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### ► To cite this version:

Anais Médieu, Fany Sardenne, Anne Lorrain, Nathalie Bodin, Chloé Pazart, et al.. Lipid-free tuna muscle samples are suitable for total mercury analysis. *Marine Environmental Research*, 2021, 169, 105385 (6p.). 10.1016/j.marenvres.2021.105385 . hal-04203480

**HAL Id: hal-04203480**

**<https://hal.science/hal-04203480>**

Submitted on 4 Jan 2024

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## Lipid-free tuna muscle samples are suitable for total mercury analysis

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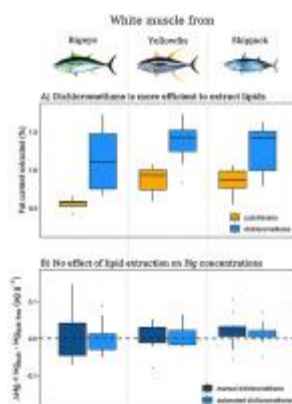
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### Abstract :

Tropical tunas are largely consumed worldwide, providing major nutritional benefits to humans, but also representing the main exposure to methylmercury, a potent neurotoxin that biomagnifies along food webs. The combination of ecological tracers (nitrogen and carbon stable isotopes,  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) to mercury concentrations in tunas is scarce yet crucial to better characterise the influence of tuna foraging ecology on mercury exposure and bioaccumulation. Given the difficulties to get modern and historical tuna samples, analyses have to be done on available and unique samples. However,  $\delta^{13}\text{C}$  values are often analysed on lipid-free samples to avoid bias related to lipid content. While lipid extraction with non-polar solvents is known to have no effect on  $\delta^{15}\text{N}$  values, its impact on mercury concentrations is still unclear. We used white muscle tissues of three tropical tuna species to evaluate the efficiency and repeatability of different lipid extraction protocols commonly used in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  analysis. Dichloromethane was more efficient than cyclohexane in extracting lipids in tuna muscle, while the automated method appeared more efficient but as repeatable as the manual method. Lipid extraction with dichloromethane had no effect on mercury concentrations. This may result from i) the affinity of methylmercury to proteins in tuna flesh, ii) the low lipid content in tropical tuna muscle samples, and iii) the non-polar nature of dichloromethane. Our study suggests that lipid-free samples, usually prepared for tropical tuna foraging ecology research, can be used equivalently to bulk samples to document in parallel mercury concentrations at a global scale.

### Graphical abstract



## Highlights

► Scarcity of tuna samples makes essential to get the most out of a single sample. ► Dichloromethane is more efficient than cyclohexane to extract lipids. ► Dichloromethane extraction has no effect on Hg levels. ► Bulk and lipid-free tropical tuna samples can be used jointly to infer Hg levels.

**Keywords** : methylmercury, fat content, delipidation, yellowfin, bigeye, skipjack

## 39 1. Introduction

40 Mercury (Hg) is a widespread heavy metal of particular concern to wildlife and human health.  
41 In oceans, it is naturally converted into methylmercury (MeHg), its organic and highest  
42 neurotoxic form, well known for its persistence and unique bioaccumulation properties in  
43 food webs (Hintelmann, 2010). Consumption of contaminated seafood is considered as the  
44 main route of human exposure to MeHg. Top predators like tunas are known to display  
45 relatively high MeHg concentrations, sometimes exceeding food safety guidelines ( $1 \mu\text{g g}^{-1}$   
46 fresh tissue) (WHO and UNEP Chemicals, 2008) depending on the oceanic basin and the tuna  
47 species. Yet, tunas are also among the most popular marine species consumed worldwide,  
48 particularly tropical species that account for more than 90% of the global tuna fishery (FAO,  
49 2018). In terms of food and nutrition security, they provide a major source of proteins,  
50 essential fatty acids, vitamins and minerals (Di Bella et al., 2015; Sirot et al., 2012).

51 Knowing their economic importance, nutritional benefits and potential impact on human  
52 health, tropical tunas have been studied broadly but at relatively small spatial scale to  
53 document their Hg exposure and characterize the processes driving Hg biomagnification  
54 along food webs (Bodin et al., 2017; Chouvelon et al., 2017; Houssard et al., 2019;  
55 Kojadinovic et al., 2006; Nicklisch et al., 2017). Complex regional interplay between physical  
56 (e.g. light intensity), geochemical (e.g. redox status), physiological (e.g. organism's length  
57 and age), and ecological factors (e.g. tuna's foraging depth) have been identified to govern Hg  
58 concentrations in these top predators (Choy et al., 2009; Houssard et al., 2019; Kojadinovic et  
59 al., 2006; Médiéu et al., 2021; Wang et al., 2019). Nevertheless, key global aspects remain  
60 poorly understood, in particular biogeochemical methylation/demethylation mechanisms  
61 controlling MeHg bioavailability at the base of the food web, as well as factors driving both  
62 fate and accumulation through the food web. Global studies combining Hg concentrations and  
63 ecological tracers are therefore needed to clarify these points, especially in the context of the

64 UNEP Minamata Convention since monitoring studies in marine biota have become essential  
65 the better characterize Hg cycle and fate in oceans.

66 Pelagic food web structuration and functioning have been broadly investigated, mainly  
67 through the use of carbon and nitrogen stable isotopes data ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values) (Fry,  
68 2006). Recently, collaborative and global studies relying upon  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values enabled  
69 identifying broad-scale patterns of trophic structure, movements and trophodynamics of tunas  
70 in relation to environmental conditions (Logan et al., 2020; Lorrain et al., 2020; Pethybridge  
71 et al., 2018). Based on individual records with associated metadata (i.e. fish length, fishing  
72 date and position) (Bodin et al., 2020), these collaborative and global studies also represent a  
73 gold mine of already collected and preserved samples to characterize spatial and/or temporal  
74 Hg trends in tunas.

