APPLIED ORGANOMETALLIC CHEMISTRY
Appl. Organometal. Chem. 2001; **15**: 566–571
DOI: 10.1002/aoc.211

Metabolism and organ distribution of arsenic in the freshwater fish *Tilapia mossambica*

Suhendrayatna,[1] Akira Ohki,[2]* Tsunenori Nakajima[2] and Shigeru Maeda[3]
[1]Department of Applied Chemistry and Chemical Engineering, Faculty of Engineering, Kagoshima University, 1-21-40 Korimoto, Kagoshima 890-0065, Japan
[2]Department of Bioengineering, Faculty of Engineering, Kagoshima University, 1-21-40 Korimoto, Kagoshima 890-0065, Japan
[3]Kagoshima National College of Technology, 1460-1 Shinkou, Hayato-cho, Kagoshima 899-5193, Japan

Arsenic concentration and arsenic speciation in the liver, intestine, ovary, bone, brain, muscle, gill and eye of the freshwater fish *Tilapia mossambica* exposed to arsenic were investigated. The profile of arsenic distribution in tissues of *T. mossambica* after exposure to a medium containing arsenate was brain > intestine > ovary > eyes > muscle > gill > bone > liver. The minimum content of arsenic is in liver tissue (2.5 μg_{As} g^{-1} dry weight), whereas the maximum content is in brain tissue (61.8 μg_{As} g^{-1} dry weight). Arsenic accumulated in liver tissue was present as methylated arsenic species, and no inorganic arsenic species were found in liver tissue. A notable exception is in brain tissue. Most arsenic accumulated in brain tissue was inorganic arsenic species, and no methylated arsenic was found in brain tissue. In a dietary exposure treatment, the maximum arsenic accumulation in the tissue of *T. mossambica* fed with *Neocaridina denticulata* dosed with arsenic from a *Chlorella vulgaris* diet (*via* the food chain) is in the ovary (7.4 μg_{As} g^{-1} dry weight), followed by gill, liver, muscle, bone, brain, eyes and intestine. Trace amounts of methylated arsenic were found in liver tissue in this treatment. Methylated arsenic in fish exposed via water was more evenly distributed in the organs compared with dietary exposure.

Keywords: arsenic; accumulation; transformation; speciation; *Tilapia mossambica*, methylarsenic

Received 6 December 2000; accepted 6 March 2001

INTRODUCTION

Arsenic species are well known as pollutants in aquatic ecosystems. This metal can be easily accumulated in nature by bacteria, fungi and algae, as well as by higher plants and animals, and is converted to a range of organic arsenic species.[1,2] It can be an especially serious pollutant in the aquatic environment, since it can be incorporated into the food chain and concentrated by aquatic organisms to a level that affects their physiological state; ultimately they pose a health hazard to humans. The accumulation of arsenic in different components of the food chain in an aquatic ecosystem depends upon the available arsenic concentration in both the water and the sediment. Inorganic arsenic is found as the predominant species in sediments and waters, whereas organoarsenic compounds predominate in organisms.[3] It was postulated that the inorganic arsenic, as the major species in aquatic ecosystems, is reduced to As(III), then converted to dimethylarsenic (DMA) compounds in freshwater microalgae.[4] DMA compounds are then transformed into trimethylarsenic (TMA) compounds in aquatic animals. However, the mechanisms of biotransformation of arsenic in aquatic organisms are not fully understood.[4] Recently, the bioaccumulation and biomagnification of arsenic compounds in organs and tissues of organisms have received considerable attention. Maher *et al.*[5] and Suner *et al.*[6] investigated arsenic distribution in the tissues of a

* Correspondence to: A. Ohki, Department of Bioengineering, Faculty of Engineering, Kagoshima University, 1-21-40 Korimoto, Kagoshima 890-0065, Japan.
Email: ohki@apc.kagoshima-u.ac.jp

fish, *Mullet*, collected from an arsenic-polluted site and reported that the concentration of arsenic in liver tissue is greater than in other tissues. The accumulation and transformation of arsenic in the organs of various animals may be speedily transferred from the surrounding environment and into the food chain. Liver is a site of detoxification, and trace metals are thought to accumulate in the liver prior to transformation and excretion.[7,8]

In the present study, we investigated the metabolism and organ distribution of arsenic in the freshwater fish *Tilapia mossambica*. Tilapia is a euryhaline fish that exists worldwide and is a dominant species in local inland waters and estuarine regions. This species is the dominant fish found in contaminated rivers and estuarine regions.[9] Fish were exposed to arsenate in laboratory experiments (aqueous exposure) and the arsenic concentration and speciation in eight tissues (liver, intestine, ovary, bone, brain, muscle, gill and eyes) were investigated. In addition, this study was designed to provide additional information on uptake and distribution following dietary and water-borne exposure under laboratory conditions.

