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# Application of the Tissue Residue Approach in Ecological Risk Assessment

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## EDITOR'S NOTE

This paper represents 1 of 6 review articles generated from a SETAC Pellston Workshop entitled “The Tissue Residue Approach for Toxicity Assessment (TRA)” (June 2007, Leavenworth, Washington, USA). The main workshop objectives were to review and evaluate the science behind using tissue residues as the dose metric for characterizing toxic responses and to explore the utility of the TRA for mixtures, guidelines or criteria, and ecological risk assessment.

## ABSTRACT

The objective of this work is to present a critical review of the application of the tissue residue approach (TRA) in ecological risk and/or impact assessment (ERA) of chemical stressors and environmental criteria development. A secondary goal is to develop a framework for integrating the TRA into ecological assessments along with traditional, exposure concentration-based assessment approaches. Although widely recognized for its toxicological appeal, the utility of the TRA in specific applications will depend on numerous factors, such as chemical properties, exposure characteristics, assessment type, availability of tissue residue-response data, and ability to quantify chemical exposure. Therefore, the decision to use the TRA should include an evaluation of the relative strengths, limitations, and uncertainties among exposure and residue-based methods for characterizing toxicological effects. Furthermore, rather than supplanting exposure concentration-based toxicity assessments, the TRA can be highly effective for evaluating and reducing uncertainty when used in a complementary manner (e.g., when evaluating multiple lines of evidence in field studies). To address limitations with the available tissue residue-response data, approaches for extrapolating residue-based toxicity data across species, tissues, and exposure durations are discussed. Some of these approaches rely on predicted residue-response relationships or toxicological models that have an implicit residue-response basis (e.g., biotic ligand model). Because risk to an organism is a function of both its exposure potential and inherent sensitivity (i.e., on a residue basis), bioaccumulation models will be required not only for translating tissue residue criteria into corresponding water and sediment criteria, but also for defining the most vulnerable species in an assemblage (i.e., highly exposed and highly sensitive species). Application of the TRA in ecological assessments and criteria development are summarized for bioaccumulative organic chemicals, TBT, and in situ bioassays using bivalve molluscs. *Integr Environ Assess Manag* 2011;7:116–140. © 2010 SETAC

**Keywords:** Tissue residue approach Critical body residues Ecological risk assessment Water quality criteria

## INTRODUCTION

In the present work, we describe the application of the tissue residue approach (TRA) within the context of ecological risk assessment, which we interpret broadly within the context of environmental regulatory programs, ecological impacts assessment, and environmental quality guideline and/

or criteria development. This context is important, not only for those responsible for such regulatory programs, but also for scientists conducting TRA-related research, in that the practical application of this research will depend on addressing issues created by the regulatory application of the TRA. The TRA, simply put, is an approach that relates toxicological responses of an organism to concentrations of a chemical that are measured or predicted in an organism's tissue. The scientific underpinnings of the tissue residue concept in toxicology are discussed at length in another paper in this series (McCarty et al. 2011).

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In describing the application of the TRA in ecological risk assessment, we emphasize a number of key themes that emerged from our discussions and deliberations. First, the methods by which the TRA is incorporated in ecological assessments vary widely. We accept as an underlying concept that the overwhelming majority of, if not all, toxicity elicited by chemical contaminants on ecological receptors are the result of their dose at a critical site or tissue within an organism. In that sense, it can be argued that all assessments of contaminant stress are, at their foundation, determined by tissue residues. However, this concept is incorporated into regulatory assessments in different ways, many of which are described in greater detail in later sections of this article. Some methods are empirical in scope (i.e., relying on measured tissue residue-response relationships); others are rooted in a more predictive basis (i.e., relying on model-predicted or implied relationships between toxicity and tissue residues). Therefore, within the context of ecological assessments, application of a tissue residue-based approach does not have a single focus or framework. It is a matter of how our understanding of residue-effect concepts are addressed and embedded in different types of assessments. As a result, the feasibility of applying the TRA and its subsequent utility relative to conventional toxicity assessment methods will depend on the form of the TRA and the context in which it is used.

