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1 Transforming acute ecotoxicity data into chronic data: a statistical method to better inform radiological
2 risk for non-human species

3

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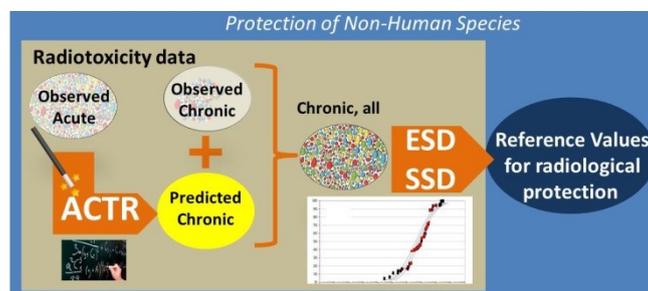
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10

11 Abstract

12 Ecotoxicity data constitute the basic information to support the derivation of ecological benchmark
13 values, whatever the stressor concerned. However the set of appropriate data may be limited,
14 especially with regard to chronic exposure conditions. The available data are often biased in favor of
15 acute data from laboratory controlled conditions, much easier to acquire. To make the best use of the
16 available knowledge and better inform effects of ionizing radiation chronic exposure on non-human
17 species, we investigated the transposition to ionizing radiation ecotoxicity of one method proposed for
18 chemicals to extrapolate chronic information from acute toxicity data. Such a method would contribute
19 to enrich chronic data sets required for the derivation of benchmark values making them more robust
20 when used as reference values for ecological risk assessment. We developed accordingly the ACTR
21 (Acute to Chronic Transformation for Radiotoxicity data) approach which we validated. We
22 introduced then the new concept of Endpoint Sensitivity Distribution (ESD). This finally allowed us to
23 compare purely chronic and ACTR-built ESDs for different taxa. For some of them, predicted and
24 observed distributions looked very similar. This promising ACTR method appeared applicable with a

25 reasonable level of confidence, but its generalization asks for improvements, some being already
26 identified.

27 **1 Introduction**

28 For any ecological risk characterization whatever the exposure situation is, the exposure level(s) of
29 animals and plants need to be compared with some form of numerical criteria which plays the role of
30 benchmark or reference value (concept of reference deviation^{1,2}). Several methods are internationally
31 recommended^{3,4} for the determination of such reference values, usually depending on the number and
32 nature of available basic ecotoxicity data, such as EC_x (Concentration giving x% change in observed
33 effect in comparison with a control). Acquisition of this type of information through laboratory testing
34 has long been subjected to constraints of all kinds, from fundings to ethics. In the field of
35 ecotoxicological research, this situation led to focus on some few model species exposed to a limited
36 set of conditions. As a consequence, the majority of existing work mainly deals with laboratory
37 experiments under a regime of acute exposure to high concentration of toxics, technically much easier
38 to realize and which guarantees a response from exposed organisms. Anyway, the ecotoxicity data
39 today available are mainly acute data, while the main operational needs relate to chronic exposure
40 situations generated by daily human activities. According to this observation, the interest of scientific
41 journals shifted recently towards long term exposure studies, and afferent results become
42 progressively more available, at least for some limited taxa. In the general shared context of resource
43 optimization, using the knowledge accumulated over years about acute toxicity requires developing
44 dedicated approaches.

45 Looking to derive robust reference values from those experimental outputs relies on the availability of
46 relevant methods. When a chronic ecotoxicity data set is satisfactory in terms of quality and quantity
47 for a given substance, the Species Sensitivity Distribution (SSD⁵) approach is recommended since
48 years now as the best method to determine ecological protection criteria such as EQSs (Environmental
49 Quality Standards^{3,4}). However, it can only be used for a small number of substances for which the
50 minimal data set required to build a chronic SSD is met. Considering that at the opposite the set of
51 acute data may be relatively large, methods have been proposed to inform chronic ecotoxicity from

