APPLIED ORGANOMETALLIC CHEMISTRY
Appl. Organometal. Chem. 2002; **16**: 692–700
Published online in Wiley InterScience (www.interscience.wiley.com). DOI:10.1002/aoc.374

Distribution of arsenic species in the freshwater crustacean *Procambarus clarkii*

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Received 3 April 2002; Accepted 19 August 2002

The concentrations of total arsenic and arsenic species in the complete organism of the crayfish *Procambarus clarkii* and its various parts (hepatopancreas, tail, and remaining parts) were analyzed in order to discover the distribution of arsenic and its species. With this information it will be possible to establish where the chemical forms of this metalloid tend to accumulate and what risks may derive from the contents and species present in the edible parts of this crustacean. The total arsenic content in the complete organism and in the various parts analyzed ranged from 2.5 to 12 μg g^{-1} dry mass (DM), with inorganic arsenic representing 18 to 34% of total arsenic. The arsenical composition varied according to the part of the crayfish considered. The hepatopancreas had the highest levels of total arsenic (9.2–12 μg g^{-1} DM) and inorganic arsenic (2.7–3.2 μg g^{-1} DM). The tail (edible part) had the lowest levels of both total arsenic (2.5–2.6 μg g^{-1} DM) and inorganic arsenic (0.46–0.64 μg g^{-1} DM). The predominant organoarsenical species were the dimethylarsinoylribosides: glycerol riboside in the hepatopancreas, sulfate riboside in the tail, and sulfonate and phosphate ribosides in the remaining parts. **Copyright © 2002 John Wiley & Sons, Ltd.**

KEYWORDS: arsenic; arsenic species; arsenosugars; freshwater; crayfish; hepatopancreas; tail muscle

INTRODUCTION

Arsenic is extensively distributed in the environment. Its presence may have a natural origin, resulting from erosion of rocks or geothermal activity (Bangladesh, Taiwan, Chile, Argentina, and the West Bengal region in India), or it may have a human origin, resulting from industrial, agricultural or animal farming activities. Freshwater ecosystems are no exception. The mean concentration of arsenic in fresh water is 1.4 μg l^{-1}, with a fairly wide range, 0.1–75 μg l^{-1}.[1] Studies of fresh water have shown the presence mainly of arsenic(V), although arsenic(III), dimethylarsinic acid (DMA), and traces of monomethylarsonic acid (MMA) may also be found.[2]

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Contract/grant sponsor: FEDER; Contract/grant number: 1FD97 1421-C03-03.
Contract/grant sponsor: Generalitat Valenciana (Conselleria de Cultura, Educació i Ciènca).
Contract/grant sponsor: Ministerio de Educación y Cultura.

Information about arsenic in freshwater organisms is scarce in comparison with the literature available on marine or even land organisms. Some studies have been carried out with a view to discovering the possible arsenic cycle in fresh water,[3,4] but the speciation of this metalloid has not been pursued. The total arsenic content in freshwater fish varies between 0.007 and 0.57 μg g^{-1} wet mass (WM),[5,6] concentrations that are similar to those present in land organisms in uncontaminated areas but which are much lower than those customarily found in marine organisms (0.1–167 μg g^{-1} WM).[7] With respect to arsenic species, Koch *et al.*[6] made a study of freshwater fish and mussels. They reported the presence in freshwater fish of arsenobetaine (AB, 9–34%), DMA (2–61%), and dimethylarsinoylriboside with phosphate as aglycone (11–12%), a pattern that differs from the arsenic profile found in marine fish, where the predominant species is AB[1], and the presence of arsenosugars has not yet been reported. In the freshwater mussels analyzed in the same study the predominant arsenic species were dimethylarsinoylribosides, and DMA and arsenic(V) were also found. The concentration of AB was low (approximately

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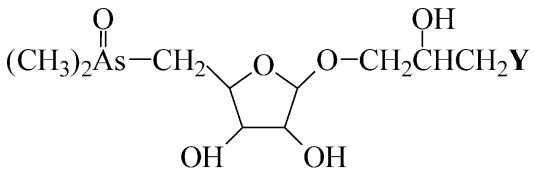

	Y
1a	-OH
1b	$-OPO_3HCH_2CH(OH)CH_2OH$
1c	$-SO_3H$
1d	$-OSO_3H$

Figure 1. Principal dimethyarsinoylribosides present in nature.

12% in muscle), contrasting with the pattern normally found in marine mussels, where AB is the predominant species.[8]

In freshwater crustaceans, previous studies of the crayfish *Procambarus clarkii* by Devesa *et al.*[9] revealed the presence of AB, the four dimethylarsinoylribosides most commonly found in nature (Fig. 1), and inorganic arsenic. However, the total arsenic content found (1.2–8.5 μg g^{-1} dry mass (DM)) was not high compared with the levels generally found in marine crustaceans (15–118 μg g^{-1} DM).[10] The concentrations of inorganic arsenic ranged between 0.34 and 5.4 μg g^{-1} DM, values that in many cases are higher than those observed in marine organisms (0.008–0.88 μg g^{-1} DM).[11] The inorganic arsenic found represented up to 50% of the total present in the sample. In the study by Devesa *et al.*,[9] however, the complete animal was analyzed, including the exoskeleton and the head, which are not edible. Some authors indicate a concentration of metals and even arsenic in the outer covering and the hepatopancreas.[12,13]

The edible part of *P. clarkii* is the tail, although sometimes the hepatopancreas is also ingested with it; therefore, it is important to study the concentrations of total arsenic and arsenic species in both parts. Consequently, the present work analyzes the various parts of *P. clarkii* (hepatopancreas, tail, and remaining parts) and the complete crayfish in order to evaluate where this metalloid accumulates predominantly, to establish whether there are differences between the various parts of the body in terms of the distribution of arsenic species, and to determine the possible risks deriving from consumption of this product.

