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Authors	O'Callaghan, Irene;Sullivan, Timothy
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3   Running head: O'CALLAGHAN & SULLIVAN: MOULTING AND METAL

4   CONCENTRATIONS

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6   Shedding the load: moulting as a cause of variability in whole-body

7   metal concentrations

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9   Irene O'Callaghan[1,2] and Timothy Sullivan[1,3]

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11   *[1] School of Biological, Earth & Environmental Sciences, University College Cork, Ireland;*

12   *[2] School of Chemistry, University College Cork, Ireland; and*

13   *[3] Environmental Research Institute, University College Cork, Ireland*

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16   *Correspondence: T. Sullivan; e-mail: timothy.sullivan@ucc.ie*

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21   ABSTRACT

22   Moulting is a biological process shared by aquatic macroinvertebrates, but while the exoskeleton

23   is believed to be a major sink of metal pollutants, the contribution of the moulting of the

24   crustacean exoskeleton to total accumulated metal concentrations is insufficiently considered.

25   We present a conceptual, qualitative model that illustrates the impact of moulting on the whole-

26   body burden of an unspecified metal analyte in a hypothetical moulting invertebrate. The model

27 demonstrates that moult stage is a contributor to the whole-body pollutant concentration, and that

28 this introduces a temporal component even in steady-state exposure conditions. The applicability

29 of this model is illustrated by comparison to published results of pre- and post-moult

30 accumulations. A solution for reducing this variability in the measurement of whole-body metal

31 concentrations is presented, and its potential application to both *ex-situ* and *in-situ* studies of

32 biomonitor species is discussed.

33

34 **Key Words**: aquatic environment, bioaccumulation, body burden, crustaceans, ecdysis,

35 measurement errors, exoskeleton, macroinvertebrates, metal pollution

36

37   INTRODUCTION

38 Biomonitoring is defined as the use of an organism, whether whole, in part, or communal, to

39 determine the quality of the environment, and it is commonly used as a method of detecting and

40 quantifying contaminants present in the environment (Markert, 2007). Biomonitoring is

41 generally employed for the quantification of environmental pollutants, wherein a measurable

42 parameter may be the abundance of an identified biomonitor species in a geographical area, or

43 under a defined set of conditions (Ketelaars & Frantzen, 1995; Bonada *et al.,* 2006;).

44   A primary application of an aquatic biomonitor is the measurement of bioavailability and

45 uptake of trace metals in the environment, and hence an indication of environmental

46 concentrations (Johnson *et al.,* 1993; Flessas *et al.,* 2000). Trace metals may enter the aquatic

47 environment by means of natural processes, such as metals leaching into rivers following a forest

48 fire in an adjacent location, or through anthropogenic disturbance (Richardson *et al.,* 2001). In

49 many instances, there is a higher ratio of metals entering the hydrosphere as a result of

50   anthropogenic activities than of metals from natural sources entering waterways (Callender,

51   2003).

52   A distinction must be made between total and bioavailable pollutant concentrations in

53   order to appreciate the advantage of biomonitoring over direct measurement of the pollutants.

54   Various physico-chemical processes can render a pollutant biologically inert. These can be

55   divided into environmental processes, that influence the "environmental availability," and

56   internal biological processes within an organism, that influence the "toxicological

57   bioavailability" of the pollutant (Peijnenburg *et al.,* 1997). While total pollutant concentrations

58   can be determined directly through chemical analysis, accounting for the concept of

59   bioavailability requires either the application of measured environmental parameters to a model

60   of the environmental processes (the *a priori* approach, often the application of partition

61   coefficients to measured total pollutant concentrations), or direct measurement of accumulated

62   concentrations within the organism of interest (the *a posteriori* approach). The classification of

63   pollutants according to their bioavailability and toxicity, such as the Priority Substances List of

64   the Water Framework Directive and subsequent amendments (European Commission, 2000,

65   2008), is a similar approach to the former. Biomonitors exemplify the latter, as the measured

66   biological concentrations are the result of both the environmental availability and toxicological

67   bioavailability processes, and, therefore, offer an insight into the results of these processes

68   without requiring knowledge of the processes themselves. This approach avoids the need to

69   simplify such processes, so long as the biomonitor is chosen such that the toxicological

70   bioavailability encountered is representative for the ecosystem under study.

71   The biomonitoring of bioavailable pollutant concentrations using biomonitor organisms

72   also presents another advantage. Metal pollutants are often concentrated within the organism of

73   interest, which can allow for the observation of concentrations of contaminants that would

74   otherwise be difficult to detect at background concentrations, especially at a high spatial or

75   temporal frequency that would otherwise be difficult and prohibitively expensive to carry out

76   using analytical methods (Bryan & Darracott, 1979). This concentration behaviour relaxes the

77   limits of detection and allows the quantification in the organism what would rarely be detected in

78   the water (Phillips, 1977).

79   There is still some debate around the extent to which crustaceans uptake and

80   bioaccumulate trace metals, and reproducibility of measured metal concentrations appears to be

81   the exception, rather than the norm (Depledge & Rainbow, 1990; O'Callaghan *et al.,* 2019). This

82   is compounded by the uncertainty regarding the most likely sources, uptake pathways or sites of

83   bioaccumulation within the organism of interest (Fleming & Richards, 1982; Elangovan *et al.,*

84   1999; Van Hattum *et al.,* 1999; Robinson *et al.,* 2003; Santoro *et al.,* 2009). Several models have

85   been proposed for the processes of uptake, bioaccumulation, and excretion of various pollutants

86   in freshwater macroinvertebrates (Rainbow & Luoma, 2011; Awrahman *et al.,* 2015), a task

87   made more complicated by the varying behaviour of different metal analytes and potential

88   interactions between said metals (O'Callaghan *et al.,* 2019). A survey of these models, however,

89   indicates that periodic moulting of the exoskeleton, a process common to many invertebrate

90   species (Lebrun *et al.,* 2011), may not have been adequately accounted for in regard to trace

91   metals loss from the whole organism upon renewal of the exoskeleton.

92   Failing to take into account exoskeletons could therefore be a substantial source of

93   variability in measurement of the bioavailability of trace metals or in biomonitoring programs.

94   Previous studies have shown that the moulted exoskeleton may contain sizable concentrations of

95   bioaccumulated pollutants, pointing to ecdysis as being a possible pathway through which

96   significant portions of the accumulated substance may be shed or lost from the organism

97   (Miramand *et al.,* 1981; Hall, 1982; Topcuoğlu *et al.,* 1987; Rauch & Morrison, 1999). Moulting

98   may ultimately influence survival of an organism, as periodic moulting may reduce or maintain

99   pollutant concentrations below critical concentrations for the organism (Bryan & Darracott,

100 1979; Bergey & Weis, 2007). Furthermore, in the context of quantifying the accumulated

101 concentrations, accuracy and applicability of existing pollutant uptake and release models could

102 be improved by consistently including moult stage as a variable.