A second theme to emerge from this review is that the TRA should not necessarily be viewed as a replacement for conventional exposure or dose-based methods for toxicity assessment. On the contrary, application of the TRA can be particularly beneficial when used to complement conventional toxicity assessment approaches, because each approach contains different strengths and limitations, the balance of which often depends on the specific chemical and assessment attributes. Methods for incorporating the TRA that have limited usefulness in some regulatory applications may have considerable value in others. For these reasons, we did not, and in fact could not, develop a single overarching decision tree for deciding when to use the TRA across all types of ecological assessments. Instead, we emphasize the key issues to consider when deciding to use the TRA as another line of evidence in ecological assessments.

To this end, we put forth a generalized framework for considering the TRA in ecological risk assessment in relation to other conventional toxicity assessment methods (e.g., external exposure and dose-based methods). This framework is followed by an overview of 3 different approaches for TR-based assessments: those based on empirical, calculated, and implicit tissue residue-effect relationships. The remainder of this article provides a review of several case studies of the TRA in different ecological assessments or guidelines development to illustrate the key issues confronting its application.

We note that chemical residues in organisms may be measured for a wide variety of reasons, not all of which are central to the focus of this chapter. Of special note are residues measured or predicted in a prey organism as a means to assess exposure of a predator organism (or human) at a higher trophic level. Although this approach can be a valuable component of ecological or human health risk assessments, it is not the focus of this paper. Another common objective of tissue residue measurements is to assess organism exposure to chemicals (i.e., bioaccumulation) because residues may reflect an integration of exposures occurring over a longer period of

time. Such applications are discussed to some degree in previous papers in this series and may be very valuable within the context of some assessments, but they are not emphasized in this paper. Rather, we focus on the acquired dose as a means to assess effects on the organism in which the residue is measured or predicted, and subsequent application to various ecological assessment scenarios.

#### *Why consider the TRA in ecological assessments?*

McCarty et al. (2011) describe a number of strengths of the TRA in response to the general question, "Why use TRA?" As part of our workshop deliberations, the list of potential strengths of the TRA was revisited within the context of regulatory applications to explore where existing regulatory needs could be addressed through the incorporation or enhancement of TRA. Table 1 summarizes these considerations; application of the TRA is shown to have the potential to reduce uncertainty associated with a wide array of issues in assessing ecological risk and developing environmental quality guidelines. Later in the present work, we describe some of the current limitations the TRA in ecological risk assessments.

#### *Regulatory history and status of TRA*

The development of chemical-specific environmental quality guidelines (e.g., water and sediment quality criteria) has become an integral component in the assessment and management of contaminants either existing in, or potentially released to, the environment. The history of environmental quality guidelines demonstrates an almost complete focus on media-based expressions of exposure-effect relationships. As outlined by Meador et al. (2011) in this series, the toxicity information on which criteria and other regulatory approaches are based generally reflects expressions of effect resulting from exposure to a specific, constant chemical concentration in water or, in more recent years, sediment. Furthermore, many regulatory protocols, such as the US Environmental Protection Agency (USEPA) guidelines for developing water quality criteria (USEPA 1985) generally exclude data where exposure was introduced or quantified via a route other than water (e.g., food). The decisions to follow this approach were likely influenced by 2 important realities: the vast majority of toxicity data were available for waterborne exposure, and in the end, it was concentrations in water that were being regulated, so expressing toxicity on the basis of water concentration avoided having to translate exposure and/or dose expressed in other ways. Nevertheless, clearly a great deal of progress in environmental management was made through the application of these programs.