75 An issue with global modern and historical datasets is that samples from different laboratories  
76 are not always processed the same way. To account for the influence of lipids on  $\delta^{13}\text{C}$  values  
77 (DeNiro and Epstein, 1977) while making a single analysis for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values,  
78  $\delta^{13}\text{C}$  values are either produced from i) bulk tissue and a mathematical correction (Sardenne et  
79 al., 2015), or from ii) lipid-free tissue, with lipids removed through various methods and  
80 solvents selected not to alter  $\delta^{15}\text{N}$  values. In the latter case, manual or automated (high  
81 temperature and pressure) methods are generally applied with solvents of low polarity such as  
82 dichloromethane and cyclohexane (Bodin et al., 2009; Ménard et al., 2007). While these  
83 methods do not affect  $\delta^{15}\text{N}$  values and provide valuable data on lipid content, nothing is  
84 known regarding their effect on Hg content, restricting the development of global studies on  
85 tuna's Hg concentration with preserved samples prepared for tuna foraging ecology research.

86 Here, we investigated i) the efficiency and repeatability of two common lipid extraction  
87 methods (manual and automated) and two neutral solvents (dichloromethane and  
88 cyclohexane) on lipid content determination (experiment A), and ii) the influence of the most

89 efficient solvent for lipid extraction on total Hg concentrations (experiment B). Experiments  
90 were carried out in three tropical tuna species, i.e. bigeye (*Thunnus obesus*), yellowfin (*T.*  
91 *albacares*) and skipjack (*Katsuwonus pelamis*), and on white muscle tissues, the commonly  
92 used tissue in studies investigating both trophic ecology and Hg bioaccumulation and the final  
93 storage for MeHg in fish. .

## 94 2. Material & methods

### 95 2.1. Sample collection

96 All bigeye, yellowfin, and skipjack tuna samples ( $n_{total} = 33$ ) were collected in the western  
97 Indian Ocean during the unloading of commercial vessels (purse seine) at Victoria port  
98 (Seychelles). To test for efficiency and repeatability of different lipid extraction protocols  
99 (experiment A), we used three individuals, one per tuna species ( $n_{exp A} = 3$ ). To test for the  
100 effect of lipid extraction on Hg concentrations (experiment B), we used 10 other individuals  
101 per tuna species ( $n_{exp B} = 30$ ), all collected from the same fishing set. For these 30 individuals,  
102 fork length (FL) was measured to the lowest cm and ranged respectively for bigeye, yellowfin  
103 and skipjack from 44 to 91 cm ( $62 \pm 17$  cm), 35 to 129 cm ( $70 \pm 35$  cm), and 34 to 64 cm ( $45$   
104  $\pm 10$  cm) (Supplementary Information Appendix S1). For both experiments, white muscle  
105 samples of around 20 g (wet weight) were collected in the front dorsal position, stored frozen  
106 at  $-20^{\circ}\text{C}$ , freeze-dried for 48 hours with an Alpha 1-4 LD freeze-dryer (Christ, Coueron,  
107 France) and finally ground to a fine homogeneous powder using a MM400 grinder (Retsch,  
108 Eragny sur Oise, France) prior to lipid extraction and total Hg analyses.

109

### 110 2.2. Lipid extraction protocols

111 Two lipid extraction methods (manual and automated) and neutral solvents (dichloromethane  
112 and cyclohexane) were first tested on five replicates of three individuals, one per tropical  
113 tuna species, to identify the most efficient and repeatable method and solvent in extracting  
114 lipids (experiment A,  $n_{exp A} = 3$ , Fig. 1A). The use of one single individual per tuna species  
115 allowed overcoming the potential bias related to inter-individual variability when estimating  
116 both efficiency and repeatability of the lipid extraction methods, as recommended and done in

117 other studies testing for different lipid extraction methods (AOCS, 2017; JCGM, 2012;  
118 Sardenne et al., 2019a). All lipid extractions were done at LEMAR (Plouzané, France).

119 **2.2.1. Manual extraction:** Following the method of Ménard et al. (2007),  $100 \pm 4$  mg of each  
120 powdered and dried aliquot were mixed with 10 mL of solvent for 1 hour using a rotary  
121 shaker. The mixture of the powder and the solvent was then separated by centrifugation at  
122 2500 rpm for 10 min at  $10^{\circ}\text{C}$ . The lipid-free powders were stored in a dry-room until total Hg  
123 analysis, while the lipid extracts were collected in a pre-weighted vial and evaporated to  
124 dryness under  $\text{N}_2$  flow with a N-evap 111 nitrogen evaporator (OA-SYS, Berlin, USA).

125 **2.2.2. Automated extraction:**  $150 \pm 10$  mg of homogenized dried aliquot were extracted with  
126 20 mL of solvent at  $100^{\circ}\text{C}$  under 1400 psi for 10 min using a ASE 350 Accelerated Solvent  
127 Extractor (Dionex, Voisins de Bretonneux, France), following the method of Bodin et al.  
128 (2009). The lipid-free powders were stored in a dry-room until total Hg analysis, while the  
129 lipid extracts were collected in a pre-weighted vial and evaporated to dryness under  $\text{N}_2$  flow  
130 with a N-evap 111 nitrogen evaporator (OA-SYS, Berlin, USA).

131 **2.2.3. Total lipid content:** For both extraction methods, lipid residuals were weighted on a  
132 Mettler Toledo MX5 analytical micro-balance (Mettler Toledo, Columbus, Ohio) to the  
133 nearest 0.001 mg to determine the total lipid content (TLC, %) of the samples, expressed on a  
134 dry weight basis (dw).

