Rethinking the utility of contaminant body-residue as the dose metric for pulsed exposures in aquatic toxicity. Jong-Hyeon Lee, 2003/04/25

Background

Standard regulatory paradigms such as water quality criteria use the environmental concentration as a surrogate for the concentration at target site. These paradigms are based on the premise that the toxicant concentration at target site is proportional to the organism concentration, which is in turn proportional to the exposure concentration. Some of the limitation of this approach includes the difficulties in determining the bioavailable fraction of the environmental concentration, multiple uptake routes and non-steady-state situations (e.g., short exposure times). If effects were based on the body residue required to produce the effect, complications arising from the uncertainty regarding bioavailability and accumulation would essentially be eliminated (Landrum et al., 1992). In addition, based on body residue approach mixture toxicity by different narcotic compounds is additive (Landrum et al., 1991). Therefore, “the utility of expressing dose on a body residue basis is a reasonably new concept that has the potential to greatly improve our ability to assess risk particularly where there are multiple routes of exposure“ and to interpret the significance of chemical residues in field-collected organisms exposed to various contaminants.

The first attempt at using the body residue approach was to establish the body residue basis for toxicity (constant Critical Body Residue (CBR) model). The CBR for acute mortality for a wide range of non-polar narcotic organics was relatively constant (McCarty and Mackay, 1993). Meanwhile, in the case of other organics with different mode of toxic action (reactive or receptor-mediated compound), the CBR values decreased even after body residue attained the steady state, which can be interpreted by the Critical Area-Under-the Curve (CAUC) model (Verhaar et al., 1999) and Dynamic Energy Budget-toxicology (DEBtox) model (Kooijman and Bedaux, 1996) . Recently, Lee et al. (2002a) reported that the CBR values for narcotic compound such as PAHs also decrease with increase of exposure time. Lee et al. (2002b) developed a Damage Assessment Model (DAM) to predict the toxicity time course for PAH in amphipod *Hyalella azteca*. The DAM assumes that death occurs when the cumulative damage reaches a critical point and is described by a combination of both first-order toxicokinetic and toxicodynamic models with assumptions that damage accumulates in proportion to the body residue and recovers in proportion to the cumulative damage. The constant CBR and CAUC model assume perfect reversible and irreversible response, respectively, whereas DAM can provide a way to estimate damage recovery rate constant from 0 to ∞ without any assumption;

Constant CBR model	$S(t) = \exp(-k_1 R(t))$
DEBtox model	$S(t) = \exp(-H(t))$, $H'(t) = k_2(R(t)-R_0)_+$
DAM	$S(t) = \exp(-k_3 D(t))$, $D'(t) = k_a R(t) - k_r D(t)$

with $S(t)$ = control-adjusted survival rate, k_1, k_2, k, k_3 = constant, $R'(t) = k_u C(t) - k_e R(t)$, R_0 = No-Effect-Concentraion, $(x)_+ = x$ if $x>0$ or 0 if $x<0$, k_u = uptake clearance rate, k_e = elimination rate

constant, k_a = damage accrual rate, k_r = damage recovery rate constant.

When body residue attains steady state, damage can be constant (constant CBR model), or linearly increase (CAUC model), or increase and reach steady state (DAM) depending on damage recovery rate. Therefore, body residue responsible for mortality depends on damage recovery rate as well as the duration of exposure due to the build up of damage in the organism with longer-term exposures. Thus, pulsed exposures may allow a period of damage recovery that would alter the interpretation of the impact of specific body residues (Fig. 1). In the case exposed to mixtures of PAHs with different elimination rate constant, the situation is so more complex that damage for each PAH accumulates and recovers at the different speed under pulsed exposures.

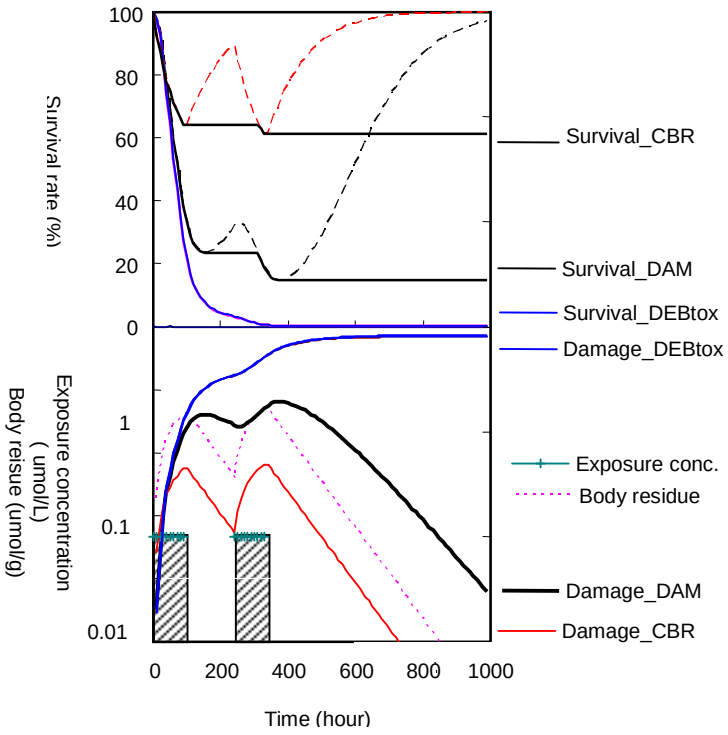

Fig. 1. Comparison of simulation results of toxic effect under two pulsed-exposures using constant Critical Body Residue (CBR) model, Dynamic Energy Budget-toxicology (DEBtox) model, and Damage Assessment Model (DAM).

