Statistical analysis of pollutant acute toxicity on marine invertebrates: a new approach by Cox's regression models

Indirect bioassay
Cox's regression models
Proportional hasard models
Polychaetes
Detergent

Essais toxicologiques indirects
Modèles de régression de Cox
Hasards proportionnels
Polychètes
Détergent

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ABSTRACT

Most of the methods used for evaluating toxicity from bioassays do not take direct account of time as one of the major factors of mortality. Semi-parametric Cox's regression models integrating time are very flexible and powerful tools for appreciating the toxicity of pollutants on marine organisms.
Investigation of the evolution of the mortality rate during experiments provides very useful information on toxicity. Besides providing estimations of lethal concentrations, this permits the study of simultaneous effects and of the interactions of several factors on mortality. The principal advantages of such a model are highlighted on the basis of data concerning the influence of detergents on survival of Polychaetes.

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RÉSUMÉ

Analyse statistique de la toxicité de polluants sur des invertébrés marins : une nouvelle approche par les modèles de régression de Cox

La plupart des méthodes utilisées dans des essais biologiques indirects pour évaluer la toxicité d'un produit chimique ne prennent pas en compte l'influence du temps écoulé comme l'un des facteurs essentiels agissant sur la mortalité. Les modèles de regression de Cox qui permettent d'intégrer le facteur temps peuvent s'adapter aisément à de nombreux modèles expérimentaux et constituent un outil puissant pour apprécier la toxicité de polluants sur des invertébrés marins.
L'analyse de l'évolution des taux de mortalité sous différentes conditions expérimentales fournit des informations essentielles sur la toxicité. Outre des estimations des concentrations létales, ils permettent d'analyser les effets simultanés et les interactions de différents facteurs sur la mortalité. Les principaux avantages de tels modèles sont mis en évidence à partir d'un exemple concernant l'influence d'un détergent non ionique sur la mortalité de Polychètes.

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INTRODUCTION

Effective control measures for toxic pollutants require that aquatic organism experiments be carried out at various concentrations and under different physical conditions (temperature, salinity, etc.). Despite the necessity of extrapolating the results to low concentrations (Hubert, 1984), these experiments are useful to assess risks to animals and humans. In such bioassays, the modifications occurring in the organisms tested are recorded once or several times during the experiment. These modifications can be biological, physiological or nutritional and may or may not be reversible. This paper deals with statistical analysis of the toxicity of a chemical by indirect bioassays when modifications occurring in an organism are irreversible, a case which corresponds to the most current bioassay type in which numbers of deaths and survival times are recorded. In most experiments, observations are performed only at one time, which is more or less arbitrarily fixed, and the response of each organism to the chemical is assumed to depend only on the concentration under external experimental conditions; it is considered that no tolerance towards the chemical occurs during the experiment. One may suppose that each organism has its own toxicity threshold; statistically that threshold

can be modelized by a random variable whose pdf (probability distribution function) describes sufficiently well the results of the action of the pollutant on the species studied. The empirical distribution of the toxicity threshold should be obtained from random observations. However, in common practice, only some fixed values of that distribution are estimated. To each p value of the probability corresponds one concentration: lethal concentration $100p$ (LC$100p$); institutional offices (APHA/AWWA/WPCF, 1985) generally admit LC50 ($p=0.5$), LC10 ($p=0.1$) and LC90 ($p=0.9$) as standard concentrations for comparison of chemical toxicities. Consequently it is often only these parameters that are estimated. The estimations of LC$100p$ can be obtained in several ways: either the family of the probability distribution of thresholds is known and one particular distribution is selected from observations; or the family of distribution is not explicited and non-parametric estimations of the LC$100p$ are performed. The former case includes the well-known Probit and Logit methods corresponding to a normal and to a logistic distribution of the tolerance threshold, respectively (Finney 1964, Ashton 1972, Hubert 1984). The latter case includes Spearman-Kärber and derivative methods, undoubtedly the most effective non-parametric method for estimating LC50 (Hamilton 1979). Although all these methods have been generalized in several directions to take into account particularities of the threshold distributions (skewness, kurtosis, etc.) (Miller and Halpern 1980), they are not convenient for analysing time to responses, the duration of the experiment being given. However, it is clear that exposure time has an effect on the response as well as concentration. Moreover the evolution of dose-response curves permits a better appreciation of the toxicity of the chemical tested. It should be noted that estimating of lethal concentrations at the times of observation by means of the previous methods would be inefficient. Indeed the dependence between the observations at successive times would thereby be neglected. Moreover, interaction between time and concentration on probability of response would be ignored. Undoubtedly, the time component has to be included in the planning and statistical analysis of indirect bioassays if the toxicities of pollutants are to be appreciated with reliability (Kalbfleisch *et al.* 1983). The development of statistical modelling techniques associated with the broad extension of computing facilities allows implementation of better suited analysis methods for investigation of toxicity data.

