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1                                   **MEASUREMENT OF TRITIUM IN THE FREE WATER OF MILK**

2                   **SPOTTING AND QUANTIFYING SOME BIASES AND PROPOSING WAYS OF IMPROVEMENT**

3  
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16  
17 **Abstract**

18 As one of the three natural isotopes of hydrogen, tritium is ubiquitous and might potentially  
19 be present in any water or organic molecule that constitutes a biological matrix. Milk is one of  
20 the most frequently monitored foodstuffs in the vicinity of chronic release of radionuclides, as  
21 it is a very common product and also because it integrates deposition on large areas of grass at  
22 a local scale. Different parameters have been studied to assess their impact on the reliability  
23 of tritium measurements. The volume of the sample, the technique used to extract the water  
24 and the level of dehydration modulate the results but in different ways: dispersion of results,  
25 under- or overestimation of the tritium activity. The influence of sample storage and  
26 preparation has also been investigated. Methodological improvements of tritium  
27 measurements in the free water of milk are proposed.  
28

29 **Key words**

30 Tritium measurement

31 Free water

32 Isotopic fractionation

33 Environment

34

35 **Highlights**

36 Biases in tritium assay are caused by the conditions in which the water is extracted

37 Isotopic fractionation does not fit with the Rayleigh formula when milk is distilled

38 Recommendations are made to improve tritium activity measurement

## 39 Introduction

40

41 *Among* the unstable isotopes released by the nuclear industry, the quantities of tritium  
42 reaching the environment are usually small and generally fit easily with regulatory limits. As  
43 tritium was massively released during the atmospheric nuclear tests between 1945 and 1980, it  
44 has become widely dispersed in the environment and in food chains. Its quantity in the  
45 atmosphere peaked in 1963 and has been decreasing ever since. It is now mainly localized in  
46 the water of oceans (about 99 %) (Jacobs, 1968; Weaver et al., 1969)(UNSCEAR, 2008).  
47 Nevertheless, tritium, along with  $^{14}\text{C}$  and noble gases remain the dominant radionuclides  
48 released into the atmosphere by the nuclear industry. The main anthropic sources are weapon  
49 facilities, nuclear power plants, reprocessing facilities, the production and use of labelled  
50 compounds for medical use, research or even self-powered lighting products and research  
51 facilities for nuclear fusion (Guétat et al., 2008; IRSN/DEI, 2010).

52

53 Being an isotope of hydrogen, tritium can be incorporated into almost all components of  
54 biological systems: water (HTO) or organic molecules (Diabaté and Strack, 1993) (so-called  
55 Organically Bound Tritium or OBT). When dealing with OBT, two categories of atomic  
56 bonds are generally distinguished:

57 - binding to a nitrogen, oxygen or sulphur atom, i.e. labile bonds. It can easily be  
58 exchanged with labile hydrogen of other functional groups or molecules in its  
59 near vicinity, especially water; this fraction is called exchangeable Organically  
60 Bound Tritium (eOBT).

61 - binding to a carbon atom. Such covalent bounds are stable and therefore  
62 hydrogen atoms (or isotopes) are incorporated in the metabolic cycle of each  
63 molecule with more or less complex and lengthy features. This latter type of

64 bound tritium is known as non-exchangeable Organically Bound Tritium  
65 (neOBT).

66

67 Exposure of individuals depends on the type of the tritiated molecule(s) incorporated as well  
68 as on its/their metabolism. When tritium originates from tritiated water release and is further  
69 integrated in the food chain by, for example, going through photosynthesis (see further details  
70 in Boyer et al., 2009), some simplifications are considered to define a single “dose per unit  
71 intake factor” (ICRP 1989, 1997):

- 72 - considering exchangeable/non exchangeable proportions to be equal,
- 73 - considering an average biological half-life of 40 days for all non-exchangeable  
74 tritium of all organic molecules.

75

76 When using liquid scintillation counting, measurements of tritium specific activity on water  
77 allow the lowest decision threshold to be reached. Laboratories that measure tritium in  
78 environmental samples frequently use a same protocol:

- 79 - extraction of the free water of the sample and measure (Free Water Tritium),  
80 then
- 81 - on the one hand: oxidisation of the dry fraction resulting in the production of  
82 combustion water, then measurement of total organic tritium (i.e. the sum of  
83 eOBT and neOBT),
- 84 - on the other hand: isotopic exchange of hydrogen isotopes by washing the dry  
85 fraction with tritiated water, thus a second extraction of water to measure (if  
86 possible) eOBT and oxidisation of the “washed” and dried fraction to measure  
87 the neOBT as combustion water.

88

89 Frequently, eOBT is not measured but deduced from the following simple relation:

90

$$91 \quad eOBT = OBT - neOBT \quad (1)$$

92

93 Every isotope or inaccuracy effect in every step of the procedure may induce errors in the  
94 measurement of the specific activity of extracted free water and of OBT (Baumgärtner and  
95 Kim, 1990; Kim and Baumgärtner, 1991).

