomethylated arsenic (MA): the intake of TMA was the largest of all the measured species. Intake of As_i characteristically and invariably occurred in each meal. Of the intake of As_i, 45–75% was methylated *in vivo* to form MA and DMA, and excreted in these forms into urine. The mean measured urinary total arsenic level in 56 healthy volunteers was 129±92.0 μg As dm^{-3}, composed of 64.6% TMA, 26.7% DMA, 6.7% As_i and 2.2% MA. The mean blood total arsenic level in the 56 volunteers was 0.73±0.57 μg dl^{-1}, composed of 73% TMA, 14% DMA and 9.6% As_i. The urinary TMA levels proved to be significantly correlated with the whole-blood TMA levels (r=0.376; P<0.01).

Keywords: Dietary arsenic, methylated arsenic compounds, arsenobetaine, toxicity, metabolism

INTRODUCTION

Arsenic occurs in foods and especially high levels in fish, shellfish and seaweeds. Generally, the total arsenic intake by people eating large amounts of marine products is high, and intake by the Japanese is higher than that by Europeans and Americans.[1] There has been no report on the chemical species of dietary arsenic, while studies for elucidating chemical species and chemical structures of arsenic contained in fish, shellfish and seaweeds have made rapid progress in recent years, with numerous reports already published.[2-5] Arsenic compounds can sometimes be taken as poisons, while arsenic is presumed to be an essential element for livestock.[6] Whether or not it is also an essential element for humans remains to be studied.

As a result of previous studies, arsenic is now known to vary in toxicity with its chemical species or chemical structure. The increase in toxicity of arsenic compounds which man is likely to ingest from food has been estimated to be in the order trimethylarsine oxide ≦ arsenobetaine < dimethylated arsenic (DMA) < monomethylated arsenic (MA) < inorganic arsenic (Asi).[7,8] In the fields of nutrition, toxicology and industrial hygiene, arsenic levels in foods and biological materials have usually been determined merely as total arsenic levels. In studies of the biological effects of arsenic and also in nutritional studies of arsenic, the measurement of each of the chemical species of arsenic (which vary in toxicity) and their evaluation will undoubtedly offer a study method of far higher precision than the previous methods.

The aim of the present study was to calculate the intake of each chemical species of dietary arsenic by the Japanese, and to determine urinary and blood levels of each species in healthy volunteers, in order to establish basal data needed for application to studies in the fields of nutrition, toxicology and industrial hygiene.

MATERIALS AND METHODS

Arsenic intake and urinary arsenic excretion

A group of 35 healthy volunteers, made up of 12 men (aged 45.5±8.3 years) and 23 women (aged 40.3±11.1 years) residing in Tokyo and

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Table 1 Daily intake of chemical species of arsenic, daily energy intake and daily intake of seafood and seaweed by 35 subjects

Group	Intake of arsenic (μg As day⁻¹)					Energy intake (kJ)	Seafood[b] (g wet wt)	Seaweed (g wet wt)
	As_i	MA	DMA	TMA	Total			
Male (1)[a]	51.4±28.8	3.45±2.73	16.7±12.1	202±336	271±336	9824±1636	65.4±47.1	21.8±20.9
(2)	8.34–101	0.16–9.63	0.36–36.6	6.60–946	19.9–1039	5661–12 012	12–151	2–64
Female (1)	24.5±17.4	1.57±2.16	10.7±10.2	120±140	155±152	7247±1280	69.8±42.7	19.7±29.2
(2)	9.18–96.7	0.42–9.18	1.26–38.0	1.95–602	15.8–635	5393–10 276	14–177	1–124
Total (1)	33.7±25.1	2.25±2.50	12.9±11.1	148±226	195±235	8130±1862	68.3±43.5	20.3±26.5
(2)	8.34–101	0.16–9.63	0.36–38.0	1.95–946	15.8–1039	5393–12 012	12–177	1–124

[a] (1) Mean±SD and (2) range of 12 male and 23 female. [b] Seafood = fish + shellfish.

