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Statistical models for biphasic dose-response relationships (hormesis) in toxicological studies

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Abstract

The dose-response phenomenon characterized by low dose stimulation and high dose toxicity has been reawakened after a long period of marginalization. This phenomenon termed hormesis is induced by biological, physical and chemical agents and occurs in all groups of living things including whole plants and animals, microorganisms, cells and tissues. Hormesis has attracted increased interest among toxicologists from diverse disciplines, resulting to emergence of new scientific tools for its study. Statistical models have been developed and used to characterize hormesis dose-response relationships. Some of these models include the classical Brain-Cousens model, the Cedergreen-Ritz-Streibig model and their reparameterizations. Other hormesis models are the bilogistic models, their modifications or extensions and the hormesis models used in allelopathy such as An-Johnson-Lovett model. These models are used to describe either U-shaped or inverted U-shaped dose-response relationships and to compute hormesis quantities. This review explored the applications of these models in toxicological studies with emphasis to their strengths and weaknesses.

Keywords: Hormesis, dose-response curves, toxicology, mathematical models, effective doses.

INTRODUCTION

Toxicology is a branch of science concerned with the study of the toxicity of biological, chemical and physical agents to living organisms. The extent of damage is established through bioassay involving whole organism (e.g animals, plants, microorganisms) or substructure of the organism such as cells, tissues or organs. One of the fundamental principles of toxicology is that there is a relationship between a toxic reaction (response) and the amount of the toxicant (dose). The study of dose-response relationships cuts across many science disciplines such as biology, medicine, pharmacology, chemistry, etc. Dose-response relationships are generally dependent on the exposure time and route. Due to complexity of biological systems, different dose-response relationship is possible for a substance after a different exposure time or route, leading to different conclusions on the effect of the toxicant/stressor under consideration (Beckon *et al*., 2008). A number of responses can be studied, often at different organizational levels (e.g population, whole animal or plant, cells, tissue). In microbiological studies for instance, responses such as cell multiplication, bioluminescence, enzyme activity and synthesis, nutrient uptake, CO_2 evolution, O_2 consumption, metabolite production etc could be used to measure the effect of a substance on microbial population. The dose-response relationship is usually shown in a dose-response curve, a 2-dimensional plot of dose on the *x*-axis and response on *y*-axis. The dose-response curve normally takes the form of a sigmoid curve (Fig.1), either a monotonically decreasing or increasing curve. The concentration at which toxicity appears is referred to as the threshold dose level. It is the concentration above which toxicity sets in.

The sigmoidal dose-response curve can be fitted with a number of monophasic dose-response models such as logistic, Weibull, Logit, Probit, Gormpertz models and their modifications (see Altenburger *et al*., 2000; SYSTAT Software Inc., 2002, 2006). These models are usually used to estimate the EC_{50} (the half maximal effective concentration or

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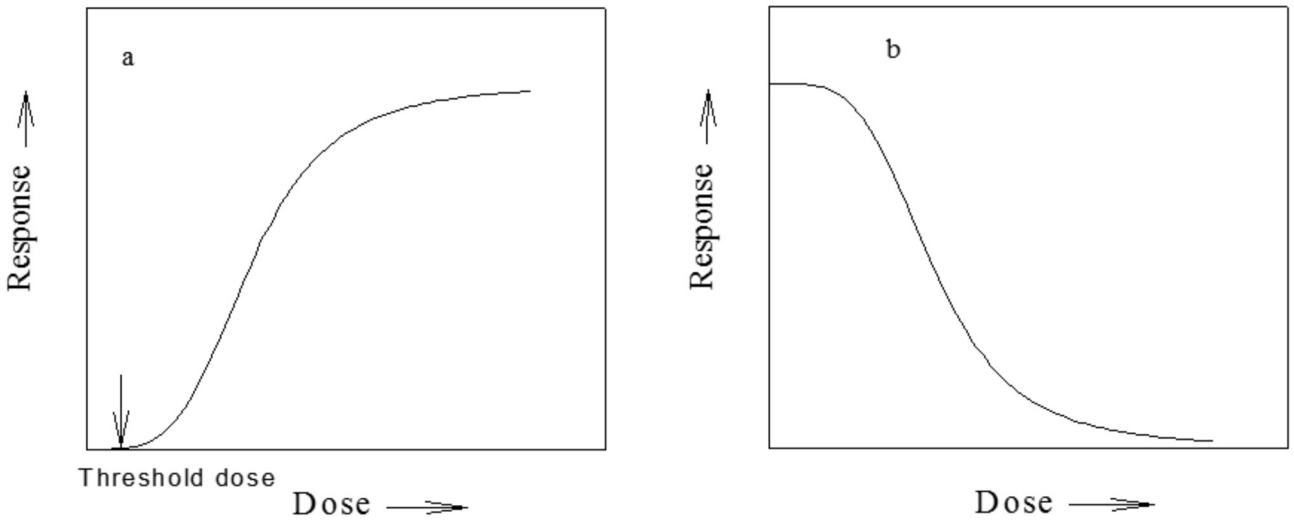

Fig.1: Typical examples of monophasic dose-response curves: (a) monotonically increasing curve, an ascending form of dose-response curve showing a positive measure of effect which increased with increase in the dose of the effecter (positive effect), (b) monotonically decreasing curve, a descending form of dose-response curve showing positive measure of effect which decreases with dose (negative effect).

the concentration that inhibited the response by 50%) of the substance under test, where EC_{50} is defined as the inflection point of the curve. However, it is important to note that some dose-response curves are not monotonous, but showing initial stimulation of response at low dose and subsequent inhibition of the response at high dose. This biphasic dose-response relationship has gained recognition as a generalizable phenomenon and appeared to be the rule rather than exception (Calabrese *et al*., 1999; Calabrese & Baldwin 2001; Stepnowski *et al*., 2004; Calabrese & Blain 2005; Cedergreen *et al*., 2005). This phenomenon termed 'hormesis' has been observed with all groups of living things for a wide range of endpoints. Hormesis is induced by physical and chemical stressors including phenolic compounds (Boyd *et al*., 1997; Sinclair *et al*. 1999; Okolo *et al*. 2007; Zaki *et al*., 2008; Nweke & Okpokwasili, 2010 a, b; Nweke *et al*., 2014, 2015), perfluorinated carboxylic acids (Mulkiewicz *et al*., 2007), mycotoxins (Li *et al*., 2014; Wang *et al*., 2014), bacteriocins (Murado & Vázquez, 2010), antibiotics (Welch *et al*., 1946; Randall *et al*., 1947, Linares *et al*., 2006; Migliore *et al*., 2010, 2013), herbicides (Cedergreen 2008a,b; Cedergreen *et al*., 2009; Belz & Cedergreen, 2010; Cedergreen & Olesen, 2010; Belz *et al*., 2011; Belz & Leberle, 2012; Nweke *et al*., 2016), Wastewater (Hoffmann & Christofi, 2001; Nwanyanwu & Abu 2010), heavy metals (Christofi *et al*., 2002; Rodea-Palomares *et al*., 2009; Shen *et al*., 2009) and ionic liquids (Cho *et al*., 2007; 2008, Wang *et al*., 2011) either as individual or as mixtures. Hormesis was commonly observed in the toxicity test on luminescent bacteria (Christofi *et al*., 2002; Brack *et al*., 2003; Fulladosa *et al*., 2005; Wang *et al*., 2011; Deng *et al*., 2012; Zhang *et al*., 2013). Calabrese and Blain (2009) reviewed the hormetic effects of inorganic and organic chemicals on plants.

Following its recognition, there has been increased interest in statistical models that describe hormesis. A number of such models have been proposed and are actually in use for statistical modeling of biphasic dose-response relationships. This review aimed at exploring the existing hormesis models as applied in the mathematical modeling of biphasic dose-response curves. The strengths and weaknesses of these models in terms of their ability to estimate hormetic quantities and other biologically-relevant parameters in toxicological studies were discussed.

Types of hormetic dose-response relationships

Three main types of biphasic dose-response relationships are known in toxicological sciences. First, the dose-response relationship characterized by increase in positive effect (desirable effect) at low doses of toxic agent and decrease in the effect at high doses. Example of this type of hormesis is the stimulation of activity of an enzyme at low doses and the inhibition of enzyme activity at high doses of a toxic agent, producing an inverted U-shaped dose-response curve. Second, the dose-response relationship characterized by low dose decrease and high dose increase in a given negative effect (undesirable effect) at high doses of a substance, producing a U-shaped dose-response curve. This type of relationship is usually observed in pharmacology. Third, a dose-response relationship produced by essential nutrients in which response (e.g. growth of an organism) approaches zero at high dose of the effecter and the effecter is required for the expression of the effect such that the response also approaches zero at low dose of the effecter. These dose-response relationships are illustrated in Fig. 2.

