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10% Body weight (gain) change as criterion for the maximum tolerated dose: A critical analysis

Letter to the Editor

Com participação do Diretor da ALAPTE
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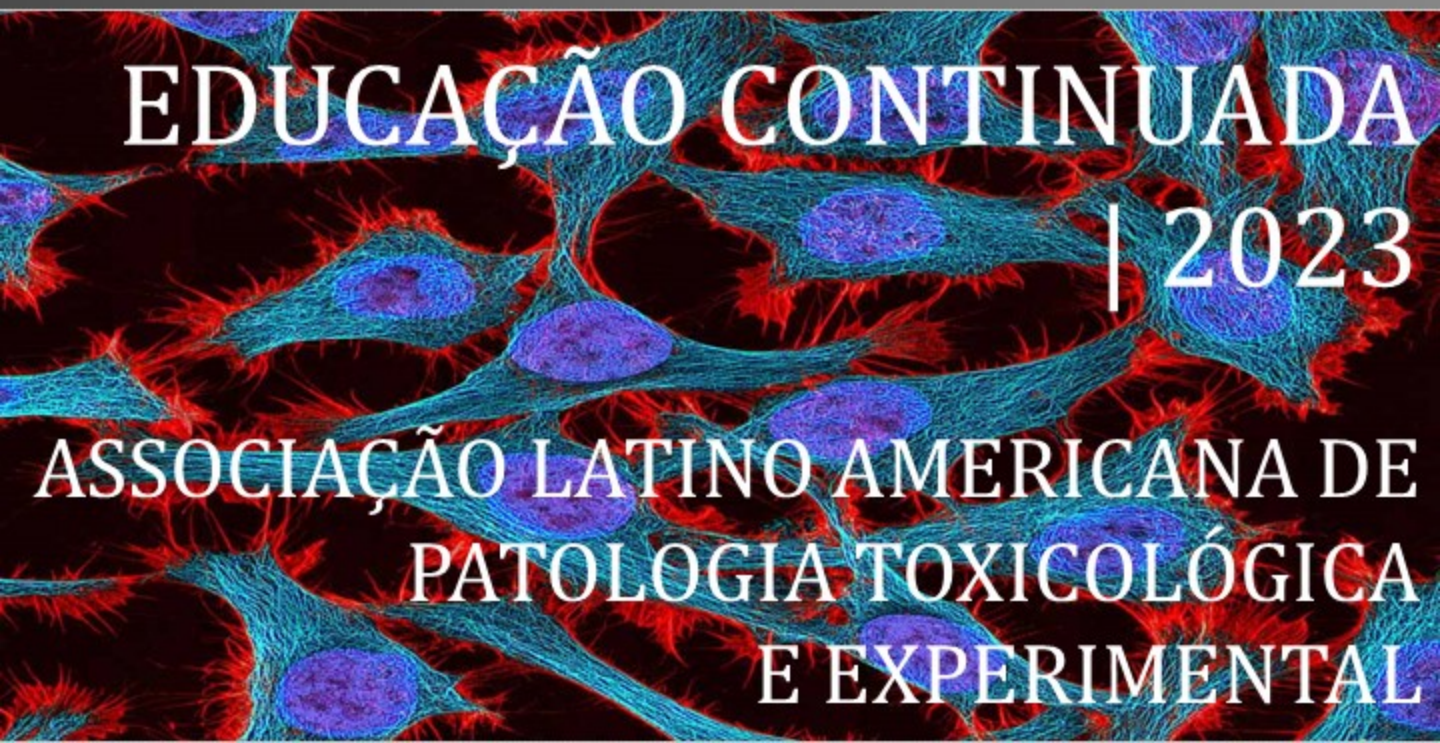

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Letter to the Editor

Letter to the Editors regarding "10% body weight (gain) change as criterion for the maximum tolerated dose: A critical analysis"

We find several reasons to disagree with the proposal by Van Berlo et al. (2022) who suggest that the predictive value of the rodent cancer bioassay might be improved by altering the way in which body weight is used in setting the highest dose in bioassays.

The variables relating to body weight measured in toxicology studies include body weight, weight gain (relative to initial weight), and the efficiency of food utilization (EFU) expressed as body weight gain per 100 g food consumed (Hoffman et al., 2002). Depending on the progression of toxicity, a 10% reduction in body weight gain may or may not be a reliable indicator that the maximum tolerated dose (MTD) is achieved. Such a reduction in terminal body weight might be achieved by a continuous marginal effect on body weight development or by losses and gains from a number of causes over a study of considerable length (Everds et al., 2013; Kemi et al., 2000; Cadoni et al., 2017; Weber, 2017). Other observations (clinical signs, hematology, clinical chemistry, histopathology) must be factored into the decision to determine whether the MTD has been reached.