MATERIALS AND METHODS

Chemicals

Deionized water (18 MΩ cm) was used for the preparation of reagents and standards. All chemicals were of *pro analysi* quality or better. For high-performance liquid chromatography coupled to inductively coupled plasma mass spectrometry (HPLC–ICP-MS) quantification, the standards were prepared in aqueous medium from the following compounds: arsenic(V) from sodium arsenate heptahydrate ($Na_2HAsO_4{\cdot}7H_2O$, Sigma); arsenic(III) from As_2O_3 (Alfa Products); monomethylarsonic acid (MMA) and DMA from $CH_3AsO(OH)_2$ (Pfalz and Bauer) and $(CH_3)_2AsO(OH)$ (Aldrich) respectively; AB from trimethylarsine following the method described by Edmonds and Francesconi.[14] For total and inorganic arsenic quantification by flow injection-hydride generation-atomic absorption spectrometry (FI-HG-AAS), a commercial standard solution of arsenic(V) (Merck Farma Química, Barcelona, Spain) was used.

TORT-2 (Lobster hepatopancreas, National Research Council Canada) was employed as certified reference material in the determination of total arsenic. This sample of TORT-2 was also used to evaluate the accuracy of the determination of inorganic arsenic, because, although inorganic arsenic is not certified in TORT-2, its concentration has been reported in the literature.[11] For identification of arsenosugar species, oyster tissue reference material (1566a, National Institute of Standards & Technology, USA) and kelp powder sample, a commercially available algae product from Eastern Canada, were employed. The arsenic species present in both samples had previously been characterized by other authors.[8,15]

Glass and plasticware were cleaned by soaking in 2% extran solution overnight, rinsing with water, then deionized water. This was followed by soaking in 0.1 mol l^{-1} HNO_3 solution overnight, rinsing with deionized water, and air drying.

Collection and preparation of samples

For the analysis of the various parts, 120 crayfish from an experimental tank were dissected. This source was selected because, in a previous study carried out by Devesa *et al.*,[9] the specimens from this source had the highest levels of inorganic arsenic, which is important from a toxicological viewpoint. The samples came from a pool of specimens that were not differentiated in terms of sex. The individual specimens were dissected and each of these parts — hepatopancreas, tail, and remainder of body (exoskeleton, gills, gut) — was analyzed separately. Three samples of crayfish were pooled and analyzed without being dissected. Once prepared, the samples were frozen at −20 °C and freeze-dried. The lyophilized samples were crushed to a fine powder in a mill. The resulting powder was stored in previously decontaminated twist-off flasks and kept at 4 °C until analysis.

Determination of total arsenic

Total arsenic was quantified in the natural samples and reference materials by FI-HG-AAS after a dry-ashing step. The lyophilized sample (0.25 ± 0.01 g) was weighed, and 1 ml of ashing aid suspension (20% m/v $Mg(NO_3){\cdot}6H_2O$ and 2% m/v MgO) and 5 ml of 50% v/v HNO_3 were added, and the mixture was evaporated on a sand bath until total