The relative response

In toxicological studies, the dose-response data are usually expressed in relative terms. The data are either expressed as percent of the control (response in the absence of toxicant)

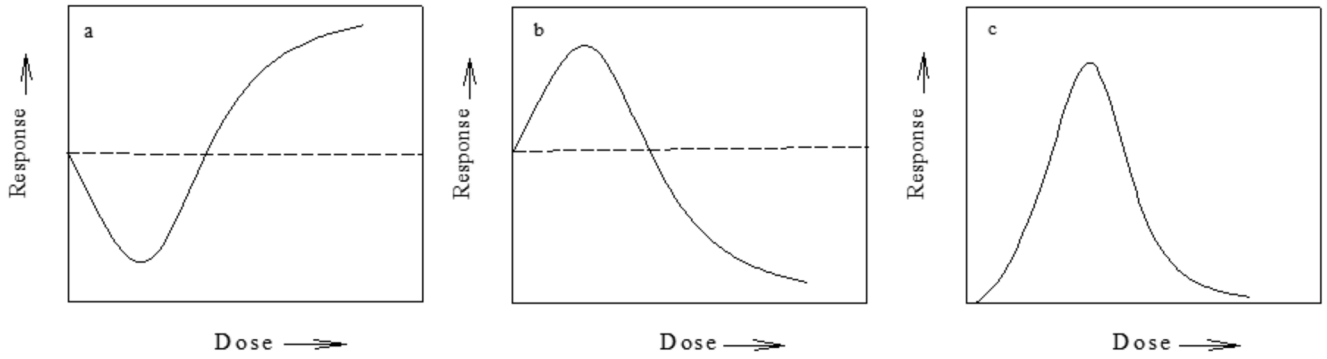

Fig 2: Biphasic dose-response curves: (a) a U-shaped (also trough or J-shaped) dose-response curve indicating low dose decrease and high dose increase of negative effect (b) an inverted U-shaped dose-response curve indicating low-dose stimulatory and high-dose inhibitory responses (c) a dose-response curve typical of such produced by essential nutrients in which response approaches zero at high dose of effecter and the effecter is required for the expression of the effect such that the response also approaches zero at low dose of the effecter. Note that if the response is normalized relative to the control such that the response becomes percent inhibition of response, then type 'a' curve can depict stimulation of response at low doses (with negative values of percent inhibition) and inhibition of response at high doses.

or as percent inhibition of response relative to control as shown in equations 1 and 2 respectively. In the former transformation, the data runs from 100% of control (y at $x = 0$) to values above 100% (indicating hormesis) before decreasing to zero as the concentration of the toxicant increases. When expressed as percent inhibition, dose-response data runs from 0% (y at $x = 0$) to values below 0% and then increases to 100% as the concentration increases (see Fig. 3). An inverted U-shaped dose-response relationship can be converted to U-shaped dose-response relationship when data are expressed as percent inhibition of response. The relative response data can be expressed as ratio by dividing the expressions by 100. This approach has been generally used in the analyses of data generated in toxicological studies.

$$\text{Relative response}\left(\%\ \text{of control}\right) = \frac{R_T}{R_C} \times 100 \quad (1)$$

$$\text{Relative response}\left(\%\,\text{Inhibition}\right) = \frac{R_C - R_T}{R_C} \times 100 \quad (2)$$

In equations 1 and 2 is the response of the control and R_T is the response in the tests (at different concentrations of the toxicant).

The Brain and Cousens hormesis model

Among the monotonic dose-response models, the log-logistic model (eq. 3) was more frequently used in dose-response studies. Several studies of dose-response relationships following exposure to toxic chemical substances use the log-logistic function (Field *et al*., 2002; Abondanzi *et al*., 2003; Nweke & Okpokwasili, 2011a, b, 2012; Azgm & Göksu, 2015). The log-logistic function expresses dose-response as a monotonically increasing or decreasing sigmoidal curve that is symmetric about its point of inflection and assuming approximately normally distributed data (Schabenberger *et al*., 1999; Cedergreen *et al*., 2005).

$$y = c + \frac{d-c}{1+\exp\left\{b\left[\ln(x)-\ln(e)\right]\right\}} = c + \frac{d-c}{1+\left(\frac{x}{e}\right)^b} \quad (3)$$

In the logistic model, y is the response, x is the dose, d represents the response of the untreated control (y at $x = 0$) and c is the response at infinite dose, e is the dose at which the value of d - c is reduced by 50% (ED_{50}) and b is the relative slope around ED_{50}.

The log-logistic model and other monotone functions produce curves that are strictly decreasing from a maximum response at zero dose (control) to lower limit at infinite dose or are strictly increasing from zero response (also control) to maximum response at infinite dose depending on whether the response or relative response (effect) is being analyzed (Cedergreen *et al*., 2005) [Fig.1]. Thus, they cannot be used to describe dose-responses with hormesis. There was need for mathematical model to describe the initial response stimulation in the analysis of dose-response relationships.

One of the earliest if not the first attempt to mathematical modeling of hormesis was made by Brain and Cousens (1989). They extended the original four-parameter logistic model (eq.3) by introducing the term fx to allow for hormesis as shown in equation 4.

$$y = c + \frac{d-c+fx}{1+\exp\left\{b\left[\ln(x)-\ln(e)\right]\right\}} = c + \frac{d-c+fx}{1+\left(\frac{x}{e}\right)^b} \quad (4)$$

In eq. 4, the parameters d and c are as defined in eq. 3. However, the parameters e and b lost their interpretations as the ED_{50} and relative slope at ED_{50} respectively and thus has no straight forward biological meaning (Schabenberger *et al*., 1999; Cedergreen *et al*., 2005). The parameter f denotes the rate of stimulation of the response at low dose. If $f = 0$, eq. 2 reduces to the four-parameter logistic model. Thus, $f > 0$ is a necessary condition for the presence of hormesis. The model can be used to describe inverted U-shaped and U-shaped curves typical of pharmaceuticals (Calabrese & Baldwin, 2001).

If the data is transformed relative to control as percentages (0 to100%) or simple ratio (0 to 1), the dose-response curve can be converted to U-shaped curve by simply subtracting

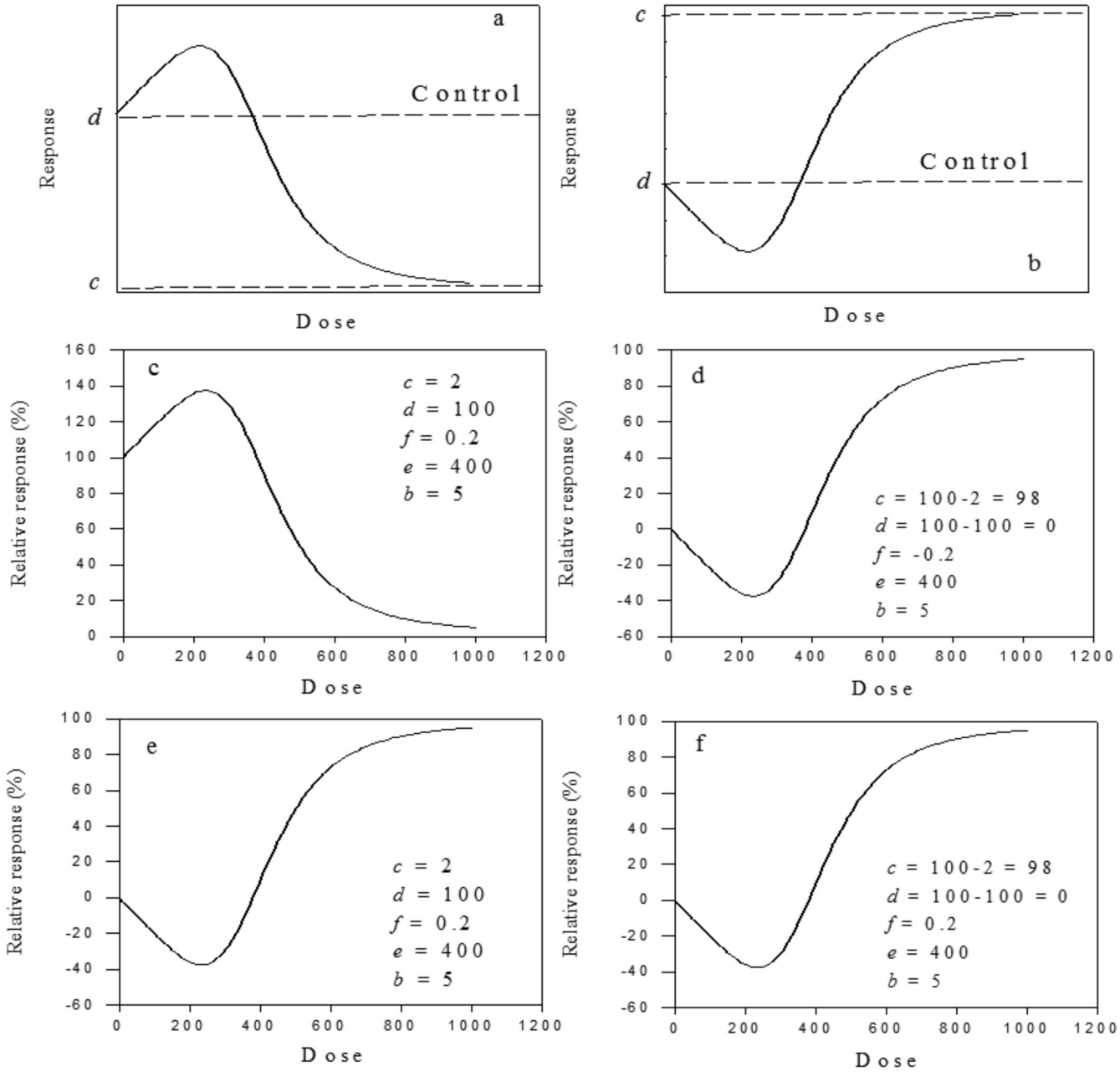

Fig. 3: Dose-response relationships generated from Brain-Cousens model: (a) an inverted U-shaped curve indicating parameters *c* and *d*, (b) U-shaped curve indicating parameters *c* and *d*, (c) an inverted U-shaped curve plotted with arbitrary values of *c*, *d*, *f*, *e* and *b* according to eq. 4 (d) a U-shaped curve plotted with arbitrary values of *c*, *d*, *f*, *e* and *b* according to eq. 2; notice negative value of *f* (e) a U-shaped curve plotted with arbitrary values of *c*, *d*, *f*, *e* and *b* according to eq. 5 (f) U-shaped curve plotted with arbitrary values of *c*, *d*, *f*, *e* and *b* according to eq. 7. Notice the relationship between the values of *c*, *d*, *f*, *e* and *b* in curves c, d, e and f.

the function from 100 or 1 as shown in equations 5 and 6 respectively.

