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EFFECTS ANALYSIS OF TIME-VARYING OR REPEATED EXPOSURES IN AQUATIC ECOLOGICAL RISK ASSESSMENT OF AGROCHEMICALS

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Abstract—Exposure to agrochemicals in the aquatic environment often occurs as time-varying or repeated pulses. Time-varying exposures may occur due to runoff events and spray drift associated with precipitation and application events. Hydrologic dilution, dispersion, and degradation also produce pulsed exposures. Standard laboratory toxicity tests using constant exposure concentrations typically do not investigate the toxicity of time-varying or repeated exposures. Detoxification, elimination, and recovery may occur within organisms or populations during the periods between exposures. The difficulty of estimating effects of realistic time-varying exposures from measurements made under constant exposure conditions is often an important source of uncertainty in ecological risk assessment of pesticides. This article discusses the criteria and tools for deciding whether time-varying exposures are relevant in a particular risk assessment, approaches for laboratory toxicity testing with time-varying exposure, modeling approaches for addressing effects of time-varying exposure, deterministic and probabilistic ecological risk characterization of time-varying exposures and toxicity, and uncertainty analysis.

Keywords—Aquatic ecological risk assessment Agrochemicals Time-varying exposure Effects analysis Repeated exposures

INTRODUCTION

Input to aquatic environments by agrochemicals often occurs in pulses rather than by continuous exposure [1]. For example, substances released through spills, application of pesticides or agrochemicals, surface runoff, precipitation of airborne pollutants, or discharge of industrial effluents result in maximum concentrations just after reaching a water body. These concentrations then change rapidly as a result of varying rates of input and dilution, changes in form, and solubility and degradation. Standard laboratory toxicity tests utilizing continuous exposure scenarios typically do not investigate the toxicity of short-term pulsed or intermittent exposures of substances to aquatic organisms [2–4]. Some level of detoxification and/or elimination (depuration) of the substance during the substance-free period may reduce the toxic effects of the earlier exposures and is dependent on the length of time between pulses (recovery interval, postevent interval).

The value of investigating the relationship between exposure duration and toxicity of compounds is relevant when evaluating the potential field effects of agrochemicals. Input to surface waters from agrochemicals typically occurs in pulses or time-varying and/or repeated exposures (application interval) due to agricultural runoff [5] and spray drift; continuous exposure may not provide an appropriate estimate of a toxic effect, especially for agrochemcials with short half-lives. Application often results in a pulse concentration in the environment, followed by an interval during which concentrations of the agrochemical may fall to below detectable levels (recovery interval) prior to the subsequent application due to hydrologic dilution, runoff events, or degradation in soil, water, and sediments (Fig. 1). Such exposures can be pulsed and nonrepeating or repeating. Effects from the exposure(s) may be cumulative or reversible.

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POTENTIAL CONSEQUENCES OF A TIME-VARYING OR PULSED EXPOSURE

Time-varying or repeated exposures can have a variety of consequences. The first pulse may select more hardy, more robust individuals (individual selection for tolerance), causing an apparent lessening in toxic response. Induced individual tolerance from previous pulses may strengthen survivors (making them more tolerant [6]) through acclimation, induction of detoxification or biotransformation enzymes, and so on (e.g., induction of cytochrome P450–dependent mono-oxygenases [7] and mixed-function oxygenases in fish [8,9]). Cumulative individual effects occur when the first pulse weakens the organism (making it less tolerant), dissipating energy and lowering organism fitness [6], or the substance irreversibly interacts with the receptor [10,11]. Postexposure, latent, or delayed effects occur after a typical study is terminated. This may be an important factor in the interpretation of toxicity studies and in risk assessment for agrochemicals [12]. Ecological recovery may occur after an exposure that affects a significant proportion of the population. The population (and higher organization) may recover adequately during the period of negligible exposure (recovery interval). Exposure–response reciprocity may occur; for example, a 2-d exposure at 2 μg/L may cause the same effects as a 1-d exposure at 4 μg/L or 4 d at 1 μg/L [13].

Independence or dependence of effects from repeated exposures

The effects of a first exposure pulse may influence the biological response to a second pulse. Using time to event

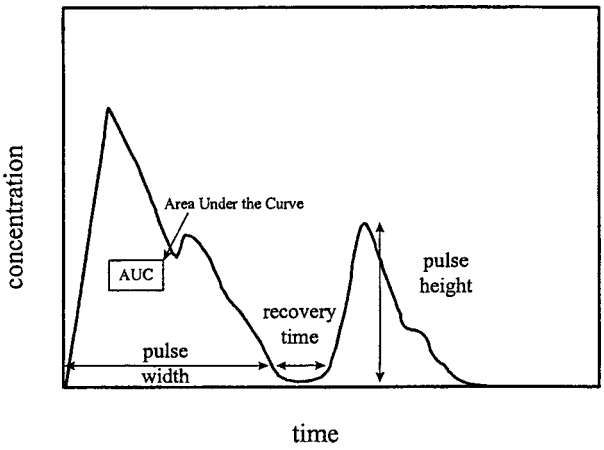

Fig. 1. Schematic showing pulse, time-varying, or repeated-exposure relationships and terminology.

approaches [14], one can determine whether enhanced, reduced, and independent effects are produced with pulsed exposures. The value of the effect parameter (e.g., mortality) at a particular time (e.g., 96 h) after several exposures can be compared with the value after a single exposure. Again, the effect may be similar, enhanced, or reduced with respect to a single exposure. The response may also depict a split probit [15,16], which is often attributed to different mode of actions occurring at different times during an exposure (e.g., pulsed exposure events) or distinct subsets of individuals with different resistance to a stressor (or pulsed exposures in this case). Note that use of the typical model (lognormal or probit) could also produce a split probit because of poor data fit [16]; alternative toxicity models should be investigated in such instances. Such a sharp break in the plot may also indicate the presence of two different substances [15].

Acute versus chronic exposure

Intermittent or pulsed exposures most often are associated with acute responses [17–19]; however, chronic effects may also be important when considering postexposure toxicity (delayed effects) such as effects on growth or when sublethal responses occur [1,19–21]. However, growth rates may not be a reliable indicator of delayed effects because reproductive failure may occur even when poor growth is not observed. Also, the relationship between cumulative mortality and exposure duration may not be proportional in intermittent exposure events when the peak concentrations are constant [1]. Naddy et al. [22] supports a curvilinear response for pulsed studies with chlorpyrifos using *Daphnia magna*. The relationship between concentration and duration of exposure was not proportional at low concentrations and longer durations; once higher concentrations and shorter exposure durations were reached, the relationship became proportional (i.e., if the concentration is halved, the exposure duration must double to produce the same effect).

Shorter exposure causes less effect

Jarvinen et al. [23] studied the acute toxicity of chlorpyrifos, endrin, and fenvalerate to fathead minnows in both single pulsed and continuous exposures. Varying combinations of pulse (1–96 h) and recovery (0–95 h) periods were used, totaling 96 h. Also, Naddy and Klaine [24] demonstrated that chlorpyrifos toxicity to *D. magna* was reduced using shorter but more frequent exposure pulses. Generally for these agrochemicals, the longer the pulse (shorter the recovery), the closer the toxicity value was to the 96-h lethal concentration (LC50) from continuous exposure.

In a study by Williams and Holdway [25], the Australian crimson spotted rainbow fish (*Melanotaenia fluviatilis*) was exposed to either 2 h or continuous levels of Cd or Zn. Larval fish (9–10 d) demonstrated 96-h LC50 values of 0.56 and 0.01 mg/L Cd and 1.57 and 0.27 mg/L of Zn for 2 h and continuous exposures, respectively. Substantially higher levels were required to produce an LC50 for the 2 h compared to the 96-h exposure.

Heming et al. [26] exposed early life stages of rainbow trout to pulsed and continuous doses of methoxychlor. The pulsed exposure scenario mimicked environmentally realistic exposures from the use of the agrochemical to control biting fly larvae in western Canadian rivers. Survival, growth, and development of early life stages were assessed for 68 d after a pulsed exposure of 580 μg/L (biota returned to clean test water after 2 h) and a spiked concentration of 30 μg/L (static design in which the substance degraded over 68 d). The methoxychlor concentration decreased due to fate processes. No biologically significant effects were observed on any growth stage for either exposure scenario, although the pulsed concentration was >10 times the spiked level.

Stuijfzand et al. [27] reported that increasing the exposure duration to diazinon from 2 to 4 d decreased the survival of midges (*Chironomus riparius*) and caddisflies (*Hydropshyche angustipennis*) by a factor of 1.4 to 1.6 and 2.2 to 8.4, respectively. In a recent study [28], the effects of fenoxycarb on growth and reproduction of *D. magna* were examined using a single pulse dose. This realistic exposure regime was based on laboratory fate data and field observations and mimicked the reduction in fenoxycarb following field application to natural waters. For both the pulsed and the continuous exposure studies, reproduction was the most sensitive endpoint. A substantial reduction in toxicity (maximum acceptable toxicant concentrations of 26 and 0.0016 μg/L from the pulsed and the continuous exposure studies, respectively) was observed from exposure to environmentally realistic levels of fenoxycarb.

Effect related to peak concentration, not duration

Curtis et al. (in [3]) reported that intermittent exposure to fenvalerate was more toxic to fish than continuous exposure when daily mean concentrations were equal. However, the results are difficult to interpret because the concentration administered intermittently was higher than that administered continuously to achieve equal daily mean concentrations and may have resulted in increased toxicity, which would not be representative of field situations.

