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Three-phase metal kinetics in terrestrial invertebrates exposed to high metal concentrations

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ABSTRACT

Models of metal toxicokinetics are critically evaluated using both newly generated data in the NoMiracle project as well as those originating from older studies. The analysis showed that the most frequently used one-compartment two-phase toxicokinetic model, with one assimilation and one elimination rate constant, does not describe correctly certain data sets pertaining particularly to the pattern of assimilation of trace elements. Using nickel toxicokinetics in carabid beetles and earthworms as examples, we showed that Ni in fact exhibits a three-phase kinetics with a short phase of fast metal accumulation immediately after exposure, followed by partial elimination to an equilibrium concentration at a later stage of a metal exposure phase, and by final elimination upon transfer to an uncontaminated food/soil. A similar phenomenon was also found for data on cadmium kinetics in ground beetles and copper kinetics in earthworms in data already published in the literature that was not accounted for in the earlier analysis of the data. The three-phase model suggests that the physiology of controlling body metal concentrations can change shortly after exposure, at least in some cases, by increasing the elimination rate and/or decreasing metal assimilation. Hence, the three-phase model, that allows for different assimilation and/or elimination rates in different phases of exposure to a toxicant, may provide insight into temporal changes in the physiology of metal handling. Consequently, this alternative model should always be tested when describing metal toxicokinetics when temporal patterns of internal metal concentration exhibit an initial "overshoot" in body metal concentrations.

1. Introduction

The ultimate effect of toxic chemicals on organisms depends principally on three factors: the extent of exposure to a toxicant as influenced by environmental availability, internal concentration as determined by rates of uptake and elimination, and interaction with receptors at the target site. While availability is determined primarily by the properties of the environment, internal concentration and the concentration at the target site are strongly influenced by the biology of the organism. As an initial line of defence against exposure to toxic chemicals, animals may reduce the transfer of the chemicals from the gut lumen or body wall to body fluids and subsequently their transfer to internal target sites. Once, however, a chemical has passed these physiological barriers, a molecule can be degraded to non-toxic or less toxic forms, as happens with many organic pollutants and pesticides, or – in the case of many metals – accumulated toxicants can be fixed in an insoluble form, preventing them thus from interacting with vital biochemical functions.

In cases where metals are accumulated in detoxified forms, it is well recognised that they can be retained within the body for a prolonged time. This means that under continuous exposure, there is a tendency for total internal body concentrations to increase as long as the animal is exposed to the metal. Such a behaviour was found for example for cadmium accumulation in pseudoscorpions (Janssen et al., 1991) and isopods (Crommentuijn et al., 1994). This strategy, however, has its limitations, since the concentration of a chemical, even if fixed in a relatively inert form, cannot increase infinitely and also there is likely to be an energetic cost to this sequestration. As a result this strategy is not applicable to all species for all metals. Indeed, many organisms have developed some ways of elimination/depuration allowing to excrete at least part of the assimilated metal. This is what happens, for example, with many metals in carabid beetles (Janssen et al., 1991; Kramarz, 1999a; Bednarska et al., 2009) and also earthworms (Vijver et al., 2005). This basic distinction of animals between those with more efficient excretion and those accumulating toxic chemicals to high concentrations has been

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recognised. Thus, Boháč and Pospíŝil (1989) distinguished three groups of animals with respect to characteristics of their metal handling, which they called "deconcentrators", "microconcentrators" and "macroconcentrators".

Although differences in metal physiology are recognised to exist between species in relation to trace metal concentration, it has to date been widely accepted that the accumulation and elimination pattern can be described with a simple one-compartment model, derived from the Atkins' (1969) equation, assuming that an animal exposed to a constant concentration of a toxic chemical assimilates the chemical with a constant rate k_a, and eliminates it with a constant rate k_e, which gives at time t an internal body concentration C_t:

$$C_t = C_0 + \frac{k_a}{k_e}(1 - e^{-k_e t}) \quad (1)$$

where the assimilation constant k_a is expressed in mass of a chemical assimilated per unit body mass per unit time (e.g., mg kg^{-1} day^{-1}), k_e is the unit-less elimination constant expressed per time (e.g., day^{-1}) and C_0 is the chemical concentration in the animal at the start of the exposure (mg^{-1} kg^{-1}).

Further, it also has commonly been assumed that if the animal is transferred to a clean (i.e., uncontaminated) medium at some time t_c, then for time $t > t_c$ the internal body concentration C_t is described by the following equation:

$$C_t = C_0 + \frac{k_a}{k_e}(1 - e^{-k_e t}) - \frac{k_a}{k_e}(1 - e^{-k_e(t - t_c)}) \quad (2)$$

(see e.g. Janssen et al., 1991; Spurgeon and Hopkin, 1999). In this paper, the model consisting of the Eqs. (1) and (2) combined to describe the kinetics of a chemical during the two different phases of a toxicokinetic experiment, these pertaining to what is usually termed an uptake and an elimination phase ($t \le t_c$ and $t > t_c$, respectively), will hereafter be called the "classic two-phase model".

