LINKING SURVIVAL AND BIOMARKER RESPONSES OVER TIME

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Abstract – The practical usefulness of biomarkers is limited by the complexity of linking their responses to life-history traits of the organisms (e.g., survival, growth, reproduction) over time. Here we present a first attempt to simultaneously model biomarker responses and survival over time with a toxicokinetic-toxicodynamic approach. Even though more work is clearly needed, the present study provides a novel direction for interpreting biomarker responses and dynamically linking them to life-history traits.

Keywords – TKTD modelling GST Biomarker Survival Copepods

INTRODUCTION

Biomarkers have been a promising tool in ecotoxicology for decades, but many problems hamper their practical usefulness [1]. One of the main problems is the difficulty in linking biomarker responses to relevant endpoints at the level of the individual (life-history traits such as survival and reproduction). Generally, the biomarker response at one time point is compared to a trait at one (sometimes even a different) time point, even though both biomarkers and life-history traits tend to change with the exposure time. Toxicokinetic-toxicodynamic (TKTD) models have proven to be very successful in explaining observed effect patterns on life-history traits such as survival [2] and sub-lethal endpoints [3] over time. Even though these models are a crude simplification of highly-complex biological and biochemical processes, they are able to provide an explanation for observed patterns, as well as educated predictions for untested situations. The essence of TKTD models is that a chemical first needs to be taken up into an organism; it is an internal concentration over time that causes toxicity. This first step thus requires a TK model. The next step is a TD model to link the internal concentration to a physiological process in the organism, such as the probability to die or the costs for somatic maintenance. The effects on such a process (over time) will eventually dictate the dynamic effects on the life-history trait.

Can we include biomarker responses in this picture? We can assume that the internal concentration is also responsible for these responses, which makes a link to a TK model a reasonable one. One consequence of this assumption is that we *a priori* expect the biomarker response to change with exposure time; as the body burden of the toxicant increases over time, so the response will change along with it. It would thus be immensely useful if biomarker responses would routinely be followed over the course of exposure, in controlled experiments. Furthermore, we need to follow individual-level traits under the same conditions, to see if the same TK parameters can describe both types of endpoints. A proper quantitative TD model for biomarker responses is still missing, and will likely depend on the biomarker of choice. The transcriptional response on glutathione S-transferase (GST), which is a phase II enzyme in PAH biotransformation, has previously shown reliable concentration- and time-dependent expression patterns in calanoid copepods exposed to single oil components [4], water soluble fractions of crude oil [5] and dispersed oil [6], and is thus a good candidate for further research.

Here we present a first attempt to simultaneously model biomarker responses and survival over time, linked to the same toxicokinetics model, with a very simple TD model. We certainly do not see this model as a definitive way to analyse such data, but we do consider this a promising avenue for further research.

METHODS

Experimental data were taken from [7] and all information regarding experiments and analysis can be found there. Briefly, experiments were performed on two copepod species (*Calanus finmarchicus* and *Calanus glacialis*). Both species were exposed to water soluble fractions (WSF) of marine diesel. Two oil:water ratios were used to generate WSFs for the acute toxicity tests (1:40 and 1:10.000). Acute toxicity tests were performed where survival was monitored over a period of 6 days (21 individuals per treatment) for both WSFs and both species. Thereafter, sub-lethal exposure experiments using an oil:water ratio of 1:40 to generate WSFs were performed, in which copepods were exposed to three concentrations, corresponding to 0.5, 5 and 50% of their 96-h LC50, for 12, 24 and 48 hours (concentrations shown in the legend to the lower panels of Figure 1 and 2). No mortality was observed in the sub-lethal experiments, but copepods were sampled for biomarker analyses (expression of glutathione S-transferase, GST). GST transcriptional levels were analysed using Q-PCR and are presented as mean

normalised expression using elongation factor 1α as reference gene [7]. Three replicates were analysed for each concentration at each time point, with each replicate consisting of a pooled sample of 25 animals (*C. finmarchicus*) or 10 animals (*C. glacialis*).

The basis of the TKTD model is formed by the framework of the General Unified Threshold model for Survival (GUTS [2]), and we assume that we can model diesel as a single compound. In reality, diesel is a complex mixture of hydrocarbons, but without more information on its composition, and the effects of the individual components on copepods, a mixture modelling approach is not feasible. In support of our choice, the study of Baas et al. [8] showed that a mixture model for a mixture of 14 polycyclic aromatic hydrocarbons could be well approximated by a model treating the mixture as a single chemical.