In a more environmentally realistic study, Schulz and Liess [29] exposed caddisfly larvae (*Limnephilus lunatus*) to three different equivalent fenvalerate concentrations for 1 or 10 h. After transfer to a stream microcosm with pesticide-free water, chronic effects (emergence, dry wt) after 240 d were more pronounced in equivalent exposures (μg/h) of 1 h compared to 10 h.

Latency (delayed effects)

Van der Hoeven and Gerritsen [10] studied the effect of chlorpyrifos on *Daphnia pulex*, reporting that the agrochemical immobilized daphnids several days before death. Even when exposure was discontinued, immobilized *D. pulex* died,

further supporting the concept of irreversible effects. However, in a study by Naddy and Klaine [24], no latent effects were observed for daphnids that survived initial chlorpyrifos exposure, provided adequate recovery time between exposures was allowed. Without an adequate recovery period, effects were observed. In Naddy and Klaine [24], the authors suspected that in the van der Hoeven and Gerritsen [10] study, a combination of higher exposure concentrations, coupled with longer exposure periods (less recovery time), allowed the daphnids to accumulate chlorpyrifos, exceeding their critical toxicity threshold.

Unless study results indicate otherwise, it may be appropriate to assume latency. Although latency is a function of the toxic substance, it is the degree of this latency that is important in the design and interpretation of the pulsed toxicity study (K.R. Solomon, 1998, ''Latency of Responses,'' ECOFRAM, unpublished data). For example, if latency does not occur, short-term exposures should be evaluated against toxicity tests with similar exposure times. In order to demonstrate latency (or lack of) in acute studies, observation intervals must continue after the exposure is completed and the organism has been removed from the stressor. For example, in Hurd et al. [12], diflubenzuron effects on aquatic macroinvertebrates were not observed until molting began, some two to four weeks after a single exposure. Lack of latency in chronic studies can be determined from observation during exposure, just as in acute studies. However, since endpoints such as growth are typically measured only at study termination, one could argue that such observations are the result of events that occurred early in the exposure period.

Often, knowledge about the mode of action of a substance may be all that is needed to determine whether a substance has latent effects (e.g., diflubenzuron). Close analogues that demonstrate the presence or absence of latency can be used to deduce the likelihood of latency occurring. Most organic substances display a baseline or narcotic mode of action [30], which is believed to be a general disruption of membrane integrity [31]. Narcosis occurs when the compound reaches a critical threshold or critical body residue (CBR) [32]; such effects are not latent and are reversible unless death has occurred. This concept can also be applied to agrochemicals where the mode of action may not be well understood in nontarget organisms. At an ecosystem level, complexity and nonlinear biological dynamics may create a latency period between the exposure event and effects [33,34]. Where it is possible to redesign bioassay protocols to determine whether latency exists, the protocols should be modified as long as the results are compatible with historical data from similar protocols.

Reversible effects with recovery between pulses

If the mode or mechanism of action for the agrochemical has noncumulative, reversible effects (e.g., triazines) or a period of recovery returns the organism to pre-exposure state (e.g., organophosphates), time-varying exposure testing may be useful to provide more realistic estimates of the effects associated with exposure. Also, for rapidly acting compounds like pyrethroids, maximum or peak concentrations are more important than the area under the curve (AUC) or cumulative dose.

Toxicity tests with the midge (*Chironomus riparius*) to organophosphorus and carbamate compounds showed that two 1-h pulses caused significantly fewer symptoms of intoxication than 2 h of continuous exposure to carbamate compounds, when at least 2 to 6 h of clean water was provided between doses [35]. Mancini et al. (in [4]) and Clark et al. [36] have also conducted tests that suggest that multiple-pulse exposures of substances are less toxic to aquatic organisms than continuous exposures of equal total duration. This suggests that some level of detoxification and/or elimination of the substance during the substance-free period can reduce the toxic effects of the earlier exposures [3] and is dependent on the length of time between pulses (Wang and Hanson, as cited in [3]).

Using algae (*Pseudokirchneriella subcapitata*, formerly *Selenastrum capricornutum*) and pulsed exposure to atrazine from the field, Klaine et al. [37] demonstrated that recovery from exposure up to 50 μg/L of atrazine was nearly instantaneous once the herbicide was removed from the overlying water. Klaine et al. [37] also showed that *D. magna* survival after exposure to chlorpyrifos was age related, with older daphnids being more sensitive. Daphnids exposed to two pulses had a higher mortality if the second pulse occurred later in their life cycle. Naddy and Klaine [24] demonstrated that provided sufficient time between exposures occurred, no effects on *D. magna* were observed. Naddy and Klaine [24] also stated that ≥72 h were needed for recovery of *D. magna* from exposure to chlorpyrifos. In some cases, recovery did not occur, and the authors hypothesized that the critical exposure threshold for the compound had been exceeded (see later section). Based on mobility data, it appeared that daphnids could recover from environmentally realistic chlorpyrifos exposures below a critical threshold.

Irreversible, additive, or cumulative effects

For some compounds, no difference in the toxicity has been observed between time-varying or repeated and continuous exposures when these exposures are additive for compounds with slow depuration times or with only slowly reversible or irreversible effects (organism memory; Breck, as cited in [3]; van der Hoeven and Gerritsen [10]). In a study by Kallander et al. [35], no difference in the toxicity of organophosphorus compounds to midges was observed between pulsed and continuous exposures, whereas chronic pulsed exposure over 28 d to bromoxynil was reported to be more toxic to *D. magna* when compared to 28-d continuous exposure [38]. Reproductive parameters and growth (weight) were adversely affected at pulsed exposure levels of 20 μg/L as compared to continuous levels of 40 (sublethal effects) to 80 (survival) μg/L.

In an investigation of five insecticides (technical permethrin, microencapsulated permethrin, fenitrothion, carbaryl, and carbofuran), mosquito larvae were exposed to the same insecticide concentrations in the pulsed (two 1-h exposures, separated from between 6 and 24 h) and continuous (2-h exposure) tests [3]. Results of this study demonstrated that double-pulse exposures were equally toxic as or more toxic than a single continuous exposure of equal total duration.

Microencapsulated permethrin was the only insecticide of the five tested that exhibited greater toxicity in the double-pulse exposure test. The investigators speculated that some of the larvae immobilized after the first 1-h exposure to microencapsulated permethrin recovered between exposures, which allowed them to ingest more capsules than those exposed continuously. With respect to the other insecticides tested in double-pulse exposures, recovery from immobilization during clean water periods between exposures did not result in lower toxicity of the compound. Therefore, either insufficient recovery intervals (duration between events) occurred (6 h for all

except carbaryl, which was tested with a 24-h period) or other irreversible toxic effects were occurring [3].

Panter et al. [39] studied plasma vitellogenin levels in fathead minnows (*Pimephales promelas*) in response to pulsed and continuous exposures of estradiol for 21 or 42 d. Intermittent exposures produced approximately equal vitellogenin concentrations to those in response to the same continuous exposures but were significantly higher than continuous exposure to the equivalent time-weighted average concentration. The intermittent exposure response was greater than expected from a simple integration of exposure and duration using the AUC.

Population recovery

In a simulation of single- and multiple-pulsed exposure on the population dynamics of *Gammarus pulex*, Kedwards and Wood (T. Kedwards and S. Wood, 1998, ''The Influence of Simulated Perturbations on the Population Dynamics of *Gammarus pulex* [L.],'' SETAC Europe Annual Meeting, Bordeaux, France, April 1998, unpublished data) observed population reductions proportional to the lack of immigration of individuals and to the magnitude of the exposure. The magnitude of effects was inversely related to the age class of the amphipod. Recovery interval of the population to original levels was also proportional to the immigration numbers and inversely to the level of the exposure.

DECISION CRITERIA FOR CONSIDERING PULSED EXPOSURE ASSESSMENT

The decision to consider pulsed exposure assessment is best addressed on a case-by-case basis with several aspects that may move one to consider pulsed testing.

Application interval and half-life

Agrochemicals with short half-lives and those applied with intervals longer than two to three half lives can easily exhibit pulsed behavior. A pulsed toxicity study, based on simple calculations of peak height and pulse duration, can be designed using conservative estimates of the expected environmental concentration in ponds and other aquatic exposure scenarios. Numerous questions should be addressed when choosing this option, such as which chemical half-life (water or soil), drift versus runoff input (or both), and which specific habitat (stream, river, pond, lake) should be considered. Lower-order streams are more susceptible to time-varying or pulsed exposures than higher-order streams and rivers, lakes, or reservoirs because their hydrologic dampening is less and these systems are more directly connected to drift and runoff events in the watershed. Ponds adjacent to agricultural areas are also susceptible to pulsed exposures. Each of these parameters may influence the pulse exposure design.

Exposure modeling

GENEEC. As currently available, output from GENEEC (generic environmental exposure concentration model; U.S. Environmental Protection Agency Office of Pesticide Programs Environmental Fate and Effects Division) can be used to indicate potential time-varying behavior. Peak and time-weighted average concentrations as well as the aquatic half-life are generated by this model. This information could be used to develop pulsed testing protocols for particular agrochemicals.

