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Simon Colas, Séverine Le Faucheur

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1 **How do biomarkers dance? Specific moves of defense and damage biomarkers for biological**
2 **interpretation of dose-response model trends.**

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4
5 Simon Colas^{1*}, Séverine Le Faucheur¹

6 ¹ Université de Pau et des Pays de l'Adour, E2S-UPPA, CNRS, IPREM, Pau, France

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8 * Corresponding author at: Université de Pau et des Pays de l'Adour, E2S-UPPA, CNRS, IPREM, Pau,
9 France.

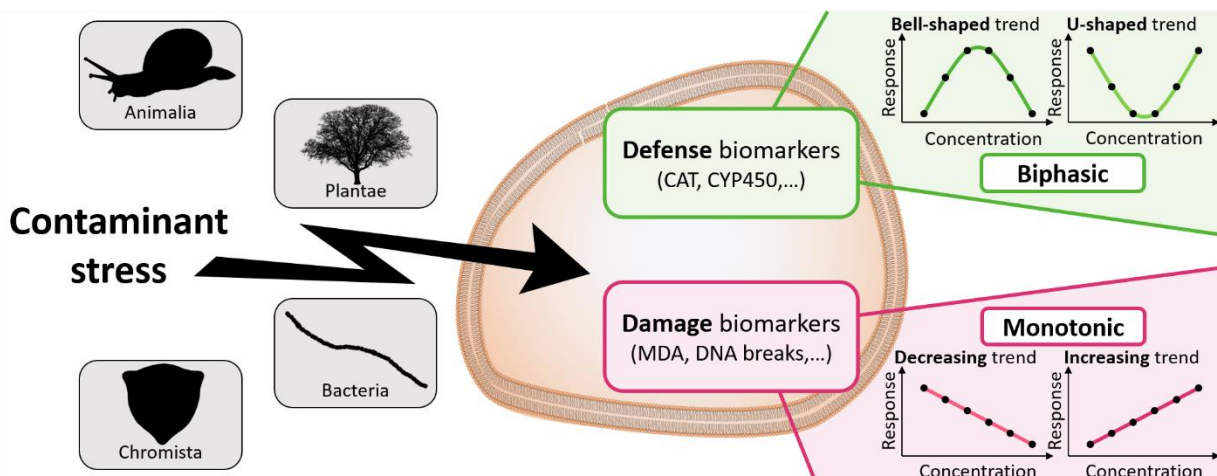
10 *E-mail address:* simon.colas@univ-pau.fr (S. Colas).

11
12 **Highlights**

- 13
14 • We interpreted 2,595 biomarker dose-response curves generated by chemical exposure.
15 • Defense biomarkers mainly describe biphasic (bell- or U-shaped) trends.
16 • Damage biomarkers mainly describe monotonic (decreasing or increasing) trends.
17 • Cellular defense and damage responses appear to have been conserved during evolution.
18 • Response trend analysis is a promising tool for environmental risk assessment.

19
20 **Graphical abstract**

21



22

23

24 **Abbreviations**

25

26 AIC, Akaike information criterion; BIC, Bayesian information criterion; CAT, catalase; CRIDaR,
27 concentration range inducing damage responses; CRIDeR, concentration range inducing defense
28 responses; HSP, heat-shock protein; LC-MS, liquid chromatography-mass spectrometry; ML,
29 maximum likelihood; N.D., non-determined; PQL, Penalized Quasi-Likelihood; RMSE, root-mean-
30 square error; SOD, superoxide dismutase.

31

32 Abstract:

33
34 Omics and multi-omics studies are currently increasingly used in ecotoxicology to highlight
35 the induction of known or new biomarkers when an organism is exposed to one (or more)
36 contaminant(s). Although it is virtually impossible to identify all biomarkers from all possible
37 organisms, biomarkers can be grouped into two categories, defense or damage biomarkers and
38 they have a limited number of response trends. Our working hypothesis is that defense and
39 damage biomarkers show different dose-response patterns. A meta-analysis of 156 articles and
40 2,595 observations of dose-response curves of well-known defense and damage biomarkers was
41 carried out in order to characterize the response trends of these biological parameters in a large
42 panel of living organisms (18 phyla) exposed to a wide variety of inorganic or organic
43 contaminants. Defense biomarkers describe biphasic responses (bell-shaped and U-shaped) to a
44 greater extent than damage biomarkers. In contrast, damage biomarkers varied mainly
45 monotonically (decreasing or increasing). Neither the nature of the contaminant nor the type of
46 organisms, whatever the kingdom (Plantae, Animalia, Chromista or Bacteria), influence these
47 specific responses. This result suggests that cellular defense and damage mechanisms are not
48 specific to stressors and are conserved throughout life. The meta-analysis results confirm the
49 usefulness of trend analysis in dose-response models as a biological interpretation of biomarkers
50 in large dataset and their application in determining the concentration ranges inducing defense
51 responses (CRIDeR) and the concentration ranges inducing damage responses (CRIDaR)
52 regardless of the contaminant tested or the organism studied.

53
54 Keywords: meta-analysis, omics analysis, CRIDaR, CRIDeR, ecotoxicology, toxicology

55 56 1. Introduction

57
58 Lethal endpoints are most commonly used to build dose-response curves to feed in risk
59 assessments of contaminants. There is however a growing interest in identifying sublethal
60 endpoints that can provide information on early effects at the individual level. Biomarkers can be
61 used as indicators of sublethal effects, to quantify a stress on an organism of interest. They can
62 be also used for environmental monitoring. A biomarker is defined as an observable and/or
63 measurable change at the molecular, biochemical, cellular, physiological or behavioral level,
64 which indicates the present or past exposure of an organism to at least one stressor[1,2]. It can
65 also provide information on early effects at the individual level[3]. The biological unity of the living
66 world means that the same types of biomarkers can be used for organisms belonging to different
67 taxonomic groups. Well-known biomarkers include catalase (CAT)[4], superoxide dismutase
68 (SOD)[5], heat-shock proteins (HSP)[6] and single-strand DNA breaks[7]. A further distinction can
69 be made between defense biomarkers and damage biomarkers[8]. Defense biomarkers result
70 from protective reactions of the organism exposed to a contaminant. Induction of these
71 mechanisms will allow the organism to cope with a stress. Damage biomarkers, on the other hand,
72 reflect a direct and harmful change caused to the exposed organism. They are the result of an
73 alteration that can lead to an inability to grow, reproduce or even survive.