We are using the special case of scaled toxicokinetics (TK), combined with stochastic death. Scaled TK is needed as there is no information about actual body residues; the single TK rate constant (the dominant elimination rate, k_e) is estimated from the effect patterns over time. This model provides us with a dose metric (the scaled internal concentration, C_i^*) over time from the external concentration (C_w):

$$C_i^*(t) = C_w(1 - e^{-k_e t}) \quad (1)$$

This dose metric can subsequently be linked to the hazard rate (the 'instantaneous probability to die'), which is the special case for stochastic death (SD) in GUTS:

$$h(t) = k_k \max(0, C_i^*(t) - z) \quad (2)$$

using two parameters: the threshold for effects (z) and a killing rate (k_k). The integrated hazard rate over time can easily be converted into a survival probability [2].

For the GST response, we assume a linear increase with the dose metric (C_i^*) above the threshold. We assume that the same threshold can be used for both survival and the biomarker response. The stress factor on the biomarker is calculated analogous to effects on metabolic processes in DEBtox [9]:

$$s = \frac{1}{C_{TG}} \max(0, C_i^*(t) - z) \quad (3)$$

And we calculate the GST response under stress as:

$$G = G_0(1 + s) \quad (4)$$

Adding the biomarker response to our model thus requires two additional parameters: the GST response in the blank (G_0) and a tolerance concentration (C_{TG}). The tolerance concentration is the proportionality constant that linearly links the scaled internal concentration above the threshold to the level of effect on the GST response. In contrast to the proportionality constant for survival (k_k), a higher value of the tolerance implies less effect.

The model parameters are estimated from the data by maximising the overall likelihood function. For each species, we have three data sets, and thus three contributions to the likelihood. For the survival data, we apply the multinomial likelihood [2], and for the GST response we assume independent normal distributions for the deviations between model and data [9]. The three data sets are treated as independent. All calculations were performed in Matlab R2010a (MathWorks).

RESULTS AND DISCUSSION

Even though the amount of information in the data is limited, these fits show that it is possible to capture both survival and biomarker responses within the same model framework. The simultaneous model fits are shown in Figure 1 and 2, and the corresponding parameter estimates are provided in Table 1. Note that for *C. glacialis*, the confidence intervals are very large because k_e can go to zero (negligible elimination). For this reason, the toxicity parameters cannot be identified (all will be tightly correlated to k_e). The same threshold for effects (z) was assumed for both the effects on survival and the biomarker response. This is generally supported by the data, although the biomarker response for *C. finmarchicus* shows a (highly variable) response at 104 µg/L, just below this threshold. This might indicate that the biomarker response is slightly more sensitive than survival, or that the threshold varies between individuals (which is supported by the large standard error). For this reason, an individual tolerance (IT) assumption might be more representative [2]. However, we were unable to fit both survival and biomarker responses satisfactorily with a pure IT model (results not shown).