Pesticide root zone model/exposure analysis modeling system. The pesticide root zone model (PRZM) [40] coupled with the exposure analysis modeling system (EXAMS) [41] are higher-tier (beyond screening-level tools and approaches) aquatic exposure models. When associated with the multiple scenario risk assessment tool (MUSCRAT) (G. Mangels and J.M. Cheplick, 1997, MUSCRAT, Ver 1.0, Beta Ver, unpublished data), numerous agrochemical-specific exposure scenarios can be run. Only those scenarios indicating substantial risk (as defined by a particular regulatory paradigm) will be subject to additional scrutiny using time-varying or pulsed exposure testing. A risk assessment tool to evaluate duration and recovery (RADAR) (M. Williams, 1998, RADAR, Ver 1.10, Waterborne Environmental, Reston, VA, USA, unpublished data), a postprocessor for PRZM/EXAMS, can be used to address time-varying exposures, providing output from PRZM/EXAMS corresponding to a particular threshold concentration (the concentration where a peak event begins and ends). The RADAR requires a complete time-series record (a consistent sampling frequency). The analysis is based on the units of the data file. If the time series is hourly, durations and recoveries are expressed in hours. If the time series is daily (as is the case here), all output is expressed in days. Note that the user needs to fill in missing data if the time-series data are not continuous (if sampling frequency is not consistent).

This threshold concentration should be a function of the toxicity of the compound. For example, the acute threshold could be 10% of the LC50, LC10, or other acute endpoint and the no-observed-effect concentration (NOEC) or other chronic endpoint (e.g., effect concentration [EC10]) for chronic effects. The slope of the exposure response could also be used to assist in determining an acceptable threshold. For each 36-year run, the generated information may appear as event, date, peak concentration, average (arithmetic mean) concentration, geometric mean concentration, duration (in days), and recovery period (in days). Recovery refers to the time between events. For each event, the following information may be generated: number of peaks, peak height/average concentration, peak height/geometric mean concentration, and concentration–time above threshold (in AUC units of concentration–day).

Hypothetically, if the peak height ratio (either arithmetic or geometric mean) is similar for all events, then one can assume that the peak shapes are similar. The concentration above the threshold is calculated using the AUC (see Fig. 1) method. These data can easily be processed into cumulative distributions, subjected to trend analysis, sorted by duration, recovery times, and so on, using RADAR to provide outputs of particular pulses (e.g., 90th-percentile pulse length, 10th-percentile recovery length, or the most likely pulse and recovery length). If an unmanageable number of values is generated (e.g., too many pulses are identified), additional nonecotoxicologically based thresholds (filters or event trigger values) may need to be added to create an interpretable data set (e.g., modify the ecotoxicologically based threshold by 10 to artificially reduce the number of events).

Figure 2 shows a modeled agrochemical concentration in a low-order headwater stream obtained using RADAR (figure text added). Most events have a duration of 1 d, and all events have a duration of 2 d or less before returning to below 2 μg/L. This is due to an extremely rapid dissipation rate due to hydrologic dilution in a fast-moving stream. Note that except for two events, when the precipitation event lasted for 2 d, the peak concentration equals the averages (single-day events). Other years have a similar multiple-pulse pattern. The number

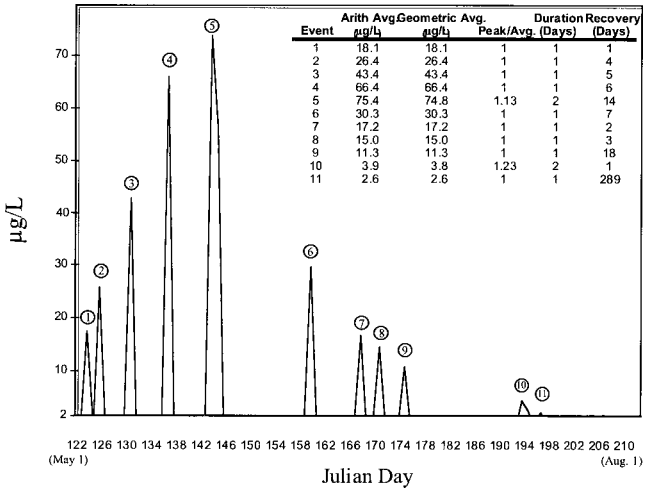

Fig. 2. Example of event threshold of 2 µg/L and influence on number of peaks, event duration, recovery duration, and shape of peak for a low-order stream in 1948 (e.g., edge of field for a headwater stream) using pesticide root zone model/exposure analysis modeling system (PRZM/EXAMS) with risk assessment tool to evaluate duration and recovery (RADAR) postprocessing.

of peaks decreases as the threshold increases (Fig. 3a). Because each event contains only one peak, the maximum number of peaks occurs at the lowest threshold concentration.

All the events are at least 1 d in length with only 20% at 2 d and longer (Fig. 3b). Peak concentrations with *p* values of 0.2 to 1.0 range from 7.5 to 3 µg/L (Fig. 3c). Most events have recovery intervals of 1 to 7 d (Fig. 3d). From these data, a laboratory study representing these exposure parameters could be designed using a 1-d pulse at 5 µg/L with a recovery interval of 4 d. Other scenarios are also possible if alternative *p* values are chosen from the probability distributions.

Similar information for a higher-order (lower-reach) stream (river) and a pond are shown in Figures 4 and 5. The same input to PRZM/EXAMS is used for these scenarios as used for the lower-order stream with the exception that the characteristics of the receiving water body are changed. Different event threshold levels (1 and 2 µg/L) and the effect on number and shape of the peaks and associated parameters are described for the higher-order stream (Fig. 4). The spikiness in the graphical outputs is an artifact of model operation and output.

As in the headwater stream, the number of events versus threshold decreases for both the higher-order stream and the pond as the threshold increases. Fewer, broader peaks are observed when compared to the headwater stream in Figures 4 and 5. Hydrologic dilution produces peaks of lower magnitude in the higher-order stream as compared to the headwater stream and similar concentrations to the pond. Pulse durations in the pond are longer than both stream scenarios because of the lack of flow in the pond system. Recovery intervals are similar between the stream scenarios (subject to similar runoff regimes); however, the pond recovery intervals can be quite long due to the lack of flow in and out of the pond. One can conclude that pulsed behavior is particularly relevant in the headwater stream and less important in the higher-order stream and pond. Postprocessor (RADAR) enhanced PRZM/EXAMS outputs allow one to observe whether the scenario produces potential time-varying responses. Using the tools described previously, decisions can be made whether additional efforts to address time-varying exposure and effects are warranted. The follow-

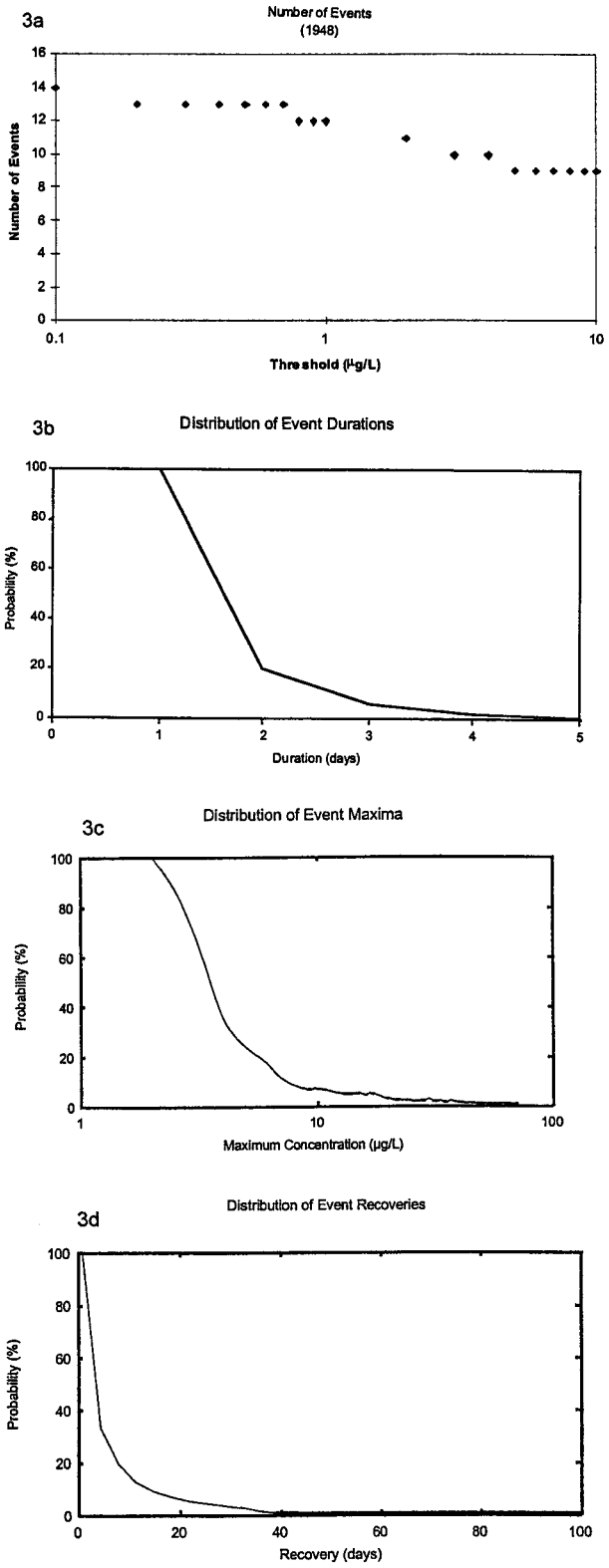

Fig. 3. (**a**) Influence of event threshold on event parameter distribution for a low-order stream (results from Fig. 2) using risk assessment tool to evaluate duration and recovery (RADAR) threshold values ranging from 0.1 to 10 µg/L. (**b**) Probability of duration for the same scenario. (**c**) Probability of peak maximum for the same scenario. (**d**) Probability of recovery over 36 years for a low-order stream with a threshold at 2 µg/L using pesticide root zone model/exposure analysis modeling system (PRZM/EXAMS) with RADAR postprocessing.