Kanagawa Prefecture, a district adjacent to Tokyo, were enrolled as subjects in the study. They were all fed breakfast, lunch, dinner and a snack between meals on the duplicate portion system for 1 day, and the contents of each meal were classified in the food classes according to the *Standard Tables of Food Composition in Japan* and weighed. Urinary arsenic excretions were calculated from the 24 h-pooled urine samples from 7 of the 23 female subjects. The study was initiated after all the subjects voided before breakfast. The Neue Jr software devised by Hakodate Junior College, Hakodate, Japan (South Electric Co., Hakodate, Japan) was used in calculating the energy intake from the breakfast, lunch, dinner and snack.

Urinary and blood arsenic levels

Urine and blood samples were taken from another group of 56 volunteers, made up of 30 men (aged 47.3±12.5 years) and 26 women (53.5±10.2 years) residing in Tokyo and Kanagawa Prefecture, between 10 and 11 a.m. on the respective sampling days. The blood samples were first measured for hematocrits, and then centrifuged at 2000*g* for 10 min to separate blood cells from plasma. The measured urinary arsenic levels were corrected with a urine specific gravity of 1.024. The urine and blood samples were all preserved frozen at −20 °C until assayed for arsenic.

Pretreatment for arsenic

A portion of each of breakfast, lunch, dinner and snack was ground in a food blender (capacity 1200 cm^3) for about 5 min to make a homogenate. If viscosity increased during the grinding of any sample, distilled water was added. The homogenized sample was transferred into a 50 cm^3 plastic container, and preserved frozen at −20 °C until assayed for arsenic. The measured levels of arsenic in the samples to which distilled water had been added were corrected for the added amounts of distilled water.

Analysis of arsenic

For the measurement of arsenic levels, 2–3 g of each food sample on a wet weight basis, 2 cm^3 of each urine sample, or 0.5 cm^3 of each blood sample was used. The sample was transferred into a 10 cm^3 polymethylpentene test-tube, and after the addition of 5 cm^3 of 2 mol l^{-1} NaOH to the food or urine sample, or 2 cm^3 to the blood sample, the mixture was heated at 95 °C for 3 h. The assay sample was stirred with a magnetic stirrer once every 30 min. The treated food or urine sample was diluted with sufficient distilled water to make 50 cm^3, or the treated blood sample was likewise diluted to make 10 cm^3, and a part of the sample was used for assay. Inorganic arsenic and methylated arsenic compounds do not undergo changes in chemical species (e.g. distribution of methyl groups) even when heated in 2 mol l^{-1} NaOH. As_i, MA, DMA and TMA were determined by atomic absorption spectrophotometry.[10]

RESULTS

Arsenic intake from diet

Table 1 shows the daily intake of each chemical species of dietary arsenic by the 35 subjects. The mean total arsenic intake by the 12 men was 271.4 (15.8–1039) μg As day^{-1}, and that by the 23 women, 155.1 (15.8–635.2) μg As day^{-1}, the intake by the male subjects tended to be higher.

Intake by the men and the women showed great individual variations. The mean total arsenic intake by the 35 subjects was 195 ± 235 μg As day^{-1}, composed of 76% TMA, 17.3% As_i, 5.8% DMA, and 0.8% MA: in other words, the intake of TMA was the greatest of all the chemical species of dietary arsenic. It was characteristic that the subjects always ingested some As_i from each meal. The intake of each chemical species of arsenic by the male subjects tended to be higher than that by the female subjects, but there was a statistically significant difference only in As_i intake between the sexes of the subjects (*t*-test, $P<0.01$).

The mean daily energy intake by the 35 subjects was 1934 ± 445 kcal (8092 ± 1862 kJ). The mean intake by the 12 male subjects was 2348 ± 391 kcal (9824 ± 1636 kJ), and that by the 23 female subjects was significantly correlated with the intake of each chemical species of arsenic: As_i ($r=0.681$; $P<0.01$), DMA ($r=0.475$; $P<0.01$), and total arsenic ($r=0.391$; $P<0.05$), with the exception of TMA.

Table 1 also shows correlations of the intake of fish and shellfish with the intake of each species of arsenic by the 35 subjects. The intake of fish and shellfish correlated significantly with the intake of TMA ($r=0.585$; $P<0.01$) and with that of total arsenic ($r=0.565$; $P<0.01$) by both the male and female subjects. The intake of seaweed was not, however, correlated with the intake of each chemical species of arsenic by either the male or the female subjects.