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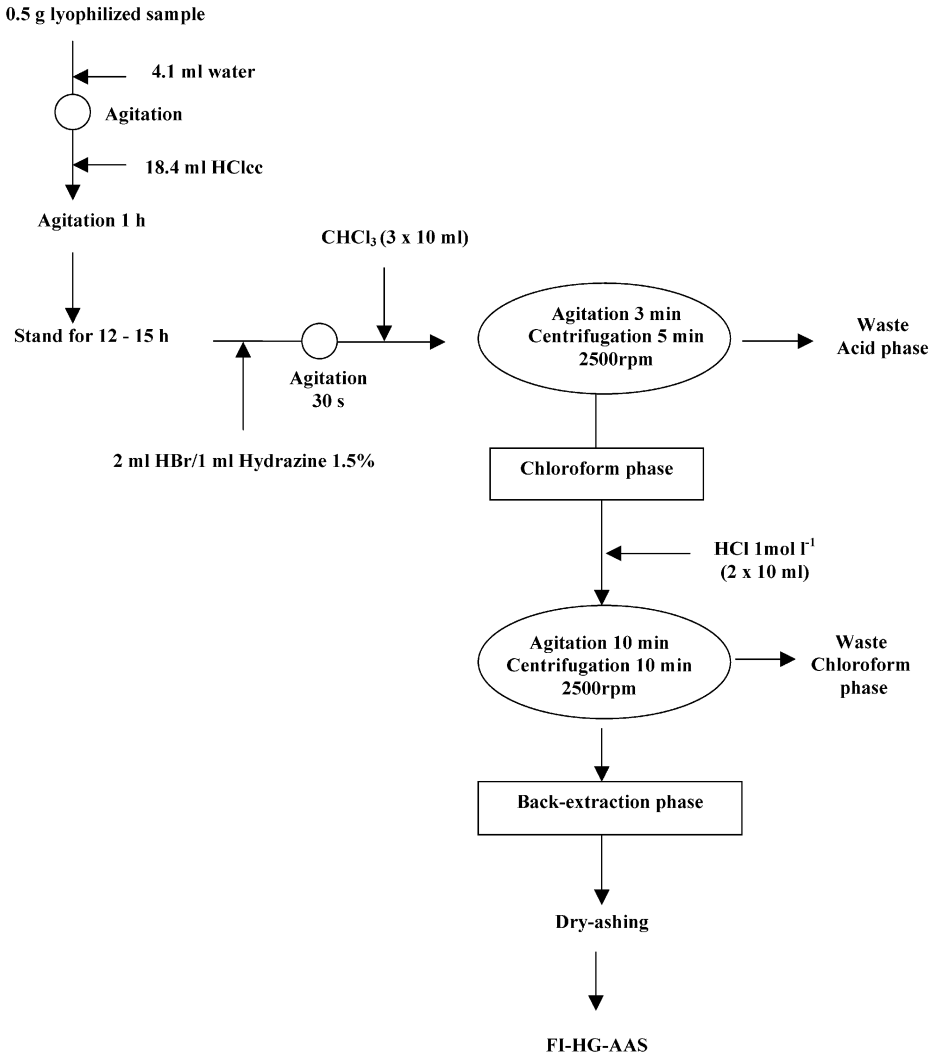

Figure 2. Scheme of inorganic arsenic extraction.

dryness. The sample was then dry ashed as described by Ybáñez *et al.*[16] The ash from the mineralized samples was dissolved in 5 ml of 50% v/v HCl and 5 ml of reducing mixture (5% m/v KI + 5% m/v ascorbic acid). After 30 min, this solution was filtered through Whatman No. 1 filter paper into a 25 ml volumetric flask and diluted to volume with water. For quantification of total arsenic an AAS Perkin–Elmer model 3300 (Perkin–Elmer, PE, Norwalk, CT, USA) equipped with a PE AS-90 autosampler and a PE FIAS-400 flow injection system was employed. The instrumental conditions used for total arsenic determination were as follows: FI-HG: loop sample, 0.5 ml; reducing agent, 0.2% m/v $NaBH_4$ in 0.05% m/v NaOH, 5 ml min^{-1} flow rate; HCl solution, 10% v/v, 10 ml min^{-1} flow rate; carrier gas, argon, 100 ml min^{-1} flow rate; AAS: wavelength 193.7 nm; spectral bandpass 0.7 nm; electrodeless discharge lamp system 2, lamp current setting 400 mA; cell temperature 900 °C.

The analytical characteristics for the total arsenic methodology are as follows. Limit of detection (LD): 0.006 μg g^{-1} WM; precision: 2%; accuracy for TORT-2 (value found: 22.3 ± 0.2 μg g^{-1}; certified value: 21.6 ± 1.8 μg g^{-1}).

Arsenic speciation analysis by HPLC–ICP-MS

Lyophilized samples were weighted into 15 ml centrifuge tubes. Dry masses of approximately 0.5–1.0 g were used. One extraction round included the addition of 5 ml of MeOH/H_2O (1:1, v/v) per 0.5 g of dry tissue, vigorous mixing, sonication for 10 min, centrifugation at 3000 rpm for 10 min, and transferring of the supernatant by a Pasteur pipette to a 100 or 250 ml round-bottom flask. The residue was extracted an additional four times following the same procedure. The five supernatant fractions were combined in the round-bottom flask, evaporated to near dryness (~1–2 ml) and made up to an exact volume of 10 ml by the addition of deionized water. Extracts were stored at −20 °C until analysis.