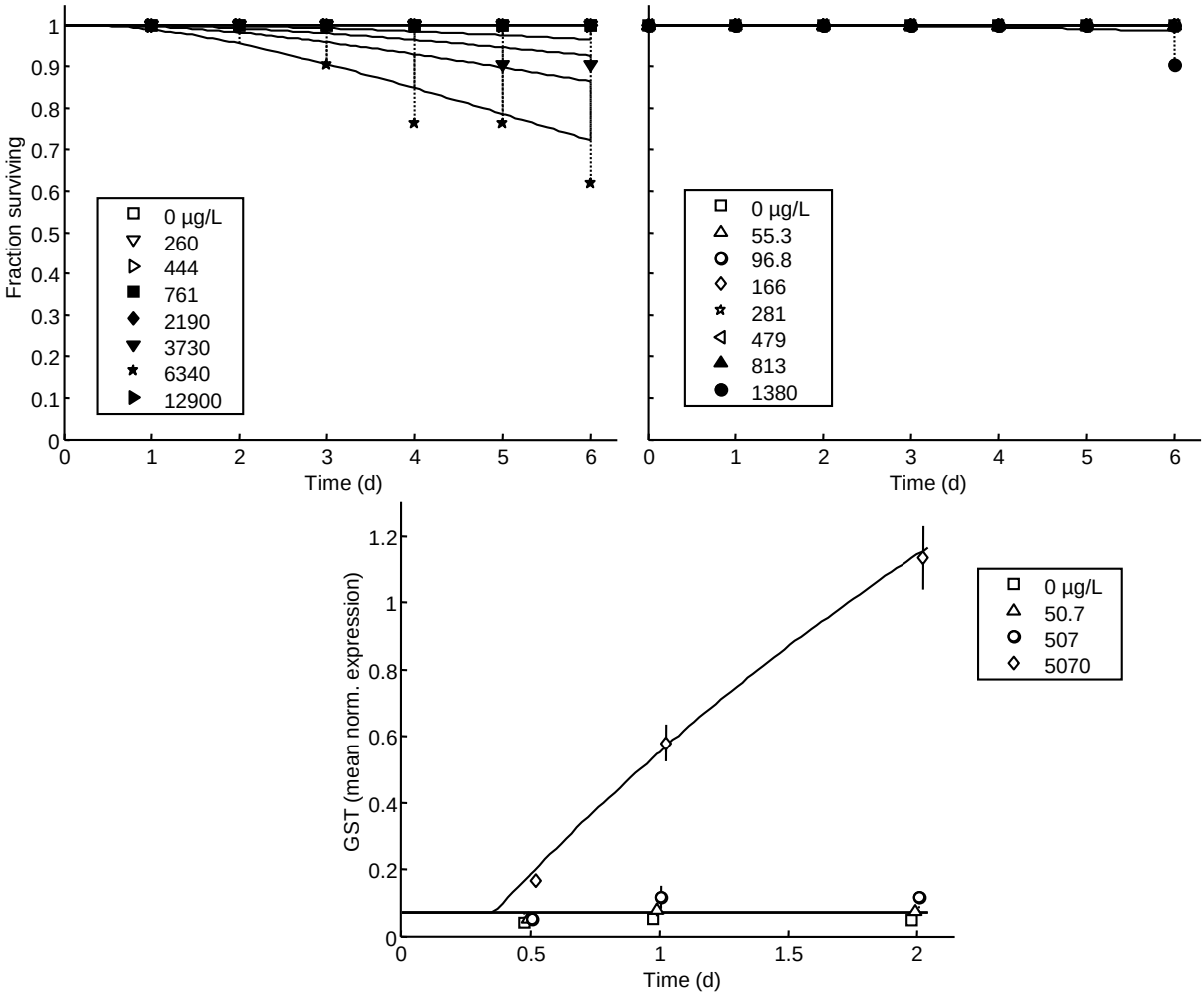

Figure 1. Survival (upper plots, dotted lines link observations to corresponding model curve) and GST (lower plot, bars are standard errors) in *C. glacialis*. GST observations are slightly shifted in time for each treatment to increase readability.

