

MODELING THE EFFECTS OF BINARY MIXTURES ON SURVIVAL IN TIME

JAN BAAS,*† BART P.P. VAN HOUTE,‡ CORNELIS A.M. VAN GESTEL,§ and SEBASTIAAN A.L.M. KOOIJMAN†

†Vrije Universiteit Amsterdam, Department of Theoretical Biology, De Boelelaan 1085, 1081 HV, Amsterdam, The Netherlands

‡Vrije Universiteit Amsterdam, Department of Molecular Cell Physiology, De Boelelaan 1085, 1081 HV, Amsterdam, The Netherlands

§Vrije Universiteit Amsterdam, Department of Animal Ecology, De Boelelaan 1085, 1081 HV, Amsterdam, The Netherlands

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Abstract—In general, effects of mixtures are difficult to describe, and most of the models in use are descriptive in nature and lack a strong mechanistic basis. The aim of this experiment was to develop a process-based model for the interpretation of mixture toxicity measurements, with effects of binary mixtures on survival as a starting point. The survival of *Folsomia candida* was monitored daily for 21 d during the exposure to six binary mixtures of cadmium, copper, lead, and zinc in a loamy sand soil. The measurements were used to develop a model to describe survival in time. The model consists of two parts: A one-compartment model that describes uptake and elimination of the compounds, and a hazard model describing survival. The model was very successful in describing the data and at finding possible interactions. The mixture of copper and lead showed a slight antagonistic effect, the other mixtures showed no interaction. The model is straightforward in its biological assumptions and does not require a mode-of-action a priori choice of the mixture that might influence the modeled interaction of the components in the mixture. The model requires measurements at intermediate time points, but runs with relatively few parameters and is robust in finding interactions. When mixture effects are considered at only one time point, care should be taken with the assignment of interactions because these may be different for different points during the time course of the experiments.

Keywords—Time modeling Survival Mixtures Interaction

INTRODUCTION

Chemical risk management, in most cases, is based on effects induced by single chemicals. In real life, however, organisms are exposed to mixtures of numerous chemicals. In addition, single chemicals may be transformed by organisms, which may lead to mixtures that do not exist in nature [1] and where metabolites may be responsible for effects [2,3]. To deal with mixture and other effects, safety factors sometimes are used to derive safe concentrations in the environment. Nevertheless there is an increasing concern about the possibility that exposure to low concentrations of multicomponent mixtures may induce unexpected effects [4–9].

Mostly organisms are exposed to mixtures of unknown composition. However, when the composition of a mixture is known, there are two basic models in use for predicting the effect of the mixture from its individual components: Concentration addition (CA) [10,11] and independent action (IA) [12]. Concentration addition is used to predict the effect of substances with a common target site and a similar mode of action. The effects of substances with dissimilar mode of action and different target sites are described by the concept of IA. Detailed descriptions of these approaches can be found elsewhere [4,13–15].

Because there is no real consensus on how the models relate conceptually to each other [16,17], they are sometimes treated as equally valid concepts [15], and one or both models may be selected depending on the chemicals in the mixture. Altenburger et al. [13] and Backhaus et al. [14] chose to analyze both concepts and compared results of both models with their data. They selected the model that best fits their data as the preferred model, though this may be difficult to decide and

possible interactions that might influence the best fitting model are not taken into account.

Interactions between the individual components in a mixture may cause severe effects [1]. For binary mixtures, the approach by Jonker et al. [15,18] is based on finding deviations (interactions) from the standard CA and IA models in a statistically sound way. They not only model synergism and antagonism, but also more complex responses, such as dose level- or dose ratio-dependent interactions. Their approach is descriptive, with the log-logistic dose-response model as a starting point using fixed time points.

Basically, effects of mixtures are difficult to model, especially when the number of components in a mixture becomes larger, causing the number of possible interactions to increase rapidly. Most of the models in use are descriptive in nature and lack a strong mechanistic basis. A need exists for a better understanding of the dynamics of the effects of mixtures, therefore measurements with intermediate time points are needed for the development of models taking into account the processes of uptake and elimination [1,19]. Furthermore, we will show that selection of a process-based model eliminates the need to predecide the components' mode of action within mixtures, as is required for use of most current standard models and thereby implicitly assigns the interpretations of potential interactions by components.

The aim of the present study was to develop a process-based model, free of choices, for the interpretation of mixture toxicity measurements, for binary mixtures, taking survival as a starting point.

MATERIALS AND METHODS

Survival was monitored in time for the collembolan *Folsomia candida* exposed to binary mixtures of toxicants in soil. We chose metals as a toxicant because they can be present in

* To whom correspondence may be addressed
(jan.baas@falw.vu.nl).

the environment at toxic levels, do not degrade (although may transform to different chemical species), and are relatively easy to amend into soil. Selecting four metals, cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn), allowed the testing of six binary mixtures of metals. The results were used to develop a process-based model for the effects of binary mixtures on survival.

