

# Association Between Contaminant Tissue Residues and Effects in Aquatic Organisms

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## I. Introduction

Tissue residues have been proposed to be a more appropriate indicator of adverse effects in aquatic biota than external water concentrations because tissue residues should represent a more toxicologically relevant "dose" (McCarty and Mackay 1993). McCarty (1986, 1987) examined quantitative structure—activity relationships (QSARs) of the acute toxicity and bioconcentration of organic chemicals and concluded that chemicals should accumulate to a critical body residue (CBR). McCarty (1986) defined the CBR as the molar tissue concentra-

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tion (e.g., mmol/kg) of a toxic chemical that consistently produces a defined toxic effect such as mortality or reduced growth. According to the theory of McCarty and others (e.g., McCarty and Mackay 1993), CBRs within a defined mode-of-action category should be relatively constant across different chemicals, species, and exposure conditions. Although the CBR concept has both theoretical and experimental support, a comprehensive evaluation of the consistency and applicability of the CBR approach for different chemical classes has not previously been completed.

This review evaluates the consistency of CBRs and the applicability of the CBR approach for eight mode-of-action and chemical classes. These mode-of-action classes encompassed a broad range of environmental contaminants and included narcotics, excitatory agents, acetylcholinesterase (AChE) inhibitors, reactives/irritants, central nervous system (CNS) seizure agents, aryl hydrocarbon (Ah) receptor agonists, inorganic metals, and organometals. The organic chemicals within these classes have been previously defined based on evaluations of chemical structure, degree of toxicity, and behavioral effects in fish (Russom et al. 1997). The primary objectives of this review are (1) to determine if representative chemicals within a defined mode-of-action class accumulate to a consistent "critical" level in different species of aquatic organisms and (2) to determine if CBRs within each mode-of-action class are relatively consistent for a variety of chemicals and exposure conditions.

This review is organized as follows. Section II provides an overview of the CBR approach and Section III presents the methodology used to evaluate CBRs in this review. Section IV discusses data related to CBRs for narcotics. This chemical class is treated separately because of the substantial amount of attention devoted to developing CBRs for chemicals with a narcosis mode of action (McCarty 1986, 1987; McCarty et al. 1993). Section V considers and evaluates tissue residue data for other classes of organic chemicals: excitatory agents, AChE inhibitors, reactive compounds, CNS seizure agents, and compounds whose toxicity has been associated with binding to the Ah receptor. Section VI discusses the more limited information available regarding metals, including both heavy metals and organometallic compounds. Section VII discusses the physical, chemical, and biological determinants of CBRs and provides conclusions and research recommendations.

## II. The Critical Body Residue Approach

### A. Theoretical and Experimental Basis for CBRs

A CBR is defined as the concentration of a chemical accumulated in tissues of an aquatic organism that corresponds to a specific toxicity endpoint such as mortality, reduced growth, or reduced reproduction. Specifically, a tissue residue concentration is considered a "critical" residue if it is consistently associated with a specific toxicity endpoint, independent of aqueous exposure conditions. The body residue for a specific chemical therefore cannot be considered a CBR if exposure conditions or other chemical, biological, or environmental variables

substantially modify the body residue concentration associated with the specific toxicity endpoint. A CBR can be defined for either individual chemicals or for classes of chemicals that share the same mode of action, and have been hypothesized to be relatively constant across a wide range of aquatic species and taxonomic groups (McCarty and Mackay 1993).

Evaluation of chemical toxicity using tissue residues rather than an evaluation of toxicity based on contaminant concentrations in water, sediment, or diet offers several potential advantages. These advantages may include providing a direct measure of the internally accumulated dose, an indication of site-specific bioavailability, and integration of contaminant exposure routes and duration. In contrast, chemical toxicity based on the aqueous exposure concentration has been shown to be modified by the duration of the exposure, the history of an organism's prior exposure, the exposure dynamics (e.g., intermittent, continuous, or pulsed exposure concentrations), the bioavailability of the chemical, and the route of exposure. These chemical, biological, and environmental variables produce toxicity values for aqueous exposures of narcotic chemicals that encompass a range of values differing by four to five orders of magnitude (McCarty 1987).

The relationship between toxicological effects and exposure can be described as a CBR if tissue residue concentrations consistently produce the same toxicological effect (Fig. 1). Figure 1a shows that both water concentration and tissue residue are associated with adverse effects. In this case, the tissue concentration may or may not causally determine effects, depending on whether the tissue residue represents the toxicologically relevant dose or whether it simply covaries with the water exposure. In Fig. 1b, tissue residue, but not water concentration, is associated with adverse effects and therefore is potentially "critical." Figure 1c illustrates the reverse situation: water concentration is associated with effects, whereas tissue residues are not. Figure 1d illustrates the CBR concept by showing that the association between water concentration and adverse effects varies with exposure conditions, pH in this illustration, whereas tissue residue is a consistent predictor of a specific adverse effect level.

The CBR concept was developed by McCarty and colleagues (McCarty 1986, 1987; McCarty et al. 1992, 1993) after they determined that tissue concentrations of many chemicals with the same mode of toxic action were relatively constant for a defined level of toxicity such as death, when expressed on a molar basis (mmol/kg). McCarty (1987) predicted that CBRs for narcotic chemicals would be 1–2 mmol/kg for acute toxicity and 0.2–0.4 mmol/kg for chronic toxicity. CBRs for chemicals with more specific modes of action such as receptor-mediated toxicity were predicted to be lower than those for narcotic chemicals. For example, McCarty (1987) predicted the CBRs for nonnarcotic chemicals to be 0.3–0.6 mmol/kg for acute toxicity and 0.03–0.2 mmol/kg for chronic toxicity.

McCarty et al. (1992, 1993) later refined the CBR estimates for narcotic chemicals into two mode-of-action subclasses: nonpolar narcotics, which do not contain an oxygen or other polar group, and polar narcotics, which contain an

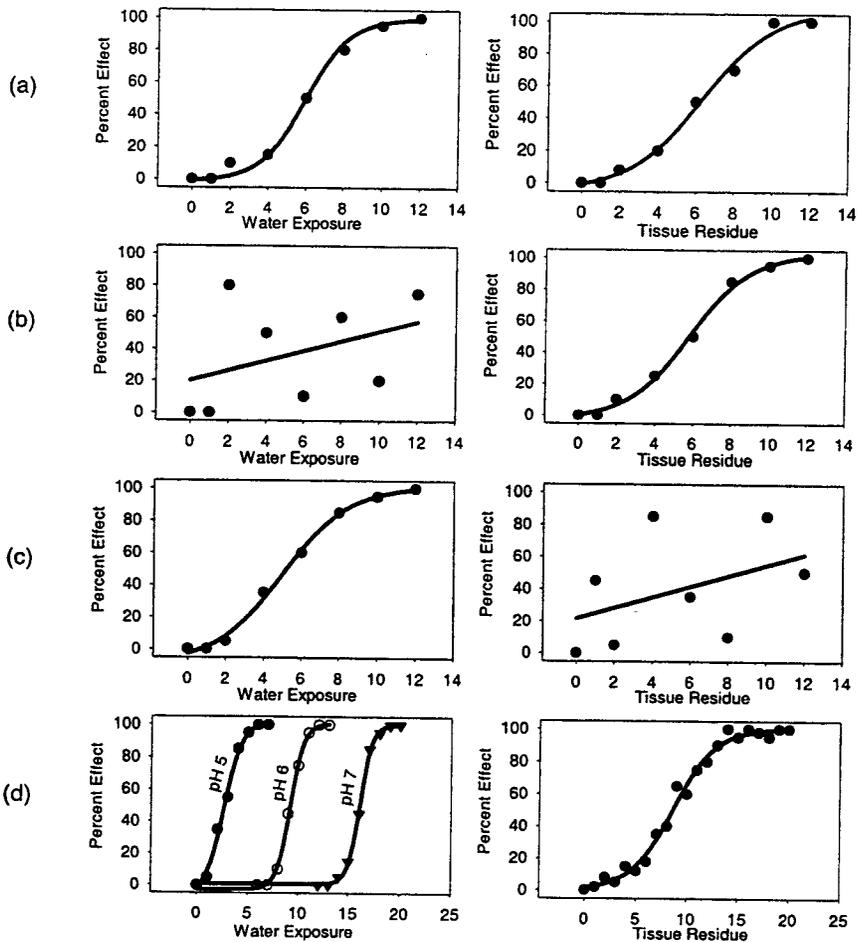


Fig. 1. Hypothetical relationships between exposure and toxicological effects in which exposure is represented as either water or tissue concentration. a, b. Potential critical body residue (CBR) relationships between dose and response. c. Relationship between dose and response that does not support the CBR concept. d. Hypothetical tissue dose-response relationship that supports the CBR concept.

ionizable polar group such as a hydroxyl. Nonpolar narcotics were predicted to have a CBR for acute lethality of 4.4 mmol/kg. The predicted CBR was supported by the relatively narrow range (>0.7–13 mmol/kg) of experimentally determined and estimated CBRs for several chlorobenzenes, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and the carbamate insecticide aminocarb (McCarty et al. 1992). McKim and Schmieder (1991) calculated lethal residues of 14 organic chemicals in large rainbow trout (*Oncorhynchus mykiss*) from chemical uptake experiments and grouped the CBR data

according to the mode-of-action characteristics of the chemical. In agreement with the CBR concept, the two nonpolar narcotics had the highest calculated CBRs (1.6 and 1.7 mmol/kg) and the polar narcotics had the lowest calculated CBRs (0.2–1 mmol/kg).

McCarty et al. (1993) estimated CBRs for 30 phenolic compounds in fathead minnows (*Pimephales promelas*) from the relationship between acute toxicity ( $LC_{50}$ ) and bioconcentration factors (BCFs). The estimated CBRs encompassed a 1000-fold range in values, which was attributed to differences in the mode of action of the compounds. Less polar alkyl phenols generally accumulated to the highest CBRs (>2 mmol/kg) and were considered by McCarty et al. (1993) to be nonpolar narcotics, whereas those with CBR values between 0.5 and 2.0 mmol/kg were considered to be polar narcotics. Phenolic compounds with CBRs less than 0.5 mmol/kg were presumed to have a nonnarcotic mode of action. McCarty et al. (1993) concluded that the CBR values estimated for fathead minnows were in general agreement with experimentally determined and theoretically estimated CBR data for other aquatic species. However, they also concluded that there was substantial uncertainty in the CBRs because of uncertainty in the type and number of modes of action of the phenolic compounds examined.

