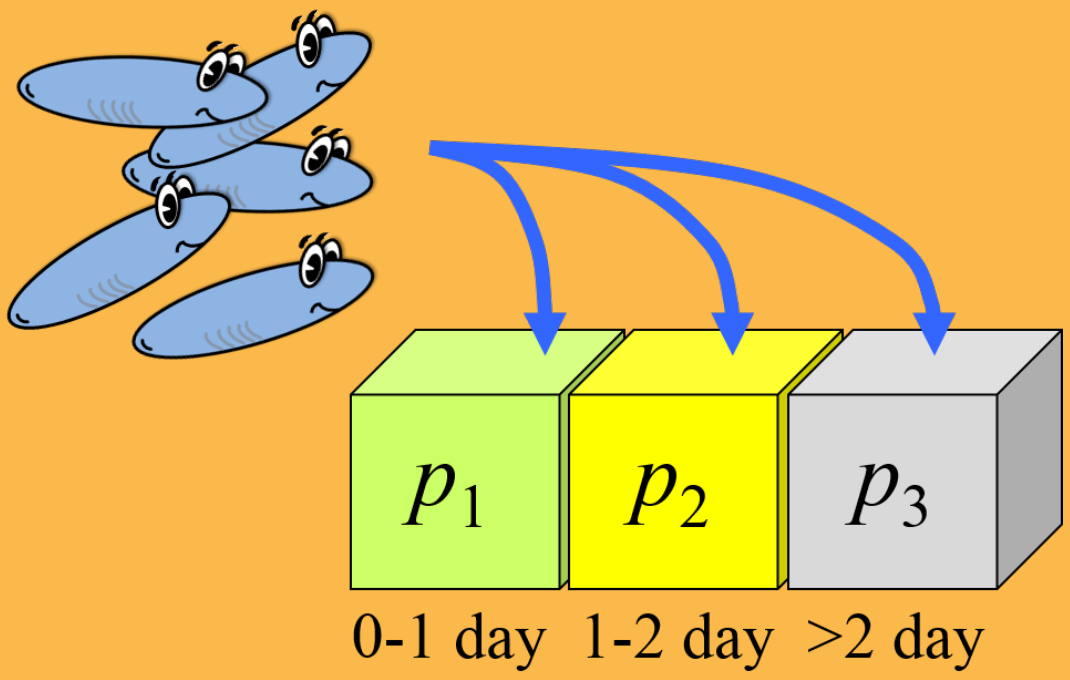
Modelling survival under chemical stress

A comprehensive guide to the GUTS framework



Tjalling Jager and Roman Ashauer

Modelling survival under chemical stress

A comprehensive guide to the GUTS framework

ISBN 978-1-9999705-1-2
Version 2.1, November 21, 2022

This e-book is available from Leanpub, and can be downloaded from https://leanpub.com/guts_book. The book can be obtained totally free of charge. However, please consider paying for it: that gives us the opportunity to continue working on this book, and on other ones. A version (and error) log can be found on the support page: http://www.debtox.info/book_guts.html. If you did not download this book from Leanpub (recently), check the landing page for the book to see if there is an update.

Original artwork by Tom Parker (drawings on Page 8, 67 and 85): https://www.facebook.com/TomParkerIllustration/.

You can cite this book as:
Jager T and R Ashauer (2022). Modelling survival under chemical stress. A comprehensive guide to the GUTS framework. Version 2.1. Toxicodynamics Ltd., York, UK. Available from Leanpub: https://leanpub.com/guts_book.

Alternatively, refer to one of the papers in the open literature. E.g., the general paper on GUTS [122].

If you spot errors (spelling, grammar, conceptual, or mathematical), please notify us by email (see addresses below).