Exposure experiment

All exposure experiments were carried out with *F. candida*. The organisms were taken from a culture that had been maintained at our laboratory for several years. The organisms were exposed on Lufa 2.2 standard soil (Speyer, Germany), a loamy sand containing 4% organic matter and a pH (KCl) of 6.1. The soil was dried and amended with aqueous solutions of four metal salts, $\text{CdCl}_2 \cdot 2\frac{1}{2}\text{H}_2\text{O}$ (Sigma-Aldrich, Darmstadt, Germany, >99.2% pure), $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ (Merck, Steinheim, Germany >99.5% pure), $\text{Pb}(\text{NO}_3)_2$ (Merck, >99.5% pure), and ZnCl_2 (Merck, 100% pure).

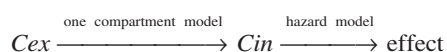
Prior to exposure, the soil moisture content was brought to 20%, corresponding to approximately 45% of water-holding capacity. The springtails were exposed in plastic jars (5 cm in diameter and 3 cm in height) filled with approximately 15 g of contaminated soil. Chronosequential measurement of *F. candida* survival was essential for proper interpretation of the data and model results. Because *F. candida* normally live in the soil, counting them each day would be difficult without disturbing the experiment. Therefore the springtails were forced to live on top of the soil by compacting to create a smooth surface.

All jars were kept in a climate room with a temperature of $18 \pm 1^\circ\text{C}$, a relative humidity of 75%, and a day:night cycle of 12:12 h. Each jar received 10 adult springtails of approximately the same size and age (acquired by sifting the springtails through a sieve of 450 μm). The organisms were fed a few grains of dry baker's yeast (Oetker BV, Veenendaal, The Netherlands). All experiments were carried out with three replicate jars for each treatment. Survival was counted on a daily basis and dead organisms were removed from the jars. Every second day the jars were weighed and water loss was compensated for with deionized water.

Before the main experiment with mixtures was started, a range-finding experiment was performed with the springtails exposed to the individual metals. In the main experiment, survival was followed for 21 d for six metal combinations (Cd/Zn, Cd/Cu, Cd/Pb, Cu/Zn, Cu/Pb, Zn/Pb). In all experiments, six concentrations (including a control concentration) per metal were used in a full factorial design. The tested concentrations were spaced a factor of two and ranged from 1.78 to 28.5 $\mu\text{mol/g}$ dry soil for Cd, 6.30 to 101 $\mu\text{mol/g}$ dry soil for Cu, 3.86 to 61.8 $\mu\text{mol/g}$ dry soil Pb, and 12.2 to 196 $\mu\text{mol/g}$ dry soil for Zn.

Modeling

Modeling effects of single compounds. The basic modeling scheme is



where Cex = external concentration ($\mu\text{mol/g}$ dry soil) and Cin = internal concentration ($\mu\text{mol/g}$ fresh wt animal).

The model assumes that the internal concentration determines the effect. Because internal concentrations rarely are

known, a one-compartment model is used to calculate internal concentrations from external concentrations. We assume that, when the internal concentration exceeds a certain threshold, the probability to die starts to deviate from the control. The external concentration that associates the internal threshold is called the no-effect concentration (NEC), i.e., the environmental concentration below which no effect occurs. The "power of the effect" is described by a killing rate. If the killing rate is higher, the compound of interest is more toxic. An elaborate description of the model can be found in Kooijman and Bedaux [20].

When exposure is to a single compound, this leads to three (time-independent) parameters to describe the effect: The killing rate, expressed in concentration⁻¹ d⁻¹; the elimination rate (k_e), expressed in d⁻¹; and the NEC, expressed in environmental concentration $\mu\text{mol/g}$ dry soil. A fourth parameter, the control mortality rate (λ) expressed in d⁻¹, is used to correct for effects seen in the controls and easily can be included in the calculations.

Because the model describes uptake and elimination, measurements in time are crucial for the parameter estimates. If the elimination rate of the compound is known (or measured separately), interpretation of data with observations at only one time point is possible.

Modeling effects of mixtures. For the exposure to two compounds simultaneously, the effects are described by the toxicity parameters of the individual compounds, extended with an interaction parameter called d_{AB} , which is expressed as

$$\text{Effect} \sim [\text{A}] + [\text{B}] + d_{AB}[\text{A}][\text{B}]$$

When d_{AB} equals zero, there is no interaction between the two components. Antagonism occurs if $d_{AB} < 0$, and synergism occurs if $d_{AB} > 0$. This leads to a total of eight parameters to be estimated from the survival dataset for each binary mixture: The control mortality rate and the interaction parameter for the mixture and the NEC, the killing rate, and the elimination rate for both components in the mixture.

More generally, for mixtures the model contains three parameters per compound describing the effect of the individual compound, a control mortality rate for the experiment, and one interaction parameter for each pair of compounds. So for a mixture of k compounds, a total of 1 (control mortality) + $3k$ (effect parameters for the three compounds) + $k(k - 1)/2$ interaction parameters are required.

