Dose-response relationships. An overview, a generative model and its application to the verification of descriptive models.

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ABSTRACT

Taking as a starting point a set of simple quantitative hypotheses regarding the possible relationships between cell receptors and effector molecules, a statistical algorithm or generative model is developed that simulates different types of dose-response profiles and whose results are used in two ways, both subject to empirical verification. In the first of these, the suitability of several common descriptive models for the study of dose-response relationships is discussed, and changes are introduced that improve their suitability for this conceptual framework, generalise their application and lead to the systematisation of possible interactions between more than one effector, as well as between the effects of self-stimulating and self-depressor mechanisms. Secondly, the generative model suggests the existence of some unexpected profiles and their possible explanations. Both the profiles and the hypotheses appear to be supported by experimental evidence.

INTRODUCTION

A dose/response model (DR) is, in general, an equation describing the variation of a representative magnitude in an object population, with the variation in the magnitude of an effector agent. A typical case, to which we will restrict ourselves here, is the inhibition of the growth of a microbial population by a chemical agent, but the same resource can often be applied, with minor changes, to stimulatory effects, to mortality or survival, to quantitative changes in cell components [1], to the characters of macro-organisms [2], and to different physical and chemical agents [3-6]. DR models, which are essential for risk assessment [7-11], are also to be found in the bibliography of widely differing areas, formalised through equations that represent with varying accuracy the

peculiarities of the system to which they are applied. Here, we will refer only to some studies of historical interest and to some recent discussions on the topic.

The typical profile of an empirical DR curve is a logical consequence of the populational level implied in experiments of this nature. If the sensitivity to an effector of the population elements varies according to a unimodal distribution (density) function, then necessarily the profile of the corresponding cumulative (mass) function will be sigmoidal. Several mathematical formulas that include parameters with interesting physical (biological) significance, or that can be reparameterised for this purpose, fit this hypothesis. Table 1 shows five of these forms, some widely used and others –by no means the least useful– only rarely used.

The sigmoidal profile is, therefore, a basic condition of DR models. Furthermore, it seems logical to require that 1) its asymptote does not necessarily imply the mortality of all the experimental population, or in other words, that the model can represent situations in which a portion of the population is resistant or immune to the effector; 2) that there is a null response to a null dose, *i.e.*, that the function lacks of intercept. This is necessary because the sigmoidal profile is the result of responses assessed in terms of the proportion of inhibition in relation to a control, which prevents the usual correction of values by means of a simple subtraction of the said control.

Thus, and if we do not intend to descend to the level of first principles, it is possible to formulate a statistical generative model that, assuming only reasonable basic hypotheses regarding the interaction between a cell population and an effector, produces simulations of DR relationships that are suitable for examining the adequacy and peculiarities of descriptive models. Such is the object of this study.

THEORY. DISCUSSION OF SOME DOSE-RESPONSE MODELS

Although all the equations in Table 1 fit the sigmoidal profile, they are also all problematical with regard to some of the above-mentioned additional conditions, and therefore may have unwanted consequences on the fit of the experimental results. There follows a brief examination, proposing changes that will suit them to the above-mentioned requirements.

Logistic equation (T1.1). Commonly used to describe growth processes [12], it is attractive because of the direct physical significance of its parameters. In fact, it is possible to reparameterise it easily so that the essential term in the DR analysis (dose producing the semimaximum response, or

$RD_{50}=m$) appears explicitly. In effect, if K is the maximum response (not necessarily 1), and making $R=K/2$, we obtain $b=\exp(\mu m)$ and :

$$R = \frac{K}{1 + e^{[\mu(m-D)]}} \quad (1)$$

where μ is the specific rate of the response (increment of R per unit of R and per unit of D , with dimensions D^{-1}).

The expression, suitable for describing the response of populations whose sensitivity to the effector follows a normal distribution, is the basis for the probit transformation [13-14], a classical resource for linearising a DR relation. Although sometimes useful, such linearisation is unsatisfactory if $K < 1$ (populations with a resistant fraction) and implies the loss of a parameter, making comparisons difficult (two cases with a different K produce indistinguishable lines), so non-linear adjustment methods are to be preferred. Even so, a non-null intercept R_0 poses problems with real data, affected by experimental errors, because the solution often implies an unacceptably high R_0 and biases in the values of μ , m and K . The problem is minimised by restricting R_0 to very low levels compared to K , but that makes the value of μ very sensitive to the experimental error, especially to an overestimation of the response to low doses, as frequently occurs in practice. Thus, it is recommended to force the fulfilment of the aforementioned condition 2, by subtracting the intercept in (1):

$$R = K \left(\frac{1}{1 + e^{[\mu(m-D)]}} - \frac{1}{1 + e^{\mu m}} \right) \quad (2)$$

so that K and m no longer represent the maximum response ($R_{\max}$) and the RD_{50} . In effect:

$$R_{\max} = \lim_{D \rightarrow \infty} R = K' = K \left(\frac{e^{\mu m}}{1 + e^{\mu m}} \right) \quad (3)$$

so that RD_{50} (abscissa for $R = K'/2$) turns out to be:

$$RD_{50} = m' = \frac{1}{\mu} \ln(2 + e^{\mu m}) \quad (4)$$

If the logistic equation is suitable in the case of normal distribution sensitivities, when distribution is assimilable to the log-normal model, the dose (and the parameter m , with dose

dimensions) must be substituted for its logarithm. The same transformations applied to the logistic equation lead to:

$$R = K \left(\frac{1}{1 + e^{[\mu(\ln m - \ln D)]}} - \frac{1}{1 + e^{\mu \ln m}} \right) \quad (5)$$

it being a common practice in this case, in order to avoid possible negative abscissas, to code the independent variable as $\ln D' = \ln(D+1)$, so that $m = m' - 1$. This model, like all those to be considered from now on, suffers from the lack of the significance of a specific response coefficient for the parameter μ , because it has the character of a dimensionless neperian logarithm. On the other hand, as in the case of the modified logistic equation, K and m are only the apparent maximum response (R_{max}) and RD_{50} , since the real ones are:

$$R_{max} = \lim_{\ln D \rightarrow \infty} R = K' = K \left(\frac{e^{\mu \ln m}}{1 + e^{\mu \ln m}} \right) \quad (6)$$

$$RD_{50} = m' = \text{EXP} \left[\frac{1}{\mu} \ln(2 + e^{\mu \ln m}) \right] \quad (7)$$