Although the classic two-phase model can indeed describe the toxicokinetics of a number of metals in many animals satisfactorily (Janssen et al., 1991; Spurgeon and Hopkin, 1999; Sterenborg et al., 2003; Vijver et al., 2006), its formulation can be questioned on physiological grounds because it assumes the same, constant elimination rate k_e both when an animal is actually exposed to a toxicant and also in the elimination phase. This flaw of the classic model was noticed earlier by Kramarz (1999ab), who used a model allowing for different elimination rates in the first ($t \le t_c$) and the second phase ($t > t_c$) of experiments. Moreover, in some studies a peculiar initial "overshoot" can be noticed (e.g., Neuhauser et al., 1995; Descamps et al., 1996; Spurgeon and Hopkin, 1999; Lagisz et al., 2005), which neither the classic nor the modified (Kramarz, 1999ab) two-phase model can handle. As examples, Neuhauser et al. (1995) found in a study on the earthworm *Allolobophora tuberculata* that from among the five studied metals (Cd, Cu, Ni, Pb and Zn), the uptake and elimination kinetics of Ni, the uptake kinetics of Pb and Cu, and the elimination kinetics of Pb displayed unexpected patterns. Especially the concentrations of Ni, Pb and Cu increased rapidly in the initial part of the uptake phase (worms transferred from uncontaminated to contaminated soil) but then slowly decreased to levels that were close to those observed in the worms before transfer to the contaminated soils. Further, in the elimination phase (worms transferred from contaminated to uncontaminated soil), Ni and Pb concentrations decreased initially but then tended to follow an irregular pattern over the rest of the study. Descamps et al. (1996), in a study of cadmium levels in centipedes *Lithobius forficatus*, also found that concentrations first increased dramatically and then decreased even though the animals were fed constantly with Cd contaminated *Chironomus* larvae. The studies of Lagisz et al. (2005) on Cd and Zn kinetics in *Pterostichus oblongopunctatus* also showed such an unexpected pattern of metal kinetics that prohibited use of the classic two-phase model to analyze their data, and Janssen et al. (1991) also observed large deviations from the classic model during the accumulation period in the carabid *Notiophilus biguttatus* fed with collembolans *Orchesella cincta* contaminated with cadmium (see original data shown in Fig. 2D in Janssen et al. (1991)). It is worth noticing that the specific pattern with an overshoot at the beginning of exposure to a metal occurs in studies on both essential (Ni, Cu) and non-essential (Cd, Pb) metals.

In the cases highlighted above, the authors generally assumed that such deviations might be due to experimental errors that were not explained by the model, with aberrant data points often treated as outliers, possibly resulting from simple analytical errors. For a single experiment, especially when sparsely sampled at the very beginning of the uptake phase, such an interpretation is reasonable. However, as more studies have shown this pattern, this apparent "overshoot" may not simply be the result of analytical errors but instead may have a wider biological basis within the different studies (Neuhauser et al., 1995; Descamps et al., 1996; Spurgeon and Hopkin, 1999; Lagisz et al., 2005). Therefore, when a similar pattern was observed across several studies on nickel toxicokinetics performed by different research teams under the umbrella of the international research project "NoMiracle", we realized that we are probably observing a more general phenomenon. In particular two invertebrate species with different exposure routes, namely ground beetles and earthworms, both exhibited a similar accumulation pattern that was divergent from the classic model. These two studies, which were both conducted to study the uptake of Ni, were both characterised by a high increase in Ni body concentrations at the very early stage of the uptake phase (the first days or even hours of exposure to Ni-contaminated food or soil) followed by a decrease of Ni body concentrations when the animals were still exposed to metal-contaminated food/soil and finally a further decrease of internal concentrations following transfer to clean media as might be expected from the classic model.

Our observations, when coupled to the literature results, therefore lead to the hypothesis that an animal, when suddenly exposed to highly elevated concentrations of a metal, may show a lag in physiological response that results in a delay in the onset of efficient elimination and/or decrease in metal assimilation. Such a lag time would explain the toxicokinetic pattern not expected from the traditionally used classic two-phase model: the initial fast increase in concentration of a chemical (high assimilation/uptake, possibly accompanied by low or non-existent elimination), followed by a decrease in body concentration leading to some equilibrium concentration (k_a balanced by k_e when either metal assimilation is significantly decreased or efficient elimination mechanisms are turned on or both phenomena act in unison), followed eventually by further decrease to the initial concentration if the animal is transferred to an uncontaminated environment and when the physiological response results in the elimination of the remaining contaminant. This would mean that the classic two-phase model neglects an important physiological mechanism, and a somewhat more complicated toxicokinetic model should be used to describe the behaviour of some toxicants in organisms.

In this study we gathered data originating from different toxicokinetic studies to test whether the modified toxicokinetic model could describe the toxicokinetics of some metals in certain species better than the classic model. The modified model should allow for: (1) an early phase in which animals assimilate a metal at a high rate and are not able to excrete efficiently, and (2) a switch to a phase characterised by lower or null assimilation rate and/or increased elimination constant, describing the different assimilation and/or elimination efficiencies after the switch-point, and (3) a final elimination phase after transfer to uncontaminated food or medium. Fit of the collected data to the modified model was compared to those