Urinary arsenic excretion

Table 2 shows arsenic intakes and urinary arsenic excretions by seven of the 23 female subjects. The mean total arsenic intake by the seven subjects was 189.9 μg As day^{-1}, and the mean urinary total arsenic excretion was 196.1 μg As day^{-1}. The correlation between the intake and the urinary excretion of each of the four chemical species of arsenic was such that the urinary excretion of As_i was less by about 45–75% than the intake of As_i in all the subjects, while the urinary excretion of MA and that of DMA were greater than the respective intakes in all the subjects.

Urinary arsenic level

Table 3 shows the measured urinary arsenic levels in the 56 subjects, and those corrected for the urine specific gravity of 1.024. The mean measured urinary total arsenic level was composed of 64.6% TMA, 26.7% DMA, 6.7% As_i, and 2.2% MA.

Table 4 shows correlations between the measured urinary arsenic levels for the 56 subjects, and those corrected for the urine specific gravity of 1.024. There was a correlation between the urinary total arsenic level and the urinary TMA level, followed by a correlation between the former and the urinary level of As_i + MA + DMA, while the correlation between the urinary As_i or TMA level and the urinary total arsenic level tended to be less than those between the urinary levels of the other chemical species.

Table 2 Daily intake and daily urinary excretion of chemical species of arsenic by seven female subjects

	Amount of arsenic (μg As day^{-1})									
	As_i		MA		DMA		TMA		Total	
Subject	I[a]	E[b]	I	E	I	E	I	E	I	E
a	28.7	15.8	0.66	3.02	10.9	36.9	39.3	53.2	79.6	108
b	17.4	4.15	nd[c]	1.86	nd	33.0	36.0	51.3	53.5	90.3
c	24.3	10.7	nd	3.84	8.5	67.0	602	483	635	565
d	18.0	4.84	0.64	1.97	nd	12.5	17.8	22.1	36.5	41.4
e	33.7	10.1	1.17	3.84	38.0	53.1	214	146	287	213
f	22.3	8.2	0.68	0.49	6.1	16.3	28.6	15.0	57.7	40.0
g	31.6	10.6	nd	1.36	32.1	58.6	72.4.	116	136	186

[a] I, Daily intake of arsenic. [b] E, Urinary excretion of arsenic. [c] nd, Not detected

Table 3 Urinary levels of chemical species of arsenic in 56 subjects

Group	Concentration of arsenic ($\mu g\ As\ l^{-1}$)					
	As_i	MA	DMA	TMA	I+M+D[b]	Total
Male (1)[a]	9.14±2.69	3.18±2.14	32.2±23.7	80.3±70.1	44.6±26.5	126±85.0
(2)[a]	4.17–15.0	0.58–9.84	1.93–89.2	2.15–301	8.43–104	18.4–390
Female (1)	8.09±3.04	2.42±1.71	36.9±33.1	86.8±80.6	47.4±35.8	134±100
(2)	4.17–20.3	0.58–8.12	2.76–98.3	1.95–277	11.6–124	12.3–358
Total (1)	8.65±2.83	2.83±1.98	34.4±28.3	83.3±74.5	45.9±31.0	129±92.0
(2)	4.17–20.3	0.58–9.84	1.93–98.3	1.95–301	8.43–124	12.3–390
Male[c] (1)	9.60±3.01	2.90±1.66	32.8±23.9	79.4±62.2	44.9±26.0	128±81.5
(2)	5.73–20.0	0.93–7.87	1.85–97.3	2.70–233	9.52–113	20.1–328
Female[c] (1)	9.45±4.10	2.58±1.47	34.9±25.0	90.3±75.3	46.9±20.2	136±81.9
(2)	5.36–25.0	0.70–6.96	3.68–78.7	2.34–269	12.2–99.2	21.5–303
Total[c] (1)	9.53±3.52	2.75±1.57	33.8±24.2	84.4±68.1	45.8±25.5	132±81.1
(2)	5.36–25.0	0.70–7.87	1.85–97.3	2.34–269	9.52–113	20.1–328

[a] (1) Mean ± SD and (2) range of 30 male and 26 female. [b] I+M+D, As_i+MA+DMA. [c] Adjusted to a specific gravity of 1.024.