Modeling the NEC. The NEC is an important parameter because it indicates the concentration in the environment under which no adverse effects occur and because it is a direct measure for an organism to cancel adverse effects. The NEC can be modeled in different ways. Pure independent NEC means that each component in the mixture has its own NEC irrespective of the other component in the mixture (so when both components are present at 75% of their NECs, no effect occurs). Pure addition implies that each component in a mixture attributes to the NEC (so when both components are present at 75% of their NECs, an effect will occur). This implies that a mixture consisting of many compounds is likely to exceed the NEC and therefore will have an effect. Then, there is a possibility to use models where fractions of the NEC are used by each compound, depending on the uptake and elimination characteristics of the specific compounds.

The data clearly show a concentration range where no effect occurs for all tested mixtures, so pure addition is not used as a model for the NEC. We tested three different models for the

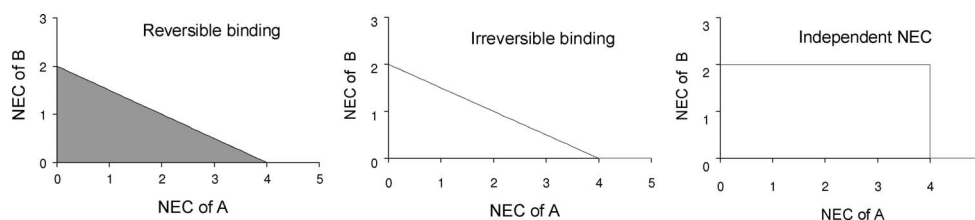


Fig. 1. Three models to cancel effects of mixtures (no-effect concentration [NEC] of compound A = 4; NEC of compound B = 2): Reversible binding (where the NEC of one compound depends on the other), irreversible binding (where the NEC remains fixed once effects show up), and the independent action model (where the NEC of one compound is independent of that for another compound).

NEC. The first model is called reversible binding, the second is called irreversible binding, and the last is called independent NEC.

In the reversible and irreversible binding models the (limited) capacity to cancel adverse effects by an organism is considered to depend on the concentration of the two compounds in the organism and each component takes fractions of the NEC. In the first model, the NEC varies with the internal concentrations of the two compounds. In a biological sense this can be seen as reversible binding to receptors. In the second model, once the NEC is exceeded, the fractions that the components in the mixture take in the NEC become constant. In a biological sense this can be viewed as irreversible binding to receptors in the organism. In the third model, each component has its own NEC irrespective of the concentration of the other.

The way in which the NEC is dealt with in the different models is shown in Figure 1. The details of the models are shown in the *Appendix*.

All models were created as freeware software in Octave (University of Wisconsin, Madison, WI, USA, 1998–2006 J.W. Eaton). MATLAB versions (Natick, MA, USA, 1994–2006 The MathWorks) are also available. All programs run on desktop computers or on UNIX systems.

RESULTS AND DISCUSSION

Measurements

The exposure of the *F. candida* was different from the International Organization for Standardization experiment [21] because the organisms were kept on top of the soil to allow a daily count of the survival. Still the median lethal concentrations for this experiment are similar to other experiments [22–24], as shown in Table 1.

The pH (KCl) of the test soil decreased from 5.76 ± 0.10 (standard deviation [SD], $n = 6$) in the control to 4.08 ± 0.01 (SD, $n = 3$) for the highest copper concentration. Individual Cd, Pb, and Zn treatments had less effect on soil pH with mean pH (KCl) values ($\pm$ SD, $n = 3$) of 6.63 ± 0.03 , 4.95 ± 0.43 , and 4.97 ± 0.16 , respectively. After three weeks of exposure,

Table 1. Median lethal concentration (LC50) values for *Folsomia candida* for this and other experiments (all values in $\mu\text{mmol/g}$ dry soil)

Metal	LC50			
	(21 d) this experiment	(28 d) [22]	(28 d) [23]	(28 d) [24]
Pb	11–42	5–13		
Cd	13–29		5–12	
Zn	49–96			11

survival in the control experiment was between 80 and 93%, which was considered acceptable [21]. The results of all measurements were consistent and there was little difference between the triplicate measurements. For the modeling of the data, the results of the triplicate measurements were combined to start with 30 live springtails per concentration.

As a typical example of the results observed, Figure 2 shows survival as a function of time for the mixture of Cu and Cd. The figure shows survival for a fixed Cd concentration of $14.2 \mu\text{mol/g}$ dry soil and an increasing concentration of Cu.

Note that care should be taken with the interpretation of the results at the high metal concentrations because anionic effects are expected to influence survival at high concentrations. For *F. candida*, nitrate proved to be more toxic than chloride [23]. For the model, the information of the highest concentrations is very limited because nearly all exposed organisms have died in a very short time. The information that is most important for the model is at lower concentrations, especially those determining the difference between no effect and a partial effect. At these concentrations we do not expect an effect of the anions on survival. Therefore possible effects of the anion on the survival of the springtails do not play a major role in the modeling work and the results of the exposure experiment were used to demonstrate the model.

