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Review

The molecular basis of simple relationships between exposure concentration and toxic effects with time

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ABSTRACT

Understanding the toxicity of chemicals to organisms requires considering the molecular mechanisms involved as well as the relationships between exposure concentration and toxic effects with time. Our current knowledge about such relationships is mainly explained from a toxicodynamic and toxicokinetic perspective. This paper re-introduces an old approach that takes into account the biochemical mode of action and their resulting biological effects over time of exposure. Empirical evidence demonstrates that the Druckrey–Küpfmüller toxicity model, which was validated for chemical carcinogens in the early 1960s, is also applicable to a wide range of toxic compounds in ecotoxicology. According to this model, the character of a poison is primarily determined by the reversibility of critical receptor binding. Chemicals showing irreversible or slowly reversible binding to specific receptors will produce cumulative effects with time of exposure, and whenever the effects are also irreversible (e.g. death) they are reinforced over time; these chemical have time-cumulative toxicity. Compounds having non-specific receptor binding, or involving slowly reversible binding to some receptors that do not contribute to toxicity, may also be time-dependent; however, their effects depend primarily on the exposure concentration, with time playing a minor role. Consequently, the mechanism of toxic action has important implications for risk assessment. Traditional risk approaches cannot predict the impacts of toxicants with time-cumulative toxicity in the environment. New assessment procedures are needed to evaluate the risk that the latter chemicals pose on humans and the environment. An example is shown to explain how the risk of time-dependent toxicants is underestimated when using current risk assessment protocols.

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1. Introduction

Understanding the toxicity of chemicals to organisms is the basis for a correct risk assessment. Given the enormous variety of chemicals that contaminate the environment, their different modes of action and mechanisms of toxicity (Escher and Hermens, 2002) in different species, quantitative studies on the relationship between exposure levels to toxicants and toxic effects are necessarily complex (Bradbury, 1995; Rubach et al., 2010) and represent a major challenge to ecotoxicologists. Relating an observed exposure concentration–effect relationship to the mechanism of toxicity of a compound, which is a prerequisite for meaningful risk assessment of chemicals, is only the first step for such an understanding. A second step involves the time-dependency of toxic effects (Baas et al., 2010), which is often forgotten in ecotoxicological research although time is considered in risk assessment protocols (e.g. chronic toxicity). Certainly, the inclusion of time is becoming more common in experimental studies (Legierse et al., 1999; Newman and McCloskey, 1996; Smit et al., 2008) and models (Lee and Landrum, 2006; Jager et al., 2011). However, the underlying mechanisms of time-dependency are best understood in the case of baseline toxicity (i.e. narcotics), but not so much in chemicals with specific modes of action (e.g. reactive electrophiles, enzyme inhibitors, etc. – for a review see Escher and Hermens (2002)).

The influence of time of exposure on toxicity was suggested a long time ago (Bliss, 1937), but it has taken decades for time-to-event analyses of ecotoxicity data to be developed (Newman and McCloskey, 1996) and applied in risk assessment (Crane et al., 2002). Unfortunately, implementation of time-dependent approaches on standard toxicity protocols and regulatory risk assessment is still lagging behind. Standard acute test protocols (e.g. OECD tests) require that toxic effects are recorded at intermediate time-points, but the derivation of LC50 and other toxicity metrics is only done at fixed times (e.g. 48 or 96 h). Consequently, most of the information obtained is not used even if it could be analyzed further using appropriate descriptive methods (Jager et al., 2006). Two different approaches can be used to analyze toxicity test data that includes time information: time-to-event procedures (Newman and McCloskey, 1996) and mechanistic models (Mackay et al., 1992; Kooijman and Bedaux, 1996; Ashauer and Escher, 2010). Time-to-event (TTE) analysis is an empirical method, which describes the time-dependent toxicity of a particular chemical to a particular species by fitting a mathematical curve to the experimental data. Often the parameters in those mathematical equations cannot be explained in biological terms, but the equations thus obtained can predict the toxicity of the chemical to a species with reasonable accuracy within the tested conditions (Zhao and Newman, 2004). Many mechanistic models have been proposed to analyze the time-dependent toxicity of chemicals, and their inclusion here is outside the scope of this paper (for a comprehensive review see Jager et al. (2011)). All these models are useful tools to describe the toxic effects observed over time. For the case of survival endpoints, the current trend is to integrate their different assumptions under a general unified threshold model of survival (GUTS) based on toxicokinetics and toxicodynamics (Jager et al., 2011). However, for these mechanistic models to be realistic they need to be based on sound toxicological concepts.

