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Estimating the range of the maximum hormetic stimulatory response

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ABSTRACT

An ever-expanding hormetic database (HDB) was used to demonstrate that the median maximal hormetic stimulatory response (MHSR) of biphasic dose-response relationships increases in value with an increase in the number of stimulatory doses/concentrations that are administered below the estimated threshold/ZEP (zero equivalent point – *i.e.,* the dose where the response crosses the control group value). With only one dose or concentration administered below the ZEP, the median MHSR for microbes (*in vitro*), animals (*in vitro* and *in vivo*), and plants (*in vitro* and *in vivo*) ranged between 120% and 125% of the control response. However, when individual agents having at least six doses below the ZEP were mined from the HDB (and a median MHSR then determined), the median MHSR increased to 160–190%. This progressive increase in the MHSR appears to be due to several factors, including (i) the enhanced capacity of additional doses in the stimulatory hormetic zone to better estimate the response optima, and (ii) enhanced variability due to the presence of more doses in the stimulatory zone. This study offers a novel perspective for improving research protocols, unraveling the limits of biological plasticity, understanding low-level stress biology, advancing human and ecological health, and enhancing human performance.

1. Introduction

The hormetic dose response is a biphasic dose response characterized by stimulatory low doses and inhibitory (toxic) doses. Over the past two decades the hormetic dose response has been the object of considerable research interest (Calabrese and Mattson, 2017; Calabrese, 2008). Assessment of many thousands of hormetic dose-response relationships has indicated that the maximal hormetic stimulatory response (MHSR) is typically modest [commonly 130–160% compared to control (*i.e.,* 100%) responses], with over 80% of the assessed responses being less than twice the control responses (Calabrese and Blain, 2011). While this 30–60% "rule" has been widely affirmed in the peer-review literature, one of the authors (EJC) suggested that the number of stimulatory hormetic doses administered might be an important factor in determining the magnitude of the MHSR and should be investigated. Agathokleous and colleagues (Agathokleous et al., 2019), assessed this hypothesis in a preliminary fashion using data limited to the effects of the rare earth element lanthanum (La) on plants. Their data suggested that the MHSR increased in a modest manner as the number of doses below the threshold increased from 1–5 to 6–10. Herein, this hypothesis is tested in a more robust (large sample size) and general manner using an expanded hormesis database (HDB) composed of > 11,000 dose responses of animals, microbes, as well as plants to many different types of stimulatory agents (Calabrese and Blain, 2011).

2. Methods

The HDB is continuously being updated and expanded (Calabrese and Blain, 2011). It was used in this study to test the hypothesis that the MHSR will increase in value as the number of hormetic doses administered below the ZEP also increases. This study used data from plants (*in vitro* and *in vivo*), animals (*in vitro* and *in vivo*) and

Abbreviation: HDB, hormetic database; La, lanthanum; MHSR, maximal hormetic stimulatory response; RSD, relative standard deviation; ZEP, zero equivalent point

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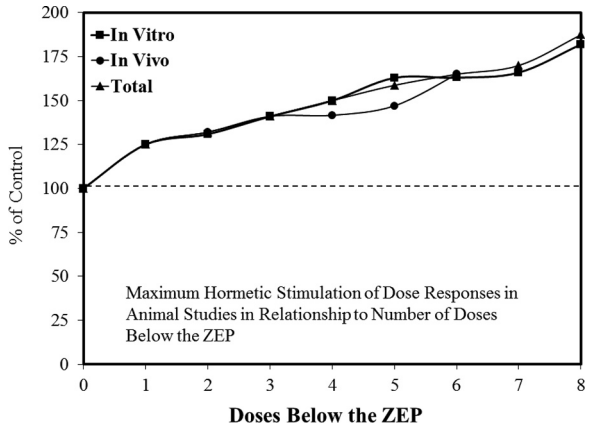

Fig. 1. Maximum hormetic stimulation of dose responses in animal studies in relationship to number of doses below the ZEP.