Despite the important role played by media-based quality guidelines in environmental regulation, advances in our understanding of toxicology are prompting consideration, if not actual implementation, of changes in the development of these guidelines to make them more rigorous and accurate barometers of potential ecological risk (e.g., USEPA 2000b, 2006; Reiley et al. 2003). A notable force in prompting reevaluation of regulatory approaches is increased awareness of the challenges presented by bioaccumulative chemicals. These substances have several features that create difficulties for approaches based solely on waterborne exposure and/or effect. In general, the effective dose of such chemicals to the receptor organism comes from multiple routes, such as diet and/or sediment, in addition to water, so that the internal

**Table 1.** Potential benefits of incorporating TRA into ecological risk assessments

Benefit	Rationale	Example Application
Integration of exposure pathways	TRA can provide an effective means of assessing toxicological effects resulting from multiple exposure routes (water, food, sediment), particularly for bioaccumulative organic compounds and organometallic compounds. For many metals, however, challenges exist in the development and application of reliable residue-response relationships (Adams et al. 2011).	PCBs: (Munns et al. 1997) 2,3,7,8-TCDD: (Cook et al. 1993) Selenium: (USEPA 2004)
Integration of exposure over time and space	Measurement of tissue residues in native or experimentally deployed organisms integrates temporal and spatial variability in exposure concentrations, which may be more cost effective compared with repeated measurement of exposure concentrations.	TBT: (Meador 2000) Monitoring and dredging programs
Facilitate exposure-response modeling for fluctuating exposure	TR concepts are used as the founding principle behind a variety of toxicokinetic and toxicodynamic modeling approaches used to predict effects from a variety of exposure profiles (Escher et al. 2011). Such predictions are often valuable for assessing risk from short-term fluctuations in exposure concentrations.	Episodic exposures such as accidental releases, stormwater runoff, pesticide drift or runoff.
Facilitate development of mixture models	Most environmental exposures involve simultaneous exposure to multiple chemicals. TRA can be useful in assessing the aggregate exposure to such mixtures, and to express the total toxic potency from multiple chemicals with the same mode of action.	PAHs: (Di Toro and McGrath 2000; USEPA 2003a) Ah receptor-active dioxins, PCBs, furans: (Steevens et al. 2005)
Ameliorate problems with analytical detection	Some chemicals, such as pyrethroid insecticides, sulfonyl urea herbicides, selected dioxins and PCBs, can be toxic at concentrations near or below the limits of analytical detection in water. Basing toxicity assessments on tissue residues rather than concentrations in water can circumvent problems associated with analytical detection for such chemicals.	2,3,7,8-TCDD: (Cook et al. 1993) TBT: (Meador 2000)
Improve assessment of bioavailability	Measurement of chemical residues in tissue reflects the portion of chemical available for uptake (bioavailable fraction) and may reduce uncertainty associated with predicting the bioavailable fraction of chemicals based on external concentrations.	Organic chemicals and metals
Aid in establishing causal relationships	Identifying causal relationships between chemical stressors and ecological effects observed in the field is often confounded by co-occurring contaminants and uncertainties regarding chemical bioavailability. Coupling measurement of chemical residues in tissue with residue-effect relationships can aid in diagnosing exposures responsible for impairments, thereby targeting management actions on those most likely to improve ecological condition.	USEPA 305b listing of impaired waters, Stressor Identification and Causal Analysis/Diagnosis Decision Information System (CADDIS) (USEPA 2000a, 2009a)

dose received by organisms in nature is additive across exposure routes. Furthermore, many bioaccumulative chemicals have comparatively slow accumulation kinetics, which can create difficulties for interpreting toxicity data, particularly for shorter-term exposures. Another focus has been the improvement of methods for incorporating bioavailability into environmental quality guidelines, particularly in the case of cationic metals. The ability to better represent the many influences of water quality on the toxicity of waterborne metals has permitted the development of guidelines that have much greater site specificity in the way they evaluate potential risk from metals. The methods developed to address these issues rely heavily on the TRA concepts. Lastly, it has been recommended previously that development and application of environmental quality guidelines harmonize and integrate media-based, external dose-based, and tissue residue-based approaches to improve their scientific foundation

and maintain consistency in their regulatory implementation (Reiley et al. 2003).

