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Probabilistic assessment method of the non-monotonic dose-responses-Part I: Methodological approach

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ABSTRACT

More and more studies aim to characterize non-monotonic dose response curves (NMDRCs). The greatest difficulty is to assess the statistical plausibility of NMDRCs from previously conducted dose response studies. This difficulty is linked to the fact that these studies present (i) few doses tested, (ii) a low sample size per dose, and (iii) the absence of any raw data.

In this study, we propose a new methodological approach to probabilistically characterize NMDRCs. The methodology is composed of three main steps: (i) sampling from summary data to cover all the possibilities that may be presented by the responses measured by dose and to obtain a new raw database, (ii) statistical analysis of each sampled dose-response curve to characterize the slopes and their signs, and (iii) characterization of these dose-response curves according to the variation of the sign in the slope.

This method allows characterizing all types of dose-response curves and can be applied both to continuous data and to discrete data.

The aim of this study is to present the general principle of this probabilistic method which allows to assess the non-monotonic dose responses curves, and to present some results.

1. Introduction

Nowadays, an increasing number of scientists call into question the paradigm according to which, in toxicology, dose-response curves are exclusively monotonic (MDRCs). Indeed, a significant increase in the number of experimental studies showing non-monotonic dose response curves (NMDRCs) was identified, especially since scientists have studied endocrine disruptors and responses at low doses (Beausoleil et al., 2013; Calabrese and Baldwin, 2001a, 2001b; Calabrese and Blain, 2005, 2011; Vandenberg, 2013). The occurrence of NMDRCs is often correlated to low doses although these two aspects are not synonymous (Vandenberg et al., 2012). These curves raise questions about the current approaches in regulatory toxicology, particularly in terms of the determination of the reference dose. The assumption that dose-response relationships are monotonic is currently at the basis of chemical assessments and allows the determination of the reference dose from the No Observed Adverse Effect Level (NOAEL), Lowest Observed Adverse Effect Level (LOAEL), etc. established through toxicity tests. However, in the case of a NMDRC, there is no certainty that the relevant reference dose will be determined. Thus, any methodology designed to study the relevance of dose-response curves in a toxicity study is of interest for regulatory toxicology.

Some studies present methods or descriptions to highlight these NMDRCs (especially the hormesis phenomenon), to explain the biological aspect or to identify the effector that could be the cause (chemical or physical) (Beausoleil et al., 2016; Calabrese and Baldwin, 2002; Conolly and Lutz, 2004; Gaylor et al., 2003; Lagarde et al., 2015; Lushchak, 2014; Nascarella and Calabrese,

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2012; Vandenberg et al., 2012).

The major difficulty is to assess the statistical plausibility of non-monotonic dose-response curves from previously conducted dose-response studies. This difficulty is linked to the fact that these studies present (i) few doses tested, (ii) a low sample size per dose (i.e. number of data), and (iii) the absence of any raw data. The methodology commonly used to evaluate the statistical plausibility of the NMDR relationship is the methodology of Calabrese and Baldwin (1997). It was originally intended to highlight the hormesis phenomenon but can be used to characterize all types of NMDRCs (Lagarde, 2012; Lagarde et al., 2015). This procedure developed by Calabrese and Baldwin is a numeric scoring assignment value based on various criteria. The total score defines the robustness of the statistical plausibility (Calabrese and Blain, 2011). Most of the criteria, e.g. the number of doses statistically different from the control, the magnitude of response, etc., are directly related to the response given by the control group i.e. the basal response.

The definition of non-monotonic dose-response is a mathematical definition of non-monotonicity: the slope of the dose-response curve changes sign at some point along the range of doses examined (Kohn and Melnick, 2002; Vandenberg, 2013). According to this definition, the use of the control response as a reference point to perform comparisons with the other experimental doses (as it is currently performed to characterize NMDRCs (Calabrese and Baldwin, 2002)) is not convincing. This methodological point does not cover the entire definition of this type of curves. In fact, it is highly probable that a dose which is not different from the control group could be different from one or more of the doses tested (Fig. 1).

The question is, with respect to the current definition of NMDR, whether a single comparison to the control group is appropriate to characterize the dose-response relationships.