$$y = 100 - \left[c + \frac{d - c + fx}{1 + \exp\left\{b\left[\ln(x) - \ln(e)\right]\right\}} \right] \quad (5)$$

$$y = 1 - \left[c + \frac{d - c + fx}{1 + \exp\left\{b\left[\ln(x) - \ln(e)\right]\right\}} \right] \quad (6)$$

This approach is generally applied in dose-response curve analysis with any dose response model. In this case, the values of the parameter estimates remain unchanged. It is important to note that when the data is normalized to ratio or percent changes relative to control value to give inverted U-shaped or U-shaped curve, Eq. 4 can be used to describe both the inverse U-shaped and U-shaped curves. However, in U-shaped curve, the hormesis parameter for hormesis (*f*) has a negative value ($f < 0$ and with changes in the value of parameters *c* and *d*) while in inverted U-shaped curve, the hormesis parameter has a positive value ($f > 0$) (Fig.3). Note that this is different from

the valley obtained by setting $f < 0$ for an inverse U-shaped curve as described by Cedergreen *et al*. (2005). The values of parameters *c* and *d* would change (not in interpretation but in values) while the values of *e* and *d* remain unchanged (see Fig. 3). The effect of this conversion is that the value of *c* in U-shaped curve becomes 100-*c*' or 1-*c*' and the value of *d* in the U-shaped curve becomes 100- *d* ' or 1- *d* ' (depending on whether the dose-response data was normalized to run between 0 and 100% or 0 and 1 respectively), where *c*' and *d* ' are values of *c* and *d* respectively in inverted U-shaped curve. Thus, in such cases, the U-shaped curves can also be described as:

$$y = c - \frac{c - d + fx}{1 + \exp\left\{b\left[\ln(x) - \ln(e)\right]\right\}} = c - \frac{c - d + fx}{1 + \left(\frac{x}{e}\right)^{b}} \qquad (7)$$

If eq. 5 is used to describe the U-shaped curve, the parameters *c*, *d*, *f*, *e* and *b* will retain their values as in eq. 4 (Fig. 3). Eq. 7 can generally be used to describe U-shaped hormetic dose-response curves.

Applications of Brain and Cousens model

Since its introduction, the Brain-Cousens model has been used to describe biphasic dose-response relationships involving wide range of chemical and physical agents. Velini *et al*. (2008) used the model to describe herbicide hormesis. Belz and Piepho (2012) compared Brain-Cousens model with its modification proposed by Cedergreen *et al*. (2005). They found Brain-Cousens model more suitable than the Cedergreen–Ritz–Streibig model for some dose-response data and that both models were equally suitable for some dose-response data.

Limitations of Brain and Cousens model

Although Brain-Cousens model has been generally used for analysis of hormetic dose-response relationships in many science fields of study, it is beset with drawbacks and was found inappropriate to some dose-response data. First, the value of *b* in eq. 4 is restricted to values greater than 1. At values of *b* smaller than 1, the model does not yield any dose-response curve (Cedergreen *et al*., 2005). Thus, fitting data with greatly sloping curves can especially be problematic. Second, Brain-Cousens model can cause problems when fitting data exhibiting a broad hormetic range and early increase in response at lower doses (Belz & Piepho, 2012). Third, in Brain-Cousens model, the portion of the model representing the effects at low doses is linear. The first derivative of the linear function is constant. Thus, Brain-Cousens model are founded on the assumption that the distribution of sensitivity thresholds for the low-dose effect of a hormetic substance is constant with respect to dose. Such a straight horizontal distribution of sensitivity thresholds seems generally unlikely (Beckon *et al*., 2008). Given that the switching function *fx* describing hormesis is linear with slope of *f* and intercept at *d* and increasing from *d*, the model will not be able to describe the initial 'no effect' at lower doses before the initial stimulation of effect at low dose or the pre-hormesis toxicity at low dose (Beckon *et al*., 2008; Belz & Piepho, 2012). Fourth, the Brain-Cousens model has no explicit formula for the ED_{50}. Thus, numerical approaches must be applied to reparameterize the model for estimation of ED_{50} and other effective doses (ED_x). According to Cedergreen *et al*. (2005), such reparameterization has several drawbacks. It requires some skill in Mathematics to be able to perform the mathematical manipulations to obtain the reparameterizations. In addition, fitting the reparameterized models require finding suitable initial estimates of the parameters to ensure convergence, which could be tedious.

Reparameterizations of Brain and Cousens model

The first attempt to reparameterization of Brain-Cousens model to estimate ED_{50} and its confidence limit was made by van Ewijk and Hoekstra (1993) by using a version of Brain and Cousens model and setting *c* at zero in eq. 4. By mathematical manipulation of the model, van Ewijk and Hoekstra (1993) derived a model (eq. 8) which has ED_{50} as one of the parameters. The model of van Ewijk and Hoekstra has the advantage of estimating ED_{50} and its confidence interval directly by non linear regression.

$$y = \frac{d(1 + fx)}{1 + (2fED_{50} + 1)\left(\frac{x}{ED_{50}}\right)^{b}} \qquad (8)$$

In eq. 8 (using van Ewijk and Hoekstra notation), *d* represents the response *y* at $x = 0$, *f* is the hormesis parameter (if $f > 0$, the curve shows an increase for low doses), *b* loses its simple interpretation.

After the publication of van Ewijk and Hoekstra (1993) article, the van Ewijk-Hoekstra model became a reference model for estimating ED_{50} and its confidence limit where subtoxic stimulation occurred. The van Ewijk-Hoekstra model has been used to analyze dose-response curves in many toxicological studies (eg. Folker-Hansen *et al*., 1996; Fairchild *et al*., 1998; Muyssen & Janssen, 2001; Groenendijk *et al*., 2002; Belgers *et al*., 2007; Cho *et al*., 2007; Mulkiewicz *et al*., 2007, De Silva & van Gestel, 2009; Castro-Ferreira *et al*., 2012).

Furthermore, Schabenberger *et al*. (1999) reparameterized the Brain-Cousens model to obtain estimates of arbitrary effective doses (ED_K), the dose at which the maximum stimulating effect occurred (M) and the limiting dose of stimulation (LDS) representing the dose at which the effect of hormesis has vanished (eqs. 9 – 12). The reparameterizations of Schabenberger and co-workers are shown in Table 1 and the parameters of the models are illustrated in Fig.4. Details of the mathematical manipulations are described in Schabenbeger *et al*. (1999) and its notations have been simplified in the outline of Belz and Piepho (2012). In the models of Schabenberger *et al*. (1999), the parameters *b*, *c*, *d* and *f* are as described in eq. 4, *k* is the percentage decrease in the term $d - c$.

Table 1: Reparameterizations of Brain-Cousens model (eq. 4) for estimation of ED_k, M and LDS (Schabenberger *et al*., 1999).

Parameterization for estimating ED_k

$$y = c + \frac{d - c + fx}{1 + \left[\frac{k}{100-k} + \left(\frac{100}{100-k}\right)\frac{fED_k}{d-c}\right]\exp\left[b.\ln\left(\frac{x}{ED_k}\right)\right]} \quad (9)$$

Parameterization for estimating ED_{50} (k=50)

$$y = c + \frac{d - c + fx}{1 + \left[1 + \frac{2fED_{50}}{d-c}\right]\exp\left[b.\ln\left(\frac{x}{ED_{50}}\right)\right]} \quad (10)$$

Parameterization for estimating LDS (k=0)

$$y = c + \frac{d - c + fx}{1 + \left(\frac{fLDS}{d-c}\right)\exp\left[b.\ln\left(\frac{x}{LDS}\right)\right]} \quad (11)$$

Parameterization for estimating M

$$y = c + \frac{d - c + fx}{1 + \left(\frac{Mf}{(d-c)b - Mf(1-b)}\right)\exp\left[b.\ln\left(\frac{x}{M}\right)\right]} \quad (12)$$

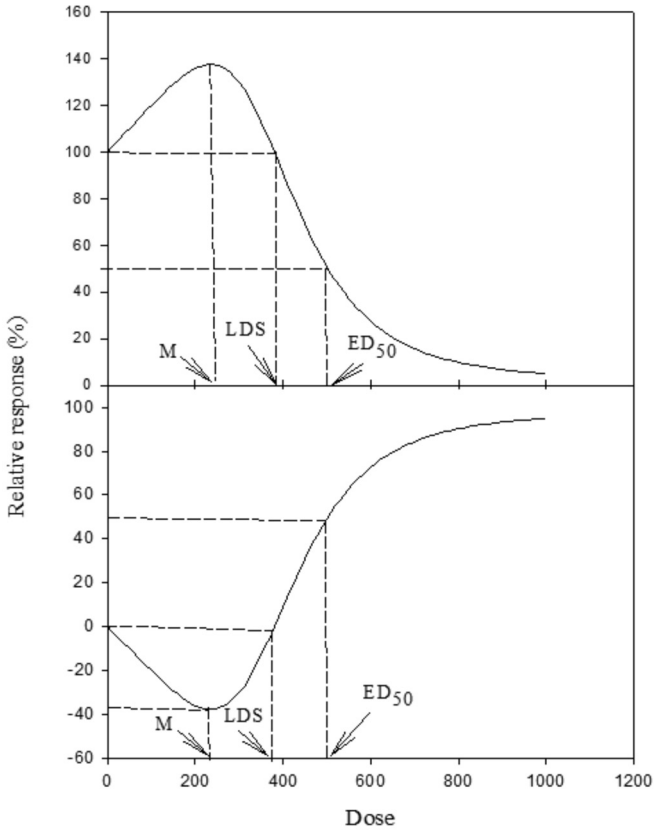

Fig. 4. U-shaped and inverted U-shaped dose-response relationships plotted from Brain-Cousens model to show the hormesis quantities M, LDS and ED_{50}

These reparameterizations of the Brain-Cousens model have been used in toxicological studies. For instance Nweke *et al*. (2014; 2015) used it to estimate the effective doses of binary mixtures of formulated glyphosate and phenols against dehydrogenase activity of *Rhizobium* species. Belz *et al*. (2008) used the reparameterized models to estimate effective doses and hormesis quantities for predicting hormesis produced by mixtures of pollutants, herbicides or allelochemicals. Hormetic effects of antibiotic mixtures have also been predicted using the reparameterized model (Zou *et al*., 2013). The model was also used to study plant hormesis in response to herbicide application (Zelaya & Owen, 2005; Belz & Cedergreen, 2010). More recently, Nweke *et al*. (2016) used the model to describe herbicide hormesis in microbial community of river water.