74 The identification of new biomarkers is an important field of research today in a context of
75 the increasing complexity of stress caused by global changes[9]. The development of “omics”
76 tools over the last decade is a promising avenue to address that challenge[10–12]. However,
77 despite the recent progress, it is still difficult to provide a biological interpretation to an
78 observation of change in specific sub-cellular or cellular parameters. This is particularly true for
79 metabolomics, for which, a large proportion of the metabolites observed cannot be identified
80 with the current information available in databases. In mass spectrometry, especially with liquid
81 chromatography-mass spectrometry (LC-MS) analyses, only 4%-5% of the peaks can be identified
82 in biological samples[13]. This gap in the reference databases is also encountered in genomics[14]
83 and proteomics[15].

84 However, even if a biomarker cannot be characterized, it is still possible to model its response
85 as a function of the dose of exposure. These dose-response curves are usually drawn following
86 exposure to increasing concentrations of a substance in order to characterize its toxicity[16]. Four
87 main types of trends can then be observed[17]. In bell-shaped curves, an increasing biomarker
88 response up to a maximum is observed at low doses, followed by a decrease at higher
89 concentrations of the exposure substance. The U-shaped curves follow the same principle but in
90 the opposite direction, *i.e.*, a decrease down to a minimum followed by an increase. These two
91 types of trends can be grouped together as biphasic responses. In contrast, monotonic responses
92 are characterized by continuously increasing or decreasing trends. The increasing curves
93 correspond to a continuous increase of the biological response studied as a function of the
94 increase of the concentrations of the substance of interest. Conversely, the decreasing curves
95 indicate a decrease in the biomarker response as a function of the increase in exposure
96 concentrations.

97 A very recent study focused on metabolomic response of biofilms exposed to cobalt[18]. The
98 metabolite dose-response curves highlighted a concentration range inducing defense responses
99 (CRIDeR), mainly characterized by bell and U-shape trends, and a concentration range inducing
100 damage responses (CRIDaR) mainly composed of increasing and decreasing trends. These two
101 distinctive ranges of concentrations were validated with the use of biomass and chlorophyll
102 measurements, confirming the usefulness of this novel approach. Our working hypothesis is then
103 that the trends in dose-response curves depend on the role of the biomarker, *i.e.*, defense or
104 damage biomarkers, which can be distinguished according to whether they describe a biphasic or
105 monotonic response. To this end, we conducted a meta-analysis in which we examined the trends
106 of biomarker dose-response curves in several phyla exposed to a contaminant (organic or
107 inorganic). We tested whether this hypothesis was valid regardless of the studied contaminant
108 and phylum. There was no *a priori* on the species in order to have a broad representation of the
109 response to contamination. The conclusions of this study could be used to interpret the dose-
110 response curves plotted from a large data set of unidentified “omics” data and support their use
111 in environmental risk assessment.

112 113 **2. Material and methods**

114 115 2.1. Biomarker compilation

116 Compilation of biomarkers was carried out searching *Google Scholar* and *Scopus* using
117 combination of keywords, *e.g.*, *biomarker AND ecotoxicology AND dose-response*. Biomarkers
118 were classified either as defense or damage according to their role in organisms (Table 1 and
119 Table S1)[19,20]. The search was performed between September 2022 and June 2023, without
120 any restriction on the publication date. The aim was to reach an equal proportion of observations
121 of biomarkers of damage and defense (around 1,000 observations for each category), as well as
122 between organic and inorganic contaminants. To achieve this, we had to refine the article search
123 by adding keywords to the initial search: (i) *damage biomarker OR defense biomarker AND*
124 *ecotoxicology AND dose-response*, (ii) *biomarker AND ecotoxicology AND dose-response AND*
125 *metal*.

126

127 2.2. Selection criteria

128 Once the article compilation was completed, selection criteria were applied after a more
129 detailed analysis of the articles: i) only articles on whole organisms (no cell lines) were considered,
130 without selection criteria on the phylum and the species studied, ii) only experiments performed
131 under controlled conditions were kept (no field studies) to have results represented as dose-
132 response curves, iii) only studies on the toxicity of organic and inorganic contaminants were
133 chosen and iv) the number of tested contaminant concentrations had to be equal to or greater
134 than four to be able to identify a trend in the dose-response studied.

135

136 2.3. Data extraction

137 For all articles meeting the selection criteria, the information used for the meta-analysis
138 was extracted as follows. First, each biomarker was classified in either as defense or damage
139 categories according to its biological role (Table 1). The studied species and its taxa were
140 documented (Table S1). Information on the contaminant was also indicated *e.g.* its name, its
141 category (organic or inorganic), the range of concentrations tested, the number of concentrations
142 tested and the exposure time. Finally, the trends of fluctuations in the response of the biomarkers
143 were examined according to the significance of the statistical tests used by the different authors.
144 Two scenarios were encountered.

145 In the first one, statistical tests were performed to compare the biological response at
146 each tested concentration. If at the highest concentration, the response of the biomarker was
147 significantly higher than that of the control and that no intermediate concentration had a
148 response significantly higher than the response at the highest concentration, then the response
149 trend was characterized as "increasing". Conversely, if at the highest concentration tested, the
150 response of the biomarker was significantly lower than that of the control and that no
151 intermediate concentration had a response significantly lower than the response at the highest
152 concentrations, then the response trend was characterized as "decreasing". On the other hand,
153 if at an intermediate concentration (or several if they followed one another), the response of the
154 biomarker studied was both significantly higher than that of the control group and that of the
155 highest concentration tested, then the trend was considered to be "bell-shaped". In the same
156 way, if at an intermediate concentration (or several if they followed one another), the response
157 of the biomarker studied was both significantly lower than that of the control group and that of
158 the highest concentration, then the trend was considered to be "U-shaped".