Process-based modeling

Parameter estimates. In three steps, eight parameters were estimated for each two metal combination. The first step is to estimate the parameter values for the individual metals by using the survival data with a concentration of the other metal

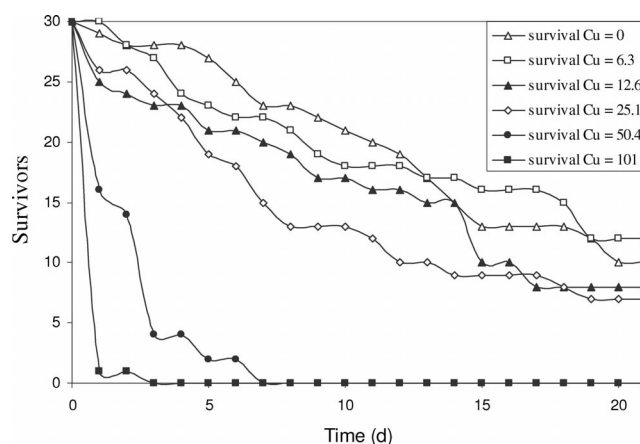


Fig. 2. Survival of *Folsomia candida* during 21 d of exposure to a copper cadmium mixture in Lufa 2.2 standard soil (Speyer, Germany). Survival is shown for a fixed cadmium concentration of $14.2 \mu\text{mol/g}$ dry soil and a copper concentration that increases from 0 to $101 \mu\text{mol/g}$ dry soil.

Table 2. Deviance of model values for the mixture of cadmium and copper for the three different ways to model the no-effect concentration (NEC)

Model	Deviance
Reversible binding	1,071.4
Irreversible binding	1,021.7
Independent NEC	1,225.8

being zero. In the second step, these parameters values were used as starting parameters for the maximum likelihood estimates for the mixtures of metals. Finally, different starting values were used to check the maximum likelihood estimates.

For each of the six combinations of metals, we had a total of 792 data points (22 d and 6 concentrations for each metal), which were used simultaneously for the parameter estimates. The difference in performance for the different models for the NEC was not very large. But the irreversible binding model gave the best results in describing the measurements as is shown in Table 2.

All further calculations were carried out with the irreversible binding model where the two metals compete to approach the limit of for the NEC, and each metal is assigned a constant fraction of the NEC once effects are statistically significant.

In Table 3, the estimates for the NEC, the killing rate, the elimination rate, and the interaction parameter are shown for the different mixture experiments. The control mortality rate was between 0.011 and 0.016 d⁻¹ for all experiments and is not shown.

The value of the elimination rate proved difficult to estimate, because it generally was high (in most cases larger than 2 d⁻¹) and could not be discriminated easily from (much) higher values. A high value for the elimination rate implies that the equilibrium between the external and the internal concentration is set rapidly. When it is higher than approximately 2 d⁻¹, the steady state is set before the first measuring point (1 d) and a further discrimination is not possible. Therefore the lowest value was chosen that did not improve the estimates, and this parameter was treated as a fixed value in the other estimates.

Modeling of effects

Calculation of survival with the parameters for the individual compounds already gave a good match with data. This was the first clue that interactions, with an influence on survival, between two metals were low. The parameter estimates, shown in Table 3, confirm this. Only for the mixture experiment with copper and lead was the parameter d_{AB} significantly ($p < 0.05$) different from zero, showing a slight antagonistic effect in the data. All other experiments showed no significant interaction (the interaction parameter was not significantly [$p < 0.05$] different from 0). In Figure 3, the results of the modeling work are shown. In the figures, a constant concentration of cadmium is shown with a varying concentration of copper.

The data were analyzed further for the occurrence of varying interactions in time, to see whether or not a pattern could be found (e.g., an interaction only when a certain internal concentration is reached, similar to the dose/response or dose/level interactions [15]). Many of these interactions would be easy to program and would include one extra parameter in the modeling work, though no such pattern could be found.

The model gave an excellent agreement between the mea-

Table 3. Parameter estimates for the no-effect concentration (NEC), killing rate, elimination rate, and interaction parameter (d_{AB}) for the toxicity of the different metals in the mixture experiments with *Folsomia candida* in Lufa 2.2 standard soil (Speyer, Germany). Numbers 1 and 2 refer to the first and the second metal in the compound, as given under mixture; * = Not significantly ($p < 0.05$) different from zero

Mix- ture	NEC ($\mu\text{m/g}$ soil)		Killing rate ($[\text{mmol/g}$ soil] ⁻¹ d ⁻¹)		Elimination rate (d ⁻¹)		d_{AB} (g soil/ μmol) ²
	1	2	1	2	1	2	
Cd Cu	32	17	33	1.7	5	1.7	0.061*
Pb Cd	3.6	38	11	0.22	5	0.5	-0.014*
Cu Pb	119	23	0.41	160	5	5	-0.935
Cu Zn	35	19	84	2.5	5	0.78	0.789*
Zn Cd	67	8.7	42	3.5	0.98	4.4	-0.176*
Pb Zn	4.2	55	25	39	0.88	3.6	-1.151*

sured and calculated survival data and allowed determination of interactions in mixtures. The approach is straightforward in its biological assumptions and does not require a great number of parameters. This process-based approach also can be extended to more than two compounds without any fundamental difficulties, but for multiple compound mixtures, the number of parameters rapidly increases and soon will become unwieldy.