Equation T1.2. Apparently unnamed, we will call it the “clinical equation”, because it is widely used in this field. It differs very little from the logarithmic version of the logistic equation, but its parameters have a more complicated relationship with the relevant terms of the DR analysis. In spite of not fulfilling conditions 1 and 2, its use is very widespread [15], being commonly found, as has been said, in clinical bioassays, and often suggested in statistical software [16]. As formulated in Table 1, the asymptotic value (*i.e.* $\lim R$ when $\ln D \rightarrow \infty$) is, in effect, 1. If the equation is modified thus:

$$R = a + \frac{K - a}{1 + e^{b - c \ln D}} \quad (8)$$

the asymptotic value is now K , therefore meeting condition 1 (suitability for cases with a fraction of immune population) The corresponding RD_{50} , would now be:

$$m = \text{EXP} \left\{ \frac{1}{c} \left[b - \ln \left(\frac{K}{K - 2a} \right) \right] \right\} \quad (9)$$

with the existence of an intercept with the value:

$$R_{(\ln D=0)} = a + \frac{K-a}{1+e^b} \quad (10)$$

To allow the model to fit condition 2, (10) must be subtracted from (8), obtaining:

$$R = (K-a) \left(\frac{1}{1+e^{b-c \ln D}} - \frac{1}{1+e^b} \right) \quad (11)$$

so that, as in the preceding cases, R_{max} and RD_{50} are therefore:

$$R_{max} = K' = \lim_{\ln D \rightarrow \infty} R = (K-a) \left(\frac{e^b}{1+e^b} \right) \quad (12)$$

$$m' = \text{EXP} \left\{ \frac{1}{c} \left[b - \ln \left(\frac{e^b}{e^b + 2} \right) \right] \right\} \quad (13)$$

Seen this way, however, the model is practically equivalent to the logistic one in its logarithmic version.

Fermi's equation (T1.3). This is but another version of the logistic equation (in which the transformation is performed by changing $D-m$ for $m-D$), whose ordinate represents a decrease in survival S instead of an increase in mortality. The same transformations applied to the logistic equation lead to the fulfilment of conditions 1 and 2. Peleg [5-6; 17] defends its usefulness because of its suitability for describing responses of populations whose sensitivity is distributed according to a Weibull density function. The problems associated with the distributions of sensitivity will be discussed in detail later. For the present we will only remark that, in that case, the Weibull cumulative function itself would be the most suitable descriptive model.

Weibull cumulative function (T1.4). A function capable of generating a wide variety of profiles, either symmetrical or not, depending on the relationships between the values of its parameters (α and β), is the distribution β :

$$f(x; \alpha, \beta) = \frac{\Gamma(\alpha + \beta)}{\Gamma(\alpha)\Gamma(\beta)} x^{\alpha-1} (1-x)^{\beta-1} ; 0 < x < 1 ; \alpha, \beta > 0$$

used basically to determine, by means of bayesian inferences, bounds or tolerance limits of different phenomena, with no need to attribute a normal distribution to the corresponding variables. The distribution β is related to the distribution Γ :

$$f(x; \alpha, \theta) = \frac{1}{\theta^\alpha \Gamma(\alpha)} x^{\alpha-1} e^{-(x/\theta)} \quad ; \quad x > 0 ; \alpha, \theta > 0$$

and through it, to a large group of distributions that are particular cases of Γ (thus χ^2 , Erlang, Poisson and negative exponential). The direct application of distributions β and Γ as equations for a fit in DR relationships encounters the difficulty of their cumulative functions containing integrals that cannot be solved in a closed form. That is the reason why, in this domain, the tendency is to use more manageable related functions [7-9; 18-20], among which the flexibility of the Weibull model stands out [10; 21]:

$$f(x; \alpha, \theta) = \frac{\alpha}{\theta^\alpha} x^{\alpha-1} e^{-(x/\theta)^\alpha} \quad ; \quad x > 0 ; \alpha, \theta > 0$$

The Weibull cumulative function, in effect, can be taken as a DR model, whose median (not its mode, sometimes used for this purpose) directly provides the required dose for the semi-maximum response m . It meets condition 2, and to meet condition 1, only needs the introduction of the maximum response K as a factor:

$$R = K \left\{ 1 - e^{-(D/\theta)^\alpha} \right\} \quad (14)$$

which is a change that does not alter the value of m given in Table 1.

Gompertz equation (T1.5). A traditional model in population dynamics [22], it only requires the subtraction of the intercept in order to meet condition 2.

$$R = K \left(e^{-e^{b-cD}} - e^{-e^b} \right) \quad (15)$$

so that, as usual, R_{max} and RD_{50} must be calculated by:

$$R_{max} = K' = \lim_{D \rightarrow \infty} R = K \left(1 - e^{-e^b} \right) \quad (16)$$

$$m' = \frac{1}{c} \left\{ b - \ln \left[\ln \left(\frac{2}{1 + e^{-eb}} \right) \right] \right\} \quad (17)$$

Zwietering et al. [23], among other authors, reparameterised the expression T1.5 to give it a useful form from the point of view of microbial growth, assimilable to the DR once the intercept has been eliminated. If r_m is the maximum rate of the response (maximum increment of R per unit of D , with dimensions RD^{-1}) and δ the threshold dose, below which there is no detectable response, it can be said that:

$$b = \frac{r_m \delta e}{K} + 1 \quad , \quad \text{or:} \quad r_m = \frac{K(b-1)}{\delta e} = \frac{cK}{e} \quad (18)$$

$$c = \frac{r_m e}{K} \quad , \quad \text{or:} \quad \delta = \frac{K(b-1)}{r_m e} \quad (19)$$

To sum up the preceding discussion, it can be said that the equations in Table 1 better suit the requirements of a DR model if they are modified according to the previously mentioned criteria, leading to the forms in Table 2. Fermi's equation, whether modified or not to meet conditions 1 and 2, is redundant due to its similarity with the logistic equation, and will not be included from now on. The equation T1.2, although problematic in its original formulation, is no longer so if it is modified, but in this case it is no improvement on the modified log-logistic equation, which contains one parameter less, is easier to manage and conceptually clearer.

MATERIALS AND METHODS

Microbiological methods

The micro-organisms used as producers of bacteriocins were *Lactococcus lactis* (CECT-539) and *Lactobacillus Casei* (CECT-4043); those used as indicators in the DR assays were *Leuconostoc mesenteroides* sp. *lysis* (kindly provided by Dr. Ray, of the University of Wyoming, Laramie, USA) and *Carnobacterium piscicola* (CECT-4020). All were stored at -50°C in a suspension of powdered milk with 25% glycerol, and were cultured for the production of biomass, nisin or both [24]: 250 ml Erlenmeyer with 50 ml of Tryptone-Glucose-Yeast Extract (TGE, DIFCO) medium [25], at 30°C , with orbital agitation at 200 rpm, and inoculated with cell

suspensions in sterile distilled water (1% of the initial volume of the culture), adjusted to an OD (700 nm) of 0.900.