Among all the possible methods, such as growth curve analysis (Carter and Hubert 1984), log-linear models for contingency tables (Holford 1980), Cox's regression models (Cox 1972) seem to be well suited for precise analysis of indirect bioassays (Pierce, Stewart and Kopecky, 1979).

MATERIAL AND METHODS

Application of Cox's regression models to toxicity of Cemulsol on the Polychaetes *Scolelepis fuliginosa*

To demonstrate the advantages of Cox's regression models in the study of the toxicity of pollutants on marine invertebrates we analysed data extracted from Stora (1972) where the precise experimental procedure can be found. In this experiment the polychaete *Scolelepis fuliginosa* was submitted to a non-ionic detergent, Cemulsol 870. The assays were performed at five concentration levels: 10, 12, 14, 16 and 20 mg$\times 1^{-1}$ and at two temperatures: 17 and 22°C. The counts of dead polychaetes under each experimental condition are recorded 24, 48, 72 and 96 hours after the start of the assay. The data are displayed on Table 1. The deaths are regrouped in 24-hour intervals.

Table 1

Observed counts of polychaetes at risk and dead 24, 48, 72, 96 *hours after the start of the bioassay under each experimental condition. Predicted mortality estimated from the retained statistical model.*

Nombres de polychètes estimés et à risque 24, 48, 72, 96 heures après le début de l'expérience dans les conditions expérimentales. Mortalité estimée à partir du modèle retenu.

Temp. (°C)	C (mg.l^{-1})	Days	Exposed	Mortality Obs.	Counts Est.
17	10	1	20	0	0.15
17	10	2	20	0	1.30
17	10	3	20	3	1.88
17	10	4	17	1	1.37
17	12	1	20	0	0.31
17	12	2	20	1	2.59
17	12	3	19	4	3.51
17	12	4	15	2	2.39
17	14	1	20	0	0.58
17	14	2	20	5	4.52
17	14	3	15	6	4.72
17	14	4	9	1	2.47
17	16	1	20	0	0.98
17	16	2	20	8	7.08
17	16	3	12	5	5.69
17	16	4	7	4	2.95
17	20	1	20	1	2.30
17	20	2	19	16	12.43
17	20	3	3	2	2.37
17	20	4	1	0	0.73
22	10	1	20	0	1.55
22	10	2	20	3	4.27
22	10	3	17	3	1.94
22	10	4	14	1	0.48
22	12	1	20	4	3.07
22	12	2	16	6	6.26
22	12	3	10	3	2.21
22	12	4	7	2	0.49
22	14	1	20	7	5.30
22	14	2	13	7	7.81
22	14	3	6	3	2.22
22	14	4	3	1	0.37
22	16	1	20	9	8.16
22	16	2	11	8	8.69
22	16	3	3	2	1.63
22	16	4	1	0	0.20
22	20	1	20	15	14.41
22	20	3	5	2	4.26

Cox's regression models

Time to death of an organism from the start of experiment will be modelized here by a random variable T. In a Cox's regression model, the probability distribution of survival times is related to one or several explicative variables by the hazard function, which can be considered as the instantaneous probability of death, at time t, for an organism having survived as far as t. A hazard function can be written (Holford 1976, Kalbfleisch and Prentice 1980):

$$\lambda(t) = \lim_{\Delta t \to 0} \left\{ \frac{\text{Probability}\,(t < T < t+\Delta t \mid T > t)}{\Delta t} \right\}.$$