Comparison of results obtained with other models

Process-based models that deal with interactions in mixtures are not (readily) available. The best-known and readily available model designed to deal with interactions in binary mixtures is the model developed by Jonker and coworkers [15,18]. They developed a way to find deviations from the two reference models, CA, and IA. The deviations are either synergism or antagonism or more complicated interactions as dose-level and dose-ratio interactions. In the latter cases, the interaction depends on the absolute dose of the toxicant or on the ratio of the applied doses of the individual compounds in the mixture.

Modeling interactions

We used an updated version of the original spreadsheet that was made suitable for survival data. The interactions obtained when applying the spreadsheet for the copper-cadmium mixture are shown in Table 4.

In the approach described by Jonker et al. [15,18], the choice of the standard model (CA or IA) must be made on the basis of assumptions about the mode of action of the compounds in the mixture. When an incorrect working mechanism is selected, supposed effects may be attributed wrongly to an interaction.

The IA model suggests a synergistic effect over the whole time period for the mixture of copper and cadmium. When time progressed, the synergistic effect became dose-level dependent, but the model still gave a synergistic effect.

When the CA model is used as a base model, the variation in interactions is much larger. During the first few days there is no interaction, then there is a synergistic effect, then again no interaction, and later in the experiment a dose-level interaction with synergism at low doses and antagonism at high doses. The switching point from antagonism to synergism is about twice the median lethal concentration value (parameter b , see Jonker et al. [15], is close to 0.5). However, care must