52 acute toxicity data: extrapolation^{6,7}, Acute to Chronic Ratio^{8,9} (ACR) or Acute to Chronic
53 Transformation¹⁰ (ACT). Such extrapolation methods are currently applied for example in the
54 framework of Life Cycle Impact Assessments^{11,12} and were implemented in operational tools for a
55 long time by regulatory agencies such as the U.S. EPA¹³ to address data gaps in species sensitivity and
56 reduce reliance on uncertainty factors in ecological risk assessment. Adopting the widely used SSD
57 approach, the ACT approach was developed to transform a data sample assumed to be representative
58 of the acute toxicity of a substance into a sample considered to be representative of the chronic toxicity
59 of the same substance. During the last decade different concepts developed to deal with ecological risk
60 assessment for chemicals have already been successfully transposed to ionizing radiation and
61 radioactive substances^{14,15,16,17,18}. This work made it possible in particular to begin to address the
62 issue of effect of stressors mixture on fauna and flora, one key aspect under discussion for regulatory
63 risk assessment. Dealing purely with radiotoxicity eliminates the problem of mixture of radioactive
64 substances as radiotoxic effects expressed as radiological doses are additive.

65 Adapting and applying such ACT method for radiotoxicity data treatment (ACTR) would expand the
66 chronic dataset from the knowledge related to acute effects, and thereby lead to obtain sufficiently
67 large and qualitative dataset to allow a proper use of statistical extrapolation method such as SSD.
68 This ACT method aims not only to increase the number of available chronic data, but also to enrich
69 qualitatively these data sets. As such, regarding ionizing radiation or radioactive substances, it would
70 make it possible to inform chronic ecotoxicity for species for which there are no experimental chronic
71 data (but only acute ones). The expected increase in the number of data but also in the number of
72 species would give more robustness in the derivation of protection criteria for non-human species
73 exposed to ionizing radiation, the paucity of chronic datasets in terms of quantity and species diversity
74 being identified for a long time as a major weakness of the process.

75 In this publication, we explore if and how the ACT method may be applied to radionuclides. The
76 related two-phase study is described hereafter, firstly explaining the methodological aspect of the
77 ACTR approach, introducing the concept of Endpoint Sensitivity Distribution (ESD), and then
78 comparing purely chronic and ACTR-built ESDs.

79 The ACT for chemicals was developed to establish a relationship between several stressors of the
80 same nature for a single organism. Its transposition to radionuclides implies somewhere a conceptual
81 shift, looking to establish a parallel between multiple taxa for a single stressor, ionizing radiation.
82 Validating the ACTR method will allow to generate new (*i.e.* predicted) radiotoxicological data from a
83 purely desk study. Such approaches make the best use of already available knowledge and fully
84 comply with the growing demand on ethical and responsible experimentation on living organisms.
85 This is a process in line with the optimization of resources, including the reduction in costs, today
86 expected from all scientist.

87 **2 Material and methods**

88 ACT type methods are based on ecotoxicity data acquired for both acute and chronic exposure to a
89 given chemical. In both cases, chemotoxicity is expressed with regard to the chemical concentration in
90 the exposure medium. Biological effects of ionizing radiation, or radiotoxicity, are expressed in terms
91 of dose (rate) that is to say with regard to the energy deposited into the exposed organisms^{19, 20, 21}.
92 Corresponding units are Gy (dose, acute exposure) and Gy per unit of time (dose rate, chronic
93 exposure), that implies significant changes in the methodological approach to convert data from acute
94 to chronic ones, as described later.

95 *2.1 Radiotoxicity data*

96 The FREDERICA database (www.frederica-online.org) in its 2014 updated version²² constitutes the
97 primary source of basic radiobiological data for non-human species. Garnier-Laplace et al. (2010)
98 proposed the process of a meta-analysis of these data to build dose (rate)-effect relationships (*i.e.* dose-
99 response curves). These curves gave access to parameters analogue to the EC_{10} (EDR_{10} , dose rate
100 giving 10% change in observed effect in comparison with a control - chronic exposure) and EC_{50}
101 (ED_{50} , dose giving 50% change in observed effect in comparison with a control - acute exposure).
102 Their standard errors were also determined.

103 In order to have internally consistent data sets, data from literature reporting observed effects were
104 restricted to external gamma irradiation of non-human species under controlled conditions (either

105 laboratory or controlled field) and categorized into acute (high-dose, short term) and chronic (low
106 dose, long term) exposure situations since these exposure regimes lead to different biological or health
107 consequences²³. This data treatment allowed building a total of about 800 and 240 dose-response
108 relationships for respectively the acute (ca. 135 species) and chronic (ca. 30 species) exposure regime,
109 confirming the large predominance of acute toxicity information.