Let $\mathbf{z}$ be a vector of explicative variables and $\mathbf{z}_0$ the vector value selected as reference. At $\mathbf{z}=\mathbf{z}_0$ the hazard function is $\lambda_0(t)$ but its form is not explicited. According to these notations a Cox's regression model can be written:

$$\lambda(t;\ \mathbf{z})=\lambda_0(t).\exp(\mathbf{z}.\boldsymbol{\beta}^t).$$

In that expression $\boldsymbol{\beta}=(\beta_1, \beta_2, \ldots, \beta_p)^t$, where « ^{t} » denotes transposition, is a vector of p unknown parameters and $\lambda(t;\ \mathbf{z})$ is the hazard function at $\mathbf{z}$. If $\mathbf{z}$ is constant over time, *i.e.*, for example, if concentration and temperature remain at fixed values during over all the experiment, $\lambda(t;\ \mathbf{z})$ and $\lambda_0(t)$ are proportional.

Exponential function permits linear expression by logarithmic transformation. Calculations are thus simplified. However, any positive continuous function could be used. Cox (1972) showed that $\boldsymbol{\beta}$ could be estimated from data without $\lambda_0(t)$ being explicited by maximization of the following partial likelihood function VP:

$$\mathrm{VP}(\boldsymbol{\beta})=\prod_{i\in D}\frac{\exp(\mathbf{z}_i.\boldsymbol{\beta}^t)}{\sum_{j\in R_i}\exp(\mathbf{z}_j.\boldsymbol{\beta}^t)}.$$

Assuming that the deaths are independent of each other, the likelihood function is obtained by the product of the probabilities of death of each subject effectively dead, conditionaly on the set at risk at this time. D is here the set of all the times of death and R_i that of the organisms under experiment just before t_i.

The estimator $\hat{\boldsymbol{\beta}}$ of $\boldsymbol{\beta}$, thus obtained, has the asymptotic distributional properties of maximum likelihood estimators. Therefore it is possible to obtain approximate confidence intervals for $\hat{\beta}_i$ and to carry out tests of hypothesis on the $\boldsymbol{\beta}_i$ components of $\boldsymbol{\beta}$ (*e.g.* $\beta_i=0$). It should be noted that while each component $\boldsymbol{\beta}_i$ of $\boldsymbol{\beta}$ expresses the importance of each explicative variable on survival however, it is dependent on the units choosen for measuring the variables and must be accordingly interpreted. Confidence regions for the vector parameter $\boldsymbol{\beta}$ can be constructed either by usual methods from the estimated asymptotic covariance matrix $\hat{\boldsymbol{\Sigma}}$ of $\hat{\boldsymbol{\beta}}$, or from log-likelihood function (Cox et Hinkley 1974); however these regions are sometimes too conservative.

In an indirect biological assay, observations are generally performed at predetermined times. Consequently, time at the response of each organism is not precisely known; only the time interval where the response (death) occurred is recorded. If the time-step between two observations is not too large, then it is possible to consider that the basal hazard function $\lambda_0(t)$ is constant over each of these intervals. Then we can assume (Prentice and Glocker 1978, Pierce *et al.* 1979, Stewart and Pierce 1982, Friedman 1982):

$$\lambda_0(t)=\lambda_j$$

for any death response time occurring in the j-th interval of time between two observations.

Let $t_1, t_2, \ldots, t_k$ be the observation times and t_0 the time at the start of the experiment. Without loss of generality we set: $t_0=0$. The organisms under experiment are randomly distributed in groups determined by the values of the explicative variables which are assumed discrete or discretized beforehand. All the individuals of the same group thus have the same descriptive vector $\mathbf{z}_h$. Let p_{hj} be the conditional probability that a subject of that group died in the j-th interval $[t_{j-1}, t_j[$, given it was living before t_{j-1}. Let q_{hj} be the complementary probability: $q_{hj}=1-p_{hj}$. If the number of deaths is y_{hj} and s_{hj} the number of organisms at risk at the beginning of this interval, a binomial model for the number of deaths can be postulated. The log-likelihood function V is:

