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JSFit: a method for the fitting and prediction of J- and S-shaped concentration–response curves†

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Most monotonic S-shaped concentration–response curves (CRCs) can be satisfactorily described by a classical Hill equation. However, the Hill equation cannot effectively describe the non-monotonic J-shaped CRCs that display stimulation at low concentrations and inhibition at high concentrations. On the other hand, the physical meaning of the model parameters in current models describing the J-shaped CRCs is not very clear. It is well known that both toxicity experiments and the fitting process inevitably produce uncertainty. To effectively deal with the J-shaped concentration–response data with uncertainty and make the model parameters meaningful, we developed a method for the fitting of the J-shaped and/or S-shaped concentration–response data (simply called JSFit). The JSFit first uses one Hill equation (S-shaped) or combines with two Hill equations (J-shaped) for fitting, then nonlinear least squares fitting is performed by means of the Levenberg–Marquardt algorithm, and finally the observation-based confidence intervals of the fitting curve are constructed by the delta procedure. For the convenience of application, we wrote a computational program (JSFit) using the MATLAB programming language and introduced automation of the initial parameters into the program. The JSFit was then successfully used in the fitting and prediction of the toxic data of pesticides, ionic liquids, antibiotics, and personal skin-care products on *Vibrio Qinghaiensis* sp.-Q67.

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Introduction

Hormesis is a biphasic concentration–response relationship exhibiting low-concentration stimulation and high-concentration inhibition.¹ Since the discovery of hormesis in the nineteenth century, many studies have described qualitatively the phenomena such as homeostasis, equilibrium, stability, and optimization throughout the scientific fields as diverse as physiology (*e.g.* temperature affecting the growth rate²), ecology (*e.g.* disturbances affecting species diversity³), and psychology (*e.g.* intensity of punishment affecting the rate of training⁴) and economics (*e.g.* tax rate affecting tax revenue⁴). In recent years, significant research has shown that hormesis can be induced by many chemicals, especially some poisonous and harmful substances such as persistent organic pollutants,⁵ pesticides,⁶ metal ions,⁷ antibiotic⁸ and ionic liquids.^{9,10} The quantitative description of the hormetic concentration–response relationship has become an important step for gaining a better understanding of hormesis and the influence and meaning of hormesis in environmental risk assessment.¹¹

Fortunately, a few models^{4,12–16} can be used to quantitatively describe the hormetic concentration–response relationship. The most widely used is the model with five parameters (simply M5) cited by Ge *et al.* in 2011.⁹ In 2013, several biphasic models were compared in terms of the goodness-of-fit (GoF) statistics of the adjusted coefficient of determination (R_{adj}^2), the root mean square error (RMSE), and the Akaike information criterion (AIC), where M5 gave the best description of hormetic data sets.¹¹ Although the GoFs of these models were satisfactory, the model coefficients were far away from the values represented by their physical meaning.

Some scholars have directly fitted hormetic data using the sigmoidal equation, but this would miss some important information at low concentration. In 2016, de Garcia *et al.* used three logistic models, from a family of sigmoidal equations, to fit hormetic data of the bacterium *Vibrio fischeri* exposed to pharmaceuticals and personal care products for description of the concentration–response behaviour.¹⁷ Since most of the stimulative effects take place at low concentration levels, such as EC_0 and EC_5 , some conclusions obtained from the sigmoid equation at these levels would not be correct.

In 2015, Di Veroli *et al.* developed an automated fitting procedure and software, named as Dr-Fit, to fit the concentration–response curves (CRCs) with multiple inflection points by a many Hill model.¹⁸ Without doubt the Dr-Fit software was a powerful tool for fitting CRC. However, because the number of data points was relatively few in the paper and as the

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experimental errors were not considered, the CRC model from the Dr-Fit can only be used to fit but not to predict.

In fact, there are some more or less errors with the experimental data points and fitting process, which are often ignored by scholars. So, it is necessary to examine the effects of experimental errors and fitting deviation on the CRC fitting in the construction of a CRC prediction model. Zhu *et al.*¹⁹ introduced confidence intervals (CIs): FCIs based on the function fitting and OCIs based on observation, into the CRC fitting. The CIs can be used to test the data points in turn and to judge whether there is a large deviation from the experimental data points, which could further improve the experimental method to a certain extent.

Because hormesis describes a biphasic concentration–response relationship, which is stimulation at low concentration and inhibition at high concentration, it is usually expressed as a J-shaped concentration–response relationship or CRC.²⁰ In 2008, Beckon *et al.* constructed a new model based on the theory that the stimulation and inhibition effects in the two phases could be separated into two different and independent sensitivity threshold probability distributions.⁴ Trogl *et al.* used two sigmoid equations to model the hormetic curves.²¹ However, the regression coefficients in the models did not have any significant physical meaning. Therefore, it is necessary to enrich the physical meaning of the model coefficients in the development of a quantitative model.

The main purpose of this paper is to develop a model for the fitting and prediction of the monotonic S-shaped and/or non-monotonic J-shaped concentration–response data. In the model development, the observed concentration–response data are fitted to a Hill function (S-shaped) or an integration function from the product of two Hill functions, and then automatic initialization of the model coefficients is performed, and the CIs of the fitted CRCs are finally constructed. On the basis of the developed model, some characteristic parameters of J-shaped CRC are given. Furthermore, the outlier and reliability of the observational data are also analyzed.

Data and methods

Concentration–effect data set

The concentration–response/effect data set (*C–E*) used in this paper comes from the literature.²² The data set consists of toxic effects and luminescence inhibition ratios (each three replications) of 1-ethylpyridinium chloride ([epy]Cl) at 12 concentration levels in seven exposure times (0.25, 2, 4, 6, 8, 10 and 12 h) on *Vibrio Qinghaiensis* sp.-Q67 (Q67) (Table S1 in the ESI†). In the set, the CRCs of [epy]Cl in the first 0.25 h and 2 h are S-shaped, while those in the latter five times (4, 6, 8, 10 and 12 h) are J-shaped (Fig. S1†).

