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1 **Effects of sample preparation on methylmercury concentrations in Arctic organisms**

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**Abstract**

The biogeochemical cycling of mercury (Hg) in the marine environment is an issue of global concern since consumption of marine fish is a major route of human exposure to the toxic specie methylmercury (MeHg). The most widely utilized and accepted technique for preparing biological tissue samples for the analysis of MeHg involves an alkaline digestion of the sample. Recent studies suggest however, that this technique is inadequate to produce satisfactory recoveries for certain biological samples, including fish, fur, feathers and other “indicator” tissues which contain relatively high levels of MeHg. Thus an improved acidic extraction method has been proven to produce more satisfactory results for a wide range of biological tissues. The present study compares the two methods on real sample material from different organisms of an Arctic marine food chain, and shows how this could lead to misinterpretation of analytical results. Results show significantly ($p < 0.05$) lower concentrations for alkaline digestion for large parts of the food chain; especially in fish and birds. The mean differences in concentrations found between the two different methods were 28, 31 and 25 % for fish (Polar and Atlantic cod), Little Auk and Kittiwake, respectively. For samples lower in the food chain (i.e. zooplankton and krill) no significant differences were found. This leads to a clear underestimation of the levels of MeHg found higher up in these food chains; the ratio of MeHg to Hg in biological samples; and thus potentially erroneous conclusions drawn from these results concerning the biological cycling of mercury species. We hypothesize that the main reasons for these differences are poor extraction efficiency and/or matrix effects on the ethylation step prior to analysis. This is the first study to examine the effects of these artefacts on real environmental samples covering a complete food chain.

**Key words**

Methylmercury, mercury speciation, sample treatment, acid extraction, alkaline digestion, Arctic food chain

57 **Introduction**

58 Inorganic mercury (Hg) can undergo methylation into the toxic and bioaccumulative specie
59 methylmercury (MeHg; [1]). MeHg is accumulated in the aquatic food chain with potential harmful
60 effects to organisms [2, 3]. For humans [4], the Hg toxicity is primarily linked to intake of Hg through
61 fish consumption due to high levels of MeHg in predatory fish at the top of the food chain [1], [5].
62 This is of particular importance in the Arctic where concentrations of Hg in marine animals are about
63 10-12 times higher than in pre-industrial times [6], and due to the high levels of fish consumption
64 amoung indegineous peoples [7]. To be able to understand the biogeochemical cycling of Hg and
65 human exposure to MeHg toxicity in the marine environment, it is important to choose suitable
66 methodology at all steps of the analytical procedure including; sample collection, sample pre-treatment
67 and measurement [8].

68 To analyse [9] and isolate [8] MeHg from biological matrices, a wide range of methods have
69 been developed. Historically, digestion of biota in a potassium hydroxide – methanol (KOH-MeOH)
70 solution has been widely employed [1, 10, 11], and has been thought to be an efficient method for
71 extraction. Due to the required dilution (i.e. only small volumes of digest possible for analysis; larger
72 volumes reduces ethylation efficiency) this approach is limited to samples containing high
73 concentrations of MeHg, but method detection limits (MDL) as low as 1 ng/g have been reported [1].
74 A relatively recent study describes optimal conditions for the alkaline digestion; 5 mL of 15-25 %
75 KOH-MeOH are added to 20 mg sample and heated at 55-60 °C for 24 hours [12].

76 However, more up to date work has elucidated the shortcomings of the alkaline digestion
77 technique, showing it inadequate to produce satisfactory recoveries for certain biological tissues [12],
78 [13]. The suggested reason for inadequate recoveries when applying alkaline digestion is interference
79 from the organic matrix [12], potentially due to presence of keratin filaments or similar substances
80 [13]. For these reasons an acidic extraction method producing adequate recoveries has been suggested
81 [12, 14]. Briefly this method involves the addition of 10 mL 4.3 M nitric acid ($HNO_3$) to 20 mg of
82 sample, which is heated at 55-60 °C for 24 hours.

83 The basis for this study is a set of samples from the Norwegian Arctic covering different
84 organisms of the food chain (zooplankton, fish and birds). All samples were analysed for MeHg

85 utilizing the alkaline digestion method and the acidic extraction technique for sample pre-treatment.
86 Generally, we hypothesize that concentrations of MeHg will be significantly lower when utilizing the
87 alkaline digestion method compared to the acid extraction method. More specifically, that differences
88 in concentrations will be largest for biological material that contain large amounts of fat, e.g. liver
89 samples, due to the increased interference seen from the organic matrix on analysis of these samples.
90

91 **Materials and methods**

92 *Study area and sampling*

93 Organisms of the pelagic food chain were collected from two fjords at the Svalbard
94 Archipelago in the Norwegian Arctic, Kongsfjorden and Liefdefjorden. Samples were collected in
95 2007 and 2008 as described in detail elsewhere [15, 16, 17]. Specifications related to collection,
96 preparation and transport are also described in the literature [15, 16, 17 and 18].