96

97 Usually, water is extracted from fresh samples or after isotopic rinsing by at least one of the  
98 following four techniques:

99

- 100 - filtration: it allows quick and easy recovery of the main part of the dry matter,  
101 except soluble molecules which are in the filtrate. The bias induced depends on  
102 the filtration technique (i.e. characteristics of the filter) and on the nature of the  
103 sample. Retentate and distillate both need further treatment prior to  
104 measurement.
- 105 - distillation: it is performed under atmospheric pressure or under reduced  
106 pressure, it allows the recovery of almost pure water. Under reduced pressure, it  
107 is possible to completely distil at lower temperature (which induces less  
108 degradation of organic samples), to prevent the risk of contamination of  
109 extracted water by pyrolytic products (Wood et al., 1993) and to limit the isotopic  
110 effect during evaporation.
- 111 - azeotropic distillation extracts water at lower temperatures than distillation. As it  
112 uses organic compounds, it is more difficult to perform and it can additionally  
113 induce contamination of the dry matter by hydrocarbons.

114 - Freeze drying: i.e. extraction of water via sublimation; it has the same  
115 advantages as distillation under reduced pressure. The size of the apparatus, the  
116 temperature of the cold trap (usually  $> -20^{\circ}\text{C}$ ) and the time required to  
117 completely extract the water may induce biases by condensing atmospheric  
118 vapour before starting or during the process.

119

120 Repetition of measurements performed in our laboratory on the free water of a given tritiated  
121 milk obtained by distillation under reduced pressure or by freeze-drying, have shown certain  
122 systematic errors and dispersion of the values beyond the basic uncertainties of the  
123 measurements. The reasons underlying these differences have been sought and improvements  
124 of the reliability of tritium measurements are proposed.

125

126 Four possible hypothetical origins of the observed differences in measured specific activities  
127 were identified:

128 - the influence of sample storage: as ambient levels of tritium at the Valduc Centre  
129 of the French Atomic Agency can be higher than those of the environment where  
130 the samples were collected, they may become significantly more tritiated during  
131 their storage.

132 - the influence of the technique of water extraction: the usual techniques of  
133 dehydration differ from each other by their conditions of pressure, temperature  
134 and the apparatus used. These different factors may lead to biases in the  
135 measurement.

136 - the influence of the mass of sample: as each water removal technique has a  
137 specific dead volume and a specific geometry, the global yield of dehydration

138 can be influenced and thus be the origin of a bias in the measurement of specific  
139 activity.  
140 - the influence of the final degree of dehydration: if isotopic fractionation occurs  
141 during water removal, the final level of dehydration will influence the specific  
142 activity measured.

143

## 144 **1 Materials and Methods**

145

146 Water was extracted from nineteen aliquots of the same milk sample (collected in the vicinity  
147 of the Valduc Centre of the French Atomic Agency) using three different dehydration  
148 techniques. The experiments were completed with twenty-one measurements performed on  
149 milks collected for our routine activity. Each time, weights of fresh milk, of dry matter and of  
150 collected water were noted.

151

### 152 ***1.1 Analytical method***

153

154 Specific tritium activities were measured by liquid scintillation counting (PerkinElmer Tri-  
155 Carb 2910 TR) with an overall precision ( $2\sigma$ ) of  $\pm 17\%$ . The scintillator used was Ultimagold  
156 LLT (Packard). Quenching effects of the measuring system were carefully examined and the  
157 results measured corrected accordingly.

158

### 159 ***1.2 Storage of samples***

160

161 The commercially available source water Volvic is considered to have very low levels of  
162 tritium. It is commonly used in laboratories as a blank. In order to check if storage of samples



163 in Valduc induced biases in the measurement of the specific activity, samples of Volvic water  
164 were stored in different conditions and their specific activity was been measured after 6 h, 16  
165 h, 24 h, 48 h, 96 h, 1 week, 2 weeks, 3 weeks or 30 days of storage.

166

167 Modifications in the conditions of storage were performed to test the influence of the  
168 temperature and the type of bottle in which the samples were stored.

169

170 Four sets of nine samples of 50 mL of Volvic water were stored in 150 mL polyethylene  
171 bottles at -25°C, 3°C, 20°C and 40°C.

172

173 Five other sets of nine samples of Volvic were stored in different kind of bottles:

174 - 50 mL of Volvic water in 150 mL high density polyethylene (HDPE)

175 bottles,

176 - 150 mL of Volvic water in the same kind of bottles,

177 - 50 mL of Volvic water in the same bottles placed in double welded

178 vinyl bags,

179 - 20 mL of Volvic water in 20 mL glass bottles,

180 - 20 mL of Volvic water in 20 mL HDPE bottles (usually used for

181 scintillation counting)

182

183 1 L of Volvic water was also stored in an open 1.5 L bottle. 10 mL were sampled after 6 h, 16

184 h, 24 h, 48 h, 96 h, 1 week and 2 weeks of storage. This experiment was shorter than the

185 others since there was no water left in the bottle after the seventh sampling (due to

186 evaporation and aliquot removal).

187

188 The results presented below are the means of four repetitions.

189

### 190 **1.3 Techniques of water removal**

#### 191 **1.3.1 Comparison of common techniques**

192 Three commonly used techniques of dehydration were compared pairwise:

- 193 - distillation under reduced pressure using a distillation bridge with a Liebig
- 194 condenser
- 195 - distillation under reduced pressure with a rotating evaporator (Buchi Rotavapor
- 196 R200 or Buchi Rotavapor RE 121 equipped with Buchi 471 Oil bath)
- 197 - freeze drying using a Heto Drywinner PL3000.