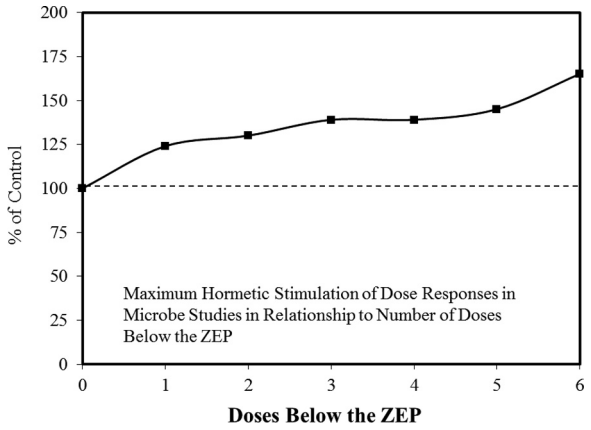

Fig. 3. Maximum hormetic stimulation of dose responses in microbe studies in relationship to number of doses below the ZEP.

microorganisms that demonstrated inverted *U*-shaped dose responses – the most common responses in the HDB (Calabrese and Blain, 2011). To ensure reasonable robustness of the data, *a priori* criteria were imposed that required each evaluative category to contain > 60 dose responses. This approach initially restricted the number of doses below the ZEP to between 6 and 8, depending on the comparison group. A further analysis was made that utilized all data, including those with > 8 doses below the ZEP, despite the lower sample size in these groups. For this situation, all dose responses with < 6 doses or > 6 doses below the ZEP were combined and compared.

3. Results

The median MHSR for animals (*in vitro* and *in vivo*), plants (*in vitro* and *in vivo*), and microbes increased as the number of doses below the ZEP increased (Figs. 1–4; Tables 1–3) and was in the 120–125% range with only one dose below the ZEP. The median MHSR associated with an increase for the animal *in vitro* studies (Table 1) is reported for eight (8) doses below the ZEP, but only six (6) such doses below the ZEP for *in vivo* studies. Both types of studies (animal *in vitro* and *in vivo*) displayed a 125% median MHSR for one dose below the ZEP (Table 1). By the sixth dose below the ZEP, the *in vitro* and *in vivo* groups displayed similar median MHSR values of 163% (*in vitro*) and 165% (*in vivo*), respectively. However, with the addition of two more doses (*i.e.,* 7 and 8) below the ZEP for the *in vitro* data, the median MHSR increased to 180%, although the sample size was progressively reduced.

Each of the median MHSR values is based on a different number of

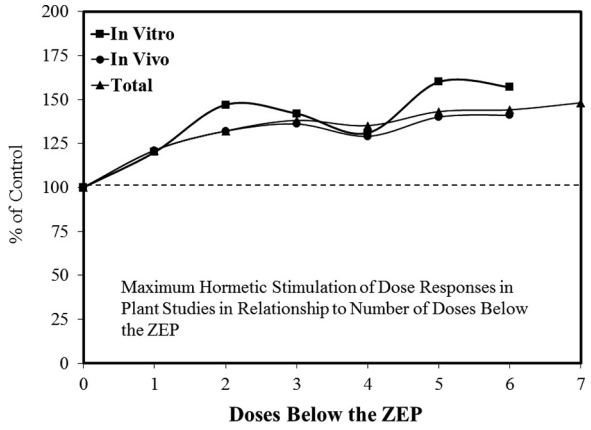

Fig. 2. Maximum hormetic stimulation of dose responses in plant studies in relationship to number of doses below the ZEP.