*TRA in North America.* Tissue residue concepts are only beginning to be implemented in environmental quality guidelines. In the United States, the biotic ligand model (BLM) approach has been incorporated into USEPA water quality criteria for Cu (USEPA 2007), and its application to additional metals in the near future is anticipated. Incorporation of BLM concepts into Canadian water quality guidelines (CCME 2007) allows for the use of the BLM in the development of water quality metals guidelines as 1 way of establishing the factors that modify bioavailability (called exposure and toxicity modifying factors) and for standardizing data to appropriate conditions for guideline development. The USEPA is also developing a TR-based approach for deriving aquatic life criteria for bioaccumulative organic

chemicals (USEPA 2006) and Se (USEPA 2004) and has published sediment quality benchmarks based on equilibrium partitioning (USEPA, 2003a, 2003b, 2003c, 2003d). In particular, the guidance for polycyclic aromatic hydrocarbons (PAH) mixtures uses the TRA modified from that reported by Di Toro and colleagues (Di Toro and McGrath 2000; Di Toro et al. 2000). Canada has used the TRA in 1 component of the development of the Canada-wide standard for petroleum hydrocarbons (CCME 2008). These soil quality guidelines (i.e., soil to groundwater to aquatic life pathway) used the narcosis model that incorporated a tissue residue basis in addition to a species sensitivity distribution approach based on external exposure concentrations.

Beyond the development of environmental guidelines, many other regulatory programs have the flexibility to incorporate chemical or site-specific procedures into their assessments. Perhaps this greater flexibility has led to the current case-by-case implementation of the TRA methods. Many site assessment programs, such as the USEPA Superfund program for evaluation and remediation of contaminated sites, have incorporated residue-based assessments into site-specific risk assessments (e.g., the Portland Harbor Superfund site). The pesticide registration process in the United States has begun to use TR-based approaches in assessments of new pesticides, particularly for those showing bioaccumulative potential (USEPA 2008, 2009b). Measurement and interpretation of tissue residues of aquatic organisms has probably seen its widest application in a variety of environmental monitoring programs (e.g., the US “Mussel Watch” program; Kimbrough et al. 2008).

*TRA in Europe.* To our knowledge, environmental policy frameworks in which the TRA is used formally by the European regulatory community are lacking. The EU Water Framework Directive is oriented primarily around traditional water-based quality standards. Within the EU Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) program on evaluation of chemicals, correction of bioavailability for metals (BLM) and PAHs (relative to black carbon) is being discussed. However, formal plans have not been undertaken to use the TRA in this regulatory program.

At a national level, examples of rudimentary TRA applications can be found. In The Netherlands, concentrations in mussels (*Dreissena polymorpha*) and eel (*Anguilla anguilla*) are routinely monitored in rivers (Hendriks et al. 1998). The levels observed are compared with “calculated” tissue quality guidelines. These are derived by multiplying water or sediment criteria, i.e., HC5 based on plants and cold-blooded animals, by the bioaccumulation factor (BAF) or the biota sediment accumulation factor (BSAF), respectively.

To address some pollutants for which direct quantitation is difficult because of very low trace levels, artificial materials such as semipermeable membrane devices (SPMDs) are used for waste water, surface water, and soil monitoring (Van Loon et al. 1997; Van Stee et al. 2002). The total residue adsorbed to the material is compared with baseline toxicity levels.