MATERIALS AND METHODS

Chemicals

Trivalent sodium arsenite [$NaAsO_2$, As(III)], pentavalent sodium arsenate [$Na_2HAsO_4{\cdot}7H_2O$, As(V)], and dimethylarsinic acid were commercial products of Wako Pure Chemical Industries, Ltd, Japan. Methylarsonic acid and arsenobetaine were obtained from Trichemical Laboratory, Japan. Arsenic standards were freshly prepared by serial dilution from stock solutions to the desired concentration just before use, and Milli-Q water was used for all dilutions. Milli-Q water (18.2 MΩ) was obtained with a Milli-Q system (Millipore S.A., 67120 Moisheim, France). The other chemicals used were reagent grade. Plankton tissue (CRM 414) and cod muscle (CRM 422) standard reference materials were purchased from the Community Bureau of Reference, Commission of the European Communities, Brussels. Glass and plastic wares were cleaned by soaking in an ultrasonic bath (Branson 3510) with a cleaning solution followed by a Milli-Q water rinse before use.

Preparation of undosed and arsenic-dosed diets

For the accumulation and transformation of arsenic via the food chain, undosed and arsenic-dosed diets were prepared by culturing *Chlorella vulgaris* in four 5 dm^3 flasks of arsenic-free modified Detmer (MD) medium[10] and MD medium containing 30 mg_{As} dm^{-3} of As(V) under sterile conditions. All flasks were inoculated with 6 mg of dry cells obtained from the stock laboratory algal culture in the log growth phase and cultured at 25–30 °C for 225 h under constant aeration (2 dm^3 min^{-1}) with continuous illumination (approximately 4000 lux around the flask for 24 h per day). Algae from all flasks were mixed prior to centrifugation to ensure homogeneity of the diet. Suspended algal cells were collected by centrifugation and washed with distilled-deionized water. The washing procedure was repeated at least twice and the washed cells were freeze-dried for approximately 48 h. The freeze-dried *C. vulgaris* algal diets were stored in a vacuum desiccator before use. Arsenic concentration measured in the arsenic-dosed diet was 250 μg g^{-1} dry weight with 9% as arsenite, 79% as arsenate, 2% as MMA, and 10% as DMA, whereas no arsenic was found in the arsenic-undosed alga diet.

Three types of artificial food were also prepared, as previously described,[11] containing no arsenic (type 1), 1.38 μg per gram dry weight of arsenite (type 2) or 1.93 μg per gram dry weight of arsenate (type 3).

Determination of total arsenic

Total arsenic was determined by mineralizing the dried organisms (*ca* 5 mg) in the presence of 50% magnesium nitrate (2 cm^3) at 60 °C for 12 h and then at 550 °C for 6 h in a furnace. The resulting ash was dissolved in 10 M HCl (10 cm^3) and 40% aqueous potassium iodide solution (1 cm^3) was added. The solution was extracted twice with chloroform (5 cm^3 each); the chloroform phase was back-extracted with 0.02% aqueous magnesium nitrate solution (2 cm^3), and the aqueous phase was analyzed for arsenic with an atomic absorption spectrophotometer (Japan Jarrel Ash AA-890) with a flameless atomizer (FLA-1000). The measured concentration of total arsenic in plankton, CRM-414 (certified as 6.82 ± 0.28 μg of arsenic per gram dry weight), was 7.1 ± 0.2 μg of arsenic per gram dry weight and that in standard cod muscle, CRM-422 (certified as 21.1 ± 0.5 μg of

arsenic per gram dry weight), was 24.1 ± 0.6 μg of arsenic per gram dry weight.

