LETTER TO THE EDITOR

Re: Gi *et al*. 2018, In vivo Positive Mutagenicity of 1,4-dioxane and Quantitative Analysis of its Mutagenicity and Carcinogenicity in Rats, *Archives of Toxicology* **92**:3207–3221

Paul A. White[1*], Andreas Zeller[2*], Stefan Pfuhler[3*], and George E. Johnson[4*]

[1]Environmental Health Science and Research Bureau, Health Canada, Ottawa, Ontario, Canada

[2]Pharmaceutical Sciences, pRED Innovation Center Basel, F. Hoffmann-La Roche Ltd, Grenzacherstrasse 124, Basel, Switzerland

[3]The Procter & Gamble Company, Mason Business Centre, Mason, Ohio, USA

[4]Swansea University Medical School, Swansea University, Swansea, Wales, UK

*Authors contributed equally

Gi et al. recently published an *in vivo* genotoxicity study of 1,4-dioxane, examining, amongst other endpoints, treatment-induced transgene mutations in the livers of *gpt* delta rats (Gi et al. 2018). The authors employed the BMDS and PROAST software packages to analyze the dose-response data and determine a point of departure (PoD) metric known as the BMD or Benchmark Dose. With respect to BMDS, the authors used one standard deviation of the concurrent control group as the benchmark response (BMR), and determined the lower confidence limit of the BMD_{1SD} (i.e., the $BMDL_{1SD}$). With respect to PROAST, they used 10% increase over the mean concurrent control group as the BMR, also known as the Critical Effect Size (CES), and determined the lower confidence limit of the BMD_{10} (i.e., the $BMDL_{10}$). The authors also examined the no observed effect level (NOEL), the highest tested dose that failed to elicit a significant increase in response relative to the concurrent control.

The authors' interpretation of the aforementioned PoD metrics involved quantitative comparisons of the NOEL, the $BMDL_{10}$, and the $BMDL_{1SD}$. Although the analyses and interpretation of the results are interesting, there are several weaknesses in the presentation and interpretation of the BMD values. The authors correctly specify the advantages of the BMD approach relative to the NOEL approach, highlighting the inability to calculate a confidence limit on a NOEL from a single study, yet they did not examine or discuss the confidence intervals associated with the BMD values (i.e., the upper and lower confidence limits). This is critically important since it provides a means for determining the precision of the BMD; moreover, it's utility for risk assessment and regulatory decision-making. More specifically, the ratio of the BMDU to the BMDL provides information about the uncertainty of the BMD, which in turn reflects the quality of the underlying dose-response data (Wills et al. 2017). Indeed, it has been suggested that the magnitude of uncertainty of the BMD estimate, as indicated by the BMDL to BMDU ratio, should be used as a tool for evaluating the statistical quality of the underlying data (Benford et al. 2010), and the utility of a BMDL as a reference PoD for regulatory decision-making (Barlow et al. 2006; Benford et al. 2010).

In order to scrutinise the precision of the BMD estimates presented by the authors, we reanalysed the data presented in Table 2, and examined the BMDL to BMDU ratios as indicators of BMD precision, and the statistical quality of the underlying experimental data. The results obtained revealed $BMDL_{10}$ to $BMDU_{10}$ ratios of 350 and 3736 for the exponential and hill models, respectively. These values indicate, in our opinion, that the dose-response data presented by the authors are not

suitable for determination of a $BMDL_{10}$ that can be used as a reference PoD for regulatory decision-making. Additionally, the authors' comparison of the NOEL and the $BMDL_{10}$ is problematic since it gives the reader the false impression that the BMD approach may yield a far more conservative PoD. Indeed, the NOEL is actually within the BMD_{10} confidence interval (i.e., $BMDL_{10}$ to $BMDU_{10}$).

Careful consideration of the aforementioned uncertainty in the BMD_{10} (i.e., the $BMDL_{10}$ to $BMDU_{10}$ ratio) reveals that the low $BMDL_{10}$ is a consequence of (1) the large amount of variability in the dose-specific responses, and (2) the low CES selected by the authors. Accordingly, the $BMDL_{10}$ should not be interpreted as conservative, but rather reflective of BMD imprecision. Furthermore, with respect to the BMD_{1SD} approach, it is not at all surprising, given the large variability in the control responses for experiment 1, that the value is much higher than the BMD_{10} (i.e., 576 ppm versus 0.98 ppm). More specifically, the ratio of the standard deviation to the mean is 0.6, corresponding to a BMR, when expressed as a percentage relative to control, of 60%. Consequently, the $BMDL_{1SD}$, which is quantitatively analogous to a $BMDL_{60}$, is far greater than the $BMDL_{10}$. Thus, as already indicated above, it is critically important to (1) examine complete BMD confidence intervals (i.e., BMDL to BMDU), and (2) select a CES that is appropriate for the endpoint under consideration. With respect to the latter, recent analyses indicate that a CES of 10% is too low for the genetic toxicity endpoint examined; moreover, that a CES in the range of 40-50% is more appropriate for the interpretation of transgenic rodent mutagenicity dose-response data (Zeller et al. 2017). Reanalysis of the Gi et al. data with a CES of 50% yields BMDL and BMDU values of 23 and 2030 ppm for the exponential model, and 171 and 2490 ppm for the hill model; resultant BMDL to BMDU ratios for the exponential and hill models are 88.3 and 14.6, respectively. Accordingly, the use of an appropriate, endpoint-specific CES yields more precise BMD values, and a BMDL values that can be deemed more suitable for regulatory decision-making; moreover, a regulatory decision based on a PoD that is not restricted to one of the studied doses.

In conclusion, although it is clear that Gi and colleagues have presented interesting, important information regarding the genetic toxicity of 1,4-dioxane, their interpretation of the BMD analysis is problematic in several respects. They only note the lower confidence limits of the BMD values, which, as noted, can give the false impression that the BMD approach yields conservative values. Relatedly, interpretation of the NOEL[1] via comparison with the lower confidence limit of the BMD_{10}, gives the contrasting false impression that the NOEL is less conservative. When using the BMD approach for regulatory interpretation of genetic toxicity dose-response data, we unequivocally recommend uniform examination of the complete BMD confidence interval, and, in particular, the BMDL to BMDU ratio. This ratio is critically important for appropriate interpretation of BMD reliability, and, by extension, for determining the regulatory utility of the underlying dose-response data. Lastly, going forward, it is clear that future work should scrutinise all available dose-response data in an effort to determine CES values that are appropriate for genetic toxicity endpoints.

[1]It should be noted that reanalysis of the data presented in Table 2 yielded a NOEL of 1000ppm. For details about the approach employed for NOEL determination see Johnson et al., 2014 (Johnson et al. 2014).

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