Tjalling Jager

tjalling@debtox.nl
DEBtox Research, Stevensweert, The Netherlands

Roman Ashauer

roman@ecotoxmodels.org
University of York, York, United Kingdom and Syngenta Crop Protection AG, Basel 4058, Switzerland

Contents

Preface

About this book

Stressors, such as toxicants, can cause mortality among organisms. Interestingly, not all individuals will die at the same time in the same exposure treatment, and the number of deaths will change with exposure time. The pattern of mortality will also depend on the exposure profile (e.g., constant versus pulsed exposure), and can be modified by the addition of other stresses (e.g., mixture toxicity). Making sense of these complex issues requires mechanism-based models, known as toxicokinetic-toxicodynamic (TKTD) models. For mortality, almost all published TKTD models can now be viewed as members of an over-arching framework, that we have christened GUTS: the General Unified Threshold model of Survival. GUTS has been published in the scientific literature [122], but a publication is obviously not the place to provide a detailed description of the model or to provide guidance on how to apply the model in practical situations. Furthermore, a publication is static, while science and its application is (hopefully) progressing. As the GUTS framework is now gaining broader interest in the scientific, regulatory and industry communities, it is time for a more in-depth treatise.

We decided to publish this book with Leanpub for several reasons. Firstly, this provides us with the opportunity to update our book as frequently as we like (a publication in the scientific literature is static). This is not only important to repair errors, but also to include new developments as soon as possible. Secondly, Leanpub allows people to access the book for free. This is important to reach a large audience, but also to allow this book to function as a guidance for the use of GUTS models in a regulatory setting.

As the users of (results of) GUTS models will have very diverse backgrounds, we only make few assumptions about the reader's knowledge regarding mathematics, statistics and ecotoxicology. Therefore, this book should also be readable for undergraduate students in the natural sciences. In any case, the conceptual basis of the model and its application can be understood without full understanding of the technical details (which we provide as well). At the end of the introduction (Section 1.6), we provide a short 'readers guide'. To refresh your knowledge of differential equations, modelling and statistics, we suggest the dedicated e-book [121].

Note that this book deals with effects on survival, and potentially other 'all-or-nothing' responses (such as immobility). The GUTS framework, however, can *not* deal with effects on graded endpoints such as growth and reproduction (see [10]). For those endpoints, an energy-budget perspective is required, such as offered by Dynamic Energy Budget (DEB) theory (see [128, 118]). For the interested, we advise the dedicated e-book on that topic [117].

Support on the web

The supporting web page for this book is http://www.debtox.info/book_guts.html, which contains a version (and error) log and information directly relating to the book. Additional information can be obtained from http://www.debtox.info (maintained by Tjalling) and http://www.ecotoxmodels.org/ (maintained by Roman). Here, you will find (links to) software to perform GUTS calculations, and (lists of) publications that apply these concepts.

About the authors

Tjalling Jager studied biology at the VU University in Amsterdam, the Netherlands, after which he started to work at the National Institute for Public Health and the Environment (RIVM). Next to his work on risk assessment systems, he wrote a PhD thesis on modelling bioaccumulation and bioavailability in earthworms, in collaboration with the University of Utrecht. After 9 years at RIVM, Tjalling moved to the VU University, were he worked for 13 years as a scientific researcher at the department of Theoretical Biology (led by Prof. Bas Kooijman) on application of DEB theory to stressors. Since 2015, he is self-employed, running the private company DEBtox Research (http://www.debtox.nl), and continuing to work on DEB- and GUTS-related topics. Tjalling has (co-)authored over 100 scientific papers, and several e-books and book chapters.

Roman Ashauer studied Geoecology at the University of Karlsruhe, Germany, and Environmental Sciences at Trent University, Canada. During his PhD at the University of York, UK, he started working on toxicokinetic-toxicodynamic modelling. From 2007 to 2012 Roman worked at the Swiss Federal Institute of Aquatic Science and Technology (Eawag) and from 2013 at the University of York, UK. There, he was Associate Professor and Deputy Head of Department by 2018 and still holds an Honorary Fellowship at the Department of Environment and Geography. In 2019 Roman joined Syngenta Crop Protection AG where he works as a Science and Technology Fellow. Roman has won the SETAC/CEFIC-LRI Innovative Science Award and published over 70 scientific papers (http://www.ecotoxmodels.org).

Acknowledgements

Firstly, we are indebted to all of the scientists that have worked on advancing our understanding of the survival process over more than a century. Their work has laid the foundation on which GUTS, and this book, are built. In Section 1.5, we attempt to piece together these historical roots. Secondly, we thank CEFIC-LRI for providing us with the funding needed to prepare the fist version of this book.[1] This book could not have been written without the broader community of GUTS users and developers, especially the

[1] Project: http://cefic-lri.org/projects/eco39-review-ring-test-and-guidance-for-tktd-modelling/.

participants of the GUTS workshops in 2010 (Kastanienbaum, Switzerland) and 2015 (Mallorca, Spain). Their inputs, and the intensive discussion at the workshops, have led to the development of GUTS in the first place, and have shaped our ideas that are now coalesced into this book. Furthermore we would like to thank the participants of the GUTS software ring test for their substantial contributions to Appendix A, and Anna-Maija Nyman for pointing out relevant REACH documents.