Determination of methylated arsenic compounds

Inorganic and methylated arsenic compounds in the dry sample were determined by hydride generation atomic absorption spectrophotometry (HG-AAS) after digestion with 2 M NaOH (5 cm^3) at 90–95 °C for 3 h in an aluminum heating block. The digest was treated with 5 cm^3 of 4% $NaBH_4$ in 0.1 M NaOH at pH 6.2 buffer solution (0.125 M Tris–HCl) to hydrogenate arsenite to arsine.[12,13] Arsenate and the methylated arsenic compounds were hydrogenated with 5 cm^3 of 10% $NaBH_4$ in 0.1 M NaOH at pH 1 buffer solution (10% oxalic acid). The arsines generated were cooled with liquid nitrogen and were collected in a U-trap. Upon warming the U-trap, the arsines volatilized in the sequence of their boiling points [b.p.: AsH_3, −55 °C; CH_3AsH_2, 2 °C; $(CH_3)_2AsH$, 35.6 °C (747 mmHg); $(CH_3)_3As$, 52 °C (736 mmHg)] and were passed through a quartz-tube atomizer and determined with an atomic absorption spectrophotometer (Nippon Jarrell Ash, AA-890). Triplicate analyses were performed for each sample. The absolute detection limits for total and arsenic speciation in a single injection were 0.5 ng and 5 ng respectively. The coefficients of variations for the total and the arsenic species were below 5%.

Experimental procedure

T. mossambica (5–8 cm in total length), visibly free of any deformities, disease or lesions, were obtained from the stock ponds of the Aquatic Chemical Nutrition Laboratory, Faculty of Fisheries, Kagoshima University, Kagoshima, Japan. *T. mossambica* were acclimatized in tap water at 21 ± 1 °C with a 12 h light–dark cycle for at least 7 days. Mortality was less than 5% of the population during acclimatization. *T. mossambica* were fed daily with the arsenic-free artificial food (type 1), with the pH ranging from 7.6 to 7.8. Before the food chain experiment, the fish were fed with an arsenic-free dried powder diet of the alga *C. vulgaris* for 5 days.

Three types of experiment were performed. In experiment I, *T. mossambica* were exposed to 15 dm^3 dilute medium containing 10 mg dm^{-3} arsenate under static conditions for 7 days. A control medium (arsenic free) was also prepared. Diluted MD medium [1/10 (v/w)] was used in this experiment with a pH range from 7.6 to 7.8. The fish were fed daily with arsenic-free *C. vulgaris* dried powder equivalent to approximately 2% of their body weight. After 7 days observation, eight tissues (liver, intestine, ovary, bone, brain, gill, muscle and eyes) were dissected from individual fish. Tissues were rinsed with deionized water and dried at 60 °C for 24 h until constant weight. The weight of dried tissues was recorded, and the arsenic species concentration was analyzed.

In experiment II, accumulation and transformation of arsenic via the three-step food chain (*C. vulgaris* → *Neocaridina denticulata* → *T. mossambica*) was investigated by feeding the arsenic-dosed alga *C. vulgaris* to freshwater organisms for 7 days. The arsenic concentration measured in the algal powder was 250 μg g^{-1} dry cells, with 9% as arsenite, 79% as arsenate, 2% as MMA, and 10% as DMA. In the first step, the arsenic-dosed algae (*C. vulgaris*; 5 mg dry weight per day; 35 mg total) were fed to shrimp (*N. denticulata*, 28 mg dry weight) for 7 days in aerated dilute MD medium, then the *N. denticulata* were collected and washed with distilled water. The arsenic concentration measured in *N. denticulata* was 25.7 μg g^{-1} dry cells, with 60% as inorganic arsenic compounds, 37% as DMA, and 3% as TMA. In the third step, three fish (*T. mossambica*, 13 g dry weight) were fed for 7 days with arsenic-dosed *N. denticulata* (56 mg dry weight per three fish a day; 390 mg total) in aerated dilute MD medium. After 7 days observation, two fish were collected, washed with distilled water and analyzed for total and species of arsenic compounds by the methods described above. A control treatment was also prepared. Fish were fed daily with *N. denticulata* that contained low levels of arsenic (2 μg g^{-1} dry weight); 10% As(III), 5% As(V) and 85% DMA.