MODIFICATIONS OF BRAIN AND COUSENS MODEL

Cedergreen-Ritz-Streibig model

Consequent upon the inadequacies of Brain-Cousens model as observed by Cedergreen and her coworkers, they modified the model by replacing the term fx in eq. 4 with $f\exp(-1/x^{\alpha})$ to introduce a six-parameter version of modified Brain-Cousens model (Cedergreen *et al*., 2005). The model function for inverted U-shaped hormetic pattern is eq. 13.

$$y = c + \frac{d - c + f\exp\left(-\frac{1}{x^{\alpha}}\right)}{1 + \exp\left\{b\left[\ln(x) - \ln(e)\right]\right\}}$$

$$= c + \frac{d - c + f\exp\left(-\frac{1}{x^{\alpha}}\right)}{1 + \left(\frac{x}{e}\right)^{b}} \quad (13)$$

The U-shaped hormetic pattern of Cedergreen–Ritz–Streibig model is shown in eq. 14 (Drage *et al*., 2012).

$$y = c + d - \frac{d - c + f\exp\left(-\frac{1}{x^{\alpha}}\right)}{1 + \exp\left\{b\left[\ln(x) - \ln(e)\right]\right\}}$$

$$= c + d - \frac{d - c + f\exp\left(-\frac{1}{x^{\alpha}}\right)}{1 + \left(\frac{x}{e}\right)^{b}} \quad (14)$$

Where f is the hormesis parameter ($f > 0$ as a necessary condition for hormesis), parameters c and are as defined in eq. 4, while parameters α, b and e has no straightforward biological interpretation. The dose-response relationships simulated from these models are shown in Fig. 5. The

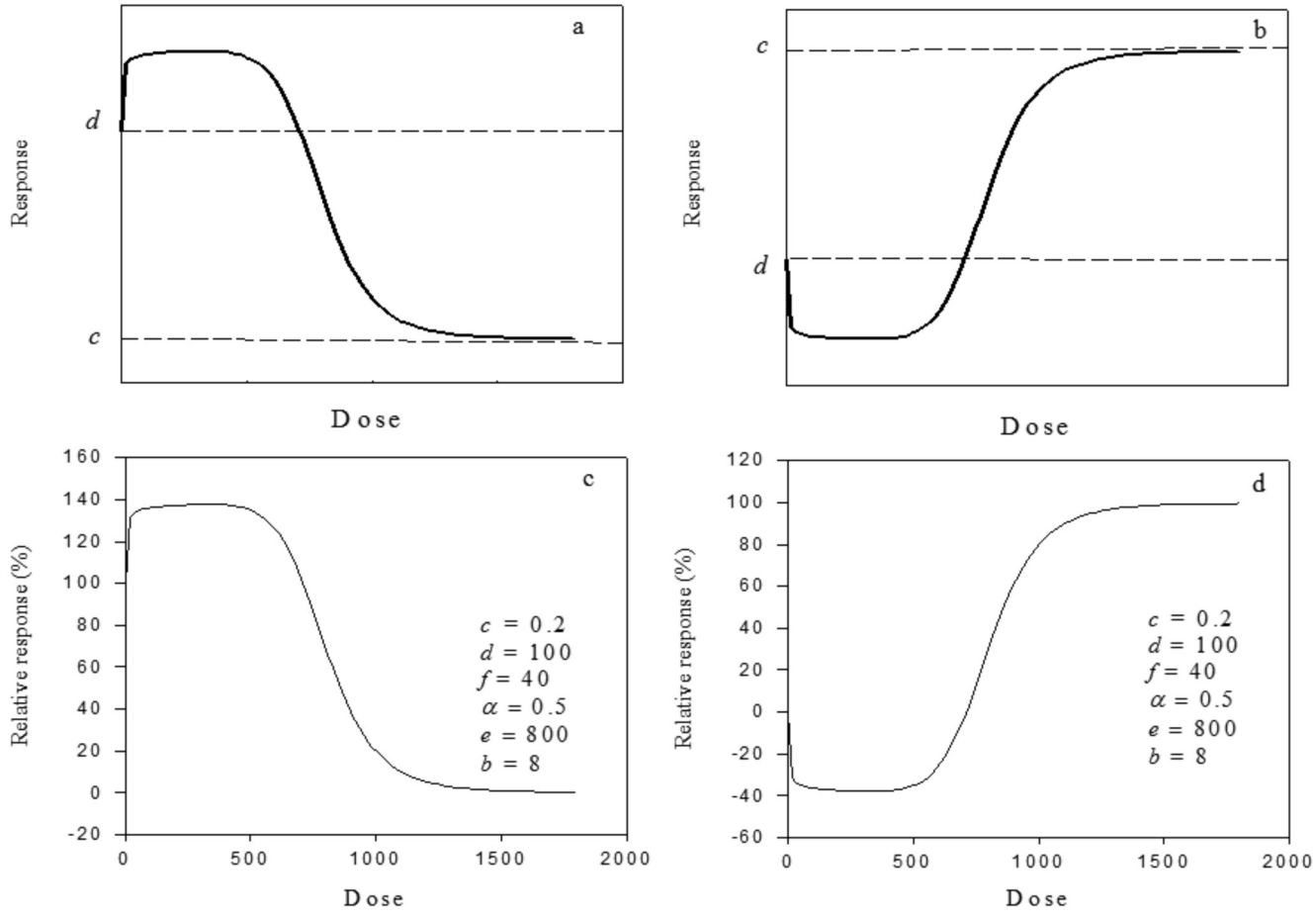

Fig. 5: Dose-response relationships generated from Cedergreen–Ritz–Streibig model: (a) an inverted U-shaped curve indicating parameters *c* and *d*, (b) U-shaped curve indicating parameters *c* and *d*, (c) an inverted U-shaped curve plotted with arbitrary values of *c*, *d*, *f*, *e* and *b* according to eq. 13 (d) a U-shaped curve plotted with arbitrary values of *c*, *d*, *f*, *e* and *b* according to eq. 14. Notice the unchanged values of *c*, *d*, *f*, *e* and *b*, the early hormetic response at low doses and the broad hormetic dose range.

Cedergreen–Ritz–Streibig model can be converted to U-shaped form as was described for Brain-Cousens model (see eq. 7).

The new model could describe curves displaying hormesis and test for the significance of the hormetic effects and is more robust both in terms of variation in data and in terms of describing both very large (of an almost 100% increase) and relatively small hormetic effect (of a 10% increase) when compared with Brain-Cousens model (Cedergreen *et al*., 2005). In addition, the model could be used to calculate the maximal hormetic response and any percentage effect dose or concentration (ED_k) and its associated standard errors from the decreasing part of the curve (Cedergreen *et al*., 2005). According to Cedergreen *et al*. (2005), the new model could be used to model the actual control values without the usual overestimation that occurs when using strictly decreasing model. Furthermore, another advantage of the Cedergreen-Ritz-Streibig model over Brain-Cousens model is that Cedergreen-Ritz-Streibig model better described data sets that were characterized by early increase in responses at low doses and a broad hormetic dose range (Belz & Piepho, 2012). The Cedergreen-Ritz-Streibig model was superior to Brain-Cousens model in terms of the graphical agreement between observed and fitted values (Belz & Piepho, 2012). Similar observation was made by Zhu *et al*. (2013), attributing the improved flexibility to the introduction of the parameter *α*. The new model was found to outperform the Brain-Cousens model in describing the hormetic data sets evaluated in Cedergreen *et al*. (2005). The Brain-Cousens and Cedergreen-Ritz-Streibig models have been frequently used in biology and have considerably helped hormesis phenomenon to earn recognition. Since its introduction, the modified model has been successfully applied in plant hormesis studies (Cedergreen *et al*., 2007; Cedergreen *et al*., 2009; Cedergreen & Olesen 2010; Belz & Leberle, 2012).

Limitations of Cedergreen Cedergreen-Ritz-Streibig model

Although the new model has found wide applications in toxicological studies, it has got its own limitations. One of the limitations of the model is that the parameter *α*, which determines the rate of increase in the hormesis zone, has to be fixed because there are rarely enough data available to determine the rate of increase statistically (Cedergreen *et al*., 2005). However, this may not be a problem if enough data at hormetic zone are available as was shown by Zhu *et al*. (2013) as well as Belz and Piepho (2012). Another

drawback of the model is that the effective doses (ED_k), the dose of maximum stimulation (*M*) and the limiting dose for stimulation (*LDS*) could not be determined explicitly from the original model. Although, Cedergreen *et al*. (2005) discouraged reparameterizations to introduce these quantities as a parameter of the model, and applied delta method and statistical software 'R' as a robust and feasible approach to estimate ED_k values with statistical properties, they could not estimate *LDS*. In addition, the maximum dose for hormesis (*M*) was obtained without standard errors and confidence intervals. Due to this limitation, ED_1 (dose causing 1% decrease in response) has been used to characterize the transition from stimulation to inhibition in studies where this model was applied (for instance in Cedergreen *et al*., 2005). The reports of Belz and Piepho (2012) indicated that the Cedergreen-Ritz-Streibig model is not infallible as it could not adequately describe some dose-response data especially those exhibiting steep curves in the inhibitory dose zone. In the study of Zhu *et al*. (2013) with five sets of biphasic dose-response relationships from three different experimental systems, the Cedergreen–Ritz–Streibig model was not the best among five hormesis models evaluated. In the study of Beckon *et al*. (2008), the Cedergreen-Ritz-Streibig model was observed to be scale-dependent, fitting the dose-response data of the effect of histamine on phagocytosis fairly well only after the data were transformed by multiplying the dose-axis by a very large factor.