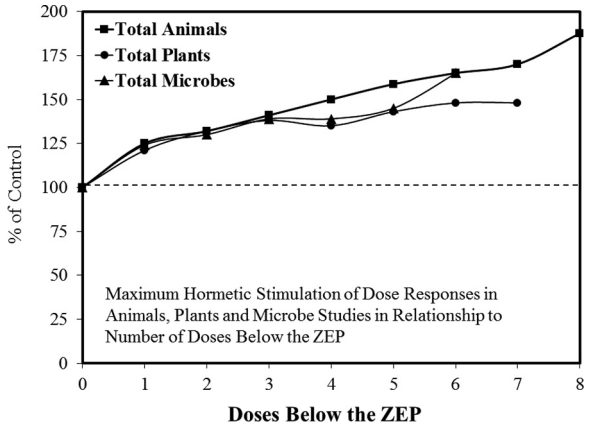

Fig. 4. Maximum hormetic stimulation of dose responses in animal, plant, and microbe studies in relationship to number of doses below the ZEP.

dose responses (Tables 1–3). Those MHSR values based on 2–4 doses below the ZEP are derived from the largest sample sizes and are therefore statistically the most robust. In comparison to the specific case of the 8th dose below the ZEP of the animal *in vitro* data, the median MHSR value is derived from the smallest sample size (*i.e.,* based on 83 dose responses) and thus is the least statistically robust. In both the *in vitro* and *in vivo* findings for animals (Table 1) and plants (Table 2), the most reliable estimate of MHSR, as based on the number of doses below the ZEP, appears to occur with the first 5 doses, with declining reliability thereafter. This pattern is similar for animals *in vivo*, as well as for plants (*in vitro* and *in vivo*) (Table 2) and microbes (Tables 1–3). That is, the most robust data occurred with 1–5 doses below the ZEP, with the dose-count dropping markedly thereafter.

One way to consider these data is to estimate the median change (*i.e.,* increase) in MHSR per number of doses below the ZEP. For example, in the cases of animal data (Table 1) and microbes (Table 3), there was an increase in the median MHSR of about 8–9% for each increase in the number of doses below the ZEP. For plants, the median rate of increase in MHSR per number of doses below the ZEP is 4.5–5.5% (Table 2).

A further analysis was undertaken that included all the available data. In this case, the separate findings of the microbes, plants, and animals with six or fewer doses were combined with those having more than six doses below the ZEP. The findings show that the median MHSR increased for each group having greater than six doses below the ZEP (plants = 17.7%; animals = 32.8%; microbes = 60.6%) (Table 4).

Table 1
The effect of the number of doses below the ZEP on the MHSR for hormetic dose responses in animal experiments (*in vitro* and *in vivo*).

	In Vitro		In Vivo		Total		Adjusted MHSR[a]	
# of Doses Below the ZEP	MHSR # of Dose Responses	MHSR Median	MHSR # of Dose Responses	MHSR Median	MHSR # of Dose Responses	MHSR Median	RSD 20%	RSD 10%
1	278 (9.1%)[b]	125	285 (14.4%)	125	503 (10.0%)	125	121.0	122.0
2	568 (18.6%)	131	525 (26.4%)	132	1098 (21.8%)	132	120.7	126.3
3	701 (23.0%)	141	507 (25.5%)	141	1208 (24.0%)	141	124.1	132.6
4	611 (20.0%)	150	340 (17.1%)	142	951 (18.9%)	150	129.4	139.7
5	383 (12.6%)	163	248 (12.5%)	147	631 (12.5%)	159	136.8	147.2
6	248 (8.1%)	163	81 (4.1%)	165	329 (6.5%)	165	139.7	152.4
7	176 (5.8%)	166			213 (4.2%)	170	143.0	156.5
8	83 (2.7%)	180			102 (2.0%)	188	159.5	173.8
Total	3048		1986		5035			

[a] False positive (*i.e.,* response due to variability) subtracted from MHSR Median Column.
[b] The 278 MHSR # of dose responses with one dose below the ZEP represents 9.1% of the 3048 *in vitro* dose responses. All other percentages in Tables 1–3 would be interpreted in a similar manner.

