APPLIED ORGANOMETALLIC CHEMISTRY
Appl. Organometal. Chem. **13**, 771–776 (1999)

Accumulation and Fate of Ingested Tetramethylarsonium Ion in the Shrimp *Crangon crangon*

Britta Bachmann,* Douglas A. Hunter and Kevin A. Francesconi
Institute of Biology, Odense University, DK-5230 Odense M, Denmark

The uptake and fate of the tetramethylarsonium ion was examined in the common shrimp *Crangon crangon*. Shrimps were fed 10 mg of food (corresponding to ~2% of body weight) containing 29, 71, 268 or 580 μg As g^{-1} wet weight in the form of tetramethylarsonium ion daily for 19 days. Two days after the last meal the shrimps were sampled and frozen until dissection. The shrimps were dissected into tail muscle, midgut gland, gills and the remaining tissues ('remainder'), and their total arsenic concentrations were measured by hydride generation–atomic absorption spectrometry (AAS). The arsenic concentration of all four tissues increased with increasing arsenic concentration in the food. The retention efficiency of arsenic in the muscle and 'remainder' tissue was related to the arsenic concentration of the food. The retention efficiency on a whole-animal basis ranged from 29 to 88%, with the highest retention efficiency found at the lowest arsenic concentration of the food. The chemical form of the retained arsenic species was shown to be unchanged tetramethylarsonium ion by co-chromatography with standard material using high-performance liquid chromatography (HPLC) with off-line detection by graphite furnace–AAS.

Keywords: tetramethylarsonium ion; shrimp; *Crangon crangon*; uptake; fate; analysis

Received 19 October 1998; accepted 27 April 1999

* Correspondence to: B. Bachmann, Institute of Biology, Odense University, DK-5230 Odense M, Denmark.

INTRODUCTION

High concentrations of arsenic in marine organisms were first reported almost 100 years ago. Because seafood contributed significantly to the human diet, there were early concerns regarding the possible toxicity of the arsenic. These concerns were allayed with the identification of arsenobetaine, a harmless organoarsenic compound, as the major chemical form of arsenic in marine animals.[1] Subsequent work demonstrated a range of organoarsenic compounds in marine algae and marine animals, and, although full toxicological data are not available for many of them, the compounds are generally regarded as being non-toxic.[2]

Recent interest in these arsenic compounds has focused on their origin in marine animals. Besides arsenobetaine, and possibly arsenosugars in some filter-feeding organisms, the most common form of arsenic in marine animals is the tetramethylarsonium ion. This compound was first reported in the clam *Meretrix lusoria*, where it occurred at particularly high concentrations in the gills.[3] Subsequent work revealed that the tetramethylarsonium ion was common in molluscs, where it often occurred as a significant arsenic-containing constituent.[4] The application of improved analytical techniques for determining arsenic species has revealed the presence of tetramethylarsonium ion in crustaceans[5,6] and fish[7,8] in addition to other molluscan species.[9]

The origin of the tetramethylarsonium ion in marine animals is not known. The tetramethylarsonium ion has not, so far, been reported in algae, seawater or sediments. The fact that it was present at significant levels in filter-feeding molluscs initially suggested that it might be selectively accumulated from low, undetectable levels in algae or seawater. This hypothesis was supported by a laboratory study[10] on the uptake of nine arsenic compounds by the mussel *Mytilus edulis*. Most of the compounds

 CCC 0268–2605/99/100771–06 $17.50

tested, including those present as natural constituents of seawater (arsenate, arsenite, dimethylarsinate and methylarsonate), were not accumulated. Arsenobetaine and, to a lesser extent, tetramethylarsonium ion were readily accumulated from the seawater by the mussels. Furthermore, the accumulation of arsenobetaine and tetramethylarsonium ion approximated to the relative concentrations of these two arsenic species found in natural populations of molluscs. These results provided an explanation, in part at least, for the observed distribution of arsenic compounds in marine molluscs.