135

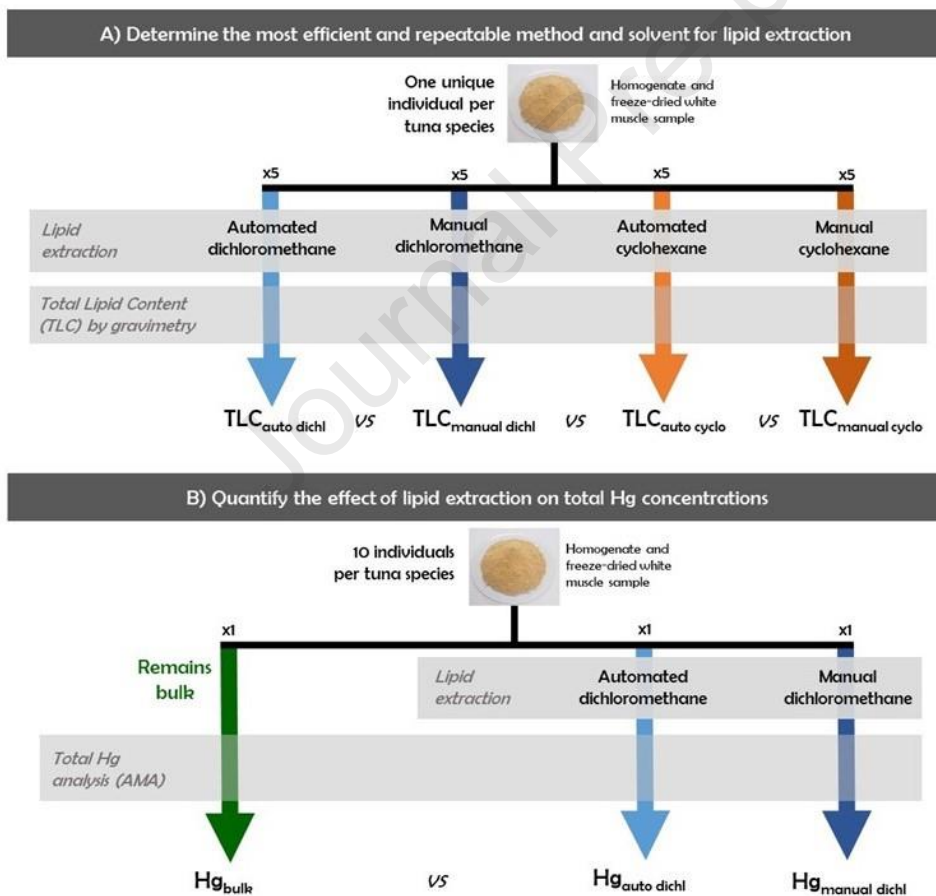
### 136 **2.3. Total mercury analysis**

137 The potential impact of lipid extraction methods on Hg concentrations was assessed on 10  
138 individuals per tuna species, with Hg concentrations determined on both bulk and lipid-free  
139 powders (Experiment B,  $n_{exp\ B} = 30$ , Fig. 1B). Total Hg concentrations were measured on  
140 powdered, dried and homogenized tissue by thermal decomposition, gold amalgamation and



141 atomic adsorption (AMA 254, Altec, Czech Republic, Száková et al., 2004) at GET  
 142 (Toulouse, France). Blanks and two biological materials, TORT-3 (lobster hepatopancreas;  
 143  $\text{Hg} = 292 \pm 22 \text{ ng g}^{-1} \text{ dw}$ ) and BCR-464 (tuna fish;  $\text{Hg} = 5240 \pm 100 \text{ ng g}^{-1} \text{ dw}$ ), covering a  
 144 wide range of Hg concentrations, were routinely used every 5-10 samples in each analytical  
 145 batch to check Hg measurement accuracy. Recovery rates (defined as the ratios of observed  
 146 value by certified value) were calculated for the two certified reference materials (TORT-3:  
 147  $100 \pm 3\%$ ; and BCR-464:  $100 \pm 2\%$ ) and variability was below 4%. Hg concentrations are  
 148 expressed in  $\mu\text{g g}^{-1}$  (equivalent to part per million, ppm), on a dw basis.

149



150

151 **Figure 1:** Outline of the two experiments testing for A) the efficiency and repeatability of each lipid extraction  
 152 protocol (manual and automated method, and dichloromethane and cyclohexane solvent) using five replicates of  
 153 the same individual per tropical tuna species ; and for B) the influence of manual and automated method with

154 dichloromethane on total Hg concentrations ( $n_{exp\ B} = 30$  individuals). TLC=Total Lipid Content. See Material and  
155 methods for details.

156

## 157 **2.4. Statistical analysis**

158 All statistical analyses were performed with the statistical open source R software 3.6.1 (R  
159 Core Team, 2018). We first evaluated the efficiency of both solvents (i.e. dichloromethane  
160 and cyclohexane) and methods (i.e. manual and automated) in extracting lipids with an  
161 analysis of variance (ANOVA) on TLC values (experiment A). The higher the TLC values,  
162 the higher the efficiency. Repeatability of each extraction experiment was assessed per  
163 tropical tuna species using the coefficient of variation (CV, defined as the ratio of standard  
164 deviation by mean) of TLC values measured on the five replicate samples. The lower the CV,  
165 the higher the repeatability. Then, we assessed the influence of manual and automated  
166 extraction methods on total Hg concentrations with two separated paired t-test on  $\Delta\text{Hg}$  values  
167 (with  $\Delta\text{Hg} = \text{Hg}_{\text{bulk}} - \text{Hg}_{\text{lipid-free}}$ ) (experiment B). For these two tests, we considered all  
168 individuals ( $n_{exp\ B} = 30$  pairs of observations), no matter the species, as  $\Delta\text{Hg}$  values do not  
169 significantly differ between species (ANOVA,  $p > 0.05$  for both methods). TLC,  $\Delta\text{Hg}$  values,  
170 and residuals of ANOVA were tested for normality with a Shapiro test. Observations were  
171 considered as outliers according to the “1.5 rule”, i.e. if they were less than  $Q1 - 1.5 \cdot \text{IQR}$ , or  
172 greater than  $Q3 + 1.5 \cdot \text{IQR}$ , where  $Q1$ ,  $Q3$  and  $\text{IQR}$  are the lower quantile, upper quantile and  
173 inter-quantile range (defined as  $Q3 - Q1$ ), respectively.