110 For comparison, the ACT method is based on three data sources (AQUIRE, the US-EPA database -
111 <http://www.epa.gov/ecotox/>; a European database - <http://www.ecetoc.org/> and a Dutch report²⁴). For
112 the 25 substances considered, the number of documented species per substance varied from 5 (12 data)
113 to 133 (977 data) and from 3 (6 data) to 45 (102 data), respectively for acute and chronic exposure
114 regime. These numbers as well as the ratio between acute and chronic information appeared very
115 similar to those characterizing the radiotoxicity data set used to develop the ACTR method.

116 *2.2 Principles of data transformation*

117 The proposed ACTR method is intended to infer the parameters of the statistical distribution of
118 chronic radiotoxicity data for a given taxonomic group of organisms (taxon) from the set of observed
119 acute radiotoxicity data available for the same group. More precisely, this statistical approach aims at
120 empirically transforming observed data of acute radiotoxicity (ED_{50}) into predicted data of chronic
121 radiotoxicity (EDR_{10}) for any given couple (species, endpoint). The method is inspired from the one
122 published by Duboudin *et al.* (2004) for chemical substances where ecotoxicity data sets are suffering
123 from similar bias in favor of acute effects data.

124 The ACTR method consists of a four-step process of statistical modelling. Basically, all observed data
125 (EDR_{10} and ED_{50}) are first log-transformed to deal with the classical skewness of radiotoxicity data.
126 Mean and standard deviation of the distributions of the two sets of transformed data are determined by
127 taxon (at the class level). Secondly different linear models are tested to predict average chronic
128 distribution parameters from the acute distribution parameters, including simultaneously all the taxa.
129 The best linear models (one model for the mean and one for the standard deviation) are selected. This
130 is performed as part of a process combining a bootstrap with a cross-validation. Thirdly the best

131 models are fitted for each taxon to the corresponding observed acute data (ED_{50}). Applying the fitted
132 models allows finally generating predicted EDR_{10} from the ED_{50} observed for a given taxon.

133 In details, the taxonomic level of interest is fixed by the identification of sets of observed radiotoxicity
134 data that contain a sufficient number of acute (ED_{50}) and chronic (EDR_{10}) data for same groups of
135 organisms (arbitrarily fixed at six data of each type at least to ensure the robustness of the predictions,
136 without constraint on the species number). The level of grouping needed is the taxonomic level to
137 adopt for applying the method. The robustness of the method relies also on the use of consistent
138 radiotoxicity data within each of such a taxon. EDR_{10} and ED_{50} values from the FREDERICA
139 database span several orders of magnitude (respectively nine and eight) due to the huge variety of
140 effects reported. For each taxon, extreme values of ED_{50} and EDR_{10} may be assimilated to potential
141 outliers which could bias our analysis. In this context, an outlier is defined as any data which value is
142 outside the range defined by one and a half time the Inter Quartile Interval (note that this factor of 1.5
143 is usually applied²⁵). We used a classical univariate detection process, *e.g.* based on boxplots, to
144 identify outliers in order to eliminate them for the rest of our work.

145 For each taxon, distribution parameters (mean and standard deviation) of the two sets of observed data
146 are estimated after their log-transformation. The transformation is also convenient for the validation
147 process, allowing the use of the log-normal distribution which is weighted according to species
148 importance. Some species are more or less commonly used for radiotoxicity testing under controlled
149 conditions although no standardized laboratory tests exist, leading to unequal number of observed data
150 per species. To give each species its deserved weight in the data sets, the procedure considered the
151 number of data per species in the data set (acute or chronic) related to a given class. The corresponding
152 weight was calculated applying the equation 1 (SI) to each class. Once observed EDR_{10} and ED_{50} data
153 selected, class distribution parameters are estimated according to the transformation and weighting
154 procedures using equations 2 to 5 (SI).