The assayed effectors were pure nisin (Applin & Barrett, Ltd), bacteriocin extracts obtained from the producer cultures, and lactic acid. The methods for the extraction of bacteriocins and their quantification by means of DR assays against the above-mentioned indicators were described in detail in a previous study [26].

Statistical methods

The generative model described in this study was produced on a *Microsoft Excel 97* spreadsheet. To obtain random numbers n_i with the specified distributions, from the random unitary numbers u_i that this spreadsheet provides, the expressions of Canavos [27] were used. Although in some of these distributions means (n) and standard deviations (σ) are not definition parameters, in all cases they were the values on which comparisons were based. For reasons that will be explained in the discussion, values of continuous distributions rounded off to integer numbers were used.

Random uniform number in the interval $[a; b]$

$$n_i \sim U[a;b] = \text{INT}\{u_i [(b+1)-a] + a\} \quad ; \quad \text{being: } n = \frac{a+b}{2} \quad ; \quad \sigma = \frac{b-a}{2\sqrt{3}} \quad (20)$$

Normal random number, $n_i \sim N: (n; \sigma)$

$$n_i = \text{INT} [(-2 \ln u_1)^{1/2} \sin (2\pi u_2) \sigma_n + n] \quad (21)$$

Log-normal random number $n_i \sim \log\text{-}N: (n; \sigma)$

$$n_i = \text{INT} \{ \text{EXP} [(-2 \ln u_1)^{1/2} \sin (2\pi u_2) \sigma' + n'] \} \quad (22)$$

Given that the log-normal distribution is the one whose neperian logarithm presents a normal distribution, numbers thus generated, with parameters n' and σ' , produce empirical values (the most useful for comparative purposes) n and σ for the mean and the standard deviation, being:

$$n = \text{EXP} \left(n' + \frac{\sigma'^2}{2} \right) \quad ; \quad \sigma_n = \text{EXP} (n' + \sigma'^2) [1 - \text{EXP} (-\sigma'^2)]^{1/2}$$

Weibull random number, $n_i \sim W: (\theta; \alpha)$

$$n_i = \text{INT} \left[\theta \left(\ln \frac{1}{1-u} \right)^{1/\alpha} \right] ; \text{ being: } n = \theta \Gamma \left(1 + \frac{1}{\alpha} \right) ; \sigma_n = \theta \left[\Gamma \left(1 + \frac{2}{\alpha} \right) - \Gamma^2 \left(1 + \frac{1}{\alpha} \right) \right]^{1/2} \quad (23)$$

The results, whether strictly experimental or obtained from a generative model, were fitted to the descriptive models by minimisation (*quasi*-Newton method) of the quadratic differences between the expected and observed values.

RESULTS AND DISCUSSION

1: A model generating dose/response relationships

Due to the existence of diverse non-equivalent mathematical formulas that generate sigmoidal curves, and in fact there are examples of DR relationships that fit these forms with different degrees of acceptability [28-29], it seems useful to attempt to establish a model that, starting from simple hypotheses, allows the response of a population against an effector to be simulated, and also to test diverse types of specific hypotheses formulated from series of experimental results. In the model proposed here, we started from the following hypothesis:

S1: Each cell has n potential receptors of the effector, and each receptor may adopt two different states (active or sensitive and inactive or immune), a being the number of active receptors.

S2: The dose D is defined as the number of effector units (molecules) present in each cell, accepting the fact that every molecule is capable of bonding with one receptor.

S3: Restricting the cell response C to two modalities: death ($C=0$) and survival ($C=1$), and assuming that the lethal effect occurs when at least p receptors are occupied, the following rule can be established:

$$\text{If } a < p, C=1 \quad \forall D. \quad \text{If } a \geq p \quad \begin{cases} \text{If } D < p, C=1 \\ \text{If } D \geq p, C=0 \end{cases} \quad (24)$$

Thus, when of a total number Y of cells, S survive at a given dose, the population response can be formulated as $R=1-(S/Y)$. It must be noted that direct comparison between D and p is not appropriate. With $a < p < D$, then $C=1$ at any dose of the effector, because $a < p$, even though $D > p$. This allows for the possibility of the existence of elements that are immune to the effector ($a < p$,

although $n > p$), which is one of the necessary conditions required for the previously mentioned descriptive models.

S4: Finally, it is possible to consider two kinds of additional factors, capable of affecting the response, as they induce changes in either the receptor or the effector or in both. When they affect the receptor, we can talk of excitors or inhibitors (h) depending on whether they raise or lower the value of a . When they affect the effector, we can talk of enhancers or antagonists (f), depending on whether the effective dose D_e they produce is higher or lower than the real one.

Under such hypotheses, and since any DR experiment implies populational values, n , a and p must be considered as random numbers –in principle discrete–, produced by the individual variability of biological entities. In addition, h and f must be assumed to be random –continuous in principle–, for reasons of experimental variability. And because the probability distributions of these numbers can affect the characteristics of the DR function, the generative model must consider, for each of them, diverse possible distributions. As its plausibility is better discussed when taking the results into account, we will now only describe the alternatives used, which can be easily formulated on a spreadsheet, according to the following steps:

M1: Generation of the random number n (number of receptors in each cell). In principle, a binomial function $B:(n,a)$, defined by n assays with a success probability of a , appears suitable for representing the number a of active receptors in a cell population with n receptors. In this case, however, the values of n and a are subject to unwanted restrictions, because the variance, $\sigma^2 = na(1-a)$, of the binomial distribution is not independent from the mean, $\mu = na$, making it impossible to test hypotheses which cannot be rejected *a priori*, as is the case of low means with high variances. The problem disappears if n and a are independent numbers, generated with continuous distributions and rounded off to integer numbers. A simple hypothesis for n is that of a normal distribution, $n_i \sim N:(n; \sigma_n)$, although given its domain $(-\infty, \infty)$, low means and high deviations may lead to a certain number of negative values n_i , which lack physical significance. This may be resolved by means of two procedures with different implications:

a) Generating a series of values n_i' and introducing the following restriction to obtain n_i :

$$\text{If } \begin{cases} n_i' \geq 0 ; n_i = n_i' \\ n_i' < 0 ; n_i = 0 \end{cases} \quad (25)$$

b) Generating n with distributions of domain $[0, \infty)$. For reasons that will be discussed later, the alternatives tested were the log-normal and Weibull distributions.

M2: Generation of the random number a (number of active receptors in each cell). Similar reasons to the ones given in the above case suggest the convenience of the same alternative hypotheses for a (normal, log-normal and Weibull distributions). Another possibility for situations lacking physical significance is here $a_i > n_i$, which can be resolved by generating a column of values a_i' , and introducing the following restriction to obtain a_i :

$$\text{If } \begin{cases} a_i' \leq n_i & ; a_i = a_i' \\ a_i' > n_i & ; a_i = n_i \end{cases} \quad (26)$$

Another alternative is to assume for a a uniform distribution between 0 and n .