198

199 Equal quantities of samples were used in each group of paired samples. In the first technique,  
200 the sample was introduced in a 1 L Erlenmeyer flask in a water bath at 55°C. The flask was  
201 fitted with a splash head (to prevent or limit the sample from spurting in the apparatus during  
202 distillation). The distillate was collected from a Liebig condenser containing a flow of 3°C  
203 thermostated water. Its dead volume was 0.69 mL ± 0.25 mL. The distillation bridge was  
204 connected to a Vacuubrand ME 2C pump working at full capacity. The condensate was  
205 recovered in an Erlenmeyer also at in a water bath at 3°C. At the end of dehydration, the first  
206 water bath was heated to 70 °C.

207

208 Distillation under reduced pressure was also conducted using rotating evaporator. This  
209 technique differs from the previous one by the apparatus used. One of the major differences  
210 between them is the geometry of the condenser: a rotating evaporator is equipped with a  
211 diagonal spiral condenser which has a dead volume of about 7 mL. Samples are introduced in  
212 a 1 L flask which is then connected to a rotating evaporator (Buchi Rotavapor R200 or Buchi

213 Rotavapor RE 121 equipped with Buchi 471 Oil bath) connected to a pump (Vacuubrand ME  
214 2C) which is also used at its full capacity. Condensed vapours are recovered in a flat-  
215 bottomed flask. As with the previous technique, the water bath was thermostated first at 55°C  
216 and then at 70°C. The water flowing in the condenser was thermostated at 3°C.

217

218 Freeze drying was performed with a modified Heto Drywinner PL 3000. Samples were  
219 introduced in acrylic pots connected to a manifold which is connected to a glass insert. The  
220 insert was placed in the cold trap of the Heto Drywinner PL 3000 (temperature: - 55 °C) to  
221 allow the required decontamination of the cold trap between samples thus avoiding “memory  
222 effects”. The manifold was also connected to the pump (Adixen Pascal 1005) used for general  
223 vacuum applications. Pressure in the system was < 0.5 hPa. After complete dehydration of the  
224 samples, the glass insert was removed from the system and immediately sealed to avoid  
225 contamination of the extracted water with atmospheric moisture until the ice has completely  
226 thawed.

227 Dehydration techniques were compared by pairwise to improve the power of the statistic tests  
228 (9 repetitions to compare freeze drying and rotating evaporator and 8 for freeze drying vs.  
229 distillation bridge and 8 for rotating evaporator vs. distillation bridge).

### 230 **1.3.2 Test of the reliability of freeze drying and distillation**

231 The specific activity of tritiated pure water (type 3 produced by RiOs 3 Water Purification  
232 System (Merck Millipore)) were measured in three cases:

- 233 - without other treatment,
- 234 - after being distilled under reduced pressure using a distillation bridge (as  
235 described above),
- 236 - after being freeze-dried (in the conditions described above). For each case, 3  
237 aliquots of  $49.9 \text{ g} \pm 0.1 \text{ g}$  were prepared.

### 238 **1.3.3 Influence of ambient atmosphere on freeze drying**

239 To detect possible external contamination, two kinds of experiments were performed:

240

241 Measurement of the specific activity of water extracted by freeze drying in two different  
242 ambient atmospheres: one in the Valduc Centre (in the conditions described above) and one in  
243 Besançon (25-France) where the specific activity of the atmosphere in HTO is below the  
244 decision threshold. The milk was separated into 7 samples of  $51.23 \text{ g} \pm 0.45 \text{ g}$ . They were  
245 frozen in Valduc in plastic bottles inserted in double welded vinyl bags. Three were freeze-  
246 dried in Valduc, three in Besançon and one was distilled using a distillation bridge in the  
247 Valduc Centre in the conditions described above. Freeze drying at Besançon was performed  
248 in a Cosmos 20k (Cryotec). Vacuum was generated by a pump (Adixen Pascal 2005-Ci)  
249 working at full capacity. After the end of freeze drying, the water was recovered by heating  
250 the condenser. The specific activity of each recovered water sample was measured in the  
251 Valduc Centre and compared.

252

253 Empty freeze drying: Drywinner Heto PL 3000 was used empty three times for 5-7 days at  
254 Valduc Centre. A commercial bubbling system (MARC 7000-SDEC France) was used to  
255 monitor the atmospheric tritium levels during the third repetition. After the end of freeze  
256 drying, the mass of the cold trap was measured and compared to its mass when empty. Then,  
257 10 mL of non-tritiated water was inserted into the cold trap to recover possible traces of water  
258 trapped during freeze drying. The specific activity of the water in the cold trap was measured,  
259 taking into account the dilution and compared to the specific activity of the water in the pots  
260 of the bubbling system.

261

### 262 **1.4 Sample mass**

263

264 To test the influence of the quantity of the sample on the reliability of measures, 6 masses  
265 were considered (about 15, 30, 60, 120, 240 and 480 g) and samples were treated by two of  
266 the three previously presented methods: distillation under reduced pressure using a distillation  
267 bridge or a rotating evaporator. Most of the sets were composed of two samples prepared with  
268 the distillation bridge and one with the rotating evaporator. The central point at 30-38 g was  
269 composed of three more samples treated with the rotating evaporator. The highest mass was  
270 only composed of one measurement since in other repetitions milk spurted throughout the  
271 apparatus until there was none left.

272

### 273 **1.5 State of dehydration**

274

275 The weight of fresh milk before treatment, and of dry matter and water after dehydration on  
276 the other hand, provide correlation between the mass of water extracted from milk and the  
277 specific activity measured.

