Design of experiments aligned with benchmark dose analyses results in significant reduction and refinement in the use laboratory animals

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Benchmark dose (BMD) modelling can significantly contribute to reduction and refinement of laboratory animal usage

- ❑ Similar dose-response and dose-effect information may be obtained from fewer animals.
- ❑ Allows fewer animals in the high response dose groups, thereby producing less toxicity-induced animal distress.

It is time to abandon the NOAEL approach as the default. BMD-aligned experiments will result in more precise risk assessments and a reduced use of laboratory animals.

BMD – a ready to use 3R-method

The use of benchmark dose (BMD) has increased substantially, with growing awareness among researchers and inclusion in regulatory testing guidance documents. The relation between dose-response assessment and the 3R (reduction, refinement and replacement) show strong benefits by using the BMD approach. In addition, the BMD approach does not need to go through the full process of validation. It is ready to use!

Reduction may be a result from two angles. (1) The information (e.g., a point of departure, PoD) that can be deduced from a given dataset is more precise when applying the BMD approach as compared to the NOAEL approach. (2) The same information (e.g., in terms of the precision of a PoD) may be obtained from fewer animals when using the BMD approach rather than the NOAEL approach.

Advantages of the BMD approach
BMD defines the level of effect. A fundamental problem of the NOAEL approach is that the size of the effect at that dose is left un-quantified.
BMD avoids false negatives. The probability that an existing effect is missed all together is much higher when the NOAEL approach is applied as compared to the BMD approach. In addition, there is a high probability of finding a NOAEL that is higher than the "true" threshold of adverse toxicity.
BMD avoids LOAEL-to-NOAEL extrapolation. Standard dose-response assessment with pairwise comparisons easily leads to a situation where the lowest dose shows significant effects (i.e. LOAEL), adding uncertainty.
BMD is less sensitive to outliers. Outliers will often remain unnoticed in the NOAEL approach, resulting in inconclusive data or lead to confusing discussions.
BMD can use combined datasets. The underlying idea is that dose-responses related to different subgroups may differ in background response and in sensitivity to the chemical but they are often similar regarding the shape of the dose-response.
BMD can use historic data of the same endpoint. Combining a dataset for a given chemical with datasets on the same endpoint (study type) for various other chemicals.

BMD-aligned experimental design

The BMD approach results in a higher statistical precision for the point of departure used to establish e.g. exposure limits. Therefore, BMD analysis is a prerequisite for optimal study performance. Here are some characteristics of optimal experimental design:

More dose groups – To minimize the probability of unfortunate dose placement, a relatively large number would be appropriate. 5-7 doses have been suggested.

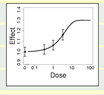
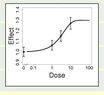
Dose spacing – with more dose groups the dose spacing can result in wider dose range without missing the threshold.

Un-equally sized dose groups – Re-distribute animals between dose groups so that fewer animals are needed in the top dose and more close to the threshold value.

Top-dose – Similar to traditional experimental designs, a substantial response are needed at the top dose also for BMD estimates.

It is a common misconception that the NOAEL reflects a dose without effects. Our simulation data show a clear risk of underestimation of the thresholds for adverse effects at the critical effect size of 5% increase from background (Table 1). In addition, the NOAEL approach easily leads to a situation where the lowest dose shows significant effects (i.e. only LOAEL).

Table 1. Percentage of continuous results generated with Monte Carlo simulations in relation to a comparison between the NOAEL and the "true" BMD at 5% increase from background. Variablility = 10%.

Dose placement	Animals/group	NOAEL>BMD_{true}	NOAEL<BMD_{true}	Only LOAEL
	5	92%	5%	3%
	10	87%	10%	3%
	20	73%	23%	4%
	50	35%	56%	9%
	5	92%	0%	7%
	10	86%	0%	15%
	20	68%	0%	31%
	50	26%	0%	74%

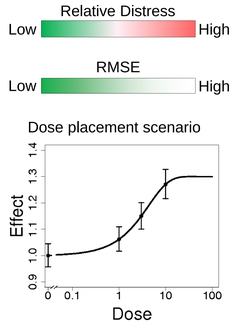
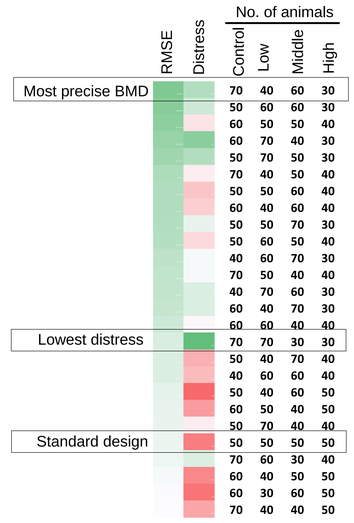


Figure 1. Top 30 ranking of dose group designs with lowest Root Mean Squared Error (RMSE). The column for distress relate to the relative level of distress, assuming that distress is proportional to dose.



Continuous experimental data were generated by Monte Carlo simulations for fixed doses and a constant number of 200 animals. In total, 85 different designs were compared. The Root Mean Squared Error (RMSE) for the BMD05 in relation to the "true" BMD05 was significantly lower than the standard design with equally sized dose groups (Figure 1). The optimal designs (minimum RMSE) also reduced animal distress under assumption that the distress is proportional to dose.

Refinement with BMD-aligned design

Toxicity is required at the top-dose according to current guidance for regulatory testing, but not so high that the study is jeopardized by morbidity or mortality.

Our simulation studies show that the probability of false negatives increases with lower responses at the top dose. As a general principle, the larger the effect at the top dose, fewer animals are needed. Since the BMD is not dependent on pairwise comparisons between equally sized groups, it is possible to re-distribute animals between dose groups so that fewer animals are needed in the top dose to increase performance of the BMD estimate. In the case of toxicologically induced distress this will be important to refine the use of laboratory animals.

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