McCarty and Mackay (1993) summarized the state of the science on the CBR approach and tabulated estimated and measured CBRs for several mode-of-action categories (Table 1). As they noted, the tabulated data were not intended to be a comprehensive compilation of all available data but rather a summary of ranges of values considered to be representative of CBRs for chemicals in various mode-of-action classes. The data summary and evaluation by McCarty and Mackay (1993) suggested that nonpolar narcotics generally had the highest CBRs and showed limited variability, that polar narcotics had lower CBRs than nonpolar narcotics, and that chemicals with more specific modes of action generally had the lowest CBRs. van Wezel and Opperhuizen (1995) examined the

Table 1. Ranges of CBRs for the acute and chronic toxicity of chemical classes.

Chemical class/ Mode of action	Lethal effects (mmol/kg)	Sublethal effects (mmol/kg)
Narcotics	1.7 to 8 (nonpolar)	0.2 to 0.8 (nonpolar)
	0.6 to 1.9 (polar)	0.2 to 0.7 (polar)
Excitatory agents	0.1 to 0.3 <sup>a</sup>	$1.5 \times 10^{-4}$ to $9 \times 10^{-2}$
AChE inhibitors	$5 \times 10^{-2}$ to 2.7	$3 \times 10^{-3}$ (data for one chemical)
Reactives/irritants	$9.4 \times 10^{-3}$ to 13	No data
CNS seizure agents	$4.8 \times 10^{-5}$ to $1.7 \times 10^{-2}$	$5 \times 10^{-4}$ to $1.5 \times 10^{-2}$
Ah-receptor agonists	$1.5 \times 10^{-7}$ to $4 \times 10^{-5}$	$3 \times 10^{-7}$ to $8 \times 10^{-6}$

CBR, critical body residue.

<sup>a</sup>Range excludes one possible outlier value for 2,4-dinitrophenol of  $1.5 \times 10^{-3}$  mmol/kg.

Source: Summarized by McCarty and Mackay (1993).

experimental and theoretical support of the CBR approach summarized by McCarty and coworkers and concluded that lethal residues of narcotics were relatively constant and varied from 2 to 8 mmol/kg. McCarty and Mackay (1993) and McKim and Schmieder (1991) speculated that there are relatively narrow ranges of CBRs that are characteristic of a particular mode of action. However, these scientists also cautioned that the CBR approach may not apply to all modes of action, such as those with receptor-mediated and surface-acting toxicity.

### B. Uncertainties in the CBR Approach

Despite its potential advantages, the CBR approach has not been comprehensively evaluated across a broad range of chemicals and multiple mode-of-action classes. Barron et al. (1997) previously noted that most of the theoretical and experimental support for the CBR approach has been limited to a relatively small number of narcotic chemicals, and most experiments have been relatively short-term exposures conducted with small fish. Barron et al. (1997) reviewed structure-activity relationships for CBRs in relation to mechanism of action, toxicity endpoints, mixture toxicity, exposure dependence, dose dependence, chemical transformation, and chemical activation, and concluded that additional evaluation was needed before CBRs could be broadly applied in ecological assessments. Sources of uncertainty in the CBR approach include extrapolating data between species, exposure regimes, and chemicals (Barron et al. 1997). Additional uncertainty and potential variability in CBRs involve the correct assignment of chemical mode of action, and effects of metabolism, excretion, and tissue disposition of chemicals in aquatic organisms.

### III. Methodology for Evaluating CBRs

This evaluation of contaminant tissue residues and adverse effects in aquatic organisms is based on an examination of existing data. The information reviewed included both primary scientific literature and a recently developed comprehensive database summarizing exposure concentrations, tissue residue concentrations, and general toxicity endpoints (Jarvinen and Ankley 1999). The Jarvinen and Ankley (1999) database contains data from more than 500 research articles and includes effects from approximately 200 organic and inorganic chemicals. The majority of the studies contained in the database were not designed explicitly to measure CBRs, and thus a range of potential relationships between tissue residue and adverse effect may exist (see Fig. 1). Also, the endpoints presented by Jarvinen and Ankley (1999) were variable in the level of effect, such as the percentage mortality observed at a measured tissue concentration; thus, a given tissue residue is not numerically associated with a specific effect level. Despite these limitations, the Jarvinen and Ankley (1999) database provided a useful and reasonable approximation of the range of tissue residues for a range of adverse effects for many compounds.

The original information source was inspected when determining the minimum and maximum values in the CBR range determined from the Jarvinen and Ankley (1999) database and supplemented with newer data from the primary literature. Values from multiple species, exposure regimes, and analytical methods were included in the range of CBRs. A large number of individual studies were also examined to determine the consistency of CBRs across species of aquatic organisms, experimental methods, and exposure regimes. Consistent with the CBR approach (McCarty 1987), reported tissue concentration data (i.e., mg/kg) were converted to molar concentrations (mmol/kg) using the molecular weight of the compound. This conversion is considered appropriate for narcotic chemicals (McCarty and Mackay 1993), but its applicability to other modes of action is unknown.

The consistency of CBRs for selected individual chemicals was evaluated by first compiling data on tissue concentrations of chemicals causing mortality to determine if CBRs were consistent between species, exposure temperatures, and other environmental, chemical, and biological variables. When studies produced consistent CBRs using different environmental, chemical, or biological variables, we concluded that the particular variable did not influence the CBR. Substantial differences in CBRs between studies provided evidence that particular environmental, chemical, or biological variables did influence the relationship between bioaccumulation and toxicity and that the chemical did not consistently accumulate to a critical level to produce toxicity.

The consistency of CBRs within chemicals having the same mode of action was evaluated by segregating tissue residue data into one of the eight mode-of-action classes for organic and inorganic chemicals identified in Table 2. The classification schemes for organic chemicals followed that of McCarty and Mackay (1993) and Russom et al. (1997). If the mode of action of the chemical was uncertain, the value was excluded from further analysis. Single extreme low or extreme high values (two orders of magnitude below or above the next value) were excluded from the reported range of CBRs. We also evaluated the range of CBRs for major chemical subgroups within a mode-of-action category (Russom et al. 1997). The chemical subgrouping was based on known mode-of-action subcategories (e.g., nonpolar versus polar narcotics) or major differences in chemical structure, such as organochlorine and pyrethroid CNS seizure agents.

## IV. Narcotic Chemicals

### A. Background

Narcotics are a structurally diverse group of low molecular weight chemicals that cause hypoactivity and anesthesia (i.e., depression of locomotor and sensory functions). They are the largest class of synthetic organic chemicals and include alkanes, benzenes, simple alcohols, phenols, ketones, and esters. Narcotic chemicals are used as solvents and chemical intermediates and are components of paints and adhesives. They are not reactive, and generally have moderate water

Table 2. Chemical classes and modes of action evaluated.<sup>a</sup>

Chemical class	Mode of action	Structural features	Example chemicals
Narcotics	Narcosis (anesthesia) Nonspecific action	Small molecules, diverse structure, nonreactive	Phenols, ketone, esters, alcohols, benzenes
Excitatory agents	Oxidative phosphorylation uncoupling	Poly halo- and nitrobenzene derivatives	Pentachlorophenol, dinitrophenols
AChE inhibitors	AChE inhibition	Organophosphorus and carbamate pesticides	Chlorpyrifos, terbufos, carbaryl
Reactives/irritants	Irritation/damage to membranes and nerve tissue	Electrophilic structures (e.g., reactive double bonds)	Aldehydes, alkenes, alkynes
CNS seizure agents	Nervous system interaction	Organochlorine and pyrethroid insecticides	DDT, fenvalerate
Ah-receptor agonists	Ah-receptor binding	Planar chlorinated aromatic compounds	TCDD, planar PCBs
Heavy metals	Ionoregulation, respiration, cell damage	Free cation and complexes with simple anions	Copper, cadmium, zinc, arsenic
Organometals	Nerve tissue damage	Metal-organic complexes	Methylmercury, tributyltin

<sup>a</sup>Chemical classes and mode-of-action categories were adapted from McCarty and Mackay (1993) and Russom et al. (1997), and were expanded to include heavy metals and organometals.

solubility, high soil mobility, high volatility, and low bioaccumulation. They partition primarily in the vapor phase and rapidly equilibrate between exposure water and tissue.

Narcotic chemicals have rapidly reversible anesthetic effects and do not appear to cause cumulative injury. Their mode of action is a nonspecific and reversible interaction with cellular lipids and proteins (van Wezel and Opperhuizen 1995). Potential mechanisms of narcosis include (a) changes in membrane fluidity, (b) altered membrane protein function through interaction with membrane lipids or direct protein binding, and (c) disruption of nerve function (van Wezel and Opperhuizen 1995; van Wezel et al. 1996). Narcotic chemicals have been grouped into two mode-of-action subcategories (i.e., nonpolar narcotics and polar narcotics) on the basis of chemical structure and general degree of toxicity (Russom et al. 1997). Russom et al. (1997) included a third subcategory of narcosis for ester narcotics, such as benzoates and acetates. However, ester narcotics are not treated separately from nonpolar narcotics in the CBR approach, and data for these chemicals are limited. In this review, only nonpolar and polar narcotics are considered. Nonpolar narcotics include the majority of chemicals causing narcosis and are characterized as having the lowest toxicity and the highest CBRs (Vaes et al. 1998). Polar narcotics are more polar chemicals that elicit initial excitatory responses followed by narcotic-like depression (Bradbury et al. 1989). Polar narcotics include phenol and phenolic compounds substituted with one to two electron-withdrawing groups such as nitro or halo substituents. Phenolic compounds containing additional electron-withdrawing groups may act as excitatory agents rather than as narcotics. The polar narcotics evaluated in this review included phenol, mono- and dihalogenated phenols, and nitrophenol. The excitatory agents evaluated in this review (Section V.A) included trichlorophenol, tetrachlorophenol, pentachlorophenol, and dinitrophenol.