155 Chronic distribution parameters (mean $wMuC.lg$ and standard deviation $wSigmaC.lg$) are assumed to
156 be predictable from a linear combination of the acute parameters (mean $wMuA.lg$ and standard

157 deviation $wSigmaA.lg$). The best model is selected among six, what we arbitrarily considered a
158 reasonable number with regard to the number of parameters to be estimated (equations 6 to 11 for the
159 mean and 12 to 17 for the standard deviation, SI). The selection is made by coupling a bootstrap
160 process with a cross validation procedure, and the selected model is the one with the smallest
161 prediction error, averaged at the taxonomic level of interest. The model is used to calculate the chronic
162 distribution parameters predicted for each taxon of this taxonomic level (as illustrated by equations 28
163 and 29, SI).

164 Knowing the predicted mean and standard deviation of the chronic distribution, EDR_{10} data can be
165 predicted from observed ED_{50} data. This step requires translating ED_{50} values in units of EDR_{10} values,
166 a procedure called standardization. Observed ED_{50} data are made dimensionless (Equation 18) and
167 transposed into the chronic dimension (Equation 19). The last step consists in the back-transformation
168 of the result (Equation 20) to obtain the predicted EDR_{10} value.

169 *2.3 Validation of the ACTR method*

170 The validation of such a method is typically done by splitting the used data into two subsets, a training
171 one and a testing one. This is possible for sufficiently large data sets, but not for the generally smaller
172 sets usually available for radiotoxicity. The problem may be solved using the same coupling of
173 bootstrap and cross validation previously mentioned, an approach that will allow limiting overfitting.

174 A first evaluation of the ACTR performances may be obtained visually by comparing for each taxon
175 the ESDs of observed and predicted EDR_{10} data. The ESD is a new concept that corresponds to the so-
176 called SSD⁵ model restricted to a given taxonomic level and integrating all quality-assessed relevant
177 endpoints per species (those that could lead to changes in population size or structure, i.e. those
178 directly relevant to population demography - mortality, morbidity, reproductive success). It is
179 suggested to build both log-empirical and log-normal ESDs as classically applied to construct SSDs.
180 The log-empirical model is the graphical representation of the empirical cumulative probability of
181 weighted data that implies to weight also this distribution. A modified version of the Hazen method is
182 proposed (Equations 21 to 23, SI). Fitting a log-normal distribution is very common when looking to

183 express the statistical distribution of ecological data. The log-normal ESD of predicted EDR_{10} may be
184 plotted by using either the predicted distribution parameters previously defined or, as it was decided
185 here, more accurately recalculating parameters from the predicted EDR_{10} data (Equations 2 to 4, SI).
186 Even giving an immediate trend, a visual comparison is always somewhat subjective. A
187 complementary validation procedure is proposed, based on numerical comparisons. HDR_5 values are
188 estimated per taxon from observed and predicted ESDs using EDR_{10} data. Agreement between these
189 values is estimated from the overlap of their 95% confidence intervals (see SI, §.4 for calculation
190 details), before to analyze their ratio.

191 *2.4 Statistics*

192 All calculations and graphics were done using the version 3.3.2 of the R language²⁶ and already
193 available packages: dplyr 0.7.6²⁷; sampling 2.8²⁸; boot 1.3.20²⁹ and ggplot2 3.0.0³⁰ for the graphs.

194 **4 Results and discussion**

195 The Acute to Chronic Transformation proposed for chemicals has been successfully transposed to
196 ionizing radiation and radioactive substances, taking into account some specificities of the stressor.
197 The ACTR method deals with a single stressor when the context of the development and application of
198 the original ACT is that of multiple stressors. Additionally the conversion of acute radiotoxicity data
199 into chronic ones implies a change in units (Gy to $\mu\text{Gy h}^{-1}$). When the ACT for chemicals established a
200 parallel between stressors for a single organism, the innovative aspect of the ACTR method is to look
201 for a parallel between taxa for a single stressor.