278

279 “Sequential distillations” of milk were performed. The apparatus used in these experiments is  
280 illustrated in **Fig. 1**. A sample of about 300 mL was introduced in a 1 L Erlenmeyer flask in a  
281 55°C bath. The flask was connected to a splash head to prevent or limit the sample from  
282 spurting into the apparatus during distillation. When the system is under reduced pressure,  
283 vapours flow to a condenser at 3°C and connected to a Vacuubrand ME 2C pump working at  
284 full capacity. The condensed vapour then falls into a dropping funnel. Each 10-30 mL (23 mL  
285 on average), it is opened to let the water flow into a 50 mL Erlenmeyer. Once the dropping  
286 funnel is empty, it is closed to collect the next aliquot and the water is collected from the 50  
287 mL Erlenmeyer and weighed. The experiment is pursued until the sample is completely dry.

288 When possible, the dry matter of the sample is freeze-dried to collect any water which could  
289 remain. The specific activity of each aliquot is measured.

290

291 Fig. 1: Apparatus used for "sequential distillations"

292

293 Sequential distillations were carried out on four different milks collected for our routine  
294 measurements.

## 295 **2 Results and discussion**

296

### 297 **2.1 Influence of samples storage**

298

299 Only one set of samples showed any significant change in its specific activity during storage:  
300 the Volvic water stored in an open bottle (**Fig. 2**). The specific activity of the water increased  
301 until it reached equilibrium with the atmospheric water vapour ( $178.5 \pm 133.0 \text{ Bq L}^{-1}$ ) after  
302 two weeks.

303

304 Fig. 2: Specific activity of Volvic water stored in an open bottle versus duration of storage. The first point  
305 having a specific activity under the limit of detection was plotted as having a specific activity of  $0 \text{ Bq L}^{-1}$ .

306

307 All the other results of the experiments remained below the decision threshold ( $2.8 \text{ Bq L}^{-1}$ )  
308 during storage.

309

310 This proves that storage in well closed plastic or glass bottles is able to prevent the marking of  
311 the samples from the laboratory environment even in the case of the relatively tritiated  
312 atmosphere of a nuclear centre. Nevertheless, to avoid any cross contamination, we decided to  
313 store samples at  $-20^\circ\text{C}$  (to preserve organic matter during storage) and in double packaging  
314 (bottle + sealed vinyl pocket or double sealed vinyl pocket) to avoid any unintentional  
315 marking.

316

## 317 **2.2 Influence of the technique of dehydration**

### 318 **2.2.1 Comparison of three common techniques**

319 The specific activity of water extracted from milks using the three techniques previously  
320 described was measured (mean: 60.4 g). The three techniques were not performed on each of  
321 the collected milk samples. **Fig. 3** shows how the results are distributed.

322

323 Fig. 3 : Comparison of the distributions of measured specific activities of water extracted using the three  
324 different methods described in this study. Central boxes represent the values from the lower to upper quartile.  
325 Middle lines represent the median. Vertical lines extend from the minimum to the maximum value of each  
326 population, which are represented by horizontal lines at their extremity. The specific activity of the water  
327 extracted by freeze drying is significantly higher than the specific activity of the water extracted by each of the  
328 two distillation methods tested (Wilcoxon test,  $p < 0.01$ ). Differences between the two techniques of distillation  
329 were not significant.

330

331 On the one hand, both methods of distillation (distillation bridge and rotating evaporator)  
332 gave similar median concentrations but the rotating evaporator technique showed a standard  
333 deviation 22 % higher than the distillation bridge. On the other hand, the measured specific  
334 activities of water extracted by freeze drying were 40% higher when compared to the results  
335 obtained with a rotating evaporator.

336 Three experiments were performed to explain these results.

337

### 338 **2.2.2 Reliability of distillation and freeze drying**

339 The specific activity of pure water was measured with or without a complementary treatment  
340 (i.e. distillation or freeze drying) performed in the Valduc Centre. The results of this  
341 experiment are presented in **Fig. 4**.



342

343 **Fig. 4 :** Comparison of specific activities measured on water with and without treatment (distillation or freeze  
344 drying)  
345

346 Distillation had no significant incidence on the measured specific activity of extracted water  
347 (+ 2.9 %) whereas freeze drying led to a clear increase (+ 42.9 %).

### 348 **2.2.3 Influence of ambient atmosphere on freeze drying**

349 First, to test the effect of ambient air during freeze drying, two sets of three 50 g-aliquots of  
350 the same milk sample were freeze-dried, one set in Besançon and one in the Valduc Centre.  
351 Freeze drying in both the Valduc Centre and Besançon led to almost complete extraction of  
352 the water, i.e. 88% of the weight of the total sample without any significant difference  
353 between samples (standard deviation: 0.09 %). Nevertheless, the comparison of the specific  
354 activities of the two sets of samples did show significant differences (see **Fig. 5**).

355

356 **Fig. 5 :** Specific activities of water extracted from milk by means of freeze drying performed in Besançon and in  
357 the Valduc Centre.  
358

359 This difference can only be explained by a (de)marking of the extracted water by the  
360 condensation of atmospheric water vapour. This phenomenon can occur during freeze drying  
361 by leaks in the apparatus, or before freeze drying by condensation of atmospheric water on the  
362 frozen sample or even after freeze drying when the vacuum is broken to recover the  
363 condensed water. Note that the observed deviation after freeze drying in Besançon is high  
364 regarding the little difference of specific activities between atmospheric water ( $< 5.8 \text{ Bq L}^{-1}$ )  
365 and free water of milk (about  $20 \text{ Bq L}^{-1}$ ).