## B. Variability of CBRs

There is both theoretical and experimental support for the presence and consistency of CBRs for narcotics, but research has been limited to a relatively small subset of chemicals such as chlorophenols, chlorobenzenes and anilines, and chlorinated alkanes (van Wezel and Opperhuizen 1995). Comprehensive evaluation of the Jarvinen and Ankley (1999) database and primary literature for a broad range of narcotic chemicals shows that there is considerable variation in CBRs between studies of the same chemicals or chemicals with nearly identical structures. For example, the lethal body residues of the nonpolar narcotic 1,2-dichlorobenzene varied by one order of magnitude between different studies: 2–3 mmol/kg (van Hoogen and Opperhuizen 1988), 0.42 mmol/kg (Pawlisz and Peters 1993a), and 1–4 mmol/kg (van Wezel et al. 1996). Lethal body residues of 1,4-dichlorobenzene differed more than 20 fold, ranging from 0.39 to 8 mmol/kg (van Wezel et al. 1996). The lethal body residue of the fluorinated isomer 1,4-difluorobenzene was 28 mmol/kg (Sijm et al. 1993). Hence, a 72-

fold difference in CBRs has been measured for two dihalogenated benzenes derived from just four studies with *Daphnia magna*, guppies (*Poecilia reticulata*), and fathead minnows. Considerable variation in reported lethal body residues was also observed for the 1,2,3- and 1,2,4-isomers of the nonpolar narcotic trichlorobenzene. CBRs in these same test species varied by one order of magnitude between several studies: 2–3 mmol/kg (van Hoogen and Opperhuizen 1988), 21 mmol/kg (Pawlisz and Peters 1993a), 5.3–20 mmol/kg (de Maagd et al. 1997), and 3–6 mmol/kg (van Wezel and Jonker 1998). This variation suggests that relatively high variation in CBRs can be observed even between studies of the same chemical. With the data available, the cause of the variation (i.e., chemical, biological, or environmental factors) could not be determined between studies of a single chemical or between studies of similar chemicals.

Examination of the Jarvinen and Ankley (1999) database shows that tissue residues of narcotics (both polar and nonpolar) that reduce survival range from 0.009 mmol/kg for 3-chlorocresol to 450 mmol/kg for butanol (tested in brown trout, *Salmo trutta*, and *Daphnia magna*, respectively). This 50,000-fold range in tissue residues incorporates 144 reported database values, which were mostly for benzenes, phenol, chlorophenols, nitrophenols, alkyl naphthalenes, ketones (e.g., acetone), and tetrachlorethanes. More specifically, the tissue residue data for nonpolar narcotics incorporated 80 reported values and ranged from 0.032 to 450 mmol/kg (Jarvinen and Ankley 1999). Tissue residues of polar narcotics that reduce survival ranged from 0.009 to 4.9 mmol/kg and comprised 64 reported values (Jarvinen and Ankley 1999). Tissue residues associated with adverse effects were generally lower for polar narcotics than for nonpolar narcotics, but the data ranges overlapped substantially. Large variation was also evident between narcotic chemicals within the same study using consistent experimental conditions. For example, Fig. 2 shows that lethal body residues exhibited a 3,500-fold range in *Daphnia magna* exposed to each of 10 narcotics, from 0.1 mmol/kg for 2-methylnaphthalene to 350 mmol/kg for butanol (Pawlisz and Peters 1993a,b). The variability in tissue residues associated with adverse effects, both within and between chemicals, indicates that the CBR approach may have limited application even for narcotic chemicals unless factors such as species sensitivity, biological variability, and exposure conditions are considered.

### C. Determinants of CBRs

According to CBR theory, lethal tissue residues of narcotic chemicals should be approximately 2 mmol/kg tissue and should be relatively independent of chemical, biological, and environmental factors. However, critical evaluation of the available data shows that the CBRs of narcotic chemicals can show substantial variability. Determinants of CBRs include chemical structure, biological factors such as biotransformation and lipid content, and environmental factors such as ultraviolet radiation (UV) and pH.

The CBRs of phenolic compounds summarized by McCarty et al. (1993)

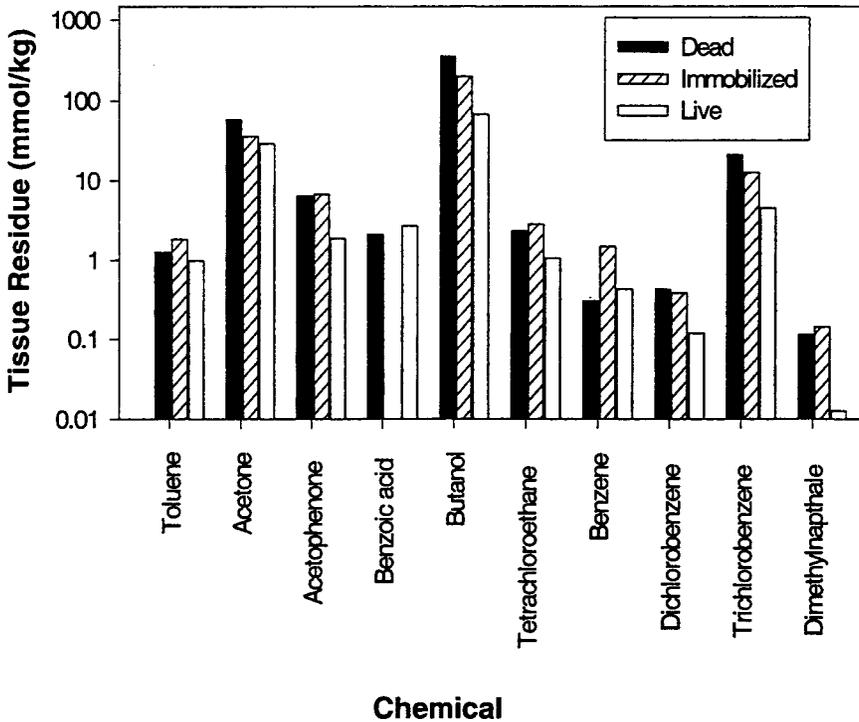


Fig. 2. Tissue residues of 10 narcotic chemicals in dead, immobilized, and live *Daphnia magna*. (Data from Pawlisz and Peters 1993a,b.)

exhibited approximately three orders of magnitude range in CBRs, similar to the variability in  $LC_{50}$ s. The addition of halogen groups appears to lower CBRs for phenols (Fig. 3), and consideration of substituent groups may provide a means of reducing variability in CBRs across individual compounds (McCarty et al. 1993; Di Toro et al. 2000). A reduction in CBR appears to be related to a change in mode of action from narcosis to excitation. Mono- and dichlorophenols are considered to be polar narcotics, whereas trichlorophenol, tetrachlorophenol, and pentachlorophenol are considered to be excitatory agents (McCarty et al. 1993; Penttinen and Kukkonen 1998). Phenols with electron-donating (e.g., alkyl) groups have higher CBRs and are considered nonpolar narcotics (McCarty et al. 1993). Some nonpolar narcotics such as halogenated benzenes have been shown to produce CBRs that appear to be relatively independent of molecular shape, molecular size, and octanol–water partition coefficient ( $K_{ow}$ ) (Sijm et al. 1993). However, most studies (e.g., Connell and Markwell 1992) have not evaluated broad groups of structurally diverse narcotics, and the variation in CBRs was not systematically evaluated to determine whether there was a relationship between chemical structure and tissue residues.

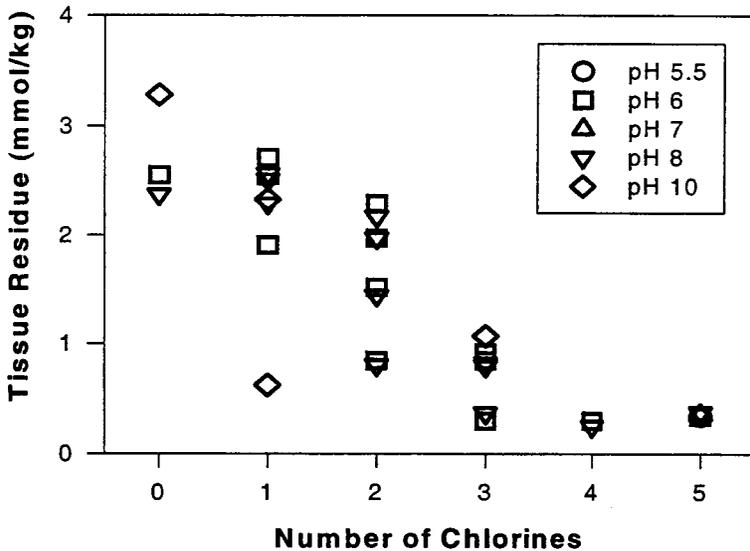


Fig. 3. Tissue residues at death in goldfish (*Carassius auratus*) exposed to phenol, monochlorophenols, dichlorophenols, trichlorophenols, 2,3,4,6-tetrachlorophenol, and pentachlorophenol. Exposures were conducted for 5 hr at pH 5.5–10. Each symbol represents the mean tissue residue of several fish samples. (Data from Kishino and Kobayashi 1995.)

Biotransformation by aquatic organisms may account for some of the variability in narcotic CBRs. For example, biotransformation of three PAHs in *Hyallorella azteca* resulted in greater accumulation of total residues (parent + metabolites) than would be expected if no biotransformation had occurred (Lee et al. 1999). Tissue residues of fluoranthene in the amphipod *Leptocheirus plumulosus* were made up of approximately 30% metabolites (Kane Driscoll et al. 1998). Tissue residues of chemicals that are biotransformed may appear less toxic because of a higher apparent CBR if tissue concentration measurements are based on total residues. This discrepancy occurs because both parent chemical and less toxic metabolites are quantified rather than only the parent compound (McCarty et al. 1993). Biotransformation may be increased by prior chemical exposure, and the induction of metabolic activity may be dependent on the species and exposure conditions.

Different species or individuals of a species may have different concentrations of lipids, which can contribute to CBR variability. CBRs for narcotics should increase in direct proportion to the lipid content of the organism (McCarty et al. 1992) because the mechanism of toxicity for narcotics appears to involve nonspecific binding to lipoidal tissues. Additionally, higher lipid content may act to lower the concentration of chemical in the target tissue. A correlation between increasing lipid content and higher CBRs has been shown by several researchers (van Wezel et al. 1995; de Maagd et al. 1997), and has

become the basis of the target lipid model of DiToro et al. (2000) and DiToro and McGrath (2000).