202 *4.1 Data analysis: taxonomic level of interest and identification of outliers*

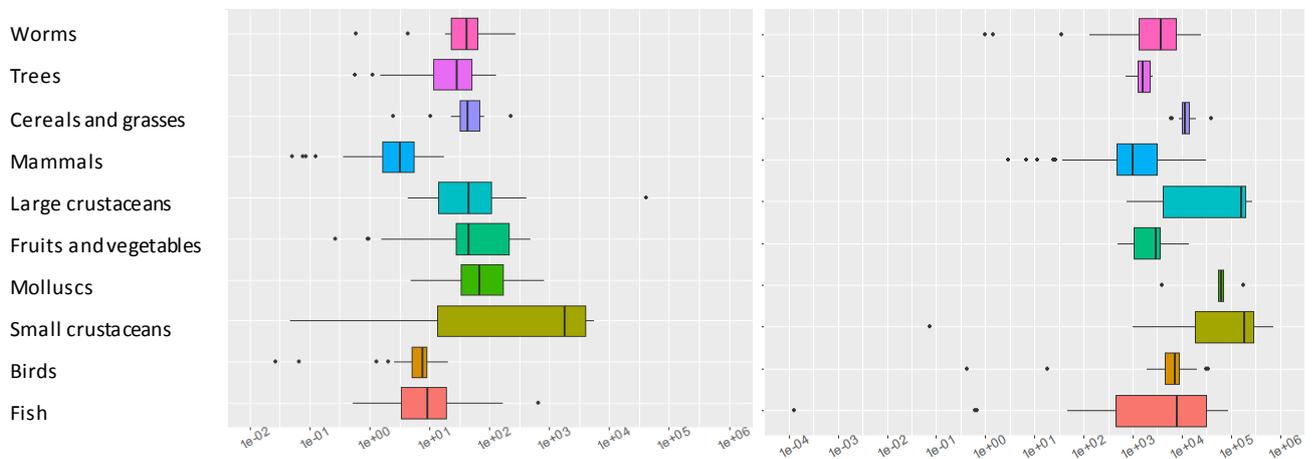
203 Applying the size criterion (at least 6 ED_{50} and EDR_{10} data per taxon) to the metadata issued from the
204 FREDERICA database (SI) led to identify the class as the lowest taxonomic level for the ACTR
205 implementation (Table 1). From these data, 22 EDR_{10} and 20 ED_{50} data were identified as outliers
206 (Fig.1) and removed. The Mollusc class, having only four EDR_{10} values (Table 2), was eliminated
207 which finally left nine classes to implement the ACTR method.

208 Radiotoxicity data were obtained on species grouped into taxa identified according to their scientific
 209 name, following the taxonomic habits. At the opposite, uses in the field of ecological risk
 210 characterization are to use common names, which additionally may differ from one to the other
 211 reference consulted. We decided to establish a link between the scientific name of the classes we
 212 considered and common names adapted from those currently employed by the IAEA³¹ and in the
 213 Wildlife Transfer Database. This correspondence has been adopted in order to facilitate to any user
 214 aggregation of data at higher levels of taxonomy.

215 Table 1. the ten classes of potential use for the implementation of the ACTR method

Scientific name	Common name
<i>Actinopterygii</i>	Fish
<i>Aves</i>	Birds
<i>Branchiopoda</i>	Small crustaceans
<i>Gastropoda</i>	Molluscs
<i>Magnoliopsida</i>	Fruits and vegetables
<i>Malacostraca</i>	Large crustaceans
<i>Mammalia</i>	Mammals
<i>Monocots</i>	Cereals and grasses
<i>Pinopsida</i>	Trees
<i>Polychaeta</i>	Worms

216



217

218 Fig.1. Identification of outliers (data outside the 1.5xInter Quartile Interval) per class in the data set of
 219 observed data (ED_{50} data on the left, EDR_{10} data on the right – x-axis: log of dose or dose rate)

220

221 Table 2. Number of radiotoxicity data per class (before outlier detection/after outlier elimination)

Class	ED_{50}		EDR_{10}	
	Data number	Species number	Data number	Species number
Fish	74/73	11/11	31/28	4/4
Birds	37/33	11/11	27/23	2/2
Small crustaceans	15/15	1/1	9/8	2/2
Molluscs	20/20	5/5	6/4	1/1
Fruits and vegetables	48/45	11/10	13/13	4/4

Large crustaceans	19/18	6/6	7/7	2/2
Mammals	80/76	4/4	63/56	5/5
Cereals and grasses	17/14	6/4	30/28	2/2
Trees	35/33	5/5	8/8	3/3
Worms	13/11	1/1	27/24	2/2

222 Grey line: class eliminated from the selection after removing outliers due to the too small number of
 223 remaining data (below 6)