103   We present a generalized description of the flow of an unspecified metal pollution in a

104 moulting aquatic macroinvertebrate, such as a crustacean. This conceptual description offers an

105 illustrative, non-specific picture of the potential impact of moulting on measured accumulations,

106 equivalent to a repeating, discontinuous depletion of total accumulated metal concentrations. We

107 demonstrate the applicability of this conceptual model by comparing to previously published pre-

108 and post-moult measurements of overall body concentrations of crustaceans, and discuss the

109 relevance of this conceptual model to the study of accumulation in biomonitor species. We also

110 present a possible technique for reducing this variability, and discuss the potential application of

111 this technique both in the field and the laboratory.

112

113   CONCEPTUALIZING THE IMPACT OF MOULTING

114 *Existing models*

115 Common types of models used to describe the accumulation of metals in aquatic invertebrates

116 include bioconcentration, bioaccumulation, and accumulation factors (BCF/BAF/ACF), the

117 biotic ligand model (BLM), the free ion activity model (FIAM), and biodynamic models (Wang

118 & Tan, 2019).

119   BCF, BAF, and ACF factors provide an intuitive and relatively uncomplicated way of

120   estimating accumulation rates, but rely on the assumption that equilibrium will be reached across

121   the organism-environment interface (McGeer *et al.,* 2003; van den Brink *et al.,* 2019). Moulting

122   consists of dynamic changes in the organism, which violates this key assumption, reducing the

123   contribution of moulting to a static correction term rather than a time-varying process.

124   BLM, as well as the related FIAM and extensions thereof, are commonly applied to

125   studies of the total accumulated concentration ionic metals in aquatic macroinvertebrates (Brown

126   & Markich, 2000; Di Toro *et al.,* 2001; van den Brink *et al.,* 2019). Both models, however, focus

127   on the interface between the environment and the proposed receptor site, and ignore the internal

128   mechanisms of translocation, transformation, and excretion (Vijver *et al.,* 2004). The

129   contribution of moulting is closely linked with the relative sequestration of metal pollutants in

130   exoskeleton and soft tissue compartments, which relies on these internal mechanisms.

131   Biodynamic models, commonly referred to as physiologically-based pharmacokinetic

132   (PBPK), are better suited to modelling the dynamic potential contribution of moulting to the

133   accumulated concentrations of contaminant (Ardestani *et al.,* 2014; van den Brink *et al.,* 2019).

134   This approach models the processes of uptake, accumulation, translocation, transformation, and

135   excretion across time, and does not rely on any steady-state assumptions. toxicokinetic-

136   toxicodynamic (TKTD) models, such as general unified threshold model of survival (GUTS)

137   (Jager *et al.,* 2011; EFSA Panel on PPR *et al.,* 2018), are an example of a biodynamic approach

138   applied to both contaminant accumulation and resultant biological effects. One of the most

139   comprehensive static metal-accumulation biodynamic models in the literature arguably shows a

140   relatively good correlation with observed results across a large number of studies (Luoma &

141   Rainbow, 2005), and GUTS is considered sufficiently developed for use in risk assessment

142 applications (EFSA Panel on PPR *et al.,* 2018). These models, however, have not yet been

143 extended to include the contribution of moulting, a correction that would have to be separately

144 determined for each organism-analyte pair.

145

146 *Choice of approach*

147 We take the approach of describing moulting using a non-specific model that is designed to

148 capture the essence of the problem, while remaining broadly applicable to any moulting aquatic

149 invertebrates such as crustaceans, whose exoskeleton may act as a significant sink for

150 contaminants, and any transition metal, metalloid, or heavy metal species. Such approach should

151 be contrasted with the common approach deriving a quantitative, predictive model described

152 above. The aim of our model is instead to illustrate certain contributions of the moulting process

153 to measured concentrations that are common to all moulting aquatic invertebrates and metal

154 analytes, without offering a prediction for the significance of these contributions in any one

155 scenario.

156 We limit the applicability of the presented model to the transition, metalloid and heavy

157 metals, as it has been observed that the accumulation of the alkali or alkaline earth metals in the

158 exoskeleton of an aquatic crustacean may differ from that of the aforementioned elements. The

159 accumulation of calcium, in particular, has been extensively investigated throughout the various

160 moult stages, and has been found to undergo a series of storage and resorption processes. This is

161 said to be linked to the use of calcium in the release of the exoskeleton and hardening of the

162 newly developing cuticle (Greenaway, 1985). The chitinous nature of the exoskeleton, and, more

163 specifically, the nitrogen groups therein, may play a role in the alternative behaviour of the

164 transition metals, metalloids, and heavy metals, as it has been noted that chitinous materials

165 show a poorer affinity towards the alkali and alkaline earth metals (Rae & Gibb, 2003). For this

166 reason, the assumptions made in the following section apply only to the accumulation of

167 transition metals, heavy metals, and the metalloids.

168

169 *Assumptions*

170 For the purpose of creating a concise and simplified conceptual model, we must introduce a

171 number of assumptions. These assumptions are chosen such that they adequately isolate the

172 impact of moulting on trace metals concentrations, while removing internal processes that are not

173 mediators of the moulting process.

174   1) In order to reduce the process to a flow network, we make the assumption that all

175 metal pathways are uni-directional from intake to depuration. This does not mean that there are

176 no bi-directional pathways, but rather that bi-directional flows can be replaced by a long-term

177 uni-directional approximation.

178   2) While the process of moulting may be complex and irregular, we assume that each

179 moulting event happens similarly and that the properties of each moulted exoskeleton are largely

180 identical, in that each sequential exoskeleton is capable of accumulating metal contaminants at a

181 fixed rate, after consideration of the growth factor. This is a simplifying assumption, and the

182 impact of moulting is qualitatively similar under non-uniform moulting behaviour.

183   3) The frequency of moulting is taken to be constant, for the purposes of illustration.

184 Again, non-constant frequency of moulting would produce qualitatively similar results.

185   4) Contaminant intake occurs solely through the processes of respiration, ingestion, and

186 adsorption. The inclusion of these three pathways is intended to make the model as general as

187 possible, and the results still hold if uptake through either ingestion or respiration does not occur,

188   and/or if uptake through adsorption does not occur. Adsorption is defined as the uptake of metal

189   contaminants directly from the overlying and interstitial waters in direct contact with the surface

190   of the exoskeleton, and results in uptake of the contaminant directly into the pre-moult

191   exoskeleton; absorption through the exoskeleton and into the body is not directly considered for

192   the reasons explained in Assumption 1.

193   5) The only process of depuration included in the model is moulting. Gut contents are not

194   taken into account, and, therefore, excretion from the alimentary tract does not reduce

195   accumulated concentrations in the model; metal pollutants are taken to enter the system when

196   they are assimilated from the alimentary tract into the biological tissues.

197   6) This model only considers the movement of trace metals and assumes that no internal

198   processes of biotransformation are taking place. This is true regardless when considering the

199   elemental concentrations, but a more complex model would be required to account for change in

200   speciation or complexation of metals due to biological processes.

201   7) In this conceptual system, we make the assumption that the rate of translocation

202   between the body and pre-moult exoskeleton is driven towards equilibrium by the presence of

203   open binding sites in the destination and high concentrations at the source. The flow of

204   translocation can therefore be approximated as proportional to the source concentrations. Other

205   models of translocation could be considered and would result in qualitatively similar results.