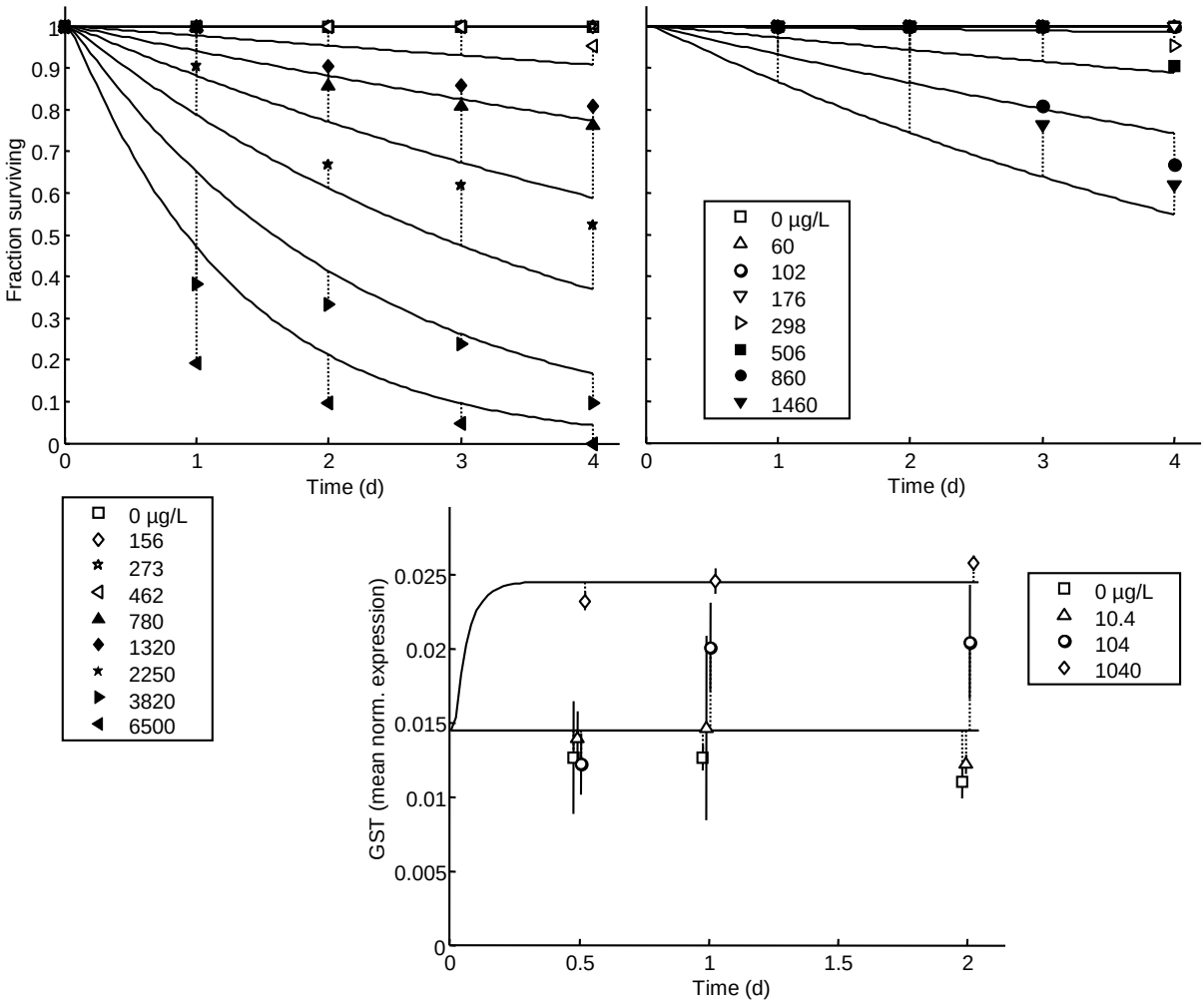

Figure 2. Survival (upper plot, dotted lines link observations to corresponding model curve) and GST (lower plot, bars are standard errors) in *C. finmarchicus*. GST observations are slightly shifted in time for each treatment to increase readability.

Table 1. Parameter estimates for the fits in Figure 1 and 2, with likelihood-based confidence intervals.

Parameter	Symbol (unit)	Estimate (95% conf.) *C. finmarchicus*	Estimate (95% conf.) *C. glacialis*
Dominant elimination rate	k_e (d^{-1})	18.8 (2.12-∞)	0.295 (<0.598)
No-effect concentration	z (µg L^{-1})	269 (170-296)	507 (<1077)
Killing rate	k_k (L µg^{-1} d^{-1})	1.28 (0.977-1.73) $\cdot 10^{-4}$	8.50 (>4.21) $\cdot 10^{-6}$
Blank value GST	G_0 (-)	0.0144 (0.0126-0.0163)	0.0696 (0.0484-0.0907)
Tolerance conc. GST	C_{TG} (µg L^{-1})	1110 (709-1890)	113 (<186)

Clearly, more dedicated experimental testing is needed before biomarker responses can be successfully integrated into TKTD modelling. Specifically, experiments need to follow both biomarker responses and effects on life history traits over time. For quantal traits (such as survival), models based on the GUTS framework can be used, but for continuous sub-lethal endpoints, other approaches should be auditioned [3].

The linear-with-threshold relationship of Equation 3 might be overly simplistic for biomarkers; for example, more bell-shaped concentration-response curves have been reported

for GST [10]. Furthermore, we reduced all of the kinetics and dynamics involved in the biomarker response into a single first-order process, with the same rate constant as for effects on survival. This basically assumes that both types of effect share the same rate-limiting process (e.g., the exchange of the compound between water and organism). Even though it is tempting to include more mechanistic details at the molecular level, we advice some restraint: processes should only be added to the model if they influence the effect dynamics, and when they can be parameterised using the available information. A complex endpoint like survival can be modelled in GUTS using a very simple general mechanism, so biomarker responses should not require something way more complex or species specific.

Even though a lot more work is needed, the present study provides a novel and promising direction for interpreting biomarker responses and dynamically linking them to life-history traits such as survival.

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