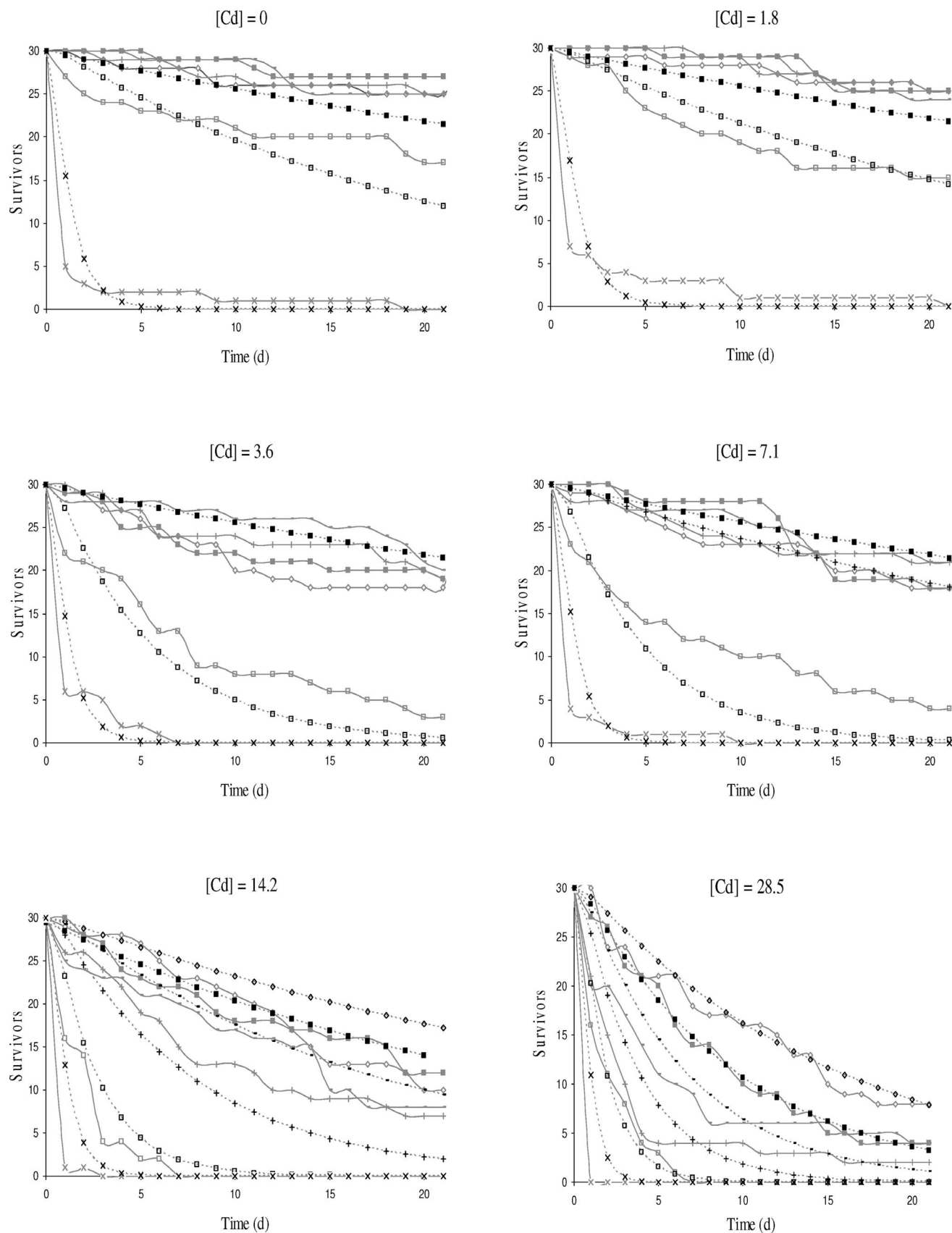


Figure 3. Comparison of measured data with the model values. In each of the individual figures, the cadmium concentration is constant and the copper concentration increases. The symbols $\diamond$, $\blacksquare$, $+$, $\square$, and $\times$ refer to a copper concentration of 0, 6.3, 12.6, 25.2, 50.4, and 101 $\mu\text{mol/g}$ dry soil, respectively. Measured survival is shown in solid lines and modeled survival is shown in dashed lines.

Table 4. Results for the interactions obtained when applying the spreadsheet model to the data for the mixture of copper and cadmium on the survival of *Folsomia candida* in Lufa 2.2 standard soil (Speyer, Germany). * = Change from antagonism to synergism at approximately 2 × median lethal concentration

Time (d)	Interactions (CA as base model) ^a	Interactions (IA as base model) ^b
2–3	No interaction, CA	Synergism
4–5	Synergism	Synergism
6	Synergism	Dose level-dependent synergism
7–9	Dose level-dependent synergism	Dose level-dependent synergism
10–15	No interaction, CA	Dose level-dependent synergism
16	Dose ratio-dependent interaction	Dose level-dependent synergism
17	No interaction, CA	Dose ratio-dependent interaction
18–21	Low doses S, high doses A*	Dose level-dependent synergism

^a CA = concentration addition model; S = synergism; A = antagonism.

^b IA = independent action model.

be taken in interpreting the data: The spreadsheet looks for deviations from the standard model and attributes the deviations to interactions. This means that (small) random variations in the survival data may change the best fitting model. The more elaborate the exposure experiment (in terms of concentration ranges and exposed organisms), the less problems random variations give. For this experiment, containing six concentrations for each metal and 30 exposed organisms, random variations can be problematic. The best fitting model can be switched from a dose level-dependent interaction to no interaction if survival is changed by only a few organisms for two or three data points. For example, if we measured 25, 26, 18, 19, 13, and 8 surviving organisms, this would lead to an interaction, whereas a measurement of 26, 25, 20, 17, 13, and 8 survivors would not.

Note that anionic effects on survival cannot be ruled out. Depending on the specific characteristics of the toxicity of the anions and compared to the metal *a* (dose dependent), the synergistic effect could be seen in the data as mortality is increased by effects of anions at high concentrations of metals and anions in both standard models. We do not see such an effect in the data we have, so we assume that all effects can be contributed to the metals.

Comparison of the spreadsheet results with the results of the process-based model

The interaction parameter in the process-based model did not significantly differ from zero, indicating that there was no interaction. Because in this case the complete dataset was modeled as a whole, minor random variations do not show up as an interaction effect. This makes the approach less sensitive to random outliers and therefore more robust.

When models are used that do not incorporate time, it is impossible to speak of the interaction in the mixture. The interaction depends on the time point that is considered.

Note that, for use of the CA or IA models, at least five parameters per time point are needed to describe the effects of the mixture when no interaction takes place. When there is an interaction, this can increase to seven parameters per time point. In the experiment described here, 119 parameters would be required to describe the results with the CA model and 133 parameters with the IA model. This number of parameters may be reduced by describing the time dependence of the median lethal concentration and slope values in the mixture (adding new parameters). The process-based model needs only eight parameters to model the complete time series though measurements in time are essential.

CONCLUSION

We developed a model that describes effects on survival of binary mixtures in time. The model was tested against an elaborate survival dataset containing six binary metal mixtures (containing copper, lead, zinc, and cadmium) and it showed excellent agreement between measured and calculated survival data. Only in the case of the mixture of copper and lead a slight antagonistic effect was found; the other mixtures showed no interaction.

The model is straightforward in its biological assumptions and does not require a mode-of-action a priori choice of the mixture, which influences possible interactions in the components in the mixture as we have shown. In addition, the model also does not require a great number of parameters. This is in contrast to nonprocess-based models, where numerous parameters are needed to give a description of a chronological measurement. The use of the model does require measurements at different intermediate time points.