Reparameterizations of Cedergreen-Ritz-Streibig model

Cedergreen *et al*. (2005) discouraged reparameterization and recommended the use of delta method and software R, with add-on package *drc* for analysis of hormetic dose-response data. However, this approach only allowed the estimation of ED_k doses with statistical properties and to extract *M* without statistical properties. Therefore, applications of their model are limited to situations where *M* estimates are sufficient without confidence limits and where *LDS* estimations in the form of ED_1 are adequate (Belz & Piepho, 2012). This approach was found applicable in most studies involving hormesis. However, it was not applicable in the study of Belz *et al*. (2008) where the *LDS* was used to predict hormesis in joint action analysis of pollutant toxicities. Furthermore, the approach as recommended by Cedergreen *et al*. (2005) may not have been easier than parameterization for some users. Researchers may find either of the approaches more competitive. This remains a pending question that is worth exploring (Belz & Piepho, 2012).

The need to evaluate the impact of hormesis model selection on effective dose estimates and other hormetic quantities necessitated the use of reparameterizations to allow for their explicit estimations. To solve this problem, Belz & Piepho (2012) provided a general method for reparameterization of the Cedergreen-Ritz-Streibig model model to allow for estimation of *M*, *LDS* and ED_k doses with their confidence interval. The reparameterizations (eqs. 15 – 17) are shown in Table 2.

Other modifications of Brain and Cousens model

Other modifications of Brain and Cousens model as introduced by Tu *et al*. (2007) are:

$$y = c + \frac{d - c + fx}{1 + \exp\left(-\frac{(x-e)}{\omega}\right)} \qquad (18)$$

$$y = c + \frac{d - c + \frac{fx}{\exp(x)}}{1 + \exp\left(-\frac{(x-e)}{\omega}\right)} \qquad (19)$$

Table 2: Reparameterizations of Cedergreen-Ritz-Streibig model (eq. 13) for estimation of ED_k, *LDS* and *M* (Belz & Piepho 2012).

Parameterization for estimating ED_K; parameter to be replaced in equation 13

$$d = \left(\frac{100-K}{100} - \frac{1}{1+\exp\left[bLn\left(\frac{ED_k}{e}\right)\right]}\right)^{-1} \times \left(\frac{-c + f\exp\left(\frac{-1}{ED_k{}^{\alpha}}\right)}{1+\exp\left[bLn\left(\frac{ED_k}{e}\right)\right]} + \frac{c(100-K)}{100}\right) \qquad (15)$$

Parameterization for estimating *LDS* ($ED_{K=0}$): parameter to be replaced in equation 13

$$d = \left(1 - \frac{1}{1+\exp\left[bLn\left(\frac{LDS}{e}\right)\right]}\right)^{-1} \times \left(\frac{-c + f\exp\left(\frac{-1}{LDS^{\alpha}}\right)}{1+\exp\left[bLn\left(\frac{LDS}{e}\right)\right]} + c\right) \qquad (16)$$

Parameterization for estimating *M* parameter to be replaced in equation 13

$$f = \left(\exp\left(\frac{-1}{M^{\alpha}}\right) \times \left(\alpha M^{-\alpha-1}\right) \times \left\{1+\exp\left[b\ln\left(\frac{M}{e}\right)\right]\right\} - \exp\left(\frac{-1}{M^{\alpha}}\right) \times \exp\left[bLn\left(\frac{M}{e}\right)\right] \times \frac{b}{M}\right)^{-1}$$
$$\times \left((a-c) \times \exp\left[bLn\left(\frac{M}{e}\right)\right] \times \frac{b}{M}\right) \qquad (17)$$

$$y = c + \frac{d - c + \frac{fx}{\exp(x)}}{1 + \exp\left(-\left(\frac{\ln(x) - \ln(e)}{\omega}\right)\right)} \quad (20)$$

In Eqs. 18, 19 and 20, the parameters *c*, *d*, *f* and *e* are as defined in eq. 4. The parameter ω is related to parameter *b* of the original Brain-Cousens model. eq. 18 is very much similar to the original Brain-Cousens model (eq. 4) in terms of its strengths and weaknesses.

Equations 19 and 20 use the non-monotonic weighting function, $fx/\exp(x)$ to weight the logistic switch-off function. This allows the hormesis effect to dominate at the early stage and gradually diminish unlike the weighting function, *fx* that never vanishes. Unlike the Cedergreen–Ritz–Streibig model, eqs. 19 and 20 are not applicable to dose-response data with broad hormetic dose range but can be used to describe early increase in responses at low doses within a narrow hormesis zone (see Fig. 6). These equations were originally used to model time-dependent hormetic growth of *Escherichia coli* (Tu *et al*., 2007). Nonetheless, they can be adopted for general application in toxicological studies. Our experience with the models indicated that equations 19 and 20 could be problematic with data having large dose values. To get around this, large doses can be divided by 1000 or be transformed to ln (dose). In addition, to prevent the models from returning unrealistic low *d* values (underestimation of *d*), *d* can be fixed to experimentally observed response at zero dose. Eq. 20 appeared to be suitable for dose-response data that had saturation effect at values far below 100% inhibition as the concentration of the toxicant increases. The transformations applied to the original Brain-Cousens model are applicable to eqs. 18 – 20. Thus, the inverted form of eq. 18 can be written as eq. 21 (to show only one) to describe U-shaped hormetic dose-response relationships.

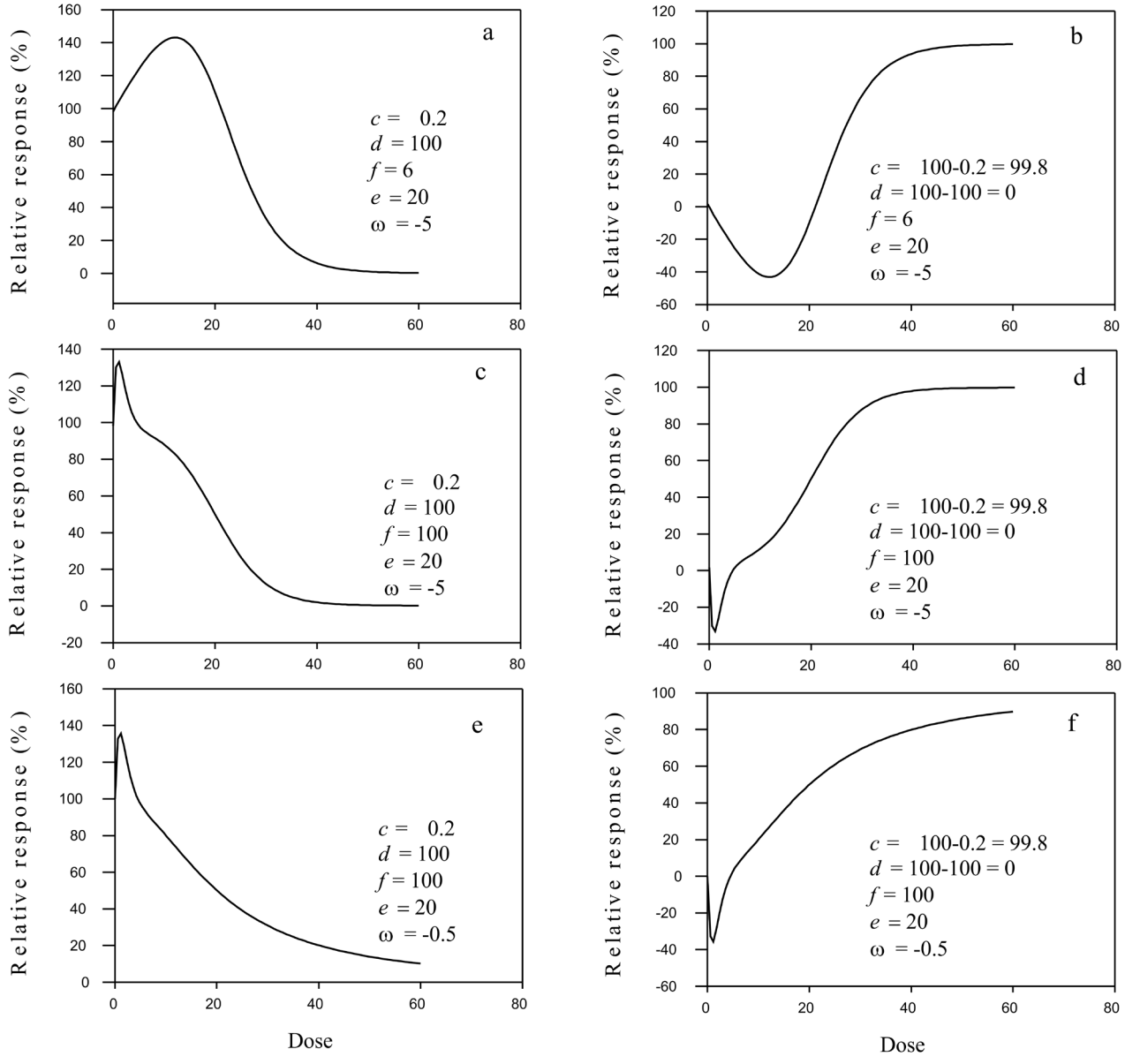

Fig. 6: Inverted U-shaped and U-shaped dose-response relationships generated from the modified Brain-Cousens model according to eq. 18 (a and b), eq. 19 (c and d) and eq 20 (e and f). Notice the early hormetic response at low doses and the narrow hormetic dose range of equations 19 and 20.

$$y = c - \frac{c - d + fx}{1 + \exp\left(-\frac{(x-e)}{\omega}\right)} \qquad (21)$$

Statistical test for hormesis

In the Brain-Cousens and Cedergreen-Ritz-Streibig models as well as their reparameterizations, the parameter *f* determines the size of hormesis. If $f = 0$, the equations are reduced to the original logistic model. Thus, $f > 0$ is an important condition for hormesis. These models permit a simple test for hormesis. To test for the statistical significance of hormesis, the models can be fitted to the experimentally-observed data using statistical software. If the 95% confidence interval for the estimate of *f* does not fall within zero (0), then the hormetic effect is statistically significant. Details of the process for statistical test for hormesis with Brain-Cousens model have been discussed by Schabenberger *et al*. (1999). Alternatively, the experimental data exhibiting hormesis can generally be compared with the control (*y* at $x = 0$) using ttest or Duncan test implemented in statistical softwares to determine if hormetic effect varied significantly with the control.