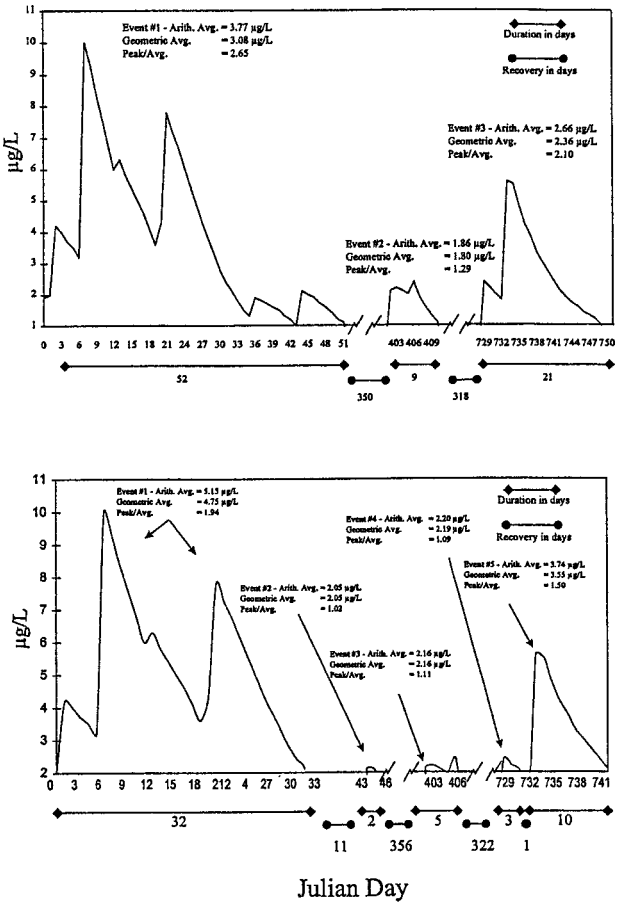

Fig. 4. Examples of event threshold values (1 [**top**] and 2 µg/L [**bottom**]) from a single year and influence on number of peaks, event duration, recovery duration, and shape of peak for a large stream (lower reach) using pesticide root zone model/exposure analysis modeling system (PRZM/EXAMS) with risk assessment tool to evaluate duration and recovery (RADAR) postprocessing.

ing section describes the next steps to move to a more detailed, higher tier or level of risk assessment.

SELECTING TOOLS FOR INVESTIGATING TIME-VARYING AND REPEATED EXPOSURE

Figure 6 provides guidance on which direction to take when assessing the effects of time-varying exposures on ecological receptors. Depending on the time-varying exposure pattern, a

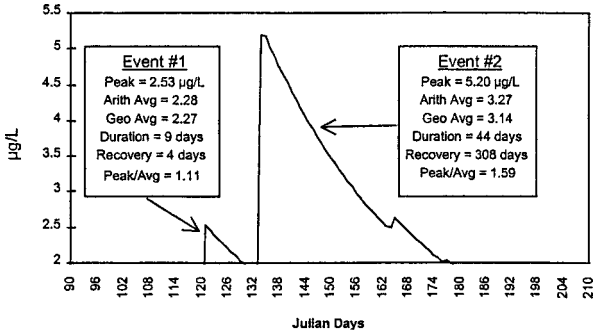

Fig. 5. Number of pulses, event duration, and recovery duration for a pond with a 2-µg/L threshold (events in 1956) using pesticide root zone model/exposure analysis modeling system (PRZM/EXAMS) with risk assessment tool to evaluate duration and recovery (RADAR) postprocessing.

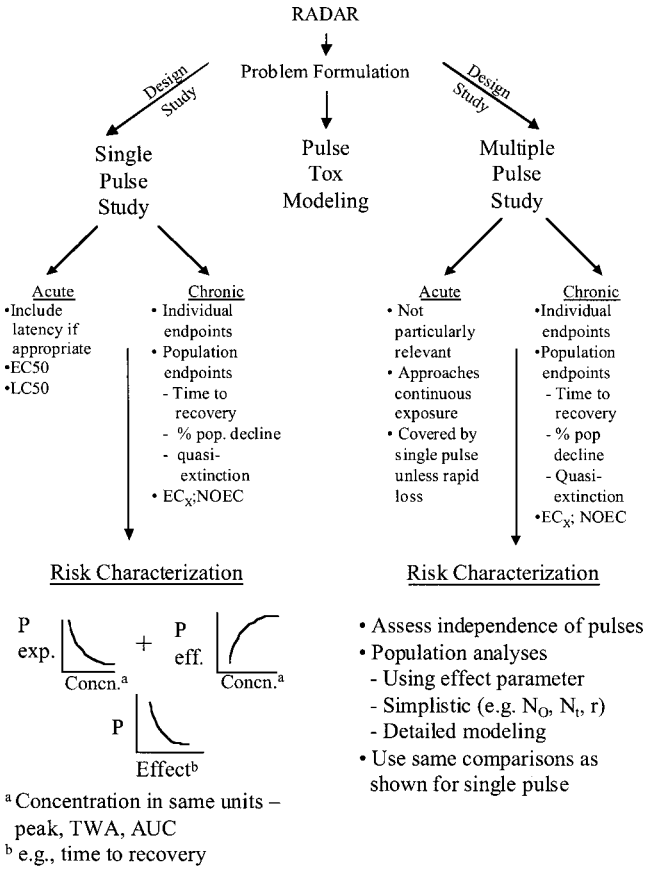

Fig. 6. Decision matrix for time-varying exposures. RADAR = risk assessment tool to evaluate duration and recovery; TWA = time-weighted average; AUC = area under the curve; NOEC = no-observed-effect concentration; EC = effective concentration.

single-pulse or multiple-pulse laboratory study could be run; if the pulses cannot be adequately or appropriately represented in the laboratory, pulse toxicity modeling could be conducted. Acute and/or chronic studies can be designed, producing measurement endpoints appropriate to the risk assessment endpoints. If expected, studies should be extended to include delayed or latent effects. Chronic studies could address either individual [28] or population endpoints (T. Kedwards and S. Wood, 1998, ''The Influence of Simulated Perturbations on the Population Dynamics of *Gammarus pulex* [L.],'' SETAC Europe Annual Meeting, Bordeaux, France, unpublished data). Note that acute studies under a multiple-pulse regime are not particularly relevant because short multiple-pulse exposures approach continuous exposures unless a rapid loss of the substance occurs in the environment and test system between pulses.

For the single-pulse case, a joint probability distribution can be used to characterize the risks (Fig. 6). In the multiple-exposure case, the pulses are assessed for their independence in order to determine whether the responses are enhanced, reduced, or independent for multiple pulses. An understanding of the independence (or lack of) of the exposures is helpful in characterizing the risks using population analysis or joint probability distribution analysis.

Laboratory approaches

Pulsed exposure toxicity studies can easily be conducted using current study designs provided the exposure profile is

known and the endpoint is defined to provide toxicologically relevant estimates [1]. Interpretation of the exposure concentrations may be problematic, as one could use the AUC, determine arithmetic or geometric averages, and so on (e.g., where on the concentration curve should the concentration be set—arithmetic mean, geometric mean, 90th percentile, and so on?). Consultation with the appropriate regulatory authority is recommended when developing pulsed toxicity test designs because these studies are not yet standardized. Also, study designs need to be determined to test the appropriate hypotheses.

Single pulses based on maximum concentration or pulse duration (width of pulse) can easily be designed in the laboratory. Designs based on degradation or hydraulic parameters are more difficult to test in the laboratory. Flow rates could be altered in a flow-through study, semistatic- or static-renewal rates could be controlled to mimic these pulses, or static designs with periodic additions of agrochemical could be used if the compound degraded rapidly. An understanding of the behavior of the substance in the test system and under environmentally realistic conditions prior to study conduct is important.

Static exposure. Some of the processes that can affect the concentration of a agrochemical in the water column, especially volatilization, hydrolysis, photolysis, and biodegradation, may also take place in the exposure medium of a toxicity test. The exposure concentration of such a compound will decline during the course of a static toxicity test. Moreover, the test organisms will be exposed to the agrochemical degradation products. If the rate of decline in the concentration is environmentally realistic, the results of a static test may be more relevant to the risk assessment than other designs; however, if rates in the exposure solution are very different from those in the environment, the results may be meaningless.

Static-renewal exposure. Virtually any temporal exposure pattern may be created in a static-renewal toxicity test, provided the renewals are frequent enough. In standard practice, test solutions are renewed daily, but exposures shorter than 24 h are certainly feasible. Static-renewal exposures would be especially suitable for generating square pulses (exposure to a constant concentration for a set duration, followed by transfer to clean water) with relatively stable test substances—a situation most relevant when real-world exposure is expected to be dominated by water flow, such as in small streams. Static-renewal designs may also be used by laboratories not equipped to run flow-through studies.

Flow-through exposure. Flow-through exposure systems (e.g., diluters) are typically used to create constant exposure concentrations in toxicity tests (especially for substances that have short half-lives), but they can be adapted to simulate pulsed exposures as well. Changes in exposure concentration can be brought about by changing the concentration of the stock solution, by changing the stock solution pumping rate, or both. The resulting changes in the exposure solution may be gradual (depending on the water replacement rate in the exposure container), not immediate, as in a static-renewal test.

Microcosms (and mesocosms). Microcosms simulating natural dissipation processes can be used to generate exposure solutions for static, static-renewal, or flow-through toxicity tests. For example, the agrochemical can be applied to an outdoor tank containing natural sediment and natural water in which it will be subjected to the influences of volatilization, photolysis, hydrolysis, biodegradation, and sorption, all under conditions more realistic than laboratory single-species studies. Water can be taken from the microcosm, periodically or continuously, for use in toxicity tests. Alternatively, organisms can be exposed in cages or other containers placed directly in the microcosm.