The experimental studies which have shown NMDRCs often have a low number of tested doses and a low sample size per dose. Furthermore, there are almost systematically no additional studies to prove the repeatability of the results (i.e. reproducibility of dose-response curves). Given these data gaps, how confident can we be in the response values per dose (mean, proportions, etc.) and therefore in the experimental dose-response curves?

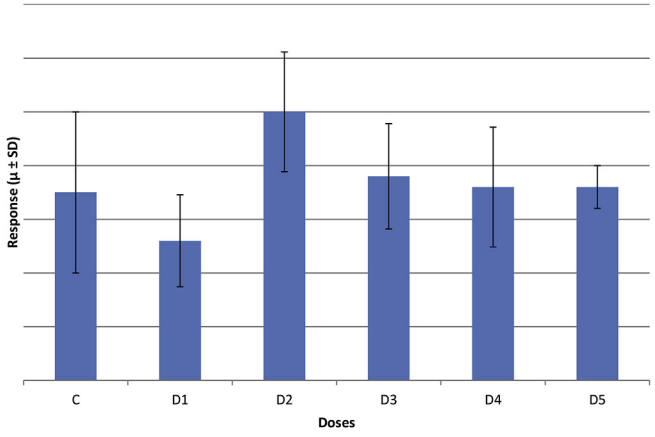

Fig. 1. Theoretical example of a dose-response curve.
In this theoretical example, it is supposed that the sample composing C (i.e. control) has no statistically significant differences compared to other doses. D1 and D5 are not different from C and other doses but they are different to D2. From a classical statistical point of view this example would have not been characterized as NMDRC, as there are no statistically significant differences compared to C. However according to the definition of a NMDRC there is a change in sign in the curve which is given by the statistical differences between D2 with D1 and D5.

The current methods to characterize dose-response curves use the data available in the experimental study (mean, proportions, etc.). These data should be considered as a sample of the true distribution and the use of a single value to define the response of a dose implies uncertainty (i.e. deterministic approach). A probabilistic approach would partially overcome this bias by taking into account the response variability per dose.

We have attempted to address these key points by developing a probabilistic method to characterize the different types of dose-response curves. Based on a sampling principle, this methodology aims to evaluate all of the response possibilities that each dose of an experimental curve could provide from its summary data. It can be used to assess all the profiles of curves that could be obtained from the original dose-response curve and, consequently, to assess the probability of a curve being a non-monotonic dose-response (NMDR), monotonic dose-response (MDR) or without a dose-response relationship (WDR).

This methodology relates only to the statistical aspect of dose responses curves and is adaptable to all curves, to continuous and discrete data.

2. Material and method

2.1. Methodology principle

The methodology is composed of three main steps: (i) sampling from summary data, (ii) comparative analysis of each dose-response curve, (iii) characterization of these dose-response curves.

Fig. 2 summarizes the methodology with its steps, the methods and the procedure used. The use and the automation of the method with R software are described in the supplementary material.

2.1.1. Step 1: Sampling

The sampling step was developed in order to address two problems often observed in studies of dose-response relationships. (i) The first is the absence of raw data. Dose-response relationship studies rarely exhibit the raw data used to characterize the dose-response curve. The data per dose are mainly available as a mean (μ) and a standard deviation (SD) for continuous data or a proportion for discrete data. These data are referred to as “summary data” in this study. It was necessary to generate a set of data that could be used for a comparative assessment. (ii) The second problem is the hypothetical uncertainty linked to the results of experimental studies. It is difficult to find available sets of experimental studies which have tested the same substance and the same endpoint in relatively close experimental conditions (i.e. repeatability tests). This hypothetical uncertainty is reinforced by a low number of doses, and/or by a low number of data per dose (i.e. sample size). We assume that in these conditions, the experimental values are a potentially false reflection of the reality. In other words, we assume the experimental values from one study should not be considered as representative of reality but as a part of the reality. Failing being able to adjust the number of values in a sample per dose (which would distort the statistics and the original conditions of the study), sampling is repeated many times while retaining the sample size defined in the study. This procedure provides an overview of all the results options that can be provided by the summary data.

The general principle is to generate raw data by pseudo-random sampling inside distributions themselves adjusted from the summary data provided in the experimental studies. Assuming that all the data included within this distribution are potentially representative of the original data, it is possible to extract a sample theoretically equivalent to the initial dataset via a random draw of values in this distribution. By repeating the operation several times,