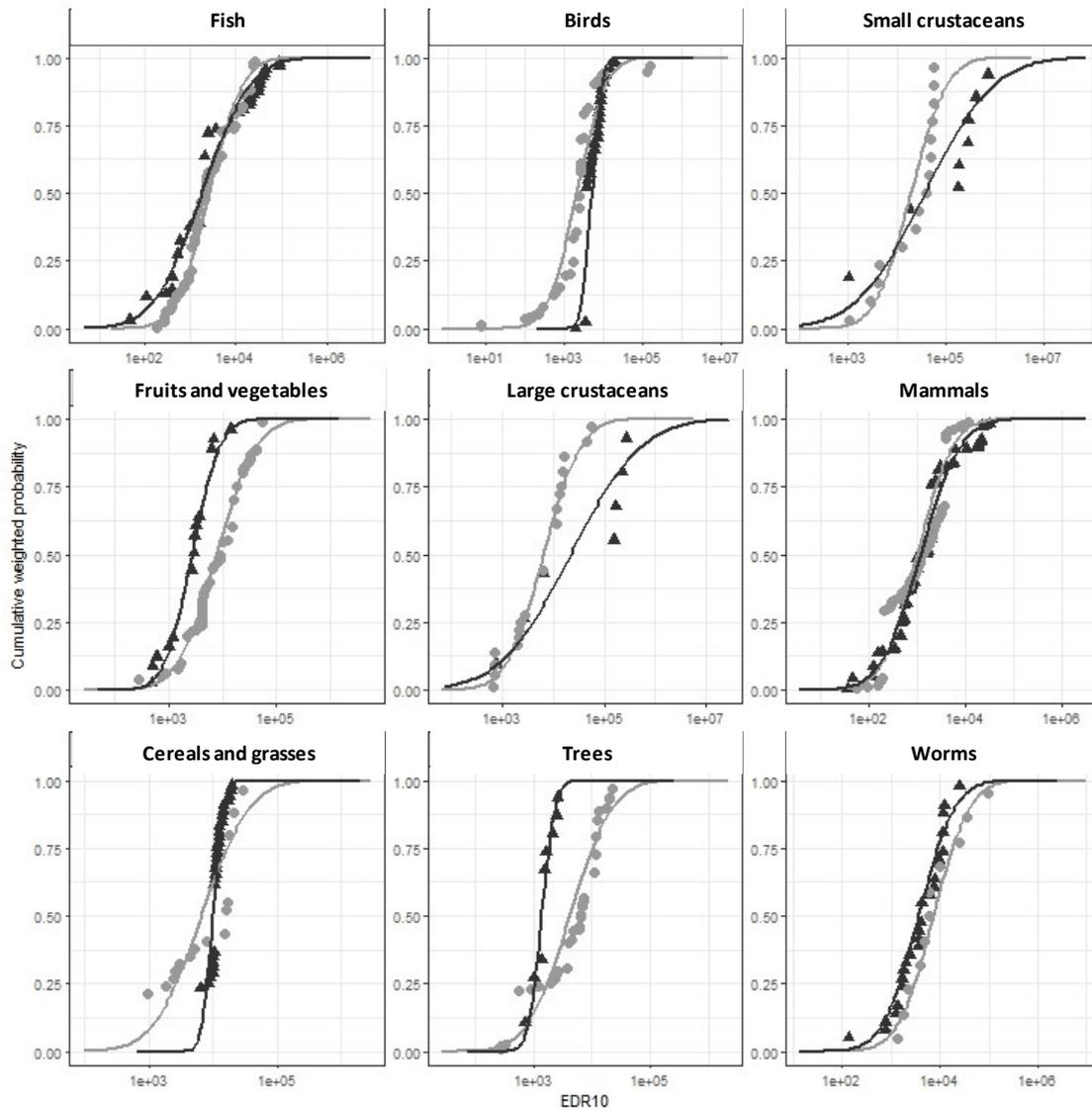
224 *4.2 Selection and fit of the model to predict chronic distribution parameters*

225 Distribution parameters were calculated for the nine classes on observed data, for both acute and
 226 chronic data sets (Table SI.1). The best models for predicting the chronic mean and standard deviation
 227 were respectively identified as Equations 7 and 12 (SI). Coefficients of these equations were fitted on
 228 the whole set of observed acute and chronic distribution parameters, resulting in Equations 28 and 29.
 229 Their application to each class generated the parameters of the predicted chronic distribution (Table
 230 SI.2).