M3: Generation of the random number p (minimum of occupied receptors for a lethal effect).

Because the number p represents the possibility of a different response to equal values of n and a , it has the significance of a cell susceptibility, whose values can once again be generated according to the three above-mentioned distributions (normal, log-normal and Weibull).

M4: Generation of the random number h (effect of excitors and inhibitors of the response).

Since h represents a factor that increases (excitor) or decreases (inhibitor) the average number of active receptors a , a simple way of formulating it is to include it in the expression of a . If a is uniform, the expression (20) can be modified as follows:

$$a_i \sim U(0; n) = \text{INT} \{ u [(n+1-h_I) - (0+h_E)] + (0+h_E) \} \quad (27)$$

it being a factor that either excites (h_E) or inhibits (h_I) the response depending on whether it helps to increase the lower bound of a or decrease the upper one. Another possible solution is to define a , whatever its distribution, $D(a; \sigma_a)$, may be, in the terms:

$$a_i \sim D(a; \sigma_a) ; \text{ being: } a = h \frac{n}{r} , \text{ with restrictions } \begin{cases} 1 < r < n \\ hn/r \leq n \end{cases} \quad (28)$$

where n/r represents the maximum number of potentially active receptors, with $h > 1$ for the excitors and $h < 1$ for the inhibitors.

M5: Generation of the random number f (effect of enhancers or antagonists of the effector). If f represents a factor that alters the probability of effective interaction between effector and receptor, it is possible to represent it in terms of an effective dose D_e , different from the nominal dose D . A simple formulation for the effective dose is:

$$D_e = f_i D \quad (29)$$

with f_i being a random number whose mean is f ($f > 1$ for enhancers and $f < 1$ for antagonists), generated according to the same three distributions (normal, log-normal and Weibull).

M6: Definition of the cell population and the effective doses D_e . If four column vectors with 1,000 values for the numbers n , a , p , h are generated under any of the above-mentioned hypotheses, we have a simulation of 1,000 cells defined by the row vectors (n_i, a_i, p_i, h_i) . Let us now generate a column vector with 1,000 values of the number f , corresponding to the 1,000 cells, and let us place a series of d increasing nominal doses D in a row of the spreadsheet. The product of these doses multiplied by the 1,000 f values therefore produces a matrix ($d \times 1,000$) of effective doses D_e , of which each i^{th} row represents the real series of doses assayed against the cell defined by the i^{th} cell vector (n_i, a_i, p_i, h_i) .

M7: Determination of cell responses C . Using the effective doses D_e instead of the nominal doses, D , rule (24) is now applied to the comparison of each cell vector with the corresponding series of doses. Thus, a 0 and 1 matrix ($d \times 1,000$) is obtained, in which each row represents the cell response to a series of effective doses, and each column, the response of 1,000 cells to the effective doses that represent a specific nominal dose. The d sums of the columns represent, then, the values of the accumulated response of the cell population to the series of effective doses D_e , and its profile will therefore represent a DR relationship.

The typical profile already appears, in effect, in populations of ~200 cells. However, when several assays under the same hypotheses were fitted to any of the models in Table 2, the variability of the parametric estimations showed a scarce sample, which was augmented in the following way: after obtaining the d totals of columns that give the response of 1,000 cells to the series of doses (21 values, including 0), with the results placed in a row z , the following macro was written:

```
ActiveCell.Offset(0, 22).Range("A1:U1").Select
For i = 1 To 9
Selection.Copy
```

```

ActiveCell.Offset(i, 0).Range("A1").Select
Selection.PasteSpecial Paste:=xlValues, Operation:=xlNone, SkipBlanks:= False, Transpose:=False
ActiveCell.Offset(-i, 0).Range("A1:U1").Select
Next i
End Sub

```

which copies this row in the next one ($z+1$), so that the sheet generates new series of random numbers. The results of the new calculation now occupy the row z , from where they are copied to row ($z+2$). Every iteration of this procedure produces a row with the response of 1,000 cells which can be added to the previous ones and, after several iterations, parameter stabilisation shows that the results are representative of the population level. Although 5,000 cells is sufficient for this stabilisation, in this study (note the macro instruction 'For $i=1$ To 9'), a sample of 10,000 was used.

2: Behaviour of the generative model according to the random properties of its factors

In order to examine the effects on the DR function of the distributions attributed to the factors n , a , p , h and f , we will analyse the general features of the response before studying the fit of the models in Table 2 in the most characteristic cases. As the basic elements in the DR relationship are the dose and the factors defining the cells, for the present we will omit both the excitor or inhibitor and the enhancer or antagonist factors.

2.1: Hypothesis of normal distributions of n , a and p

According to what was noted in M1 to M3, we start by accepting that $n_i \sim N(n; \sigma_n)$, $a_i \sim N(a; \sigma_a)$ and $p_i \sim N(p; \sigma_p)$ are random, normal and independent numbers. Of course, if the three deviations are null, then there is no probabilistic behaviour: the DR function is null if $a < p$ or stepped (in $D=p$) to an asymptotic value $K=1$ if $a \geq p$. Its stepped nature remains even when the variances are non-null in n and a , which give variability only to the asymptotic value, and, if $\sigma_a \neq 0$, they permit a non-null response even when $a < p$. Variations in the number of receptors n , or of the active receptors a , do not therefore produce, either separately or jointly, the smooth profile commonly found in experiments, whose key is the random nature of the susceptibility p . However, n and a contribute to change this profile once p becomes random.

If none of the deviations are null, the relations between the parameters of the respective distributions create a more complex casuistry. Fig. 1 (A-F) systematises the six possible cases according to the relations between the values of the means, with arbitrary variances that make the probability of negative values of n , a and p negligible. It must be noted that:

i) The light-shaded areas typify the characteristics of the fraction of the population in which the lethal effect occurs, but do not represent it quantitatively. That would happen if the random numbers n , a and p were not independent, *i.e.*, if a specific value of n would condition the probabilities of the values a and p . However, since we start from the more general hypothesis of independence, the accumulated effect can not be assessed analytically, but only by means of the proposed generative model, which provides the superposed profiles in the same figures.

ii) Cases such as those of B, E and F in Fig. 1, in which the average value of a exceeds that of n , are not likely to be rejected, because they only mean that in cells with a high number of receptors, there is a high probability that most of them are in an active state. However it seems reasonable to avoid these cases, as has been said, by means of restriction (26). In this case it must be noted that one part of the distribution of a (hard-shaded areas in Fig. 1) is reassigned to a distribution identical to that of n , creating a further obstacle to the analytical solution of the accumulated effect.

iii) Finally, any of the cases A-F can be found in a G-type situation. As was also noted, the normal distribution domain $(-\infty, \infty)$ leads, in the hypothesis of low means with high variances, to high probabilities of negative values, with no physical significance, of the factor involved. This creates truncated distributions, producing DR curves that would require the acceptance of an intercept that is also uncertain (lethal effect even with a null dose) which must therefore be considered as artefactual. Thus, in order to preserve the factual content of the model, recourse must be had to distributions of the domain $[0, \infty)$, as we will see next.