4. Discussion

With data mined from the HDB, this study demonstrated that the median MHSR of a hormetic dose response increases in magnitude with an increase in the number of doses administered in the hormetic range below the ZEP and, as such supports the main hypothesis upon which this study was designed to test.

Although the hypothesis of this study has nominally been validated, the apparent mechanism by which increasing the number of doses below the ZEP translates into increasing MHSR values has not been directly addressed. It appears that for plants (*in vitro* and *in vivo*) the median MHSR values leveled off or peaked after 5 to 6 doses (Table 2) while for animals they were slightly higher at 8 doses (*in vitro*) and 6 doses (*in vivo*) below the ZEP, with some uncertainty about having yet peaked. However, the animal dose responses having 8 and 6 doses below the ZEP are far fewer in number than the animal dose responses having 1–4 doses below the ZEP. This numerical asymmetry was also true for microbes. Such a limitation provided an incentive to lump plant, animal and microbe data together into 1–6 doses and > 6 doses below the ZEP to produce two groups each for plants, animals and microbes (for a total of 6 groups) and then compare the ranges of the MHSR values for each of the 6 groups. Lumping doses in this manner enhanced the robustness of all group responses, especially for the groups having > 6 doses below the ZEP. These findings indicated that the median MHSR increased across each of the plant, animal and microbe groups with > 6 doses below the ZEP, with the median MHSR values for the plant and animal groups within the 157–186% range. The higher value for microbes may have been affected to some extent by the instability of a lower sample size.

Table 3
The effect of the number of doses below the ZEP on the MHSR for hormetic dose responses in microbes.

# of Doses Below the ZEP	MHSR # of Dose Responses	MHSR Median	Adjusted[a] MHSR	
			RSD 20%	RSD 10%
1	267 (15.8%)	124	118.0	121.0
2	417 (24.7%)	130	118.7	124.3
3	431 (25.6%)	139	122.1	130.6
4	285 (16.9%)	139	118.4	128.7
5	211 (12.5%)	145	121.3	133.2
6	75 (4.4%)	165	139.7	152.4
	1686			

[a] False positive (*i.e.,* response due to variability) subtracted from MHSR Median Column.

4.1. Role of background variation

An alternative explanation is that the increase in median MHSR is related to random variation. Numerous computer simulation exercises by our group have revealed that hormetic-like dose responses can occur by chance (*i.e.,* when no treatment effect is assumed or treatments do not differ from control). The frequency and magnitude of these false-positive hormetic responses are a function of the assumed background variation. This suggests that the median MHSR would also be superimposed on the background variation of normal controls, that it would increase as a function of the number of doses below the ZEP, and that it would therefore enhance the possibility of a more variable MHSR. Such a falsely positive statistical enhancement would become asymptotically

Table 2
The effect of the number of doses below the ZEP on the MHSR for hormetic dose responses in plant experiments (*in vitro* and *in vivo*).

	In Vitro		In Vivo		Total		Adjusted MHSR[a]	
# of Doses Below the ZEP	MHSR # of Dose Responses	MHSR Median	MHSR # of Dose Responses	MHSR Median	RSD 20%	RSD 20%	RSD 20%	RSD 10%
1	136 (15.4%)	120	301 (13.4%)	121	437 (13.6%)	121	115.0	118.0
2	221 (25.0%)	147	532 (23.7%)	132	753 (23.4%)	132	120.7	126.3
3	196 (22.2%)	142	589 (26.3%)	136	787 (24.5%)	138	121.1	129.6
4	155 (17.6%)	131	397 (17.7%)	129	552 (17.2%)	135	114.4	124.7
5	108 (12.2%)	160	291 (13.0%)	140	391 (12.2%)	143	119.3	130.6
6	67 (7.6%)	157	131 (5.8%)	141	199 (6.2%)	148	122.7	135.4
7					93 (2.9%)	148	121.0	134.5
Total	883		2241		3212			

[a] False positive (*i.e.,* response due to variability) subtracted from MHSR Median Column.