In a recent critical review of CBRs of 33 species exposed to 156 narcotic chemicals, DiToro et al. (2000) also found CBRs to be highly variable. These authors concluded that nonpolar narcotic chemicals had variable chemical potency, with aliphatics, ethers, alcohols, and aromatics having similar potency, and ketones, halogenated chemicals, and PAHs having much higher potency. DiToro et al. concluded that variability in CBRs for narcotics could be reduced by recognizing this differential potency, by incorporating the percent lipid concentration of organisms into a QSAR model, and by explicitly recognizing differential species sensitivity. Although the resulting target lipid model of DiToro et al. accounted for much of the variation of CBRs for the narcotic chemicals examined, there is still considerable variation associated with observed body residues. On average, the target lipid model was found to increase the accuracy of predicted mean CBRs for individual species, but individual measurements of nonpolar narcotic chemicals within a species still varied by a factor of 10 or more (DiToro et al. 2000).

Environmental exposure conditions such as differences in temperature, pH, and salinity may alter CBRs through their influence on metabolism, chemical bioavailability, and degree of chemical ionization. For example, higher temperatures were shown to reduce the CBR for 1,2,4-trichlorobenzene (van Wezel and Jonker 1998), and the CBR of 4-nitrophenol was influenced by both the temperature and pH of the solution (Howe et al. 1994). Alternatively, pH had only a minor effect on the lethal body residues of 12 chlorophenols (Kishino and Kobayashi 1995). Although data are limited, the route of exposure may be a less important source of variability of CBR variability. For example, the CBR in amphipods exposed to fluoranthene in sediment were 0.3 mmol/kg, similar to the 0.4 mmol/kg in amphipods exposed in a water-only medium (Kane Driscoll et al. 1998).

Photoenhanced toxicity, which is a 2- to 1000-fold increase in toxicity observed under UV compared to fluorescent lighting (minimal UV), can alter the CBRs for specific PAH compounds with three to five benzene or substituted benzene rings. For example, photoactivated fluoranthene at 0.12 mmol/kg caused 98% mortality, whereas nonphotoactivated fluoranthene at 1.25 mmol/kg had no effects on survival (Ankley et al. 1995). Most CBR data have been derived from laboratory studies with minimal UV, and the CBR ranges summarized in this review do not include chemicals that exhibit photoenhanced toxicity. CBRs of photoactivated PAHs are dependent on the degree, duration, and spectrum of UV exposure, and not only on the tissue residue of the PAH (Barron et al. 2000). Application of the CBR approach to photoactivated chemicals may be problematic because the UV exposure of the organisms must be determined in addition to tissue residues.

Empirically derived CBRs have been found to vary considerably for many narcotic chemicals. Causes of this variability include differences in chemical structure, species responses, and exposure conditions. The variability in the re-

ported CBRs may also relate to the fact that relatively few of the available data were derived from studies aimed specifically at determining CBRs. Also, there is some uncertainty associated with the mode of action of the chemicals considered to be narcotics.

## V. Nonnarcotic Organic Chemicals

Chemicals with more specific modes of action include excitatory agents, AChE inhibitors, reactive chemicals, CNS seizure agents, and Ah receptor agonists. With the exception of a few investigations of excitatory agents, research specifically evaluating the chemical, biological, and environmental determinants of CBRs for nonnarcotic chemicals is limited. The CBR concept was developed for narcotic chemicals, and was only postulated to apply to other chemical groups.

### A. Excitatory Agents

Excitatory agents are a smaller group of substituted phenolic chemicals that cause hyperactivity and overreaction to outside stimuli. Generally, this group is composed of chemicals with a single aromatic ring (e.g., phenols, anilines, pyridines) containing multiple electron-withdrawing substituents (e.g., halogens and nitro groups). These chemicals include some chlorophenols such as pentachlorophenol (PCP) and some nitrophenols such as dinitrophenol that are used as solvents, chemical intermediates, and biocides. Trichlorophenols were grouped in the excitatory agent category in agreement with the evaluations of Penttinen and Kukkonen (1998), although these chemicals may also act as polar narcotics (Russom et al. 1997). Chlorophenols, particularly PCP, have also been widely used as pesticides and wood preservatives. The excitatory agents generally have lower water solubility, higher affinity for sediments and soil, and less volatility, and are more persistent in the environment than narcotic agents. The mechanism of action of the excitatory agents is an uncoupling of oxidative phosphorylation (i.e., dissociation of the electron transport process from the generation of ATP).

In general, CBRs for excitatory agents appear to be lower than those for polar narcotics, despite their structural similarities. The number and type of electron-withdrawing groups on a phenol determine its mode of action and thus influence the magnitude of the CBR. On average, phenols with four and five chlorines are considered excitatory agents and have been found to cause death at tissue residues of approximately 0.3 mmol/kg, whereas monochlorophenols and dichlorophenols are considered narcotics and generally caused death at tissue residue concentrations greater than 1 mmol/kg (see Fig. 2). The range of tissue residues associated with mortality is highly variable between several excitatory chemicals. Tissue residues of PCP associated with reduced survival in fish and invertebrates range from 0.0004 mmol/kg (Fisher et al. 1999) to 0.9 mmol/kg (Landrum and Dupuis 1990), which represents a 2300-fold range in CBRs for just one chemical. However, within related taxa such as fish, CBRs for PCP were similar and ranged from 0.05 mmol/kg for rainbow trout (McKim

and Schmieder 1991) to 0.3 mmol/kg for fathead minnows (Arthur 1991; Hickie et al. 1995) to 0.4 mmol/kg for goldfish (*Carassius auratus*; Kishino and Kobayashi 1995). For 2,4-dinitrophenol, CBRs from the same study ranged from 0.11 to 4.4 mmol/kg, which is a 40-fold range in the amphipod *Gammarus pseudolimnaeus*, and from 0.034 to 0.36 mmol/kg, a 10-fold range, in rainbow trout depending on exposure pH and temperature (Jarvinen and Ankley 1999).

Exposure temperature and pH both appear to account for some of the variability of CBRs for excitatory agents, whereas other environmental variables appear to have less influence. In a study of amphipods exposed to 2,4-dinitrophenol, CBRs at pH 6.5 were 30 times lower than those at pH 8.5, whereas at a constant pH, CBRs were within a sixfold range at different temperatures (Jarvinen and Ankley 1999). A combination of higher pH and lower temperature substantially increased PCP CBRs in zebra mussels, *Dreissena polymorpha* (Fisher et al. 1999) (Fig. 4). In the Fisher et al. (1999) study, CBRs for 50% mortality increased eight times with increasing pH and decreasing temperature, whereas  $LC_{50}$ s ( $\mu\text{mol/L}$ ) increased 380 times. In another study of chlorophenols, pH had only a minor effect on the CBRs for mortality in goldfish (Kishino and Kobayashi 1995). CBRs were shown to be independent of exposure time for PCP and for 2,4,5- and 2,4,6-trichlorophenol (Klee 1998). Similarly, few differences in CBRs for PCP were observed between constant exposure concentrations and pulsed exposure concentrations (Hickie et al. 1995). However, because of the limited research on excitatory agents, additional data are required to ex-

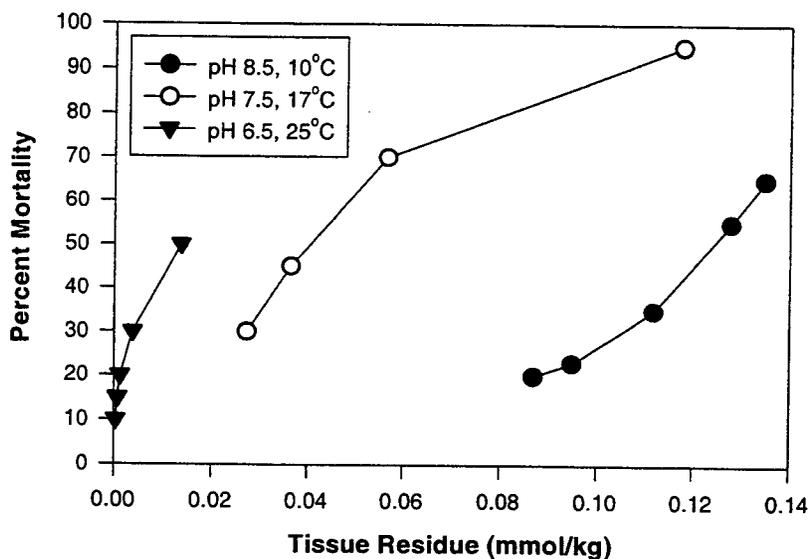


Fig. 4. Relationship between tissue residues of PCP and mortality of zebra mussels (*Dreissena polymorpha*) when exposed at different pH values and temperatures. (Data from Fisher et al. 1999.)

plore the effects of these and other environmental, chemical, and biological variables on CBRs of these chemicals.

Limited data were available for assessing the sublethal effects of CBRs for excitatory agents. In general, sublethal effects were observed at lower body residues than lethal effects, but with similar variability (Jarvinen and Ankley 1999). Figure 5 shows that fathead minnows exposed to PCP for 28 days exhibited a general trend of decreasing growth with increasing tissue residues, but data for individual fish were highly variable (Arthur 1991). Penttinen and Kukkonen (1998) quantified the disturbance of normal energy metabolism as the rate of heat dissipation by the excitatory agents 2,4-dichlorophenol (DNP), 2,4,5-trichlorophenol (TCP), and PCP in two species of freshwater invertebrates: chironomid larvae (*Chironomus riparius*) and an oligochaete worm (*Lumbriculus variegatus*). Heat output was unaffected at low body residues, but increased linearly with tissue residue concentrations when tissue residues were in the range of 0.2 to 2 mmol/kg (Penttinen and Kukkonen 1998).

Overall, data on the application of CBR approaches to excitatory agents suggest that CBRs may be relatively constant for some compounds such as PCP, although differences in species sensitivity are apparent. However, CBRs appear to vary as a function of environmental exposure factors such as temperature and pH, which limit the environmental applications of the approach. Additional focused research on CBRs for key compounds is necessary to more adequately evaluate application of the CBR concept for excitatory agents.