2.2: Other possible distributions of n , a and p .

Strictly speaking, none of the cases A-F is implausible, given the mechanics of the generative model, which prevents us from rejecting *a priori* the hypothesis of normality, with all its consequences. However, situation G is different, given the unacceptability of the intercept. Nevertheless, an argument can be made for the possible existence of factors capable of interacting with either the receptor or the effector or with both, with “filtering” effects equivalent to the truncation of the distribution of a that produces a response to a null dose. But in that case, and if the experimental data really suggest an intercept, it would be necessary to introduce those factors into the model as independent variables to be considered in addition to the dose. On the other hand, the formulation of a given in (28) neither solves the problem nor provides new information.

Thus, in a single variable DR model it is hard to avoid the idea that the distributions of n , a and p , especially if high variances are accepted, must be of a domain $[0, \infty)$. In this regard, the most

reasonable candidates are the Weibull and log-normal distributions, which are suitable for describing phenomena in which there intervene factors linked by relations that are not merely additive (the normal distribution is suitable for describing phenomena derived from additive relations). Both approach the normal distribution as the mean increases, but differ in the sign of their bias: always to the right in the log-normal, and from right to left as the mean increases in the case of the Weibull model. Fig. 2 shows examples of these functions, with the same means and variances used in the normal distributions in Fig. 1, in cases equivalent to A (high means and variances), and G (low means and high variances).

If means and variances are high (in any of the situations A-F), the DR curves are sigmoidal, and differ little from those resulting from normal distributions. If means are low and variances are high (cases G in Fig. 2), the domain $[0, \infty)$ corrects, as is to be expected, the artefactual intercept. But now the section of the decreasing slope tends to prevail over that of the increasing slope, which is why in the absence of data at very low doses, asymptotic profiles with a ever-decreasing slope can occur, which can apparently be described by a von Bertalanffy's equation:

$$R = K + (R_0 - K)e^{-\mu D} \quad (30)$$

which, with a null intercept ($R_0=0$), becomes:

$$R = K(1 - e^{-\mu D}) \quad ; \quad \text{with } RD_{50}: \quad m = \frac{\ln(2)}{\mu} \quad (31)$$

As the conditions under which this kind of response seems to occur are acceptable from a realistic point of view, it will be convenient to provisionally add the expression (31) to those of Table 2 when it comes to verifying the adequacy of the corresponding models.

2.3: Effects on the DR curve of the relationships between the factors a and p

Although the adequacy of the models in Table 2 will be examined later, it can already be said that all of them coincide in the results in Table 3, independently of the distributions accepted for n , a and p . As n is only the upper bound of a , the table is limited to the effects of the relationships between the distribution parameters of a and p (for every value of n) on the basic parameters of any DR model (maximum response K , specific rate of the increase in response μ , and dose for the semi-maximum response m).

2.4: Effects of the factors h and f

In any of the formulations (27) or (28) proposed in M4, the number h is limited to increasing (excitor) or decreasing (inhibitor) the average number of active receptors a , and its effects can therefore be reduced to those of the variations in a and σ_a shown in Table 3. In the case of f , however, there are further implications. If its distribution is normal, once again the domain $(-\infty, \infty)$ produces, in hypotheses with high variances, a certain number of negative values f_i of f . Furthermore, the DR profiles depend not only on f , but also on the distributions of n , a and p .

If n , a and p are also normal, negative values f_i of f , although of an uncertain physical significance (the modifier would make the dose negative), only mean, given the rule (24), a dose without any effect. The result is not problematical, and has well defined implications for the mechanics of the generative model, since even if the effects of a certain number of doses are randomly annulled, the probability of an effect being in any way null is higher when the doses are low than when they are high. Thus, the DR profile, relatively unaffected by low doses, tends to reach the asymptote more smoothly. The section of the decreasing slope prevails over the section of the increasing slope (Fig. 3A), and again, in the absence of data at low doses, profiles arise that can apparently be described by means of von Bertalanffy's equation (31).

If, when n , p and a are normal, the distribution of f is of a domain $[0, \infty)$, the increase of σ_f produces the consequences shown in Fig. 3B, explainable as follows: Increasing σ_f means increasing the interval of effective doses D_e that corresponds, with finite probability, to a nominal dose D . Thus, a dose with a mean D will include, with finite probability, effective doses lower than the mean ($D_e < D$), against which some cells that would die at a mean dose will now survive. But it will also include cases when $D_e > D$, in which cells that would survive with the mean dose now die. As the increase in survival predominates at higher doses and that in mortality at lower doses, maximum mortality decreases, and a dose interval occurs in which the superposition of both effects creates a brief response stabilisation and generates a DR profile of the same nature as one produced by a population with a bimodal sensitivity distribution.

The “bimodal” profile depends on the asymmetry and the standard deviation of f , and varies little, whether f has a log-normal or a Weibull distribution (both are asymmetrical, and both biased to the right if the mean is low, as is the case given definition (29)). In addition, and due to reasons formally analogue to those discussed in the preceding paragraph, the same type of profile appears, even if the distribution of f is symmetrical, when at least one of the remaining factors (n , p or a) is considered to have an asymmetrical distribution.

Should this profile be considered artefactual? Since its key is the random nature of f , it should be remembered that the notion of molecular activity is assimilable to the notion of an internal energy, which –like the average rate in a system of particles, described by a Maxwell distribution– is a random variable. In the usual DR experiments, the distributions of f will tend to be not very asymmetrical, given the moderation of the conditions and the presumably high means of factors such as n , a and p , whose distributions, even when they are Weibull or log-normal, will be close to a normal distribution. Thus, and as the “bimodal” profile smoothes out as σ_f decreases (Fig. 3B), in practice it may often be hidden by experimental error. Nevertheless, it will probably show up in the case of complex effectors that can adopt many states conditioned by variables such as temperature or pH. The states of ionisation in lateral chains of protein molecules could be an example of this, and in fact, we will present experimental evidence, repeatedly obtained with nisin, that show a small wave at the beginning of the logistic profile, very similar to the wave in Fig. 3B.

3: Fits of the descriptive models to the results of the generative model.