97 A total of 63 samples were used in the present study (Table 1), specifically zooplankton and
98 krill samples (*Calanus finnmarchicus, Calanus hyperboreus, Thiemisto libellula, Thiemisto abysorum*;
99 n = 15), fish samples (Polar cod (*Boreogadus saida*) and Atlantic cod (*Gadus morhua*); n = 20) and
100 marine bird samples (Little Auk (*Alle alle*); n = 16, and Kittiwake (*Rissa tridactyla*); n = 15). The
101 zooplankton and krill samples were extracted and digested as whole species, the Polar cod as whole
102 individual fish, the Atlantic cod and the Little Auk as muscle samples and the Kittiwake as both
103 muscle and liver samples (Specifications in Table 1).

104 All samples were analysed freeze dried.
105

106 *Sample preparation*

107 All real samples were extracted and analysed utilizing both an alkaline digestion method and
108 an acid extraction method. Several acids have previously been tested for the purpose of MeHg
109 extraction from different matrices, including $HNO_3$ [12, 14, 19], sulphuric acid [12], and hydrochloric
110 acid [19, 20]. Our chosen method of acid extraction is based on [12]. In short, samples (minimum 0.03
111 g) were weighed out, added 10 mL 30 % $HNO_3$ and heated at 60 °C overnight (approx. 15 hours).
112 Before analysis the extraction solution was added 10 mL DI water. 0.050 mL extraction solution was

113 neutralized with 0.050 mL 15 % KOH and ethylated before purge/trap and gas chromatography – cold
114 vapor atomic fluorescence spectrometry (GC-CVAFS) analysis and detection as described below.

115 Two different alkaline digestion methods were tested on reference materials. Alkaline
116 digestion method 1 is based on traditional methods of MeHg biota digestion; described in previous
117 literature [1, 8, 19]. Optimal conditions for this method are described in [12]; samples (minimum 0.02
118 g) are weighed out, added 5 mL 20 % KOH-MeOH and heated at 55 °C for 24 hours. 50 µL digestion
119 solutions were analysed without dilution, ethylated before purge/trap and GC-CVAFS analysis and
120 detection.

121 The other alkaline digestion method that was tested (alkaline digestion method 2) is similar to
122 method 1 and described in [13]. In short, samples are weighed out (minimum 0.02 g), added 1 mL 25
123 % MeOH-KOH and heated at 65 °C for 3-4 hours. After heating samples are diluted to 2.5 mL with
124 MeOH before analysing a maximum of 0.030 mL of digestion solution. Samples were then ethylated
125 before purge/trap and GC-CVAFS analysis and detection.

126 The two alkaline digestion methods 1 and 2 were compared by analysing various certified
127 reference materials (CRM; n = 4 parallels; DORM-3 fish protein, TORT-2 lobster hepatopancreas and
128 SRM-2976 mussel tissue). Additionally, the CRMs were also extracted utilizing the acid extraction
129 method for comparison.

130 Of the alkaline digestion techniques, only method 1 was used when digesting and analysing
131 real sample material.

132

133 *MeHg analysis*

134 The analysis method for MeHg is based on USEPA Method 1630 [21] for determining MeHg
135 in water by distillation, aqueous ethylation, purge and trap, and CVAFS. Automated systems were
136 used for analysis (Brooks Rand Labs MERX automated systems with Model III AFS Detector). The
137 automated MERX setup is standardised for MeHg analysis, including run duration, heating duration,
138 cooling duration and purge duration of all samples corresponding to 5 minutes, 9.9 seconds, 3 minutes
139 and 6 minutes, respectively. All calibrations standards (covering 0.5 – 1000 pg) were within a
140 recovery of 100 ± 15 %, and produces a relative standard deviation (RSD) of < 8 % for the calibration

coefficients (leading to a $r^2$ for the calibration curve > 0.9999). Average calibration blanks (< 0.5 pg) and standard deviation of calibration blanks (< 1.0 pg), were also satisfactory.

For every batch of MeHg analysis (n = 30 individual samples) quality assurance and quality control (QA/QC) measures included method blanks (n = 4), sample duplicates (n = 3), matrix spikes (n = 3) and CRMs (n = 6). The certified MeHg concentrations of the CRMs used were 0.355 ± 0.056 mg/kg (± uncertainty), 0.152 ± 0.013 mg/kg and 28.09 ± 0.31 µg/kg for DORM-3, TORT-2 and SRM-2976, respectively. Samples that were analysed in duplicates were also used for matrix spike samples. Samples chosen for matrix spikes were added 1000 pg (low concentration samples; 1.0 – 100 ng/g; 0.1 mL of 10.0 ng/mL methylmercury hydroxide; MeHgOH) or 10 000 pg (higher concentration samples; 100 – 1000 ng/g; 1.0 mL of 10.0 ng/mL MeHgOH) depending on the concentration of the biological sample. Real sample data were not corrected for CRM recoveries.