The HPLC system consisted of a Waters Model 510 delivery

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pump, a Rheodyne Model 7010 injector valve with a 20 µl sample loop, and a reverse phase C_{18}-column (Inertsil ODS, GL Sciences, Japan, 250 mm × 4.6 mm). A guard column (C_{18}, Supelco) was used preceding the analytical column. The mobile phase contained 10 mmol l^{-1} tetraethylammonium hydroxide, 4.5 mmol l^{-1} malonic acid and 0.1% methanol. The pH was adjusted to 6.8 by using 3% nitric acid. The HPLC system was connected to the ICP nebulizer via a PTFE tube (20 cm × 0.4 mm) and appropriate fittings. A VG Plasma Quad 2 Turbo Plus ICP mass spectrometer (VG Elemental, Fisons Instrument), equipped with an SX 300 quadrupole mass analyzer, a standard ICP torch, and a de Galan V-groove nebulizer, was used. Samples (20 µl) were analyzed in the 'time-resolved analysis' mode. Standard interference corrections for $^{40}Ar^{35}Cl^+$ at m/z 75, i.e. at the same mass as ^{75}As, were made by monitoring m/z 75, 77 ($^{40}Ar^{37}Cl^+$ and $^{77}Se^+$) and 82 ($^{82}Se^+$). Signals were corrected according to the internal rhodium standard. Assignment of arsenic compounds to the peaks in the chromatograms was performed by matching the retention times to the arsenic species in the extracts of the oyster tissue standard reference material and the kelp powder for the identification of arsenosugars, and to standards containing arsenic(III), arsenic(V), MMA, DMA and AB for the identification of the remaining species. A series of standards containing [As] = 10, 25, 50 and 100 ng ml^{-1} of arsenic(III), arsenic(V), MMA, DMA and AB was used to quantify the resulting chromatograms. The arsenosugars were quantified by using the calibration curve of DMA, because in a previous study in algae using HPLC–ICP-MS,[17] with a system similar to the one used in the present study, Shibata *et al.* showed that the response was proportional to the quantity of arsenic and not to the chemical form in which it appeared. All samples were filtered (25 mm Acrodisc Syringe Filter 0.45 µm, HT Tuffryn membrane, Pall, Gelman Laboratory) prior to injection onto the column.

The analytical characteristics of the method are as follows. LD: 1 ng g^{-1} DM for AB; 9.7 ng g^{-1} DM for arsenosugar 1a; 4.8 ng g^{-1} DM for arsenosugar 1b; 2.6 ng g^{-1} DM for arsenosugar 1c; 12.3 ng g^{-1} DM for arsenosugar 1d; and 5.7 ng g^{-1} DM for arsenic(III); precision was 3%.

Determination of inorganic arsenic

The method employed was developed previously by Muñoz *et al.*[18] (Fig. 2). The lyophilized sample (0.50 ± 0.01 g) was weighed into a 50 ml screw-top centrifuge tube, 4.1 ml of water was added, and the sample was agitated until it was completely moistened. Then 18.4 ml of concentrated HCl was added, and the sample was agitated again for 1 h, and then left to stand for 12–15 h (overnight). A reducing agent (2 ml of HBr and 1 ml of hydrazine sulfate 1.5% m/v) was added and, after agitation for 30 s, 10 ml of $CHCl_3$ was added and the mixture was agitated for 3 min. The phases were separated by centrifuging at 2000 rpm for 5 min. The chloroform phase separated by aspiration was poured into another tube. The extraction process was repeated two more times and the chloroform phases were combined and centrifuged again. The remnants of the acid phase were eliminated by aspiration, and possible remnants of solid material in the chloroform phase by passing it through Whatman GD/X syringe filters with a 25 mm PTFE membrane (Merck). The chloroform phase, containing the species arsenic(III), arsenic(V), and MMA, was back-extracted by agitating for 3 min with 10 ml of HCl (1 mol l^{-1}). The phases were separated by centrifuging and the aqueous phase was aspirated and poured into a beaker. This stage was repeated once more and the back-extraction phases obtained were combined. Subsequently, 2.5 ml of ashing aid suspension and 10 ml of concentrated HNO_3 were added to the acid phase. After evaporation on a sand bath (PL 5125 model, Raypa, Scharlau S.L., Barcelona, Spain) until total dryness, the samples were treated in the same way as for total arsenic, described previously.

The analytical characteristics of the method, evaluated in a previous study,[18] are as follows. LD: 13 ng g^{-1} DM or 3 ng g^{-1} WM; precision: 3–5%; quantitative recovery for arsenic (III) (99%) and arsenic(V) (96%). The application of the methodology was carried out with a sample certified for total arsenic, TORT-2 (Lobster hepatopancreas), because of the non-existence of samples certified for inorganic arsenic. The results obtained were 0.581 ± 0.055 µg g^{-1} DM.

Table 1. Total arsenic contents, inorganic arsenic contents, percentages of inorganic arsenic with respect to total arsenic, and moisture contents in *P. clarkii*

Sample	[As] (µg g^{-1} DM)		Inorganic arsenic (%)	Moisture (%)
	Total	Inorganic		
Whole body	4.0	1.2	30	69
	4.4	1.0	23	70
	4.1	1.0	24	71
Hepatopancreas	11	3.2	29	72
	12	3.0	25	75
	9.2	2.7	29	73
Remaining parts	2.8	0.77	28	11
	3.4	0.61	18	67
	3.1	0.76	25	68
Tail	2.5	0.51	20	81
	2.5	0.64	26	81
	2.6	0.46	18	81

RESULTS AND DISCUSSION

Table 1 shows the concentrations of total and inorganic arsenic found in the complete organism and in the different parts of the crayfish. It also shows the percentages of inorganic arsenic with respect to total arsenic, and the moisture of the samples.