General procedure of modelling

The modelling flow chart is shown in Fig. 1, and involves the steps: (1) for the monotonic and hormetic data, a suitable mathematical model is selected, such as a single Hill model or a product of two Hill models. (2) Calculus is used to analyze the

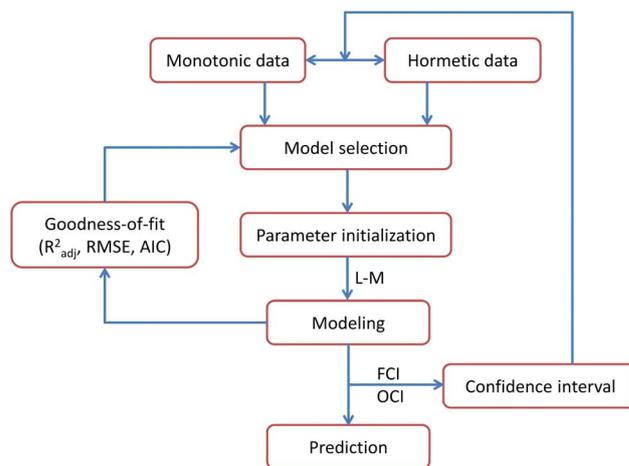


Fig. 1 The technical route used in this study, where R_{adj}^2 refers to the adjusted coefficient of determination, RMSE to the root mean square error, and AIC to the Akaike information criterion, while FCIs and OCIs are the function-based confidence intervals and observation-based confidence intervals, and L–M is an acronym for “the Levenberg–Marquardt algorithm”.

specific meaning of each model coefficient, where its initial values are estimated by combining the information in the original experimental data (initialization). (3) On the basis of the Levenberg–Marquardt algorithm²³ in MATLAB, experimental data are fitted by the selected model to obtain the model coefficients. (4) The GoFs, such as R_{adj}^2 , RMSE and AIC, of the model are calculated to evaluate the fitting quality. (5) On the basis of the fitted CRC and experimental data, the FCIs and OCIs are constructed. The reliability of the experimental data is analyzed and outliers in the original experimental data are picked out. (6) Using the hold-out method,²⁴ the predictive potential of the model is evaluated.

Model coefficient estimation

For a monotonic concentration–response relationship, the CRC may be described quantitatively by the classical Hill equation.^{25,26} For a given concentration, C , the response/effect, E , can be calculated by the Hill equation with four parameters:

$$E_{Hill}(C, E_{\infty}, E_0, EC_{mid}, H) = E_0 + \frac{E_{\infty} - E_0}{1 + (EC_{mid}/C)^H} \quad (1)$$

where H is the Hill exponent, E_0 is the effect at the concentration of 0, E_{∞} is the effect at the concentration large enough and EC_{mid} is the concentration corresponding to the effect of $(E_{\infty} + E_0)/2$.

For a non-monotonic concentration–response relationship, the CRC can be described by an integration model combined with two Hill equations. The hormetic concentration (C)–effect (E) relationship has two inflection points,¹⁸ which determine the number of equations to be integrated, and one minimum, which is break point of the integration model (Fig. 2).

Therefore, by means of the break point, a hormetic concentration–response curve (HRC) could be split into two independent and separate Hill equations, named as two phases:



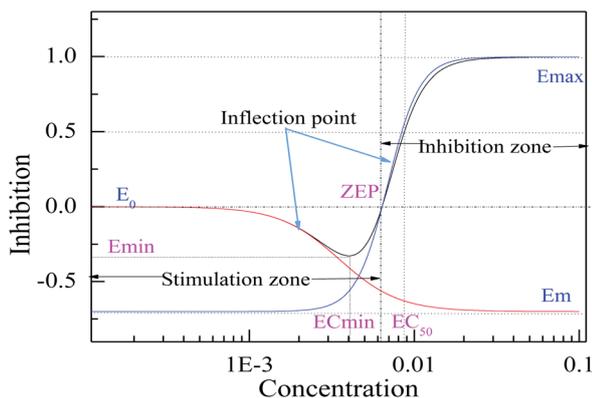


Fig. 2 The hormetic concentration–response curve (CRC) and its related parameters (model coefficients and characteristic parameters) diagram, where the black line refers to the CRC fitted by the model integrating with two Hill functions, the red line to the CRC fitted by the first Hill model for the first (decreasing) phase, the dark blue line to the CRC fitted by the second Hill model for the second (ascending) phase, E_0 and E_m are the minimum and maximum stimulative effect parameters of the first Hill model, E_m and E_{max} are the minimum and maximum inhibition effect parameters of the second Hill model, E_{min} and EC_{min} are the minimum effect or maximum stimulative effect and corresponding concentration and ZEP is the zero effect point, where the effect is 0 and the concentration is ZEP. The effects at the lower left of ZEP are the “stimulation zone”, while those at the upper right are the “inhibition zone”. EC_{50} is the concentrations where the inhibitory effects are 50%.

one descendant phase (first phase) and one ascendant phase (second phase) (Fig. 2).