The design of the pulsed study (pulse magnitude, duration, frequency) could be based on the application interval and half-life, the GENEEC output, or PRZM/EXAMS–RADAR outputs. These studies can be conducted when pulses in exposure are either expected or observed. The results would be used to characterize the exposure–response relationship in terms of pulse height (maximum concentration), pulse width (duration), frequency, or other exposure metrics.

Time-to-event analysis

If the maximum toxic response occurs primarily in a period shorter than the duration of the typical toxicity study (96 h for fish, 48 h for daphnids), time-to-event (TTE) analysis may be useful for designing and interpreting pulsed toxicity studies. Exposure duration, a critical determinant of exposure consequences, is explicitly included in TTE, affording possibly more accurate prediction of effects for different exposure durations. Using conventional testing methods, TTE methods draw from an experimental design in which groups of individual organisms are monitored through time (M.C. Newman, 1997, ''Noting Time-to-Event Enhances Prediction from Toxicity Test Data,'' ECOFRAM, unpublished data). The times (specific time or interval) until some event occurs are recorded for each individual. Time-to-event analysis may provide more ecologically meaningful estimates of lethal (and sublethal) effects than exposure–response methods alone [42]. Such TTE analyses include both exposure intensity and duration as well as other covariates [14], allowing for design of a study to specifically test pulse durations and intensities that may alter the response of an organism to a more realistic exposure regime.

Results from a TTE analysis could also be used to estimate time-varying exposure model parameters discussed later in this article or even to project possible consequences in the field. Time-to-event analysis may assist in determining whether the events are independent, antagonistic, additive, or synergistic. If independent, time-varying exposure testing would not be needed, as each pulse event occurs as a singular event, without organism memory or effect overlap. Slopes can be compared or the effect endpoint at time t can be compared using a chi-square analysis. Time-to-event analysis can also be used to model the proportion of the population affected by an exposure, based on

$$\log \text{TTE} = a + b(\log \text{concn.}) + \sigma(W) \quad \text{or}$$

$$W = \frac{\log \text{TTE} - a - b(\log \text{concn.})}{\sigma}$$

where W is a characteristic of the specific underlying model reflecting the proportion affected (e.g., W for 50% affected [0.5] would be 0 for the lognormal model).

As a closely related alternative, Mancini [43] proposed a method to use data from a classical (constant exposure concentration) toxicity study to calculate the effects from time-variable exposures. This approach assumes reversibility of effects and can be used for both acute and chronic toxicity studies. The method is able to calculate the probable mortality and/or the percentage of mortality expected due to any temporally

variable substance level. See Mancini [43] for details on the approach.

Time-varying toxicokinetic models

Pulsed exposure toxicity tests can be coupled with toxicokinetic modeling as a solution to the problems associated with predicting substance effects in non-steady-state field exposures. Such models may be useful for long recovery intervals, long pulses, or numerous repeated exposures (exposure scenarios not easily duplicated in the lab). Toxicokinetic models may be compartment, physiological, or bioenergetic based. These models can be useful tools in estimating changes in tissue concentrations resulting from absorption, distribution, metabolism, and elimination of a substance from an organism. Compartment-based models describe substance movement among compartments. For example, a simple two-compartment model may contain water (source) and organism (sink) compartments. Physiological-based models describe the accumulation and internal distribution of substances among multiple tissues. They can account for different rates of elimination from various tissues but can require a great deal of detailed physiological data, some of which may not be available. Bioenergetic-based models describe substance accumulation and loss in terms of the organism's energy requirements where the organism is treated as a single compartment and substance uptake is a function of the flux of water across the gills or food/sediment through the gut. These models become particularly important in estimating the effect of a substance when evaluating non-steady-state, nonequilibrium exposures that may vary temporally or spatially. Understanding substance accumulation and distribution in an organism ultimately contributes to predicting its toxic effect [44].

To be able to define toxic effects based on body residue levels would also eliminate uncertainties associated with the bioavailability of the substance in question. While a number of poorly defined species-specific and site-specific variables still affect the refinement of toxicokinetic models, they are gaining more widespread use in ecotoxicological evaluations [44].

In this article, three relatively simple toxicokinetic models are described: the food and gill exchange of toxic substances model (FGETS), PULSETOX (as defined in the following), and the dynamic energy budget toxicology model (DEBtox). Their use in pulsed exposure testing and assessment is addressed. The decision as to which of these kinetic models is most appropriate will depend on data availability; however, use of the simplest model that will adequately address the study question will minimize potential errors [44].

Pulsed exposure toxicity tests can be coupled with toxicokinetic modeling as a solution to the problems associated with predicting substance effects in non-steady-state field exposures. Such models may be useful for long recovery intervals, long pulses, or numerous repeated exposures (difficult to test the exposure scenario in the lab due to cost and logistics). The results of these models will be useful in a weight-of-evidence approach to help understand the compound dynamics in time-varying exposures and the potential for effects.

FGETS. This model is an example of a bioenergetic-based toxicokinetic model that addresses the chemical uptake in fish from both food and environmental pathways [45] using thermodynamic potential. It models the chemical exchange between fish and the aqueous environment that occurs across gill membranes and across gut walls from ingestion of substances

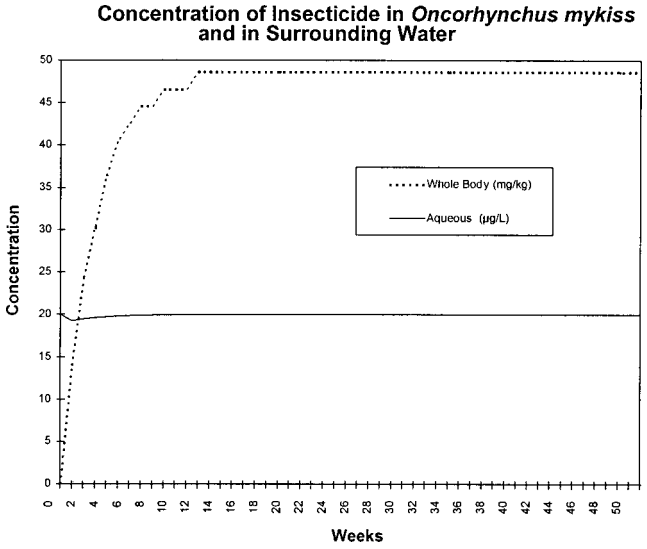

Fig. 7. Output from the food and gill exchange of toxic substances model (FGETS)—aqueous and fish concentrations.

(bioaccumulation from water and food) (Fig. 7). An important aspect of FGETS is that it is a chemical–biological mechanistic model in which uptake is not considered an arbitrary action. The model can calculate the time to reach lethality in fish assuming that the substance has a narcotic mode of action. The FGETS model can be run in three modes: laboratory, food chain (simulates one or two fish) and food web (simulates more realistic predator–prey interactions). Inputs for the three modes and various model assumptions are found in the Appendix. The FGETS model incorporates data on the composition, structure, and morphology of the organism in calculating uptakes. An important aspect to this is tracking individual organism weight as a dynamic variable. Fundamental to the formulation of a dynamic energetic-based model is the relationship between the type of chemical, its mode of action or partitioning capability, and the physiological and genetic characteristics of the individual [46].

A similar model, modified from FGETS, was developed for *Daphnia* spp. [47]. Acute effects (mortality) of a lipophilic narcotic on a dynamic daphnid population are modeled, using uptake from both water and food. The model is based on the Lassiter and Hallam [48] survival of the fattest static theory in which the effect of a toxic exposure is analyzed by relating the K_{ow} to the partition coefficient of the fat (lipid) and aqueous phases in the aquatic organism. For exposure of equal chemical activity, increasing lipid content and hydrophobicity increase the exposure duration tolerance without an effect [48]. A dynamic approach was developed in order to couple the dynamic behavior of individuals with chronic or multiple acute substance (e.g., pulsed) exposures. The authors note that ecological risk assessment needs to be based not only on the attributes of the substance but also on the biology of the exposed organism and its population dynamics.

PULSETOX. Another model, PULSETOX, is a residue-based, pulse exposure toxicokinetic model for aquatic toxicology based on a simple one-compartment first-order kinetics (1CFOK) equation [2,44]. This model tracks the whole-body accumulation of a chemical in fish and predicts acute toxicity using previously established relationships among aqueous concentrations, whole-body residues, and lethality using the nonpolar narcosis CBR theory (Fig. 8). This simple toxicokinetic

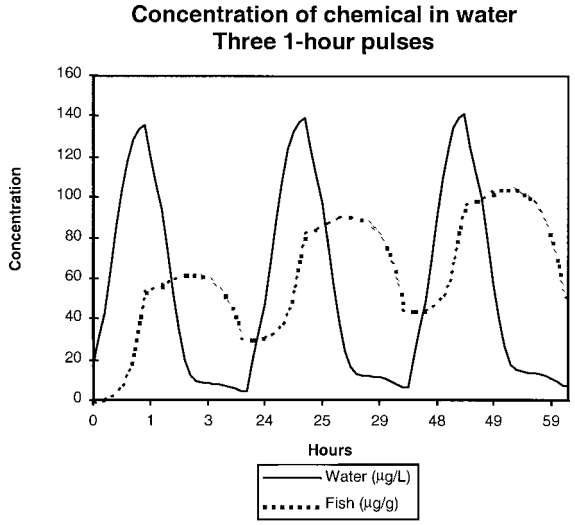

Fig. 8. Pulsed exposure toxicity test (PULSETOX) output—aqueous and fish concentrations.

model accounts for the effect of non-steady-state, nonequilibrium accumulation from temporally varying exposures on toxicity using a fugacity approach. The model can be run in two modes: repeating exposure and cyclic or random exposure (see Appendix). The only loss mechanism from the system is via tank outflow; volatilization, degradation, and so on are not considered.