206   8) We assume that the described processes are not influenced by any biological damage

207   that may occur, and we do not account for the possibility of mortality as part of the model.

208

209   *The conceptual model*

210   Based on the above assumptions, we present a simplified three-input, two-compartment model of

211   metal accumulation in a hypothetical moulting aquatic invertebrate (Fig. 1). The corresponding

212   rate diagram is shown in Figure 2. The model compartmentalizes metal concentrations

213   accumulated within (and on) the pre-moult exoskeleton (Compartment E in Figure 2), which is

214   defined as the part of the body that is removed entirely during the moulting process, and

215   concentrations accumulated in the remainder of the body (Compartment B in Figure 2). For the

216   purposes of simplifying the model, the body is inclusive of all non-moulting parts (gills, legs,

217   hepatopancreas, and other organs), but not the gut contents as explained in Assumption 5.

218   *<Figs. 1 & 2>*

219   Contaminants may enter Compartment B through ingestion or standard respiration, where

220   i denotes the concentration of contaminant present in the ingestate, r the concentration of

221   contaminant in the water overlying the gill regions, and k_i and k_r the rate constants of the

222   respective processes. Contaminants may enter Compartment E through surface adsorption

223   directly from overlying and interstitial waters in contact with the exoskeleton, where a denotes

224   the concentration of contaminant present in these waters, and k_a the corresponding rate constant.

225   Contaminants may also flow from Compartment B to Compartment E through the process of

226   internal translocation or sequestration, where B is the concentration of contaminant in

227   Compartment B, and k_t the rate constant of translocation. The features of this flow are

228   summarized in Assumption 7.

229   Moulting, or ecdysis, refers to the regular removal of the outer exoskeleton. As the

230   exoskeleton is shed, a new exoskeleton develops beneath it (see Drach, 1967). The loss of the

231   moulted exoskeleton cannot be modelled as a continuous flow of contaminants as it occurs

232   suddenly and periodically, and, therefore, it is not included in the process diagram in Figure 2.

233   Instead, the diagram describes the inter-moult movement of contaminants, and moulting is

234   implemented externally as a periodic discontinuous removal of the contaminants within

235   Compartment *E*. The moult period, or the rate at which an organism moults, will vary greatly

236   with species and other factors. It should be noted that occasional consumption of the organism's

237   own moulted exoskeleton has been observed in some macroinvertebrate species (Elangovan *et*

238   *al.*, 1999), although there is consensus that metals bound within a chitinous exoskeleton are less

239   bioavailable than other forms of analyte (Khan *et al.*, 2010). The possibility of such an

240   occurrence is not considered in this model but would result in qualitatively similar results.

241

242   *Impact of moulting*

243   The conceptual two-compartment model of Figure 1 can be converted into a causal diagram

244   describing the relationship between the variable of interest, namely the environmental

245   concentration of bioavailable metal, and the measured metal concentration. This diagram is

246   shown in Figure 3. The effect of environmental concentration on measured concentration occurs

247   through the mediation variables of body and exoskeleton concentration. The hypothesis that the

248   measured whole-body concentration is an accurate estimator for the bioavailable environmental

249   concentration, given an acceptable measurement error, is, therefore, weakened by the direct

250   effect of moult stage on exoskeleton concentration.

251   *<Fig. 3>*

252   It can be directly observed from Figure 3 that the effect of the moult event on the overall

253   accumulated metal concentration depends greatly on the ratio of exoskeleton metal concentration

254   to body metal concentration. If a simplifying assumption is made that the moult stage of different

255 organisms is uncorrelated, then sampling more organisms should reduce, but not eliminate, the

256 influence of moulting.

257

258 DERIVATION OF KEY EQUATIONS

259 *Differential rate equations*

260 The key aspects of the model are described by the following pair of differential rate equations,

261 which describe the change in inter-moult concentrations of metal contaminant in each

262 compartment.

$$\frac{\mathrm{d}[B]}{\mathrm{d}t} = k_i[i] + k_r[r] - k_t[B]$$ (1)

263

264

$$\frac{\mathrm{d}[E]}{\mathrm{d}t} = k_t[B] + k_a[a]$$ (2)

265

266

267

268 *Growth factor*

269 The growth aspect is one of significant variability, as growth may indicate linear growth, lateral

270 growth, or increasing thickness of the exoskeleton. Growth rate will vary considerably with

271 species, as well as with the life stage of the organism. In order to account for the growth

272 uncertainty, we use a growth factor, G. Equations 1 and 2 can, hence, be extended to account for

273 growth by dividing each k_x term by G.

274

275 *Closed-form expressions*

276 Equations 1 and 2 can be solved for instantaneous compartment concentrations, assuming all

277 concentrations are 0 at time $t = 0$ and ignoring the effects of moulting. This produces the

278 following closed-form expressions:

279 $$[B](t) = (U/k_t)(1 - e^{-t/\tau}) \tag{3}$$

$$[E](t) = (U + k_a[a])t - U\tau(1 - e^{-t/\tau}) \tag{4}$$

281 where the uptake rate is given by $U = k_i\,[i] + k_r\,[r]$, and the process time constant is $\tau = G/k_t$.

282   Note that the concentrations in Compartment B can be modelled as a first-order

283 underdamped system, where the concentration approaches an equilibrium value of U/k_t, while

284 the concentration in Compartment E increases indefinitely as the loss due to moulting is not yet

285 accounted for.

286

287 *Steady-state equations*

288 Steady-state is reached when $t \gg \tau$ in all the above equations. This results in expressions for the

289 final, steady-state accumulated concentration in Compartment B in an environmental

290 equilibrium.

291 $$[B] = U/k_t \tag{5}$$

292 As moulting happens periodically, the steady-state equivalent in the case of the Compartment E

293 has the appearance of a sawtooth pattern, rather than a fixed value. The period of the

294 concentration in Compartment E is equal to the moulting period, T_M, while the peak

295 concentration is given by:

296 $$[E]_{\mathrm{MAX}} = (U + k_a[a])T_M \tag{6}$$

297 The variance of the corresponding error due to moulting is therefore given by:

298 $$\sigma^2 = \frac{([E]_{\mathrm{MAX}})^2}{12} \tag{7}$$

299   More explicit derivation of the above equations is presented in Supplementary material File S11.

300

301   SIMULATION

302   *Objectives*

303   The conceptual model is designed to offer insights into the contribution of moulting to whole-

304   body concentrations in the general case. The following simulation is intended to provide an

305   example of how the model can describe the impact of moulting in an existing experimental

306   study. Its specificity to a particular organism and metal pollutant should not be taken to be a

307   statement about the limitations of the conceptual model, but rather an indication of how the

308   generalized model can be applied to a specific case. The implementation of the model employed

309   in the following sections is described in full in Supplementary material File S12.

310

311   *Simulation parameters*

312   The simulation parameters shown in Table 1 were derived from studies of the uptake of

313   vanadium by the caridean shrimp *Lysmata seticaudata* (Risso, 1816) (Miramand *et al.,* 1981).