The experiments with *Mytilus* provide the only data so far on the bioaccumulation of tetramethylarsonium ion by a marine animal. That study was limited to uptake from water. The investigation reported here extends that work by examining the uptake of tetramethylarsonium ion from food by the shrimp *Crangon crangon*. The advantages of using *C. crangon* in such experiments have been reported previously.[6]

MATERIALS AND METHODS

Collection and maintenance

Common shrimp, *C. crangon*, were caught at Kerteminde (Denmark) in October 1997 and kept at constant temperature (14.1 ± 0.1 °C) with a 12 h light–dark cycle. Shrimps of 37–50 mm body length (measured from the tip of the rostrum to the tip of the telson) were used in the experiment. The shrimps were held individually in polypropylene beakers (1 dm^3) containing approximately 700 cm^3 of seawater. The seawater was gently aerated and changed daily. The shrimps were allowed to acclimatize to laboratory conditions for seven days and were not fed during this period.

Experimental design

Six groups of *C. crangon* (10 individuals in each) were daily fed chicken meat containing either no added arsenic or arsenic added in the form of tetramethylarsonium ion (as the iodide salt) or arsenobetaine. The food was prepared as described by Hunter *et al.*.[6] The arsenic concentration of the food was measured in five samples from each food batch by hydride generation–AAS, as described below for the tissues. Arsenic concentrations (mean $\pm$ SD) of the food batches were 29 ± 1.2, 71 ± 3.7, 268 ± 7.4 and 580 ± 17 $\mu g\ As\ g^{-1}$ wet weight for tetramethylarsonium ion, 188 ± 15 $\mu g\ As\ g^{-1}$ wet weight for arsenobetaine and 0.2 ± 0.1 $\mu g\ As\ g^{-1}$ wet weight for the control group. The accumulation efficiency of arsenobetaine has previously been determined,[6] and shrimps receiving arsenobetaine in the present study served as a positive control group. The shrimps were fed 10 mg food, corresponding to ~2% of the body weight, daily for 19 days. Food not taken by the shrimp was removed from the container after 2 min, frozen and offered again next day, in addition to the regular food. The water was changed daily 1–2 h after feeding; the salinity ranged from 22.2 to 25.4‰.

Sampling and dissection

Shrimps were sampled 48 h after their last meal and stored at −80 °C until dissection. The length, weight and sex of each individual were recorded. The shrimps were carefully dissected into muscle tissue, gills and midgut gland. The remaining tissues, including appendages and exoskeleton, were pooled as 'remainder' tissue. All tissues were frozen, freeze-dried and stored in a desiccator until analysis.

Total arsenic analysis

Arsenic concentrations were determined in individual samples of muscle and 'remainder' tissue, and in pooled samples of midgut gland and gills for each treatment group. The freeze-dried tissues were ground to a powder and a portion was digested by dry-ashing using a modification of the method described by Penrose *et al.*.[11] Tissue samples of approx. 10 mg (weighed to $\pm$ 0.01 mg) were weighed into crucibles and 1.00 cm^3 of slurry was added. The slurry was prepared by adding 30 g of magnesium nitrate to 500 cm^3 of stirred milli-Q water, followed by 50 g of magnesium oxide. The samples were dried overnight at 80 °C and digested in a muffle furnace by heating the samples for 1 h at 200 °C, 1 h at 300 °C then 8 h at 500 °C. The cooled samples were dissolved by adding 2.50 cm^3 of 6 M HCl followed by 2.50 cm^3 of milli-Q water. Total arsenic was measured by hydride generation–AAS using a Perkin-Elmer MHS-20 mercury/hydride system coupled to a Perkin-Elmer 2380 AAS. Portions (10 mg) of standard reference material DORM-1 (certified arsenic concentration: 17.7 ± 2.1 $\mu g\ As\ g^{-1}$) were analysed in triplicate together with each of six sets of samples. For five of the six sets, the arsenic concentrations obtained for DORM-1 were within the acceptable range (overall mean $\pm$ SD was 17.5 ± 0.9 $\mu g\ As\ g^{-1}$, $n = 15$). For

one set of samples, however, the DORM-1 arsenic values were low (12.1 ± 0.5 μg As g^{-1}, $n = 3$). This set of samples consisted of the pooled gill tissues from all the treatment groups (there was insufficient material to repeat these analyses). Based on the DORM-1 results, the arsenic concentrations obtained for the gill samples are all likely to be lower than their true values. Thus, comparison of arsenic concentrations between gill samples was valid, but comparison of gill tissue with other tissues from *C. crangon* could only be qualitative.