This model also can be extended to more than two compounds without any fundamental difficulties, but for a multiple-compound mixture the number of interaction parameters becomes unwieldy (as with the CA/IA models).

Because the model works with the dataset as a whole, it is less sensitive to random outliers that will be appointed to an interaction than when concentration addition or independent action are used as the base models. These models look at data per time point, whereas the process-based approach looks at the complete time series at once. With concentration addition or independent action models, time should be specified when the interaction in the mixture is considered because the interaction may vary in time.

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APPENDIX

Modeling the no-effect concentration

In this appendix, the three ways to model the no-effect concentration (NEC) are worked out. The basic idea is that

the organism is supposed to be able to cancel effects of compounds with some limited capacity. We propose three different ways to cancel effects: Reversible binding, irreversible binding, and independent NEC. We start with the conceptually most straightforward case, the reversible binding, and then consider numerically more friendly alternatives. All models are based on internal concentrations, which are converted to external concentrations by a one-compartment model.

List of symbols

Symbol	Units	Meaning
c_A, c_B	mM	External concentration for compound A, B
Q_A, Q_B	mmol C-mol ⁻¹	Internal concentration for A and B
k_A, k_B	d ⁻¹	Elimination rate for A, B
P_{Ad}, P_{Bd}	l C-mol ⁻¹	Bioconcentration factor for A, B
Q_A^0, Q_B^0	mmol C-mol ⁻¹	Internal NEC for A and B
C_A^0, C_B^0	mM	External NEC for A and B
B_A, B_B	C-mol mmol ⁻¹ d ⁻¹	Killing rate for internal compound A, B
b_A, b_B	mM ⁻¹ d ⁻¹	Killing rate for external compound A, B
D_{AB}	C-mol ² mmol ⁻² d ⁻²	Internal interaction rate between A, B
d_{AB}	mM ⁻² d ⁻²	External interaction rate between A, B
h_0	d ⁻¹	Hazard rate in the blank
h_c	d ⁻¹	Hazard rate as function of toxicant concentration
$h(t)$	d ⁻¹	Total hazard rate
t_0	d	The no-effect time

Description of the three models for the NEC

The reversible binding model. Suppose that compounds A and B compete for capacity to cancel effects and that Q_A^0 and Q_B^0 are the internal NECs. No effects occur if

$$1 > Q_A/Q_A^0 + Q_B/Q_B^0$$

If this condition is not fulfilled, compounds A and B take fractions of the effect cancel capacity

$$w_A = (Q_A/Q_A^0)(Q_A/Q_A^0 + Q_B/Q_B^0)^{-1}$$

$$w_B = (Q_B/Q_B^0)(Q_A/Q_A^0 + Q_B/Q_B^0)^{-1}$$

The internal concentrations of A and B that cause effect are

$$Q_A^e = \max(0, Q_A - w_A Q_A^0)$$

$$Q_B^e = \max(0, Q_B - w_B Q_B^0)$$

Notice that the internal concentrations Q_A and Q_B change in time, so the concentrations that do not cause effects change in time, because the different compounds compete for the effect-canceling capacity.

The hazard model is used for effects on survival. The hazard rate relates to the internal concentrations as

$$h_c(t) = B_A Q_A^e(t) + B_B Q_B^e(t) + D_{AB} Q_A^e(t) Q_B^e(t)$$

Compounds A and B do not interact if $D_{AB} = 0$. This formulation of interaction corresponds to the standard in the analysis of variance and rests on the first term of the Taylor approximation of the general differentiable function of two variables. The implication is that the approximation is accurate as long as we focus on small effects. The interaction parameter is a rate, and therefore we call it the interaction rate. If it is positive we have synergism, if negative antagonism.

If the external concentrations c_A and c_B are constant, and the compounds follow (independently) a one-compartment model for the uptake and elimination, the internal concentrations are given by (assuming that the organisms were not exposed previously)

$$Q_A(t) = c_A P_{Ad} [1 - \exp(-tk_A)]$$

$$Q_B(t) = c_B P_{Bd} [1 - \exp(-tk_B)]$$

The internal concentrations typically are not known, so we define a concentration that is proportional to the internal concentration, but has the dimension of an external concentration, using the bio concentration factors P_{Ad} and P_{Bd} and substitute