314   The measurements extracted from Miramand *et al.* (1981) is available in Supplementary material

315   Table S1. Further details of how these parameters were derived are presented in Supplementary

316   material File S13 and compared with the cited measurements in Supplementary material Fig. S8.

317   *<Table 1>*

318

319   *Simulation results*

320   Figure 4 shows the bioaccumulated concentrations of metal contaminant in Compartment B,

321   when the environmental conditions are in equilibrium. The black dashed lines indicate the

322   steady-state values.

323   *<Fig. 4>*

324   As expected of a first-order system, the concentration in Compartment B reaches steady-

325   state at a speed dictated by the rate of internal translocation of the contaminant. Once the internal

326   concentration reaches steady-state, there is no significant change in contaminant concentration

327   without a corresponding change in the environmental conditions.

328   Figure 5 shows the bioaccumulated concentration of the contaminant in Compartment E.

329   Moulting forces the pre-moult exoskeleton concentration to 0 every T_M days. This produces a

330   periodic pattern with increasing amplitude, approaching a sawtooth pattern as Compartment B

331   reaches steady-state.

332   *<Fig. 5>*

333   The total concentration of contaminant in the organism as a whole is shown in Figure 6.

334   The lower black dashed line shows the steady-state concentration for Compartment B, whereas

335   the upper black dashed line includes the peak concentration for Compartment E. Despite the

336   concentration in Compartment B reaching steady-state, the influence of moulting is still

337   significant. This results in a time variation in the overall concentration.

338   *<Fig. 6>*

339

340   DISCUSSION

341   *Contribution of moulting*

342 Studies measuring the bioaccumulation of trace metals in freshwater macroinvertebrates

343 typically rely on the assumption that the accumulated concentrations of metals are relatively

344 time-invariant. Many models of pollutant uptake likewise rely on the steady-state assumption. In

345 both cases, individual measurements of total accumulated pollutant concentrations provide an

346 accurate quantification of the time-averaged accumulation flux.

347   The contribution of the conceptual model presented herein is to explain how periodic

348 processes, exemplified by moulting, can produce fluctuations in the total accumulated whole-

349 body concentrations. The *L. seticaudata* example demonstrates the significant effect this can

350 have on the measured whole-body concentration. Seeing as discontinuous loss occurs during

351 each moult, steady-state is not reached; the internal concentrations settle into a periodic

352 oscillation, with the total concentration varying between minimum and maximum values. The

353 error introduced by the continuous approximation is described in Supplementary material File

354 S14, and illustrated by Supplementary material Figs. S9, S10. From an experimental perspective,

355 this introduces a source of variability in the measurements, as the measured value depends not

356 only on the mean total concentration, but also on the moult stage at the time at which the

357 measurement is taken.

358

359 *Validity of simulated example*

360 Example parameters, listed in Table 1, were used for the purposes of demonstrating the effects of

361 moulting on the measured whole-body concentration. This raises the question of whether the

362 chosen parameters produce results that fairly represent realistic pollutant accumulations. This can

363 only be answered through comparison with measured concentrations.

364 The model predicts that approximately 74% of the accumulated metal contaminant concentration

365 is lost during the process of moulting. Table 2 presents published measurements of analyte

366 concentrations accumulated within the exoskeleton of various species, expressed as a percentage

367 of whole-body accumulated concentrations. In cases where data extraction and/or post-

368 processing was required to obtain the values given in Table 2, further details are given in

369 Supplementary material Tables S4–S7. It must be emphasized, when interpreting these figures,

370 that most of the cited studies do not account for the effects of moulting on overall concentrations

371 we describe. Therefore, by assuming steady-state whole-body concentrations it would be

372 expected that the measured values presented herein represent approximately half of the

373 exoskeleton concentration at the time of moulting. Under these same assumptions, the

374 exoskeleton would contribute approximately 37% of the whole-body concentration using the

375 model parameters presented herein.

376   As is to be expected and considering the wide range of biological species, analytes, and

377 environmental or experimental conditions, there is a broad variation in reported exoskeleton

378 concentrations. Despite this variation, it is clear that such concentrations are a significant fraction

379 of the whole-body accumulated concentration. Our model would, therefore, be correct in

380 attributing a significant role to the contribution of moulting to whole-body pollutant

381 concentrations.

382   *<Table 2>*

383   If the results of the model are valid, the question then arises as to whether the

384 mechanisms described in the model are also valid. Hall (1982) presented measurements of the

385 accumulated concentrations of nickel in the cladoceran *Daphnia magna* Straus, 1820, presented

386 here in Supplementary material Tables S2, S3. Figure 7 shows the measured soft-tissue and

387   exoskeleton concentrations in individuals that have not moulted. It shows that the soft-tissue

388   concentrations rapidly reach steady-state, while the exoskeleton concentrations continue to

389   increase in the absence of moulting. A corresponding fit of the model is shown, with a value of *P*

390   < 0.001 for both datasets. *In-vivo* measurements of whole-body nickel concentrations of one

391   individual over time are shown in Figure 8. A moulting event occurred between t = 20 h and t =

392   49 h, depleting the whole-body concentration. Our model correctly describes the effects due to

393   this moulting behaviour. These results indicate that our description of the processes that result in

394   depuration via moulting is likely valid. Further details of the derivation of these parameters is

395   given in Supplementary material File S15.

396   *<Figs. 7 & 8>*

397

398   *Reducing variability due to moulting*

399   Figure 9 shows how accumulated concentrations can fluctuate through time. For most of the

400   moult period it is unclear at what point in the period the organism lies. In the context of

401   crustaceans, however, it is usually relatively easy to identify if the organism is immediately at a

402   pre-moult or post-moult stage (Drach, 1967; Buchholz, 1982). The pre-moult stage is often,

403   depending on the species, associated with visual changes to the exoskeleton, such as changing

404   colour or texture (Drach, 1967). The post-moult stage is, at the very least, signalled by the

405   appearance of a shed cuticle. Both these stages correspond to the maximum and minimum

406   exoskeleton concentrations, respectively. In the context of bioaccumulation studies, we therefore

407   propose that sampling could be undertaken synchronously with moulting (moult-synchronous

408   sampling) to ensure the robustness of the measurement by reducing the variability due to

409   exoskeleton concentration fluctuations. This would take the form of ensuring only specimens

410   which are immediately pre-moult or post-moult are sampled.

411   *<Fig. 9>*

412   In the context of *ex-situ* studies, implementation of moult-synchronous sampling quite

413   simply takes the form of delaying sampling until the desired moult stage has been reached for

414   each organism. Implementation for *in-situ* studies, however, is less straightforward due to the

415   requirement that samples be taken when the site is visited. In a case such as this, we propose

416   acquiring the organisms as normal, but holding them in a suitable tank until the desired moult

417   stage has been reached. This approach assumes that minimal depuration through other means

418   occurs between acquisition and moulting.