366

367 The apparatus used in the Valduc Centre avoided the risk of exchange during melting of  
368 frozen condensate but this was not the case in Besançon. This can explain why the impact of  
369 condensed atmospheric vapour was so significant in Besançon whereas the differences in

370 specific activities remained small. In laboratories specifically equipped for measurement of  
371 tritium (for example with a small cold trap which can be isolated from the atmosphere while  
372 the condensed water is recovered), the biases would be, at worst, in the measurement  
373 uncertainty interval.

374

375 Secondly, empty freeze dryings were run in the Valduc Centre. They showed recovery of  
376 water with a significant specific activity. The results are presented in Table 1.

377

378 **Table 1: Mass and specific activity of water recovered after empty freeze dryings performed in the Valduc**  
379 **Centre**

Duration of freeze drying (h)	Mass of recovered water (g)	Specific activity of the recovered water (Bq L <sup>-1</sup> )
168	0.4	200
168	0.0	No measurement
146	0.8	257

380

381 The results of the first and third freeze dryings fit well with the results presented in **Fig. 4**. For  
382 example, 0.8 mL of “parasite” water with a specific activity of 257 Bq L<sup>-1</sup> can explain an  
383 overestimation of the specific activity of about + 4 Bq L<sup>-1</sup> in a sample of 47.5 mL in which the  
384 specific activity is about 23 Bq L<sup>-1</sup>.

385

386 Nevertheless, the mass of water collected during freeze drying appears variable, as does its  
387 specific activity. This indicates that an intermittent mechanism (most probably depending on  
388 atmospheric conditions) leads to the pollution phenomenon.

389

390 In the third repetition, the specific activity of the recovered water was about  $257 \text{ Bq L}^{-1}$  where  
391 the specific activity of atmospheric vapour measured by bubbling during freeze drying was  
392 only  $90.8 \text{ Bq L}^{-1}$ . This means in our opinion that, in addition to a possible leak in the  
393 apparatus (before the cold trap), four other ways of contamination might be suspected:

- 394 - as the sample is frozen before freeze drying, atmospheric water is able  
395 to condense at its surface in the time lapse between storage and  
396 introduction into the freeze drying system;
- 397 - the water vapour in the air present in the freeze drying system (about 6  
398 L in the case of the Heto Drywinner PL 3000) is prone to condense  
399 starting from the moment when the cold trap is at its set-point  
400 temperature to the instant when vacuum is established in the system;
- 401 - the water vapour in air that fills the freeze drying system when the  
402 vacuum is broken at the end of freeze drying can also condense in the  
403 cold trap;
- 404 - some exchanges may occur from the atmosphere to the condensed water  
405 while the water melts in the cold trap for recovery.

406

407 It appears that during the different steps of a freeze drying process, some atmospheric water  
408 can be condensed or exchange and therefore be mixed to the extracted free water of the  
409 samples. The efficiency measured in water recovery is of the order of 97 %. The mass of  
410 external water cannot be identified in the different tests because it surely compensates sample  
411 water which is lost at the same time. This phenomenon was fortunately discovered due to the  
412 ambiance in the Valduc Centre that is sufficiently tritiated to be measured. Nevertheless,  
413 marking of extracted water during freeze drying may occur in other laboratories with very low

414 tritiated atmosphere. In that case, the water extracted would be “demarked” with non-tritiated  
415 water, even if it is within the uncertainty interval of measure.

416

417 One way to limit the deviation due to this pollution of the extracted water would be to freeze  
418 dry larger samples to dilute the effect in the water extracted from the sample or to operate  
419 freeze drying in a dry atmosphere.

420

### 421 **2.3 Influence of the mass of sample in distillation techniques**

422

423 The free water of nineteen aliquots from the same milk sample was extracted by distillation  
424 using a rotating evaporator or a distillation bridge. The results of this series of experiments are  
425 presented in **Fig. 6**. As expected, the nineteen specific activities measured are well described  
426 by a Gaussian distribution. Uncertainties ranged from 14 % for higher specific activities to  
427 17 % for lower specific activities.

428

429 Fig. 6: Specific activity of extracted water as a function of the mass of treated samples and  
430 normality of the distribution of density of measured specific activities

431

432 Small samples (< 60 g) and large samples ( $\geq 60$  g) present almost the same average value,  
433 respectively  $22.3 \text{ Bq L}^{-1}$  and  $22.8 \text{ Bq L}^{-1}$ , but different standard deviations:  $2.17 \text{ Bq L}^{-1}$  and  
434  $0.88 \text{ Bq L}^{-1}$ . The variation of these average values may be explained by differences in the  
435 proportions of water extracted in each case (respectively 84.4% vs. 85.9 %).

436

437 Treating very large samples (> 100 g) is not easy: experimentally we observe that the milk is  
438 boiling and spurting very rapidly after the beginning of each repetition. This phenomenon is  
439 prone to contaminate both extracted and condensed water. Treating small samples allowed the

440 use of larger vessels compared to the sample volume: a difference of a factor of 5 between the  
441 volumes of the Erlenmeyer flask and the sample is sufficient to limit spurting.

442 Each distillation technique also showed its own limitations with regard to the mass of the  
443 samples treated.

444

445 **Fig. 7:** Proportion of water extracted (in % of weight of total sample) versus mass of sample treated. The mean  
446 proportion of free water obtained was evaluated by measuring the dry mass of each sample remaining after 5 h in  
447 a forced-air oven at 102°C.  
448

449 While the rotating evaporator efficiently dehydrates only samples between 100 g and 400 g,  
450 the distillation bridge is efficient for a larger range of sample volumes (only one dehydration  
451 was not complete as it was interrupted to soon) (Fig. 7).