Blood arsenic levels

Table 5 shows the blood arsenic levels for the 56 subjects. The chemical species of arsenic detected in blood comprised As_i, DMA, and TMA, while no MA was detected. The mean blood total arsenic level in the 56 volunteers was $0.73 \pm 0.57\ \mu g\ dl^{-1}$, composed of 73% TMA, 14% DMA, and 9.6% As_i. As_i and TMA were detected in all the blood samples, while DMA was detected in only 57% of the samples. The major portion of each of the three chemical species of arsenic detected in the blood samples, i.e. As_i, DMA and TMA, occurred in blood cells rather than in plasma.

Table 4 Correlations between measured urinary levels of each chemical species of arsenic, and those corrected for a urine specific gravity of 1.024 in 56 subjects

	As_i	MA	DMA	TMA	I+M+D[a]
MA	0.741**				
MA[b]	0.241				
DMA	0.512**	0.453**			
DMA[b]	0.078	0.319*			
TMA	0.291*	0.304*	0.379**		
TMA[b]	0.035	0.155	0.222		
I+M+D	0.606**	0.548**	0.992**	0.396**	
I+M+D[b]	0.236	0.398**	0.984**	0.237	
Total	0.431**	0.421**	0.633**	0.946**	0.649**
Total[b]	0.126	0.277*	0.502**	0.927**	0.522**

[a] I+M+D, As_i+MA+DMA. [b] Adjusted to a specific gravity of 1.024. **, $P<0.01$; *, $P<0.05$.

There was a significant correlation between whole-blood and urinary TMA levels ($r=0.407$; $P<0.01$), but not between whole-blood levels and urinary levels of As_i, DMA or total arsenic (Fig. 1).

DISCUSSION

As the Japanese eat large amounts of seaweed, fish and shellfish, their arsenic intake is relatively greater than intake by Europeans and Americans. There has been no report on the intake of each chemical species of arsenic from dietary sources. Ishizaki *et al.*[11] reported a daily total arsenic intake of $126\ \mu g\ As\ day^{-1}$ by the Japanese, and Ikebe *et al.*[12] also reported $273.2 \pm 262.8\ \mu g\ As\ day^{-1}$. These values are in good agreement with our data given in Table 1.

The present study revealed that the intake of chemical species of dietary arsenic by man was in the order TMA > As_i > DMA > MA: the intake of TMA was predominant. TMA may not occur in seaweed but it is found in fish and shellfish at high levels. Trimethylated arsenic compounds whose chemical structures have previously been elucidated are arsenobetaine,[3] arsenocholine[4] and trimethylarsine oxide.[5] Because arsenocholine and trimethylarsine oxide are rarely detected in fish and shellfish, we may hypothesise that the most predominant trimethylated arsenic compound ingested by man is arsenobetaine, which

Table 5 Blood levels of chemical species of arsenic in 56 subjects

	Concentrations of arsenic (μg As dl^{-1})				
Group	As_i	MA	DMA	TMA	Total
Male[a]	0.08±0.04	nd	0.11±0.05	0.41±0.27	0.60±0.31
Male[b]	0.04–0.19		0.05–0.21	0.06–0.94	0.11–1.20
Female[a]	0.06±0.03	nd	0.08±0.03	0.73±0.70	0.87±0.74
Female[b]	0.02–0.10		0.05–0.13	0.10–2.68	0.14–2.89
Total[a]	0.07±0.03	nd	0.10±0.04	0.56±0.54	0.73±0.57
Total[b]	0.02–0.19		0.05–0.21	0.06–2.68	0.11–2.89

[a] Mean±SD. [b] Range of 30 male and 26 female.

having an oral LD_{50} of 10.0 g kg^{-1} in mice,[7] is of low toxicity among arsenic compounds. It is clear that arsenobetaine is not produced in the human body,[10, 13] and that it is not degraded *in vivo* into other chemical species of arsenic.[14, 15] It was normal to find As_i in each meal. This finding appeared to derive from the occurrence of As_i in all food.[2] The finding that intake of MA was the least of the four chemical species of arsenic may be explained by the results in Table 1: DMA, occurring abundantly in seaweed, fish and shellfish, is ingested more often than MA.