Thanks to LaTeX for providing the platform to write this book, and thanks to WikiPedia (http://www.wikipedia.org) for many useful facts and figures. And finally, a special thanks to Tom Parker for making dedicated drawings for this book (Page 8, 67 and 85).

Warnings

We noticed that some of Tjalling's e-books are offered through other websites, after removal of the title page. Please download this e-book from Leanpub, which is the only place where it is legitimately offered (even for free). That will ensure that you have a complete and correct version, and that you are notified as soon as a new version is available.

This book *will* inevitably contain errors. If you spot one, please let us know so that we can include it in the version/error log on the support page, and make corrections in an update of this book. We do not accept liability or responsibility for any damage or costs incurred as a result of these errors.

Chapter 1

Introduction

1.1 Relevance of survival

Why are we interested in survival in the first place? Survival is a relevant endpoint for many questions related to the effects of chemicals in the environment. Which species, populations and communities are most vulnerable to chemical pollutants? Which chemicals are most likely to pose a risk to the environment? Knowing if a chemical affects the survival of organisms, and in which concentration range, is central to answering these questions. Furthermore, survival is easy to measure in short toxicity tests, which are often termed acute toxicity tests, and has a clear and unambiguous relation with population dynamics of a species. Often, the organism's sensitivity to lethal effects is used as a proxy for toxicity in general, and acute toxicity data are required in virtually every framework for chemical risk assessment [180, 213]. Therefore, acute toxicity tests have been, and still are, routinely carried out for thousands of chemicals, for example using fish or water flea as the test organism. Clearly, it is imperative to make the best use of this data and the information contained in it, and model analysis is the only way to achieve that. Finally, the knowledge that we gain, and the concepts that we advance, through modelling survival can later be transferred to modelling other kinds of data.

In ecotoxicology, mortality due to chemical stress is an important endpoint, both from a scientific and regulatory perspective. The GUTS framework originated in ecotoxicology, and this is where we currently see most applications. However, we believe that the GUTS framework, and this book, will be of interest to readers from other fields of science as well. Mortality due to chemicals is also of interest in human toxicology and epidemiology [207], pharmacology and medical research [7, 138]. We can further generalise from mortality due to chemical stress to failure or any binary event due to other stressors or external factors. This means that modelling survival, and hence GUTS, becomes relevant and applicable to problems in a diverse range of fields. This includes biological problems [52, 87, 88, 168] and engineering [78], where the survival of mechanical parts is modelled using reliability theory [162], as well as social sciences where the occurrence of certain events is analysed using event history analysis [4, 48, 102]. We believe that GUTS, or elements of it, can help to generate new insights into a wide range of problems. All that is needed is to replace the death of test organisms used as examples throughout this book with the binary event of interest and replacing the toxicant concentration, which is the stressor used here, with the intensity of any other

Table 1.1: Examples of stressors and stressed entities in different fields of inquiry. All of those fields are interested in the analysis of survival (of the stressed entity) over time. In the social sciences it would be more appropriate to talk about influence instead of stress.

Field of inquiry	**Stressor examples**	**Stressed entity**	**Relevant terms used**
Ecotoxicology	Toxicant	Organisms in the environment	Survival analysis, hazard modelling, toxicodynamics
Toxicology	Toxicant	Humans	Survival analysis, hazard modelling, toxicodynamics
Epidemiology	Toxicant, pathogens, environmental factors	Humans	Survival analysis, hazard modelling
Medical research and pharmacology	Toxicant, pharmaceutical, medical treatment (e.g., operation, radiation)	Humans, rats, mice	Survival analysis, event history analysis, pharmacodynamics
Biology	Environmental factors	Organisms in the environment, humans	Reliability theory, mortality, hazard
Actuarial science	Environmental, social and economic factors	Humans	Force of mortality, mortality rate, death rate
Engineering	Mechanical forces, heat, radiation, electricity	Machine parts or products	Reliability theory, failure probability, accelerated life testing
Social sciences	Policy, social & economic factors	Social systems, organizations, human behavior	Event history analysis, transition analysis, duration analysis

stressor or causative factor.