452

453 In these experiments, distillations of 60-100 g milk samples under reduced pressure using a  
454 distillation bridge gave the best results with a limited dispersion of the measured specific  
455 activities.

## 456 **2.4 Influence of the state of dehydration**

457

458 The specific activities measured were viewed with respect to the state in which each  
459 experiment was ended. The results are presented in Fig. 8. The specific activity appears to  
460 increase slightly with the degree of dehydration but neither Spearman's nor Student's  
461 correlation tests revealed a correlation between the two parameters.

462

463 **Fig. 8 :** Specific activity of extracted water versus the degree of dehydration

464 The line shows the same relationship modelled by relation (10) with  $p = 0.04$ ,  $\alpha = 1.14$ , and  $\beta = 11.7$ .

465

466 Further experiments were then carried out with sequential distillations performed on different  
 467 milks. The results are shown in Fig. 9 and Fig. 10. To facilitate comparison of the results,  
 468 specific activities are expressed as relative activities (1 corresponds to the mean specific  
 469 activity at the end of each sequential distillation) and levels of water extraction are expressed  
 470 as a % of the total mass of sample at the end of each distillation. In Fig. 9 the specific activity  
 471 of each aliquot seems to follow a two-component function.

472

473 **Fig. 9** : Specific activity of aliquots of extracted water versus the proportion of water extracted

474 The line plots the variation modelled with equation (11) with  $p = 0.04$ ,  $\alpha = 1.14$ , and  $\beta = 11.7$ . Each type of label  
 475 represents a set of repetition. Four repetitions were performed on one sample of milk (squares), two on a second  
 476 sample (triangles) and lozenges represent a set performed on a third sample. Mean specific activity of extracted  
 477 water = 1.

478

479 **Fig. 10** : Mean specific activity versus proportion of water extracted. The solid line was computed using relation  
 480 (10) with  $p = 0.04$ ,  $\alpha = 1.14$ , and  $\beta = 11.7$ . Each type of label represents a repetition set. Four repetitions were  
 481 performed on one sample of milk (squares), two on a second (triangles) and lozenges represent a set performed  
 482 on a third. Mean specific activity of extracted water = 1

483

484 Isotopic fractionation during distillation is generally described by means of the Rayleigh  
 485 equation that was first derived for fractional distillation of mixed liquids (Rayleigh and Strutt,  
 486 1902).

$$487 \quad \frac{X}{X_0} = \left( \frac{n}{n_0} \right)^{\alpha} \quad (2)$$

488 where:

489  $n_0$ : is the initial number of all moles of all species in the sample

490  $n$ : is the number of all moles of all species in the residual sample

491  $X_0$ : is the initial mole fraction of HTO in the sample

492 X: is the mole fraction of HTO in the residual sample

493  $\alpha$ : is the vapour-liquid fractionation factor

494

495 Kim and Baumgärtner (1997) reported that tritium enrichment on distillation of pure

496 HTO/H<sub>2</sub>O can be calculated using the Rayleigh formula taken under the following form (3):

497 
$$A_r V_r = A_0 V_0 \left( \frac{V_0}{V_r} \right)^\alpha \quad (3)$$

498 Where:

499  $A_r$  : is the specific activity of residual water

500  $A_0$  : is the initial specific activity of the water sample

501  $V_r$ : is the volume of residual water

502  $V_0$ : is the initial volume of water in the sample

503  $\alpha$ : is the vapour-liquid fractionation factor

504

505 Under equilibrium vaporization conditions,  $\alpha$  may be equivalent to the vapour pressure

506 isotope effect (VPIE) that can be calculated theoretically (Van Hook, 1968) or determined

507 experimentally (Baumgärtner and Kim, 1990) under given approximations. The VPIE

508 corresponds to the definition provided in (4) and is considered equivalent to the separation

509 factor ignoring the corrections accounting for both a non-ideal liquid and gas phases (Jancso

510 and Van Hook, 1974; Kakiuchi, 2000):

511

512 
$$VPIE = \frac{P_{H_2O}}{P_{HTO}} \approx \alpha = \frac{(X_T / X_H)_L}{(X_T / X_H)_V} \quad (4)$$

513

514  $P_{H_2O}$  and  $P_{HTO}$  are the vapour pressures of pure water and pure tritiated water, respectively,  
 515  $X_T$  and  $X_H$  stand respectively for the molar fractions of HTO and water, in the liquid (L) and  
 516 vapour (V) phase.

517

518 In order to fit with our experiments, residual volume ( $V_r$ ) and specific activity ( $A_r$ ) were  
 519 replaced in (3) by extracted volume ( $V_e$ ) and specific activity ( $A_e$ ) using relations (5) and (6).