419   An argument could be put forward that the variability due to moulting could be reduced

420   by sampling multiple specimens. This argument relies on the assumption that the moult stage of

421   each specimen is uncorrelated; however, moulting can be induced or accelerated by

422   environmental stressors (Fowler *et al.,* 1971; Nugegoda & Rainbow, 1987), therefore, it could

423   happen that specimens in similar conditions can moult together. For this reason, performing

424   measurements on multiple specimens held in the same conditions may not be sufficient to

425   overcome the effects of moulting on accumulated concentrations.

426   Even ignoring the possibility of correlated moult stage within the population under study,

427   moult-synchronous sampling can offer a more efficient approach to the determination of mean

428   total bioaccumulated concentrations. Both increased sampling size and moult-synchronous

429   sampling aim to reduce the measurement error in the overall measured accumulated

430   concentration due to moulting. Equation 7 quantifies the variance of the measurement error due

431   to moulting. In the example of Figure 6, this corresponds to a mean error variance of $\sigma^2 = 14$

432   ppb. If measurements were only made within the first post-moult day, this would reduce the

433   mean error variance of a single measurement to $\sigma^2 = 0.14$ ppb. In lay terms, the resulting increase

434   in statistical accuracy corresponds to that which would be obtained by increasing the number of

435   specimens by a factor of 100. Figure 9 shows the reduction of measurement error variance due to

436   moulting from restricting the sampling window. Moult-synchronous sampling is, therefore, a

437   more efficient means of reducing the measurement error due to pollutant loss during moulting

438   than simply increasing the sampling size.

439

440   ACKNOWLEDGEMENTS

446

447   SUPPLEMENTARY MATERIAL

448   Supplementary material is available at *Journal of Crustacean Biology* online.

449   S1 Table. Accumulated vanadium concentration values derived from Miramand *et al.* (1981).

450   S2 Table. Accumulated nickel concentration values derived from Figure 6 of Hall (1982).

451   S3 Table. Accumulated nickel concentration values derived from Figure 2a of Hall (1982).

452   S4 Table. Accumulated concentration values derived from Bergey & Weis (2007).

453   S5 Table. Accumulated concentration values derived from Hennig (1984).

454   S6 Table. Accumulated concentration values derived from Keteles & Fleeger (2001).

455   S7 Table. Accumulated vanadium concentration values derived from Reinecke *et al.* (2003).

456   S8 Figure. Accumulation data from Miramand *et al.* (1981) (CF actual), and fit to Equation 3

457   (CF fit), with $T_M = 21$ days and $G = 1$.

458   S9 Figure. Simulated exoskeleton compartment pollutant concentration, with continuous

459   moulting approximation (given by Equation S14) overlaid in dashed black.

460   S10 Figure. Error in whole-body pollutant concentration when modelling moulting as a

461   continuous process.

462   S11 File. Expanded derivations of the model equations.

463   S12 File. Implementation of the model code.

464   S13 File. Derivation of model parameters from data presented in Miramand *et al.* (1981).

465   S14 File. Description of the continuous approximation assumption and resulting error.

466   S15 File. Derivation of model parameters from data presented in Hall (1982).

467

468   REFERENCES

469   Ardestani, M.M., van Straalen, N.M. & van Gestel, C.A. 2014. Uptake and elimination

470   kinetics of metals in soil invertebrates: a review. *Environmental Pollution*, **193**: 277–295.

471   Awrahman, Z.A., Rainbow, P.S., Smith, B.D., Khan, F.R., Bury, N.R. & Fialkowski, W.

472   2015. Bioaccumulation of arsenic and silver by the caddisfly larvae *Hydropsyche siltalai*

473   and *H. pellucidula*: A biodynamic modeling approach. *Aquatic Toxicology*, **161**: 196–

474   207.

475   Bergey, L.L. & Weis, J.S. 2007. Molting as a mechanism of depuration of metals in the

476   fiddler crab, *Uca pugnax*. *Marine Environmental Research,* **64**: 556–562.

477   Bertine, K.K. & Goldberg, E.D. 1972. Trace elements in clams, mussels, and shrimp.

478   *Limnology and Oceanography,* **17**: 877–884.

479   Bonada, N., Prat, N., Resh, V.H. & Statzner, B. 2006. Developments in aquatic insect

480   biomonitoring: a comparative analysis of recent approaches. *Annual Review of*

481   *Entomology*, **51**: 495–523.

482   Brown, P.L. & Markich, S.J. 2000. Evaluation of the free ion activity model of metal-

483   organism interaction: extension of the conceptual model. *Aquatic Toxicology,* **51**: 177–

484   194.

485   Bryan, G.W. & Darracott, A. 1979. Bioaccumulation of marine pollutants. *Philosophical*

486   *Transactions of the Royal Society of London B: Biological Sciences*, **286**: 483–505.

487   Buchholz, F. 1982. Drach's molt staging system adapted for euphausiids. *Marine Biology*,

488   **66**: 301–305.

489   Callender, E. 2003. Heavy metals in the environment – historical trends. *Treatise on*

490   *Geochemistry*, **9**: 67–105.

491   Depledge, M.H. & Rainbow, P.S. 1990. Models of regulation and accumulation of trace

492   metals in marine invertebrates. *Comparative Biochemistry and Physiology C*, **97**: 1–7.

493   Di Toro, D.M., Allen, H.E., Bergman, H.L., Meyer, J.S., Paquin, P.R. & Santore, R.C. 2001.

494   Biotic ligand model of the acute toxicity of metals. 1. Technical basis. *Environmental*

495   *Toxicology and Chemistry,* **20**: 2383–2396.

496   Drach, P. 1967. Sur la méthode de détermination des stades d'intermue et son application

497   générale aux crustacés. *Vie et Milieu, Série A: Biologie marine*, **18**: 595–610.

498   EFSA Panel on Plant Protection Products and their Residues (PPR), Ockleford, C.,

499   Adriaanse, P., Berny, P., Brock, T., Duquesne, S., Grilli, S., Hernandez-Jerez, A.F.,

500   Hougaard Bennekou, S., Klein, M., Kuhl, T., Laskowski, R., Machera, K., Pelkonen, O.,

501   Pieper, S., Smith, R.H., Stemmer, M., Sundh, I., Tiktak, A., Topping, C.J., Wolterink, G.,

502   Cedergreen, N., Charles, S., Focks, A., Reed, M., Arena, M., Ippolito, A., Byers, H. &

503   Teodorovic, I. 2018. Scientific Opinion on the state of the art of

504   Toxicokinetic/Toxicodynamic (TKTD) effect models for regulatory risk assessment of

505   pesticides for aquatic organisms. *EFSA Journal*, **16**: e05377 [doi: 10.

506   2903/j.efsa.2018.5377].

507   Elangovan, R., Ballance, S., White, K.N., McCrohan, C.R. & Powell, J.J. 1999.

508   Accumulation of aluminium by the freshwater crustacean *Asellus aquaticus* in neutral

509   water. *Environmental Pollution*, **106**: 257–263.

510   Eriksson, S.P. & Baden, S.P. 1998. Manganese in the haemolymph and tissues of the

511   Norway lobster, *Nephrops norvegicus* (L.), along the Swedish west coast, 1993–1995.

512   *Hydrobiologia*, **375/376**: 255–264.