231 *4.3 Prediction of EDR_{10} values from ED_{50} data*

232 The ACTR method has no other ambition than to offer a pragmatic way of transforming acute data
 233 into chronic data, with the most “fit for purpose” approach. Therefore we only discuss the quality of
 234 the mathematical representativeness of the results obtained.

235 The EDR_{10} values predicted by applying the ACTR method to the observed ED_{50} data available per
 236 class are presented as all other data in the attached Excel® file (SI). The whole set of EDR_{10} data was
 237 used to build ESDs per class, fitting both a log-empirical and a log-normal distribution to the observed
 238 and predicted data (Fig.2). Parameters of the log-normal distribution were re-calculated, to improve
 239 the accuracy of the fitting process (Table SI.3).



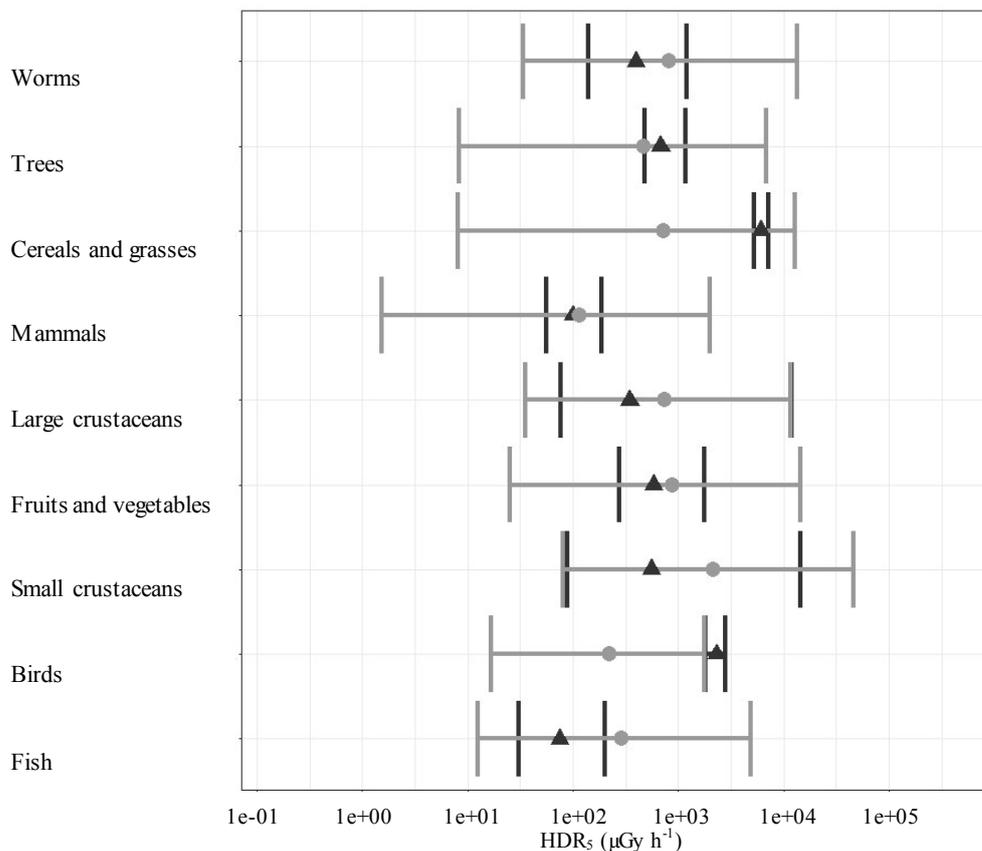
240

241 Fig.2. Log-empirical (symbols) and log-normal (lines) ESDs at the class level for observed (black) and
 242 predicted (grey) EDR_{10} data (x-axis: $\mu\text{Gy h}^{-1}$, y-axis: dimensionless)

243 Predicted and observed ESDs look very similar for some classes (Fish, Mammals, Worms) but much
 244 less for others (cereals and grasses, crustaceans).