The suitability of the models in Table 2 for describing the results of the generative model was verified in the hypotheses that, according to the preceding discussion, generate more differentiated DR profiles. Figs. 4 and 5 show such profiles and some representative fits, and Table 4 specifies their hypotheses, the RD_{50} directly derived from the generative model and the parametric estimations obtained with the equation in the same table. The conclusions are:

1: Apart from the expected lack of fitting (since they do not meet the conditions $K \leq 1$ and $R_0 = 0$) of the models in Table 1 in all cases, von Bertalanffy's equation (31) also fails in the case of P1 and P5 profiles, in spite of its previously mentioned apparent suitability.

2: P6 “bimodal” profiles provide a satisfactory fit for the sum of two equations in the same way, with different parameters. Thus, taking the Weibull model (which provides the best fit) as our example, we would have:

$$R = K \{1 - e^{[-(D/\theta)^\alpha]}\} + K' \{1 - e^{[-(D/\theta')^{\alpha'}]}\} \quad (32)$$

3: A general selection criteria based on the correlation coefficient between expected and observed values in all cases would provide the increasing order: log-logistic (LL) < Gompertz (G) < logistic (L) < Weibull (W), which, however, does not reflect the precision of the estimations of such an important parameter as m , which in the case of Gompertz's equation can be underestimated by 50%. A stricter analysis can be based on the fact that with 10,000 cells, the generative model

produces stable values (*i.e.* populational values, equivalent to excluding experimental error), and hence the residual distributions (expected values minus observed values) only describe cases of lack of fit that can be attributed to the inadequacy of the models.

Fig. 6 shows such residual distributions in two typical cases, and demonstrates (apart from the absolute values) its lack of a random nature along the D axis, as well as the fact that residues from different models “couple” or not, according to the DR profile being considered. This means that, in spite of an initial satisfactory fit of any of the descriptive models to all the profiles, none of them exactly translates the simulations of the generative model. If the realism of the latter is accepted, the conclusion is that none of the equations is a rigorous DR model, and that all of them, although good operative models, can produce biases in parametric estimations. Finally, if a normal random error that has been simulated with a realistic variance with an experimental perspective is added to the response, the variability of the estimations increases in every equation as well as between the different equations.

Under such conditions, the most acceptable model is simply the most robust, *i.e.*, the one whose estimations are most independent of the distributions supposed for the factors that determine the response. Due to its importance in the DR analysis, and its low sensitivity to experimental error, the parameter m is in this regard a good criterion. Its application leads to the order $G < L < LL \approx W$, whilst the introduction of a moderate experimental error increases the differences between them but does not modify their order. Thus, for example, 100 estimations of m in the profile P3, under a normal homoscedastic error with a standard deviation equivalent to 5% of the maximum response, produce variation coefficients of 7.6_(W), 8.0_(LL), 10.6_(L) and 15.7_(G) %. Everything indicates, therefore, that the most robust model is the Weibull model, and that Gompertz's can be excluded, as under any hypothesis does it improve on the other models. The main reason for not omitting the logistic equation will now be discussed.

4: Advantages of the logistic model, and several additional modifications.

The main advantage of the logistic equation is to be found in the meaning of its parameters, which represent concepts that are directly applicable to DR analysis. According to the results of the generative model, m can be understood as the inverse of the affinity between the effector and the receptor, a magnitude that decreases when the probability of interaction between them increases; μ is the set of factors that define cell susceptibility, which increases with the number of active receptors in each cell, and K represents the efficiency of the effector, taken as the accumulated total of the

affected cells. Thus, the logistic structure facilitates the introduction of response-modifying terms, to which a precise factual significance can also be attributed. In effect, if we abbreviate the equation (2), making only its parameters explicit:

$$R = \phi(K, m, \mu) \quad (33)$$

diverse modifications of the response due to interactions of the effector or receptors with other factors can be easily defined, providing that:

a) The effects on the response can be represented by means of terms such as:

$$B = 1 + bX \quad (34)$$

where X is the concentration of the modifying factor, and b a coefficient with the dimensions of a reciprocal concentration. Naturally, $b=0$ or $X=0 \Rightarrow B=1$.

b) B -type terms are introduced in (33) as factors or divisors of any parameter (thus denoted B_k , B_m , B_μ), with each case corresponding to a specific kind of interaction.

Thus, variations in the response attributable to a factor X can be typified according to the criteria in Table 5. The analysis presents a clear formal analogy with that of enzyme inhibition mechanisms (competitive, uncompetitive, acompetitive, by substrate), by means of the well known modifications of the Michaelis-Menten model. And such an analogy suggests that –as in inhibition by substrate– the concentration of the effector itself might also act here as a response-modifying factor, and hence the dose (D) would substitute the concentration of the modifier (X) in (34), which would then become:

$$A = 1 + aD \quad (35)$$

allowing six more cases similar to the cases in Table 5 to be defined, by replacing B with A .

These twelve situations are plausible, can be simulated in the generative model, and for some of them empirical evidence will be put forward. To continue with the formal discussion, it can be noted that there appears to be no doubt as to the interactions between two effectors described in Table 5: they can be accounted for by numerous chemical and biochemical mechanisms, and their empirical detection is only a matter of carrying out several DR series with one effector, in the

presence of an increasing level of a second. The analysis can be limited to comparisons similar to those in Fig. 7, or the bivariate character of the system can be assumed (see Fig. 9), if the parameters of the corresponding equation in Table 5 are calculated by any nonlinear fit method, in all the combinations of both factors that have been assayed. The problem becomes more complicated if the modifier is the effector itself, a hypothesis under which the analysis of the six plausible cases (Fig. 7) allows us to establish that:

1: The term A affects K : 1.1: If A_k multiplies K , when $D \rightarrow \infty$, $R \rightarrow \infty$. The mortality of all the population is attained without any possibility of defining an asymptotic value. 1.2: If A_k divides K , when $D \rightarrow \infty$, $R \rightarrow 0$. The mortality decreases starting from a maximum.

2: The term A affects m : 2.1: If A_m multiplies m , when $D \rightarrow \infty$, $R \rightarrow 0$ (as in 1.2). 2.2: If A_m divides m , when $D \rightarrow \infty$, $R \rightarrow K/2$. An asymptote is reached, but the DR function shows a profile analogue to the one in 1.2, even if the fit with the same model is unsatisfactory.

3: The term A affects μ : 3.1: If A_μ multiplies μ , when $D \rightarrow \infty$, $R \rightarrow K$. Without variation in the RD_{50} , the asymptote is reached at lower values of the dose. 3.2: If A_μ divides μ , when $D \rightarrow \infty$, $R \rightarrow K [1 - e^{-(\tilde{y}/b)} / 2(1 + e^{-(\tilde{y}/b)})]$. An asymptotic value is obtained that progressively decreases as a_μ increases (in fact, when $a_\mu \rightarrow \infty$, $R \rightarrow 0$).