### 174 3. Results & discussion

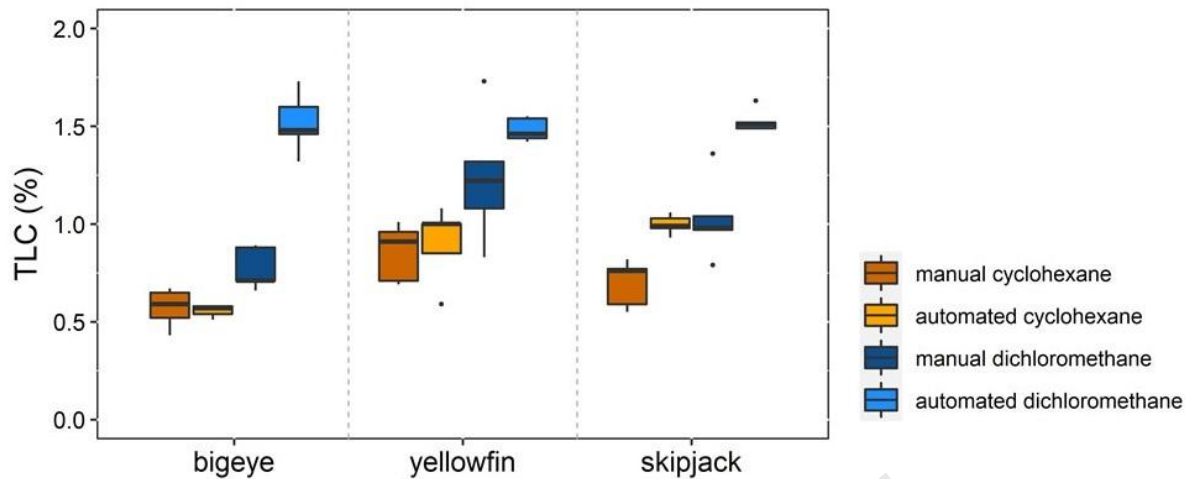
175 **3.1. Efficiency and repeatability of lipid extraction methods and solvents:** TLC values  
176 measured in white muscle varied between 0.4 and 1.7% dw (Supplementary Information  
177 Appendix S1). These values are similar to levels reported in same tropical tuna species from  
178 the Indian (~3%, Sardenne et al., 2016), Atlantic (~6%, Sardenne et al., 2019) and Pacific  
179 (~2%, Peng et al., 2013) Oceans, which confirms tropical tunas are lean species.

180 For the three tropical tuna species, TLC values were significantly higher when lipids were  
181 extracted with dichloromethane than with cyclohexane (Fig. 2; ANOVA,  $p < 0.01$ ).  
182 Dichloromethane and cyclohexane are both non-polar solvents recommended for routine  
183 analysis of lipids/fatty acids and/or prior  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  analysis (Bodin et al., 2009; Ménard  
184 et al., 2007). Here, the higher efficiency of dichloromethane may result from its slightly  
185 higher Snyder polarity index (3.1) compared to cyclohexane (0.2) and therefore to the fact  
186 that dichloromethane, although a neutral solvent is likely to extract some membrane polar  
187 lipids (e.g. phospholipids and sphingolipids) too, increasing TLC values. Nevertheless, this  
188 might be of minor importance regarding the protein fraction as this solvent has been shown to  
189 have negligible effects on the  $\delta^{15}\text{N}$  values across tissues and tuna species (Sardenne et al.,  
190 2015).

191 Regarding differences between manual and automated methods, TLC values were  
192 significantly higher for bigeye and skipjack replicates for the automated extraction (Fig. 2;  
193 ANOVA,  $p < 0.05$ ). For bigeye replicates, interaction between solvent and method was also  
194 significant, showing that the efficiency of the extraction method depends on the solvent used,  
195 with automated extraction with dichloromethane being the most efficient protocol to extract  
196 lipids (Fig. 2, ANOVA  $p < 0.05$ ). Conversely, TLC values did not differ significantly between  
197 the two extraction methods for yellowfin replicates (Fig. 2; ANOVA,  $p > 0.05$ ). The higher

198 efficiency of the automated method may be explained by its combined conditions of elevated  
199 temperature and pressure that maintain solvents near their supercritical region where they  
200 have high extraction properties (Ramos et al., 2002). This suggests that lipid extraction with  
201 automated method would be preferred over manual method. On the other hand, this automated  
202 method is twice more costly in polluting solvent, and its cleaning steps are longer compared  
203 to the manual method. Considering the low difference in extraction efficiency and the fact that  
204 is was significant only for the skipjack in-house reference sample, both manual and automated  
205 methods are relevant to extract lipids from lean tuna muscle tissues.

206 The repeatability of the different experiments varied between the protocols between 4 and  
207 27%, but remained overall lower than 18% (Supplementary Information Appendix 2).  
208 Acceptable repeatability often ranges between 5 and 10%, but for lean tissues like white  
209 muscle of tropical tunas, repeatability down 20-30% is considered acceptable too, as found in  
210 other studies (Bodin et al., 2009; Dodds et al., 2004). As expected, automated method showed  
211 generally lower CV values, as ASE 350 has been developed to reduce handling steps and to  
212 ensure increased reproducibility. This would be another argument to prefer the automated  
213 method; yet given the pros and cons arguments listed above, both manual and automated  
214 methods remain acceptable for the lipid extraction from white muscle tissue of tropical tunas.