$$\mathrm{V}(\boldsymbol{\beta}, \lambda_1, \lambda_2, \ldots, \lambda_k)=\sum_{h,\ j}(y_{hj}.\mathrm{Log}\,p_{hj}+s_{hj}.\mathrm{Log}\,(1-p_{hj}))$$

where the probability p_{hj} is function of λ_j and $\boldsymbol{\beta}$:

$$q_{hj}=1-p_{hj}=\exp\left\{-\int_{t_{j-1}}^{t_j}\lambda_j(t).dt\right\}$$

$$q_{hj}=\exp\left\{-\lambda_j.\exp(\boldsymbol{\beta}^t.z_{hj})\right\}.$$

Maximization of the likelihood function leads to estimates $\hat{\boldsymbol{\beta}}$, $\hat{\lambda}_j$. Several ways can be selected for maximization. The method proposed by Prentice and Gloecker (1978) requires calculating the first and second derivatives of log-likelihood function. Undoubtedly the easiest way (as signified to the authors by Pierce) is the utilization of the theory of generalized linear models (Thomson 1977, Thomson 1981, McCullagh and Nelder 1983). In this aim, the number of positive responses in each group is modelized by a binomial random variable. Estimates of $\boldsymbol{\beta}$ and $\boldsymbol{\lambda}$ can be obtained using a Log-Log complementary link function (Whitehead 1980, Aitkin *et al.* 1988).

We can set:

$$\mathrm{Log}\,(-\mathrm{Log}\,(1-p_{hj}))=\mathrm{Log}\,(\lambda_j)+\boldsymbol{\beta}^t.\mathbf{z}_{hj}$$

or:

$$q_{hj}=1-p_{hj}=\exp\,(-\exp\,(\lambda_j+\boldsymbol{\beta}^t.\mathbf{z}_{hj})).$$

Implementation of a procedure performing adjustment to data can be easily carried out with different softwares such as GLIM (Payne 1985) or SAS IML (1985). For testing adequation of the fitted model to the data, we used "Deviance" (McCullagh and Nelder, 1983) corresponding in the present case to the likelihood ratio test statistic for the model fitted compared to the saturated model with a parameter for each combination of the variables. The contribution of one or several terms to the goodness of fit can be appreciated by the difference between the deviances related to the models with and without these terms. If the model is adequate to resume the data, then deviance is asymptotically distributed as a chi2 variable with degrees of freedom equal to the number of parameters not fixed by the model. From the estimated asymptotic covariance matrix of $\hat{\boldsymbol{\beta}}$, $\hat{\boldsymbol{\Sigma}}$, it is possible to obtain LC100p approximate confidence intervals.

Indeed the Log of the LC100p is a linear combination of the $\hat{\beta}_i$ estimates of β_i and the estimate of a linear combination is the linear combination of the estimates; moreover the variance of that linear combination can be computed from: $\boldsymbol{\beta}^t . \hat{\Sigma} . \hat{\boldsymbol{\beta}}$.

RESULTS

From the data on polychaetes (tab. 1) it is easy to note the influences of concentration, temperature and time on survival. However the importance of each factor and the form of the dependence is not clear. We have analysed the data by means of Cox's regression models. Calculations were performed by using of GLIM. Time, included in this model as a factor, corresponds to the time factor in analysis of variance terminology: at each time-interval a binary (0-1) variable is associated. Its value is zero everywhere except for the observations performed in this time-interval.

In a first step, only the time factor was included in a Cox's regression model. The goodness of fit between the data and the model, measured by "Deviance", is equal to 167.27 with 34 degrees of freedom (d. f.). Under the hypothesis of validity of the model, this value has to be compared with the quantiles of a central chi-square random variable with 34 d. f. ($p < 0.000\,1$). This comparison led to reject the tested model: time is not sufficient to explain the observed variations of the survival probability. Subsequently, the logarithm of the concentration and the temperature were then included separately in a Cox's regression model. Both variables, specially concentration, highly improved goodness of fit criterion (Tab. 2); the diminution of deviance was respectively equal to 76.85 and 17.63 with one degree of freedom. However the log-concentration of Cemulsol displayed a more important effect on survival than did temperature (Tab. 2). After inclusion of concentration and temperature, the residual deviance is equal to 58.63 with 32 d. f. ($p < 0.001$). The proposed model is not satisfactory. Therefore three interaction terms were considered:

- Log-concentration × Temperature;
- Time × Log-concentration;
- Time × Temperature.