A pulse LC50 defines the duration and magnitude of the exposure required for an organism to accumulate a dose equivalent to the lethal or critical body residue. The CBR is the minimum tissue concentration associated with an adverse effect. The concentration can be based on the whole organism or on a particular target organ and can provide a more direct measure of a predicted adverse effect than can external exposure concentrations [49]. Such an approach may be an inappropriate indicator if the whole-body residue is not representative of accumulation in specific target tissues [1]. Hickie et al. [2] demonstrated that the toxicity resulting from pulsed exposures is controlled primarily by the rate of substance accumulation and depuration rate in the exposed fish and that the level of biological response is associated more with the accumulated dose than exposure concentration. Because the model is mechanistically based, it is useful in illustrating the effects on toxicity resulting from interactions among the number, duration, and frequency of pulses. Although inadequate information exists relating biological responses with CBRs, the model assumes that the CBR is an acceptable, but not ideal, surrogate to the lethal dose and that residues predicted by PULSETOX can be interpreted in relation to lethal effects. The CBR-based techniques are useful to see if a particular exposure or series of exposures approach the residue level where effects may be realized without having to test an organism in difficult laboratory study designs or prior to beginning laboratory testing programs.

Klee [50] presented a successful validation trial of the basic modeling algorithm used in PULSETOX, the 1CFOK model [44]; the uptake, depuration, and the bioconcentration factor (BCF) of chlorinated benzenes and phenols in rainbow trout were adequately described by the model when compared to laboratory measurements using predicted versus measured concentrations.

The PULSETOX model provided a useful deterministic approach for predicting effects of pulsed exposures and provided a clear link between pharmacokinetics of the chemical and the time course of toxicity both within standard continuous toxicity tests and in independent pulse toxicity tests. Such models may not be useful for chemicals that are highly degradable, are reactive, or do not readily bioconcentrate. Although the model is thought to be less effective for substances that cause cumulative damage where toxicity could increase through time even though peak fish residues do not change with successive pulses, Meyer et al. (in [2]) have modified the toxicokinetic model by the addition of a power term that was effective in describing the intermittent exposure toxicity with this type of substance.

DEBtox. Another model that may be used for pulse toxicity results and body residue analysis is the dynamic energy budget toxicology model (DEBtox), an energetics-based model [51]. The DEBtox (Ver 1.0) software package is designed to analyze results from standard aquatic toxicity tests. Results from tests on acute and chronic survival studies, fish early life stage studies, *D. magna* 21-d reproduction studies, and algal growth inhibition can be input. Outputs from the program are estimates of study parameters with standard deviations and correlation coefficients using different models for differing modes of action; goodness of fit for the selected model with graphical presentation; graphic presentation of the likelihood function of the no-effect concentration with confidence intervals; calculation of time-, concentration-, and/or response-dependent EC values; statistical analysis of any single or combination of parameters based on the likelihood ratio test; and an analysis of residuals, including plots as functions of concentration, exposure time, or response.

The DEBtox model may prove useful for analysis of data generated from time-varying or repeated-exposure studies. The program estimates primarily parameter values by maximizing a nonlinear likelihood function for a survival experiment combined with a weighted least-squares method. For growth and reproduction, a set of differential equations at initial conditions is solved using a fourth-order Runge Kutta numerical method. An elimination rate is essential to this analysis and dictates how fast a response occurs during exposure. In addition to various tabular outputs, graphs of time profiles, concentration profiles, the likelihood function for the no-effect concentration, and various response surfaces (e.g., effect surface) are produced.

Use of time-varying models

Models such as PULSETOX can assist in the interpretation of time-varying or repeated-exposure studies or to estimate effects from long pulses, long recovery intervals, or numerous repeated exposures that would be difficult to test in the laboratory. The results from this modeling are not used directly in risk characterization but are useful in understanding the dynamics of the compound in environmental receptors. For agrochemicals with log K_{ow} at or above 3, results from a fish bioconcentration study will usually be available. A bioconcentration factor, often at 1 or 2 concentrations, a K_u and K_d (uptake and depuration rate constants, respectively) will be available from this study. Using these inputs, as well as others for PULSETOX, the time to reach a lethal body residue can be estimated using default CBRs [11,44]. Estimates of body residues and/or time to lethality can be estimated for other exposure concentrations or pulse durations. If a compound has a log K_{ow} less than 3, FGETS could be used to determine the

depuration rate from the uptake rate (slope of the uptake curve) and the BCF (calculated from K_{ow} or water solubility). According to Spacie et al. [52], the BCF = K_u/K_d.

Besides measuring the BCF for compounds, numerous equations are available that can be used [52–54] to calculate the BCF. Such quantitative structure–activity relationship (QSAR) equations can be based on many or specific classes of chemicals and are based on one or more measured parameters (e.g., K_{ow}, water solubility). Several equations for calculating fish BCF values are listed by the European Center for Ecotoxicology and Toxicology of Chemicals (ECETOC) [53] based on general chemicals (see ECETOC document for specific references) using either log K_{ow} or water solubility (e.g., BCF = -1.32 + log K_{ow} and BCF = 2.02 − 0.47 log WS [water solubility in mg/L], respectively).

The correlation coefficients for the equations based on water solubility (0.7–0.87) are lower than those based on log K_{ow} (0.93–0.97). Additional equations, some specific for a particular class of compounds and others that use parameters such as molecular connectivity indices, may also be found in the previously cited references.

According to ECETOC [53], uptake from food is important only if the log K_{ow} >4.5 and the concentration in food is about 10^5 greater than water. Also, for compounds with log K_{ow} <4, the typical time to equilibrium is less than 28 d. Therefore, studies in which the uptake phase is conducted for 28 d (e.g., Federal Insecticide Fungicide and Rodenticide Act [FIFRA]—Office of Pollution Prevention and Toxic Substances [OPPTS] 850.1730, Organization for Economic Cooperation and Development 305) can produce meaningful steady-state BCF values for compounds between log K_{ow} of 3 and 4. Above log K_{ow} of 4, BCF values may be underestimated because equilibrium may not have been achieved. Additionally, uptake of compounds with molecular weights greater than 700 g/mole may not be predicted by log K_{ow} because of steric hindrances [53].

As an alternative to calculating the CBR, the whole-body concentration may be measured during a typical toxicity study consistent with the time of death (or other effect). This is obviously a more costly method than using empirically derived ranges, calculating the value using TTE and FGETS, or other methods mentioned previously. Although the true CBR for a particular effect would be measured, the cost and potential time lag between death and analysis of the body residue may be problematic.

Additional considerations exist when working with CBRs. The CBR may be based on a threshold (instantaneous) or cumulative body residue (refer to [11]). A threshold is probably the typical response since many compounds are considered nonpolar narcotics. In these cases, the arithmetic or geometric mean could be used to represent both the exposure concentration and the body residue. For cumulative CBRs, derived from reactive, irreversible, or slowly reversible target interactions, the body residue should be determined using AUC or critical area under the curve. Verhaar et al. [11] have found this parameter to be constant and independent of exposure time for particular single species–compound interactions. For receptor-mediated or reactive toxicity, LC50 versus time values decrease after achieving steady-state bioconcentration, and the incipient LC50 will be substantially lower than the 96-h LC50 for many compounds.

Physiologically based pharmacokinetic or toxicokinetic models

Physiologically based pharmacokinetic or toxicokinetic models would be employed only at higher levels of risk assessment refinement (higher tiers) due to costs and complexity and typically only for specialty products or special cases of need. Considering all the information presented, significant factors need to be considered that greatly affect the interpretation of substance residues in wildlife tissues and highlight the important role of physiologically based toxicokinetic models to elucidate the intraspecies and interspecies differences affecting the development of CBRs. The coupling of these more complex kinetic models (more compartments, more linkages, real model of an organism) with CBRs allows for the estimation of when a chronic or acute toxic response is expected to occur under various exposure scenarios. This type of modeling also predicts the time course for the toxic effect. Such methods should be able to elucidate the intraspecies and interspecies differences affecting the development of CBRs, making them useful for extrapolating responses to other species. Besides being an effective tool for predicting acute toxicity from pulse exposures, the concept of CBRs and toxicokinetics could be applied to other cases where exposure–response relationships are complex, for instance, exposure via sediments, diet, and for chemical mixtures [44,55].

As with any model, the user needs to have a good understanding of the assumptions of the particular toxicokinetic model being used. For instance, many models will assume steady-state conditions whereby all organisms are assumed to internally act on a substance in the same way when exposed to the same source of chemical activity. When these assumptions are clearly not appropriate, more complex toxicokinetic modeling should be investigated [56]. Pharmacokinetic models in general could be improved by a better understanding of CBRs, true dose–response distributions, and the link between CBRs and modifying factors such as fat content, which may alter the toxicokinetics and toxicodynamics of a substance [2].

Pharmacokinetic modeling is the process of developing mathematical descriptions of absorption, distribution, metabolism, and excretion of chemicals in biota [57]. Such models can be used for interpolation but should not be used for extrapolation outside the dose ranges, routes of exposure, and species used to develop the model without an explicit understanding of the model constraints. Models have been developed to describe the disposition of more than 100 chemicals in mammalian species, and although more limited in scope than mammals, models for nonmammalian vertebrate species such as fish have also been developed [55,58–63; K.J. Clark, W.L. Hayton, W.H. Gingerich, and G.R. Stehly, ''Pharmacokinetics, Metabolism and a PBPK Model of Benzocaine in Channel Catfish,'' SETAC Annual Meeting, November 1997, San Francisco, CA, USA, unpublished data] and aquatic invertebrates [47].