513   European Commission. 2000. *Directive 2000/60/EC of the European Parliament and of the*

514   *Council of 23 October 2000 establishing a framework for Community action in the field*

515   *of water policy.* (OJ L 327, 22.12.2000, pp. 1–73). Office for Official Publications of the

516   European Communities, Luxembourg.

517   European Commission. 2008. *Directive 2008/105/EC of the European Parliament and of*

518   *the Council of 16 December 2008 on environmental quality standards in the field of*

519   *water policy, amending and subsequently repealing Council Directives 82/176/EEC,*

520   *83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive*

521   *2000/60/EC of the European Parliament and of the Council.* (OJ L 348, 24.12.2008, p.

522   84). Office for Official Publications of the European Communities, Luxembourg.

523   Fleming, T.P. & Richards, K.S. 1982. Uptake and surface adsorption of zinc by the

524   freshwater tubificid oligochaete *Tubifex tubifex*. *Comparative Biochemistry and*

525   *Physiology C*, **71**: 69–75.

526   Flessas, C., Couillard, Y., Pinel-Alloul, B., St-Cyr, L. & Campbell, P.G. 2000. Metal

527   concentrations in two freshwater gastropods (Mollusca) in the St. Lawrence River and

528   relationships with environmental contamination. *Canadian Journal of Fisheries and*

529   *Aquatic Sciences*, **57**: 126–137.

530   Fowler, S.W., Small, L.F. & Kečkeš, S. 1971. Effects of temperature and size on molting of

531   euphausiid crustaceans. *Marine Biology*, **11**: 45–51.

532   Greenaway, P. 1985. Calcium balance and moulting in the crustacea. *Biological Reviews*,

533   **60**: 425–454.

534   Guary, J.C. & Fowler, S.W. 1990. Experimental study of the transfer of transuranium

535   nuclides in marine decapod crustaceans. *Marine Ecology Progress Series*, **60**: 253–270.

536   Hall, T.M. 1982. Free ionic nickel accumulation and localization in the freshwater

537   zooplankter, *Daphnia magna*. *Limnology and Oceanography*, **27**: 718–727.

538   Hennig, H.F.-K.O. 1984. *Baseline surveys and metal binding proteins as metal pollution*

539   *indicators*. Ph.D. thesis, University of Cape Town, Cape Town, South Africa.

540   Jager, T., Albert, A., Preuss, T.G. & Ashauer, R. 2011. General Unified Threshold Model of

541   Survival – a toxicokinetic-toxicodynamic framework for ecotoxicology.

542   *Environmental Science & Technology*, **45**: 2529–2540.

543   Johnson, R.K., Wiederholm, T. & Rosenberg, D.M. 1993. Freshwater biomonitoring using

544   individual organisms, populations, and species assemblages of benthic

545 macroinvertebrates. In: *Freshwater biomonitoring and benthic macroinvertebrates* (D.M.

546 Rosenberg & V.H. Resh, eds.), pp. 40–158. Chapman & Hall, London.

547 Ketelaars, H.A. & Frantzen, N.M. 1995. One decade of benthic macroinvertebrate

548 biomonitoring in the River Meuse. *Netherland Journal of Aquatic Ecology*, **29**: 121–133.

549 Keteles, K.A. & Fleeger, J.W. 2001. The contribution of ecdysis to the fate of copper, zinc

550 and cadmium in grass shrimp, *Palaemonetes pugio* Holthius. *Marine Pollution Bulletin*,

551 **42**: 1397–1402.

552 Khan, F.R., Bury, N.R. & Hogstrand, C. 2010. Cadmium bound to metal rich granules and

553 exoskeleton from *Gammarus pulex* causes increased gut lipid peroxidation in zebrafish

554 following single dietary exposure. *Aquatic Toxicology*, **96**: 124–129.

555 Lebrun, J.D., Perret, M., Uher, E., Tusseau-Vuillemin, M.H. & Gourlay-Francé, C. 2011.

556 Waterborne nickel bioaccumulation in *Gammarus pulex*: Comparison of mechanistic

557 models and influence of water cationic composition. *Aquatic Toxicology*, **104**: 161–167.

558 Luoma, S.N. & Rainbow, P.S. 2005. Why Is metal bioaccumulation so variable?

559 Biodynamics as a unifying concept. *Environmental Science & Technology*, **39**: 1921–

560 1931.

561 Markert, B. 2007. Definitions and principles for bioindication and biomonitoring of trace

562 metals in the environment. *Journal of Trace Elements in Medicine and Biology*, **21**: 77–

563 82.

564 McGeer, J.C., Brix, K.V., Skeaff, J.M., DeForest, D.K., Brigham, S.I., Adams, W.J. &

565 Green, A. 2003. Inverse relationship between bioconcentration factor and exposure

566 concentration for metals: implications for hazard assessment of metals in the aquatic

567 environment. *Environmental Toxicology and Chemistry*, **22**: 1017–1037.

568 Miramand, P., Guary, J.C. & Fowler, S.W. 1981. Uptake, assimilation, and excretion of

569   vanadium in the shrimp, *Lysmata seticaudata* (Risso), and the crab, *Carcinus maenas*

570   (L.). *Journal of Experimental Marine Biology and Ecology*, **49**: 267–287.

571 Nugegoda, D. & Rainbow, P.S. 1987. The effect of temperature on zinc regulation by the

572   decapod crustacean *Palaemon elegans* Rathke. *Ophelia*, **27**: 17–30.

573 O'Callaghan, I., Harrison, S.S.C., Fitzpatrick, D. & Sullivan, T. 2019. The freshwater isopod

574   *Asellus aquaticus* as a model biomonitor of environmental pollution: A review.

575   *Chemosphere*, **235**: 498–509.

576 Peijnenburg, W.J.G.M., Posthuma, L.H.J.P., Eijsackers, H.J.P. & Allen, H.E. 1997. A

577   conceptual framework for implementation of bioavailability of metals for environmental

578   management purposes. *Ecotoxicology and Environmental Safety*, **37**: 163–172.

579 Phillips, D.J. 1977. The use of biological indicator organisms to monitor trace metal

580   pollution in marine and estuarine environments – a review. *Environmental Pollution*, **13**:

581   281–317.

582 Rae, I.B. & Gibb, S.W. 2003. Removal of metals from aqueous solutions using natural

583   chitinous materials. *Water Science & Technology*, **47**: 189–196.

584 Rainbow, P.S. & Luoma, S.N. 2011. Metal toxicity, uptake and bioaccumulation in aquatic

585   invertebrates – modelling zinc in crustaceans. *Aquatic Toxicology*, **105**: 455–465.

586 Rauch, S. & Morrison, G.M. 1999. Platinum uptake by the freshwater isopod *Asellus*

587   *aquaticus* in urban rivers. *Science of the Total Environment*, **235**: 261–268.