5: Empirical evidences.

5.1: Self-inhibition and interactions between two effectors

The last six functions discussed describe, then, effectors with second order actions, classified as either self-stimulating or self-depressor with an effect on different parameters of the primary response. It is difficult to decide whether all of them correspond to actual facts; but, returning to the analogy with enzyme inhibition by substrate, in some cases at least it would be possible to propose explanatory mechanisms, such as the modification of the diffusional attributes of the medium by the effector, or the existence of different types of cell receptors with a different effect on mortality. Given the profiles of the resulting functions, the cases least likely to go unnoticed are those where self-inhibition of the efficiency or the affinity occurs, because they can create difficulties that are much more evident than the rest when an attempt is made to fit them to the usual equations. This is:

$$R = \phi(K/A, m, \mu) \quad (36)$$

$$R = \phi(K, mA, \mu) \quad (37)$$

In fact, in a previous study [26] focused on the bioassay of nisin and lactic acid against the bacteria *Leuconostoc mesenteroides* sp. *lysis*, we find that:

- a) The effect of nisin (Fig. 8A) could be satisfactorily described by a logistic equation, modified as shown in Table 2.
- b) The effect of lactic acid (Fig. 8B) could be fitted to an equation like (36).
- c) The total effect of lactic acid (L) and nisin (N) was accounted for, if we admit that L affected the parameters K and μ of the equation corresponding to the effect of N , and that N affected the parameter μ of the equation corresponding to the effect of L . In the shortened notation we have been using since (27), the description (Fig. 9) could be formulated, with ϕ_N and ϕ_L being logistic functions, as:

$$R_{NL} = \phi_N \left(\frac{K_N}{B_{k(L)}}, \frac{\mu_N}{B_{\mu(L)}}; m_N \right) + \phi_L \left(\frac{K_L}{A_k}, \frac{\mu_L}{B_{\mu(N)}}; m_L \right) \quad (38)$$

When we carried out this study we checked, on the other hand, that none of the remaining options here discussed provided satisfactory results, nor permitted the formulation of a combined model such as (38).

5.2: "Bimodal" profiles

The discussion in point 2.4 about this type of profiles was in part suggested by their persistent detection in the bioassay of bacteriocins against indicator microorganisms such as *Leuconostoc mesenteroides* and *Carnobacterium piscicola*. Although the anomaly was of little quantitative importance (Fig. 10 shows two representative examples) and could be treated as an experimental error without seriously affecting the RD_{50} , its systematic character seems to require some other explanation, perhaps similar to the one suggested in the above-mentioned section. As the examples in Fig. 10 correspond to impure extracts of bacteriocins, it could be assumed that their peculiar profiles derive from the purely additive effect of two effectors. However, analogue profiles are also obtained with pure nisin. Thus, the interpretation based upon the distribution of states of the effector would lead us to expect the effect to be sensitive to temperature, a sensitivity that was examined by

carrying out, in quadruplicate, three simultaneous assays at 25, 30 and 35° C, with increasing doses of pure nisin and in strictly equivalent conditions with regard to the remaining variables.

The results are shown in Fig. 11, and although again they are not very spectacular in quantitative terms, they confirm the tendency expected with the increase in temperature, for which we can find no explanation other than the above-mentioned hypothesis of the distribution of the states of the effector.

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Symbolic notations used

EQUATION	PARAMETER
Logistic	R, D : response and dose, respectively. K : maximum response. Dimensions R . μ : specific rate of response. Dimensions D^{-1} . m : dose producing semimaximum response RD_{50} . Dimensions D .
Modified logistic	R, D : response and dose, respectively. K : parameter to be empirically determined. Dimensions R . K' : maximum response. Dimensions R . m : parameter to be empirically determined. Dimensions D . m' : dose producing semimaximum response RD_{50} . Dimensions D . μ : parameter to be empirically determined. Dimensions D^{-1} .
Logarithmic logistic	$R, \ln D$: response and logarithm of the dose, respectively. K : parameter to be empirically determined. Dimensions R . K' : maximum response. Dimensions R . $\ln m$: parameter to be empirically determined. Dimensionless. $\ln m'$: logarithm of the dose producing semimaximum response RD_{50} . μ : parameter to be empirically determined. Dimensionless.
'Clinical'	$R, \ln D$: response and logarithm of the dose, respectively. K : maximum response. Dimensions R . a : parameter to be empirically determined. Dimensionless. b : " " " c : " " "
Modified 'clinical'	$R, \ln D$: response and logarithm of the dose, respectively. K : parameter to be empirically determined. Dimensionless. K' : maximum response. Dimensions R . a : parameter to be empirically determined. Dimensionless. b : " " " c : " " "
Weibull and modified Weibull	R, D : response and dose, respectively. K : maximum response. Dimensions R . Γ : gamma function. α, β, θ : Weibull's distribution parameters
Gompertz	R, D : response and dose, respectively. K : maximum response. Dimensions R . b : parameter to be empirically determined. Dimensionless. c : " " " . Dimensions D^{-1} .
Modified Gompertz	R, D : response and dose, respectively. K : parameter to be empirically determined. Dimensionless. K' : maximum response. Dimensions R . m' : dose producing semimaximum response RD_{50} . Dimensions D . b : parameter to be empirically determined. Dimensionless. c : " " " . Dimensions D^{-1} .
Terms of the generative model	n : number of potential receptors per cell. a : number of active receptors. D : dose, number of effector units (molecules). C : cell response. R : response. Y : total number of cells. S : survivor cells at a given dose. D_e : effective dose. h : factor affecting the receptor (inhibitor or excitor). f : factor affecting the effector (antagonist or enhancer).

TABLE 1: Five common formulations of dose-response relationships.

Equation	RD ₅₀ = m	Asymptote (R_{max} or R_{min}) and intercept (R_0 or S_0)
T1.1: Logistic $R = \frac{K}{1 + be^{-\mu D}}$	$m = \frac{\ln b}{\mu}$	$R_{max}=K$; $R_0 = \frac{K}{1+b}$
T1.2: “Clinic” $R = a + \frac{1-a}{1 + e^{b-c \ln D}}$	$m = e^{b/c} (1-2a)^{1/c}$	$R_{max}=1$; $R_{(\ln D=0)} = a + \frac{1-a}{1+e^b}$
T1.3: Fermi $S = \frac{1}{1 + e^{[(D-m)/a]}}$	m	$R_{min}=0$; $S_0 = \frac{1}{1+e^{-m/a}}$
T1.4: Weibull $R = 1 - e^{[-(D/\theta)^\alpha]}$	$m = \theta (\ln 2)^{1/\alpha}$	$R_{max}=1$; $R_0=0$
T1.5: Gompertz $R = Ke^{-e^{b-cD}}$	$m = \frac{b - \ln(\ln 2)}{c}$	$R_{max}=K$; $R_0 = Ke^{-e^b}$

TABLE 2: Models of Table 1, modified so that they meet the conditions $K \leq 1$ and $R_0=0$. K^* and m^* represent the maximum response and the dose for the semi-maximum response, respectively.