245 To deeper analyze the ACTR results, HDR_5 values from observed and predicted ESDs were calculated
 246 (Table SI.4). Error values added to predicted parameters were obtained from the log-normal
 247 distributions fitted with a mean of zero and the standard deviation of residuals, *i.e.* 0.371 for the mean
 248 and 0.377 for the standard deviation. There is a good overlap of 95% confidence intervals of observed
 249 and predicted HDR_5 values (Fig.3). For most classes of organisms, the range of predicted values
 250 encompasses the variation of observed data. For six of the classes (*i.e.* 67%), the predicted HDR_5 value
 251 (HDR_{5_actr}) is included in the 95% confidence interval of the observed one (HDR_{5_obs}). For the classes

252 Cereals and grasses as well as Birds, the predicted HDR_5 value is lower than the lower bound of the
 253 interval of observed data, which shows that the ACTR approach is conservative. There is finally only
 254 one case for which the comparison does not meet the expectation. The HDR_{5_actr} value predicted for
 255 Fish is close to, but higher, the upper bound of the observed data. However, the ratio HDR_{5_actr}
 256 $/HDR_{5_obs}$ is about 4 for Fish as for the small crustaceans (Table SI.4), for which the ACTR method
 257 seems to give good results. The uncertainty introduced by the prediction is similar, whatever the
 258 relative location of the predicted value with regard to the interval of observed data. It should be
 259 acknowledged that this ratio for HDR_5 (i.e. the prediction is 4 times higher than the observation) is the
 260 highest of all those calculated.



261
 262 Fig. 3. Overlap between 95% confidence intervals (lines) of observed (black triangle) and predicted
 263 (grey dot) HDR_5 with their confidence intervals

264 Both validation processes gave the same general trend. The numerical comparisons of HDR_{5_actr} and
 265 HDR_{5_obs} values confirm the visual comparison of the EDR_{10} distributions (Fig.2) and argue in favor of
 266 the ACTR method.

267 The ACTR method appeared promising and seemed to be applicable with a reasonable level of
268 confidence. More precisely, the application of this method to radiotoxicity data led to enrich the
269 observed chronic data set by a factor of two at least for Fish and Mammals. Even more interesting, it
270 allowed increasing also the number of species represented in these enlarged data sets. Such a gain is
271 highly valuable for example to derive protection criteria, as it may permit to move from the very
272 conservative safety factor method to a more realistic statistical approach like the ESD and SSD. In
273 addition to the realism brought by these approaches, the associated transparency should be
274 emphasized. All the available data can be visualized, those taken into account as those identified as
275 outliers and consequently discarded from the treatment. A third benefit of using transparent statistical
276 treatments is the possibility of a continued improvement of their results by introducing new data as
277 they become available. Nevertheless, its generalization comes up against three limitations. Firstly,
278 removing radiotoxicity data identified as outliers leads to ignore the information they contain and can
279 skew the relationship formalizing the ACTR approach. Secondly, the choice of fitting a log-normal
280 distribution as the “right” cumulated probability function was made *a priori*, without posterior testing.
281 Lastly, distribution parameters had been estimated from small data sets (less than 10) that could be
282 considered insufficient to obtain unbiased estimates.

283 Our study provided a first brick in the demonstration of the concept of ACT-R that deserves to
284 be more robustly supported. The empirical relationship established here relies on sets of data that
285 could be improved by more research. The ideal data set would include both acute and chronic
286 radiotoxicity data acquired on a same species in the same experimental conditions and, in a perfect
287 world, by the same research team for a sufficient number of representative species. This will certainly
288 not happen, due to too many obstacles on this path (ethic, economic, logistic...). But any new
289 complementary radiotoxicity data will help at least to strengthen the predictive power of our empirical
290 approach that, as any palliative method, will never totally replace experimental acquisition of
291 knowledge.

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387 Competing interest statement

388 The authors declare no competing interest

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390 Supporting information

391 Additional information is provided in two complementary files. A text file named

392 ACTR_method_SI_equations contains all the equations used by the method and methodological

393 elements related to the calculation of the confidence intervals. The second file is an Excel® file

394 named ACTR_method_SI_dataset.xlsx that includes all the datasets supporting the development and

395 the validation of the ACTR method.