An investigation into the relationship between arsenic intake and fish, shellfish, seaweed and energy intake gave the following results. As TMA is the predominant chemical species of arsenic occurring in fish and shellfish, there proved to be a significant correlation between fish and shellfish intake, and TMA intake ($P<0.01$). As As_i occurs abundantly in seaweed, it was presumed that As_i intake would possibly increase with the intake of seaweed, but the findings in the present study showed no correlation to exist between the two parameters. The possible reason for this result may be that seaweed, usually supplied in a dried form, is soaked in water to soften it before it is ingested, the apparent intake in weight of the seaweed increases, but the arsenic contained in it partly dissolves and thus is lost in the water, leading to a decreased net As_i intake.

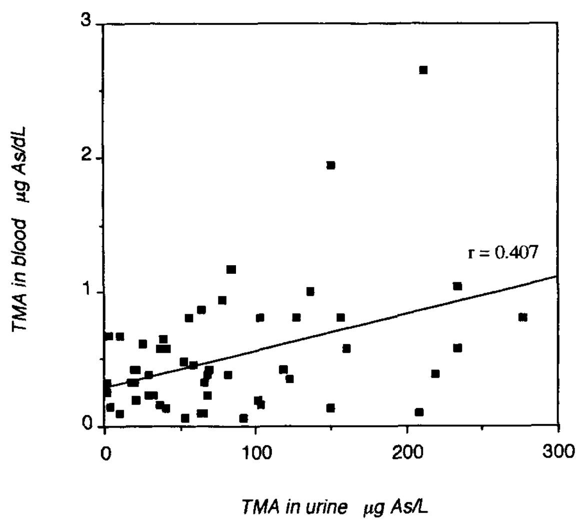

Figure 1 Correlations between urinary and blood TMA levels in 56 subjects (30 male and 26 female). The coefficient, *r*, was 0.401 ($P<0.01$) between the urinary and the blood levels of TMA.

Energy intake by male subjects tended to be slightly greater than that by female subjects. Significant correlations proved to exist between energy intake and intake of each chemical species of arsenic other than TMA by the male subjects. The finding that there was no significant correlation between energy intake and TMA intake by male subjects appeared to have resulted from the presence of a large variation in TMA content in fish and shellfish. It was impossible, however, to explain explicitly the reason why there existed no such correlation between the intakes by the female subjects.

The intake and urinary excretion of arsenic by the seven female subjects, which are given in Table 2, were mostly approximately equal to each other. This finding results from rapid elimination of arsenic compounds from the body. There have been reports on rapid elimination patterns for As_i,[16–18] MA,[18] DMA[18] and TMA[19, 20] in man. The intake–excretion patterns of the four chemical species of arsenic were such that the urinary excretion of As_i was 45–75% less than the intake of As_i, while the urinary excretion of MA and DMA was conversely increased in equivalent amounts. *In vivo* methylation of As_i would support the published data on As_i.

There is only a limited number of reports on urinary levels of chemical species of arsenic in man, viz. our previous report on levels in Japanese,[21] another report by Yamato,[22] one by Fao *et al.* on levels in Italians,[23] and another by Vahter *et al.* on levels in Swedes.[24] These reports include those in which TMA was not precisely assayed because of analytical problems involved in the assay for this species of arsenic. Urinary levels tend to vary greatly with differences in dietary habits in each country. From their study of the levels of chemical species of arsenic in urine samples from Swedes residing in Stockholm, Vahter *et al.*[24] reported that the mean urinary level of $As_i + MA + DMA$ was $12.0 \pm 10.8\ \mu g\ g^{-1}$ creatinine. They described a trend of high urinary organic arsenic (most probably TMA) levels in people eating much fish and shellfish, and our findings in the present study support this conclusion.