1.2 The descriptive approach

Why do organisms die, or better: why don't all individuals die at the same time under the same conditions? Over the years, scientists and professionals interested in the process of mortality (biologists, toxicologists, actuaries, etc.) have generally avoided this question and settled for a description (see Fig. 1.1 for two examples). These descriptions have a number of problems associated with them, all related to the fact that they are used to *describe* the data rather than to *explain* or *understand* them. To focus on (eco)toxicology: in this field, it is common practice to fit a standard curve (such as the log-normal or the log-logistic) to the data for survival versus concentration at a single time point, generally at the end of the experimental test (see right panel of Fig. 1.1). From this curve, one can deduce the concentration at which 50% of the individuals survive, and this concentration is promoted to the status of 'summary statistic': all of the information of the toxicity test is reduced to a single value. This value goes under different names, such as LC50 (when exposure is expressed as a concentration), LD50 (when exposure is expressed as a dose), EC50 (when the effect is not mortality but e.g., immobility), etc. Of course, one can also calculate an LC10 or an LC90, or in general an LCx for any x between 0 and 100%.[1] In toxicology, such summary statistics from a dose-response fit are often referred to as the 'benchmark dose' [61].

It has long been known that LC50s depend on exposure time: under constant exposure, they generally decrease gradually over time, and asymptotically approach a stable

[1] Generally excluding the boundaries, depending on the curve that is selected to describe the data. For distributions like the log-normal and log-logistic, the LC0 will always be 0, and the LC100 will always be infinite, irrespective of the mean and standard deviation.

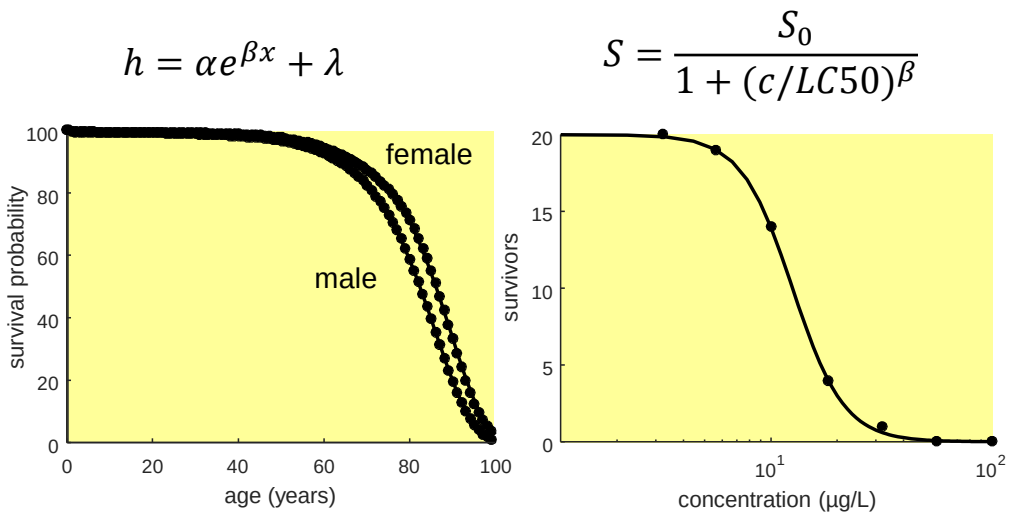


Figure 1.1: Two examples of descriptive survival functions. Left: survival curves for males and females in the Netherlands in 2012 (data from 'Nationaal Kompas'). Right: survival of guppies after 4-day exposure to dieldrin (data from [40]). Equations for the Gompertz-Makeham hazard function (left, hazard explained in Chapter 2, and see Appendix B.3.6) and the log-logistic dose response are provided here without further explanation.

value, designated the 'incipient LC50' (see e.g., [211]). Unfortunately, the time needed to achieve this incipient LC50 depends on properties of the chemical (e.g., mode of action and hydrophobicity) and the organism (e.g., species and body size). As soon as we select a standard exposure time for the LC50 (e.g., 4 days in acute fish tests and 2 days for *Daphnia*) we have generated a problem: we cannot meaningfully compare toxicity between chemicals and species anymore. An example plot is shown in Figure 1.2; which of the two chemicals is 'more toxic' to this species? The answer to that question depends on the exposure time, which is arbitrarily standardised in toxicity tests. Similarly, if the two curves in Figure 1.2 would represent two species exposed to the same chemical, we would have difficulties selecting the 'most sensitive' species.