In experiment III, the accumulation and transformation of arsenic via food was also investigated by feeding the arsenic-dosed artificial diet to *T. mossambica* for 7 days under the conditions described above. Fish were divided into three groups based on their food. Group 1: fish were fed daily with an arsenic-free artificial diet; group 2: fish were fed with an arsenite-dosed artificial diet (diet type 2); group 3: fish were fed with an arsenate-dosed artificial diet (diet type 3). The medium was changed daily. After 7 days observation, fish were dissected and rinsed with deionized water and dried at 60 °C for 24 h until constant weight. The weight of dried tissues was recorded, and the arsenic species concentration was analyzed.

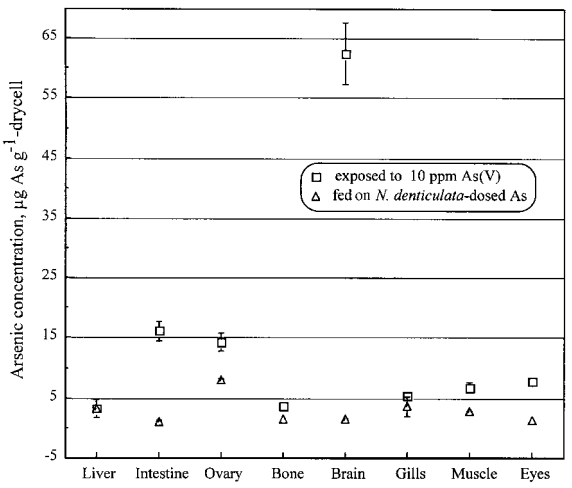

Figure 1 Arsenic concentrations in different *T. mossambica* tissues after exposure to arsenate and fed on *N. denticulata* dosed with arsenic.

RESULTS AND DISCUSSION

Water-borne exposure of arsenate to 10 mg_{As} dm^{-3} for a duration of 7 days, showed the presence of arsenic in all eight tissues of *T. mossambica* in a range from 2.5 to 61.8 μg_{As} g^{-1} dry weight (Fig. 1). Differential accumulation of arsenic in the various tissues of *T. mossambica* was observed. The minimum content was in the liver tissue (2.5 μg_{As} g^{-1} dry weight), whereas the maximum content was in the brain (61.8 μg_{As} g^{-1} dry weight). Arsenic concentrations varied in the order: brain > intestine > ovary > eyes > muscle > gill > bone > liver. The higher concentrations of arsenic found in brain tissue relative to other tissues were consistent with other study of heavy metals. Deb and Sandra[12] found the highest chromium concentrations in brain tissue of *T. mossambica*. It should be noted that some studies have reported results that do not fit with a single pattern. Some environmental data show that the higher concentrations of arsenic are found in liver tissue relative to other tissues. Maher *et al.*[5] found higher concentrations of arsenic in liver tissue of the sea mullet *Mullet cephalus* collected from Lake Macquarie NSW (1.9 μg_{As} g^{-1} dry weight) than in other tissues. Also, Suner *et al.*[6] found higher concentrations of arsenic in liver tissue than muscle tissue of the mullet *L. ramada* collected from a polluted site on the River Guadalquivir, Spain (3.63 μg_{As} g^{-1} dry weight). Hongxia *et al.*[13] also found the highest tributyltin concentrations in viscera tissues of *T. mossambica*. Not all tissues will receive the same blood flow, and the distribution of arsenic in the various tissues will be different. Arsenic accumulation in tissues will be a function of uptake and clearance rates of the individual organs. The significant correlations of arsenic concentrations between tissues indicated that co-accumulation of arsenic is occurring in tissues in a form suitable for accumulation.

In the dietary exposure experiment, the range of total arsenic content in the tissues of *T. mossambica* fed with *N. denticulata* dosed with arsenic from a *C. vulgaris* diet (*via* the food chain) varied between 0.5 and 7.4 μg_{As} g^{-1} dry weight. The maximum arsenic content of the fish on this treatment is in the ovary (7.4 μg_{As} g^{-1} dry weight), whereas the minimum arsenic content was in intestine tissue (0.5 μg_{As} g^{-1} dry weight). The profile of the arsenic

Table 1 Accumulation and distribution of total arsenic and arsenic compounds in the tissues of *T. mossambica* exposed to 10 mg_{As} dm^{-3} of arsenate for 7 days