588 Reinecke, A.J., Snyman, R.G. & Nel, J.A.J. 2003. Uptake and distribution of lead (Pb) and

589   cadmium (Cd) in the freshwater crab, *Potamonautes perlatus* (Crustacea) in the Eerste

590   River, South Africa. *Water, Air, and Soil Pollution*, **145**: 395–408.

591   Richardson, G.M., Garrett, R., Mitchell, I., Mah-Poulson, M. & Hackbarth, T. 2001. *Critical*

592   *review on natural global and regional emissions of six trace metals to the atmosphere*.

593   Final Report. Prepared for the International Lead Zinc Research Organisation, the

594   International Copper Association, and the Nickel Producers Environmental Research

595   Association

596   [https://www.echa.europa.eu/documents/10162/13630/vrar_appendix_p2_en.pdf].

597   Robinson, K.A., Baird, D.J. & Wrona, F.J. 2003. Surface metal adsorption on zooplankton

598   carapaces: implications for exposure and effects in consumer organisms. *Environmental*

599   *Pollution*, **122**: 159–167.

600   Santoro, A., Blo, G., Mastrolitti, S. & Fagioli, F. 2009. Bioaccumulation of heavy metals by

601   aquatic macroinvertebrates along the Basento River in the South of Italy. *Water, Air, and*

602   *Soil Pollution*, **201**: 19–31.

603   Topcuoğlu, S., Birol, E. & Ünlü, M.Y. 1987. Factors affecting the accumulation and

604   elimination of silver (110mAg) in marine isopods. *Marine Environmental Research*, **21**:

605   189–198.

606   van den Brink, N.W., Kokalj, A.J., Silva, P.V., Lahive, E., Norrfors, K., Baccaro, M.,

607   Khodaparast, Z., Loureiro, S., Drobne, D., Cornelis, G. and Lofts, S. 2019. Tools and

608   rules for modelling uptake and bioaccumulation of nanomaterials in invertebrate

609   organisms. *Environmental Science: Nano*, **6**: 1985–2001.

610   Van Hattum, B., De Voogt, P., Van den Bosch, L., Van Straalen, N.M., Joosse, E.N.G. &

611   Govers, H. 1989. Bioaccumulation of cadmium by the freshwater isopod *Asellus*

612   *aquaticus* (L.) from aqueous and dietary sources. *Environmental Pollution*, **62**: 129–151.

613   Vijver, M.G., Van Gestel, C.A., Lanno, R.P., Van Straalen, N.M. & Peijnenburg, W.J. 2004.

614   Internal metal sequestration and its ecotoxicological relevance: a review. *Environmental*

615   *Science & Technology,* **38**: 4705–4712.

616   Wang, W.-X. & Tan, Q.-G. 2019. Applications of dynamic models in predicting the

617   bioaccumulation, transport and toxicity of trace metals in aquatic organisms.

618   *Environmental Pollution*, **252**: 1561–1573.

619

620   FIGURE CAPTIONS

621   **Figure 1.** Schematic representation of a general overview of the major pathways of trace metal

622   pollutant uptake (via ingestion, respiration and adsorption), translocation, and loss (via moulting)

623   in a moulting aquatic organism. The dashed arrow represents the elimination of accumulated

624   pollutants at the time of moulting, which is modelled as a repeating instantaneous event, while

625   the solid arrows represent the continuous pollutant flux.

626   **Figure 2.** Rate diagram of pollutant flux into and out of the body (B) and moulting exoskeleton

627   (E) compartments. Processes illustrated are respiration (r), ingestion (i), adsorption (a), and

628   internal translocation (t); r, i, and a represent the concentrations from which respiration,

629   ingestion and adsorption, respectively, occur, B the body compartment concentration. The k_x

630   parameters represent the respective rate constants.

631   **Figure 3.** Causal diagram showing the connection between bioavailable environmental metal

632   concentrations and measured whole-body metal concentrations, showing the influence of moult

633   stage on the exoskeleton concentration mediator.

634 **Figure 4.** Simulation of theoretical concentration of a metal pollutant in Compartment B (the

635 body of the organism, excluding the moulting exoskeleton) versus time, from application of the

636 described parameters. The dashed black line represents the equilibrium value given by Equation

637 5 (see text). The lighter lines represent the effects of different values of k_t for the same

638 equilibrium value.

639 **Figure 5.** Simulation of concentration of a metal pollutant in Compartment E (the moulting

640 exoskeleton of the organism) versus time, from application of the described parameters. Unlike

641 with the concentration in the body compartment, B (see Fig. 2), the concentration does not reach

642 an equilibrium state, but oscillates between 0 (complete absence, due to moulting) and a

643 maximum value. The equilibrium maximum value is given by Equation 6 (see text), and is

644 denoted here by the dashed black line.

645 **Figure 6.** Simulation of overall concentration of a metal pollutant in the organism *versus* time.

646 The overall concentration is a mass-weighted combination of the concentrations in the body and

647 moulting exoskeleton compartments, B and E. This simulation represents the evolution of the

648 actual measured whole-body concentration over time, where the variation in the concentration

649 beyond day 200 is entirely due to contaminant loss through moulting. The equilibrium minimum

650 and maximum are given by the dashed black lines, and are derived from Equations 5 and 6 (see

651 text) after accounting for body mass.

652 **Figure 7.** Accumulated concentrations of nickel in the soft-tissue (B) and exoskeleton (E) of

653 multiple *Daphnia magna* individuals (from Hall, 1982). The corresponding fit of the model

654 qualitatively matches the behaviour seen, where B saturates, but E continues to accumulate

655 indefinitely in the absence of moulting. Hall (1982) also observed indefinite accumulation in the
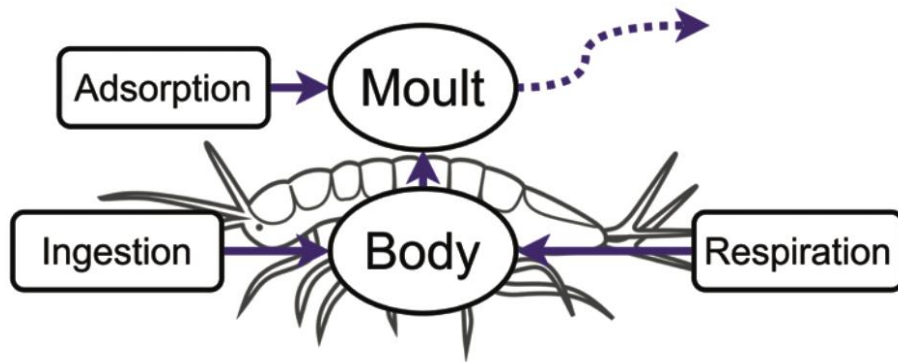
656   filtering appendages, which contain parts of exoskeleton and soft tissue, so have not been

657   included in this figure.

658   **Figure 8.** Accumulated concentrations, across time, of nickel in an individual specimen of

659   *Daphnia magna* (from Hall, 1982). A moulting event occurred between $t = 20h$ and $t = 49h$. A fit

660   of the model, accounting for a moulting event just before $t = 49$ h, accurately describes the

661   observed behaviour, despite the relative simplicity of the model.