215

216 **Figure 2:** Boxplots of total lipid content (TLC, % dry weight) associated to the four lipid extraction protocols  
 217 and measured in five replicates of each single individual per tuna species. Thick bar is the median value, points  
 218 are outliers of five replicates, and the box contains 50% of the data.

219

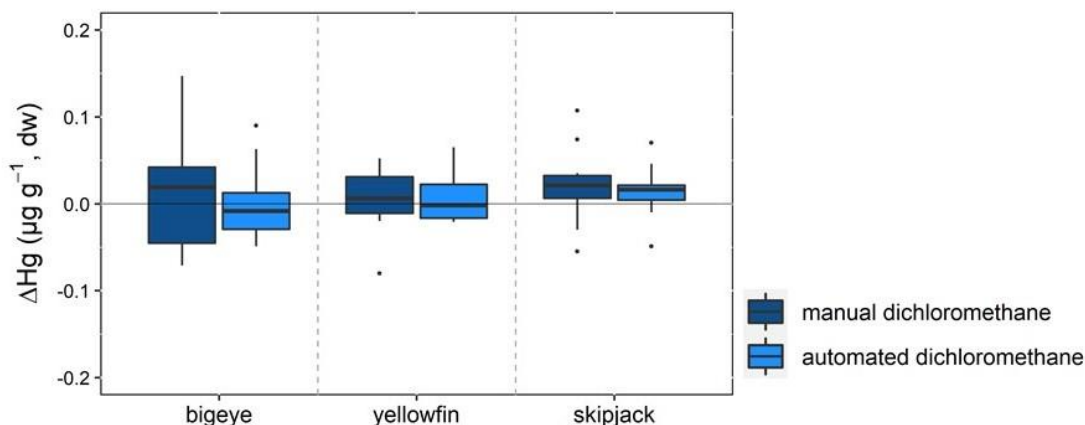
220 **3.2. Influence of lipid extraction on total Hg concentrations:** Bulk total Hg concentrations  
 221 (mean  $\pm$  sd, min-max, dw) varied between tuna species (Kruskal-Wallis,  $p < 0.05$ ), with  
 222 significantly higher levels in bigeye ( $0.81 \pm 0.47 \mu\text{g g}^{-1}$ , 0.20-1.37  $\mu\text{g g}^{-1}$ ; Dunn's test,  
 223  $p < 0.05$ ) than yellowfin ( $0.38 \pm 0.27 \mu\text{g g}^{-1}$ , 0.12-1.03  $\mu\text{g g}^{-1}$ ) and skipjack ( $0.30 \pm 0.17 \mu\text{g g}^{-1}$ ,  
 224 0.12-0.61  $\mu\text{g g}^{-1}$ ) (Supplementary Information Appendix S1). Those levels and differences are  
 225 similar to those reported previously in the western Indian Ocean or in both north and south  
 226 Pacific Oceans for similar fish size ranges (Bodin et al., 2017; Choy et al., 2009; Médieu et  
 227 al., 2021). The highest Hg concentrations in bigeye are likely to result from confounding  
 228 effects: a higher trophic position, a deeper vertical habitat giving access to mesopelagic  
 229 species with higher Hg concentrations, and a longer lifespan compared to the two other  
 230 tropical tunas (Choy et al., 2009; Olson et al., 2016).

231 Manual and automated lipid extractions with dichloromethane did not significantly affect the  
 232 Hg concentrations in white muscle samples of the three tuna species (Fig. 3; paired t-test,  
 233  $p > 0.05$ ). This agrees with Cipro et al. (2017) where no difference of Hg levels was found

234 between bulk and lipid-free muscle samples of two marine fish (i.e. *Notothenia coriiceps* and  
235 *N. rossii*). This may be related to the particular chemical properties of MeHg in fish flesh and  
236 the polarity of the solvent used for lipid extraction. In tuna white muscle, most of total Hg  
237 (>91%) is in its methylated form (Bloom, 1992; Houssard et al., 2019), which is known to  
238 bioaccumulate in the protein fraction of the muscle, mostly by forming complexes with the  
239 amino acid cysteine (Amlund et al., 2007; Leaner and Mason, 2004; Manceau et al., 2021).  
240 On the other hand, we used dichloromethane, a neutral solvent that does not affect the protein  
241 fraction by limiting the removal of proteins bound to membrane lipids (Bodin et al., 2009).  
242 Thus, by preserving the Hg-protein fraction in muscle tissues during lipid extraction, we  
243 assumed to have preserved MeHg concentrations of the samples. The absence of a significant  
244 effect of lipid extraction on Hg concentrations may be due also to the low lipid content of  
245 tropical tuna species (<6%), as measured in our experiment and showed in other studies (Peng  
246 et al., 2013; Sardenne et al., 2019b, 2016), and therefore to an unchanged mass balance in the  
247 samples. Finally, when discussing the effects of the different protocols tested, it is worthwhile  
248 mentioning that high temperature and pressure conditions of the automated extraction did not  
249 significantly affect total Hg concentrations neither, suggesting no thermal and pressure  
250 degradation of the total Hg pool in the samples. This confirms that MeHg is bound up tightly  
251 to the protein fraction in fish muscle tissues, where it is known to remain for a long time  
252 (Leaner and Mason, 2004)..

253

254



255

256 **Figure 3:** Boxplots of the differences of Hg concentrations ( $\mu\text{g g}^{-1}$ , dry weight) between bulk and lipid-free  
 257 samples. Box colours display lipid extraction methods (manual and automated). Thick bar is the median value,  
 258 points are outliers, and the box contains 50% of the data.

259

## 260 4. Conclusion

261 This study reveals the higher efficiency of dichloromethane compared to cyclohexane to  
 262 extract lipids in tropical tuna white muscle tissue, which may be attributed to its slightly  
 263 higher polarity index. Automated method appeared more efficient than manual method to  
 264 extract lipids, especially when used with dichloromethane, which may be attributed the  
 265 combined conditions of elevated temperature and pressure. Repeatability of all experiments  
 266 were in acceptable ranges considering that we were working on lean samples.