From Table 3, we can see that only the Time × Temperature interaction term improves goodness of fit significantly. The goodness of fit as measured by the deviance is equal to 36.33 with 31 d. f. Therefore the data do not carry enough information against this model (subsequently A model) to reject it. The plot of the raw residuals against estimated numbers of deaths displays no particular trend (Fig. 1), and it is not necessary to include another interaction term. Moreover, the plot of standardized residuals against the percentiles of the standard normal distribution (Fig. 2) evidences a satisfying linear trend showing that the distributional assumptions (normality) for residuals are verified (McCullagh and Nelder 1983). The estimations of the parameters of the model are displayed in Table 4. If Log-concentration and temperature-effect terms are positive (mortality increases with both variables), the Time × Temperature interaction term is negative; consequently temperature-effect decreased with the time elapsed from the start of experiment. From the theoretical model we can estimate the number of deaths under each experimental condition (Tab. 1) and the survival

Table 2

Cox's regression model. Effect on death of concentration (C) and temperature (Θ), isolated or combined. The model including only the time factor (D) is here taken as the reference.
Deviance, Log-likelihood ratio statistic, has to be compared with the chi-square variable quantiles with corresponding degrees of freedom.

Modèle de régression de Cox. Effet de la Concentration (C) et de la Température (Θ), isolés ou combinés, sur la probabilité de décès. Le modèle qui inclut seulement le facteur temps (D) est pris ici comme référence.
La deviance, statistique du Logarithme du rapport de vraisemblance, doit-être comparée aux quantiles d'une loi du chi^2 avec le nombre adéquat de degrés de liberté.

Effects	Deviance	d. f.	p	Δ Deviance	d. f.	p
D	167.27	34	10^{-6}	–	–	–
D+C	90.42	33	10^{-6}	76.85	1	1×10^{-6}
D+Θ	149.64	33	10^{-6}	17.63	1	2×10^{-4}
D+C+Θ	58.63	32	3×10^{-3}	125.40	5	1×10^{-6}

Table 3

Cox's regression model. Effects on death probability of the three second-order interaction terms between time (D), concentration (C) and temperature (Θ) in addition to simple effects (mod 1).

Modèle de regression de Cox. Effets sur la probabilité de décès des interactions du second ordre entre le Temps (D), la Concentration (C) et la Température (Θ) en plus des effets simples (mod 1).

Interaction	Deviance	d. f.	p	Δ Deviance	d. f.	p
Mod 1	58.63	32	10^{-6}	–	–	–
Mod 1+(Θ×C)	58.63	31	10^{-6}	0.0	1	1
Mod 1+(D×C)	56.23	31	4×10^{-3}	2.4	1	0.12
Mod 1+(D×Θ)	36.53	31	0.23	22.21	1	10^{-6}

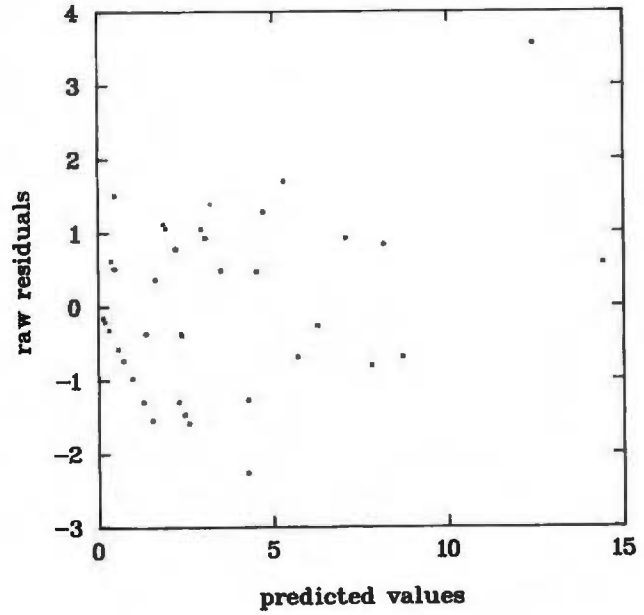

Figure 1

Cox's regression model. Plot of raw residuals against the corresponding estimated numbers of deaths.