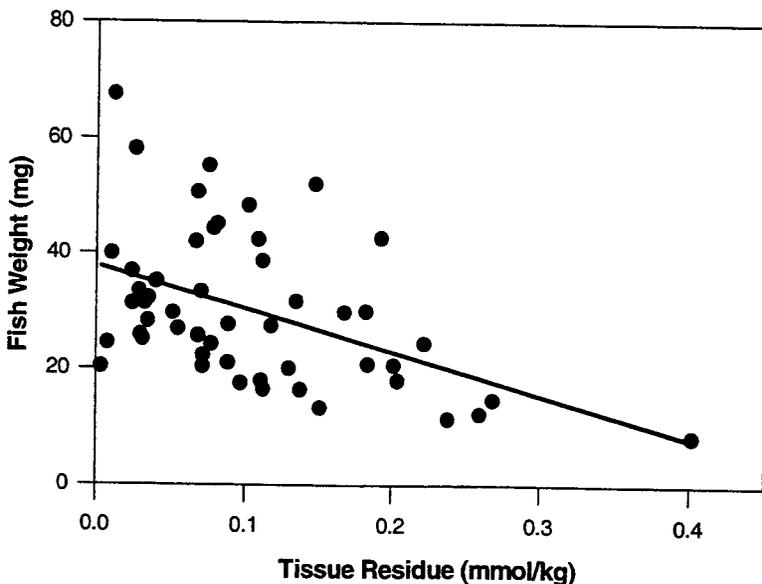


Fig. 5. Tissue residues of pentachlorophenol (PCP) and growth of fathead minnows (*Pimephales promelas*) after 28 d exposure. (Data from Arthur 1991.)

## B. Acetylcholinesterase Inhibitors

Acetylcholinesterase (AChE) inhibitors are organophosphate insecticides (OPs) such as terbufos, trichlorfon, and chlorpyrifos, and carbamate insecticides such as carbofuran and carbaryl. AChE inhibitors cause toxicity through inhibition of AChE, an enzyme that degrades the neurotransmitter acetylcholine. In contrast to the nonspecific toxicity of narcotics, AChE inhibitors cause toxicity through a specific receptor-mediated mechanism (Legierse et al. 1999). OPs and carbamates are applied in agricultural and residential uses, and can also be accidentally introduced into the environment through overspray, aerial drift, or runoff. They are typically produced as esters, amides, or thiol derivatives, which affect their physical and chemical properties and environmental fate. In general, they have low solubility in water, high soil binding, and low volatility. OPs and carbamates are less persistent and less bioaccumulative than the chlorinated insecticides such as DDT and dieldrin. Carbamate insecticides are generally less persistent in the environment and less hydrophobic than OPs.

Tissue residues of AChE inhibitors associated with reduced survival appear to be highly variable, ranging from 0.00004 mmol/kg for trichlorfon to 29 mmol/kg for dichlofenthion (Deneer et al. 1999; Jarvinen and Ankley 1999). This range encompasses 115 reported values with a 725,000-fold range in CBRs, and includes 17 OPs and 1 carbamate insecticide. Tests were conducted with multiple species, including amphipods (*Gammarus pseudolimnaeus*), grass shrimp (*Palaemonetes pugio*), sheepshead minnows (*Cyprinodon variegatus*), rainbow trout, and guppies. However, the majority of the residue data were for the OPs terbufos and trichlorfon. One extreme low value ( $10^{-6}$  mmol terbufos/kg) was excluded from the range of CBRs because it was 28 fold lower than any other value found in the database (Jarvinen and Ankley 1999).

High variation in lethal body residues of AChE inhibitors was observed both within single studies of the same chemical and across studies of multiple chemicals. Lethal residues of the OP chlorthion in pond snails (*Lymnaea stagnalis*) ranged from 0.015 to 0.63 mmol/kg, which is a 40-fold range of CBRs. There was no apparent correlation between lethal tissue residues and lipid content (Legierse et al. 1999). Similarly, lethal residues of 13 OPs in guppies exhibited a 2600-fold range in CBRs of 0.011 to 29 mmol/kg, with no apparent correlation with  $K_{ow}$  or exposure duration (Deneer et al. 1999).

Variability in lethal body residues of AChE inhibitors may be attributable to species differences and exposure duration, although further research may indicate influences of other variables. Differences in intrinsic species sensitivity to AChE inhibitors is caused by different kinetics of AChE binding such as receptor number or affinity or different rates of biotransformation (e.g., detoxification versus bioactivation rates; Barron and Woodburn 1995). Other research has shown that lethal tissue residues are lower at lower exposure concentrations and longer exposure durations (Legierse et al. 1999). Using this relationship, Legierse et al. (1999) developed a time-dependent model of the toxicity of OPs that incorporates time-dependent metabolic bioactivation and receptor binding

and AChE inhibition. Although some AChE inhibitors require metabolic activation to elicit toxicity, studies have not clearly shown that metabolic activation affects CBR values. For example, similar CBRs were reported in grass shrimp and sheepshead minnows for terbufos, which requires metabolic activation, and trichlorfon, which does not (Jarvinen and Ankley 1999).

The high variation of tissue residue concentrations associated with lethality to AChE inhibitors leads to the conclusion that the CBR concept does not apply to this class of chemicals. This conclusion is strengthened by the observations of differences in species sensitivity and differences in lethal tissue residues attributable to exposure duration. The nearly 1,000,000-fold range in lethal tissue residues for AChE inhibitors makes environmental applications of the CBR approach problematic for this class of chemicals.

### C. Reactive Chemicals

Reactive chemicals are a small group of low molecular weight compounds such as aldehydes, unsaturated aliphatics (e.g., alkenes, alkynes), and alcohols (e.g., propargylics). These chemicals contain structural features (e.g., reactive double bonds) that allow electrophilic or proelectrophilic reaction with nucleophilic structures such as amino and thiol groups on cellular macromolecules. They can react with DNA, enzymes, and other proteins, causing irritation or damage to mucous membranes and nerve tissues. The reaction between the chemical and tissue alters the functionality of cellular components, resulting in a diversity of behavioral and neurotoxic responses, including narcosis, hyperactivity, and convulsions (Russom et al. 1997). Sources of reactive chemicals include biocides such as acrolein; chemical intermediates from chemical production; products of organic pyrolysis such as automobile emissions, manufacturing, pulp and paper effluents; solvents; and industrial fluids. Because of their various chemical structures, reactive chemicals can have low (e.g., chlorinated alkenes) to high (e.g., acrolein) water solubility, moderate volatility, and low to high soil binding.

Tissue residues of reactive chemicals associated with reduced survival range from 0.094 mmol/kg for acrolein to 13 mmol/kg for benzaldehyde (McKim and Schmieder 1991; Jarvinen and Ankley 1999). This range encompasses only eight reported values with a 140-fold range in CBRs, with data for only acrolein, benzaldehyde, and hexachloro-1,3-butadiene. Other chemicals including PAHs may be bioactivated to reactive metabolites such as dihydrodiol epoxides of benzo(a)pyrene but were not considered as reactive chemicals in this review.

Although only very limited data on CBRs for reactive chemicals are available, the CBR approach does not appear applicable to this class of chemicals. Verhaar et al. (1999) presented theoretical arguments that CBRs will not be constant for chemicals with a mechanism of action that includes irreversible or partially irreversible effects. Verhaar et al. also argued that CBRs will not be constant for reactive and receptor-mediated chemicals, and that the CBRs may decline with exposure time. McKim and Schmieder (1991) concluded that acro-

lein and benzaldehyde were irritants that acted at the surface of the gills and that whole-body residues were not important in determining their toxicity. An additional complication is that the acute effects of more hydrophobic reactive chemicals may also be associated with narcosis, possibly because of high affinity for membrane lipids (Hermens 1990). This affinity may explain some of the relatively high lethal tissue residues for some reactive chemicals (McKim and Schmieder 1991; Jarvinen and Ankley 1999).

#### D. CNS Seizure Agents

The CNS seizure agents include two general classes that cause tremors and convulsions by damaging nerve tissues: the organochlorine pesticides (OCs) and the pyrethroid insecticides such as fenvalerate, permethrin, and cypermethrin. The OCs include ethanes such as DDT, cyclodienes such as chlordane and dieldrin, hexachlorocyclohexane (lindane), the chlorinated camphene toxaphene, mirex, and chlordecone (kepone). The OCs are a group of chlorinated broad-spectrum insecticides produced commercially beginning in the 1940s for agricultural and residential use. Most uses of OCs have been banned worldwide because of extreme environmental persistence (half-lives of months to years) and extensive biomagnification in the food web. Characteristics of OCs include high lipid solubility, low water solubility, and high affinity for sediments and soils. They are mobilized by soil runoff, airborne dusts, and suspended sediments. Pyrethroids are substantially less persistent in the environment than the OCs and are less hydrophobic.

Data in Jarvinen and Ankley (1999) indicate that tissue residues associated with mortality were highly variable for CNS seizure agents. CBRs for reduced survival ranged from 0.00002 mmol/kg for cypermethrin to 1.1 mmol/kg for DDT. This range incorporated 125 reported values and demonstrates a 55,000-fold range in CBRs. Tests were performed with multiple species, including pink shrimp (*Penaeus duorarum*), white shrimp (*Penaeus setiferus*), midges (*Chironomus riparius*), several crab species, sheepshead minnows, *Daphnia magna*, chinook salmon (*Oncorhynchus tshawytscha*), brook trout (*Salvelinus fontinalis*), goldfish, fathead minnows, and other fish species. High variability was observed even within structurally similar chemicals. Lethal tissue residues for OCs ranged from 0.00007 to 1.1 mmol/kg, and those for pyrethroids ranged from 0.00002 to 0.012 mmol/kg (Jarvinen and Ankley 1999).

Factors contributing to the broad range in lethal tissue residues appear to include chemical structure, species differences, exposure dynamics, and tissue-specific accumulation. Different chemicals and stereoisomers of the same chemical have been shown to have substantially different CBRs. For example, Bradbury et al. (1987) observed more than a 30-fold difference in the lethal body residues of isomers of fenvalerate in fathead minnows. The 2*S* stereoisomer of fenvalerate caused mortality within 32 hr of exposure and tissue concentrations ranged from 0.0005 to 0.004 mmol/kg, whereas the 2*R* stereoisomer did not cause mortality within 48 hr and average tissue residues were 0.017 mmol/kg.