267 We show no significant effect of lipid extraction with dichloromethane on total Hg  
 268 concentrations in tropical tuna white muscle tissue. This may be related to i) the affinity of  
 269 MeHg (i.e. the most abundant Hg chemical form in tuna muscles) to proteins, ii) the relative  
 270 low lipid content in white muscle tissue of tropical tuna species, and iii) the non-polar  
 271 characteristics of dichloromethane, known to efficiently extract storage lipids without  
 272 affecting the protein fraction. This suggests that white muscle samples that have undergone  
 273 lipid extraction with dichloromethane can be used equivalently to bulk samples to investigate

274 Hg bioaccumulation in these pelagic top predators from the western Indian Ocean. Knowing  
275 that tropical tunas in the Atlantic, Indian and Pacific Oceans all have relatively low lipid  
276 content in white muscle tissue (<6%, Peng et al., 2013; Sardenne et al., 2019, 2016), this  
277 suggests that our results might be valid at a global scale, but further studies are needed to  
278 confirm this statement. As lipid content can vary between tissues, species and oceanic basins  
279 because of differences in diet and/or in energy allocation strategies distributed among  
280 metabolic functions (i.e. growth, reproduction and maintenance), we recommend using bulk  
281 samples to infer precisely Hg concentrations in marine fish other than tropical tunas. Further  
282 studies would be particularly needed for tissues and species with high lipid content (e.g.  
283 swordfish white muscle tissue, between 20-40% dw, Young et al., 2010).



## 284 **Acknowledgments**

285 We thank the fishermen and crews of the SAPMER fishing company who gave the tuna  
286 samples. We are grateful to Jean-Marie Munaron from LEMAR (Plouzané, France) for his  
287 help with lipid extractions and the access to his lab.

288

289 **Funding:** Funding was provided by ANR-17-CE34-0010, MERTOX from the French Agence  
290 Nationale de la Recherche.

291

292 **Conflicts of interest/Competing interests:** The authors declare that they have no known  
293 competing financial interests or personal relationships that could have appeared to influence  
294 the work reported in this paper.

295

296 **Author contribution:** Anaïs Médiéu: Conceptualization, Methodology, Investigation,  
297 Writing – original draft. Fany Sardenne: Conceptualization, Methodology, Supervision,  
298 Writing – review & editing. Anne Lorrain: Conceptualization, Supervision, Writing – review  
299 & editing. Nathalie Bodin: Conceptualization, Methodology, Resources, Writing – review &  
300 editing. Chloé Pazart: Investigation. Hervé Le Delliou: Investigation, Resources. David Point:  
301 Conceptualization, Supervision, Resources, Writing – review & editing.

302

303

## 304 **References**

305

306 Amlund, H., Lundebye, A.-K., Berntssen, M.H.G., 2007. Accumulation and elimination of  
307 methylmercury in Atlantic cod (*Gadus morhua* L.) following dietary exposure.  
308 *Aquatic Toxicology* 83, 323–330. <https://doi.org/10.1016/j.aquatox.2007.05.008>  
309 AOCS, 2017. Determination of Precision of Analytical Methods. Evaluation and design of  
310 test methods.

- 311 Bloom, N.S., 1992. On the Chemical Form of Mercury in Edible Fish and Marine Invertebrate  
312 Tissue. *Canadian Journal of Fisheries and Aquatic Sciences* 49, 1010–1017.  
313 <https://doi.org/10.1139/f92-113>
- 314 Bodin, N., Budzinski, H., Le Ménach, K., Tapie, N., 2009. ASE extraction method for  
315 simultaneous carbon and nitrogen stable isotope analysis in soft tissues of aquatic  
316 organisms. *Analytica Chimica Acta* 643, 54–60.  
317 <https://doi.org/10.1016/j.aca.2009.03.048>
- 318 Bodin, N., Lesperance, D., Albert, R., Hollanda, S., Michaud, P., Degroote, M., Churlaud, C.,  
319 Bustamante, P., 2017. Trace elements in oceanic pelagic communities in the western  
320 Indian Ocean. *Chemosphere* 174, 354–362.  
321 <https://doi.org/10.1016/j.chemosphere.2017.01.099>
- 322 Bodin, N., Pethybridge, H., Duffy, L.M., Lorrain, A., Allain, V., Logan, J.M., Ménard, F.,  
323 Graham, B., Choy, C.A., Somes, C.J., Olson, R.J., Young, J.W., 2020. Global data set  
324 for nitrogen and carbon stable isotopes of tunas. *Ecology*.  
325 <https://doi.org/10.1002/ecy.3265>
- 326 Chouvelon, T., Brach-Papa, C., Auger, D., Bodin, N., Bruzac, S., Crochet, S., Degroote, M.,  
327 Hollanda, S.J., Hubert, C., Knoery, J., Munsch, C., Puech, A., Rozuel, E., Thomas,  
328 B., West, W., Bourjea, J., Nikolic, N., 2017. Chemical contaminants (trace metals,  
329 persistent organic pollutants) in albacore tuna from western Indian and south-eastern  
330 Atlantic Oceans: Trophic influence and potential as tracers of populations. *Science of  
331 The Total Environment* 596–597, 481–495.  
332 <https://doi.org/10.1016/j.scitotenv.2017.04.048>
- 333 Choy, C.A., Popp, B.N., Kaneko, J.J., Drazen, J.C., 2009. The influence of depth on mercury  
334 levels in pelagic fishes and their prey. *Proceedings of the National Academy of  
335 Sciences* 106, 13865–13869. <https://doi.org/10.1073/pnas.0900711106>
- 336 Cipro, C.V.Z., Bustamante, P., Montone, R.C., 2017. Influence of Delipidation on Hg  
337 Analyses in Biological Tissues: A Case Study for an Antarctic Ecosystem. *Water Air  
338 Soil Pollut* 228, 188. <https://doi.org/10.1007/s11270-017-3367-8>
- 339 DeNiro, M.J., Epstein, S., 1977. Mechanism of carbon isotope fractionation associated with  
340 lipid synthesis. *Science* 197, 261–263. <https://doi.org/10.1126/science.327543>
- 341 Di Bella, G., Potortì, A.G., Lo Turco, V., Bua, D., Licata, P., Cicero, N., Dugo, G., 2015.  
342 Trace elements in *Thunnus Thynnus* from Mediterranean Sea: benefit-risk assessment  
343 for consumer. *Food Additives & Contaminants: Part B* 8, 175–181.  
344 <https://doi.org/10.1080/19393210.2015.1030347>
- 345 Dodds, E.D., McCoy, M.R., Geldenhuys, A., Rea, L.D., Kennish, J.M., 2004. Microscale  
346 recovery of total lipids from fish tissue by accelerated solvent extraction. *J Amer Oil  
347 Chem Soc* 81, 835–840. <https://doi.org/10.1007/s11746-004-0988-2>
- 348 FAO (Ed.), 2018. The state of world fisheries and aquaculture 2018 - Meeting the sustainable  
349 development goals. Rome.
- 350 Fry, B., 2006. *Stable isotope ecology*. Springer, New York, NY.
- 351 Hintelmann, H., 2010. Organomercurials. Their Formation and Pathways in the Environment,  
352 in: *Organometallics in Environment and Toxicology: Metal Ions in Life Sciences*. pp.  
353 365–401. <https://doi.org/10.1039/9781849730822-00365>
- 354 Houssard, P., Point, D., Tremblay-Boyer, L., Allain, V., Pethybridge, H., Masbou, J., Ferriss,  
355 B.E., Baya, P.A., Lagane, C., Menkes, C.E., Letourneur, Y., Lorrain, A., 2019. A  
356 Model of Mercury Distribution in Tuna from the Western and Central Pacific Ocean:  
357 Influence of Physiology, Ecology and Environmental Factors. *Environmental Science  
358 & Technology* 53, 1422–1431. <https://doi.org/10.1021/acs.est.8b06058>
- 359 JCGM, 2012. *International vocabulary of metrology - Basic and general concepts and  
360 associated terms (VIM)*.