Analysis of arsenic species

Arsenic species were determined in the muscle tissue and 'remainder' tissue of *C. crangon* fed food containing 580 μg As g^{-1} wet weight as tetramethylarsonium ion or 188 μg As g^{-1} wet weight as arsenobetaine. Each of these four tissues was treated as follows; Dried tissue (5.0 mg) from each of the 10 individuals in each group was pooled and a 30 mg portion was extracted with 3.00 cm^3 of milli-Q water and sonicated for 2×30 s. The solution was centrifuged for 10 min at 50 000 g and the supernatant collected. The pellet was re-extracted with 2.00 cm^3 water and centrifuged as before. The two supernatants were pooled and a 1.00 cm^3 portion was analysed for total arsenic. The pellet was dried under reduced pressure at 30 °C in a centrifugal lyophilizer and analysed for total arsenic. The remaining supernatant was similarly dried and the resultant residue stored in a desiccator until analysis of arsenic species.

The dried extracts were redissolved in 1.00 cm^3 milli-Q water and an aliquot of the resultant solution was diluted in milli-Q water to obtain an arsenic concentration of approximately 0.5 μg As g^{-1}. This solution was filtered (0.2 μm) to remove the small amount of insoluble material. The arsenic compounds in the tissue extracts were identified by comparing their HPLC retention volumes with those from a mixture of standard compounds each at a concentration of 1 μg As cm^{-3} (dimethylarsinate, arsenobetaine, trimethylarsine oxide, arsenocholine and tetramethylarsonium ion)). The diluted extracts or the standard solution (100 μl portions) were injected into the high-performance liquid chromabography (HPLC) system, which consisted of a Jasco PU-980 intelligent HPLC-pump coupled to a Jasco DG-980-50 degasser and a Rheodyne 9125 syringe-loading sample injector with a 500 μl injection loop. The separations were made on an Ionosphere-C cation-exchange column (Chrompack) eluted with 5 mM pyridinium ion and 3% (v/v) methanol in water at pH 2.7. The flow rate of the pump was 0.4 cm^3 min^{-1} and fractions of 0.2 cm^3 were collected in a fraction collector. The arsenic concentrations of the fractions were determined off-line on a Perkin-Elmer Zeeman/3030 AAS graphite furnace.

Data handling and statistics

Arsenic concentrations in muscle tissue and 'remainder' tissue were log-transformed and the arsenic concentrations of the treatment groups were compared with the arsenic concentration of the control group by one-way ANOVA followed by a Tukey test. The percentage of the dose that was retained in the tissues (retention efficiency) was defined by Eq. [1],

$$\text{Retention efficiency} = 100 \times (\text{quantity of arsenic retained})/\text{quantity of arsenic consumed} \quad [1]$$

where

$$\text{Quantity of arsenic retained} = ([\text{As}] \text{ in treatment tissue}) - ([\text{As}] \text{ in control tissue}) \times \text{weight of treatment tissue}$$

RESULTS

Crangon crangon showed no signs of toxicity (e.g. inactivity, reduced escape response or 'loss of appetite') during the experiment. Two animals died in the experiment, one in the group receiving food containing 29 μg As g^{-1} and the other in the group receiving food containing 580 μg As g^{-1}. The dead animals were omitted from the study.

Total arsenic concentration in the shrimp

The shrimps receiving arsenobetaine served as a positive control in the experiments, and the uptake and distribution of this compound (midgut > muscle ~ 'remainder', *ca* 2.5:1:1; see Table 1) agreed well with the data previously reported for *C. crangon*.[6]

For shrimp fed tetramethylarsonium ion, arsenic concentrations in muscle tissue, 'remainder' tissue, midgut gland (and gills) showed the same pattern of increasing tissue concentration with increasing arsenic concentration in the food (Table 1). The distribution of arsenic was different from that