520 Equation (7) is thus obtained:

$$521 \quad V_e = V_0 - V_r \quad (5)$$

$$522 \quad V_e A_e = A_0 V_0 - A_r V_r \quad (6)$$

523

524 By considering (3):

$$525 \quad V_e A_e = A_0 V_0 \left( 1 - \left( \frac{V_0}{V_0 - V_e} \right)^{\frac{-1}{\alpha}} \right) \quad (7)$$

526 Lastly, each aliquot sampled during the distillation has a specific activity ( $A_{(1-2)}$ ) which is the  
 527 mean value of (7) between  $V_1$  and  $V_2$ , respectively the volume of water extracted at the  
 528 beginning and at the end of the extraction of the given aliquot (8):

529

$$530 \quad A_{(1-2)} = \frac{A_0 V_0}{V_2 - V_1} \left[ \left( 1 - \left( \frac{V_0}{V_0 - V_2} \right)^{\frac{-1}{\alpha}} \right) - \left( 1 - \left( \frac{V_0}{V_0 - V_1} \right)^{\frac{-1}{\alpha}} \right) \right] \quad (8)$$

531

532 Equation (8) fits experimental data provided by distillation of pure HTO/H<sub>2</sub>O (Fig. 11). In  
 533 these experiments,  $\alpha$  was evaluated at 1.14 which is 6.7 % higher than expected in our  
 534 experimental conditions (Baumgärtner and Kim, 1990). This difference may be explained by  
 535 the specificities of the apparatus used which seem to increase the height by the equivalent of a  
 536 theoretical plate (HETP) and thus  $\alpha \square$  (Fukada, 2004)  $\square$



537

538 **Fig. 11 :** Extraction of HTO during distillation of pure HTO/H<sub>2</sub>O versus proportion of free water extracted  
539 Lines are calculated by equations (7) and (9). Triangles and circles correspond to experimental values. Mean  
540 specific activity of extracted water = 1  
541

542 Conversely, with samples of milk, at percentages of free water extracted higher than ~ 95 %,  
543 (7) and (8) generally fail at modelling the observed experimental behaviour of the relative  
544 activity of extracted water, due to the drastic increase in relative activity measured at the end  
545 of the water extraction process (Fig. 9). When most of the water is extracted from the milk,  
546 the remaining part of the sample in the boiler is likely to behave as a non-ideal solution. It is  
547 thus necessary to introduce a correction in (7) and thus in (8). Such a correction may be  
548 obtained in two main ways: a complete theoretical description of the sources of non-ideality  
549 or a blind parameterization of the observed effect. As for the theoretical description, in its  
550 simplest form a model may at least take account of two kinds of water, cosphere (hydration)  
551 water in the immediate neighbourhood of solute particles or molecules and bulk water which  
552 retains the properties of the pure solvent (Jancso and Van Hook, 1974). In this part of the  
553 study, we will try to parameterize the observed effect and confine our work to the  
554 consequences of this behaviour on the metrology of tritium specific activity.

555

556 To take account of the drastic increase observed at the end of the water extraction, equation  
557 (7) is parameterized by introducing two dimensionless free parameters  $p$  and  $\beta$  in the  
558 following manner:

559  (9)

560 The specific activity of an aliquot is thus given by (10):

561  (10)

562

563 The experimental data presented in Fig. 9 can be modelled using relation (10). The best  
564 values for  $p$ ,  $\alpha$ , and  $\beta$  (which are respectively 0.04, 1.14, 11.7) were estimated by a function in  
565 the R software (R Core Team, 2012) which carries out minimization of a function ( $f$ ) using a  
566 Newton-type algorithm. In R software, this function is called `nlm`. Each first aliquot of the  
567 different repetitions has a variable specific activity as compared to the mean final specific  
568 activity of the set. This is most probably an artefact linked to the experimental conditions. It is  
569 noticeable in Fig. 10 that this value has a perceptible impact on the mean specific activity of,  
570 say the 4-5 first aliquots. Using the model based on relation (9) we learn that even with a  
571 (hypothetically) perfect dehydration apparatus, if the dehydration is interrupted when 10% of  
572 water remains (a situation that may happen if the temperature is too low, the pressure too high  
573 or the dehydration simply is uncompleted); the measured specific activity of the extracted  
574 water should thus be underestimated by about 9 %.

## 575 ***2.5 The dead volume, an example of a combination of sources of*** 576 ***biases***

577

578 In light samples (ranging from 15 g to 60 g), the measured specific activities of water  
579 extracted with a rotating evaporator were systematically lower than those obtained with a  
580 distillation bridge. Additional experiments were performed to explain this particular point.  
581 The rotating evaporator condenser indeed has a dead volume estimated to be  $7.0 \text{ mL} \pm 1.2$   
582 mL. In other words, 7 mL must reach the condenser before the first drop of distillate is  
583 observed and 7 mL remain in the condenser at the end of distillation. A model of the time-  
584 course of the specific activities in the condenser and in the distillate during distillation based  
585 on an isotopic fractionation was set up.

586

587 Let us now consider the following assumptions as axiomatic:

- 588 - as described above isotopic fractionation exists,
- 589 - in the condenser of the rotating evaporator, equilibrium between vapour and
- 590 condensed phase is instantaneous,
- 591 - each mass of vapour that flows up from the boiler to the condenser gets rid of
- 592 the same mass of condensed vapour to the distillate.

593

594 Now, introducing the subscript “cond.” to denote the activity (or the mass) in the condenser;  
 595 from the beginning of the distillation to the extraction of a mass of water equal to the mass of  
 596 water in the dead volume, we have:

597

$$598 \quad A_{cond} = A_{ext} \quad (11)$$

599

600 As soon as the dead volume of the condenser is filled:

601

$$602 \quad \frac{V_{cond} A_{cond}}{V_{ext} A_{ext}} = 1 \quad (12)$$

603

604 We consider that no water flows into the distillate until the dead volume of the condenser is  
 605 full and introduce the subscript “dist.” to denote a specific activity of the distillate, thus:

606

$$607 \quad \frac{V_{dist} A_{dist}}{V_{cond} A_{cond}} = 1 \quad (13)$$

608

609 This model was tested on the different data sets of our results as well as with an experiment  
 610 where the condenser was first saturated with untritiated water before distilling tritiated water.  
 611 All sets of results are well-fitted by the present model. It shows how the measured specific

612 activity (measured in the distillate) is modified by the loss of water in the dead volume (Fig.  
613 12) for a known dead volume (7 mL in this example).