Table 4
Comparison of the median MHSR for 1–6 doses below ZEP vs > 6 doses below ZEP.

	All Plants: ≤ 6doses below the ZEP	All Plants: greater than 6 doses below the ZEP	All Animals: ≤ 6 doses below the ZEP	All Animals: greater than 6 doses below the ZEP	All Microbes: ≤ 6 doses below the ZEP	All Microbes: greater than 6 doses below the ZEP
Count	3119	258	4780	467	449	96
Median	134	157	140	186	136	218.5
Percent Increase		17.2%		32.8%		60.1%

less as the number of doses below the ZEP increases. Our group tested this idea earlier in a 13-strain yeast study of nearly 2200 chemicals at four concentrations (plus control) per chemical treatment (Nascarella et al., 2009). That study revealed that a high proportion of anti-tumor agents induced hormetic-like concentration responses and that the median MHSR increased with each additional concentration below the ZEP. The increases in MHSR coincided with the increased variation of the mean responses as measured by the standard deviation for doses below the ZEP.

Findings of our yeast study agree with results from a stochastic model that was used to estimate how the number of responses below the ZEP would affect the MHSR (see Appendix 1). This analysis [using an assumed 20% relative standard deviation (RSD)] indicates that random variability would account for an incrementally and cumulatively larger fraction of the MHSR for each dose that is successively added below the ZEP. For example, variability accounted for approximately 6% of the MHSR at one dose, 16.9% at three doses, and 29.7% at nine doses below the ZEP. (Note that one dose below ZEP yields 6% variability per dose while nine doses below ZEP yield only 3.3% variability per dose). This means that a greater number of doses below the ZEP will translate into both a greater variability and a greater fraction of the MHSR that is falsely inflated. If the RSD were reduced to 10% then these falsely inflated values would be reduced by 50%. This analysis suggests that a certain fraction of the MHSR can be attributed to variation and that the size of this fraction is a function of the number of doses below the ZEP.

Fig. 5 shows the extent to which background variation may contribute to the MHSR for estimates with an RSD of 10% and 20%. Without taking this variation into account, the MHSR with eight doses below the ZEP for animal (*in vitro/in vivo*) data would be 188% (as compared to the 100% for control values). However, if background variation were adjusted to assume an RSD of 10% and 20% then a theoretical net MHSR of 173.75% and 159.5%, respectively, would be predicted. This concept of variability, however, becomes relatively less significant as the number of doses below the ZEP decreases (compare variability/dose at 1 *versus* 9 doses below ZEP). This essentially means that the MHSR still continues to increase, even after the variability adjustments for animals, plants, and microbes. Despite an increase in variability with increasing number of doses below the ZEP, increasing the number of doses still enhances the accuracy of MHSR estimates.

Fig. 6 provides a schematic representation of how more doses in the stimulatory zone can enhance the likelihood of detecting the MHSR. Overall, these findings indicate that the MHSR is greater than 120% and less than 190% as compared to the control group (100%). In light of the present analysis, the 130–160% "rule" seems to be a fair approximation of the median MHSR while having a tendency to underestimate.

Although administering multiple treatments below the ZEP is

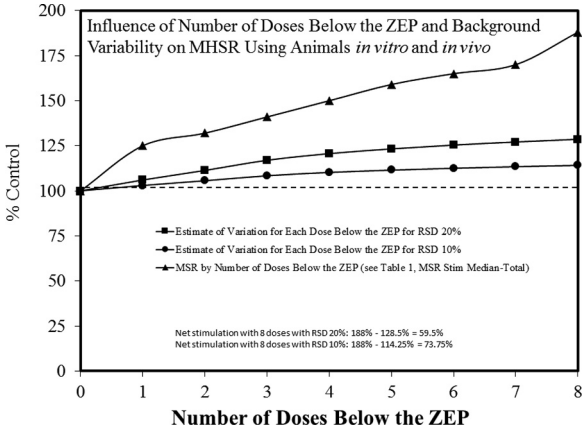

Fig. 5. Influence of number of doses below the ZEP and background variability on MHSR using animals *in vitro* and *in vivo*.