High dosage increases the possibility of overdosing leading to saturation of metabolic and reparative pathways with no relevance to human exposure levels. Effects seen at dose levels from which humans are conservatively protected by regulation (i.e.: those inducing toxicity or exceeding the capacity of the metabolic system to deal with them) do not add to the protection goal, but have a price in animal suffering in the study itself and often trigger further unnecessary investigations. Mortality in control groups of rat standard cancer bioassays may reach 75% (Charles River, 2009; Weber et al., 2011); thus it is not surprising that regulatory bodies consider a study with high mortality at the top dose as unreliable and may ask for it to be repeated - a risk that increases substantially if doses higher than those that satisfy the criteria outlined above are used. Haseman from the National Toxicology Program has pointed out that, ' … (in) designing long-term rodent carcinogenicity studies, measures should be taken to minimize potential body weight differences between dosed and control groups … ' (Haseman et al., 1997).

Hazard-based testing assumes that results obtained at high doses are indicative of results that will occur at lower, environmentally relevant, doses. This assumption, underlying the publication of van Berlo et al., fails to acknowledge the general acceptance of the questionable relevance of findings in animal studies where homeostasis is disturbed by toxicity, and ignores the view, expressed in a number of publications, that the time has come to acknowledge that the standard 2-year rodent bioassay has limited predictive value in evaluating human risk (Cohen and Arnold 2011; Cohen 2017; Goodman 2018; Berry et al., 2019).

The rodent cancer bioassay is generally reliable for identification of genotoxic carcinogens. For non-genotoxins, the same claim cannot be made (Ames and Swirsky Gold, 1990; Berry et al., 2019). The many differences between rodents and humans in metabolism, cell cycle times and division rates, as well as longevity, ensure that the predictive power of the bioassay will decrease with an increase in toxicity. The key issue, the uncertainty intrinsic to testing in rodents and extrapolating to humans (Olson et al., 2000; Tamaki et al., 2013; Ahuja et al., 2017) cannot be overcome by raising doses. Current short-term animal and *in vitro* assays have a scientific base that supports this assertion.

We can confidently identify direct and indirect mutagens (clastogens, DNA damaging agents) without using animals. These agents are then treated as non-threshold carcinogens with as low as reasonably achievable exposure paradigms (ALARA), which is supported by societal consensus as a reasonable and precautionary regulatory approach.

The central gateway of non-genotoxic carcinogenicity is cell proliferation, with the consequence of increased probability of errors in DNA replication (Knudson, 1971; Moolgavkar and Knudson, 1981; Greenfield et al., 1984; Cohen and Ellwein, 1990; Wood et al., 2015; Tomasetti et al., 2017; Smith and Perfetti, 2018). Cell proliferation of many tissues can also be induced non-specifically by systemic toxicity to the whole organism and the higher the level of such toxicity, the greater the risk of enhanced tumour formation. Typically, tumours induced by non-genotoxic chemicals in rodent bioassays are found at greater than normal background levels exclusively at exaggerated dose levels. Importantly, cell proliferation mechanisms are threshold in type and the levels of the various thresholds are determined by different underlying stimuli. The stimuli will vary according to the nature of the mechanisms involved in damaging cells with consequent regeneration, or by changes induced as a result of activation of mitogenic cell signaling pathways (hormones, receptors). Preventing the trigger for cell proliferation will lower the chance of tumour induction to natural background levels.

In the context of current efforts to reduce the amount of animal testing, attempts to improve the performance of the rodent cancer bioassay itself should be critically analysed. The low specificity of the life-time bioassay makes clear that it is important to be sure which public health problem we intend to solve by increasing animal use.

Current initiatives are directed to reducing animal use in regulatory toxicology and in defining modes of action operative in organisms below toxic levels. This is not possible for conditions which alter homeostasis significantly over a period of time. The proposal (van Berlo et al., 2022) lags behind our current understanding of human oncogenicity. Testing needs to approach the maximum tolerated dose, but not induce

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conditions that induce pathological conditions or overwhelm metabolic clearance systems. Disturbance of homeostasis in DNA repair and other cellular components is a fundamental problem in cancer risk assessment; the predictive value of information from animals in which growth and weight gain are deliberately disturbed is unlikely to be informative. We are of the view that studies approaching toxicity may be valid and they should not be rejected/repeated on strict mathematical criteria.

The toxicological community increasingly recognizes that the 2-year bioassay is no longer needed and a number of efforts are underway to build confidence in alternative approaches that are more relevant and protective (Sistare et al., 2011; Craig et al., 2019). Hence efforts should be focused on achieving better human health protection with less animal use rather than attempt to improve the performance of the rodent cancer bioassay. Genotoxic carcinogens can be confidently identified *in vitro*. For non-genotoxic carcinogens *in silico/in vitro* non-animal methods as well as animal short-term assays are being developed for regulatory use (Strupp et al., 2012; Peffer et al., 2018; Smith et al., 2018; Jacobs et al., 2020; Corton et al., 2022). Retirement of the rodent cancer bioassay is highly desirable in order to reach better human health protection by refined methods with less suffering and more sensible use of vertebrates.

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Data availability

No data was used for the research described in the article.

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