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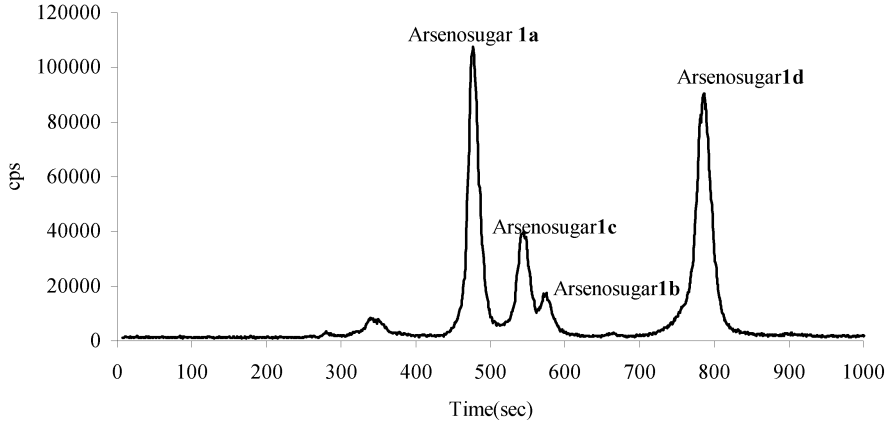

Figure 3. Reversed-phase C_{18} chromatographic separation of arsenic species in kelp powder sample.

Total arsenic contents

The concentrations of total arsenic present in the complete organisms ranged between 4.0 and 4.4 μg g^{-1} DM. These concentrations are of the same order as those reported in a previous study performed with crayfish obtained from the same sampling station (2.2–7 μg g^{-1} DM).[9]

With respect to the distribution in the various parts, the highest concentration was observed in the hepatopancreas (9.2–12 μg g^{-1} DM), followed by the remaining parts of the body (2.8–3.4 μg g^{-1} DM), which included the exoskeleton, gills and gut. The tail had a lower concentration (2.5–2.6 μg g^{-1} DM), in line with the tendency observed by other authors.[12,13]

Numerous studies have shown that in decapods the hepatopancreas acts as an organ for the storage and/or detoxification of a large number of metals (copper, cadmium, zinc, lead),[19] which might explain why the concentration of the metalloid arsenic in the hepatopancreas was much greater than in the rest of the body.

The arsenic observed in the remaining parts of the body may have been a combination of arsenic present in the exoskeleton, gills and gut. Part of this arsenic may have been in the exoskeleton—specifically, at the cuticular level. A large number of metals from the environment are adsorbed passively on the outer surface of the cuticle, although most of the metals observed on the cuticle come from within the animal itself and are part of material ingested and subsequently absorbed.[19] The gills and the gut might also contain substantial concentrations of arsenic. Some authors indicate that the gills may act as biomarkers for contamination,[13] and that mainly metals accumulate in them. The concentrations of metals in the gut may represent an important part of the total concentrations in the body in crustaceans that ingest contaminated sediments.[19]

With respect to the tail, the abdominal muscle has consistently been found in the literature to be the tissue containing the lowest concentration of metals.[13]

Inorganic arsenic contents

The concentrations of inorganic arsenic in the complete animals analyzed varied between 1.0 and 1.2 μg g^{-1} DM, values lower than those found in a previous study,[9] where the concentrations of inorganic arsenic ranged between 0.34 and 5.4 μg g^{-1} DM.

The distribution of inorganic arsenic in the various parts of the crayfish followed the same pattern as that of total arsenic. The organ with the highest concentration was the hepatopancreas (2.7–3.2 μg g^{-1} DM), sometimes being three times the concentration in the complete organism; it was followed by the remaining parts of the body (0.61–0.77 μg g^{-1} DM), and finally the tail (0.46–0.64 μg g^{-1} DM). The fact that inorganic arsenic accumulates mainly in the hepatopancreas may be connected with the detoxification process. It is known that the hepatopancreas and the green gland are the principal organs responsible for the metabolism and detoxification of metals in crustaceans.[20] Thus, the inorganic arsenic that is methylated during the detoxification process might accumulate in the hepatopancreas and be methylated to a less toxic form and subsequently excreted.

The percentage of inorganic arsenic was similar in all the organs, ranging between 18 and 31%. This agrees with the percentage found in the complete organism prior to dissection (24–34%). As in an earlier study performed on *P. clarkii*,[9] the percentages of inorganic arsenic represent a substantial part of the total arsenic, more than in the case of marine organisms (0.5–11%).[11]

With respect to the toxicological implications that ingestion of this crustacean might involve, the consumption of a portion of tail (approximately 100 g) would represent a maximum intake of 5 μg (DM) on the basis of the concentrations found in the present study. This intake would be equivalent to 7% of the provisional tolerable weekly intake (PTWI) for inorganic arsenic proposed by the WHO (2.2 μg day^{-1} per kilogram of body weight, i.e. 143 μg day^{-1} assuming a mean body weight of 65 kg).[21] Consequently,