T. mossambica organ	Dry mass (mg)	Total arsenic[a] (μg_{As} g^{-1} dry wt)	Arsenic concentration[b] (as percentage of total arsenic)				
			As(III)	As(V)	MMA	DMA	TMA
Liver	121	2.5 ± 1.5	–	–	28	28	44
Intestine	194	15.5 ± 1.6	3	4	35	26	32
Ovary	35	13.5 ± 1.5	24	25	15	13	23
Bone	7830	3.0 ± 0.3	–	3	13	17	67
Brain	49	61.8 ± 5.1	48	52	–	–	tr
Muscle	3790	6.0 ± 0.9	37	38	tr	3	22
Gill	476	4.8 ± 0.3	23	25	12	11	29
Eyes	209	7.1 ± 0.5	15	17	7	9	52

[a] Average of data from three replicated series of observations.
[b] As(III), arsenite; As(V), arsenate; MMA, monomethylarsenic compounds; DMA, dimethylarsenic compounds; TMA, trimethylarsenic compounds; tr, trace amount detected; –, not detected.

Table 2 Accumulation and distribution of total arsenic and arsenic compounds in the tissues of *T. mossambica* fed *N. denticulata* dosed with arsenic from *C. vulgaris* for 7 days[a]

T. mossambica organ	Dry mass (mg)	Total arsenic[b] ($\mu g_{As}\ g^{-1}$ dry wt)	Arsenic concentration[c] (as percentage of total arsenic)				
			As(III)	As(V)	MMA	DMA	TMA
Liver	114	2.5 ± 0.1	16	84	–	tr	tr
Intestine	291	0.5 ± 0.2	tr	100	–	–	–
Ovary	50	7.4 ± 0.2	tr	100	–	–	–
Bone	6470	0.9 ± 0.04	tr	91	–	–	9
Brain	23	0.8 ± 0.1	tr	100	–	–	tr
Muscle	3030	2.1 ± 0.1	tr	64	–	–	36
Gill	458	2.9 ± 1.7	tr	99	–	–	1
Eyes	154	0.7 ± 0.02	tr	93	–	–	7

[a] *C. vulgaris* cultured in MD medium containing 30 $mg_{As}\ dm^{-3}$ of arsenate for 10 days. *N. denticulata* were fed for 7 days with the dried powder of arsenic-dosed *C. vulgaris* (249 $\mu g_{As}\ g^{-1}$ dry cells); *T. mossambica* were fed for 7 days with the arsenic-dosed *N. denticulata* (25.7 $\mu g_{As}\ g^{-1}$ dry tissues).
[b] Average of data from three replicated series of observations.
[c] As(III), As(V), MMA, DMA and TMA; see Table 1.

distribution in the tissues of *T. mossambica* was the ovary > gill > liver > muscle > bone > brain > eyes > intestine. In comparison, the arsenic concentration in *T. mossambica* muscle tissue was on average, three times less after accumulation *via* the food chain ($2.1 \pm 0.1\ \mu g_{As}\ g^{-1}$ dry weight) than by direct accumulation from the medium ($6.0 \pm 0.9\ \mu g_{As}\ g^{-1}$ dry weight). Furthermore, only a small amount of arsenic was found in the muscle tissue ($1.3 \pm 0.1\ \mu g_{As}\ g^{-1}$ dry weight) and bone tissue ($0.5 \pm 0.1\ \mu g_{As}\ g^{-1}$ dry weight) of *T. mossambica* exposed to the arsenic-free medium and fed on undosed arsenic *N. denticulata*.

Table 1 provides details of arsenic speciation in the eight tissues of *T. mossambica* after exposure to 10 $mg_{As}\ dm^{-3}$ of arsenate. Most tissues contained a large proportion of methylated arsenic as TMA (22–67%), which is probably the end product of arsenic accumulated in the *T. mossambica* tissues. All of the arsenic accumulated in the liver (a site of detoxification) is present as methylated arsenic species. No inorganic arsenic species were found in liver tissues. A notable exception is in the brain tissue. Most of the arsenic was accumulated in brain tissue as inorganic arsenic species, and no methylated arsenic was found in brain tissue. It seems that brain tissue easily accumulates arsenic prior to methylation and excretion.