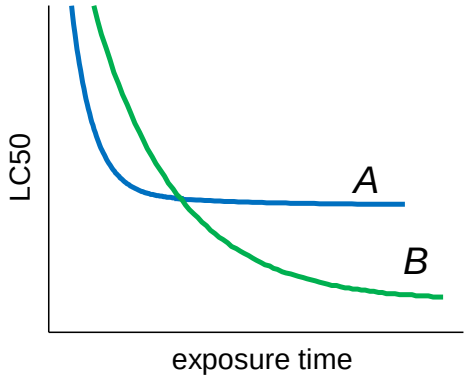


Figure 1.2: Example of LC50-time relationship for two hypothetical chemicals A and B in the same species. Depending on where to standardise the exposure time, a different conclusion of the 'most toxic chemical' will be derived. Similarly, if A and B are different species for the same chemical, it will be hard to select the 'most sensitive species'.

The LC50 describes the effect resulting from a constant exposure concentration for

a specific exposure duration. This implies two constraints: the LC50 is meaningful only when the exposure concentration in the test system has been kept constant, and secondly, that the LC50 is relevant for field situations only as long as the exposure situation is the same as that in the test (constant exposure over the same duration as the test, and similar environmental conditions). When the chemical concentration in the toxicity test is varying over time, the LC50 represents *that particular* time pattern of exposure only. If we have an LC50 for constant exposure but want to assess the effects of a time-varying exposure pattern, we have a problem: the 4-day LC50 cannot say anything useful about the consequences of a 1-day pulse exposure. This problem is often addressed in a rather *ad hoc* fashion: taking the peak exposure concentration, or the time-weighted average concentration, and use that as if it were a constant exposure.

The problems with the factor of exposure time and time-varying exposures, explained above, highlight the fact that the LC50 is merely a description. It describes the effects in a certain setting: constant exposure over a specific number of days, under standardised test conditions. Descriptions may be very useful, as long as one does not need to extrapolate beyond the conditions used to generate the data set. Unfortunately, in environmental risk assessment, we are usually interested in situations that are very different from those in the standardised toxicity tests. For example, for plant-protection products, constant exposure in the field would be an exception, and in any case, one is usually interested in effects over longer durations than those used in acute toxicity testing.

1.3 General aspects of TKTD modelling

Solving the issues raised in the previous section requires a more mechanistic approach, that explicitly includes the factor of 'time'. So why does the effect of a toxicant depend on the exposure time? The first step to answering this question is to realise that it is generally not the external concentration that directly produces the toxicity, but that chemicals first need to be taken up into the body before they can exert an effect. We thus need a toxicokinetic (TK) model. The link between internal concentrations and effect comes with its own time dependencies, which is why we additionally require a toxicodynamic (TD) model.[2] The term TKTD modelling applies to all models that include a mechanism-based representation of toxicokinetics (from external to internal concentrations) and toxicodynamics (from internal concentrations to effects over time). This is schematically drawn in Figure 1.3. GUTS also falls within this category of models.

The application of TKTD models offers a wide range of advantages over descriptive approaches, which has been discussed in detail elsewhere [128, 18, 117]. Most importantly, these models are the only way to deal with the fact that toxicity is a process in time, and they are the only way to make educated extrapolations beyond the conditions of the experimental test. Furthermore, they are the only way to learn something useful from experimental data, which will eventually lead to possibilities to extrapolate between chemicals and between species. At this moment, TKTD models are not yet routinely used in risk assessment, as far as we know. However, serious steps have been taken towards their implementation: TKTD models have been included in OECD/ISO

[2]The use of the terms 'kinetics' and 'dynamics' in this way has been a tradition in toxicology and pharmacology, but differs from the classical use of those terms in other fields such as physics.

guidance for the analysis of dose-response data [185], are mentioned as complementary method for tier-2 assessments of plant protection products by EFSA [72], and more recently, a dedicated scientific opinion was produced by EFSA [76]. We will return to the practical applications in detail in Chapter 6.