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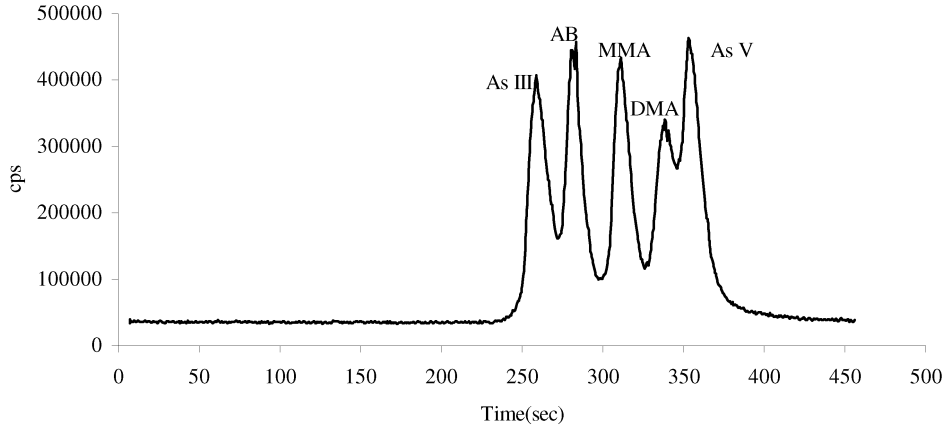

Figure 4. Reversed-phase C_{18} chromatographic separation of arsenic species solution.

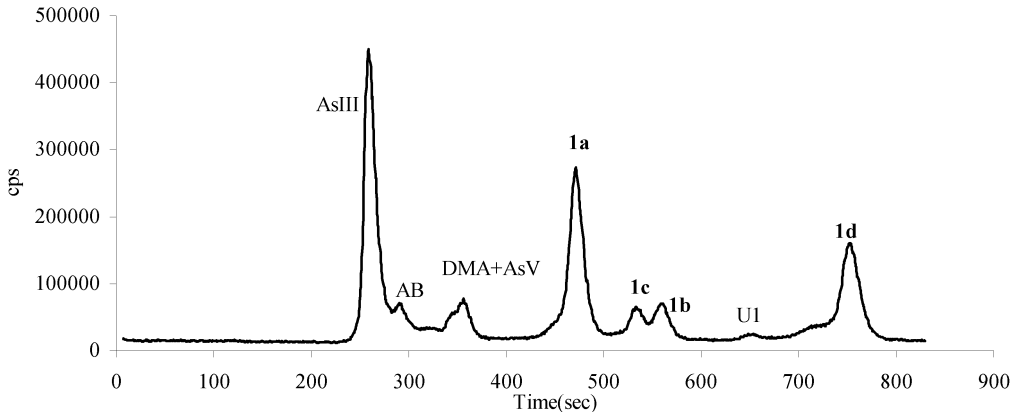

Figure 5. Reversed-phase C_{18} chromatographic separation of arsenical species in hepatopancreas sample of *P. clarkii*.

the concentrations of inorganic arsenic found in the edible part of the tail do not seem to involve a risk for the consumer.

It must not be forgotten, however, that the tail is not eaten fresh but is first subjected to a heating process (boiling, baking, etc.). These processes might modify the concentrations of the arsenic species present in the raw product, as was seen during heat treatment of certain fish of marine origin.[22,23] Moreover, the hepatopancreas is sometimes used

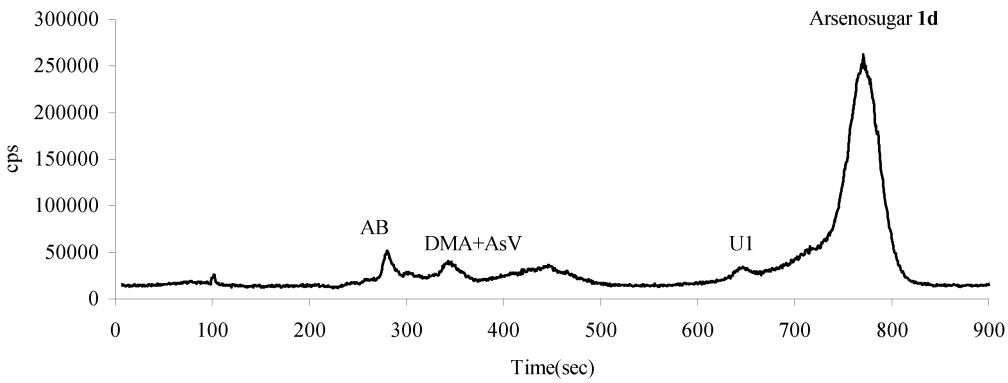

Figure 6. Reversed-phase C_{18} chromatographic separation of arsenical species in muscle tail sample of *P. clarkii*.

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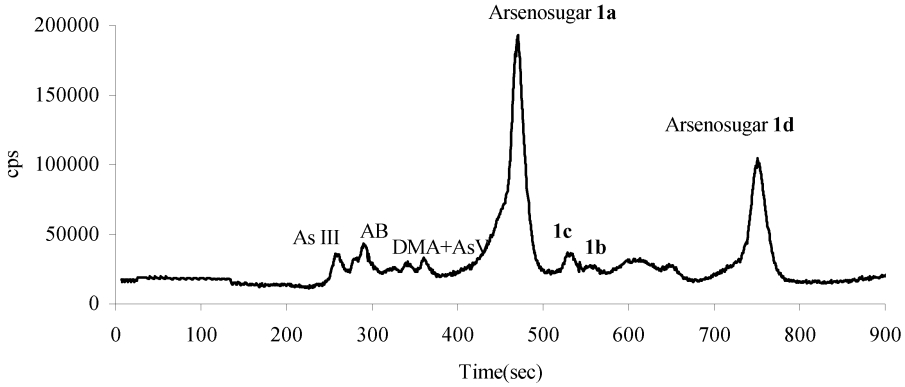

Figure 7. Reversed-phase C_{18} chromatographic separation of arsenical species in the remainder parts of *P. clarkii.*

as an ingredient in the preparation of crayfish tails for consumption, which might alter the possible toxicity of the final product.