- 361 Kojadinovic, J., Potier, M., Le Corre, M., Cosson, R.P., Bustamante, P., 2006. Mercury  
362 content in commercial pelagic fish and its risk assessment in the Western Indian  
363 Ocean. *Science of The Total Environment* 366, 688–700.  
364 <https://doi.org/10.1016/j.scitotenv.2006.02.006>
- 365 Leaner, J.J., Mason, R.P., 2004. Methylmercury uptake and distribution kinetics in  
366 Sheepshead Minnows, *Cyprinodon Variegatus*, after exposure to  $\text{CH}_3\text{Hg}$ -spiked food.  
367 *Environ Toxicol Chem* 23, 2138. <https://doi.org/10.1897/03-258>
- 368 Logan, J.M., Pethybridge, H., Lorrain, A., Somes, C., Allain, V., Bodin, N., Choy, C.A.,  
369 Duffy, L., Goñi, N., Graham, B., Langlais, C., Ménard, F., Olson, R., Young, J., 2020.  
370 Global patterns and inferences of tuna movements and trophodynamics. *Deep Sea*  
371 *Research Part II: Topical Studies in Oceanography*.  
372 <https://doi.org/10.1016/j.dsr2.2020.104775>
- 373 Lorrain, A., Pethybridge, H., Cassar, N., Receveur, A., Allain, V., Bodin, N., Bopp, L., Choy,  
374 C.A., Duffy, L., Fry, B., Goñi, N., Graham, B.S., Hobday, A.J., Logan, J.M., Ménard,  
375 F., Menkes, C.E., Olson, R.J., Pagendam, D.E., Point, D., Revill, A.T., Somes, C.J.,  
376 Young, J.W., 2020. Trends in tuna carbon isotopes suggest global changes in pelagic  
377 phytoplankton communities. *Glob Change Biol* 26, 458–470.  
378 <https://doi.org/10.1111/gcb.14858>
- 379 Manceau, A., Gaillot, A.-C., Glatzel, P., Cherel, Y., Bustamante, P., 2021. In Vivo Formation  
380 of  $\text{HgSe}$  Nanoparticles and  $\text{Hg}$ -Tetrarselenolate Complex from Methylmercury in  
381 Seabirds—Implications for the  $\text{Hg}$ -Se Antagonism. *Environ. Sci. Technol.*  
382 <https://doi.org/10.1021/acs.est.0c06269>
- 383 Médieu, A., Point, D., Receveur, A., Gauthier, O., Allain, V., Pethybridge, H., Menkes, C.E.,  
384 Gillikin, D.P., Revill, A.T., Somes, C.J., Collin, J., Lorrain, A., 2021. Stable mercury  
385 concentrations of tropical tuna in the south western Pacific ocean: An 18-year  
386 monitoring study. *Chemosphere* 263, 128024.  
387 <https://doi.org/10.1016/j.chemosphere.2020.128024>
- 388 Ménard, F., Lorrain, A., Potier, M., Marsac, F., 2007. Isotopic evidence of distinct feeding  
389 ecologies and movement patterns in two migratory predators (yellowfin tuna and  
390 swordfish) of the western Indian Ocean. *Marine Biology* 153, 141–152.  
391 <https://doi.org/10.1007/s00227-007-0789-7>
- 392 Nicklisch, S.C.T., Bonito, L.T., Sandin, S., Hamdoun, A., 2017. Mercury levels of yellowfin  
393 tuna (*Thunnus albacares*) are associated with capture location. *Environmental*  
394 *Pollution* 229, 87–93. <https://doi.org/10.1016/j.envpol.2017.05.070>
- 395 Olson, R.J., Young, J.W., Ménard, F., Potier, M., Allain, V., Goñi, N., Logan, J.M., Galván-  
396 Magaña, F., 2016. Chapter Four: Bioenergetics, Trophic Ecology, and Niche  
397 Separation of Tunas, in: *Advances in Marine Biology*. Elsevier, pp. 199–344.  
398 <https://doi.org/10.1016/bs.amb.2016.06.002>
- 399 Peng, S., Chen, C., Shi, Z., Wang, L., 2013. Amino Acid and Fatty Acid Composition of the  
400 Muscle Tissue of Yellowfin Tuna (*Thunnus Albacares*) and Bigeye Tuna (*Thunnus*  
401 *Obesus*). *Journal of Food and Nutrition Research* 4.
- 402 Pethybridge, H., Choy, C.A., Logan, J.M., Allain, V., Lorrain, A., Bodin, N., Somes, C.J.,  
403 Young, J., Ménard, F., Langlais, C., Duffy, L., Hobday, A.J., Kuhnert, P., Fry, B.,  
404 Menkes, C., Olson, R.J., 2018. A global meta-analysis of marine predator nitrogen  
405 stable isotopes: Relationships between trophic structure and environmental conditions.  
406 *Global Ecology and Biogeography* 27, 1043–1055. <https://doi.org/10.1111/geb.12763>
- 407 R Core Team, 2018. R: A language and environment for statistical computing; 2015. Vienna,  
408 Austria.
- 409 Ramos, L., Kristenson, E.M., Brinkman, U.A.T., 2002. Current use of pressurised liquid  
410 extraction and subcritical water extraction in environmental analysis. *Journal of*