Bi-logistic hormesis models

Brain-Cousens model in the original versions are not suitable for substances that are essential to the test organism, for instance essential nutrients that gives beneficial effects at low doses (such that $d = 0$), and inhibitory effects as the dose increases. In the Brain-Cousens model types, the baseline effect from which the ED_K is calculated is the zero dose asymptotes *d*, and thus is not suitable for essential effecters. Beckon *et al*. (2008) proposed a general approach to describe biphasic relationships for essential effects (with $d = 0$) and effecters with nonzero asymptote ($d > 0$). The general model for essential substances is a multiplicative combination of log-logistic function such as equation 22, based on the assumption that both negative and positive effect of the substance may be well described by log-normal distributions. Equation 22 has slopes of opposite sign, one for the upslope (+ slope) and the other for the downslope (- slope) of the biphasic dose-response relationship.

$$y = \left(\frac{1}{1 + \left(\frac{x_{Up}}{x}\right)^{\beta_{Up}}}\right) \times \left(\frac{1}{1 + \left(\frac{x_{Dn}}{x}\right)^{\beta_{Dn}}}\right) \qquad (22)$$

$$\beta_{Up} > 0, \beta_{Dn} < 0$$

In eq. 22, β_{Up} represent the rising slope (+), x_{Up} is the dose at midpoint of the rising slope, β_{Dn} represent the falling slope (-), x_{Dn} is the dose at midpoint of the falling slope. The multiplicative model describes a dose response relationship in which *y* is a positive measure of effect such as growth of an organism. At sufficiently high concentrations of the substance, the response y approaches zero and also *y* approaches zero at very low concentration (the substance being essential to the expression of the effect). It is important to note that eq. 22 could be used when the maximum measured effect equals 1, or when the effects have been normalized relative to the maximum value to run from 0 to 1 (or 0% to 100%) in the rising curve or from 1 to 0 (or 100% to 0%) in the falling curve. This transformation of dose-response data can be done as shown in equation 23.

$$y = \frac{y(dose)}{a} \qquad (23)$$

Where *y* is the relative measured positive effect, *y* (*dose*) is the effect at a given dose of the substance and *a* is the maximum effect. The relative effects can be transformed to percentages by multiplying the term by 100. When the data is not transformed, equation 22 can be rewritten as 5-parameter version shown in eq. 24. Thus, eq. 22 also holds for non transformed data if the maximum effect is 1 (ie $a = 1$).

$$y = \left(\frac{a}{1 + \left(\frac{x_{Up}}{x}\right)^{\beta_{Up}}}\right) \times \left(\frac{a}{1 + \left(\frac{x_{Dn}}{x}\right)^{\beta_{Dn}}}\right) \qquad (24)$$

$$\beta_{Up} > 0, \beta_{Dn} < 0$$

The parameters of equation 24 are as defined in equations 22 and 23.

Beckon *et al*. (2008) generalized eq. 22 to accommodate non-zero low and high dose asymptote (eq. 25) similar to the Brain-Cousens model as illustrated in Fig. 7.

$$y = c + \frac{d - c + \frac{Max - d}{1 + \left(\frac{x_{Up}}{x}\right)^{\beta_{UP}}}}{1 + \left(\frac{x_{Dn}}{x}\right)^{\beta_{Dn}}} \qquad (25)$$

$$\beta_{Up} > 0, \beta_{Dn} < 0$$

In eq. 25, parameters *d* and *c* are as defined in eq. 3 and the parameter *Max* is the theoretical maximum that would be approached asymptotically (by the rising component of the equation) in the absence of the descending component (or vice versa), x_{Up}, β_{Up}, x_{Dn} and β_{Dn} are as defined in eq. 22. Equation 25 is a modification of the original Brain-Cousens model by introduction of a logistic weighting function at the hormesis region which describes the rising curve of the hormetic dose-response model. Equation 25 describes dose-response relationships with hill-shaped curves (Fig. 7). It can be rearranged to produce a function (eq. 26) that describes dose-response relationships for negative measure of effect or dose-response data transformed to percent inhibitions, which has U-shaped curves.

In eq. 26, the parameters are as defined in equations 22, 24 and 25, *Min* is the minimum effect that would be approached by the downslope in the absence of the upslope. Such U-shaped dose-response curves can also be described by equations formed by subtracting eq. 25 from 100 or 1 (as

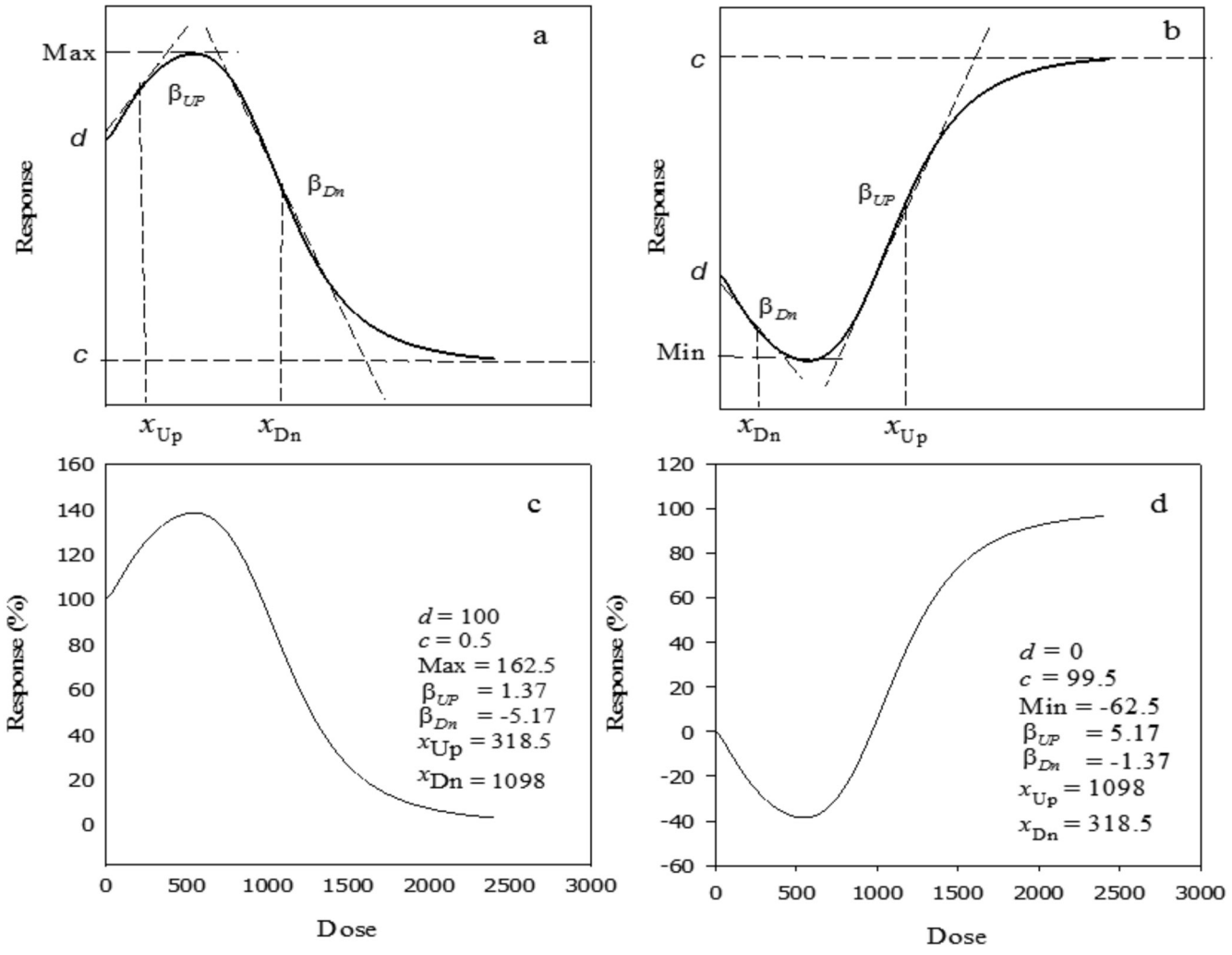

Fig. 7. Inverted U-shaped and U-shaped dose-response relationships plotted from the bilogistic models eq. 25 and eq. 26 respectively, to illustrate model variables (a and b) and plotted with arbitrary parameter values to illustrate the relationship between U-shaped and inverted U-shaped dose-response relationships (c and d).

was done in eqs. 5 and 6) depending on whether the data was transformed to percent inhibition or ratio respectively, relative to the control.