All analyses were performed at NIVA's laboratory in Oslo, Norway. MDLs are indicated in the Results as appropriate.

*Statistical analysis*

To test for differences between the results obtained by the alkaline digestion method and the acid extraction method, Student's t-tests were used. All t-tests shown in this study were two-tailed and homogeneity of variance was tested by F-tests. All statistical analysis and calculations were done in JMP 9.0 (SAS) with a significance level $\alpha = 0.05$, unless otherwise mentioned.

## Results

*Quality assurance and quality control*

Comparison of QA/QC for the two alkaline digestion methods 1 and 2 and the acid extraction method shows little variation. Concentrations of MeHg in blank digestions were 1.6 ± 0.8 pg/mL (mean ± 1 standard deviation), 1.6 ± 1.4 pg/mL and 1.3 ± 0.7 pg/mL for the acid extraction, alkaline digestion 1 and alkaline digestion 2, respectively (Table 2). This translates to detection limits (DL) of 1 pg/mL or better (3 standard deviations of blank concentrations). The actual limit of detection (LOD) and limit of quantification (LOQ) will vary depending on the weight of sample available for analysis.

169 For sample weights (0.02 – 0.1 g) and sample treatment methods included in this study, the LOD is in
170 the range of 0.2 – 1.0 ng/g (3 standard deviations) and LOQ in the range of 0.3 – 3.0 ng/g (10 standard
171 deviations). There were no significant differences in blank concentrations between the two alkaline
172 digestion methods 1 and 2 ($p = 0.21$), or between either of the two alkaline digestion methods 1 ($p =$
173 $0.81$) or 2 ($p = 0.09$) and the acid extraction method (t-test on difference of mean blank
174 concentrations).The results showing the efficiency of the extraction and digestion methods on CRMs
175 are presented in Table 2. Recovery of MeHg for all extractions and digestions were found to be
176 satisfactory (75 – 125 %; Table 2) and no significant difference was seen individually between the
177 three sample treatment methods ($p > 0.05$). The recovery for the three CRMs, DORM-3, TORT-2 and
178 SRM-2976, were within the expected concentration ranges; 0.299 – 0.411 mg/kg, 139 – 165 mg/kg
179 and 27.78 – 28.40 µg/kg, respectively, for all samples (Table 2).

180 Matrix spikes and sample duplicates values were similar between the acid extraction and
181 alkaline digestion 1 methods. Spike recoveries were 95.9 ± 11.6 % (acid extraction; n = 8) and 90.6 ±
182 10.8 % (alkaline digestion 1; n = 8) for the two methods. On average, the relative per cent difference
183 (RPD) between duplicate samples was 11.7 ± 8.9 % (n = 8) and 7.6 ± 7.0 % (n = 8) for the acid
184 extraction (range 1.4 – 26.6 %) and alkaline digestion 1 (range 0.4 – 18.4 %), respectively. No
185 significant difference was seen between the digestion and extraction, neither for spike recoveries ($p =$
186 $0.37$) or sample duplicate RPD ($p = 0.33$).

187

188 *Comparison of biological samples data*

189 Concentrations of MeHg in the biological samples ranged from 1.4 – 1050 ng/g. Increasing
190 concentrations were found following the food chain from plankton (including zooplankton and krill;
191 1.4 – 8.1 ng/g) through fish (Polar and Atlantic cod; 11.4 – 109 ng/g) and Little Auk (140 – 649 ng/g)
192 to Kittiwake (46.7 – 1050 ng/g; individual specie concentration data discussed in Ruus et al. [18]). The
193 mean concentrations of MeHg in plankton (zooplankton and krill), fish (Polar and Atlantic cod), Little
194 Auk and Kittiwake was 3.8 ± 2.1 ng/g, 45.2 ± 27.2 ng/g, 323.5 ± 152.1 ng/g and 545.1 ± 416.8 ng/g,
195 respectively (acid extraction data). See Ruus et al., [18] for a detailed discussion of the biological
196 implications of MeHg concentrations and biomagnification through this Arctic marine food chain.

197 From Figure 1 it is clear how the acid extraction method generally produced higher
198 concentrations than the alkaline digestion method (levels of MeHg shown as concentration obtained
199 by the acid extraction method divided by concentrations obtained by the alkaline digestion method). In
200 Figure 1 data is grouped in the following manner; 1) plankton (covering both zooplankton and krill);
201 2) fish (Polar and Atlantic cod); 3) Little Auk and; 4) Kittiwake. The individual sample differences
202 (Table 1) show that the concentrations obtained by the alkaline digestion method averaged 1.7, 28.4,
203 31.0 and 24.9 % lower than the concentrations obtained by the acid extraction method for the four
204 groups 1) – 4), respectively. No significant difference between the two methods was seen when
205 comparing mean concentrations of the four species groups individually ($p > 0.05$ for all groups), but
206 concentrations obtained by the alkaline digestion method were lowest in 56 out of 66 samples (Figure
207 1).