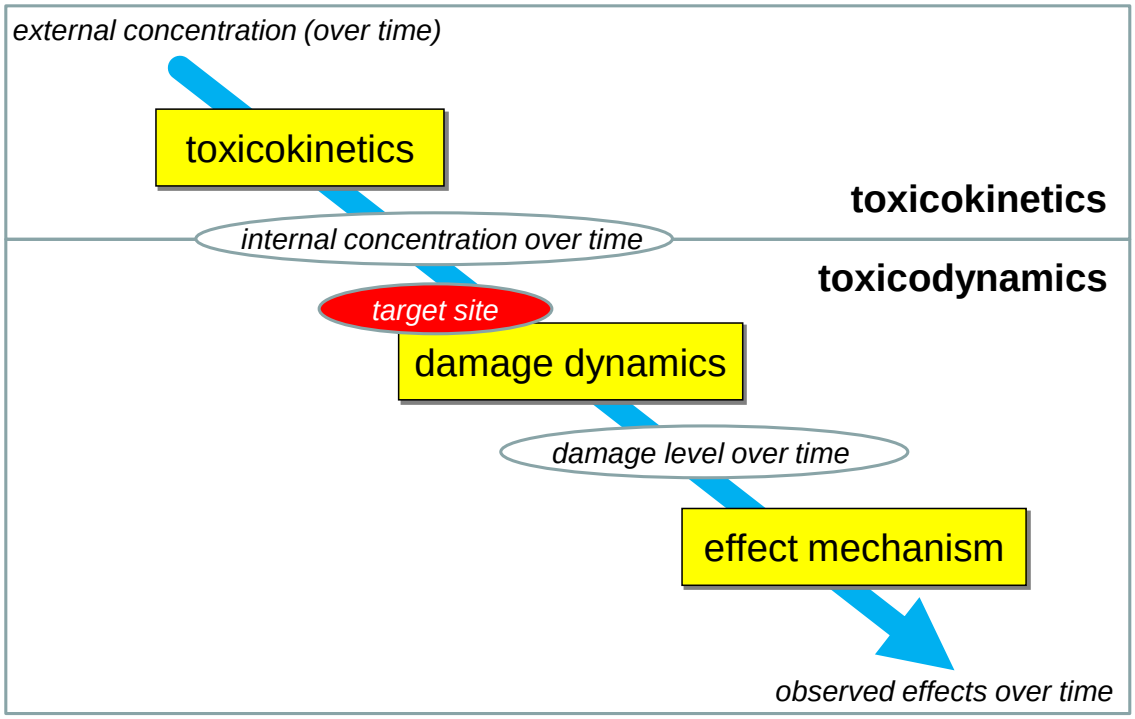


Figure 1.3: Toxicokinetic and toxicodynamic models are used to translate (time-varying) external concentrations to time patterns of effects. Toxicodynamics is split up in a 'damage' module and the actual mechanism that links damage to the effect on the observable endpoint (in our case: survival).

The TK module in TKTD may range from a simple one-compartment model to physiologically-based models, including a representation of organs and tissues. For applications in ecotoxicology, the one-compartment model is the starting point as we generally lack the information to parameterise more complex models. Furthermore, it is good to realise that physiologically-based TK models will usually simplify to one-compartment behaviour when the internal redistribution between the tissues is fast, relative to the exchange with the exposure medium. Apart from adding compartments to represent different parts of the organism, such an extension may also be warranted for organic chemicals that are biotransformed (see examples in ecotoxicology in [20, 150, 201]). For metals, more complexity in TK thinking is needed, as the relevant internal concentration for toxicity appears to be the fraction of metal inside the organism that is metabolically available, and in excess of physiological requirements (see e.g., [196, 65, 220]). It is good to stress already here that TKTD modelling is possible even in the complete absence of information about body residues (we will come back to this aspect in the next chapter of this book).

The TD module is a representation of some aspects of the organism's physiology, as far as relevant for the effect that we are interested in. As shown in Figure 1.3, the interface between TK and TD is formed by an internal concentration of a chemical: the