Modèle de régression de Cox. Représentation graphique des résidus bruts en fonction du nombre estimé de décès.

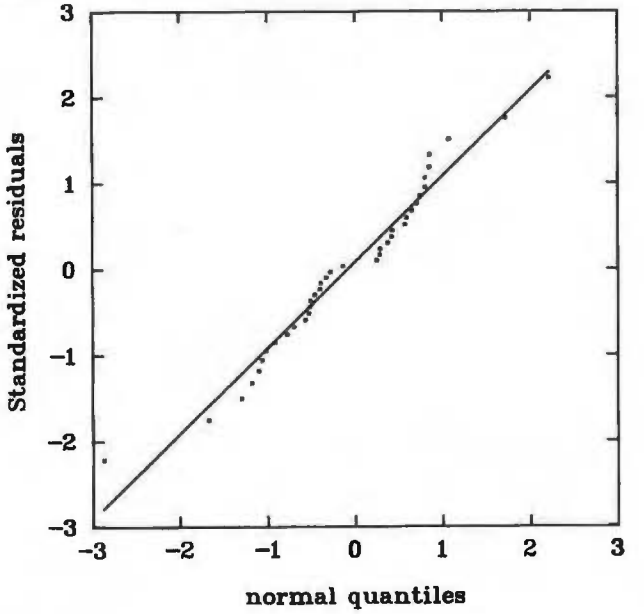

Figure 2

Cox's regression model. Normal plot of residuals. Display of standardized residuals in function of the quantiles of a standard normal distribution. Goodness of fit can be appreciated by the proximities of points around the first bisectrix.

Modèle de regression de Cox. Représentation graphique de résidus standardisés en fonction des quantiles de la loi normale centrée réduite. La qualité de l'ajustement peut être appréciée par la proximité des points et de la première bissectrice.

probabilities (Tab. 5). Moreover we can obtain estimations of particular lethal concentrations such as LC10, LC50, LC90 (Tab. 6) by interpolation from estimated survival probabilities given by:

$$p = \text{Proba}(T > t_j) = \prod_{i=1}^{i=j-1} \exp(-\exp(\lambda_i + 3.98\,\text{Log}(C) + 0.68\,\Theta - 0.21\,D \times \Theta)$$

where C is set for concentration, Θ for temperature and D for day.

DISCUSSION

In indirect bioassays, the duration of exposure is a major factor on mortality. Clearly, the observation of the evolution of mortality rate during the experiment provides more convenient information on toxicity than do the non-time varying methods (Probit, Logit, etc.) (Koïjman 1981) generally used for estimating toxicity by using lethal concentrations. In Cox's regression models applied to bioassays, all the data participate in the estimation of one particular lethal concentration; the estimates are more reliable in this latter case. Indeed, knowledge of a general mortality trend is more relevant for evaluating toxicity than is a small number of observed mortalities at only one given time. Moreover another advantage of this method lies in the possible inclusion of several variables and/or factors in a particular model. Expressing the variation of survival as a function of time and other factors can be a powerful tool for appreciating the real impact of a pollutant on living marine organisms.

For example, analysis by Cox's regression models of the toxicity of Cemulsol on *Scolelepis fuliginosa* permitted investigation not only of the individual effects of the concentration and temperature but also of the interactions: time × temperature; time × concentration; and temperature × concentration.

In this way, the absence of two statistically significant interaction terms (time × concentration and concentration × temperature) shows that the effects of concentration on survival remain constant with time and with temperature. In other words, the probability of death during a constant time interval does not vary with the duration of exposure at a given concentration. The effect of the concentration over the time of the experiment is constant, there is no sign of any sort of

Table 4

Cox's regression model. Estimated parameters for equation (A) according to retained statistical model.

Modèle de régression de Cox. Estimation des paramètres de l'équation (A) fournie par le modèle statistique retenu.