$$y = d + \frac{Min - d + \dfrac{c - Min}{1 + \left(\dfrac{x_{Up}}{x}\right)^{\beta_{UP}}}}{1 + \left(\dfrac{x}{x_{Dn}}\right)^{\beta_{Dn}}} \qquad (26)$$

$$\beta_{Up} > 0, \beta_{Dn} < 0$$

Equations 22, 25 and 26 can be used for statistical test for hormesis as described by Brain and Cousens (1989) since the equations reduce to log-logistic model if either of the slope parameter is zero. When compared with Brain-Cousens and Cedergreen-Ritz-Streibig models, the logistic models proposed by Beckon *et al*. (2008) provided better description of hormetic dose-response relationship when tested with empirical data (see Beckon *et al*. (2008) for more details). Zhu *et al*. (2013) compared the model of Beckon *et al*. (eq. 26) with other hormesis models and found that equation 26 provided better descriptions of hormetic data sets than Brain-Cousens model, its reparameterized version of van Ewijk and Hoekstra (1993) and its modification by Cedergreen *et al*. (2005). However, the model has its own limitations. Like other hormesis models, the model of Beckon *et al*. has no explicit parameter for the effective doses. The mathematical manipulations required to reparameterize this model for effective doses computation could be laborious. In addition, Beckon's model could have disagreement between the interpretation of some parameters and their corresponding fitted values. The parameter *Max* is only the estimate of peak and does no represent the exact peak of the curve. The value of *Max* depends on the slopes of the dose-response curve. Also, the parameters x_{Up} and x_{Dn} lose their interpretive utility if one or both slopes approach 0 (Beckon *et al*., 2008). By making reference to the hormetic quantities shown in Fig. 7b, eq. 25 can also be written for U-shaped curves as (eq. 27).

Beckon and co-workers also introduced an additive bilogistic model (eq. 28) to describe biphasic dose-response data. According to Beckon *et al*. (2008), equation 28 is as

good as the multiplicative model yielding almost identical parameter estimates.

$$y = c + \frac{Min - c + \frac{d - Min}{1 + \left(\frac{x_{Dn}}{x}\right)^{\beta_{Dn}}}}{1 + \left(\frac{x}{x_{Up}}\right)^{\beta_{Up}}} \qquad (27)$$

$$\beta_{Up} > 0, \beta_{Dn} < 0$$

$$y = d + \frac{Max - d}{1 + \left(\frac{x_{Up}}{x}\right)^{\beta_{Up}}} - \frac{Max - c}{1 + \left(\frac{x}{x_{Dn}}\right)^{\beta_{Dn}}} \qquad (28)$$

$$\beta_{Up} > 0, \beta_{Dn} < 0$$

Where the parameters are as defined in eq. 25. During curve fitting, care must be taken in order to select appropriate initial estimates (especially for *d* and *Max*) for realistic final estimates.

An equation (eq. 29) similar to eq. 28 for U-shaped dose-response curve was proposed by Deng *et al* (2012). The model is a bilogistic model since it was obtained through algebraic addition of two logistic functions representing the stimulation and inhibition zones of the biphasic curve.

$$y = c - \frac{d + Min}{1 + \left(\frac{x}{x_{Dn}}\right)^{\beta_{Dn}}} + \frac{d - c + Min}{1 + \left(\frac{x}{x_{Up}}\right)^{\beta_{UP}}} \qquad (29)$$

$$\beta_{Up} > 0, \beta_{Dn} > 0$$

Where *y* is the response, *x* is the dose, *c* is the response at infinite dose of the toxicant, *d* is response at the zero dose. x_{Dn} and β_{Dn} are the mid point and slope respectively of the down sloping curve while x_{Up} and β_{Up} are the midpoint and slope respectively of the upsloping curve. The model is generally used for dose-response data that is transformed to percentage inhibitions as described earlier.

In eq. 29, the inhibition at the control (*d*) and at the largest concentration (*c*) of the toxicant can be set at 0 and 100% respectively and eq. 29 can be simplified as shown in eq. 30 (Deng *et al* 2012; Li *et al*., 2014).

$$y = 100 - \frac{Min}{1 + \left(\frac{x}{x_{Dn}}\right)^{\beta_{Dn}}} + \frac{Min - 100}{1 + \left(\frac{x}{x_{Up}}\right)^{\beta_{UP}}} \qquad (30)$$

$$\beta_{Up} > 0, \beta_{Dn} > 0$$

Another bilogistic hormesis model worthy of note is eq. 31 (OriginLab Corporation). Eq. 31 is similar to eq. 29 and is good for describing U-shaped dose-response curves of the type found in Pharmacology and the curves normalized relative to controls.

$$y = Min + \frac{d - Min}{1 + 10^{(x - x_{Dn})\beta_{Dn}}} + \frac{c - Min}{1 + 10^{(x_{Up} - x)\beta_{Up}}} \qquad (31)$$

$$\beta_{Up} > 0, \beta_{Dn} < 0$$

Where *d* is the untreated control (*y* at *x* = 0), *c* is the expected response at infinite dose, *Min* is the minimum effect that would be approached by the downslope in the absence of the upslope, β_{Up} is the steepness of the rising (positive) slope, x_{Up} is the dose at the midpoint of the rising slope, β_{Dn} is the steepness of the falling (negative) slope and x_{Dn} is the concentration at the midpoint of the falling slope (see Fig. 7b).

Eq. 31 has been found to be robust in fitting biphasic dose response data. Out of five biphasic dose-response functions evaluated by Zhu *et al*. (2013) on experimental dose-response data set, Eq. 31 gave the best description of data in terms of the goodness-of-fit statistics. In addition, the value of *Min* estimated by this function was more reasonable in terms of its interpretation when compared with eq. 26 of Beckon *et al*. (2008). Setting *d* and *c* to zero and 100% respectively, eq. 31 can be simplified as (eq. 32):

$$y = Min - \frac{Min}{1 + 10^{(x - x_{Dn})\beta_{Dn}}} + \frac{100 - Min}{1 + 10^{(x_{Up} - x)\beta_{Up}}} \qquad (32)$$

$$\beta_{Up} > 0, \beta_{Dn} < 0$$

Ge *et al*. (2011) and Chen *et al*. (2015) used this simplified version of eq. 31 to adequately describe the hormetic effects of mixtures of ionic liquids on luciferase activity.

The algebraic addition of two models can be applied to other monotonic dose-response models to obtain hormesis function. This approach was used by Murado and Vazguez (2010) to describe hormetic effects of antimicrobial agents on microbial growth.

Extended logistic hormesis model

Beckon *et al*., (2008) extended the bi-logistic model (eq. 25) to obtain a model (eq. 33) accommodating two positive and two negative effects (four phases).

$$y = c + \frac{d + \frac{Max - d}{1 + \left(\frac{x_{Up2}}{x}\right)^{\beta_{Up2}}} - c + \frac{Max - d + \frac{Max - d}{1 + \left(\frac{x_{Up2}}{x}\right)^{\beta_{Up2}}}}{1 + \left(\frac{x_{Up1}}{x}\right)^{\beta_{Up1}}}}{\left(1 + \left(\frac{x_{Dn1}}{x}\right)^{\beta_{Dn1}}\right) \times \left(1 + \left(\frac{x_{Dn2}}{x}\right)^{\beta_{Dn2}}\right)} \qquad (33)$$

$$\beta_{Up1}, \beta_{Up2} > 0$$

$$\beta_{Dn1}, \beta_{Dn2} < 0$$

The extended model gave a better fit than eq. 25 when tested with an experimental data set on the effects of histamine on phagocytosis of the protozoan *Tetrahymena pyriformis* (see Beckon *et al*., 2008). In these models, *c* can be fixed at zero and *d* can be fixed at the control value (*y* at zero dose). The bilogistic and extended logistic models can generally be used to describe broad hormesis zone and longer pre-hormesis 'no effect' doses.

The multiplicative models based on 4-parameter logistic function have been further developed by Di Veroli *et al*.

(2015) to describe dose-response curves with multiphasic features (including stimulation at low doses). There model is of the form:

$$E(x) = \prod_{i=1}^{n} E(x_i) \qquad (34)$$

Equation 34 can be rewritten as:

$$y = \prod_{i=1}^{n} \left[d + \frac{a_i - d}{1 + \left(\frac{e_i}{x} \right)^{b_i}} \right] \qquad (35)$$

Where $E(x)$ is the effect (y) obtained at a given concentration x, $E(x_i)$ is the effect obtained at a given concentration C (x) in the ith phase of the dose-response curve, n is the number of phases, e_i is the relative 50% effective concentration of the phase, b_i is the hill exponent (slope function), a_i is the maximum effect and d is the effect in the absence of effecter (control).

The model enables interpreting each phase of the dose-response relationship as an independent dose-dependent process and thus provides a robust approach to fit dose-response curves with various degree of complexity (Di Veroli *et al*., 2015). An algorithm was developed, which could automatically generate and rank dose-response models with varying degree of multiphasic features typical of pharmacological studies. This algorithm can be implemented in the freely available software, *Dr fit* (sourceforge.net/projects/drfit/).

However, finding good initial estimates for the parameters is a tedious exercise in statistical softwares, probably because the response at zero dose (d) appeared in all the phases with the same value for all observable phases in a multiphasic dose-response curve. We observed that varying the value of the parameter d in the different phases of multiphasic dose-response curve (as different parameters in each phase) simplifies the curve fitting process. Nevertheless, there could be disagreements between the interpretation of some parameters and their fitted values. Whether this problem can be solved by putting constraints on the parameters or by modifying the model is a subject for further study.

HORMESIS MODELS DERIVED FROM OTHER MONOTONIC SIGMOID FUNCTIONS

The principle behind the addition of hormetic behaviour to Brain-Cousens model can be extended to other monotonic functions. A typical example is the extension of Gompertz model to include hormesis term as shown in eq. 36 (Cedergreen *et al*., 2005).

$$y = c + (d - c + fx)\exp(-\exp(b[\ln(x) - \ln(e)]) \qquad (36)$$

Where the parameters are as described in equation 4.