662   **Figure 9.** Proportional decrease in moult-induced measurement error with increasing accuracy of

663   moult-synchronous sampling, where a "100%" sampling window is equivalent to ignoring moult

664   stage when sampling. Using a sampling window of 10% of the moult period, for example, would

665   reduce the variance by a factor of 100, for the same sample size.

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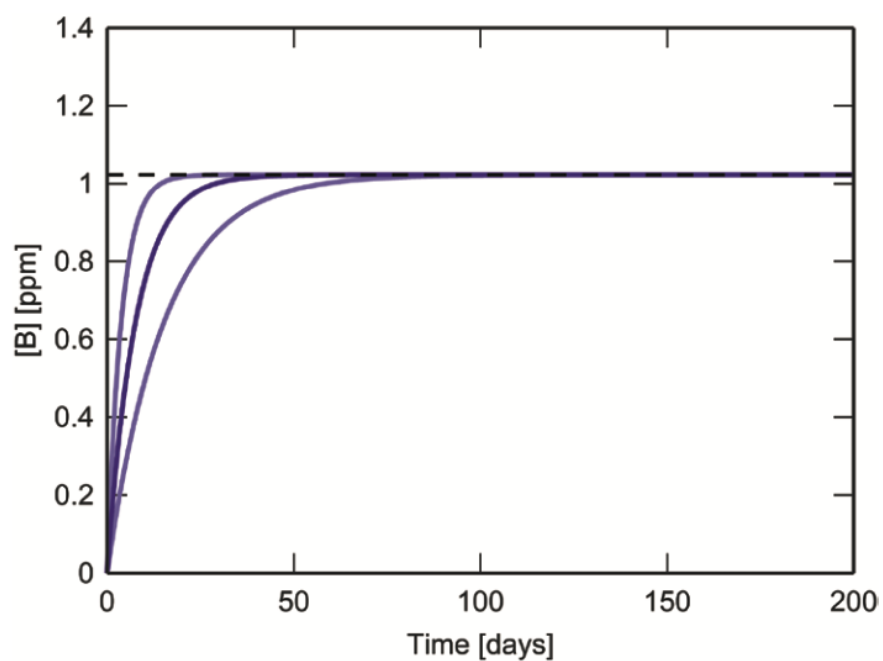669   **Figure 1.**

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671   **Figure 2.**
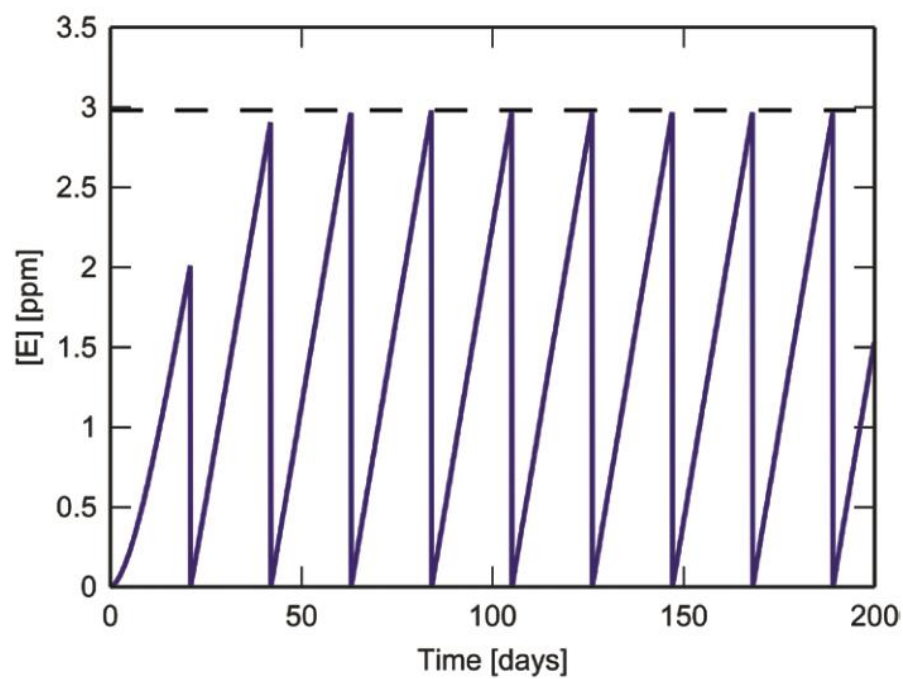

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673   **Figure 3.**

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675   **Figure 4.**
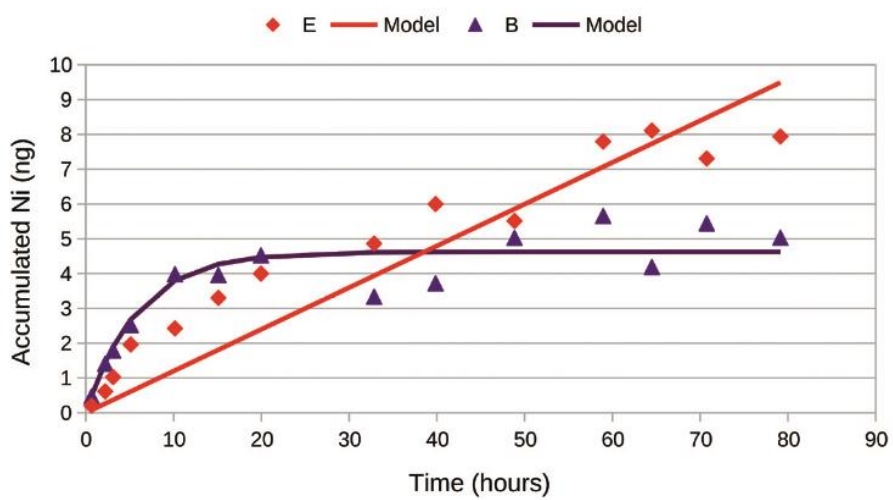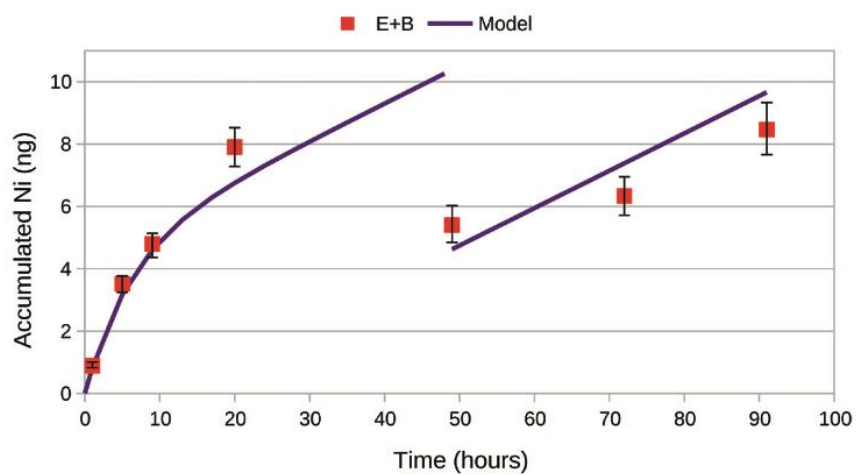
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Figure 5.

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685 **Figure 9.**

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