Concentrations of organoarsenical species

Figures 3–7 show the arsenic species chromatograms in the following samples: kelp powder (Fig. 3), the sample used to characterize the arsenosugars; a solution of standards of the arsenic species arsenic(III), arsenic(V), AB, MMA and DMA (Fig. 4); hepatopancreas (Fig. 5); tail (Fig. 6), and the remainder of the body (Fig. 7) of *P. clarkii*. Table 2 shows the concentrations of the various species of arsenic found in the complete crayfish and the different parts analyzed.

In these chromatograms, attention must be drawn to the coelution of the species DMA and arsenic(V), as a result of which it was not possible to quantify them separately with the methodology employed. Also, the concentrations of arsenic(III) determined after extraction with MeOH–H_2O may have been underestimated, as other authors have reported a low extraction of arsenic(III) with this extractant.[18] This fact and the coelution of arsenic(V) led us to consider that the only data valid for the determination of arsenic(III) and arsenic(V) were the values found for inorganic arsenic by the chloroform extraction method described earlier in this paper. Finally, we must mention the appearance of two unknown compounds, U1 and U2, which elute before and after arsenosugar **1d**, and which were reported in a previous study performed with crayfish.[9]

Figure 8 shows pie charts of the percentages that the various arsenic species represent with respect to total arsenic, together with the percentage of arsenic not extracted with MeOH–H_2O. This chart shows that there were differences in the quantities of the arsenic species extracted with MeOH–H_2O, depending on the part considered. The

Table 2. Contents of organoarsenical species as arsenic (μg g^{-1} DM) in *P. clarkii*

Sample	As(III)	AB	DMA + As(V)	Arsenosugar				U1	U2	Others
				1a	**1b**	**1c**	**1d**			
Whole body	0.058	0.069	0.083	0.26	0.071	0.060	0.19	<LD	1.6	0.073
	0.058	0.072	0.085	0.41	0.079	0.058	0.50	0.087	0.86	0.098
	0.060	0.044	0.11	0.40	0.045	0.047	0.57	0.056	1.2	0.29
Hepatopancreas	1.3	0.16	0.26	1.0	0.22	0.17	0.73	0.046	<LD	<LD
	1.2	0.31	0.50	1.2	0.21	0.21	1.3	0.069	0.069	1.3
	1.0	0.21	0.47	1.5	0.19	0.11	0.22	0.11	0.25	<LD
Remaining parts	0.037	0.11	0.075	0.45	0.006	0.037	0.31	<LD	0.16	<LD
	0.050	0.087	0.081	0.96	0.053	0.069	0.48	0.040	0.81	0.14
	0.15	0.14	0.048	0.37	0.032	0.027	0.44	0.067	0.58	0.10
Tail	<LD	0.054	0.087	<LD	<LD	<LD	1.6	0.015	<LD	<LD
	<LD	0.043	0.068	<LD	<LD	<LD	2.2	0.088	0.32	0.22
	<LD	0.10	0.17	<LD	<LD	<LD	2.4	0.089	<LD	0.43