159 In the second case, the statistical tests compared the biological response only to the
160 control group. Then the trends were determined as follows: the trend was characterized as
161 "increasing" when (i) only the biomarker response at the highest concentration was significantly
162 higher than that of the control or (ii) the biomarker response at the highest concentration was
163 significantly higher than that of the control. All the lower limits of the standard deviations of the
164 response at the previous intermediate concentrations, which were significantly higher than that
165 of the control, had to be lower than the upper standard deviation of the highest concentration.
166 In addition, no response at any intermediate concentrations was to be significantly lower than
167 that of the control. Conversely, the trend was characterized as "decreasing" when (i) only the
168 biomarker response at the highest concentration was significantly lower than that of the control
169 or (ii) the biomarker response at the highest concentration was significantly lower than that of
170 the control. All the upper limits of the standard deviations at the previous intermediate
171 concentrations, which were significantly lower than that of the control, had to be greater than
172 the lower standard deviation of the highest concentration. In addition, no response at any
173 intermediate concentrations was to be significantly higher than that of the control. The trend was
174 characterized as "bell-shaped" when (i) the response of the biomarker at an intermediate
175 concentration (or several if they followed one another) was significantly greater than that of the
176 control group, and (ii) the lower limit of the standard deviation of the response at the
177 intermediate concentration was greater than the upper limit of the standard deviation of the
178 response of the group exposed to the highest concentration. In the same way, when (i) the
179 response of the biomarker at an intermediate concentration (or several if they followed each
180 other) was significantly smaller than that of the control group, and (ii) its upper limit of the
181 standard deviation was smaller than the lower limit of the standard deviation of the response at
182 the highest concentration, then the trend was characterized as "U-shaped".

183 In cases where there were no significant response differences between the tested
184 concentrations, then the trend was characterized as "constant". In all other cases, for example
185 when the response seemed to show more than two trends, it could not be characterized and was
186 tagged as "non-determined" (N.D.). These last two categories of trends (constant and N.D.) were
187 not considered in the meta-analysis.
188

189 2.4. Data analysis

190 First, the effect of biomarker type on response trends was investigated. To that end,
191 multinomial logit models were fitted to model the proportion of response change as groups of
192 monotonic or biphasic trends in relation to the biomarker type -damage as reference or defense-
193 ("Effect"), the contaminant studied -inorganic as reference or organic- ("Contaminant") and the
194 number of studied concentrations ("scale(Concentration)"). Random effect variables were
195 introduced into the basic model, including the bibliographic references, *e.g.*, selected articles,
196 ("Ref"), exposure time ("Time_exposure") and phylum ("Phylum"). Each combination of random
197 effect variables was tested and the model with the best AIC (Akaike Information Criterion) was
198 chosen[21]. Secondly, a multinomial logit model was also fitted to model the proportion of

199 response trends (divided into four categories: bell-shaped, decreasing, increasing and U-shaped)
200 in relation to the biomarker type (damage as reference or defense), the contaminant studied
201 (inorganic as reference or organic). As before, random effect variables were added to the basic
202 model, including the bibliographic reference, exposure time and phylum information. Each
203 combination of random effect variables was also tested and the model with the best AIC was
204 chosen.

205 Finally, a multinomial logit model was also fitted to study the type of response (monotonic
206 or biphasic trends) of the most represented phyla (at least 50 observations) along four kingdoms
207 (Animalia, Plantae, Chromista and Bacteria) in the analysis, *i.e.*, Annelida (552), Arthropoda (191),
208 Chlorophyta (323), Chordata (283), Cyanobacteria (51), Magnoliophyta (688), Mollusca (278) and
209 Ochrophyta (97), in relation to the biomarker type (damage as reference or defense), the
210 contaminant studied (inorganic as reference or organic) and the number of studied
211 concentrations with “bibliographic reference” as a random effect variable. Multinomial logit
212 models were fitted using the function *mblogit* in the *mclgfit* package (0.9.7.)[22] with the
213 maximum likelihood (ML) as an estimator of variance components and the Penalized Quasi-
214 Likelihood (PQL) method for modeling the random effects set. Statistical analyses were
215 performed on R software.

216

217 2.5. Bias and *a posteriori* check

218 The authors developed the strategy for article selection and subsequent data extraction.
219 One author carried out the selection and data extraction. In order to assess the possible
220 interpretation bias in the data extraction, *i.e.*, in the determination of the trend in biological
221 responses, 20% of randomly selected articles (34/156) was additionally analyzed by the second
222 author. A percentage of similarity was calculated between the author 1’s and the author 2’s data
223 extractions, and corrections were made where necessary.

224

225 3. Results

226

227 3.1. Data compilation

228

229 The process for selecting studies and selected extracted data observations (identification,
230 screening, eligibility and inclusion in the meta-analysis) is reported in a flow diagram (Fig. S1). The
231 global build-up database of the meta-analysis is available in the supplementary information
232 section (Table S1). On 4,126 identified articles, a total of 156 articles were selected for this meta-
233 analysis and 3,176 observations of variation of biomarkers could be extracted. Among them, 581
234 categorized as “constant” or “N.D.” were subsequently removed. One hundred and nineteen
235 species are represented along 18 phyla (Annelida: 552 observations, Arthropoda: 191,
236 Ascomycota: 6, Cercozoa: 4, Charophyta: 32, Chlorophyta: 332, Chordata: 283, Ciliophora: 16,
237 Cnidaria: 7, Cyanobacteria: 51, Echinodermata: 4, Euglenozoa: 3, Haptophyta: 24, Mollusca: 278,
238 Myzozoa: 6, Ochrophyta: 97, Porifera: 6, Magnoliophyta: 688) and communities (biofilm: 15). The
239 impacts of 176 contaminants (or contaminant combinations) including personal care products,
240 pesticides, herbicides, nanoparticles or metals were studied (Table S1).

241

242 3.2. Data interpretation

243

244 In total, 20% of the total observations were re-extracted (636/3,176) out of the 34
245 randomly selected articles for comparison of trend analysis. The percentage of data extraction
246 similarity between the two investigators was 97.6%.

247

248 3.3. Model selection

249

250 Based on the AIC values (equations 5 and 13 in Table S2), the best-fit models to explain
251 the variations in the biphasic (bell-shaped and U-shaped) and monotonic (decreasing and
252 increasing) trends were for the fixed effects: the type of biomarker (“Effect”), the type of
253 contaminant (“Contaminant”) and the number of concentrations tested (“scale(Concentration)”)
254 and, for the random effects, the article selected (“Ref”).