The monotonic CRC of each phase can be modelled by a Hill equation. For the first phase (the descendant phase), the Hill equation (eqn (1)) can be rewritten as follows:

$$E_1(C, E_m, E_0, EC_{mid1}, H_1) = E_0 + \frac{E_m - E_0}{1 + (EC_{mid1}/C)^{H_1}} \quad (2)$$

For the second phase (the ascendant phase), the Hill equation (eqn (1)) can be rewritten as follows:

$$E_2(C, E_{max}, E_m, EC_{mid2}, H_2) = E_m + \frac{E_{max} - E_m}{1 + (EC_{mid2}/C)^{H_2}} \quad (3)$$

Combining eqn (2) with eqn (3), an integration model with seven parameters (called HM7-1 in this paper) can be obtained to describe the HRC. Since the minimum effect value of eqn (2) and (3) is E_m (Fig. 2), the integration model needs to be divided by E_m .

$$E(C) = \frac{E_1(C) \times E_2(C)}{E_m} = \frac{\left(E_0 + \frac{E_m - E_0}{1 + (EC_{mid1}/C)^{H_1}}\right) \times \left(E_m + \frac{E_{max} - E_m}{1 + (EC_{mid2}/C)^{H_2}}\right)}{E_m} \quad (4)$$

where $E(C)$ is the fitted effect at the concentration of C and $E_1(C)$ and $E_2(C)$ are the effects fitted by the first phase Hill model (eqn (2)) and the second phase Hill model (eqn (3)), respectively.

In many cases, E_0 is often approximately zero and E_{max} approximately equals to 1. In this case, eqn (4) can be reduced to eqn (5) (called HM5).

$$E(C) = \frac{1}{1 + (EC_{mid1}/C)^{H_1}} \times \left(E_m + \frac{1 - E_m}{1 + (EC_{mid2}/C)^{H_2}}\right) \quad (5)$$

However, due to some reasons, like solubility, E_{max} is often less than 1, and eqn (5) is transformed into eqn (6) (called HM6).

$$E(C) = \frac{1}{1 + (EC_{mid1}/C)^{H_1}} \times \left(E_m + \frac{E_{max} - E_m}{1 + (EC_{mid2}/C)^{H_2}}\right) \quad (6)$$

If E_0 is not zero, the HRC is upward translated E_0 units. Thus, eqn (6) is transformed into eqn (7) with seven parameters (called HM7-2):⁴

$$E(C) = \frac{1}{1 + (EC_{mid1}/C)^{H_1}} \times \left(E'_m + \frac{E'_{max} - E'_m}{1 + (EC_{mid2}/C)^{H_2}}\right) + E_0 \quad (7)$$

Model coefficient initialization

In the integration model, HM7-1 (eqn (4)), there are seven parameters: E_0 , E_m , E_{max} , EC_{mid1} , EC_{mid2} , H_1 and H_2 , to be estimated. In this paper, these parameters were called the model coefficients (MC_j , $j = 1, 2, \dots, 7$). Currently, the estimation of the coefficients in the existing literature often requires the user to provide appropriate initial values to perform the iteration operations, which is very difficult to do for users who are not familiar with the algorithm and HRC. In this paper, an automatic initialization method was thus given on the basis of the specific meaning of the model coefficients.

At first, coefficient estimation expressions were built by calculus and then the model parameters were estimated with discrete observation data points. The concentration–effect data set (C_i, E_i ; $i = 1, 2, \dots, n$) was sorted by concentration from small to large, and then the initial values ($MC_{j,0}$) of the seven model coefficients (MC_j) were automatically assigned as follows:

$$E_{0,0} = 0 \text{ or } E_{0,0} = E_1 \quad (8a)$$

$$E_{max,0} = 1 \text{ or } E_{max,0} = E_n \quad (8b)$$

$$E_{m,0} = \min(E_i) \quad (8c)$$

$$H_{1,0} = \frac{4}{\ln 10 E_{m,0}} \min(k_j) \quad (8d)$$

$$EC_{mid1,0} = C_{j(\min(k_j))} \quad (8e)$$

$$H_{2,0} = \frac{4}{\ln 10(1 - E_{m,0})} \max(k_k) \quad (8f)$$



$$EC_{\text{mid}2,0} = C_{k(\max(k_k))} \quad (8g)$$

where $E_{0,0}$, $E_{m,0}$, $E_{\max,0}$, $EC_{\text{mid}1,0}$, $EC_{\text{mid}2,0}$, $H_{1,0}$ and $H_{2,0}$ are the initial values of E_0 , E_m , E_{\max} , $EC_{\text{mid}1}$, $EC_{\text{mid}2}$, H_1 and H_2 , respectively, and $j(\min(k_j))$ and $k(\min(k_k))$ are series numbers of concentrations, where $\min(k_j)$ and $\max(k_k)$ are, respectively, the minimum slope in the descendant phase and the maximum slope in the ascendant phase. Here, the slope k_i ($i = 2, 3, \dots, n-1$) is defined by the central difference method as follows:²⁷

$$k_i = \frac{E_{i+1} - E_{i-1}}{\log_{10}(C_{i+1}) - \log_{10}(C_{i-1})} \quad (9)$$

Model prediction evaluation

The hold-out method²⁴ is used to evaluate the prediction ability of a model. The experimental C - E data were divided into two sets: a training set of no. 1, 3, 4, 6, 7, 9, 10 and 12 and a test set of no. 2, 5, 8 and 10. The training set was used to develop a model and the test set was used to validate the model's predictive power, which is expressed by the predictive determination coefficient, q_{ext}^2 , defined as follows:

$$q_{\text{ext}}^2 = 1 - \frac{\sum_{i=1}^{\text{pn}} (y_i - \hat{y}_i)^2}{\sum_{i=1}^{\text{pn}} (y_i - \bar{y}_{\text{tr}})^2} \quad (10)$$

where pn is the number of samples in the test set, y_i and \hat{y}_i is the experimental and predictive effect of the i th sample, \bar{y}_{tr} is the average value of experimental effects of samples in the training set. The closer the q_{ext}^2 is to 1, the stronger the predictive power of the model is.