208 When comparing individual concentration values, the data obtained from the two sample
209 treatments are significantly different for both all data treated together ($p < 0.0001$) and when grouped
210 as fish ($p < 0.0001$), Little Auk ($p < 0.0001$) and Kittiwake ($p < 0.001$) individually (t-test on
211 difference of paired samples, significance level $\alpha = 0.05$, Wilcoxon Signed Rank). For plankton no
212 significant difference was found ($p = 0.81$).

213 No significant relationship was found between concentrations of MeHg and average difference
214 between the two different sample treatment methods when all samples were included ($r^2 = 0.05$). The
215 same applies to analysing the relationship on individual species groups ($r^2 < 0.10$) and when MeHg
216 concentrations are studied on a logarithmic scale ($r^2 < 0.10$; data not shown).

217

218 **Discussion**

219 *Quality assurance and quality control*

220 Comparison of the two alkaline digestion methods and the acid extraction method indicates
221 similar performance for all three methods regarding QA/QC data, including method blanks, sample
222 duplicates and matrix spikes. The similar performance related to MeHg concentrations in all quality
223 data indicate that both methods theoretically should be suitable for determining concentrations of
224 MeHg in biological samples.

225 Based on the blank concentrations (Table 2) and weight of biological samples (0.02 – 0.1 g) 226 we derive LODs of 0.2 – 1.0 ng/g for both the alkaline digestion method 1 and the acid extraction 227 method (3 standard deviations of method blanks). This is low enough for most biological samples 228 from Arctic food chains and similar to what other studies are documenting utilizing the same sample 229 treatment methods (alkaline digestion; [1, 8, 12, 19], acid extraction; [12]) and analytical techniques. 230 However, six of our samples are below the LOQ (S2, S4, S5, S7, S8 and S9; Table 1). These are all 231 plankton samples and are still included in the study (Figure 1). They do not affect the interpretation 232 and main conclusion of our findings.

233 Our study also shows that there is no significant difference in the recovery efficiency between 234 the different extraction methods for the three CRMs that we used; DORM-3, TORT-2 and SRM-2976. 235 Compared to the biological data in the present manuscript, we conclude that our three CRMs represent 236 appropriate concentration ranges for MeHg bioaccumulation studies of Arctic food chains. Thus in 237 studies where the alkaline digestion is used, acceptable CRM recoveries may provide false confidence 238 in actual sample extraction recoveries. Differences between CRM recoveries and actual sample 239 recoveries are likely to be largest where the two biological matrixes are most different, for example 240 feather samples versus fish muscle tissue (DORM-3). In the absence of CRMs for all matrices this 241 means that care must be taken when interpreting results from one type of sample when the CRM is 242 formulated from a different type of sample. An alternative approach may be to investigate the use of a 243 surrogate internal standard, more usually applied in classical organic chemistry analysis. Both ethyl 244 and propyl mercury have been used for this purpose, especially when applying GC-MS analysis [22, 245 23], although this approach is also not without its challenges.

246 While DORM-3 is widely used in studies of MeHg in biological matrices, use of the other two 247 CRMs we utilized is less prevalent. However, when analysing low concentrations of MeHg in 248 biological material, the relatively low concentrations of the SRM-2976 means it is a good option for 249 quality control.

250

251 *Comparison of biological samples data*

252 Concentrations of MeHg found in our biological samples from Svalbard are of similar levels
253 that are previously shown for Arctic food chains [6], and representative of marine food chains. No
254 samples were below MDL and concentrations span 3 orders of magnitude; 1.4 – 1050 ng/g. This gives
255 a good basis for studying the different efficiency of sample treatment methods; i.e. alkaline digestion
256 versus acid extractions.

257 In the present study our data shows how concentrations of MeHg obtained from the alkaline
258 digestion method are always lower than concentrations obtained by the acid extraction method as long
259 as concentrations exceed 10 ng/g (specific concentrations not shown here; see Ruus et al. [18] for
260 details). For low level samples (< 10 ng/g) the difference is not significant ($p > 0.05$). This could be
261 due to either the nature of the low concentration samples (plankton and krill) or the fact that
262 concentrations are close to MDL levels in general. The same pattern of small or no difference between
263 the two sample treatment methods can be seen also for samples of fish and Kittiwake with low
264 concentrations.