255

256 3.4. Response trends of biomarkers according to their category: defense or damage

257

258 In the compiled database, biomarkers distribute almost equally between defense
259 biomarkers (1,325) and damage biomarkers (1,270) (Table S1). Among the defense biomarkers,
260 402 (30.3%) had a bell-shaped trend, 299 (22.6%) had a decreasing trend, 529 (39.9%) had an
261 increasing trend and 95 (7.2%) had a U-shaped trend. This distribution was different for damage
262 biomarkers, i.e., 139 (10.9%) had a bell-shaped trend, 439 (34.6%) had a decreasing trend, 644
263 (50.7%) had an increasing trend and 48 (3.8%) had a U-shaped trend (Fig. 1A and Table 2). The
264 proportion of biomarkers with a biphasic trend (bell-shaped or U-shaped) is significantly higher
265 for defense biomarkers than for damage biomarkers (Table 2) (p -value < $2e-16$) whereas the
266 proportion of biomarkers with a monotonic trend (decreasing or increasing) is significantly
267 greater for damage biomarkers than for defense biomarkers (Table 2) (p -value < $2e-16$). More
268 precisely, the proportion of bell-shaped trends is significantly greater than that of decreasing and
269 increasing trends for defense biomarkers (Tables 3A-C). For clarity, here and in the rest of the
270 text, p -values of the models are only given in their corresponding tables. Conversely, the
271 proportions of decreasing and increasing trends are significantly greater than the bell-shaped
272 trends for damage biomarkers (Tables 3A-C). The same observation was made for the proportions
273 of U-shaped trends compared with those of decreasing and increasing trends (Tables 3B-D).

274

275 3.5. Response trends of biomarkers according to the type of contaminant: organic or 276 inorganic

277

278 A total of 1,321 observations in the meta-analysis database relates to the effect of organic
279 contaminants and 1,274 to the effect of inorganic contaminants. Biomarkers concerning organic
280 contaminant exposure were either categorized as defense biomarkers (646) or damage
281 biomarkers (675) (Table S1). Among the defense biomarkers, 223 (34.5%) had a bell-shaped trend,
282 150 (23.2%) had a decreasing trend, 208 (32.26%) had an increasing trend and 65 (10.1%) had a
283 U-shaped trend. The observations were different for damage biomarkers, i.e., 78 (11.6%) had a
284 bell-shaped trend, 224 (33.2%) had a decreasing trend, 342 (50.7%) had an increasing trend and

285 31 (4.6%) had a U-shaped trend (Fig. 1B). The different biomarkers following inorganic
286 contaminant exposure were distributed with 679 as defense biomarkers and 595 as damage
287 biomarkers (Table S4). Among the defense biomarkers, 179 (26.4 %) had a bell-shaped trend, 149
288 (21.9%) had a decreasing trend, 321 (47.3%) had an increasing trend and 30 (4.4%) had a U-
289 shaped trend. For damage biomarkers, the observations were different with 614 (10.3%) with a
290 bell-shaped trend, 215 (36.1%) with a decreasing trend, 302 (50.8%) with an increasing trend and
291 17 (2.9%) with a U-shaped trend (Fig. 1C). The proportion of biomarkers with a biphasic trend
292 (bell-shaped or U-shaped) and the proportion of biomarkers with a monotonic trend (decreasing
293 or increasing) were not affected by the type of contaminant (Tables 2, 3, S3-6 and S8-10). The
294 type of contaminant had only an influence on the proportion of U-shaped trends (Table 3D), with
295 more U-shaped response after an exposure to organic contaminants. In all other cases, the
296 contaminant type had no influence on the type of trends.

297

298 3.6. Biomarker response trends according to phylum

299

300 To determine the effects of taxa on the biomarker response trends, bell- and U-shaped
301 trends were grouped as a biphasic response whereas the increasing and decreasing trends were
302 classified as a monotonic response. These groupings into two main categories were made to have
303 a sufficient number of data for each type of response in each phylum. For the selected phyla, a
304 uniform response is obtained. Defense biomarkers have significantly higher proportions of
305 biphasic trends than damage biomarkers (Fig. 2, and Tables S3-6 and S8-10), except for
306 Cyanobacteria where there is no significant difference among groupings (Table S7). Conversely,
307 damage biomarkers have significantly higher proportions of monotonic trends than defense
308 biomarkers. The type of contaminant (inorganic or organic) had no influence on the response
309 trends.

310

311 3.7. Biomarker response trends according to the number of tested concentrations

312

313 The number of concentrations used has a significant influence on the observation of
314 biphasic trends. Indeed, the larger it is, the greater the proportion of biphasic trends (bell- and U-
315 shaped) compared to monotonic trends (Tables 2 and 3A-D). Overall, 40% of our observations
316 came from studies that had tested four concentrations, 30% of the observations from five
317 concentrations, 18% from six concentrations and the remainder between 7 and 15
318 concentrations.

319

320 4. Discussion

321

322 4.1. Biomarkers have different response patterns depending on their biological role

323

324 The present meta-analysis highlights that defense biomarkers can mainly be described by
325 a bell- or a U-shape dose-response curve, *i.e.*, by a biphasic trend, whereas the damage
326 biomarkers are mainly characterized by a linear trend, either increasing or decreasing, *i.e.*, by a
327 monotonic trend (Fig. 1A). This difference in response trend could be related to the intrinsic
328 mechanisms of these biomarkers. Molecular, sub-cellular and cellular processes involved in the

329 defense of organisms against stress (here exposure to a contaminant) are induced at low
330 concentrations. Then, the concentrations of defense biomarkers describing a bell-shape curve
331 increase as a function of the increase of contaminant concentration in an attempt to maintain
332 cellular integrity. When the concentrations of the contaminant are too high, these defense
333 mechanisms are overwhelmed and a reduction in their response at the highest concentrations is
334 then often gradually observed[23]. Defense biomarkers with a U-shaped dose-response would
335 follow the same principle of biphasic response but in reverse[23]. The induction of damage
336 biomarkers would be initiated mainly once the defense mechanisms are overwhelmed and would
337 result from the continuous degradation of cellular and sub-cellular compounds, hence their
338 increasing and decreasing trend are proportional to the intensity of the stress[24].