These higher-level, more detailed models require more species-specific or habitat (environment)-specific information and assumptions; because they are data intensive, the amount of parameterization needed may be prohibitively costly for most applications, and the cumulative uncertainty in these models can be large if parameters are poorly specified or understood [64]. These models must be interpretable in terms of physicochemical, biochemical, and physiological properties of the organism. They still represent significant simplifications of the

true complexities of biological systems. Such detailed models are often simplified to one- or two-compartment models as described previously in order to reduce data and parameterization needs and possibly increase their utility [58].

The principal application of PBPK models is the prediction of a dose to a target tissue, a body residue for a parent chemical or reactive metabolite, or chemical concentration time course for specific tissues and organs [57,58]. The PBPK models can help reduce uncertainty associated with conventional dose extrapolation, dose–effect relationships, and CBR estimation methods. However, in many cases, it might be more feasible and cost effective to conduct a microcosm or mesocosm experiment designed with specific effects endpoints or to monitor associated effects in the aquatic environment.

Interpreting tissue concentrations of substances in aquatic organisms

A number of difficulties exist in the interpretation of substance residues in wildlife tissues. Toxicity due to the body residue of a contaminant will depend on the age, sex, fat content, and other variables of an individual within a species. Interspecies differences are also expected to be large. For instance, many toxicokinetic models assume steady-state conditions whereby all organisms are assumed to internally act on a substance in the same way when exposed to the same source of chemical activity [56].

Van Loon et al. [65] addresses the problem associated with threshold levels for individual substances through the identification of CBRs for a chemical class of compounds. It is generally thought that narcosis-type substances are completely concentration additive and are intrinsically all equally toxic [65]. Therefore, body residue levels that would cause a certain effect would be the same for all substances within this toxicological class (toxic equivalence factor of 1.0). The differences in aqueous effect concentrations within this class are related to differences in bioconcentration factors. As reported in Van Loon et al. [65], no-effect CBRs (mmol/kg lipid) for baseline toxicity are mortality (fish), 25; sublethal effects (fish), 5.0; and, estimated ecosystem no-effect level, 0.25.

Total body residues may be quantified by measuring the total molar concentrations of chemical mixtures in one measurement. An advantage of working with CBRs is that for chemicals and chemical mixtures with only baseline toxicity, the CBR is relatively constant for a certain endpoint [65].

Landrum et al. [44] also investigated CBRs for the narcosis-type class of compounds (also cited in [66]). Their findings indicated that residues that yield 50% mortality for acute exposures range from 2 to 6 mmol/kg for small fish and invertebrates to a wide range of neutral narcotics. If a tissue concentration required to produce 50% acute mortality is below 0.5 mmol/kg, the substance acts by a specific mode of action that is indeterminate between 0.5 and 2 mmol/kg; however, recent data with halobenzenes and fish indicate that CBRs are not constant and decrease with greater exposure time [67,68]. Likewise, Deneer et al. [69] found that the CBRs in the guppy for 13 organophosphorus insecticides differed widely, partially due to hydrophobicity. These results make the sole use of CBRs more complicated for compounds with specific modes of action and cannot replace the LC50 in such instances. Residue concentrations needed to elicit chronic effects are much lower than those needed for acute mortality. For 50% mortality, the residue concentration for chronic exposure to nonpolar narcotics is about 10% of that required to elicit an acute response [44].

This approach also appears to be useful with sediment-bound narcotic chemicals. Driscoll and Landrum [70] exposed *Hyalella azteca* and *Diporeia* spp. to varying levels of fluoranthene in sediments. Rough estimates of the CBR for *H. azteca* after only 10-d exposures were 3.6 to 5.6 and 1 μmol/g after 30 d. Because *H. azteca* is known to metabolize fluoranthene to more polar and possibly more toxic metabolites, fluoranthene was not acting solely as a narcotic compound in this study. For *Diporeia*, however, body residues of 2.7 to 6.5 μmol/g after 30-d exposure resulted in very little toxicity. *Diporeia* is known not to be able to readily metabolize fluoranthene and has a higher lipid content (which facilitates sequestration of nonpolar narcotics) than *H. azteca*, resulting in higher body residues and negligible toxicity in *Diporeia*.

One method for determining CBRs for substances in aquatic organisms assumes that the LC50 multiplied by the bioconcentration factor represents the lethal or effect concentration in tissue (L. S. McCarty, as cited in [53,71]). Actual body residues are expected to be lower due to either non-steady-state conditions or incomplete transfer of the substance from the water column into the organism. Also, according to McKim and Schmeider [72], BCF values are considered at steady state, a condition that may not be reached in toxicity tests of short duration with chemicals having $\log K_{ow} > 3$. Note that a recent publication [73] calculates the CBR for marine organisms using the NOEC from an acute study and not the LC50.

McCarty et al. [74] investigated the estimation of CBR values in fathead minnows for the polar narcotic compounds, substituted phenols. The ability to estimate CBRs from the experimental data was affected by pH-dependent ionization. When ionization-corrected toxicity data were assessed, several categories of CBRs were observed that were apparently related to different modes of action and generally fit the CBR classification schemes presented earlier in this article. In addition, Vaes et al. [75] reports that CBRs and LC50s for nonpolar (narcosis I) and polar narcotic (narcosis II) chemicals are different with the higher toxicity of the polar narcotic chemicals often related to the polar moeity on the molecule. However, these researchers have found that membrane–water partition coefficients (based on dimyristoyl phosphatidyl-choline) are higher for polar narcotics and generally are better able to predict the LC50 or CBR for these chemicals than using the log K_{ow}. Differences in toxicity between the compounds can be explained by different membrane lipid partitioning.

Although additional analytical measurement is required, estimated CBRs can be compared with tissue concentrations from laboratory studies to verify these data. Critical body residues may also be compared to tissue levels from field-collected specimens in monitoring programs or incidents. Critical body residues can be useful components in ecological risk assessment [49] when used in a weight-of-evidence approach.

Population analysis

Life history or age-stage models can be used to design pulsed toxicity tests a priori. In screening or initial levels of ecological risk assessment, generic life history tables for several life history strategies in aquatic invertebrates and fish could be developed. For each of these strategies, a particular period of growth suppression and/or lethality and its corresponding impact on population dynamics can be described. A pulsed toxicity study could be conducted for a particular ex-

posure period or pulse that causes an ecologically significant effect on a population. Age-specific mortality or reproduction information can be used to determine the time required for recovery of a particular population assuming that delayed effects do not occur and the effects are reversible and not cumulative. These analyses can address questions such as, Are single- or multiple-pulsed studies relevant, and are recovery intervals sufficient to allow biological recovery? For example, if the recovery period is not sufficient, a time-varying study may be warranted. Such a study could be conducted regardless of the application interval and half-life of a compound or the output from specific exposure models.

These models may be used a posteriori to address whether the results from a pulsed toxicity study could be ecologically significant, that is, cause ecologically meaningful changes in population numbers. Information on the recovery times could be integrated with population analysis results, indicating required recovery intervals for particular populations and percentage reductions in some ecological parameter. They could also be used to project and interpret responses or results for other populations.

USE OF TIME-VARYING RESULTS IN RISK CHARACTERIZATION

Figure 6 describes the risk characterization step for both single- and multiple-pulsed exposures. This step is consistent with current U.S. Environmental Protection Agency guidance, such as the guidelines for ecological risk assessment [76]. The probability of an exposure, derived from RADAR, for the time-varying exposure is then compared to the probability of an effect magnitude from a pulse exposure toxicity study or to the result from PULSETOX if additional effects testing was not conducted. In the former comparison, a probability of an effect curve is generated. Concentration units for both exposure and effects must be the same (e.g., peak, time-weighted average, AUC). In the latter comparison, a weight-of-evidence approach is used to decide whether the probability of exposures will cause a CBR to be reached, producing an effect.

Laboratory-based approach

Results from laboratory-based pulsed exposure studies can be used in this risk assessment scheme in the same manner as typical toxicity studies. Effects based on peak concentration or AUC integration can be used and compared to exposure concentrations derived in the same manner (units) (Fig. 9).

In Figure 9, exposure, characterized by a distribution of the probability of a particular concentration (peak concentration [shown] using a 2-μg/L threshold; AUC, concentration–pulse duration; geometric mean; or arithmetic mean) occurring, coupled with the effect (e.g., percentage mortality) versus concentration (peak concentration [shown], AUC, geometric mean, or arithmetic mean), generates a probability curve of the p of a particular effect occurring. In this example, there is a $p = 0.8$ that a 20% effect will occur in *D. magna* (acute toxicity in this case) in this exposure scenario and a 50% ($p = 0.5$) probability that 90% of the daphnids will be affected.

Consistent with Figure 9, time-to-event analysis can be used to characterize the risks from time-varying exposures. Time-to-event analysis assists in determining whether the events are independent, antagonistic, additive, or synergistic. If independent, time-varying exposure testing would not be needed, as each pulse event occurs as a singular event, without organism memory or effect overlap. Slopes can be compared, or the effect endpoint at time t can be compared using a chi-square analysis.