265 For samples exceeding 10 ng/g the concentrations obtained by the alkaline digestion are
266 significantly lower than for the acid extraction concentrations. There is relatively little variation in
267 RPD between concentrations for the different species groups of the food chain; fish (including both
268 Polar and Atlantic cod), Little Auk and Kittiwake; 28.4, 31.0 and 24.9 %, respectively. Previous
269 studies suggest that lower recoveries are seen for the alkaline digestion method due to interference
270 from the organic matrix [12, 13]. Hintelmann and Nguyen [12] suggests that the alkaline digestion
271 method does not completely decompose the organic matrix. An incomplete decomposition leads to
272 intact functional groups in the solution which later interefere with the analytical procedure.

273 However, if such a matrix effect exists, this effect should potentially increase down the food
274 chain as the ratio of animal protein to intereferring substance (i.e. organic matrix) potentially
275 decreases. In this study we do not observere such a pattern. In fact no relationship was found between
276 concentrations of MeHg and average difference between the two different sample treatment methods
277 ($r^2 < 0.1$ when all samples were included and for individual species groups). This indicates that the
278 relatively poor recovery of MeHg when using the alkaline digestion method compared to the acid
279 extraction is relatively unaffected by concentration magnitude. I.e. the alkaline digestion sample pre-

280 treatment method always produces unsatisfactory and significantly lower results compared to the acid
281 extraction method. However, it can be seen that the largest absolute differences are found when
282 concentrations of MeHg are the highest.

283 Interestingly, there is no or little difference seen in extraction efficiency when comparing the
284 Atlantic cod results from this study (Figure 1), whereas for Polar Cod there were significant
285 differences for concentrations > 20 ng/g (n = 12; $p < 0.001$). The Polar Cod (n = 16) samples were
286 composed from whole individuals, whereas the Atlantic cod samples (n = 4) were taken from muscle
287 tissue only. This appears to support the idea that the alkaline extraction method is not efficient for
288 matrices which are more complex than fish muscle.

289 Comparing the two sample preparation techniques for Kittiwake samples reveals that 5
290 samples are responsible for the largest absolute differences seen between the two techniques. These 5
291 samples (marked with asterisk in Figure 1) are all liver samples and therefore have a relatively high fat
292 content. Again, this corresponds with the idea that the efficiency of the alkaline digestion technique
293 will decrease with increasing "other organic matrix". Although not relevant for liver samples, one such
294 suggested interference is keratin filaments. These may be present in large amounts in certain types of
295 biological samples, such as hair, fur and feathers [13]. Whilst these matrices are not considered in the
296 present study this is an important point as these types of sample are widely analysed for MeHg due to
297 the desirability of non-destructive sampling. In some cases this interference or poor extraction may be
298 sufficient as to result in MeHg not being detected.

299 Although comparison of QA/QC for the alkaline digestion method 1 and the acid extraction
300 method shows little variation, we do acknowledge that artefact methylation during the extraction
301 procedure could possibly explain the differences seen for our real samples. Artefact methylation is
302 reviewed in Leermakers et al. [8], where it is stated that previous assessments of the potential to
303 generate MeHg from inorganic Hg during sample preparation are mainly related to distillation-based
304 methods. Our acid extraction method is based on Hintelmann and Nguyen, [12], which does not point
305 to any problems of artefact methylation. Neither is it mentioned as a problem by other studies utilizing
306 this method [13, 14]. For sediment and soil samples both the positive (artefact formation of MeHg)
307 and negative bias (incomplete leaching, and/or decomposition of MeHg) were investigated for a

308 similar acid extraction method in Liang et al. [24]. In Liang et al. [24], the conclusion is that while the
309 distillation process shows artefact formation of MeHg when concentrations of Hg reaches 2000 ng/g,
310 the nitric acid extraction is independent of Hg concentrations. This confirms the discussion in Liang et
311 al. [8], and while we cannot exclude artefact methylation completely, we conclude that the acid
312 extraction technique is a good option for MeHg concentration ranges found in Arctic marine food
313 chains.

314 As most studies examining MeHg in aquatic food chains (freshwater and marine), today and
315 historically, use powdered fish or other similar CRMs to imply extraction efficiency, the effect
316 highlighted in the present study go unnoticed. Where total Hg (TotHg) and MeHg are both measured
317 in the same sample and concentrations of the organic form are found to be lower, then it is assumed
318 that this is a real difference. Thus it is widely reported that whilst almost all Hg in fish (muscle) is
319 present in the organic form [1], in many other biological samples the ratio is lower [5]. This may
320 however, just be an artefact from the extraction method chosen and thus may easily lead to a
321 significant underestimation overall of MeHg concentrations through the food chain. We suggest
322 therefore that levels of MeHg may in general be under-reported in the literature, where the alkaline
323 digestion method has been applied. This in turn may result in erroneous conclusions about the fate of
324 mercury species in biological food chains, and our understanding of the bio-geochemical cycling of
325 MeHg in the environment in general.