339

340 4.2. Contaminants trigger similar defense and damage mechanisms in organisms

341

342 Trends in defense and damage biomarkers were demonstrated to be independent of the
343 nature of the contaminants (organic or inorganic). Indeed, except for the U-shaped response
344 trends after exposure to an inorganic contaminant (Tables 3A-D), the biomarkers involved in
345 defense mechanisms were mainly described by an increase in bell-shaped and U-shaped trends
346 whereas those involved in damage processes mainly vary in a linear way (Fig. 1B and 1C). This
347 observation supports the tenet that cellular defense mechanisms are not specific to a particular
348 stress[25] but can respond to a multitude of disturbances. In this study, the impact of organic and
349 inorganic contaminants was assessed but similar results might be observed with other stressors
350 such as pH, UV irradiation or temperature. For example, increasing temperature resulted in a GST
351 activity describing a bell-shaped trend in the fish *Sparus aurata*[26] and U-shaped trend in the
352 fresh crab *Aegla longirostri*[27]. In both organisms, CAT activity in their livers and hepatopancreas
353 followed a bell-shaped trend whereas MDA levels in their muscles showed an increasing trend.
354 Cocktail-effects studies also show these patterns of fluctuation specific to defense and damage
355 biomarkers. For example, EROD (CYP450), GST and SOD activities in goldfish exposed to a mixture
356 of norfloxacin and sulfamethoxazole described a bell-shaped response trend with increasing
357 exposure concentrations whereas DNA damage increased strictly monotonically with
358 contaminant concentrations[28].

359 Here, the parameters considered for each contaminant were the exposure concentrations
360 and the modification of biomarker responses. However, similar trends could also be observed by
361 studying exposure time (for a given concentration). In earthworms (*Eisenia fetida*) exposed to
362 perfluorooctane sulfonamide for 10 days, two defense biomarkers (CYP450 and GST) showed bell-
363 shaped response trends over time[29]. The biomarkers of damage (MDA level and AChE activity)
364 in *Bellamyia aeruginosa* exposed for 28 days to different concentrations of Cd and Pb (alone or in
365 a mixture) always varied monotonically (increase) as a function of time whereas defense
366 biomarkers (SOD, CAT, GPx or metallothioneins) described bell- or U-shaped trends over time at
367 certain concentrations[30]. Nevertheless, further analysis of the literature remains to be done to
368 conclude on these trends regarding other stresses, cocktail-effects and exposure time.

369

370 4.3. Defense and damage mechanisms are preserved throughout life

371

372 The specific study of each of the most represented phyla (Annelida, Arthropoda,
373 Chlorophyta, Chordata, Cyanobacteria Magnoliophyta, Mollusca and Ochrophyta) allowed us to
374 observe the same type of response of defense and damage biomarkers. Indeed, the defense
375 biomarkers showed significantly more biphasic responses than the damage biomarkers.
376 Conversely, they showed significantly less monotonic responses (Fig. 2). This was observed for all
377 phyla considered in the analysis (Tables S3-10). As such, Plantae (unicellular and pluricellular)
378 mainly responded to contaminant exposure (organic and inorganic) in a similar way as Animalia.
379 Although the number of observations was lower for Chromista and Bacteria, the responses to
380 exposure to contaminants were also comparable to organisms from the other kingdoms studied.
381 These observations allow to support, that the processes of cellular defense have been conserved
382 during the evolution within different kingdom[31] and that the organizational similarity of cells
383 leads to comparable cell damage between different species.

384

385 4.4. Importance of a number of tested concentrations in ecotoxicology

386

387 This study also demonstrated the importance of having several tested concentrations in
388 ecotoxicology tests. A large range of tested concentrations increases the possibility of observing
389 biphasic trends, potentially leading to a better understanding of the underlying toxicity
390 mechanisms. This is particularly true if the concentration range also includes “low”
391 concentrations[32]. Adding intermediary concentrations with similar concentration intervals
392 would provide a best modelling of the dose-response curves.

393

394

395 4.5. Implications for the use of metabolomics in ecotoxicology

396

397 Part of the biomarkers compiled in the present meta-analysis are metabolites of low
398 molecular weight. With the increase use of metabolomics and non-targeted approaches in
399 particular, a large number of metabolites as well as their variation can be measured in organisms
400 upon exposure to a contaminant. However, the identification of these metabolites remain
401 challenging as many metabolites remain unannotated in the currently available databases[13].
402 Assessing their variations via the use of tools such as DRomics for example[17] and analysing their
403 dose-response trends such as performed in the present meta-analysis is an innovative way to
404 evaluate stress in organisms without losing the interest of omics studies and their large number
405 of data[18].

406

407 5. Conclusion

408

409 The present meta-analysis of biomarker response trends showed that the defense
410 mechanisms of living organisms (Animalia, Bacteria, Chromista and Plantae) exposed to
411 different types of contaminants (organic and inorganic) predominantly described biphasic (bell-
412 and U-shaped) dose-responses. In contrast, the damage processes induced by these
413 contaminants were mostly monotonic (increasing and decreasing). The meta-analysis confirms

414 the relevance of dose-response trend analysis as a new omics data processing approach and the
415 identification of CRIDeR and CRIDaR for environmental risk assessment.