$$C_A = Q_A/P_{Ad} \quad C_B = Q_B/P_{Bd} \quad b_A = B_A P_{Ad}$$

$$b_B = B_B P_{Bd} \quad d_{AB} = D_{AB} P_{Ad} P_{Bd}$$

$$C_A(t) = c_A [1 - \exp(-tk_A)] \quad C_B(t) = c_B [1 - \exp(-tk_B)]$$

$$w_A(t) = C_A(t)/C_A^0 [C_A(t)/C_A^0 + C_B(t)/C_B^0]^{-1} \quad \text{and}$$

$$w_B(t) = C_B(t)/C_B^0 [C_A(t)/C_A^0 + C_B(t)/C_B^0]^{-1}$$

$$C_A^e(t) = \max[0, c_A(t) - w_A(t)C_A^0]$$

$$C_B^e(t) = \max[0, c_B(t) - w_B(t)C_B^0]$$

$$h_c(t) = b_A C_A^e(t) + b_B C_B^e(t) + d_{AB} C_A^e(t) C_B^e(t)$$

We now have the hazard rate expressed in external parameters. The complete hazard rate is given by $h(t) = h_0 + h_c(t)$, where h_0 is the hazard rate in the blank. Effects occur at finite no-effect t_0 if $1 < c_A/C_A^0 + c_B/C_B^0$.

A consequence of this competition model for canceling capacity is that $C_A^e > 0$ if $C_B^e > 0$, and vice versa. This occurs at time t_0 , where

$$1 = C_A(t_0)/C_A^0 + C_B(t_0)/C_B^0$$

$$= [1 - \exp(-t_0 k_A)]c_A/C_A^0 + [1 - \exp(-t_0 k_B)]c_B/C_B^0$$

This time point t_0 , the no-effect time, must be obtained numerically.

From hazard rate, we move to survival probability. The survivor probability is given by $S(t) = \exp[-\int_0^t h(s) ds] = \exp(-h_0 t)$ for $t < t_0$.

For $t > t_0$, the integration of the hazard rate should be done numerically. For relative, large negative values of the interaction rate, the hazard rate can become negative, which means that we have to take the maximum of zero and the specified value.

The irreversible binding model. Alternatively the capacity to cancel effects can be frozen at the moment effects show up. Biologically this could be seen as an irreversible binding of the toxicant to a receptor. Numerically this is much simpler than the reversible binding model. Once the no-effect time is exceeded, the no-effect thresholds for the internal concentrations remain fixed, irrespective of the varying internal con-

centrations. These levels, therefore, depend on the external concentrations and the elimination rates of the compounds. We then have constant values for

$$C_A^{0B} = [1 - \exp(-t_0 k_A)]c_A$$

$$C_B^{0A} = [1 - \exp(-t_0 k_B)]c_B$$

$$w_A = [1 - \exp(-t_0 k_A)]c_A/C_A^0$$

$$w_B = [1 - \exp(-t_0 k_B)]c_B/C_B^0$$

The survivor probability can now be evaluated analytically (given the numerically obtained value for t_0) for $t > t_0$: $S(t) = \exp[-h_0 t - b_A g_A(t) - b_B g_B(t) - d_{AB} g_{AB}(t)]$ with

$$g_A(t) = -c_A t_A + (c_A - C_A^{0B})(t - t_0)$$

$$g_B(t) = -c_B t_B + (c_B - C_B^{0A})(t - t_0)$$

$$g_{AB}(t) = c_A c_B t_{AB} - c_A (c_B - C_B^{0A}) t_A$$

$$- (c_A - C_A^{0B}) c_B t_B + (c_A - C_A^{0B})(c_B - C_B^{0A})(t - t_0)$$

$$t_A = 1/k_A [\exp(-k_A t_0) - \exp(-k_A t)]$$

$$t_B = 1/k_B [\exp(-k_B t_0) - \exp(-k_B t)]$$

$$t_{AB} = (k_A + k_B)^{-1} [\exp(-(k_A + k_B)t_0) - \exp(-(k_A + k_B)t)]$$

The Independent NEC model. A third alternative is to use fixed NEC values. These values do not depend on the concentration of other compounds. The no-effect time is given by

$$t_0^A = -\log(1 - C_A^{0B}/c_A)/k_A$$

$$t_0^B = -\log(1 - C_B^{0A}/c_B)/k_B$$

$$t_0 = \max(t_0^A, t_0^B)$$

The expressions for $S(t)$, $g_A(t)$, $g_B(t)$, $g_{AB}(t)$, t_A , t_B and t_{AB} are the same as for the irreversible binding model.

Implementation

These models are implemented in software package DEB-tool, which is available for free download from <http://www.bio.vu.nl/thb/deb/deblab/> (debtool/tox/fomort2, Reversible binding; debtool/tox/fomort2r, Irreversible binding; debtool/tox/fomort2i, Independent NEC).

The script file mydata fomort2 illustrates how to use it, including the generation of Monte Carlo data (using debtool/lib/prob/surv count), and the formal statistical test $d_{AB} = 0$. The routines /debtool/lib/regt/gasurv3, nmsurv3 and scsurv3 have been written to estimate parameter values on the basis of the maximum likelihood principle. The routines psurv3, dev3 evaluate variances, covariances, and goodness of fit. Note: The numerical derivatives in scsurv3 are found to be not accurate enough for the numerical integrations in fomort2r, so please use gasurv3 and nmsurv3 only for this model.