326

327 **Conclusions**

328 Results from the present study show significantly different MeHg concentrations in biological
329 samples, depending on the extraction method used. Although the two methods produce comparable
330 QA/QC results (blanks, duplicates, spikes and available CRMs) the concentrations found in most
331 biological matrices, especially fish and bird liver, are significantly different. This led to an
332 underestimation of between 24 and 31 % of the MeHg concentrations in the studied food chain. Such
333 differences may have consequences for our understanding of the bioaccumulation of Hg species, their
334 speciation in biota, and thus their biogeochemical cycling in general. Based on present results we
335 conclude that care must be taken when choosing the sample treatment method for analysis of MeHg in

biological samples, and that interpretation of results from alkaline digestions should be carried out with caution.

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## Tables

***Table 1.*** Individual sample specifications.

***Table 2.*** QC/QA for the sample treatment techniques.

466 ***Table 1.*** Specifications (species group, species name and analytical sample specification) with levels
467 of MeHg shown as concentration obtained by the acid extraction method divided by concentrations
468 obtained by the alkaline digestion method. Individual concentration differences are shown as relative
469 percent difference (RPD). ID numbers refer to sample ID used throughout the paper. Sample IDs in
470 bold are below limit of quantification (LOQ, n = 6).

| ID | Species group | Species name | Analytical sample specification | [Acid extraction] : [Alkaline digestion] | RPD (%) |
|---|---|---|---|---|---|
| S1 | Plankton | Calanus finmarchicus | Whole individuals | 1.0 | 3.4 |
| **S2** | Plankton | Calanus hyperboreus | Whole individuals | 0.6 | 45.1 |
| S3 | Plankton | Calanus sp | Whole individuals | 11.4 | 167.7 |
| **S4** | Plankton | Calanus sp | Whole individuals | 0.7 | 34.7 |
| **S5** | Plankton | Calanus sp | Whole individuals | 0.5 | 65.6 |
| S6 | Plankton | Calanus sp | Whole individuals | 1.0 | 1.1 |
| **S7** | Plankton | Calanus sp | Whole individuals | 1.2 | 16.4 |
| **S8** | Plankton | Calanus hyperboreus | Whole individuals | 0.8 | 21.8 |
| **S9** | Plankton | Calanus hyperboreus | Whole individuals | 0.5 | 69.7 |
| S10 | Plankton | Themisto libellula | Whole individuals | 0.9 | 11.0 |
| S11 | Plankton | Themisto libellula | Whole individuals | 1.4 | 35.7 |
| S12 | Plankton | Themisto abysorum | Whole individuals | 1.1 | 8.0 |
| S13 | Plankton | Calanus sp | Whole individuals | 1.5 | 41.6 |
| S14 | Plankton | Calanus sp | Whole individuals | 1.1 | 5.3 |
| S15 | Bird | Kittiwake (Rissa tridactyla) | Muscle | 1.0 | 0.4 |
| S16 | Bird | Kittiwake (Rissa tridactyla) | Muscle | 1.0 | 2.2 |
| S17 | Bird | Kittiwake (Rissa tridactyla) | Muscle | 1.4 | 31.4 |
| S18 | Bird | Kittiwake (Rissa tridactyla) | Muscle | 1.2 | 20.9 |
| S19 | Bird | Kittiwake (Rissa tridactyla) | Muscle | 1.1 | 9.5 |
| S20 | Bird | Kittiwake (Rissa tridactyla) | Muscle | 1.1 | 10.2 |
| S21 | Bird | Kittiwake (Rissa tridactyla) | Muscle | 0.7 | 33.2 |
| S22 | Bird | Kittiwake (Rissa tridactyla) | Muscle | 1.1 | 13.3 |
| S23 | Bird | Kittiwake (Rissa tridactyla) | Muscle | 1.7 | 52.0 |
| S24 | Bird | Kittiwake (Rissa tridactyla) | Muscle | 1.5 | 37.5 |
| S25 | Bird | Kittiwake (Rissa tridactyla) | Liver | 1.7 | 49.3 |
| S26 | Bird | Kittiwake (Rissa tridactyla) | Liver | 1.2 | 22.0 |
| S27 | Bird | Kittiwake (Rissa tridactyla) | Liver | 1.8 | 59.0 |
| S28 | Bird | Kittiwake (Rissa tridactyla) | Liver | 1.7 | 50.1 |
| S29 | Bird | Kittiwake (Rissa tridactyla) | Liver | 1.7 | 49.4 |
| S30 | Bird | Little Auk (Alle alle) | Muscle | 1.3 | 28.0 |
| S31 | Bird | Little Auk (Alle alle) | Muscle | 1.3 | 27.3 |
| S32 | Bird | Little Auk (Alle alle) | Muscle | 1.3 | 27.7 |
| S33 | Bird | Little Auk (Alle alle) | Muscle | 1.2 | 18.7 |
| S34 | Bird | Little Auk (Alle alle) | Muscle | 1.4 | 34.4 |
| S35 | Bird | Little Auk (Alle alle) | Muscle | 1.1 | 13.0 |
| S36 | Bird | Little Auk (Alle alle) | Muscle | 1.4 | 30.7 |
| S37 | Bird | Little Auk (Alle alle) | Muscle | 1.3 | 26.6 |
| S38 | Bird | Little Auk (Alle alle) | Muscle | 1.3 | 27.0 |
| S39 | Bird | Little Auk (Alle alle) | Muscle | 1.2 | 21.6 |
| S40 | Bird | Little Auk (Alle alle) | Muscle | 1.6 | 44.0 |
| S41 | Bird | Little Auk (Alle alle) | Muscle | 1.5 | 41.0 |
| S42 | Bird | Little Auk (Alle alle) | Muscle | 1.6 | 48.7 |
| S43 | Bird | Little Auk (Alle alle) | Muscle | 1.5 | 42.9 |
| S44 | Bird | Little Auk (Alle alle) | Muscle | 1.4 | 33.3 |
| S45 | Fish | Polar cod (Boreogadus saida) | Whole individuals | 1.3 | 25.8 |
| S46 | Fish | Polar cod (Boreogadus saida) | Whole individuals | 1.6 | 45.6 |
| S47 | Fish | Polar cod (Boreogadus saida) | Whole individuals | 1.5 | 38.3 |
| S48 | Fish | Polar cod (Boreogadus saida) | Whole individuals | 2.5 | 87.1 |
| S49 | Fish | Polar cod (Boreogadus saida) | Whole individuals | 1.7 | 50.2 |
| S50 | Fish | Polar cod (Boreogadus saida) | Whole individuals | 1.5 | 38.6 |
| S51 | Fish | Polar cod (Boreogadus saida) | Whole individuals | 1.6 | 46.5 |
| S52 | Fish | Polar cod (Boreogadus saida) | Whole individuals | 1.3 | 26.8 |
| S53 | Fish | Polar cod (Boreogadus saida) | Whole individuals | 1.1 | 13.1 |
| S54 | Fish | Polar cod (Boreogadus saida) | Whole individuals | 1.4 | 31.0 |
| S55 | Fish | Polar cod (Boreogadus saida) | Whole individuals | 1.4 | 32.3 |
| S56 | Fish | Polar cod (Boreogadus saida) | Whole individuals | 1.2 | 15.2 |
| S57 | Fish | Polar cod (Boreogadus saida) | Whole individuals | 1.2 | 15.5 |
| S58 | Fish | Polar cod (Boreogadus saida) | Whole individuals | 1.0 | 1.5 |
| S59 | Fish | Polar cod (Boreogadus saida) | Whole individuals | 1.4 | 35.9 |
| S60 | Fish | Atlantic cod (Gadus morhua) | Muscle | 1.3 | 22.7 |
| S61 | Fish | Atlantic cod (Gadus morhua) | Muscle | 1.1 | 5.3 |
| S62 | Fish | Atlantic cod (Gadus morhua) | Muscle | 1.2 | 16.3 |
| S63 | Fish | Atlantic cod (Gadus morhua) | Muscle | 0.9 | 5.6 |