- 411 Chromatography A, Sample Handling 975, 3–29. <https://doi.org/10.1016/S0021->  
412 9673(02)01336-5
- 413 Sardenne, F., Bodin, N., Chassot, E., Amiel, A., Fouché, E., Degroote, M., Hollanda, S.,  
414 Pethybridge, H., Lebreton, B., Guillou, G., Ménard, F., 2016. Trophic niches of  
415 sympatric tropical tuna in the Western Indian Ocean inferred by stable isotopes and  
416 neutral fatty acids. *Progress in Oceanography* 146, 75–88.  
417 <https://doi.org/10.1016/j.pocean.2016.06.001>
- 418 Sardenne, F., Bodin, N., Metral, L., Crottier, A., Le Grand, F., Bideau, A., Brisset, B.,  
419 Bourjea, J., Saraux, C., Bonhommeau, S., Kerzérho, V., Bernard, S., Rouyer, T.,  
420 2019a. Effects of extraction method and storage of dry tissue on marine lipids and  
421 fatty acids. *Analytica Chimica Acta* 1051, 82–93.  
422 <https://doi.org/10.1016/j.aca.2018.11.012>
- 423 Sardenne, F., Diaha, N.C., Amandé, M.J., Zudaire, I., Couturier, L.I.E., Metral, L., Le Grand,  
424 F., Bodin, N., 2019b. Seasonal habitat and length influence on the trophic niche of co-  
425 occurring tropical tunas in the eastern Atlantic Ocean. *Canadian Journal of Fisheries*  
426 *and Aquatic Sciences* 76, 69–80. <https://doi.org/10.1139/cjfas-2017-0368>
- 427 Sardenne, F., Ménard, F., Degroote, M., Fouché, E., Guillou, G., Lebreton, B., Hollanda, S.J.,  
428 Bodin, N., 2015. Methods of lipid-normalization for multi-tissue stable isotope  
429 analyses in tropical tuna: Lipid-normalization for multi-tissue SIA in tuna. *Rapid*  
430 *Communications in Mass Spectrometry* 29, 1253–1267.  
431 <https://doi.org/10.1002/rcm.7215>
- 432 Sirot, V., Leblanc, J.-C., Margaritis, I., 2012. A risk–benefit analysis approach to seafood  
433 intake to determine optimal consumption. *Br J Nutr* 107, 1812–1822.  
434 <https://doi.org/10.1017/S0007114511005010>
- 435 Száková, J., Koliňová, D., Miholová, D., Mader, P., 2004. Single-Purpose Atomic Absorption  
436 Spectrometer AMA-254 for Mercury Determination and its Performance in Analysis  
437 of Agricultural and Environmental Materials. *Chem. Pap.* 5.
- 438 Wang, F., Outridge, P.M., Feng, X., Meng, B., Heimbürger-Boavida, L.-E., Mason, R.P.,  
439 2019. How closely do mercury trends in fish and other aquatic wildlife track those in  
440 the atmosphere? - Implications for evaluating the effectiveness of the Minamata  
441 Convention. *Science of the Total Environment* 13.
- 442 World Health Organization, UNEP Chemicals, 2008. Guidance for identifying populations at  
443 risk from mercury exposure. UNEP DTIE Chemicals Branch and WHO Department of  
444 Food Safety, Zoonoses and Foodborne Diseases, Geneva, Switzerland.
- 445 Young, J.W., Guest, M.A., Lansdell, M., Phleger, C.F., Nichols, P.D., 2010. Discrimination  
446 of prey species of juvenile swordfish *Xiphias gladius* (Linnaeus, 1758) using signature  
447 fatty acid analyses. *Progress in Oceanography, CLimate Impacts on Oceanic TOp*  
448 *Predators (CLIOTOP)* 86, 139–151. <https://doi.org/10.1016/j.pocean.2010.04.028>  
449

## Highlights

- Scarcity of tuna samples makes essential to get the most out of a single sample
- Dichloromethane is more efficient than cyclohexane to extract lipids
- Dichloromethane extraction has no effect on Hg levels
- Bulk and lipid-free tropical tuna samples can be used jointly to infer Hg levels

### **Competing interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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