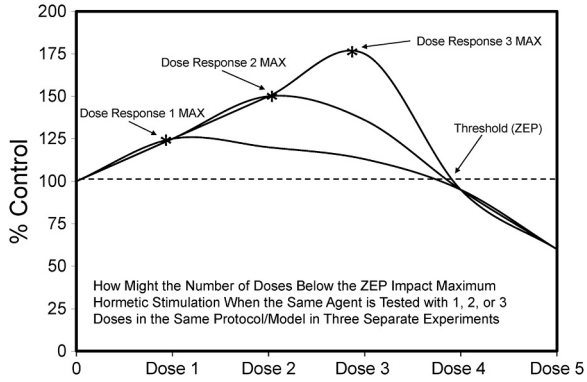

Fig. 6. How might the number of doses below the ZEP impact maximum hormetic stimulation when the same agent is tested with 1, 2, or 3 doses in the same protocol/model in three separate experiments.

necessary to enhance the accuracy of an MHSR (as this study has argued), it is, however, insufficient. To estimate a "true" MHSR, it is also necessary to measure the MHSR at an optimal time following the administration of any treatment dose. A certain amount of time is biologically required to sense a stimulus, to transmit and process information about a stimulus, and to organize and execute an adaptive response to a stimulus. Simply put, if the duration between a stimulus and the measurement of a response to that stimulus is either too short or too long then the temporal window that is required for the expression and measurement of an optimal response will be either completely or partially missed, resulting in no response or, possibly a response significantly less than a "true" MHSR. Thus, the "true" MHSR is associated not only with an optimal dose but also with an optimal temporal framework that enables a more complete expression of the MHSR (Agathokleous et al., 2019). It is clear that the "true" MHSR is a dynamic parameter that can be affected by different variables over time.

Given the above, an MHSR identified outside of the optimal temporal framework cannot therefore be a "true" MHSR. Since the ~70,000 dose responses (contained in the HDB and other hormetic databases) have been optimized neither for multiple treatment doses below the ZEP nor for their occurrence within an optimal temporal framework of response, it is reasonable to conclude that most of these hormetic responses are not really representative of a "true" MHSR. Importantly, this also means that the many previous references citing a 30–60% range for MHSR have tended to underestimate the "true" MHSR. As noted earlier, some 20% of the HDB entries exceeded 100% (*i.e.,* double) of the control response. These 20% of higher hormetic responses may reflect the minority of instances within the HDB where both the stimulatory doses and the response times have been more or less optimized to reflect a more accurate estimate of MHSR. In addition, some proportion of these dose responses may also reflect heightened variation or even possibly preliminary biological dysregulation due to degradation of regulatory control elements.

It should be noted that other experimental factors involving dosing and timing of response (besides number of doses below the ZEP and optimal time to response) could be important in affecting the MHSR. For instance, the appropriate and closer spacing of doses below the ZEP, according to log, semi-log or other possible approaches, could yield better estimates of the MHSR. Furthermore, if multiple and repetitive hormetic treatments were to be considered instead of the singular treatments examined in this study then the duration of the temporal intervals between repetitive treatments as well as the number of repetitive treatments themselves could be important factors in determining an accumulative and "true" MHSR (Leak et al., 2018). However, as a practical matter, the implementation of certain experimental factors to estimate a "true" MHSR, such as increasing the number of doses and repetitive hormetic treatments as well as their temporal distributions, may be expensive and thus limited by available resources.