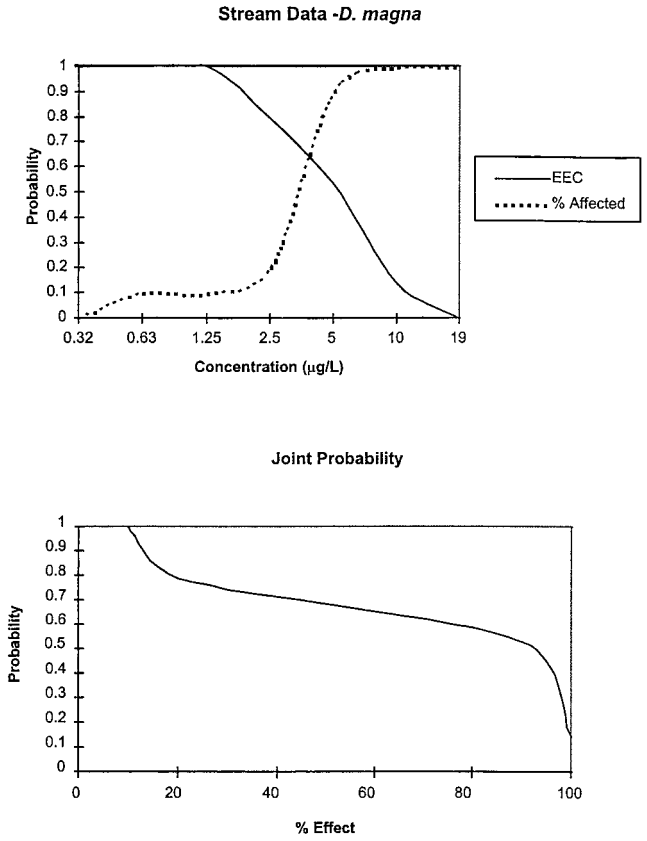

Fig. 9. Joint probability distribution: use of time-varying results in risk characterization (peak concentrations in a lower-reach stream using a threshold of 2 μg/L [**bottom**]). Exposure data are from risk assessment tool to evaluate duration and recovery (RADAR; see Fig. 4); effects data are hypothetical *Daphnia magna* survival results (30-d study) (**top**).

Population analysis approaches can be used a posteriori to address whether the results from a pulsed toxicity study could be ecologically significant, that is, cause ecologically meaningful changes in population numbers. Age-structured models can be used to extrapolate acute or chronic measurement endpoints to population effects. Risk characterization relates the exposure distribution to the population responses to determine a time to recovery, probability of a percentage of population decline, probability that the population will decline below a quasi-extinction threshold, and so on.

Toxicokinetic modeling-based approach

As mentioned earlier, time-varying models, such as PULSETOX, may be used to assist in the interpretation of effects from long pulses, long recovery intervals, or numerous repeated exposures that would be difficult to test in the laboratory. The time to reach a lethal body residue can be estimated using default CBRs. Estimates of body residues and/or time to lethality can be estimated for other exposure concentrations or pulse durations. The results from this modeling are not used directly in the risk characterization but are useful in understanding the dynamics of the compound in environmental receptors. The proximity of these concentrations to the CBR would be used in a weight-of-evidence approach in deciding whether effects are expected based on the particular exposure scenario.

Risk characterization in higher tiers

Risk characterization in higher tiers (or levels of refinement) would be subject to professional judgment and not a direct comparison of exposure and effects distributions as in lower tiers. Particular aspects of the pulsed exposure, either via monitoring (exposure and/or effects) or higher-level modeling (e.g., PBPK), would be refined and used in a weight-of-evidence approach to assessing the ecological risks.

UNCERTAINTY ANALYSIS

Uncertainties are grouped into laboratory and modeling categories.

Laboratory studies

Several issues and uncertainties are associated with pulse exposure toxicity testing. The exposure regime used in the laboratory should represent that encountered in the field. Pulse exposure toxicity testing may not be able to test pulses of long duration and/or long recovery intervals. The relationship between exposure time and acute toxicity will differ among substances. The postexposure observation period may reveal that some test organisms recovered from their toxic effects [3].

Toxicokinetic models

Uncertainties associated with and barriers to the use of toxicokinetic models in predicting toxic effects in aquatic organisms from whole-body residues are that (1) the toxicodynamics of the compound must be considered and are frequently poorly defined; (2) the distribution of the compound between tissue compartments must be at a steady state; (3) if equilibrium is not met, then the residue approach may not work for very short-term exposures; (4) defining toxic effects based on body residue levels reduces uncertainties associated with the bioavailability of the substance in question, but a number of poorly defined species-specific and site-specific variables exist that affect the refinement of toxicokinetic models; (5) considerable research is needed to generate data in support of the establishment of CBRs for the most common environmental contaminants and for a wide range of receptors, including sensitive species [44]; (6) when measuring CBRs, an operational lag time exists between when the effect occurs and the organism is retrieved and analyzed [44]; (7) high K_{ow} compounds will be more difficult to predict due to bioavailability limitations; (8) the development of CBRs for compounds with specific mechanisms of action will need to include exposures to a range of taxa to account for differences in sensitive species [44]; (9) bioaccumulation is a complex process involving biological and chemical factors, including kinetics, equilibrium levels, tissue translocation, sequestration, excretion, and depuration (body residues depend on age, health, sex, reproductive state, lipid content, trophic level, feeding habits, and ambient tissue concentrations); (10) analytical factors such as matrix interferences, extraction efficiency, methodological sensitivity, instrumentation, and variation in methodology will effect measured body residue levels [77]; and (11) a threshold level in tissue will generally refer to a single chemical. However, toxic potentials are associated with residues resulting from mixtures of compounds that will not be adequately described by developing threshold levels for individual compounds. For instance, DDT may stimulate the metabolism of dieldrin in animals, and different organochlorines may interact to effect the accumulation of residues in fish [65].

RECOMMENDATIONS

Recommendations supported in this document are that (1) time-varying exposure testing and modeling should be considered if exposure profiles and chemical behavior suggest pulsed scenarios (more realistic exposures); (2) time-varying exposures should be considered if the application interval, compound half-life, and/or exposure modeling indicate that such exposures may occur; (3) pulse testing methods or guidelines (both acute and chronic) should be developed and validated for general use; (4) where possible to redesign bioassay protocols to determine whether latency exists, the protocols should be modified as long as the results are compatible with historical data from the same protocol; (5) time-to-event analyses of time-varying responses are useful tools for interpreting population effects; (6) PULSETOX is useful when laboratory testing of time-varying exposure is not practical (e.g., long pulses, long recovery intervals, or numerous repeated exposures); (7) detailed PBPK models may be useful at higher tiers of ecological risk assessment, but monitoring is often more cost effective and less uncertain; (8) PBPK models are useful for extrapolation to other species, especially larger species where contaminant dynamics are not considered instantaneous; and (9) consideration of time-varying exposure results will reduce uncertainty and help refine the determinations of the risks associated with the use of many agrochemicals.

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APPENDIX

Run modes, input parameters, and assumptions for food and gill exchange of toxic substances model (FGETS)

Common inputs to the laboratory, food chain, and food web modes include the following:

- Molecular weight, volume
- Melting point
- Log K_{ow}
- Fish species
- Fish weight
- Water temperature
- Assume: food is in thermodynamic equilibrium with the water

Laboratory is defined as an aquarium with constant in- and outflows of water.

- Tank flow rate, volume
- Number of fish
- Concentration of chemical in food
- Length of study
- Assume: aquarium with constant in- and outflows of water and volume of the tank and the number of fish from a single species remains constant

Food chain simulates one or two fish interaction. With one fish, the fish is a predator that feeds on either plankton, benthic organisms, or generic fish. With two fish, one fish is the prey of the other. The prey species feeds on either plankton, benthic organisms, or generic fish, and the predator feeds on the prey species according to a specific length–length relationship.

- Plankton or benthic chemical concentration (constant)
- Prey identification (only in two-fish system)
- Predator identification
- Assume: concentration of chemical in water, plankton, benthos, and generic fish as well as water is constant during the length of simulation

Food web is designed to describe more realistic predator–prey interactions. Fish may feed on each other, plankton, or benthos, according to a user-specified diet. The exposure conditions (water temperature and concentrations of chemicals in plankton, benthos, and water) are arbitrary.

- Percentage diet makeup of prey (time or weight dependent)
- Percentage diet makeup of predator (time or weight dependent)
- Assume: unlimited prey resources and the physiologically active fraction of the gill is constant across all year classes for a particular species

Pulsed exposure toxicity tests (PULSETOX) modes and input parameters

For both cyclic and random exposures:

- Henry's law constant
- Volume of organism
- Volume fraction of lipid
- K_{ow}
- Molecular weight
- Bioconcentration factor
- Uptake and clearance rate constants
- Test chamber volume
- Water flow rate
- Substance stream flow rate and concentration (from GENEEC or pesticide root zone model/exposure analysis modeling system [PRZM/EXAMS])
- Number, duration, and interval of pulses
- Assume: the test chamber acts as a well-mixed vessel; the rate of change and absolute water concentration (C_w) at any point in time is a function of the test chamber volume, the flow rates of water, and the substance stream flow rate and concentration; the only clearance of chemical from the system is via the tank outflow; the organism is a single compartment (works well for smaller organisms [50]); toxicokinetic rate uptake and depuration constants, K_u and K_d, are independent of exposure concentration and do not change when the organism is intoxicated; the substance concentration in the organism reaches steady state if the external substance concentration is constant; the population of organisms responds in classic dose–response manner; individual response near instantaneous when the organism achieves its critical whole-body dose; and the whole-body substance concentration is an adequate surrogate for the dose at the site(s) of action.

For the random exposure mode, the exposure conditions must be described for each exposure phase.