Aims and methodology

In this study, we will investigate how we can predict toxic effect in organisms intermittently exposed to 1) one contaminant, 2) two contaminants with different elimination rate constant and the same mode of toxic action, 3) two contaminants with different elimination rate constant and different the mode of toxic action. And then we will be prepared to address further question, “How can we interpret the significance of chemical residues in field-collected organisms intermittently exposed to various contaminants through multiple routes of exposure?”

Issues on pulsed-exposures

Pulsed exposure experiments can provide additional information such as the potential for recovery, cumulative effects or resistance by induction of detoxification or biotransformation enzyme (Reinert et al., 2002). In addition, it has been generally reported that latent effect was observed after terminating exposure in pulsed exposure experiments (references in Reinert et al., 2002). For example, following short exposures to the toxicants, ranging from a few minutes to a few hours, mortalities continued to occur for up to 2 weeks in the case of cadmium and permethrin, although delayed mortalities ceased within 40 h with cyanide (Abel and Garner, 1986). So post-exposure observation is essential to determine delayed effect, which can be expressed by PE-LET50 (Post-Exposure Lethal Exposure Time for 50% of the population) defined as a measure of the exposure duration to a sample that produces 50% lethality of the test population during post-exposure observation (Brent and Herricks, 1999). Similarly, PE-LEC50 (Post-Exposure Lethal Exposure concentration for 50% of the population) can be also determined.

Experimental Scheme

Variables experimentally that can be controlled in pulsed exposure experiment include exposure timing, depuration interval, shape of pulse (symmetric and asymmetric pulse with different rate of increase and decrease) as well as exposure intensity, duration, frequency. So exposure scenarios used in this study are 1) single pulsed exposure with equivalent dose conditions (different intensities, durations and frequencies), 2) multiple pulsed exposures with equivalent dose conditions (different timings and depurations intervals including long depuration interval that toxic effect can be recovered and long exposure duration that cumulative damage reach steady state), 3) multiple pulsed exposures with different combinations of exposure concentrations, duration and frequency. Experiments protocols in this study will follow Lee et al. (2002a): 1) toxicokinetic experiments to estimate toxicokinetic parameters, 2) toxicity experiments at multiple exposure times to estimate toxicodynamic parameters, 3) body residue measurement in toxicity experiment to measure critical body residue at multiple exposure times. Especially, since it has been reported that long exposure from 2 to 10 days to high concentrations of fluoranthene may result in a decrease in the water-only conditional uptake clearance rate in *H. azteca* (Kane Driscoll et al., 1997), toxicokinetic experiments should be conducted for pre-exposures with selected durations and concentration in the above experiments.

We will use *Hyalella azteca* under static-renewal exposure system. Exposure concentrations in test water sampled before and after renewing test water will be measured after passing a C_{18} reverse-phase cartridge. Chemicals for these experiments are C^{14}-labeled phenanthrene, fluoranthene and DDT. In multiple exposures experiments, dual-labeled technique will be used to measure concentrations of two compounds radio-labeled by C^{14} or H^3. In previous studies, toxicokinetic and toxicodynamic parameters for fluorene and pyrene have been already determined in *Hyalella azteca* (Lee et al., 2002a and b). Toxicokinetic parameters and LC50(t) values for phenanthrene and fluoranthene can be estimated by QSAR model (Lee, 2001). In the case of DDT, toxicokinetic parameters and critical body residue values were determined (Lotufo

et al., 2000), but toxicodynamic parameters. Detailed experimental designs will be determined by simulation studies for comparison of constant CBR and DEBtox models and DAM for pulsed exposures.

Modeling approach using DAM

In previous study, damage accrual rate and damage recovery rate constant were estimated from LT50(c) data set measured in several exposure concentrations and times (Lee et al., 2002b). In this study, these parameters will be estimated from time-to-death curve in each exposure concentration. In addition, we will try to estimate toxicokinetic- and toxicodynamic parameters under the pulsed-exposures. It is another challenge how to use real effect size measured through post-exposure observation in multiple pulsed-exposures for estimation of toxicodynamic parameters.

Expected results and their significance and application

In this study, several issues in pulsed-exposure studies such as delayed effect, reversible and irreversible effects, and selection of endpoints appropriated for pulsed exposures will be re-investigated according to DAM. The current modeling approaches for pulsed exposures such as PULSETOX based on constant CBR model (Hickie et al., 1995) and DEBtox (Péry et al., 2001) can be compared with DAM using the same dataset from this study. Mixture effect from contaminants with the same or different mode of toxic action will be measured under the pulsed exposure and able to be described by damage additivity approach as well as by the current approaches such as the concentration additivity or the response additivity models.

In many cases, risk assessment decisions are based on experimental data obtained when animals are exposed to a test substance for one duration of time. However, we must assess the risk of exposure for aquatic life which is exposed to a substance for varying time intervals. Therefore, risk assessors need strategies for determining how the ecological risk associated with exposure change as duration of exposure changes. Current risk assessment duration adjustments, such as time-weighted average (TWA) concentration as a default option, are based on Haber's rule ($c \times t = k$; c= concentration, t= exposure time, k= constant at a fixed effect level) (Rozman and Doull, 2000). According to the DAM, we can assume the minimal exposure condition (worst case) of dose and time that will produce a specific adverse effect that a species is continuously exposed to a contaminant with too small elimination rate constant and damage recovery rate constant. In this case, the relationship between concentration and time at a fixed effect level is given by $(1/2)c \times t^2 = k$ (Lee, 2001). Therefore, duration adjustment such as TWA concentration may not be protective. So it is expected that DAM approach can provide more protective and predictive strategies than current approach for determining how the ecological risk associated with exposure change as duration of exposure changes.

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