Whatever experimental design factors may be used, the "true" MHSR and its optimal time to response are framed and constrained fundamentally by both the genetic makeup of an organism and its epigenetic capacity to respond and adapt to a multitude of altered external and internal conditions. For example, the availability of nutrients, the partial pressure of oxygen, the age of an organism, the state of health/disease, the temperature and day/night (diurnal) time of treatment, among others, may all affect the epigenetic capacity of an organism, the fullness and consistency of its genetic expression, and ultimately the magnitude and duration of a hormetic response, *i.e.,* the MHSR. This study thoroughly investigated only one of many experimental factors involving dose and timing of response that could affect the "true" MHSR (*i.e.,* the number of doses below the ZEP) and further recognizes that ubiquitous environmental and physiological factors, *via* the dynamics of genetic and epigenetic processing, may modulate and thus influence the magnitude of a "true" MHSR.

Taken together, these considerations suggest limitations in current understandings of the quantitative features of the MHSR. If the MHSR defines and describes the limits of biological plasticity at multiple levels of biological organization, then an improved understanding of hormetic factors affecting the range of the MHSR should become a significant research priority. The current examination represents a significant step forward in underscoring and understanding the importance of designing hormetic studies (as per number of doses below the ZEP, rates of background variation, and timing of response measurements) that estimate MHSR with reasonable accuracy. A more accurate MHSR may further enhance the translation of experimental hormesis for improved practical applications and future advancements in many fields of biology, such as agriculture, medicine, public health, and performance enhancement.

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Declaration of interest

None.

Appendix A. Role of uncertainty in estimating the maximal hormetic stimulatory response (MHSR)

A.1. Introduction

The maximal hormetic stimulatory response (MHSR) is the maximum hormetic response of a biphasic dose response and is typically expressed as a percent of the control response (*i.e.,* 100%). The MHSR is thought to range from 130% to 160%. To gain an accurate estimate of the MHSR it is necessary to examine a number of doses yielding a hormetic response. The number of doses in the hormetic range varies by study, but is usually between 1 and 8.

Typically, it is important to consider variability when assessing a response. Because variability can introduce error and artificially inflate or deflate the size of an actual response, estimating and correcting for this error will ensure that any differences in the magnitudes of the responses are real, and not just due to chance. As variability depends on the number of times a response is measured, optimal sample sizes should be chosen to minimize the relative standard deviation (RSD) and thereby enhance the accuracy in estimating the MHSR. Often the sample size will result in a RSD that is less than 20% of the control mean response. A simple stochastic model is explained and used to illustrate the effect of sample size on improving an estimate of the MHSR.

A.2. The model

Let n represent the number of responses in the hormetic range, and assume a stochastic model for the response $i = 1, \ldots, n$ is given by

$$Y_i = \mu + E_i$$

where μ represents the expected control response (equal to 100%), and E_i represents response error. For any given study, responses can be ordered from smallest to largest. Let the smallest response be represented by $Y_{(1)}$, the second largest response by $Y_{(2)}$, continuing to the maximum response represented by $Y_{(n)}$. These statistics are called order statistics, and the maximum value is represented by $Y_{(n)}$.

When there is a hormetic response, the simple model given above is inadequate. Restricting the model to the hormetic region, a simple quadratic model may fit, given by

$$Y_i = \beta_0 + \beta_1 X_i + \beta_2 X_i^2 + E_i.$$

The parameters in the model, β_0, β_1, and β_2, characterize the hormetic response, and if known, could be used to calculate exactly the maximum hormetic response. If these parameters are estimated, their values could be used to estimate the maximum hormetic response. This would provide an unbiased estimate, under the assumption that the model for response in the hormetic region is correct.

The assumption that the model for response in the hormetic region is correct is a strong assumption, since there is often little data on response in the hormetic region. For this reason, the maximum hormetic response is often not determined by estimating coefficients in an assumed response model, but rather through direct use of the order statistic, $Y_{(n)}$, for the study. Our interest lies in the expected value of $Y_{(n)}$. In general, the expected value will depend on the distribution of the response error.