416

417

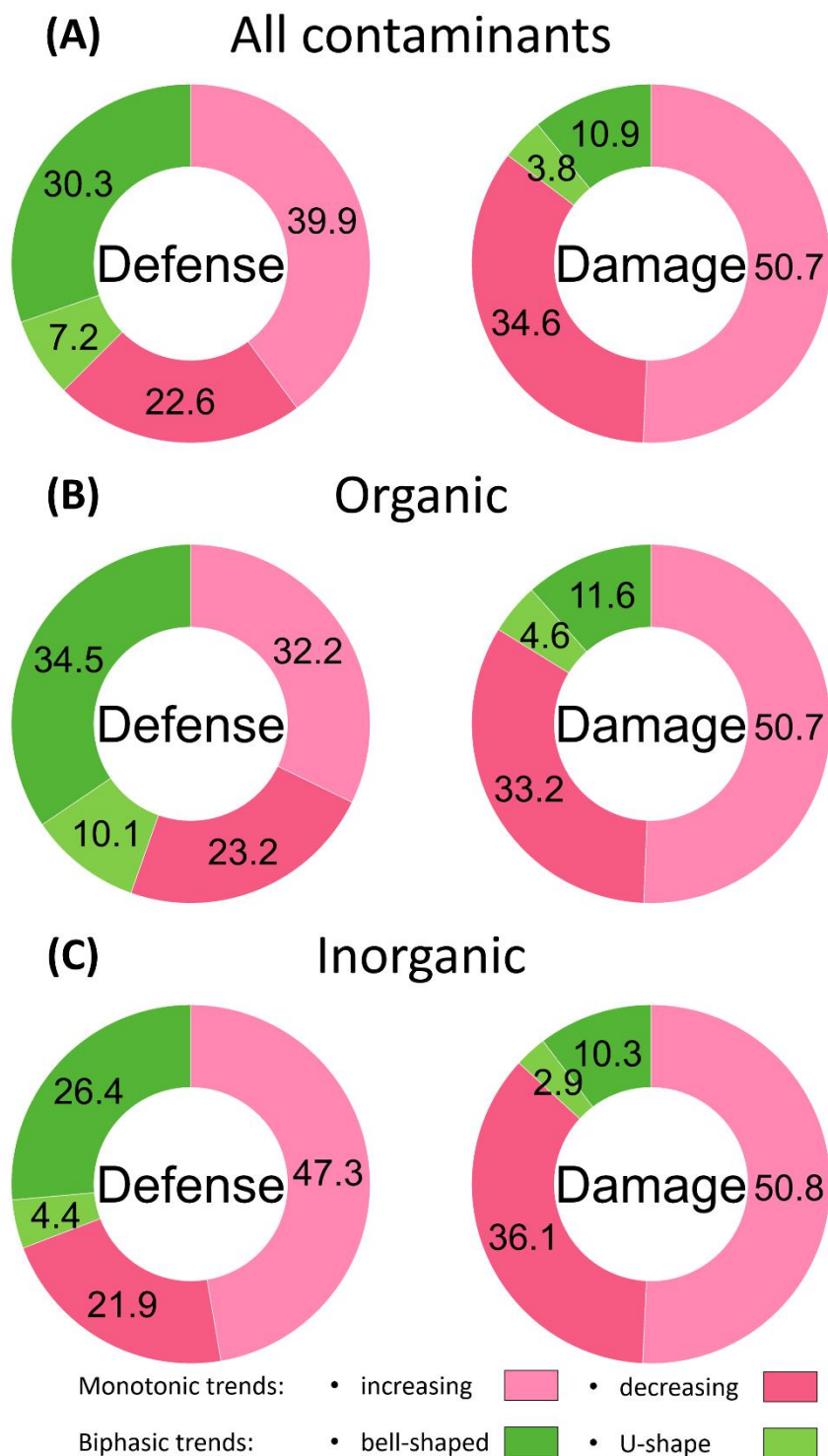
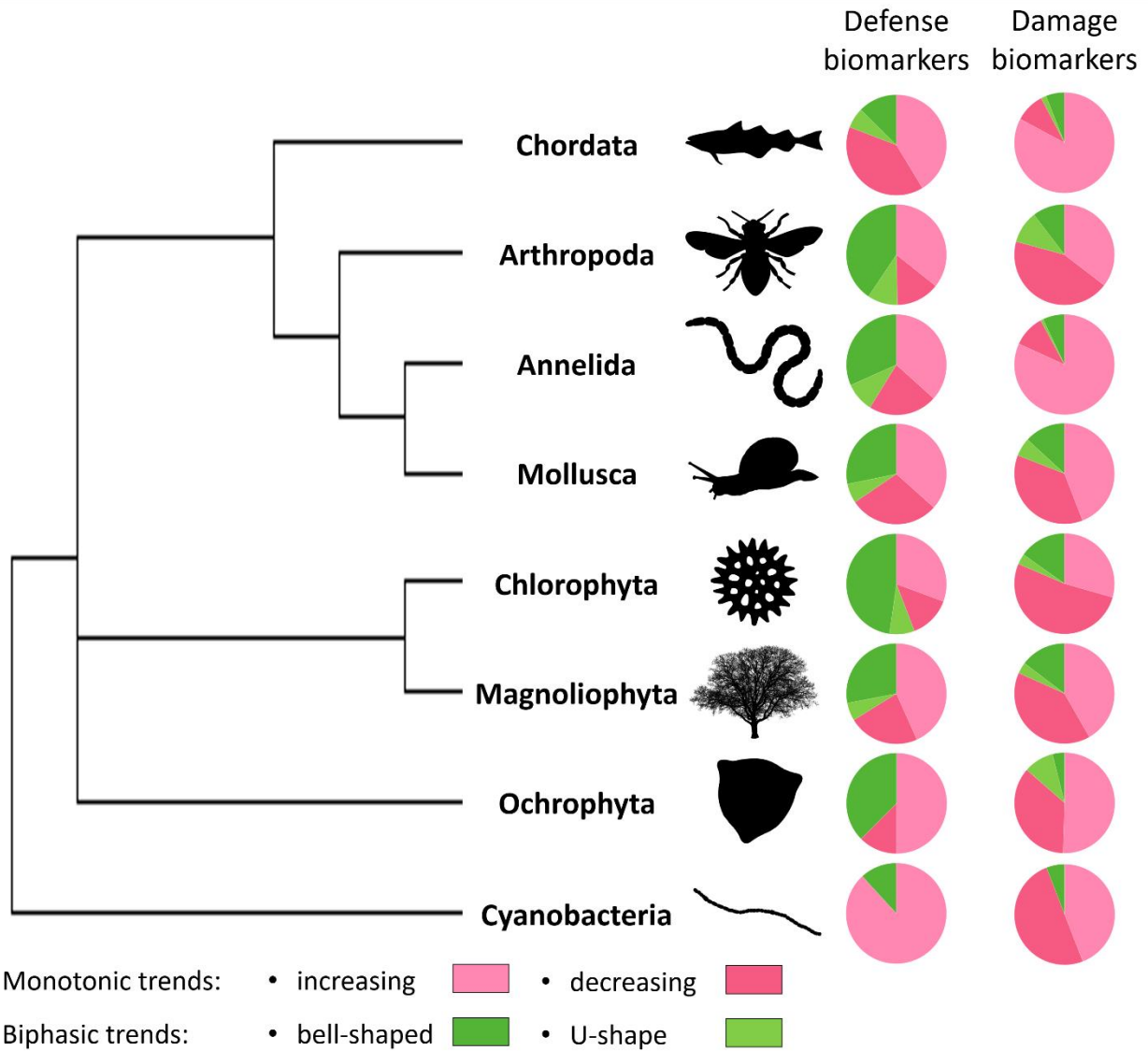


Figure 1: Proportions of biomarker response trends by response mechanism (defense or damage). (A) Influence of the category of biomarkers on the response trends to a contaminant exposure; (B) to an organic contaminant exposure; (C) to an inorganic contaminant exposure.



