Dear Editors,

Please find enclosed our revised manuscript with the new title « **DRomics, a workflow to exploit dose-response omics data in ecotoxicology** ».

First of all, we must tell you that we made a great mistake on the first version submitted to biorxiv when formatting our paper using the PCI template: we forgot one page of the part "DRomics original features compared to other tools dedicated to dose-response omics data". We are really sorry for this mistake as this part may have helped the reviewers to get an overall view of the manuscript. We reintroduced this part in the revised version of the manuscript (lines 101-149.
Moreover we found the referees comments highly valuable and we did our best to account for them in the whole manuscript. You will find our responses in bold to the reviewers below. Our modifications are highlighted in yellow in the revised manuscript in its TrackChange version in our responses to the revwiewers comments . We hope that our revised paper will meet your approval and will now be recommendable by PCI Ecotox Env Chem.

With many thanks,
Sincerely yours,

For the authors,
Marie Laure Delignette-Muller

Response to reviewers

Referee 1 -Reviewed by Jean Armengaud, 25 Mar 2023 23:12

The manuscript entitled "DRomics, a workflow to model and make sense of dose-response (multi-)omics data in (eco)toxicology" presents the latest version of this tool well suited for the interpretation of dose-response omics experiments, especially for the field of ecotoxicology. The authors have inserted new options in their software and have also developed a new module for biological interpretation. With the new web interface and all these new features, there is no doubt that the DRomics tool will be widely used by the scientific community interested in mono- or multi-omics. I recommend the publication of this interesting manuscript which is well argued and well documented. A few points should be considered by the authors to improve the presentation of the manuscript.

1. I am not sure I understand what the authors mean by "experimental versus in situ data". I assume that the in situ data is also collected experimentally…. Please rephrase by using "in natura" to describe the conditions present in a non-laboratory environment if this is what you mean here.

We removed the terminology "in situ data" in the whole manuscript. We replaced it by "situation with no experimental replicates" line 24, by "designs with no replicates" line 261, and lines 169-175 (that was rewritten differently to answer comments of referee 3 point 1) it was just removed. In the part we reintroduced, lines 111-114 were also changed to omit the ambiguous term *in situ*: "DRomics was at the root designed to be able to

analyze data from typical DR design, favoring the number of doses over the number of replicates per dose, or even for datasets with no experimental replicates. This situation of DR approach with no replicates is met in some field studies (one dose per sample) and in some screening studies as illustrated in Rollin *et al.* (2023).”

2. Some of the information in the abstract does not seem very relevant (the fact that an R package was released in its first version in 2019 seems to be a detail).

We removed what was in brackets as suggested, and just add “the R package” before “DRomics” in the abstract.

3. It would make sense to organize Table 1 by first release date (first publication) for each tool.

We think it is indeed a good idea and we permutated the columns of this table as suggested.

4. It is important that the link to the web version appears in the abstract.

We added the link to the institutional web page of DRomics, gathering all the links relative to the use of DRomics, especially the links on the two shiny apps at the end of the abstract lines 30-31: “The institutional web page https://lbbe.univ-lyon1.fr/fr/dromics gathers links to all resources related to DRomics, including the two shiny applications.”

5. The keywords should be revised. AOP and MoA are mentioned but these two concepts are not really developed in the manuscript. Ditto for toxicogenomics.

We removed the keyword “toxicogenomics” that seems not so important for our paper, but we would like to keep the two others, as the new functionalities of DRomics were really developed to help an AOP approach. Moreover, we added examples of use of DRomics on this purpose to answer referee 3 point 2 and also referee 2 point 2 (lines 54-69): “Studies implementing DR (multi-)omics approaches sometimes aim at a mechanistic understanding of adverse effects (Adverse Outcome Pathway perspective - AOP). They could identify potential Modes of Action of pollutants (MoAs) at the molecular level, that generally need to be validated in a second step using targeted experiments (Andersen *et al.* 2018). Among those making use of our R package DRomics (“Dose Response for Omics”), we can cite the following ones as examples. Larras *et al.* (2020), from transcriptomics and metabolomics analyses in *Scenedesmus vacuolatus* exposed to triclosan, pointed lipid metabolism as the most sensitive pathway, in accordance with the mode of action known in bacteria. Gust *et al.* (2021) evaluated the mode of action for reduced reproduction in *Daphnia pulex* exposed to MeNQ by identifying particularly sensitive KEGG pathways at the transcriptome level. Vokuev *et al.* (2021) using metabolomics analyses in rat urine confirmed that sarin poisoning starts with inhibition of acetylcholinesterase that triggers a complex toxicodynamic response. Lips *et al.* (2022) and Larras *et al.* (2022) illustrated how community transcriptomics and metabolomics provide insights into mechanisms of pollution-induced community tolerance of periphyton exposed to diuron. Song *et al.* (2023) showed how DR modelling and estimation of points of departure at several omics and apical levels can be mapped to an AOP network for

ionizing radiation in *Daphnia magna*. Those applications of DRomics especially motivated us to develop new R functions and a new shiny application to help the biological interpretation of DR modeling of omics data."