Equation	Parameters of interest in the DR analysis
Logistic $R = K \left(\frac{1}{1 + e^{[\mu(m-D)]}} - \frac{1}{1 + e^{\mu m}} \right)$	$K^* = K \left(\frac{e^{\mu m}}{1 + e^{\mu m}} \right)$; $m^* = \frac{1}{\mu} \ln(2 + e^{\mu m})$; μ
Logistic (lnD) $R = K \left(\frac{1}{1 + e^{[\mu(\ln m - \ln D)]}} - \frac{1}{1 + e^{\mu \ln m}} \right)$	$K^* = K \left(\frac{e^{\mu \ln m}}{1 + e^{\mu \ln m}} \right)$ $m^* = \text{EXP} \left[\frac{1}{\mu} \ln(2 + e^{\mu \ln m}) \right]$; μ^1
“Clinic” $R = (K - a) \left(\frac{1}{1 + e^{b-c \ln D}} - \frac{1}{1 + e^b} \right)$	$K^* = (K - a) \left(\frac{e^b}{1 + e^b} \right)$ $m^* = \text{EXP} \left\{ \frac{1}{c} \left[b - \ln \left(\frac{e^b}{e^b + 2} \right) \right] \right\}$; μ^1
Weibull $R = K \left\{ 1 - e^{[-(D/\theta)^\alpha]} \right\}$	$K^*=K$; $m^* = m = \theta (\ln 2)^{1/\alpha}$
Gompertz $R = K \left(e^{-e^{b-cD}} - e^{-e^b} \right)$	$K^* = K(1 - e^{-e^b})$ $m^* = \frac{1}{c} \left\{ b - \ln \left[\ln \left(\frac{2}{1 + e^{-e^b}} \right) \right] \right\}$

⁽¹⁾ : here μ lacks the significance of a response specific coefficient.

TABLE 3: Effects of means and variances of the distributions of a and p ($\forall n$) on the basic magnitudes of a DR model. K : maximum response, μ : specific rate of the increase in the response with the increase of the dose, m : dose for the semi-maximum response.

		K	μ	m
IF $a > p$	IF a increases	increases	decreases	increases
	IF p increases	decreases	increases	increases
	IF σ_a increases	decreases	decreases	decreases
	IF σ_p increases	decreases	decreases	decreases
IF $a < p$	IF a increases	increases	decreases	increases
	IF p increases	decreases	decreases	increases
	IF σ_a increases	increases	decreases	increases
	IF σ_p increases	increases	decreases	decreases

TABLE 4: Fit of the profiles P1 to P6 (see figure 5) to the four models indicated, according to the modified expressions in table 2. K : maximum response; RD_{50} and m : doses for a semimaximum response in the generative and descriptive models, respectively; r : correlation coefficient between expected and observed results. In the case of the “bimodal” profile (P6), the parameters corresponding to the second sumands of the equations, which are the most interesting in the DR analysis, are given.

		Logistic	Logistic (lnD)	Gompertz	Weibull	
<i>P1</i>	$\left\{ \begin{array}{l} n \sim N : (100;18) \\ a \sim N : (85;15) \\ p \sim N : (70;11) \\ f = 1 \end{array} \right\}$	K	,759	,764	,769	,752
		m	67,1	66,9	66,5	67,4
		μ	,168	-	-	-
		$r \times 10^4$	9999,42	9997,60	9991,57	9998,87
RD₅₀ = 67,0						
<i>P2</i>	$\left\{ \begin{array}{l} n \sim N : (100;18) \\ a \sim N : (85;15) \\ p \sim N : (70;11) \\ f \sim N : (1;0.5) \end{array} \right\}$	K	,613	,658	,639	,617
		m	61,9	62,9	62,0	62,1
		μ	,069	-	-	-
		$r \times 10^4$	9969,44	9993,61	9993,68	9974,96
RD₅₀ = 65,3						
<i>P3</i>	$\left\{ \begin{array}{l} n \sim N : (100;18) \\ a \sim N : (85;15) \\ p \sim N : (70;11) \\ f \sim N : (1;5) \end{array} \right\}$	K	,397	,409	,398	,397
		m	17,4	17,3	17,4	17,3
		μ	,072	-	-	-
		$r \times 10^4$	9928,63	9976,77	9935,29	9938,13
RD₅₀ = 17,3						
<i>P4</i>	$\left\{ \begin{array}{l} n \sim W : (40;18) \\ a \sim W : (25;15) \\ p \sim W : (10;8) \\ f = 1 \end{array} \right\}$	K	,802	,809	,802	,804
		m	6,7	6,4	6,6	6,5
		μ	,215	-	-	-
		$r \times 10^4$	9999,24	9984,97	9999,14	9999,77
RD₅₀ = 6,6						
<i>P5</i>	$\left\{ \begin{array}{l} n \sim L : (40;18) \\ a \sim L : (25;15) \\ p \sim L : (10;8) \\ f = 1 \end{array} \right\}$	K	,872	,874	,872	,872
		m	7,1	6,9	7,1	7,1
		μ	,277	-	-	-
		$r \times 10^4$	9999,35	9998,46	9999,84	9998,88
RD₅₀ = 6,9						
<i>P6</i>	$\left\{ \begin{array}{l} n \sim N : (100;18) \\ a \sim W : (85;15) \\ p \sim N : (70;11) \\ f \sim N : (1;0.9) \end{array} \right\}$	K	,384	,385	,386	,382
		m	67,4	67,9	68,4	67,1
		μ	,167	-	-	-
		$r \times 10^4$	9999,58	9999,00	9997,67	9999,71
RD₅₀ = 62,7						

TABLE 5: Systematics of the variations in response due to a modifier with specific effects on the parameters of the logistic model. The subscripts of the term B indicate the logistic parameter affected by the action of the modifier.

EFFECT ON	the parameter decreases	the parameter increases
K : effector efficiency (enhancers or antagonists)	$R = \phi(K/B_k; m; \mu)$	$R = \phi(KB_k; m; \mu)$
μ : susceptibility of the receptor (exciters or inhibitors)	$R = \phi(K; m; \mu/B_\mu)$	$R = \phi(K; m; \mu B_\mu)$
m : affinity receptor-effector	$R = \phi(K; m/B_m; \mu)$	$R = \phi(K; mB_m; \mu)$

Murado et al., Figure 1

Murado et al., Figure 2

Murado et al., Figure 3

Murado et al., Figure 4

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