418 **Figure 2:** Representation of an unrooted tree of the most represented phyla (with a species example representation) and their proportions of trend of biomarker responses by response mechanism (defense or damage).

419 **Table 1:** Examples of biomarker classification (defense and damage) as a function of their
 420 molecular, sub-cellular and cellular role.

Class of biomarkers	Roles	Biomarkers	Reference
Defense biomarkers	Biotransformation enzymes and associated compounds	Cytochrome P450 (CYP450)	[33]Stegeman et al. (1992)
		Glutathione S-transferase (GST)	[34]George (1994)
		Glutathione (GSH)	[34]George (1994)
	Toxic efflux	P-glycoproteins	[35]Bard (2000)
		Multixenobiotic resistance systems (MXR)	[35]Bard (2000)
	Metals detoxication	Metallothioneins	[36]Hall (2002)
		Phytochelatins	[36]Hall (2002)
	Oxidative stress response enzymes and molecules	Superoxide dismutase (SOD)	[33]Stegeman et al. (1992)
		Catalase (CAT)	[33]Stegeman et al. (1992)
		Glutathione peroxidase (GPx)	[37]Lauterburg et al. (1982)
Glutathione reductase (GR)		[19]Van der Oost et al. (2003)	
Chaperone proteins	Heat shock proteins (HSP)	[38]Feder and Hofmann (1999)	
Damage biomarkers	Molecular damages	Acetylcholinesterase (AChE)	[39]Payne et al. (1996)
		Vitellogenin	[40]Matozzo et al. (2008)
		Malondialdehyde (MDA)	[41]Janero (1990)
	DNA damages	Photosynthetic pigments (chlorophyll <i>a/b</i> , carotenoids)	[42]Pikula et al. (2019)
		DNA adducts	[43]La and Swenberg (1996)
		Single-strand DNA breaks	[7]Dhawan and Bajpayee (2009)
	Subcellular and cellular damages	Double-strand DNA breaks	[44]Heddle et al. (1983)
		Lysosomal membrane stability	[45]Lowe and Pipe (1994)
	Energetic metabolism damages	Adenylate energy charge (CEA)	[46]Lagadic et al. (1994)
	ROS production	Hydrogen peroxide	[47]Halliwell and Gutteridge (1989)
Superoxide radical		[47]Halliwell and Gutteridge (1989)	
Hydroxyl radical		[47]Halliwell and Gutteridge (1989)	

421

422

423 **Table 2:** Results of the Multinomial Logistic Regression to examine the effect of the type of
 424 biomarkers (Defense vs. Damage), the type of contaminants (Inorganic vs. Organic) and the
 425 number of tested concentrations on response trends (Biphasic or monotonic). Significant fixed
 426 effects are depicted in bold font and indicated with asterisks as follows: * $p < 0.5$, ** $p < 0.01$, ***
 427 $p < 0.001$.

Fixed Effects					
Contrast	Effect	Estimate	Std.Error	z	p
monotonic vs biphasic	Intercept	2.1344	0.1818	11.739	< 2e-16 ***
	Effect-Damage (ref)				
	Effect-Defense	-1.3108	0.1194	-10.980	< 2e-16 ***
	Contaminant-Inorganic (ref)				
	Contaminant-Organic	-0.3764	0.2287	-1.646	0.0997
	scale(Concentration)	-0.4664	0.1067	-4.370	1.24e-05 ***
Random Effects					
Ref	~1	Estimate	Std. Error	Approximate residual deviance:	2,471
				Number of Fisher scoring iterations:	6
biphasic~1		0.7488	0.05249	Number of observations	Groups by references: 156
monotonic ~1		0.613	0.02514	Individual observations:	2,595
Model performance					
AIC	BIC	RMSE	Sigma		
2,483.06	2,518.229	0.37	0.977		

428

429

430 **Table 3:** Results of the Multinomial Logistic Regression to examine the effect of the type of
 431 biomarkers (Defense vs. Damage), the type of contaminants (Inorganic vs. Organic) and the
 432 number of tested concentrations on response trends (bell-shaped, decreasing, increasing and U-
 433 shaped).(A) Model with bell-shaped trend as referent, (B) model with decreasing trend as
 434 referent, (C) model with increasing trend as referent and (D) model with U-shaped as referent.
 435 Significant fixed effects are depicted in bold font and indicated with asterisks as follows: * p
 436 < 0.05 , ** $p < 0.01$, *** $p < 0.001$.

(A)		Fixed Effects (bell-shaped as referent)			
Contrast	Effect	Estimate	Std.Error	z	p
decrease vs bell	Intercept	1.32218	0.22965	5.757	8.55e-09 ***
	Effect-Damage (ref)				
	Effect-Defense	-1.34802	0.14858	-9.071	<2e-16 ***
	Contaminant-Inorganic (ref)				
	Contaminant-Organic	-0.08871	0.29334	-0.302	0.76233
increase vs bell	scale(Concentration)	-0.44673	0.14127	-3.162	0.00157 **
	Intercept	1.8892	0.2206	8.565	<2e-16 ***
	Effect-Damage (ref)				
	Effect-Defense	-1.3884	0.1392	-9.975	<2e-16 ***
	Contaminant-Inorganic (ref)				
U vs bell	Contaminant-Organic	-0.3710	0.2828	-1.312	0.189
	scale(Concentration)	-0.6043	0.1328	-4.550	5.36e-06 ***
	Intercept	-1.0918	0.2639	-4.137	3.51e-05 ***
	Effect-Damage (ref)				
	Effect-Defense	-0.3638	0.2194	-1.658	0.0973
U vs bell	Contaminant-Inorganic (ref)				
	Contaminant-Organic	0.5589	0.2840	1.968	0.0491 *
	scale(Concentration)	-0.3030	0.1297	-2.337	0.0194 *
	Intercept				
	Effect-Damage (ref)				
Random Effects					
Ref	.~1				
	Estimate	Std. Error	Approximate residual deviance: 5,384		
bell~1	1.063	0.2159	Number of Fisher scoring iterations: 7		
decrease ~1	1.12	0.2511	Number of observations		
increase ~1	1.052	0.1818	Groups by references: 156		
U ~1	1.119e-06	1.123e-19	Individual observations: 2,595		
Model performance					
AIC	BIC	RMSE	Sigma		
5,415.505	5,509.287	0.357	1.444		