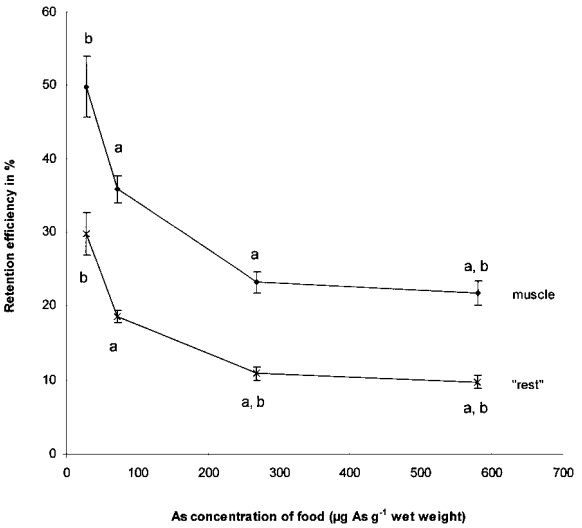

Figure 1 Retention efficiency (mean ± SEM, $n = 10$) of arsenic in the muscle tissue and 'rest' tissue of *C. crangon* fed food (~10 mg d^{-1}) with an arsenic concentration of 29, 71, 268 or 580 μg As g^{-1} (wet wt) as tetramethylarsonium ion for 19 days. Retention efficiencies significantly different from the retention efficiency of shrimp fed 29 μg As g^{-1} are marked 'a' ($P < 0.001$), while those differing from 71 μg As g^{-1} are marked 'b' ($P < 0.05$).

observed for arsenobetaine-fed shrimp. The highest arsenic concentrations were found in the muscle of tetramethylarsonium-fed shrimp. The distribution of arsenic within the shrimp appeared to be dose-related with the ratio of muscle [As]: midgut [As] increasing at higher doses.

Arsenic concentrations in the tail and 'remainder' tissue of all groups were significantly higher than in the control group ($P < 0.001$) and the arsenic concentrations were significantly different between all treatment groups ($P < 0.001$) except for the animals fed 29 and 71 μg As g^{-1}.

The retention efficiency for tetramethylarsonium in both muscle and 'remainder' tissue showed essentially the same relationship to the dose (Fig. 1).

Retention efficiencies on the whole-animal basis (Table 2) show that retention increases with decreasing concentration of tetramethylarsonium ion in the food. The contribution of the gill to the total retention is included in the calculated retention values; this contribution was low (<2%) due to the gills' small size and thus the likely underestimate of the gill arsenic concentrations (see experimental) would not significantly affect these values.

Arsenic speciation

The method used for extracting the arsenic from the tissues was very efficient, extracting about 97% of the arsenic in the muscle tissue and more than 99% of the arsenic in the 'remainder' tissue. The arsenic species were separated on HPLC using cation-exchange conditions, and identified by comparison of their retention volumes with those of standard compounds (Fig. 2). In the standard chromatogram tetramethylarsonium ion elutes at 5.7 cm^3 and arsenobetaine, the arsenic species predominant in natural *C. crangon*, elutes at 3.0 cm^3. HPLC of extracts from shrimp fed arsenobetaine (data not shown) demonstrated that they accumulated this form of arsenic unchanged, a result consistent with the earlier study with *C. crangon*.[6] The chromatograms of extracts of the muscle and 'remainder' tissue from the tetramethylarsonium-fed shrimp both showed a single peak eluting at 5.6 cm^3. A portion of the extract of 'remainder' tissue spiked with tetramethylarsonium ion standard resulted again in a single peak at 5.6 cm^3, confirming the identity of the peak (Fig. 2). No arsenobetaine peak is seen in the chromatograms of the animals being fed tetramethylarsonium ion because dilution of the sample before HPLC analysis decreased the

Table 1 Arsenic concentrations (μg As g^{-1} dry wt ± SD) in four tissues of *C. crangon.*

Arsenic compound fed[a]	Muscle tissue	'Rest' tissue	Midgut gland	Gills
Control	8.5 ± 2.2	6.0 ± 1.5	13	13
AB (188)	187 ± 35	167 ± 36	478	364
Tetra (29)	62 ± 11	31 ± 6.5	58	58
Tetra (71)	85 ± 16	39 ± 8.4	58	66
Tetra (268)	223 ± 72	91 ± 29	140	119
Tetra (580)	422 ± 137	147 ± 39	242	225

[a] AB, arsenobetaine; Tetra, tetramethylarsonium ion. The weights of arsenic fed (μg As g^{-1} wet wt) are given in parentheses.