There have been only a few studies on arsenic levels in blood samples from healthy volunteers, and no reports have been published on the measurement of chemical species of arsenic in such blood samples. The results of our present study revealed that TMA was the predominant chemical species of arsenic human blood, which was in no way contradictory to observed urinary arsenic excretion and arsenic intake patterns. The arsenic distribution pattern in blood following oral administration of arsenobetaine to experimental animals[15] is such that the plasma level of arsenic is high at early stages, or a few hours after the administration, and its blood-cell level tends to rise with time. In the present study, blood samples were taken in the morning because TMA, the dominant chemical species of arsenic in blood, was ingested about after 12 h or more following an evening meal rich in fish and shellfish. The results appear to reflect the timing of blood sampling.

As arsenic is generally accepted as a poison among people eating much fish, shellfish and seaweed, the increase in daily total arsenic intake may give rise to worries about impairment of health by arsenic. However, no poisoning with arsenic of dietary origin has occurred. The present study revealed that arsenobetaine, a trimethylated arsenic compound of low toxicity, accounted for about 80% of the daily total arsenic intake from food. The present study revealed further that As_i would be methylated to form less toxic chemical species of arsenic *in vivo* before it was excreted into urine.

REFERENCES

1. Ishinishi, N, Tsuchiya, K, Vahter, M and Fowler, B A *Handbook on the Toxicology of Metals*, Elsevier, Amsterdam, 1986, pp 43–83
2. Yamauchi, H and Yamamuta, Y *Jpn. J. Public Health*, 1980, 27: 647
3. Cannon, J R, Edmonds, J S, Francesconi, K A, Raston, C L, Saunders, J B, Skelton, B W and White, A H *Aust. J. Chem.*, 1981, 34: 787
4. Norin, H, Rhyhage, R, Christakopoulos, A and Sandstrom, M *Chemosphere*, 1983, 12: 299
5. Norin, H, Christakopoulos, A, Sandstrom, M and Rhyhage, R *Chemosphere*, 1985, 14: 313
6. Nielsen, F H, Givand, S H and Myron, D Y *Fed. Proc.*, 1975, 34: 923
7. Kaise, T, Watanabe, S and Ito, K *Chemosphere*, 1985, 14: 1327
8. Kaise, T, Yamauchi, H, Horiguchi, Y, Tani, T, Watanabe, S, Hirayama, T and Fukui, S *Appl. Organomet. Chem.*, 1989, 3: 273
9. Resources Council, Science and Technology Agency, the Government of Japan *Standard Tables of Food Composition in Japan*, 4th revised edn, Ministry of Finance Printing Bureau, Tokyo, 1982
10. Yamauchi, H and Yamamura, Y *Toxicol. Appl. Pharmacol.*, 1984, 74: 134
11. Ishizaki, M *Jap. J. Hyg.*, 1979, 34: 605
12. Ikebe, K, Tanaka, Y and Tanaka, R *J. Food Hyg. Soc. Japan*, 1988, 29: 52
13. Marafante, E, Vahter, M, Norin, H, Envall, J, Sandstrom, M, Christakopoulos, A and Rhyhage, R *J. Appl. Toxicol.*, 1987, 7: 111
14. Vahter, M, Marafante, E and Dencker, L *Sci. Total Environ.*, 1983, 30: 197
15. Yamauchi, H, Kaise, T and Yamamura, Y *Bull. Environ. Contam. Toxicol.*, 1986, 36: 350
16. Yamauchi, H and Yamamura, Y *Ind. Health*, 1979, 17: 79
17. Tam, G K H, Charbonneau, S M, Bryce, F, Pomroy, C and Sandi, E *Toxicol. Appl. Pharmacol.*, 1979, 50: 319
18. Buchet, J P, Lauwerys, R and Roels, H *Int. Arch. Occup. Environ. Health*, 1981, 48: 71
19. Yamauchi, H and Yamamura, Y *Bull. Environ. Contam. Toxicol.*, 1984, 32: 682
20. Luten, J B, Riekwel-Booy, G and Rauchbaar, A *Environ. Health Perspect.*, 1982, 45: 165
21. Yamamura, Y and Yamauchi, H *Ind. Health*, 1980, 18: 203
22. Yamato, N *Bull. Environ. Contam. Toxicol.*, 1988, 40: 633
23. Foa, V, Colombi, A, Maroni, M, Buratti, M and Calzaferri, G *Sci. Total Environ.*, 1984, 34: 241
24. Vahter, M and Lind, B *Sci. Total Environ.*, 1986, 54: 1