437

(B)					
Fixed Effects (decreasing as referent)					
Contrast	Effect	Estimate	Std.Error	z	p
bell vs decrease	Intercept	-1.32218	0.22965	-5.757	8.55e-09 ***
	Effect-Damage (ref)				
	Effect-Defense	1.34802	0.14858	9.072	<2e-16 ***
	Contaminant-Inorganic (ref)				
	Contaminant-Organic	0.08871	0.29334	0.302	0.76233
increase vs decrease	scale(Concentration)	0.44673	0.14127	3.162	0.00157 **
	Intercept	0.56699	0.19998	2.835	0.00458 **
	Effect-Damage (ref)				
	Effect-Defense	-0.04039	0.11752	-0.344	0.73108
	Contaminant-Inorganic (ref)				
U vs decrease	Contaminant-Organic	-0.28232	0.27445	-1.029	0.30363
	scale(Concentration)	-0.15753	0.13920	-1.132	0.25779
	Intercept	-2.4140	0.2515	-9.598	<2e-16 ***
	Effect-Damage (ref)				
	Effect-Defense	0.9842	0.2095	4.698	2.63e-06 ***
U vs decrease	Contaminant-Inorganic (ref)				
	Contaminant-Organic	0.6476	0.2804	2.310	0.0209 *
	scale(Concentration)	0.1437	0.1395	1.030	0.3031
	Intercept				
Random Effects					
Ref	.~1				
	Estimate	Std. Error	Approximate residual deviance: 5,384		
decrease~1	1.12	0.2511	Number of Fisher scoring iterations: 7		
bell ~1	1.063	0.2159	Number of observations		
increase ~1	1.052	0.1818	Groups by references: 156		
U ~1	1.033e-06	8.832e-20	Individual observations: 2,595		
Model performance					
AIC	BIC	RMSE	Sigma		
5,415.505	5,509.287	0.357	1.444		

438

(C) Fixed Effects (increasing as referent)					
Contrast	Effect	Estimate	Std.Error	z	p
bell vs increase	Intercept	-1.8892	0.2206	-8.565	<2e-16 ***
	Effect-Damage (ref)				
	Effect-Defense	1.3884	0.1392	9.975	<2e-16 ***
	Contaminant-Inorganic (ref)				
	Contaminant-Organic	0.3710	0.2828	1.312	0.189
decrease vs increase	scale(Concentration)	0.6043	0.1328	4.550	5.36e-06 ***
	Intercept	-0.56699	0.19998	-2.835	0.00458 **
	Effect-Damage (ref)				
	Effect-Defense	0.04039	0.11752	0.344	0.73108
	Contaminant-Inorganic (ref)				
U vs increase	Contaminant-Organic	0.28232	0.27445	1.029	0.30363
	scale(Concentration)	0.15753	0.13920	1.132	0.25779
	Intercept	-2.9810	0.2443	-12.200	<2e-16 ***
	Effect-Damage (ref)				
	Effect-Defense	1.0246	0.2039	5.026	5.01e-07 ***
U vs increase	Contaminant-Inorganic (ref)				
	Contaminant-Organic	0.9299	0.2706	3.436	0.000589 ***
	scale(Concentration)	0.3012	0.1307	2.305	0.021169 *
Random Effects					
Ref	.~1				
	Estimate	Std. Error			
increase~1	1.052	0.1818	Approximate residual deviance: 5,384		
bell ~1	1.063	0.2159	Number of Fisher scoring iterations: 7		
decrease ~1	1.12	0.2511	Number of observations		
U ~1	1.33e-06	1.884e-19	Groups by references: 156		
			Individual observations: 2,595		
Model performance					
AIC	BIC	RMSE	Sigma		
5,415.505	5,509.287	0.357	1.444		

439

(D) Fixed Effects (U-shaped as referent)					
Contrast	Effect	Estimate	Std.Error	z	p
bell vs U	Intercept	1.0918	0.2639	4.137	3.51e-05 ***
	Effect-Damage (ref)				
	Effect-Defense	0.3638	0.2194	1.658	0.0973
	Contaminant-Inorganic (ref)				
	Contaminant-Organic	-0.5589	0.2840	-1.968	0.0491 *
decrease vs U	scale(Concentration)	0.3030	0.1297	2.337	0.0194 *
	Intercept	2.4140	0.2515	9.598	<2e-16 ***
	Effect-Damage (ref)				
	Effect-Defense	-0.9842	0.2095	-4.698	2.63e-06 ***
	Contaminant-Inorganic (ref)				
increase vs U	Contaminant-Organic	-0.6476	0.2804	-2.310	0.0209 *
	scale(Concentration)	-0.1437	0.1395	-1.030	0.3031
	Intercept	2.9810	0.2443	12.200	<2e-16 ***
	Effect-Damage (ref)				
	Effect-Defense	-1.0246	0.2039	-5.026	5.01e-07 ***
increase vs U	Contaminant-Inorganic (ref)				
	Contaminant-Organic	-0.9299	0.2706	-3.436	0.000589 ***
	scale(Concentration)	-0.3012	0.1307	-2.305	0.021169 *
	Intercept	2.9810	0.2443	12.200	<2e-16 ***
Random Effects					
Ref	.~1		Approximate residual deviance: 5,384 Number of Fisher scoring iterations: 7 Number of observations: 156 Groups by references: 156 Individual observations: 2,595		
	Estimate	Std. Error			
U~1	1.21e-06	1.417e-19			
bell ~1	1.063	0.2159			
decrease ~1	1.12	0.2511			
increase ~1	1.052	0.1818			
Model performance					
AIC	BIC	RMSE	Sigma		
5,415.505	5,509.287	0.357	1.444		

440

441

442 **Associated content**

443
444 The detailed dataset used for the meta-analysis, the parameters used to select the random
445 variables in the models and the results of the logistic multinomial regressions on the effects of
446 the type of biomarker, contaminant and number of doses tested on response trends within the
447 different phyla considered, as well as its PRISMA flow diagram are available in the supplementary
448 information section.

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456
457 **Simon Colas:** Conceptualization; Data curation; Formal analysis; Investigation;
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460 administration; Resources; Supervision; Validation; Writing – original draft; Writing – review &
461 editing.

462
463 **Declaration of Competing Interest**

464
465 The authors declare no competing financial interest.

466
467

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