Figure 6 shows that across several studies the lethal tissue residues for DDT in 11 species of aquatic invertebrates and fish varied by a factor of 10,000 (Jarvinen and Ankley 1999). Exposure dynamics such as continuous versus pulsed exposures have also been shown to affect both lethal and sublethal CBRs of CNS seizure agents (Fig. 7). Tissue residues associated with reduced survival can also vary substantially in individual tissues. For example, Fig. 8 shows that no-effect concentrations of DDT in intestine and liver were higher than lethal levels of DDT residues in brain, heart, spleen gill, muscle, and carcass. These data indicate that application of the CBR concept to the overall class of CNS seizure agents is problematic.

### E. Aryl Hydrocarbon Receptor Agonists

Aryl hydrocarbon (Ah) receptor agonists are compounds that have high affinity for the Ah receptor, which is a protein complex in the cell nucleus. Chemical binding to the Ah receptor elicits a complex cascade of biochemical and physiological responses including induction of the P-450 oxidative enzyme system. Ah-receptor agonists include halogenated aromatics such as PCBs, dibenzo-*p*-dioxins (PCDDs), and dibenzofurans (PCDFs). The potency of these polycyclic aromatic compounds is determined by the chemical structure such as position of chlorine atoms and molecular conformation including the ability to form a planar configuration. For example, the most potent PCBs, PCDDs, and PCDFs have outward (meta, para) halogen substitutions rather than inward (ortho) substitutions, which increases binding affinity for the Ah receptor. Al-

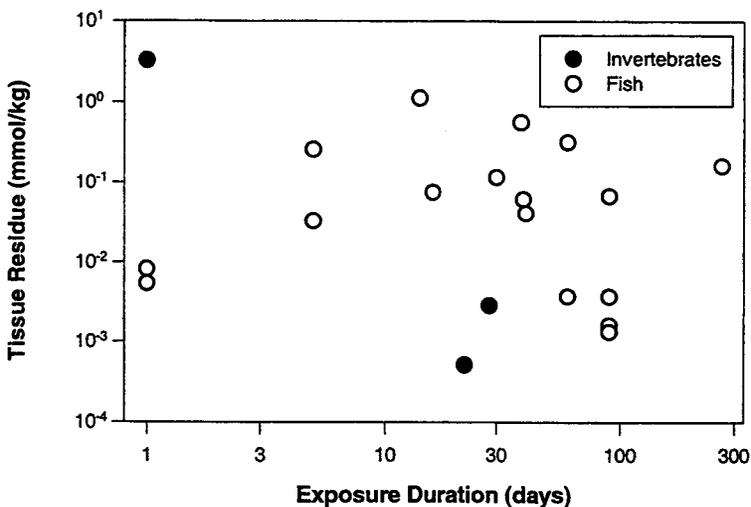


Fig. 6. Tissue residues for reduced survival in 11 species of aquatic invertebrates and fish exposed to DDT. Exposure duration ranged from 1 to 266 d. (Data from Jarvinen and Ankley 1999.)

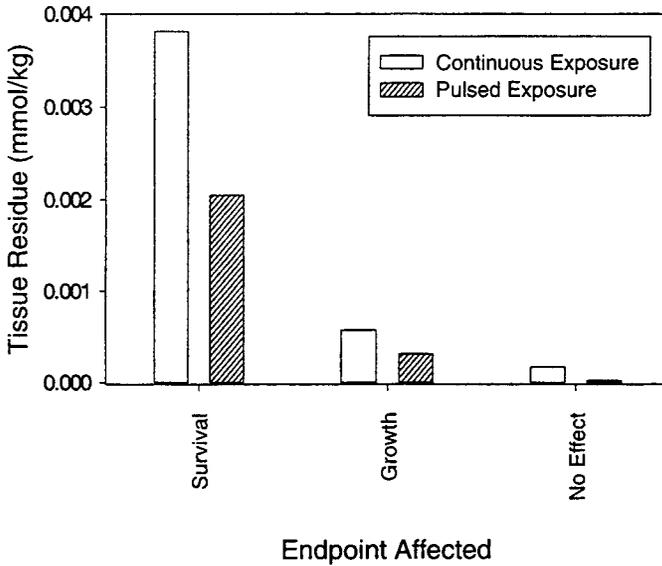


Fig. 7. Tissue residues of fenvalerate in rainbow trout (*Oncorhynchus mykiss*) exhibiting reduced survival, growth, or no adverse effects during continuous or pulse exposures. (Data from Jarvinen and Ankley 1999.)

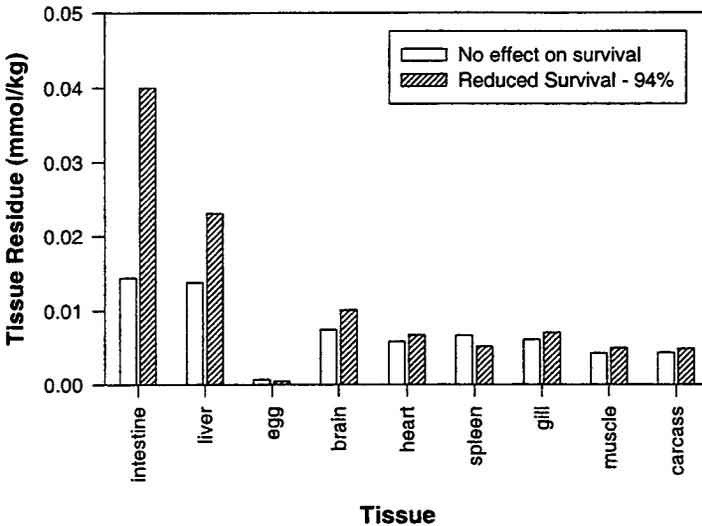


Fig. 8. Tissue residues of mummichogs (*Fundulus heteroclitus*) showing no effects and reduced survival following exposure to waterborne DDT for 1–2 d. (Data from Jarvinen and Ankley 1999.)

though these chemicals have specific mechanisms of action, some Ah-receptor agonists such as PCBs may exhibit a narcosis mode of action during short-term exposures (van Wezel and Opperhuizen 1995).

PCDDs and PCDFs, including TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin), are contaminants produced in chemical (e.g., Kraft process pulp and paper mills, chemical industries) and combustion (e.g., waste incineration) processes. PCBs were introduced into the environment as commercial mixtures of more than 100 different congeners, with the precise congener composition dependent on the manufacturing process. PCDDs and PCDFs are also composed of hundreds of individual congeners, differing in the number and pattern of chlorine substitution. In general, these compounds have low water solubility, low volatility, and high affinity for sediments and soil. Increasing the number of halogens generally increases environmental persistence and bioaccumulative properties, and non-ortho halogen substitutions generally increase toxicity.

Tissue residues of Ah receptor agonists associated with reduced survival range from 0.0000002 mmol/kg (TCDD) to 0.78 mmol/kg (2,2',3,3',5,5'-hexachlorobiphenyl; Johnson et al. 1998; Jarvinen and Ankley 1999). This range encompasses 20 reported values for polyhalogenated dioxin, furan, and biphenyl congeners, and excludes studies reporting only residue levels in eggs or residue levels of total PCBs. Tests were primarily conducted in salmonids, including chinook salmon, coho salmon (*Oncorhynchus kisutch*), rainbow trout, and lake trout (*Salvelinus namaycush*). Other species included carp (*Cyprinus carpio*), guppies, medaka (*Oryzias latipes*), mummichog (*Fundulus heteroclitus*), goldfish, and *Gambusia*. Tissue residues of TCDD that reduced survival ranged from 0.0000002 to 0.0068 mmol/kg (Johnson et al. 1998; Jarvinen and Ankley 1999), a nearly 340,000-fold variation in CBRs for one chemical.

Chemicals with a mechanism of action that requires receptor binding may be extremely species specific and dependent on life stage or environmental modulation because of differences in receptor affinity or number of receptors (Barron et al. 1997). The toxicity of chemicals that bind to the Ah receptor has been shown to depend on Ah receptor expression (Whitlock 1993) such that organisms with the highest concentrations of the Ah receptor will generally be the most sensitive to TCDD toxicity (Willett et al. 2000). In evaluating the Jarvinen and Ankley (1999) database, no clear differences in CBRs were observed between invertebrates and fish, despite speculation that invertebrates are less sensitive to TCDD because of lower concentrations of the Ah receptor. In fish, differences in sensitivity between life stages and species have clearly been shown, such as the brook trout studies of Johnson et al. (1998) and Tietge et al. (1998). Tissue concentrations of TCDD that reduced survival by 50% in swim-up fry were 5–6 pg/g fish in dead swim-up fry compared to 0.5–2 pg/g in live swim-up fry (Johnson et al. 1998). In comparison, 1200 pg/g in adult fish had no effect on survival, growth, gonadal development, and egg production (Tietge et al. 1998). Egg injection studies with TCDD demonstrate that CBRs can exhibit substantial species differences even under nearly identical exposure regimes. For example, TCDD concentrations in fish eggs causing lethality to fry varied

by approximately 40 fold between fish species (Elonen et al. 1998). Thus, species and life-stage differences in sensitivity to Ah-receptor agonists may account for much of the range in CBRs.

Mehrle et al. (1988) examined the time dependence and latency period of TCDD toxicity in rainbow trout. Tissue residues of TCDD associated with reduced survival of rainbow trout declined with increasing exposure time both during and after TCDD exposure. After 14 d exposure to TCDD, rainbow trout survival was significantly reduced at 70 nmol/kg (0.8 ng/L TCDD treatment). After 28 d TCDD exposure (0.038 ng/L treatment) and 28 d in clean water, trout survival was significantly reduced at 3 nmol/kg. Reduction in TCDD CBRs with exposure concentration and exposure time demonstrates a latency period for TCDD toxicity. In contrast, the tissue concentrations of PCBs required for mortality of fathead minnows appeared to increase with exposure time between 0 to 12 d (van Wezel et al. 1995). Application of the CBR concept across multiple species of aquatic organisms does not appear to be warranted for Ah agonists because of the extreme variability in CBRs.

## VI. Metals

Metals occur both in inorganic forms and as organometallic complexes. The inorganic form of toxic metals can include both the free metal ion and complexed aqueous chemical species. Organometallic complexes (e.g., methylmercury and tributyltin) have very different properties from inorganic metal forms and are evaluated separately in this review.