Parameter		Estimate	Estimated parameter covariance						
Day (1)	λ_1	−22.00	5.04	–	–	–	–	–	–
Day (2)	λ_2	−16.20	3.15	2.63	–	–	–	–	–
Day (3)	λ_3	−12.18	1.62	2.23	2.89	–	–	–	–
Day (4)	λ_4	− 8.70	0.22	1.85	3.35	4.71	–	–	–
Log C	β_1	3.98	−0.68	−0.61	−0.57	−0.52	0.19	–	–
Temp.	β_2	0.68	−0.22	−0.08	0.03	0.14	0.01	0.01	–
Day × Temp.	β_3	− 0.21	0.08	0.02	−0.03	−0.08	0.00	−0.01	0.00

Table 5
Estimations of median lethal concentrations (LC50) by interpolation of the predicted survival probabilities.
Estimations des concentrations létales 50 (LC50) par interpolation des probabilités de survie.

	Concentrations ($mg.l^{-1}$)									
	10		12		14		16		20	
°C	17	22	17	22	17	22	17	22	17	22
Hours										
24	0.99	0.92	0.98	0.85	0.97	0.73	0.95	0.59	0.88	0.28
48	0.93	0.73	0.86	0.51	0.75	0.29	0.61	0.12	0.31	0.04
72	0.84	0.64	0.70	0.40	0.51	0.18	0.32	0.06	0.06	0.00
96	0.77	0.62	0.59	0.37	0.37	0.16	0.19	0.05	0.02	0.00

Table 6
Estimated survival probabilities under each experimental condition.
Probabilités de décès estimées sous chacune des conditions expérimentales.

Estimates Lethal Concentration 50			95 percent confidence interval	
			Lower limit	Upper limit
17° C	24 h	29.56	24.09	36.22
	48 h	17.44	14.22	21.43
	72 h	14.25	10.93	18.69
	96 h	12.78	8.53	19.15
22° C	24 h	17.06	13.90	20.89
	48 h	12.22	9.27	16.02
	72 h	11.20	7.90	15.84
	96 h	10.94	4.98	23.92

tolerance to the pollutant at the given scale of observation. Whatever the temperature level, the concentration effect is the same. On the other hand, the presence of a negative interaction term "time × temperature" shows that the survival curve decreases more at 22 than at 17° but the rates tend towards equality with time. It seems that the effect of temperature disappears when the duration of experiment progresses.

More generally, other variables can be taken into account in indirect bioassays, eg., simultaneous presence of pollutants at different concentrations or modifications of salinity and temperature. The present method can be easily adaptated to cases where the values of variables change during the assay (Kalbfleisch and Prentice 1980, Reish and Oshida 1986).

This flexibility is particularly convenient in static or semi-static experiments where the concentrations vary with time.

This model takes into account censored observations at any time not only at the end of the experiment where they generally come from the organisms remaining at risk (surviving fraction). In this way it would be possible, under not too restrictive conditions, to select some samples for further biological analysis. The chosen observations have to be randomly selected to prevent any relationship between lethal mechanisms and censoring (Kalbfleisch and Prentice 1980).

Cox's regression models are a very flexible distribution-free method for investigating the toxicity of pollutants or the sensitivity of species to pollutants under various experimental conditions. The constancy on time of the ratio of hazard functions, depending only on explicative variables (the logarithm of this ratio is an difference between two linear combinations of variables), is undoubtedly the most restraining hypothesis (McCullagh and Nelder 1983). However, as seen in our example, it is possible to overcome in part this difficulty by inclusion in the model of time-variable interaction terms. The basal hazard function (otherwise arbitrary) must not, to any great extent with, vary on each observation interval; in all cases it is necessary to select the interval widths in function of the occurrence rate of responses.

In this approach, the dynamic aspect of an experiment is taken into account, a clear advantage over classical methods used in marine environmental toxicology such as Logit or Probit methods; moreover the time variations of survival under different conditions are modelized without expliciting its form (the form of hazard function is not required). All things considered, this method appears well suited for a variety of bioassays and particularly for bioassays on marine invertebrates.

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