A.3. Expected value of the maximum when response error is normally distributed

When response error is normally distributed, Headrick and Pant (2012) have evaluated the expected value of the order statistics for small sample sizes. We summarize their results for $n = 2, \ldots, 9$ in Table A1 when the distribution of response error is normal, with mean 0 and variance equal to 1.

Table A1
Expected Value of the Maximum, $Y_{(n)}$, by Sample Size (*i.e.* Doses in the Hormetic range) under a Standard Normal Distribution.
Source: Headrick and Pant, Tables 1,3,6,7,8.

n	$E[Y_{(n)}]$
2	0.5642
3	0.8463
4	1.0294
5	1.1630
6	1.2671
7	1.3522
8	1.4236
9	1.4850

The expected values give in Table 1 are represented as a multiple of the standard deviation, where the standard deviation is equal to 1. In the context of hormetic studies, the standard deviation of response error is seldom explicitly reported. However, dose response studies are usually conducted so that control response is reliable, typically with sample sizes large enough so that the relative standard error for control is 20% or less. When expressed on a relative scale, this corresponds to a relative standard error of 0.2. Multiplying the expected values given in Table 1 by this relative standard error, we can determine the expected increase in the hormetic response, relative to control that would occur due to response error if the same relative standard error occurred at each dose. The result is given in Table A2.

Table A2 illustrates the biasing effect produced by response error that is expected to occur if response error is normally distributed. An additional important assumption is that at each dose in the hormetic range, response is measured to the same level of error as control response. The results in

Table A2
Expected Increase in Relative Response (percent) for the Maximum, $Y_{(n)}$, by Sample Size (*i.e.* Doses in the Hormetic range) assuming the Relative Standard Deviation is 0.2.

n	$E[Y_{(n)}]$(percent)
2	11.3%
3	16.9%
4	20.6%
5	23.2%
6	25.3%
7	27.0%
8	28.5%
9	29.7%

Table A2 correspond to an assumption that the relative standard deviation for control response is 20%. If the relative standard deviation is only 10%, then the results in Table A2 would be half the reported values.

Details provided in individual dose response studies may not be adequate to determine the relative effect of response error on the maximum hormetic response. Nevertheless, this exercise suggests that response error may have an appreciable effect on interpretation of the maximum hormetic response.

References

Agathokleous, E., Kitao, M., Calabrese, E.J., 2019. Hormetic dose responses induced by lanthanum in plants. Environ. Poll. 244, 332–341.

Calabrese, E.J., 2008. Hormesis: why it is important to toxicology and toxicologists. Environ. Toxicol. Chem. 27 (7), 1451–1474.

Calabrese, E.J., Blain, R.B., 2011. The hormesis database: the occurrence of hormetic dose response in the toxicological literature. Reg. Toxicol. Pharmacol. 61 (1), 73–81.

Calabrese, E.J., Mattson, M.P., 2017. How does hormesis impact biology, toxicology, and medicine? NPJ Aging Mech. Dis. 3 (, article number UNSP 13).

Headrick, Todd C., Pant, Mohan D., 2012. On the order statistics of standard normal-based power method distributions (Article ID 945627). ISRN Appl. Math. 2012, 13. https://doi.org/10.5402/2012/945627.

Leak, R.K., Calabrese, E.J., Kozumbo, W.J., Gidday, J.M., Johnson, T.E., Mitchell, J.R., Ozaki, C.K., Wetzker, R., Bast, A., Belz, R.G., Botker, H.E., Koch, S., Mattson, M.P., Simon, R.P., Jirtle, R.L., Andersen, M.E., 2018. Enhancing and extending biological performance and resilience. Dose-Response 2018, 1–24. https://doi.org/10.1177/1559325818784501.

Nascarella, M.A., Stanek III, E.J., Hoffmann, G.R., Calabrese, E.J., 2009. Quantification of hormesis in anticancer-agent dose responses. Dose-Response 7 (2), 160–171.