614

615 **Fig. 12:** Modelled evolution of specific activity of water in different compartments during the distillation of a  
616 20 g sample of milk (Mean specific activity of extracted water = 1).

617

618 This influence depends on the mass of the sample and on its specific activity (Fig. 13). For  
619 samples of milk lower than 10 g, the bias is less than 3 %. Actually, as there is only 8.7 g of  
620 water in 10 g of milk and the dead volume of the condenser being estimated to 7 mL (7 g), the  
621 few drops that flow out from the condenser has a specific activity that is fully representative  
622 of water extracted from the sample. For samples with  $15 \text{ g} \leq M \leq 75 \text{ g}$ , the underestimation of  
623 the specific activity is about - 6 %. The latter is in good agreement with the results presented  
624 in Fig. 8 which represents how specific activities of different samples are distributed as a  
625 function of their final rate of dehydration. Lastly, using samples larger than 200 g is a  
626 necessary condition to obtain a deviation that remains below 3 % when using a rotating  
627 evaporator.

628

629 **Fig. 13:** Bias due to the rotating evaporator versus mass of sample. The model described above was tested with  
630 different masses of sample (from 7 g to 10 000 g) to determine how the modelled bias induced by the dead

631 volume of the condenser  evolves in this range of mass.

632

### 633 3 Conclusion

634

635 In this study we show that each methodological aspect tested (water removal technique, mass  
636 of sample and final state of dehydration) is able to induce a bias in the specific activity  
637 measured in the extracted water. In most environmental monitoring situations, these biases

638 remain close to the uncertainty of measurement when liquid scintillation is used in the usual  
639 conditions (i.e. about 15% when measuring a sample of 10 mL with an activity of 20 Bq.L<sup>-1</sup>  
640 water mixed with 10 mL of UltimaGold LLT (Pointurier et al., 2003) for 200 minutes).  
641 Nevertheless, some of these biases can produce systematic underestimations of the actual  
642 specific activity. First, it was shown that in the conditions of this study the water extracted by  
643 freeze drying had a specific activity significantly higher than the water extracted using a  
644 distillation technique based on the same milk sample. This is explained by a pollution of the  
645 extracted water by atmospheric water before, after or during the freeze drying process. This  
646 particular behaviour became apparent due to the fact that atmospheric water in the Valduc  
647 centre is has slightly elevated ambient levels of tritium. Caution must thus be taken to avoid  
648 marking during freeze drying, especially when the specific activity of the sample is not of the  
649 same order of magnitude as the specific activity of the atmospheric vapour. An easy and  
650 economic way to prevent this phenomenon would be to freeze dry larger samples (at least 100  
651 g) which would dilute the effect of atmospheric water condensation. A more suitable way  
652 nevertheless would be to operate freeze drying in a dry atmosphere and to break the vacuum  
653 with dry gas.

654

655 When dehydration is performed by distillation under reduced pressure, the dead volumes of  
656 the devices (especially those of the condenser) have to be limited as they can induce a bias in  
657 the estimation of the mass of extracted water as well as and in the measurement of specific  
658 activities. It appears that using a distillation bridge suits a wider range of samples masses than  
659 a rotating evaporator, the latter should be preferred for large samples (> 300 mL).

660

661 Lastly, in this study a fractionation effect during dehydration proved to be more significant  
662 than expected. This shows the necessity to perform dehydration until there is no water left in

663 the sample. When a fraction of water cannot be extracted without taking the risk of damaging  
664 the dry matter, the residual fraction of water should be estimated and the specific activity  
665 measured corrected using formula (9) proposed in this work.

666

667 The effects of the different sources of biases must be summed. For instance, if a distillation  
668 using a rotating evaporator (dead volume of 7 mL) of a sample of 20 mL of milk is  
669 interrupted when 10 % of water remains in the matrix, the measured specific activity would  
670 be about 91 % of the real specific activity and the standard deviation of this result would be  
671 11 % additionally increase by the uncertainty of measure (about 15 % in usual conditions).  
672 Overall, this would lead to a global underestimation of about 10 % and an uncertainty of  $\pm 26$   
673 %.

674

675 In the literature, VPIE has been shown to decrease while temperature increases without  
676 differences being measured between vaporization and sublimation (Baumgärtner and Kim,  
677 1990). It has also been shown that a link exists between isotopic fractionation during  
678 extraction of water and a three-layer model for bound water (Kim and Baumgärtner, 1997)  
679 (described by Drost-Hansen). The results gathered herein using milk samples show  
680 fractionation behaviour which can be described by a two-component formula, each  
681 component being based on specific Rayleigh distillation processes. These results bring to  
682 mind the features of two fractions of water that coexist in milk: the first one (96%) that acts as  
683 pure water (free water) and the second (4%) which presents an isotopic separation factor  $\beta$   
684 equal to 11.7 much higher than that of pure water;  $\alpha = 1.14$ . The fraction described here by  
685 the isotope separation factor  $\beta$  may be bound water.

686

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