### A. Inorganic Metals

Many metals such as potassium, calcium, sodium, and magnesium are essential elements to aquatic biota and are relatively nontoxic. Toxic metals can be essential in trace amounts, such as copper, nickel, and zinc, or can be nonessential elements such as cadmium, lead, and mercury (Mason and Jenkins 1995). Metals produce toxicity through several mechanisms including ionoregulatory disturbance (McDonald and Wood 1993; Playle 1997), respiratory disturbance (Playle 1997), changes in enzyme activity (Weis and Weis 1992), and cellular damage (Mason and Jenkins 1995). The acute toxicity of metals is dependent on the concentrations at the gill surface, rather than on whole-body or internal tissue concentrations (MacRae et al. 1999). In contrast, the chronic toxicity of metals may be determined by internal concentrations and the propensity for binding cellular molecules such as metallothionein that can sequester metals (Mason and Jenkins 1995).

Many studies on both marine and freshwater invertebrates and fish have shown that the rate of metal accumulation is more important than total metal accumulation. This rate-limiting relationship has been shown for effects on survival (Ahsanullah and Williams 1991; Borgmann et al. 1991; Kraak et al. 1992; Niimi and Kissoon 1994; Absil et al. 1996), growth (Rombough and Garside 1982; Borgmann and Norwood 1997), and reproduction (Spehar 1976). There-

fore, acute toxicity is related to the rate of accumulation on the gill or respiratory surface of the organism, whereas chronic toxicity appears to be related to the rate of accumulation in internal tissues or organs. Boese et al. (1999) reported that mortality in *Lumbriculus variegatus* was correlated with whole-body copper accumulation but not with total aqueous copper or calculated cupric ion ( $\text{Cu}^{2+}$ ) in exposure water. However, tissue concentrations of copper were not collected from longer accumulation times, and the effect of uptake rate is unclear.

Borgmann and Norwood (1995) observed an association between copper and zinc uptake and mortality in *Hyalella*. Uptake of metal was rapid followed by internal regulation of the metal, which resulted in a gradual decline of metal concentrations toward preexposure levels after the first 7 d of metal exposure. The measurement of relevant metal residues in aquatic organisms can be confounded by metal binding to exoskeleton and accumulation in the digestive tract (Borgmann and Norwood 1999; Neumann et al. 1999). Despite these caveats, association between toxicity and whole-body residues of certain metals have been shown in some aquatic invertebrates (e.g., *Hyalella* and *Lumbriculus*; Borgmann and Norwood 1995; Boese et al. 1999), and between toxicity and gill tissue residues in some species of fish (Playle et al. 1992, 1993a,b).

Variables that have been shown to influence the tissue residues associated with toxic effects of metals include the exposure route and conditions, intrinsic species differences, and diet before or during exposures. Numerous studies have shown that the aqueous bioavailability of metals affects the degree of toxicity, even though the organisms were exposed to and accumulated similar concentrations of the metal (Hamilton et al. 1990; Bianchini and Gilles 1996; Olge and Knight 1996; Stouthard et al. 1996; Borgmann and Norwood 1997). Different exposure routes such as water or diet can result in tissue concentrations either showing no effects or impaired growth (Besser et al. 1993; Nebeker et al. 1995). Continuous or intermittent exposure regimes have been shown to result in different CBRs (Seim et al. 1984), and closely related species have been shown to accumulate vastly different tissue residues associated with toxic effects (Berlin et al. 1981; Carr et al. 1985). The quality and quantity of food fed before and during an exposure have been shown to influence bioaccumulation as well as residues associated with toxicity in a variety of species (Dixon and Hilton 1985; Meador 1993; Pelgrom et al. 1994; Absil et al. 1996). The influence of these variables on accumulation and toxicity associated with tissue residues greatly limits the application of the CBR concept to most inorganic metals.

Accumulation of metals in fish is relatively slow, and toxicity appears to be related to the rate of metal uptake rather than a critical metal residue (Marr et al. 1996; Hansen et al. 2001). For example, growth reduction in rainbow trout was correlated to exposure time and tissue accumulation of copper in studies using different water hardness and metal exposure concentrations (Marr et al. 1996; Hansen et al. 2001). Arsenate ( $\text{As}^{5+}$ ) CBRs for lethality differed between chronic (0.053 mmol/kg) and acute (0.108–0.115 mmol/kg) exposures (McGeachy and Dixon 1990, 1992). The difference in CBR concentrations may indicate either that there are different mechanisms of mortality for acute and

chronic exposures or that the rate of accumulation is important. The relationship between toxic effects and exposure duration diminishes the applicability of the CBR approach for metals in aquatic environments. Overall, the applicability of the CBR concept to inorganic metals appears to be greatly limited. Arsenic CBRs appear to be relatively constant, whereas the CBR approach does not appear to be applicable to copper, cadmium, zinc, mercury, and selenium. Insufficient data were available to evaluate lead, nickel, aluminum, antimony, chromium, and other metals.

### B. Organometallic Chemicals

Organometallic chemicals are organic forms of metals that are hydrophobic and can be extensively bioaccumulated. This review focused on important environmental contaminants that have sufficient information for evaluating CBRs: methylmercury and organotin compounds. Methylmercury is produced by bacterial methylation of inorganic mercury (Stumm and Morgan 1981) is highly persistent in the aqueous environment. Methylmercury is soluble in water, but upon entering the organism converts to the methylmercuric ion, which can bind to sulfhydryl groups of proteins in cell membranes (Spacie et al. 1995). Organotins are produced by industrial processes for use as biocides. The most common organotins are tributyltin ( $[\text{CH}_3(\text{CH}_2)_3]_3\text{Sn}^+$ ) which has been widely used in anti-fouling paints for watercraft, and triphenyltin ( $[\text{C}_6\text{H}_5]_3\text{Sn}^+$ ) which is widely used as a fungicide (Tas et al. 1991, 1996). Both organotin compounds are primarily cationic, moderately hydrophobic, and highly lipophilic.

Methylmercury does not appear to accumulate to a critical concentration in tissues. As with inorganic metals, the rate of accumulation seems much more important than the terminal body residue concentration (Phillips and Buhler 1978; Niimi and Kisson 1994). Measurements taken in rainbow trout tissues at death revealed that lower mercury concentrations in kidney, liver, spleen, brain, and muscle were found after exposure to higher methylmercury concentrations (Niimi and Kisson 1994). Therefore, the measured residues at death were inversely proportional to the exposure concentration. The gill was the only tissue that contained similar mercury concentrations at death for all exposures, but the study design did not allow a conclusion of a CBR for gill tissue. The available data suggest that the CBR concept does not apply to methylmercury. However, further research in this area should be conducted to investigate fish gills and organisms other than fish.

Tissue residues of organotin compounds associated with adverse effects appear to be relatively consistent, based on five studies that specifically investigated the CBR concept (Moore et al. 1991; Tas et al. 1991, 1996; Meador 1993, 1997). Meador (1997) reported CBRs for tributyltin between 0.140 and 0.210 mmol/kg for three marine amphipods (*Rhepoxynius abronius*, *Eohaustorius estuarius*, and *E. washingtonianus*), one marine polychaete (*Armandia brevis*), and one marine flatfish (*Platichthys stellatus*). CBRs for tributyltin in guppies ranged from 0.010 and 0.030 mmol/kg (Tas et al. 1996), and from 0.103 and

0.245 mmol/kg in two amphipods, *R. abronius* and *E. estuarius* (Meador 1993). Moore et al. (1991) reported a lethal tissue residue for tributyltin of 0.062 mmol/kg for dietary exposures of the marine polychaete *Neanthes arenaceodentata*. CBRs for reduced survival span a range of one order of magnitude. Both exposure concentration and internal dose appear to be correlated with specific toxic effects, but between species tissue residue was a much better indicator of toxicity than  $LC_{50}$  values (Meador 1997). Accumulation of 0.022 mmol/kg of tributyltin reduced growth and reproduction (Moore et al. 1991), and in two studies CBRs for lethality to triphenyltin ranged from 0.017 to 0.025 mmol/kg (Tas et al. 1991, 1996).

The effect of lipid content on the bioaccumulation of organotin compounds is uncertain. Meador (1993) found that the CBRs for tributyltin mortality were different for amphipods depending on holding time in the laboratory before testing. Measured lipid content in these organisms was also dependent on holding time, and CBRs were more similar when normalized against lipid content. However using a similar study design, Meador (1997) concluded that lipid content was not important, which was attributed to the moderate hydrophobicity and the ionic nature of tributyltin. Overall, these conflicting results introduce considerable uncertainty regarding the importance of lipid content on CBRs for organotin compounds. Evidence for CBRs for triphenyltin effects on survival and tributyltin effects on growth and reproduction is more limited, but are consistent with the CBR concept. Lethal body burdens of tributyltin have been shown to be independent of exposure concentration or exposure duration (Tas et al. 1991), and CBRs for different exposure routes were similar (Moore et al. 1991; Tas et al. 1996; Meador 1997).

## VII. Discussion

Environmental applications of the CBR approach will require that the dependence on chemical, biological, and environmental factors is minimal or that the determinants of CBRs can be consistently defined. Previous examinations of the consistency and applicability of CBRs (McCarty and Mackay 1993; Barron et al. 1997) concluded that the primary determinant of a CBR is the mode of action of the chemical. For example, Table 1 shows a 10-million-fold difference in lethal tissue residues for various modes of toxicity, with narcotic chemicals showing the highest CBRs and Ah receptor agonists the lowest CBRs. The current review demonstrated that there is an association between tissue residues and adverse effects in aquatic organisms, but the variability in reported CBRs is extremely large. Expansion of the data reviewed by McCarty and Mackay (1993) to include newer literature and the residue effects database of Jarvinen and Ankley (1999) revealed a 14,000-fold range in CBRs affecting survival for nonpolar narcotics and a 39-million-fold range in CBRs for Ah-receptor agonists (Table 3).

Table 3. Ranges of CBRs affecting survival for organic chemical mode-of-action classes.