Goodness-of-fit

The goodness-of-fit (GoF) of a model is often expressed by the coefficient of determination (R^2), R_{adj}^2 , RMSE or AIC.²⁸ The GoFs are defined as follows:

$$R^2 = 1 - \frac{\sum_{i=1}^n (y_i - \hat{y})^2}{\sum_{i=1}^n (y_i - \bar{y})^2} \quad (11)$$

$$R_{\text{adj}}^2 = 1 - \frac{(n-1) \sum_{i=1}^n (y_i - \hat{y})^2}{(n-m-1) \sum_{i=1}^n (y_i - \bar{y})^2} \quad (12)$$

$$\text{RMSE} = \sqrt{\frac{\sum_{i=1}^n (y_i - \hat{y})^2}{n-m}} \quad (13)$$

$$\text{AIC} = n \log \left(\frac{\sum_{i=1}^n (y_i - \hat{y})^2}{n} \right) + 2(m+1) \quad (14)$$

where y_i is the experimental effect, \hat{y} is the fitted effect, \bar{y} is the average effect, n is the number of samples, and m is the number of model coefficients.

At present, there are five models: M1,¹² M2,¹³ M3,¹⁵ M4 (ref. 4) and M5 (ref. 9) that can describe the J-shaped CRC (Table S2†). It has been proved that the M5 model is the best model among the five models.¹¹ The M5 model with five model coefficients can be written as follows:

$$E = \min - \frac{\min}{1 + 10^{b(C-a)}} + \frac{1 - \min}{1 + 10^{q(p-C)}} \quad (15)$$

where min is the minimum effect, b is the steepness of the ascendant phase, a is the concentration at the midpoint of the ascendant phase, q is the steepness of the descendant phase and p is the concentration at the midpoint of the descendant phase. When the concentration is 0, the fitting effect of the M5 model tends to 0, and when the concentration is large enough, the fitting effect of the M5 model tends to 100%.

Confidence intervals

There are two kinds of CIs: the FCI based on function and the OCI based on observation. The FCI describes the uncertainty of the function fitting,²⁹ and the OCI describes the uncertainty of both the experimental observation and the function fitting. The FCIs and OCIs can be calculated by the delta procedure as follows:^{19,30}

$$\text{FCI} = \hat{y} \pm t \left(n-m, \frac{\alpha}{2} \right) \sqrt{v \cdot \text{CM} \cdot v^T} \quad (16)$$

$$\text{OCI} = \hat{y} \pm t \left(n-m, \frac{\alpha}{2} \right) \sqrt{\text{RMSE}^2 + v \cdot \text{CM} \cdot v^T} \quad (17)$$

where α is the significant level ($\alpha = 0.05$), t is the critical value searching in the t distribution table where the degree of freedom is $n-m$ and the significance level is α , v is the row vector of the Jacobi matrix corresponding to the independent variable and CM is the covariance matrix of the model coefficient estimation by nonlinear least squares regression.

All the computations were implemented on the JSFit program developed in our laboratory and written using the MATLAB language.

Results

The fitting model of J- and S-shaped CRCs

Using the JSFit program, the CRC models of [epy]Cl at seven exposure time-points to Q67 were developed. The models included 20 J-shaped CRC models at the times of 4, 6, 8, 10 and 12 h and two S-shaped CRC models at the times of 0.25 h and 2 h (Table 1).

From Table 1, all the fitted R_{adj}^2 s are larger than 0.99, while the RMSEs are less than 0.045 and AICs less than -21 , which shows that all the CRC models had a good fitting ability to the observed data. In particular, the seven-parameter models, HM7-1 and HM7-2, were better than HM6 and HM5, with the R_{adj}^2 s of HM7-1 and HM7-2 being larger than 0.998, RMSEs less than



Table 1 Model coefficients and GoFs of 22 fitting CRC models for the luminescence inhibition effects of [epy]Cl against Q67

Time	Model	E_m/E'_m	EC_{mid1} (E-3) ^a	H_1	E_{max}/E'_{max}	EC_{mid2} (E-3) ^a	H_2	E_0	R_{adj}^2	RMSE	AIC
12 h	HM6	-1.901	10.79	1.402	1.189	7.354	2.677		0.9955	0.03584	-23.26
	HM7-1	-0.481	2.987	4.223	1.085	12.33	2.328	-0.066	0.9972	0.02832	-25.71
	HM7-2	-0.409	2.999	4.298	1.151	12.36	2.334	-0.066	0.9972	0.02826	-25.73
	HM5	-0.526	3.095	1.815		11.25	2.759		0.9931	0.04449	-21.00
10 h	HM6	-1.450	8.095	1.544	1.094	6.908	2.809		0.9944	0.04082	-21.90
	HM7-1	-0.394	2.752	6.731	1.027	10.87	2.629	-0.068	0.9985	0.02135	-28.66
	HM7-2	-0.322	2.763	6.904	1.094	10.89	2.636	-0.068	0.9985	0.02132	-28.67
	HM5	-0.602	3.612	1.872		9.291	2.709		0.9934	0.04439	-21.03
8 h	HM6	-4.333	9.165	2.374	1.006	4.159	3.069		0.9963	0.03309	-24.09
	HM7-1	-0.354	2.774	6.368	0.987	9.562	2.840	-0.046	0.9989	0.01761	-30.66
	HM7-2	-0.305	2.783	6.451	1.033	9.574	2.846	-0.046	0.9989	0.01764	-30.65
	HM5	-0.913	4.776	2.202		7.006	2.488		0.9962	0.03353	-23.95
6 h	HM6	-0.432	3.525	2.431	0.939	8.009	3.141		0.9987	0.01920	-29.76
	HM7-1	-0.263	2.671	5.591	0.931	8.979	3.407	-0.028	0.9995	0.01199	-34.67
	HM7-2	-0.234	2.676	5.613	0.959	8.983	3.409	-0.028	0.9995	0.01198	-34.68
	HM5	-0.688	6.369	1.645		6.942	3.882		0.9979	0.02427	-27.32
4 h	HM6	-0.622	6.174	1.873	0.923	5.957	4.793		0.9987	0.01846	-30.17
	HM7-1	-111.662	5.517	4.384	0.919	0.3921	1.822	-0.095	0.9994	0.01209	-34.58
	HM7-2	-6.706	5.413	4.388	0.940	1.894	1.907	-0.024	0.9993	0.01348	-33.45
	HM5	-153.843	4.900	4.367		0.1314	1.359		0.9960	0.03220	-24.38
2 h	Hill	-0.038			0.753	8.006	2.869		0.9917	0.03686	-22.96
0.25 h	Hill	-0.056			0.884	5.456	1.555		0.9967	0.02342	-27.69