Eq. 36 possess weakness similar to those of the Brain-Cousens model for shallow dose response curve (Cedergreen *et al*., 2005) Another example is the extension of the sigmoid function (eq. 37) to include hormesis behaviour by Lyles *et al*. (2008) as shown in eq. 38.

$$y = \frac{a}{1 + \exp(b + ex)} \qquad (37)$$

$$y = \frac{a(1 + f\exp(x))}{1 + \exp(b + ex)} \qquad (38)$$

Where y is the response at dose x, a is the theoretical maximum response at $x = 0$, b is the slope parameter and e is related to ED_{50}. The parameter a is only the estimate of the maximum response at $x = 0$ and does not represent the exact response at zero dose. Equation 38 does not allow determination of ED_{50} as a parameter in the model. To allow explicit determination of ED_{50} as model parameter, eq. 38 was reparameterized to give eq. 39 (Lyles *et al*. 2008).

$$y = \frac{a(1 + f\exp(x))}{\left\{1 + \exp\left[\frac{b + x \times \ln\left(\frac{2(1+\exp(-b))(1+f\exp(ED_{50})}{1+f} - \exp(-b)\right)}{ED_{50}}\right]\right\}} \qquad (39)$$

Fitting equations 38 and 39 to dose-response data with large doses (x) could be problematic. In order to simplify curve fitting process, it is important to transform x to $\ln(x)$ or divide by large numbers when large x values are encountered. It is also important to note that these extended models do possess weaknesses similar to those of Brain-Cousens model for shallow dose-response curves (Cedergreen *et al*., 2005).

Models used for allelopathic hormesis

Alternative models used to describe hormetic effects of chemicals are the models originally proposed for the description of allelopathic hormesis. Allelopathy is a biological phenomenon by which an organism produces one or more biochemical substances which have detrimental or occasionally beneficial effects on another organism. This phenomenon has been extensively studied in plants. Allelochemicals are well known to induce hormesis, stimulating plant growth at low concentration and inhibiting plant growth at high concentrations (Rice, 1984; Lovett *et al*., 1989). To describe allelopathic hormesis, An *et al*. (1993) proposed a model (eq. 40) based on the hypothesis that the response to allelochemicals is simultaneously stimulatory and inhibitory in nature. The An-Johnson-Lovett model gave good simulation of hormetic response to allelopathic chemicals, for a wide range of experimental conditions (An *et al*., 1993).

$$y = y_o + \frac{S_m x^q}{K_s{}^q + x^q} - \frac{I_m x^q}{K_i{}^q + x^q} \qquad (40)$$

In equation 40, y is the biological response at a given concentration of the chemical substance x, y_o is the response at zero dose (y at $x = 0$), S_m is the maximum stimulatory response and K_s is a constant that describes the response of stimulation to increment of the limiting factor, I_m and K_i are the respective parameters of the inhibitory attribute. Parameter q is a constant.

In the study of allelopathy, the responses to allelochemical are usually expressed as percent of control. Thus, with the control (%) set at 100%, the response, y% of control is given as:

$$y = 100 + \frac{S_m x^q}{K_s{}^q + x^q} - \frac{I_m x^q}{K_i{}^q + x^q} \qquad (41)$$

Liu *et al*. (2011) modified the An-Johnson-Lovett model and introduced three new models based on ecological-limiting factor model of Monod (eq. 42), Mitscherlich (eq. 43) and logistic growth (eq. 44).

$$y = y_o + S_m\left(\frac{x}{K_s + x}\right)q - I_m\left(\frac{x}{K_i + x}\right)q \qquad (42)$$

$$y = y_o + S_m\left(1 - \exp(-K_s x)\right) - I_m\left(1 - \exp(-K_i x)\right) \qquad (43)$$

$$y = y_o + \frac{S_m}{1 + \left(a\exp(-K_s x)\right)} - \frac{I_m}{1 + \left(b\exp(-K_i x)\right)} \qquad (44)$$

In equations 42, 43 and 44, y_o, S_m, I_m are as described in eq. 40. Parameters a, b and q are constants. When compared with equations 42 and 43, equations 40 and 44 can be used to describe broader hormetic dose range. In addition, the An-Johnson-Lovett model (eq. 40) can describe the initial 'no effect' doses before the initial stimulation of effect at low doses (see Fig. 8c), the advantage it shares with the bilogistic or the extended logistic models (see Alloisio *et al*., 2015) but not the Brain-Cousens or Cedergreen-Ritz-Streibig models. Unlike equations 42 and 43, the An-Johnson-Lovett model had good agreement between the interpretation of the effect at zero dose (y_o) and their corresponding fitted value. Although these models were originally used in the study of allelochemical hormesis in plants, they can be adopted for the study of biphasic responses of other organisms to hormetic chemicals. The An-Johnson-Lovett model has been used widely in modeling plant allelopathy. Belz and Piepho (2012) used it as an alternative model to describe dose-response relationship for the effect of 2-phenylethyl-isothiocyanate on root elongation of *Amaranthus hybridus* where the Brain-Cousens and Cedergreen-Ritz-Streibig models were both unsuitable. Although the model proved a significant hormetic effect unlike the logistic models, and gave satisfactory fit pseudo R^2 value, the graphical comparison between the experimental values and the predicted curve suggests a risk of overestimating the actual hormetic effect (Belz & Piepho, 2012). It is therefore important to have many experimental data within the hormetic dose zone when applying the model.

CONCLUSION

In this paper, we reviewed the existing statistical models for description and analyses of hormetic dose-response relationships. It appears that no model has the capability to describe all forms of hormetic dose response patterns that are possibly generated in toxicological studies. Each model has its own strengths and limitations. Thus, application of a

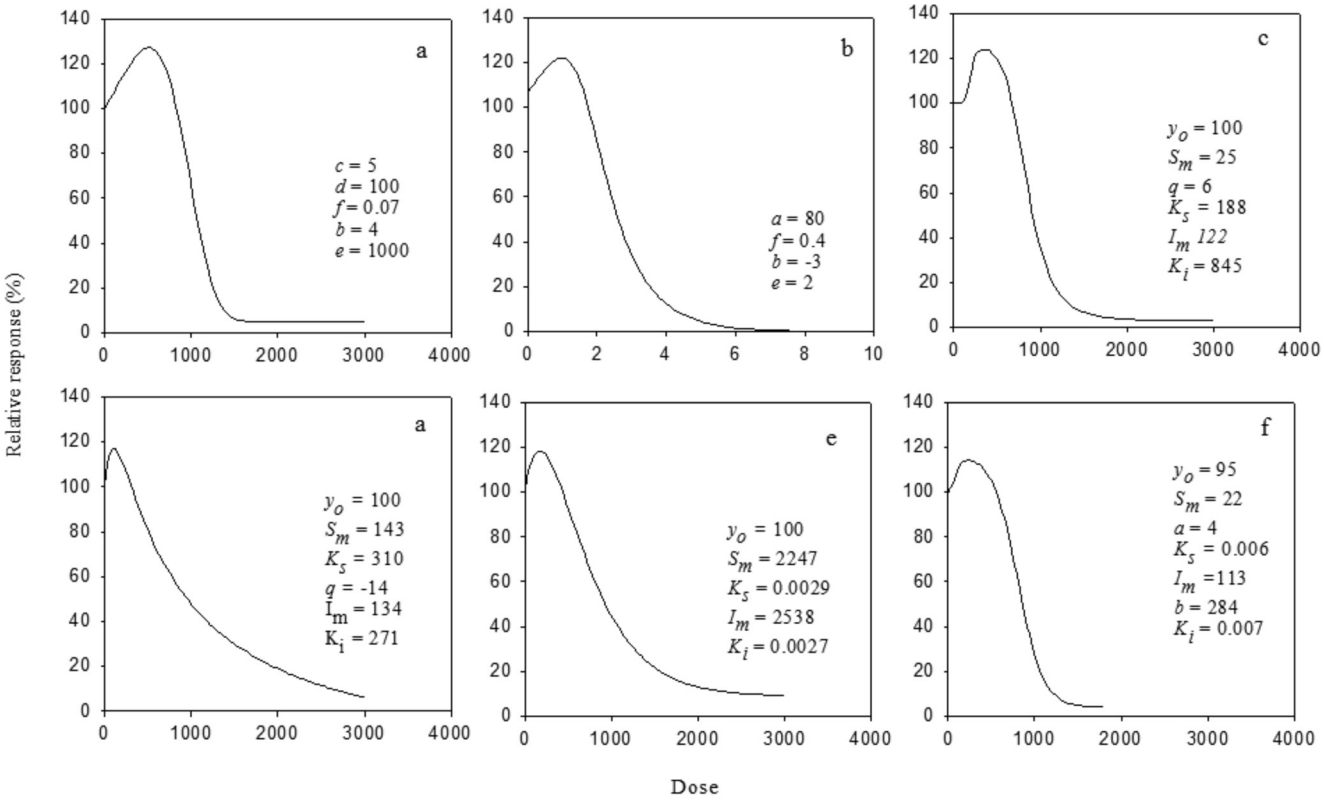

Fig. 8: Dose-response relationships generated from equations 36 (a), 38 (b), 40 (c), 42 (d), 43 (e) and 44 (f). Curves are plotted with arbitrary values of the parameters to show characteristic features of the models.

particular model may depend on the need of the investigator(s) and the peculiarities of the data under analysis. However, the multiplicative and the extended models based on algebraic multiplication or addition of logistic functions for analyses of multiphasic dose-response relationship appeared to be versatile models for description of dose-response curves involving stimulation and toxicity. Be it as it may, more research efforts should look forward to expanding possibilities, in terms of application of existing models and development of new mathematical models for hormesis.

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