Chemical class	CBR range (mmol/kg)	Number of values	Max : min ratio	Data source
Narcotics <sup>a</sup>	$9 \times 10^{-3}$ to 450 (all) NP: $3.2 \times 10^{-2}$ to 450 polar: $9 \times 10^{-3}$ to 4.9	144 (all) NP: 80 polar: 64	50,000 (all) NP: 14,000 polar: 540	Jarvinen and Ankley (1999)
Excitatory agents <sup>b</sup>	$4 \times 10^{-4}$ to 0.91	72	2300	Jarvinen and Ankley (1999) Fisher et al. (1999)
ACHE inhibitors <sup>c</sup>	$4 \times 10^{-5}$ to 29	115	730,000	Jarvinen and Ankley (1999) Deneer et al. (1999)
Reactive chemicals	$9.4 \times 10^{-2}$ to 13	8	140	Jarvinen and Ankley (1999) McKim and Schmieder (1991)
CNS seizure agents	$2 \times 10^{-5}$ to 1.1 (all) OCs: $7 \times 10^{-5}$ to 1.1 py <sup>d</sup> : $2 \times 10^{-5}$ to $1.2 \times 10^{-2}$	125 (all) OCs: 106 py <sup>d</sup> : 19	55,000 (all) OCs: 16,000 py <sup>d</sup> : 600	Jarvinen and Ankley (1999)
Ah receptor agonists <sup>e</sup>	$2 \times 10^{-8}$ to 0.78	20	39,000,000	Jarvinen and Ankley (1999) Johnson et al. (1998)

<sup>a</sup>Excludes trichlorophenols and three ring or larger PAHs; NP, nonpolar narcotics; polar, polar narcotics; <sup>b</sup>Includes trichlorophenols; <sup>c</sup>Range, number of values, and max:min ratio exclude one extreme low value (see text for discussion); <sup>d</sup>Separate range, number of values, and max:min ratio determined for organochlorine pesticides (OCs) and pyrethroids (py); <sup>e</sup>Range for TCDD and PCB congeners; excludes studies where only egg residues were reported.

Factors contributing to the variability in CBRs include exposure conditions and chemical structure. The CBRs can also vary substantially in different species and life stages of aquatic organisms, likely because of differences in the disposition of the chemical within the organism, biotransformation, and intrinsic toxicity or inherent sensitivity (Barron et al. 1997; DiToro et al. 2000). The current CBR approach does not incorporate species differences and predicts that CBRs should be relatively constant across taxonomic groups. Some chemicals may accumulate to a greater extent in certain tissues, or may cause toxicity in specific tissues, and metals and reactive chemicals may cause acute toxicity at the gill surface rather than within the organism. Therefore, the CBR approach may not apply to whole-body chemical residue analysis but may be more applicable to specific tissues. Because of the substantial dependence of CBRs on the species, exposure regime, and chemical structure, environmental applications of CBRs do not appear practical at this time.

According to the CBR concept, aquatic organisms with higher lipid content should require higher tissue residues for mortality (McCarty et al. 1992), but lipid content is not directly a part of the CBR model nor is it expressed in the predicted range of CBRs for various chemicals (see Table 1). The target lipid model (DiToro et al. 2000) directly accounts for the lipid content in an exposed organism by expressing the body burden of a chemical in mmol/kg lipid. The reduced variation in narcotic chemical CBRs observed in this model may result from lipid normalization, from accounting for differences in species sensitivity, or from categorizing narcotic chemicals into smaller subclasses that better account for differences in chemical potency. However, lipid normalization may only be applicable to chemicals such as narcotics that have a mechanism of action associated with binding to the lipid components of cells and nerve tissue.

The CBR concept predicts that tissue residues will exhibit a dose-response relationship with adverse effects; that is, adverse effects will increase as tissue residues increase above a threshold concentration of chemical. In general, a dose-response relationship between tissue residues and effects is evident for survival of aquatic organisms exposed to narcotics and some other chemical classes (Figs. 4,5). However, some studies have failed to show a dose-response relationship between tissue residues and adverse effects (Fig. 9), and CBRs for reduced survival can be very similar to no-effect concentrations (see Fig. 2). Nevertheless, a number of individual chemicals appear to accumulate to a CBR that is independent of chemical, biological, and environmental variables, suggesting that tissue residue-based measures of toxicity could offer an appropriate alternative to simple measures of aqueous exposure for some chemicals. However, additional research is needed to confirm CBRs of individual chemicals in controlled laboratory studies, to identify interspecific differences in sensitivity, and to ensure that environmental variables such as temperature and pH do not confound the application of CBRs for individual chemicals.

Overall, it appears that the magnitude of variability of adverse effects of tissue residues, both between chemicals and between taxa, is too large within

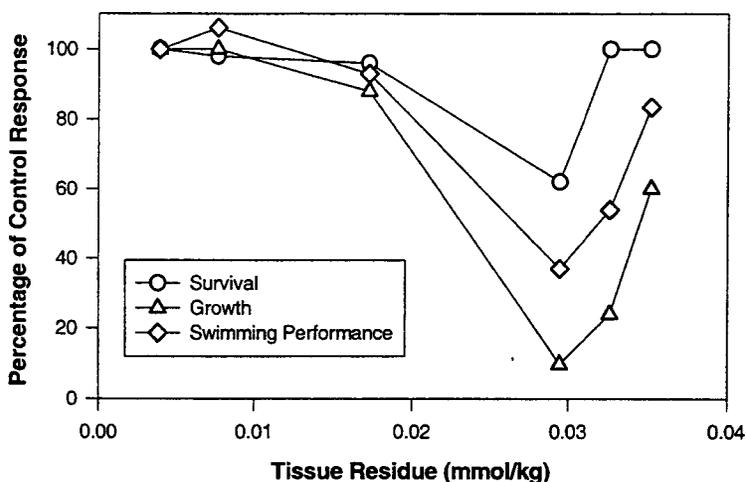


Fig. 9. Dose-response relationships between tissue residues of total naphthalenes and survival, growth, and swimming performance of rainbow trout (*Oncorhynchus mykiss*) exposed to oil for 90 d. (Data from Woodward et al. 1983.)

chemical classes to warrant extensive further investigation and validation of the CBR concept as outlined by McCarty et al. (1993). However, focused QSAR-type approaches such as the target lipid model (DiToro et al. 2000) that incorporate differences in chemical potency, lipid content, and species sensitivity appear to offer potential advances in the applicability of the CBR approach. Additional research is recommended to further reduce variability in predicted versus measured CBRs, focusing on smaller subsets of chemicals that are environmentally important rather than on broad classes of chemicals. Empirical studies should be designed to explicitly test the hypothesis of whether tissue residues can be deemed “critical” rather than assuming that a measured tissue burden is a CBR. This approach requires that laboratory studies be designed to measure tissue residues of both parent compounds and potential metabolites, ideally for whole body and different target organs, across different aqueous exposure conditions (e.g., environmentally relevant pH, hardness/alkalinity, temperature conditions) and for different exposure durations. Adverse effects then should be quantitatively related to tissue levels using dose-response models. Further, testing should be performed for several species to identify interspecific differences in sensitivity.

### Summary

Associations between tissue residues and toxicity to aquatic organisms were examined to evaluate the applicability of the critical body residue (CBR) ap-

proach across different chemical classes. Chemical classes and mode of action categories evaluated included narcotics (polar and nonpolar), excitatory agents, AChE inhibitors, reactives/irritants, CNS seizure agents, aryl hydrocarbon (Ah) receptor agonists, and inorganic metals and organometals. This evaluation indicated that empirical data do not support broad application of the CBR concept across chemical classes. This conclusion is particularly important for polar and nonpolar narcotics because the CBR concept was specifically developed for these chemical classes. The variability observed in tissue residues between chemicals within a given mode-of-action class appears to be generally of the same order of magnitude as the variability of aqueous measures of toxicity such as  $LC_{50}$  values (Table 3; Fig. 10). This observation suggests that either (a) the reported tissue residues were dependent on the aqueous dosing regime; (b) the tissue measurements do not accurately reflect the internal dose at target organs with substantially greater precision than water exposure measurements; or (c) many of the same sources of variability associated with aqueous exposures, such as chemical structure, individual species sensitivity, biotransformation processes, and lipid content, also apply to tissue-based measures of exposure. An additional source of uncertainty of CBRs is whether a chemical has been correctly assigned to a mode of action category.

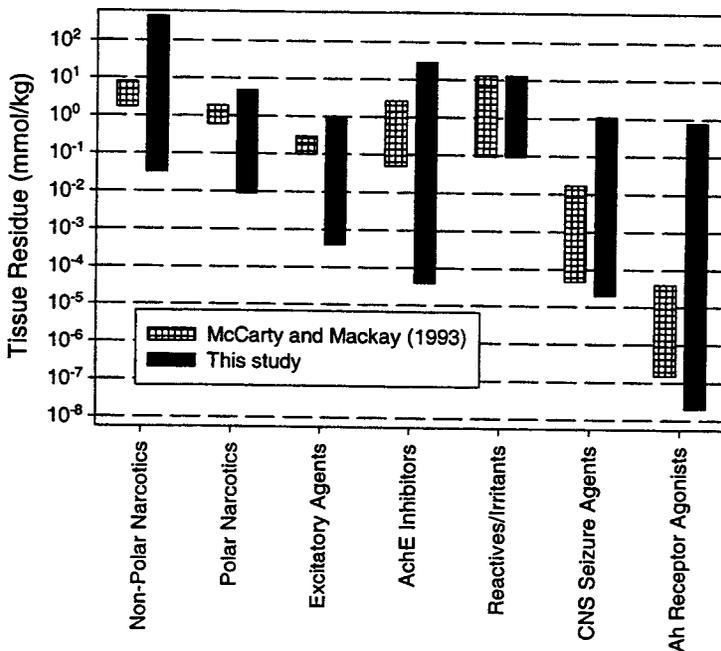


Fig. 10. Comparison of CBRs reported by McCarty and Mackay (1993) with CBR ranges determined in the current study.

The CBR approach outlined by McCarty (1986, 1987) and McCarty et al. (1993) underlines an important concept in aquatic toxicology, i.e., that internal chemical dose is the true measure of toxicity for many chemicals rather than imputed dose based on aqueous exposure. Nevertheless, without more refined and accurate examination of that actual internal dose and without additional consideration of differences in sensitivity between species, differences in toxic potency between chemicals, and differences in toxicity of environmentally modified or biotransformed compounds, the CBR approach may not offer practical advantages over conventional media-based exposure assessment.

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