^a E-3 represents the unit of data as 10^{-3} mol L⁻¹.

0.022 and AICs less than -28.66, except for the models at 12 h. As shown above, the integrated HM7-1 or HM7-2 model could describe the J-shaped CRCs well, while the single Hill model could describe the S-shaped CRCs well.

The characteristic parameters of J-shaped CRCs

For the monotonic S-shaped CRC that can be described by one Hill equation with four model coefficients (E_m , E_{max} , EC_{mid2} and H_2), the four coefficients can fully characterize the shape and location of the S-shaped CRC, and the coefficients are the characteristic parameters. However, seven model coefficients in the integration model come from the two Hill equations and cannot really depict the specific shape and location of a J-shaped CRC. We think that at least five parameters, such as the median stimulative effect concentration in the descendant phase (left) (EC_{msL}), the minimum effect concentration (EC_{min}), the minimum effect or maximum stimulative effect (E_{min}), the median effect concentration (EC_{50}) and the zero effect concentration point (ZEP), are needed to characterize the specific shape and location of a J-shaped CRC or hormetic CRC (HRC). Here, EC_{min} can be solved by letting the derivative of the J-shaped CRC to the concentration variable be zero: $\partial E(C)/\partial C = 0$. Then, E_{min} can be solved by substituting the EC_{min} into eqn (4) or (7). The values of EC_{msL} , EC_{50} and ZEP can be solved by dichotomy, where we let the effect be $E_{min}/2$, 50% and 0, respectively. We called the four concentrations and one effect the characteristic parameters of a J-shaped CRC, and the characteristic parameters of [epy]Cl obtained from the HM7-1 or HM7-2 are shown in Table 2. The characteristic parameters are some important points on a J-shaped CRC and show the change regulation of the effect with the concentration, where each characteristic parameter is part of the J-shaped CRCs. However,

the model coefficients were only used to construct the model, and they only indirectly reflect the change regulation of the effect with the concentration, but generally speaking, the model coefficients can't reflect the substantive characteristics of a J-shaped CRC.

In JSFit program, at least five model coefficients, one effect (E_m), two concentrations (EC_{mid1} and EC_{mid2}) and two slopes (H_1 and H_2) are needed to construct a model, which implies that at least five characteristic parameters can fully characterize the J-shaped CRC, and the combination of four concentrations and one effect proposed in this paper is an optimal selection. When the characteristic parameter E_{min} is determined, the shape and location of the descendant phase can be characterized by two concentrations (EC_{min} and EC_{msL}), while the shape and location of the ascendant phase can be characterized by two concentrations (ZEP and EC_{50}).

Whether a single model coefficient can reflect the change of the data depends on the model coefficient E_m . E_m is a virtual parameter that can reflect the size of E_{min} on a J-shaped CRC to a certain extent, but it is always less than the real E_{min} . When E_m is closer to E_{min} , the model coefficient will be closer to the value (including characteristic parameters) on the J-shaped CRC. For example, at 12 h, when the model coefficient E_m (from -190.1% to -48.1%) is close to E_{min} (about -30%), the model coefficient EC_{mid1} , from 10.79×10^{-3} mol L⁻¹ to 2.987×10^{-3} mol L⁻¹, is close to the characteristic parameters of EC_{msL} (about 2.0×10^{-3} mol L⁻¹), and the other model coefficients are close to the corresponding value for the J-shaped CRC.

Model coefficient initialization

On the one hand, because solution of the model coefficients is an iterative process, each coefficient to be solved requires an



Table 2 Five characteristic parameters of J-shaped CRCs of [epy]Cl against Q67

Time	Model	EC _{msL} (E-3) ^a	ZEP (E-3) ^a	EC ₅₀ (E-3) ^a	EC _{min} (E-3) ^a	E _{min}
12 h	HM6	1.843	8.765	15.29	4.467	-0.283
	HM7-1	2.241	8.696	15.40	4.169	-0.303
	HM7-2	2.249	8.693	15.40	4.169	-0.304
	HM5	1.767	8.909	15.05	4.365	-0.274
10 h	HM6	1.817	7.635	13.16	4.121	-0.252
	HM7-1	2.241	7.542	13.29	3.715	-0.283
	HM7-2	2.351	7.542	13.29	3.715	-0.283
	HM5	1.796	7.702	13.07	4.074	-0.248
8 h	HM6	2.119	6.694	11.60	3.890	-0.224
	HM7-1	2.361	6.663	11.65	3.631	-0.237
	HM7-2	2.363	6.661	11.65	3.631	-0.237
	HM5	2.011	6.754	11.55	3.936	-0.215
6 h	HM6	2.020	6.255	10.57	3.802	-0.170
	HM7-1	2.261	6.195	10.62	3.589	-0.182
	HM7-2	2.267	6.193	10.62	3.589	-0.182
	HM5	1.893	6.305	10.52	3.936	-0.162
4 h	HM6	2.001	5.485	9.087	3.715	-0.133
	HM7-1	2.225	5.468	9.120	3.758	-0.139
	HM7-2	2.227	5.470	9.124	3.802	-0.140
	HM5	2.273	5.348	9.342	5.470	-0.146