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472 ***Table 2.*** Certified reference material QC/QA for the comparison of the three different sample
473 treatment methods; acid extraction (AE), alkaline digestion 1 (AD 1) and alkaline digestion 2 (AD 2).

| **Sample** | **Sample treatment method** | **n** | **MeHg concentration** | | **% recovery (average ± 1 standard deviation)** |
|---|---|---|---|---|---|
| | | | **Average** | **Standard deviation** | |
| CRM (DORM-3) | AE | 15 | 0.325 mg/kg | 0.027 mg/kg | 91.6 ± 7.7 |
| | AD 1 | 8 | 0.313 mg/kg | 0.024 mg/kg | 88.2 ± 6.7 |
| | AD 2 | 4 | 0.298 mg/kg | 0.024 mg/kg | 83.9 ± 6.7 |
| CRM (TORT-2) | AE | 15 | 0.158 mg/kg | 0.011 mg/kg | 104.2 ± 7.3 |
| | AD 1 | 8 | 0.146 mg/kg | 0.009 mg/kg | 95.8 ± 5.6 |
| | AD 2 | 4 | 0.148 mg/kg | 0.010 mg/kg | 97.6 ± 6.9 |
| CRM (SRM-2976) | AE | 15 | 27.25 µg/kg | 0.89 µg/kg | 97.0 ± 3.2 |
| | AD 1 | 8 | 24.62 µg/kg | 1.38 µg/kg | 87.6 ± 4.9 |
| | AD 2 | 4 | 23.62 µg/kg | 1.66 µg/kg | 84.1 ± 5.9 |