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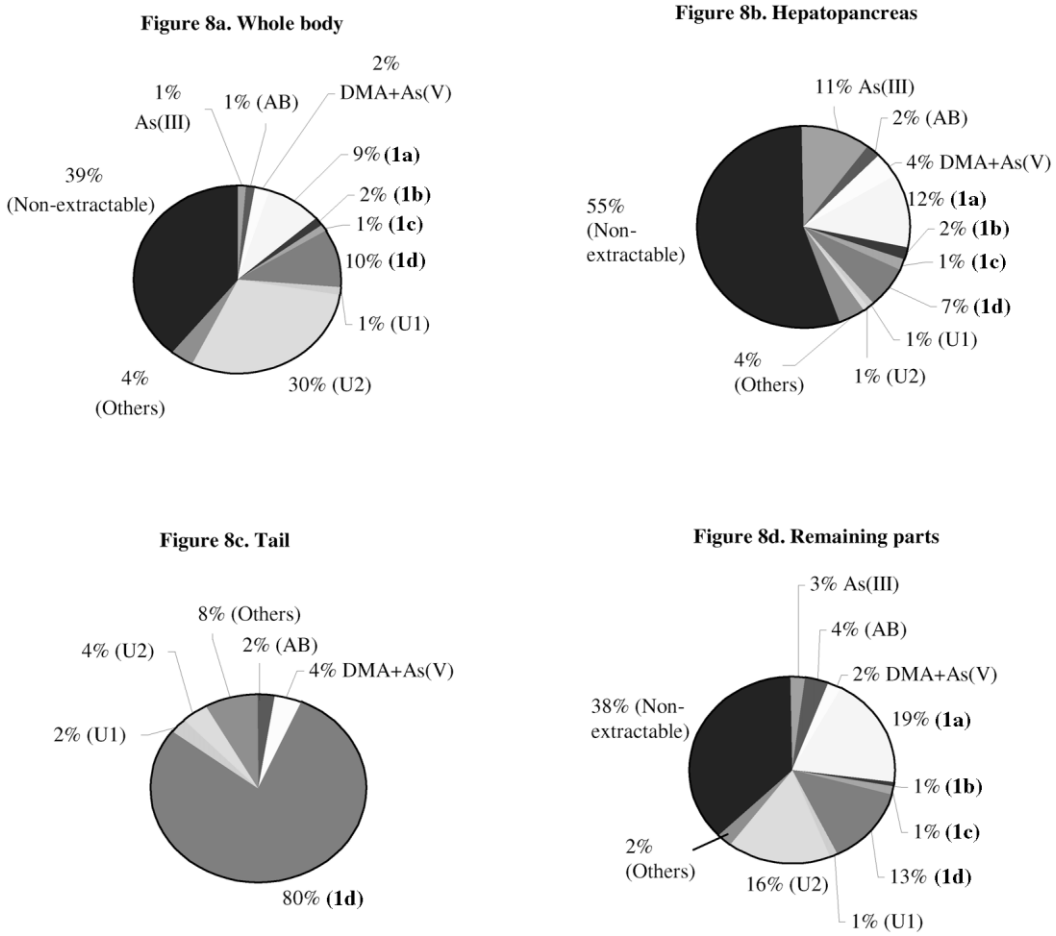

Figure 8. Percentages of arsenic species with respect to total arsenic in the complete organism and the various parts of the crayfish *P. clarkii*.

lowest extraction of arsenic with MeOH–H_2O was in the hepatopancreas, followed by the remaining parts of the body and the complete organism, whereas the arsenic in the tail was extracted in substantial proportions. In the hepatopancreas, this low extraction percentage might be because part of the arsenic was bound to metallothioneins by disulfur bonds, as happens with other metals,[19] and the MeOH–H_2O mixture does not permit the breaking of these bonds. Another possibility that must not be discounted is that the high fat content of the hepatopancreas may be the cause of the low extraction with MeOH–H_2O. In the complete organism, and in the remaining parts, the low efficiency in the extraction process might be due to the arsenic present in the exoskeleton. This arsenic might be associated with the chitin present in the outer covering,[9] a molecule that is very hard to extract from.[24]

As reported in a previous study,[9] there is a great variety of arsenic species in the complete body of the crayfish *P. clarkii* (Fig. 8a). The most abundant species of those that can be extracted with MeOH–H_2O was the unknown compound U2, followed by arsenosugars **1a** and **1d**. The concentrations of the other species were lower and very similar to one another (Table 2). The arsenical composition found differed slightly from the pattern observed previously in this type of crustacean,[9] in which the unidentified species U2 was not one of the most abundant species. This difference might be connected with the point in the biological cycle at which they were caught.

Each part of the body had a characteristic distribution of arsenic. In the hepatopancreas (Fig. 8b), there was a predominance of the hydroxylated arsenosugar and arsenic (III). In the tail (Fig. 8c), the arsenosugar with the sulfate group 1d was the most abundant species, whereas in the remaining parts of the body (Fig. 8d) there was a predominance of arsenosugars **1a** and **1d**. The presence of arsenic(III) in such a notable form in the hepatopancreas and its very low accumulation in the rest of the organism, and also the greater concentration of DMA + arsenic(V) in that organ, may be the result of the detoxification process. Excess arsenic(III) obtained from the environment, from food, or

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from reduction of arsenic(V) present in the organism may be stored in the hepatopancreas, possibly in the form of metallothioneins, and it may be in this organ that it is gradually methylated to DMA, following the methylation mechanism proposed by Challenger.[25]

CONCLUSIONS

Each of the parts of the crayfish analyzed had a characteristic arsenic composition. In all cases there was a great predominance of arsenosugars and a notable percentage of the total arsenic was attributable to inorganic arsenic.

Consumption of crayfish tail does not present a risk for the consumer, given the low concentrations of inorganic arsenic that it contains. However, the toxicity of arsenosugar is unknown, and no studies on the thermal stability of this arsenic species have been carried out. It must be taken into account that the arsenosugar **1d** is the major species in crayfish tail. It would be useful for this reason to establish the effect that the processing or cooking of this product might have on arsenosugar 1d and on the other arsenic species present in this edible part of the crayfish.

Acknowledgements

The authors are grateful to Sergio Algora, Victoria Benito, María Jesús Clemente, Maite de la Flor, and Eon Ting for their technical support and Bert Mueller for his help with ICP-MS measurements. This research was supported by the project FEDER 1FD97 1421-C03-03, for which the authors are deeply indebted. V. Devesa and M.A. Súñer received Spanish Research Personnel Training Grants from the Generalitat Valenciana (Conselleria de Cultura, Educació i Ciència) and the Ministerio de Educación y Cultura respectively.

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