*TRA in Australia and New Zealand.* Similar to the North American and European examples described previously, environmental quality guidelines in Australia and New Zealand are derived using exposure-based toxicity data and are expressed in terms of concentrations in various environ-

mental media. For example, the current Australian and New Zealand water and sediment quality guidelines (ANZECC 1992; ANZECC and ARMCANZ 2000) and the soil-based ecological investigation levels (EILs) (NEPC 1999) were all derived using toxicity data solely expressed in terms of aqueous (e.g., mg/L), sediment or soil concentrations (e.g., mg/kg). Following the development and application of aquatic BLMs for metals such as Cu by the USEPA, it is likely that the BLM approach would be reviewed and considered for adoption. Although both countries have tissue-based regulations, these are designed to protect human health and are principally related to foods (so-called dietary-based tissue guidelines).

Australia and New Zealand do have TR-based biomonitoring programs in place for organisms such as mussels and oysters. Dioxin tissue levels were also monitored as part of the Australian National Dioxin Survey (ADEH 2005). However, at least within Australia, TR-based biomonitoring does not have a national approach, and the tissue concentrations are seldom linked to toxicological effects at the individual or higher levels of organization. The TR results are principally used to determine trends in organism exposure to establish baseline concentrations, to identify pristine sites or hotspots of contamination. Passive sampling devices that are used as surrogates for tissue have been extensively used. However, they have generally not been linked to toxicological effects, due in part to a lack of knowledge of how the kinetics of chemical uptake and elimination differ between passive samplers and aquatic organisms. Rather, passive sampling devices have been used primarily to overcome the limitations of grab samples to identify chemicals present in water bodies to which organisms are being exposed.

## CONCEPTUAL FRAMEWORK FOR APPLICATION OF TRA

Discussion of TRA applications led our workgroup to the realization that the utility, applicability, and interpretability of different TRA methods vary across the types of assessments. These differences exist at many levels, but a major distinction lies between retrospective versus predictive assessments. Retrospective assessments are those intended to assess ecological effects resulting from existing exposures (Suter 1993). Examples include baseline risk assessments of sites with legacy contamination (e.g., under the USEPA Superfund Program) or interpretation of data from ecological monitoring programs. Predictive assessments are those that forecast potential risks predicted to result from future actions or inaction. Examples might include permitting a new industrial facility or decisions regarding the licensing of a new agricultural pesticide. Some regulatory processes include elements of both, such as contaminated site cleanups that assess both effects from the existing contamination and changes in risk resulting from various remedial alternatives. With respect to TR-based assessment, a key difference is that retrospective assessments generally have the ability to directly measure residues in exposed animals. The challenge then becomes one of interpreting those residues in terms of their potential to cause adverse effects and assigning causality to the chemical stressors of concern. In predictive assessment, the challenge is to apply what we know about residues and residue-effect relationships to forecast effects, even though the residues themselves are not measurable.

A framework for considering how tissue residues and a residue-based effects assessment fit into an ecological risk assessment is illustrated in Figure 1. As shown at the left of the diagram, loadings of chemicals to the ecosystem result in a distribution of chemicals across the abiotic compartments of water, sediment, and interstitial water. The upper right of the diagram shows effects on populations, communities, and ecosystems, which are common assessment endpoints. Effects at these higher levels of biological organization are mediated through responses of individual organisms, which are the most common measurement endpoints for risk assessments. The connection between exposure concentrations in media and effects on individuals can be made via several routes, as illustrated by the parallel paths shown in Figure 1. Exposure to contaminated media creates doses to an organism through water, sediment, and/or food. These doses are then distributed across tissues within the organisms, which, upon accumulation to toxic levels, result in adverse effects to the individual. In predictive risk assessments, effects may be predicted using different combinations of these elements. In some instances, effects may be predicted directly from exposure, such as occurs when traditional water column toxicity tests are used to derive exposure and/or response endpoints (e.g., LC50 or EC20 values). In others, the aggregate doses of a chemical may be quantified, such as through concentrations in diet, which are then related to a level of effect through a dose-response relationship (e.g., ingested dose associated with 50% lethality or LD50). Alternatively, the administered dose may be translated to an internal residue (acquired dose based on whole body or a specific tissue), which is then translated using the applicable residue/response relationship (e.g., residue associated with 50% lethality or LR50; Meador et al. 2011). This would be the direct application of TR-based assessment discussed above.