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486 **Figures**
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488 ***Figure 1.*** MeHg ratios in the biological samples.
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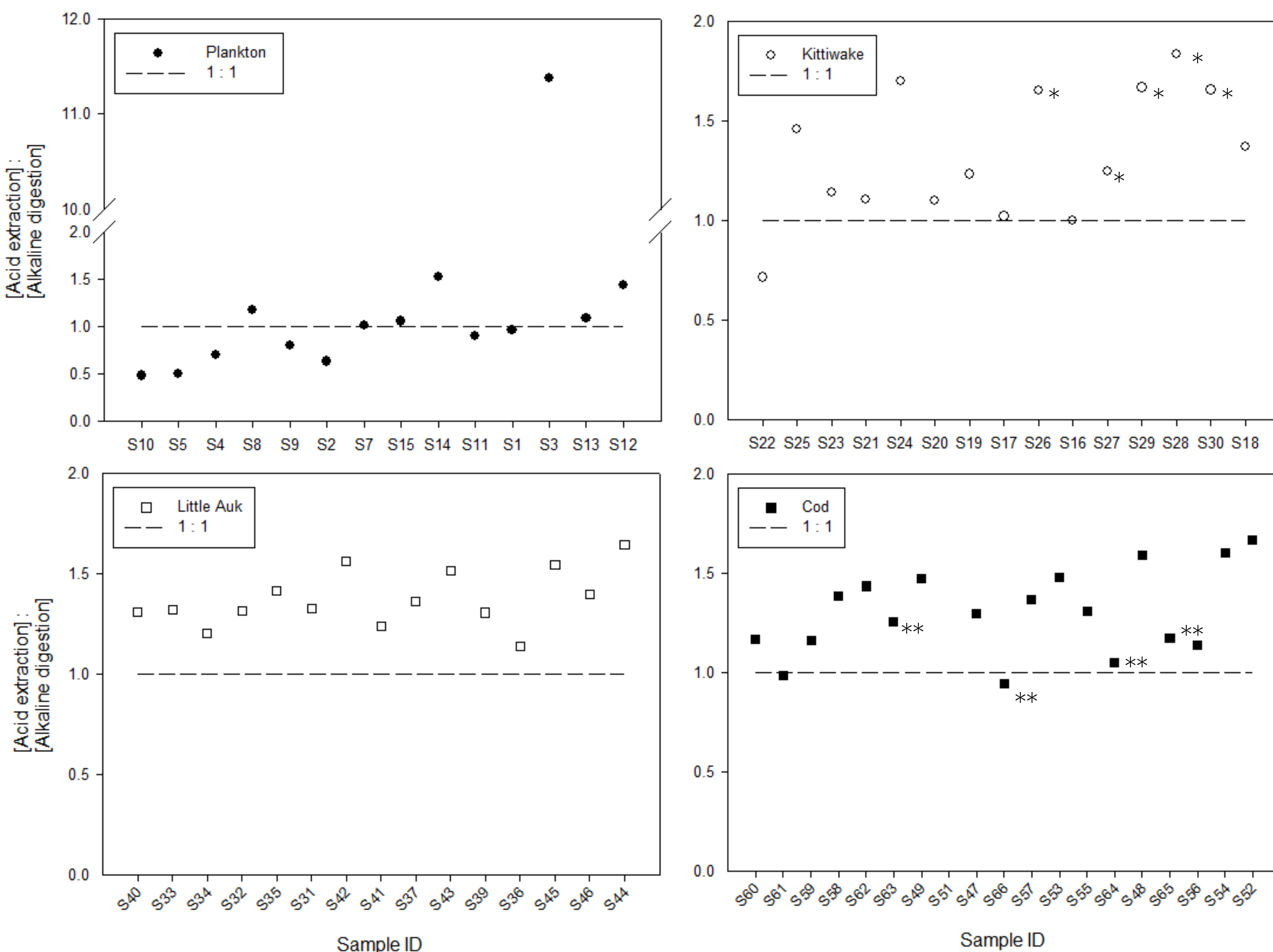


534 ***Figure 1.*** Levels of MeHg in the biological samples as concentration obtained by the acid extraction
535 divided by concentrations obtained by the alkaline digestion. Figure shows plankton samples (top left),
536 Kittiwake samples (top right), Little Auk samples (bottom left) and Polar and Atlantic cod samples
537 (bottom right). The dotted horizontal lines represent the 1:1 relationship between the concentrations
538 obtained by the two sample treatment techniques. Samples are sorted by increasing concentrations of
539 MeHg obtained by the acid extraction method from left to right. Kittiwake liver samples (n = 5) are
540 indicated by a single asterisk and Atlantic cod samples (n = 4) are indicated by two asterisks.

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