Table 2 Retention efficiency of arsenic in the individual tissues of *C. crangon* fed tetramethylarsonium ion or arsenobetaine for 19 days[a]

Tissue	29 μg As g^{-1} as Tetra	71 μg As g^{-1} as Tetra	268 μg As g^{-1} as Tetra	580 μg As g^{-1} as Tetra	188 μg As g^{-1} as AB
Tail muscle	53.8 (59.0)	28.6 (62.8)	22.9 (65.6)	19.5 (66.5)	25.5 (44.0)
Gills	0.9 (1.1)	0.4 (1.0)	0.4 (1.3)	0.2 (0.6)	1.0 (1.7)
Midgut gland	1.6 (1.9)	0.6 (1.5)	0.5 (1.5)	0.3 (1.1)	2.8 (5.0)
Rest	32.0 (38.0)	14.8 (34.8)	10.8 (31.6)	9.0 (31.8)	29.5 (49.7)
Whole animal	88.3 (100)	44.4 (100)	34.6 (100)	29.0 (100)	58.8 (100)

[a] The percentage of the arsenic body burden in each tissue is shown in parentheses. Tetra, tetramethylarsonium ion; AB, arsenobetaine.

arsenobetaine concentration to a level below the detection limit.

DISCUSSION

Tetramethylarsonium ion is a common constituent of marine animals, having been reported in molluscs,[9] crustaceans,[6] fish,[7] echinoderms[12] and coelenterates.[13] It is a significant constituent of bivalve molluscs, particularly in the gills, where it can be the major arsenic compound.[3] Uptake studies with mussels showed that the tetramethylarsonium ion was accumulated more readily, with the exception of arsenobetaine, than any of the other naturally occurring arsenic species. These results were consistent with the observed distribution of the various arsenic compounds in molluscs. In most marine animals, however, tetramethylarsonium is a minor arsenic constituent, which suggests either that it is present at only trace levels in the environment, or that uptake from food is low. The high retention efficiency of the tetramethylarsonium ion in *C. crangon* is interesting. Wild *C. crangon* contain >90% of their total arsenic as arsenobetaine, and tetramethylarsonium ion constitutes only about 1% of the total.[6] Yet, when exposed to the two compounds in their food, *C. crangon* accumulate both of them with comparable efficiency. The efficiency with which *C. crangon* accumulate the tetramethylarsonium ion suggests that little tetramethylarsonium ion is present in the natural prey of *C. crangon*, otherwise it might be expected to make a greater contribution in wild *C. crangon*. Higher concentrations of tetramethylarsonium ion in clams compared with the levels in *C. crangon* is unlikely to be explained by differences in retention efficiencies or metabolism, because *C. crangon* shows very efficient retention and negligible transformation of tetramethylarsonium ion. Possibly, the clams extract the tetramethylarsonium ion from the large volume of seawater they pass over their gills. Although the tetramethylarsonium ion has not yet been detected in seawater, it may be present at low levels, as the usual method for determining arsenic species in seawater (formation of volatile hydrides) is not suitable for that compound.

The within-animal distribution of arsenic was different for shrimp fed the tetramethylarsonium ion compared with both the arsenobetaine-fed group and wild *C. crangon* (Table 1). For *C. crangon* fed arsenobetaine, the arsenic concentrations in the midgut gland were 2.5-fold higher than those in the muscle. For *Crangon* fed tetramethylarsonium ion, the arsenic concentrations in the midgut gland were always lower than those in the muscle. The distribution pattern appeared to be dependent on the concentration, with the relative difference increasing at higher dose.

HPLC analysis of extracts from the shrimp tissue indicated that the tetramethylarsonium ion was accumulated unchanged. The method of detection employed in this study (off-line graphite furnace–AAS) was not sufficiently sensitive to detect any minor metabolites that might have been formed. We estimate a detection limit of about 5% of the total arsenic in the extract. In the previous study with *C. crangon*, the ingested trimethylarsine oxide was readily demethylated to dimethylarsinate, but we detected no evidence of such a demethylation pathway for tetramethylarsonium ion. Possible transformation of tetramethylarsonium ion into arsenobetaine by direct addition of CO_2 has been discussed briefly.[14] We could see no indication of such a change in our experiments.