One important point of the framework figure is that the various types of toxicity models illustrated (i.e., media-, dose-, and tissue-based) are all designed to make the same translation between risk (effects) on organisms and environmental concentrations. The media and dose-based toxicity models represent surrogate methods for depicting the underlying toxicological processes, which involve the accumulation of a biologically active chemical residue at the target sites in order for a toxic response to occur. Although the TRA is the more direct representation of this toxicological process, the relative benefit of each type of toxicity modeling approach will vary, depending on the assessment. When applying the TRA, the following factors should be considered:

- 1) Assessment type, scope, and goals (e.g., retrospective vs predictive and site-specific vs national-scale assessment)
- 2) Availability and reliability of tissue-residue effect data (i.e., how well can we relate accumulated residue to toxicological effects?)
- 3) Exposure characteristics for the receptor organisms (e.g., the importance of multiple exposure routes or time-variable exposures)
- 4) Bioavailability and bioaccumulation differences across sites
- 5) Mode and mechanism of action in the organism
- 6) Variability in toxicant response for a given tissue concentration across taxa and life stages
- 7) Types of organisms and ecosystems at risk

In some situations, reliance on a media or dose-based toxicity assessment model may be more desirable because of the greater availability of data compared with measured residue-response relationships. In other situations (e.g., when multiple exposure routes are toxicologically important),

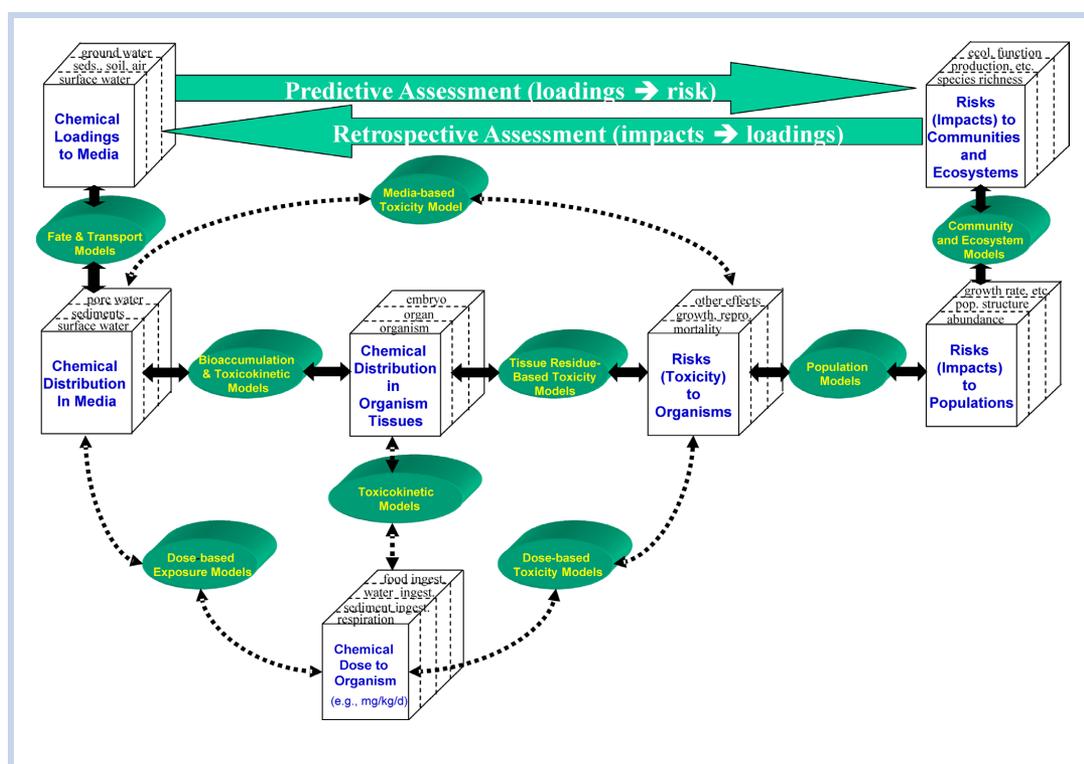


Figure 1. Framework for integrating media-, dose- and tissue residue-based approaches in ecological risk assessment.