As regards arsenic dietary intake, the speciation of arsenic in the eight tissues of *T. mossambica* fed on shrimp in a food chain based on *C. vulgaris* is presented in Table 2. Most tissues contained a large proportion of inorganic arsenic compounds as arsenate (64–100%). Smaller amounts of TMA (1–36%) were found in gill, eyes, bone and muscle tissues. TMA was found in liver and brain tissues in trace amounts. The highest amount of TMA was found in muscle tissue (36%). Trace amounts of DMA were also found in liver tissue. Significant results were found for dietary exposure in which *T. mossambica* were fed on an artificial diet containing only arsenate or arsenite in experiment III

Table 3 Arsenic species in muscle tissues of *T. mossambica* exposed to arsenic-dosed artificial diets for 7 days

Arsenic in diet	Dry mass (mg)	Total arsenic ($\mu g_{As}\ g^{-1}$ dry wt)	Arsenic concentration (as percentage of total arsenic)				
			As(III)	As(V)	MMA	DMA	TMA
As-free (control)	–	–	–	–	–	–	–
As(III) 1.38 $\mu g\ g^{-1}$	696	1.76 ± 0.2	46	26	–	tr	28
As(V) 1.93 $\mu g\ g^{-1}$	1020	0.46 ± 0.8	24	46	–	–	30

[a] Average of data from three replicated series of observations.
[b] As(III), As(V), MMA, DMA and TMA; see Table 1.

(Table 3). TMA was the predominant species among the methylated arsenic species found in muscle tissue.

In conclusion, the profile of arsenic distribution in tissues of *T. mossambica* after exposing to a medium containing arsenate is the brain > intestine > ovary > eyes > muscle > gill > bone > liver. The minimum content is in the liver tissue (2.5 $\mu g_{As}\ g^{-1}$ dry weight), whereas the maximum content is in the brain (61.8 $\mu g_{As}\ g^{-1}$ dry weight). On the other hand, maximum arsenic accumulation in the tissues of *T. mossambica* fed on *N. denticulata* dosed with arsenic from a *C. vulgaris* diet (*via* the food chain) is in the ovary (7.4 $\mu g_{As}\ g^{-1}$ dry weight) followed by the gill, liver, muscle, bone, brain, eyes and intestine. Our results show that methylated arsenic in fish exposed via water was more evenly distributed in the organs compared with the dietary exposure.

Acknowledgements The authors are sincerely grateful to Professor S. Koshio, Faculty of Fisheries, Kagoshima University, for providing *T. mossambica*. We also thank Professor K. Somekawa, Faculty of Engineering, Kagoshima University, for his helpful discussions.

REFERENCES

1. Cullen WR, Reimer KJ. *Chem. Rev.* 1989; **89**: 713.
2. Francesconi KA, Edmonds JS. *Oceanogr. Mar. Biol. Annu. Rev.* 1994; **31**: 111.
3. Francesconi KA, Hunter DA, Bachmann B, Raber G, Goessler W, Crangon C. *Appl. Organomet. Chem.* 1999; **13**: 669.
4. Kaise T, Fujiwara S, Tsuzuki M, Sakurai T, Saitoh T, Mastubara C. *Appl. Organomet. Chem.* 1999; **13**: 107.
5. Maher W, Goessler W, Kirby J, Raber G. *Mar. Chem.* 1999; **68**: 169.
6. Suner MA, Devesa V, Munoz O, Lopez F, Montoro R, Arias AM, Blasco J. *Sci. Tot. Environ.* 1999; **242**: 261.
7. Sorense EMB. Arsenic. In *Metal Poisoning in Fish*. CRC Press: Boca Raton, FL, 1991; chapter 2, 61.
8. Bodgan GM, Sampayo RA, Aposhian HV. *Toxicology* 1994; **93**: 175.
9. Lam KL, Ko PW, Wong JKY, Chan KM. *Mar. Environ. Res.* 1998; **46**: 563.
10. Suhendrayatna, Ohki A, Kuroiwa T, Maeda S. *Appl. Organomet. Chem.* 1999; **13**: 128.
11. Kuroiwa T, Yoshihiko I, Ohki A, Naka K, Maeda S. *Appl. Organomet. Chem.* 1995; **9**: 517.
12. Deb SC, Santra SC. *Environmentalist* 1997; **17**: 27.
13. Hongxia L, Guolan H, Shugui D. *Appl. Organomet. Chem.* 1998; **12**: 109.
14. Andreae MO. *Anal. Chem.* 1977; **49**: 820.
15. Anderson RK, Thompson M, Culbard E. *Analyst* 1986; **111**: 1143.