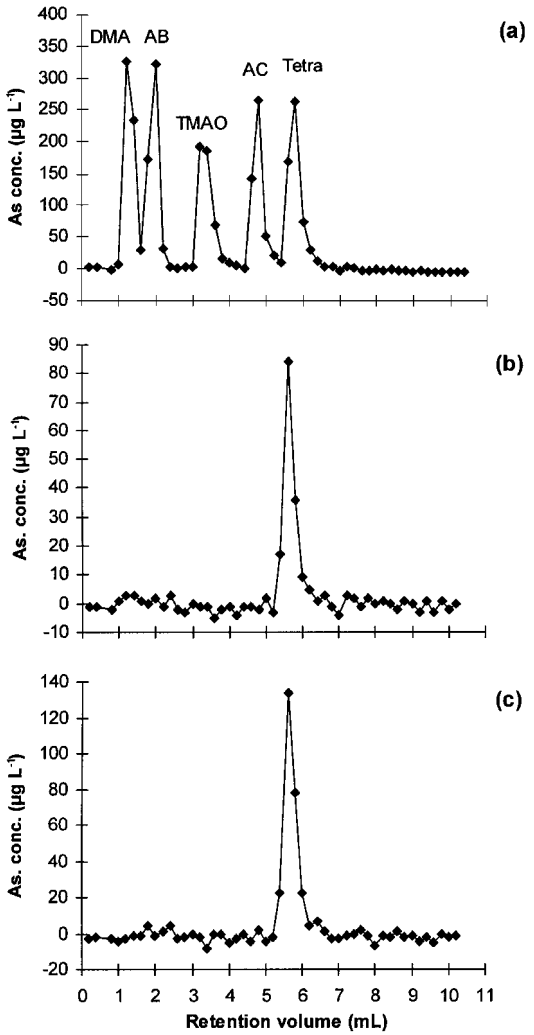

Figure 2 Representative chromatograms from HPLC–GFAAS analysis. (a) Mixture of standard compounds: DMA, dimethylarsinate; AB, arsenobetaine; TMAO, trimethylarsine oxide; AC, arsenocholine; Tetra, tetramethylarsonium ion. (b) Chromatogram of extract from 'rest' tissue of *C. crangon* fed Tetra. (c) As in (b), but spiked with Tetra standard solution.

Acknowledgements We thank Vibeke Eriksen and Carsten Frederiksen for their assistance with the analysis of samples.

REFERENCES

1. J. S. Edmonds, K. A. Francesconi, J. R. Cannon, C. L. Raston, B. W. Skelton and A. H. White, *Tetrahedron Lett.* **18**, 1543 (1977).
2. K. A. Francesconi and J. S. Edmonds, *Adv. Inorg. Chem.* **44**, 147 (1997).
3. K. Shiomi, Y. Kakehashi, H. Yamanaka and T. Kikuchi, *Appl. Organomet. Chem.* **1**, 177 (1987).
4. W. R. Cullen and M. Dodd, *Appl. Organomet. Chem.* **3**, 79 (1989).
5. E. H. Larsen, G. Pritzl and S. H. Hansen, *J. Anal. At. Spectrom.* **8**, 1075 (1993).
6. D. A. Hunter, W. Goessler and K. A. Francesconi, *Mar. Biol.* **131**, 543 (1998).
7. E. H. Larsen, G. A Pedersen and J. W. McLaren, *J. Analyt. Atom. Spectrom.* **12**, 963 (1997).
8. J. S. Edmonds, Y. Shibata, K. A. Francesconi, R. J. Rippingale and M. Morita, *Appl. Organomet. Chem.* **11**, 1 (1997).
9. W. Goessler, W. Maher, K. J. Irgolic, D. Kuehnelt, C. Schlagenhaufen and T. Kaise, *Fresenius J. Anal. Chem.* **359**, 434 (1997).
10. J. Gailer, K. A. Francesconi, J. S. Edmonds and K. J. Irgolic, *Appl. Organomet. Chem.* **9**, 341 (1995).
11. W. R. Penrose, R. Black and M. J. Hayward, *J. Fish. Res. Board. Can.* **32**, 1275 (1975).
12. M. Morita and Y. Shibata, *Analyt. Sci.* **3**, 575 (1987).
13. K. Shiomi, M. Aoyama, H. Yamanaka and T. Kikuchi, *Comp. Biochem. Physiol.* **90C**, 361 (1988).
14. J. S. Edmonds and K. A. Francesconi, Arsenic metabolism in aquatic ecosystems. In: *Metal Metabolism in Aquatic Environments*, Langston, W. J. and Bebianno, M. J. (eds), Chapman & Hall, London 1998, pp. 159–183.