^a E-3 represents the unit of data as 10⁻³ mol L⁻¹.

initial value. On the other hand, it is more difficult for people who are not familiar with programming to set the initial values. In this study, according to the physical meaning of the model coefficient, several reliable initial value equations were provided by combining discrete data with calculus (eqn (8(a-g))). According to the equations, the corresponding initial coefficients could be calculated. For a certain time, selecting the average value of observed effects (three replications), the slope vectors, k_i ($i = 2, 3, \dots, n-1$) were calculated using eqn (9), and the initial values (MC_{*j,0*}) could be obtained by eqn (8(a-g)). The initial values of the model coefficients are listed in Table 3.

From Table 3, the initial values (MC_{*j,0*}) of HM7-1 and HM7-2 calculated by eqn (8(a-g)) have a strong regularity. For the J-shaped data set, $E_{0,0}$ is 0 all the time, and $E_{max,0}$ is the twelfth effect (average value), which corresponds to the maximum concentration. Because the sixth concentration point of the 12 concentration levels has the minimum inhibitory effect, so $E_{m,0}$ is the sixth effect of the data set. EC_{mid1,0} is the fifth concentration, and the corresponding slope is $H_{1,0}$, because the slope of the fifth concentration point is the minimum in the descendant phase. At 4, 6 and 8 h, the EC_{mid2,0} is the eighth concentration, and the corresponding

slope is $H_{2,0}$, while for 10 and 12 h, EC_{mid2,0} is the ninth concentration and the corresponding slope is $H_{2,0}$ based on the maximum slope of the ascendant phase. For S-shaped CRC, $E_{m,0}$ and $E_{max,0}$ are the first and twelfth effects, which correspond to the minimum and maximum concentrations, respectively. Because the slope of the eighth concentration point is the maximum, EC_{mid2,0} is the eighth concentration, its corresponding slope is $H_{2,0}$. For the HM6 and HM5 models, the corresponding values are chosen as the initial values of the model coefficients.

Model selecting

For the monotonic S-shaped concentration-effect data, there are two manifestations: the effect increasing with concentration (monotonic increasing S-shaped) and the effect decreasing with concentration (monotonic decreasing S-shaped). For the non-monotonic J-shaped/hormetic concentration-effect data, there are three categories:

Hdata 1: the effect tends to 0 at low concentration and to 1 at high concentration.

Hdata 2: the effect tends to 0 at low concentration and is less than 1 at high concentration.

Table 3 Initial values of model coefficients of [epy]Cl against Q67

Time	$E_{m,0}/E'_{m}$	EC _{mid1,0} (E-3)	$H_{1,0}$	$E_{max,0}/E'_{max}$	EC _{mid2,0} (E-3)	$H_{2,0}$	$E_{0,0}$
12	-0.314	2.637	3.4036	0.979	12.41	2.671	0
10	-0.286	2.637	3.5626	0.979	12.41	2.698	0
8	-0.234	2.637	3.7687	0.963	8.531	2.843	0
6	-0.179	2.637	3.8234	0.933	8.531	3.233	0
4	-0.137	2.637	3.8365	0.904	8.531	3.285	0
2	-0.048			0.793	8.531	2.410	0
0.25	-0.038			0.841	8.531	1.730	0



Hdata 3: the effect is larger than 0 at low concentration and less than 1 at high concentration.

According to the characteristics of the concentration–effect data, the appropriate model should be selected in the JSFit program. For monotonic increasing S-shaped data, the ascending Hill is chosen, while for monotonic descending S-shaped data, the descending Hill is selected. The ascending Hill and the descending Hill have different initial values for the model coefficients. For non-monotonic hormetic data, the model with fewer parameters should be chosen as much as possible to avoid overfitting. Therefore, the HM5 model is selected for Hdata 1, the HM6 model for Hdata 2, and the HM7-1 or HM7-2 model for Hdata 3.

Application of JSFit

JSFit can be used to fit the concentration–effect data with S-shaped and/or J-shaped CRC profiles, whether the data are from a single pollutant or mixture. Fig. S3† provides the experimental concentration–effect scatters and the fitting CRCs for some chemical and mixture examples, including pesticide metalaxyl (Met) using the ascending Hill model, ionic liquid [epy]Br using the HM5 model (belonging to Hdata 1), antibiotic polymyxin B sulfate (POL) using the HM7-1 model (Hdata 3), and a complex personal care product mixture using the HM6 model (Hdata 2). From Fig. S3,† various CRCs could be well fitted to the experimental data.

Discussion

Model prediction evaluation

Due to the limited number of observation data, 1/3 of the J-shaped data was equidistantly selected as a test set, and the remaining 2/3 concentration–effect data were used as training sets. Because the effect at low concentration was near to 0, the model coefficient E_0 could be ignored. Furthermore, it was found that HM6 was better than HM5. Therefore, HM6 was chosen to analyze the model prediction ability. The training set was used to model the J-shape data, and the effects of various concentrations in the test set were predicted by the training set model. The obtained q_{ext}^2 values are listed in Table S3.† Fig. 3 depicts the relationships between the experimental points and the fitting curves of [epy]Cl at 12, 10, 8 and 6 h. As can be clearly seen, apart from 4 h, all the experimental points at the other times almost fall on the fitting curves. The predictive q_{ext}^2 values at 12, 10, 8 and 6 h were 0.9884, 0.9940, 0.9989 and 0.9954, respectively, which were the same as the estimated R^2 (0.9978, 0.9954, 0.9966 and 0.9992), showing a good prediction ability of HM6.