6. Last but not least, the title could be much more attractive if it was simplified as "DRomics, a workflow to exploit dose-response omics data in toxicology". Ecotoxicology is part of toxicology, so why try to differentiate the two words. Most users interested in multi-omics might simply be alerted by the introduction of "multi-omics" as a keyword.

The title was simplified as suggested, multi-omics was added in keywords, in place of toxicogenomics, but we chose to keep the term ecotoxicology (and not toxicology) as DRomics was developed especially to address needs expressed by ecotoxicologists, even if it can also be used in toxicology.

Referee 2 - Reviewed by anonymous reviewer, 13 Mar 2023 12:53

This paper presents the DRomics tool, an R package designed to analyse multi-omics data obtained in ecotoxicology. The tool was first developed in 2019. The paper presents the new functionalities recently implemented and compare the DRomics tool to other existing tools.

1. The paper is very easy to read and very clear. I have very few comments. In Table 1, the authors should indicate that DRomics can deal with proteomic and metabolomic data. It is indicated "continuous omics data", but precisions could be added in order to make a clear difference with the other tools compared.

We added "metabolomics, proteomics, …" in brackets after "continuous omics data" in Table 1

2. Some illustrations are presented of data explored with the DRomics tool. As it is new, maybe a table presenting an exhaustive list of the data set already analysed by DRomics can be added, in order to see if several type of data (transcriptomics, proteomics etc) have already been analysed.

We added few lines with examples in the introduction, both to give some examples of already analysed and published data and to introduce the use in an AOP context, to also answer to referee 3 point 2 and referee 1 point 5 (lines 54-69 : already reported previously in our response to referee 1 point 5).

Referee 3. Reviewed by Rebecca Beauvais, 24 Mar 2023 16:19

First of all, I would like to thank the recommender for giving me the opportunity to review this paper. The topic of the paper fits perfectly with the subject I am currently conducting some experiments on. Even though I knew this tool before I am still enthusiastic about its usefulness and the new possibilities developed and presented here.

I appreciated reading this paper because of two main strengths: the clarity and the conciseness of the text and the overview table, which is quite complete and well designed.

1. I noticed one major flaw that challenged me. The "in situ data" are not sufficiently explained. One or a few example(s) of applications could illustrate what the authors mean by

this. However, this addition is however a nice added value compared to other tools. On the same topic, you mention that the number of doses could be less than 4/5 but what does this mean? Listing the data format requirements in this paper could help ecotoxicologists to better design their experiment. This means that from my reading, lines 116 to 119 are not clear enough.

This part was rewritten for clarification and addition of a point on the minimal design considering the number of doses. See lines 169-175: "Moreover, we performed modifications in the modeling workflow to ensure a better robustness of results on data with a low number of doses. For example, we changed the default information criterion used for best model selection from the AIC to the AICc, as recommended by Burnham and Anderson (2004), and limited the set of models for weak designs with few doses (4 or 5). Despite this care one should favor optimal dose-response designs with more doses (at least 6-7, and never less than 4) and less (or no) replicates as recommended by statisticians in toxicology (Moore and Caux, 1997; Ritz, 2010; Larras *et al.*, 2018; Ewald *et al.*, 2022)."

Moreover, the reader will find in the part we omitted in the first submitted version (especially lines 111-114) an introduction to the necessity to take into account designs with no replicates ("DRomics was at the root designed to be able to analyze data from typical DR design, favoring the number of doses over the number of replicates per dose, or even for datasets with no experimental replicates. This situation of DR approach with no replicates is met in some field studies (one dose per sample) and in some screening studies as illustrated in Rollin *et al.* (2023).") **and as explained in answer to referee 1 point 1, the term "in situ" is no more used in the manuscript.**

I make a few suggestions below that would help to improve the full understanding of all the possibilities offered by the tool.

2. In the abstract, you mention "understand the mode(s) of action of pollutants". It sounds too general to me. To solve this, you could give some examples, here or in the introduction.

As explained in our answer to referee 1 point 5 we added some examples of application of DRomics to understand the modes of actions of pollutants (lines 54-69: already reported previously in our response to referee 1 point 5)

3. Regarding the figures, for Figure 2, I would recommend adding "(contigs)" after transcriptomics and "(metabolites)" after metabolomics and a point at the end of the caption;

This was done.

4. for Figure 3, I suggest to write "dose response (DR)" instead of DR in the caption.

This was done.

5. Also, many readers may also not fully understand your explanation of "The signal was shifted by…" I would suggest explaining this better in the body text or deleting it is not crucial to the purpose of the figure.

We removed it from the legend, considering users will find the details in the reference manual or the vignette.

6. In the same figure, have you forgotten to give a name to the x and y axes?

We replaced " x " by "Dose" and " y " by "Theoretical signal (fitted curve)".

In case of a second run of review, I would be happy to participate.