The objective of this paper is three-fold: firstly, a short and critical review of current approaches to time-dependent toxicity is made in order to provide a background. Secondly, an old approach developed by Druckrey and Küpfmüller (1949) to study the toxicity of carcinogenic substances (Druckrey et al., 1963) will be introduced, as it is almost unknown among ecotoxicologists. Recent experimental evidence with aquatic and terrestrial organisms confirm that relatively simple exposure concentration–effect relationships are identical to those derived from the theoretical (mathematical) approaches of Druckrey and Küpfmüller (Tennekes, 2010). Thus, the observed exposure concentration–effect relationship can be related to the mechanism of action of a toxicant. The third objective is to show a number of case studies taken from the literature that confirm the validity of this old approach, followed by a brief discussion of the mechanisms involved in each case. Finally, some suggestions for new risk assessment procedures are made, using an example to explain how the risk of toxicants with time-cumulative toxicity, i.e. those for which toxic effects are greatly enhanced by exposure time, is underestimated in current risk assessment protocols.

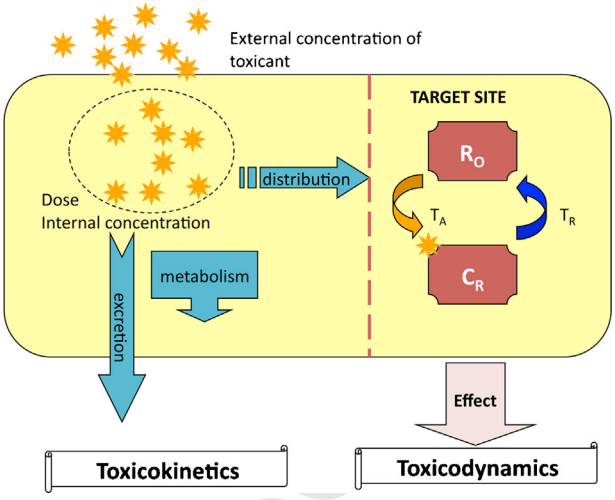

Fig. 1. Structure of the Druckrey–Küpfmüller model. The internal concentration or dose is determined by the toxicokinetic processes that take place inside the organisms. Only the toxicant molecules that reach the target receptors (R_0) can have a toxic effect. The toxicodynamics are based on binding of toxicant molecules to the target receptors (C_R), a process that takes place in time and depends on the relative velocities for association (T_A) and dissociation (T_R) to and from the receptor.

1.1. Current status on time-dependent ecotoxicity

Most of the research aimed at explaining the toxicity of chemicals in organisms is based on toxicokinetics, that is the processes of uptake, distribution within an organism, biotransformation (metabolism) and elimination (Fig. 1). Toxicokinetics determine the relationship between exposure concentration of a toxicant in the external media (or dose ingested in dietary exposures) and its concentration at the site of action, as well as its time course. Therefore, information on all aspects of the kinetics of toxicants is of particular relevance for understanding and predicting the toxicity of chemicals (Escher and Hermens, 2002). However, it is the concentration of the toxicant at the site of action that is of major interest, since this concentration determines critical receptor binding that may eventually elicit a toxic effect. The extent of the effect is assumed to be proportional to the abundance of target sites. A linear relationship between exposure levels to toxicants and their toxic effect, therefore, requires strict proportionality for each process.

More recently the concept of toxicodynamics, that is the interactions that link the internal concentration to an effect in an individual organism over time, has been incorporated as well (Ashauer and Brow, 2008; Voicu et al., 2010). Several interactions have been proposed, including damage-repair mechanisms (Lee et al., 2002), killing rates and recovery constants (Ashauer et al., 2007), which are appropriate for narcotics and some chemicals with specific mode of action. For the latter chemicals, the Druckrey–Küpfmüller model uses the relative velocities of association

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