It is noted that the fitting curve (solid blue) of the lower left panel in Fig. 3 partly deviates from the dashed line based on all experimental data. However, if one data point (○) in the test set is adjusted, the fitting curve based on the training set overlaps with the curve based on all the experimental data (see the lower right panel in Fig. 3), which explains that the selection of the training set affects the predictive potential of HM6. So, the training set to be used to develop a predictable model has to

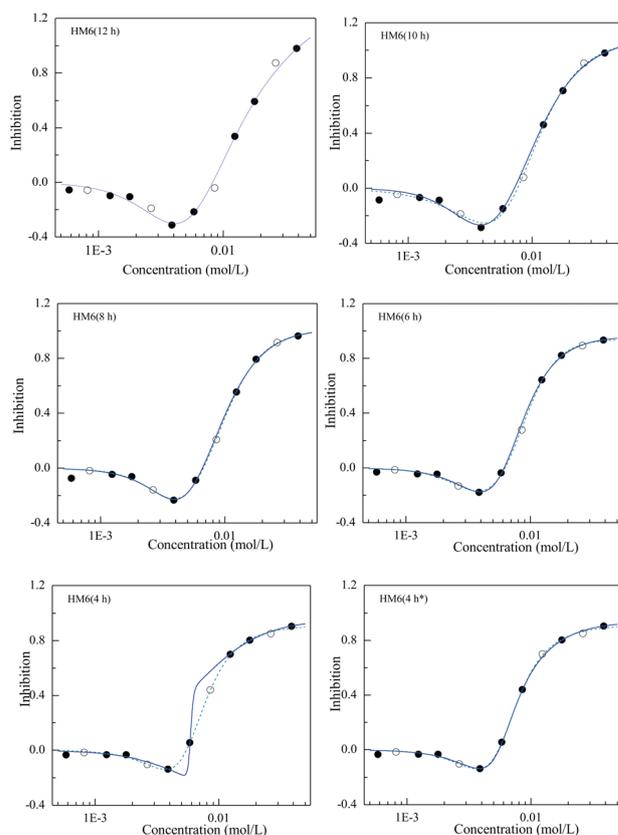


Fig. 3 The CRCs of [epy]Cl against Q67 at different times of 4, 6, 8, 10 and 12 h where “●” refers to the scatters of the training set, “○” to the scatters of the test set, “—” to the fitted CRC based on the training set and “⋯” to the fitted CRC based on all the experimental data.

contain enough data points and cannot lack some important information of CRC, such as the minimum effect or the up and down slope. In particular, the number of model coefficients selected should be as little as possible, *i.e.* using a simple model rather than a complex model¹⁸ and enough concentration–effect data to develop the CRC model.

Model comparison analysis

Currently, compared with other models (M1, M2, M3 and M4), the M5 model, which has been widely used to describe hormetically the concentration–response relationship, has a better fitting quality.^{9,11,22} However, the model coefficients obtained by the M5 model fitting the concentration–effect of [epy]Cl on *Vibrio Qinghaiensis* Q67 distort the physical meaning. For example, for the model coefficient min, the description of the minimum inhibitory ratio of hormetic CRCs is more than 0 sometimes.²² Although, the M5 model has such shortcomings, the fitting quality of M5 to the hormetic concentration–effect data is acceptable, which is the reason why it is so widely used.

Five characteristic parameters (EC_{msL} , EC_{min} , E_{min} , EC_{50} and ZEP) propounded in this paper can characterize the specific shape and location of a J-shaped CRC or hormetic CRC (HRC). The model coefficient E_m in HM5 is similar to min in M5, but the characteristic parameter E_{min} truly reflects the minimum effect of the J-shaped CRC. On the other hand, the fitting quality



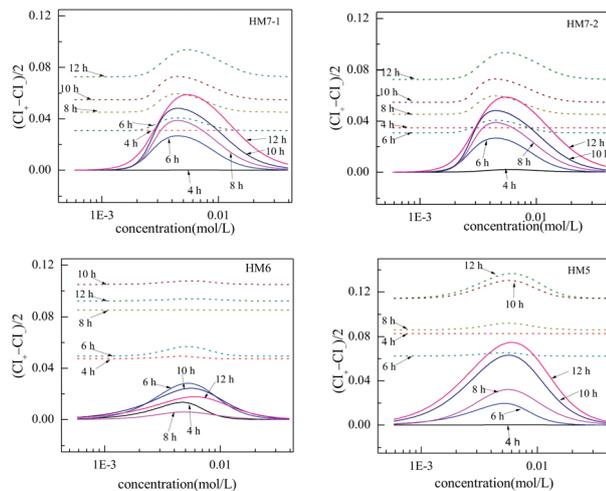


Fig. 4 Plot of the width of confident intervals, $(CI_+ - CI_-)/2$, versus the concentration, where “—” and “···” are based on FCIs and OCIs of the fitted CRCs of [epy]Cl, respectively.

of HM5 is more optimal than M5, as seen from the results of three GoFs, R_{adj}^2 , RMSE and AIC in Table 4 (also see Fig. S4†).

Analysis of the confidence intervals

The confident intervals (CIs), FCIs or OCIs, can describe the uncertainty in toxicity experiments and the function fit. The confident interval width (CIW), defined as $(CI_+ - CI_-)/2$, can quantitatively reflect the fitting quality. The CIWs from some models, such as HM6, HM7-1, HM7-2 and HM5, change with the concentration, as shown in Fig. 4.

It can clearly be seen that the variation regularity of CIW of HM7-1 is similar to that of HM7-2, whether the CIW is based on FCI or OCI. The CIW from HM6 was the minimum among the four models, while the CIW from HM5 was the maximum. For example, the CIW based on FCIs from HM6 at 12 h was 0.017, while that from HM5 was 0.075, and the CIW from HM5 is 4.4 times that from HM6. On the other hand, the changes of CIWs with concentration from the four models had a similar variation regularity, that is, the CIW based on FCIs increased from 0 to the maximum and then decreased from the maximum to 0, while the CIW based on OCIs increased from a small value to a maximum and then decreased from the maximum to another small value. On the whole, the CIW based FCIs values are less than those based on OCIs.

Although the variation regularity of the CIW based on OCIs from different models with concentration was similar to that on FCI, the steepness of change was smoother and the CIW value

was larger. Since OCI is more dependent on RMSE, the difference in the CIW based on OCIs from different models can be estimated simply according to RMSE, thus the choice of model based on RMSE is equivalent to the choice of model based on OCIs.

Of course, we can also test the reliability and identify outliers in the original observation data. There is a way to judge outliers by utilizing an expanded uncertainty,³¹ however, this method is only used with repeat measurements made at the same concentration, and the more data are measured, the more accurate the test will be. For the CRCs data, the number of measurement effects at the same concentration was limited, and the variation regularity of the CRCs data with the change of concentration is known, so we cannot test the reliability and identify outliers by this method. Therefore, a method based on OCIs to identify outliers was developed. A confidence intervals relative error (RE_{OCI}) was used to quantitatively describe the relative deviation of the predictive value from the experimental value. The RE_{OCI} is defined as follows.

$$RE_{OCI} = \frac{y - \hat{y}}{(OCI_+ - OCI_-)/2} \quad (18)$$

Substituting eqn (17) into eqn (18) gives:

$$|y - \hat{y}| = |RE_{OCI}(OCI_+ - OCI_-)/2| \\ = \left| RE_{OCI} t_{(n-m, \frac{\alpha}{2})} \sqrt{RMSE^2 + v \cdot CM \cdot v^T} \right| \quad (19)$$

Referring to the expanded uncertainty³¹ and introducing an adjusting factor (λ) gives:

$$|y - \hat{y}| = \left| RE_{OCI} t_{(n-m, \frac{\alpha}{2})} \sqrt{RMSE^2 + v \cdot CM \cdot v^T} \right| > \lambda \cdot K \cdot RMSE \quad (20)$$

where K is a coverage factor whose value is often 2 (confident probability of 95%) or 3 (99%).

Therefore, when the following inequality is true, the corresponding data point can be considered as an outlier:

$$|RE_{OCI}| > \max \left(\frac{\lambda \cdot K \cdot RMSE}{t_{(n-m, \frac{\alpha}{2})} \sqrt{RMSE^2 + v \cdot CM \cdot v^T}} \right) = \lambda \frac{K}{t_{(n-m, \frac{\alpha}{2})}} \quad (21)$$

The ratio of the number of outliers in a CRC to all the data, P_{out} , and the ratio of the number of non-outliers to all the data, P_{rel} , are respectively used to describe the proportion of outliers and the reliability of the CRC data.

Table 4 Three goodness-of-fit parameters (GoFs) of two models (HM5 and M5) in five different times

GoFs	4 h		6 h		8 h		10 h		12 h	
	HM5	M5	HM5	M5	HM5	M5	HM5	M5	HM5	M5
R_{adj}^2	0.9960	0.9607	0.9979	0.9842	0.9962	0.9879	0.9934	0.9892	0.9931	0.9922
RMSE	0.03220	0.08343	0.02427	0.05475	0.03353	0.04939	0.04439	0.0469	0.04449	0.03906
AIC	-24.38	-14.46	-27.32	-18.85	-23.95	-19.92	-21.03	-20.46	-21.00	-22.37



$$p_{\text{out}} = \frac{\text{num}(|\text{RE}_{\text{OCI}}| > \lambda \cdot K / t_{(n-m, \alpha/2)})}{n} \quad (22)$$

$$p_{\text{rel}} = \frac{\text{num}(|\text{RE}_{\text{OCI}}| \leq \lambda \cdot K / t_{(n-m, \alpha/2)})}{n} \quad (23)$$

where $\text{num}(Y)$ refers to the number of data when Y is true. If $P_{\text{rel}} \geq P_a$ or $P_{\text{out}} < \alpha$, the original CRC data are reliable. Here, $\alpha = 0.05$ and $P_a = 0.95$ when $\lambda = 2$ and $K = 2$. For a given model, the value of $\lambda K / t_{(n-m, \alpha/2)}$ is a constant, being called the threshold. The threshold values were 1.556, 1.635, 1.692 and 1.692 for HM5, HM6, HM7-1 and HM7-2.

The above method based on OCIs was used to analyze the experimental concentration–effect data of [epy]Cl at four exposure times, and the results are listed in Table S4.† Although some P_{out} values were slightly larger than 0.05, the theoretical outliers were at most 1.8 of 36 experimental data points, while the number of outliers calculated by all the models was not more than 2, which still shows that the experimental data were reliable and this set of data was believable. The way to deal with these outliers is to use the average of other data instead of the outliers when computing.

Conclusions

To process the J-shaped concentration–response data with uncertainty, the JSFit method or program was developed to quantitatively and rationally fit the J-shaped (and the S-shaped) CRC. JSFit could automatically initialize the model coefficients on the basis of the specific meaning of each model coefficient and then constructs the observation-based confident intervals (OCIs) of the CRC. Also, it was used to analyze the reliability and outliers of the experimental CRC data.

Conflicts of interest

There are no conflicts to declare.

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