reliance on a single media-based toxicity model may not adequately describe toxicity under natural settings, and thus a tissue residue or dose-based approach will be needed. In many cases, we believe that the use of multiple approaches or toxicity models as illustrated in Figure 1 can be more effective for assessing ecological risks (either in a predictive or retrospective mode) compared with reliance on any single approach.

### METHODS FOR INCORPORATING THE TRA IN ECOLOGICAL RISK ASSESSMENT

In the introduction, we noted that the TRA can be implemented in various ways. One important aspect of implementing the TRA relates to how tissue residue-response models are developed. As depicted in Figure 2, the top oval represents relationships based on measured residues associated with toxic effects (i.e., explicit use of TRA). The middle oval represents relationships based on calculated effect residues inferred from measured media effect concentrations and separate knowledge of the relationship between media and tissue concentrations (i.e., bioaccumulation). The bottom oval represents relationships that use TRA concepts, but directly relates effects to media concentrations with only an implied knowledge of effect residues (i.e., implicit use of TRA). Inputs to toxicity models that are based on measured or calculated tissue-residue response relationships can come from either measured or predicted concentrations in organisms (top and middle rectangles on the left). Inputs to toxicity models based on implicit tissue-residue response relationship take the form of chemical concentrations in external media (e.g., the BLM). The following sections provide some examples of and perspectives on these 3 approaches.

#### TRA based on measured residue-effect relationships

Currently, application of the TRA is limited by the quantity and quality of critical residue data (Steevens et al.

2004; Beckvar et al. 2005; Hendriks et al. 2005). In general, tissue-based toxicity evaluations have been carried out under conditions that are less well standardized than water-based toxicity tests. Comprehensive compilations of critical tissue residue data can be found in 2 databases: the US Army Corps of Engineers Environmental Residue-Effects Database (ERED) (Bridges and Lutz 2002) and the USEPA toxicity/residue database (Jarvinen and Ankley 1999). A third database (the USEPA PCBRes database; USEPA 2009d) focuses on dioxin-like PCB congeners, dioxins, and furans. An analysis of the unique data contained in both the ERED and toxicity/residue databases was conducted as part of an USEPA Science Advisory Board consultation on revisions to the USEPA chemical water quality criteria guidelines (USEPA 2006). This analysis demonstrates that despite the large number of chemicals and species represented by the 2 databases at that time (nearly 400 substances and over 300 species), the distribution of tissue residue-based toxicity values (i.e., critical residues) for multiple species of aquatic organisms is highly skewed to a relatively small subset of chemicals (Figure 3). Specifically, only 14% of the chemicals represented in the combined databases contained critical residues for 6 or more species (Figure 3A). For chemicals whose specific modes of toxicity are of concern, this finding would seem to greatly limit the ability to evaluate the distribution of species sensitivity in terms of critical residues and set critical residue thresholds that would be protective of aquatic assemblages. Further, relatively few freshwater and saltwater species have been widely evaluated in terms of critical residues (i.e., for 10 or more chemicals, Figure 3B, C and D).

In addition, other limitations in the availability of critical residue data for aquatic organisms include the type of endpoints, life stages, and tissues represented (Figure 4). More than 50% of the studies examined lethality, followed by growth (18%) and reproduction (7%). Adult organisms represent the most common life stage tested, and by far the most common tissue in which critical residues are measured in fish is whole body (60%). The lack of residue-response data

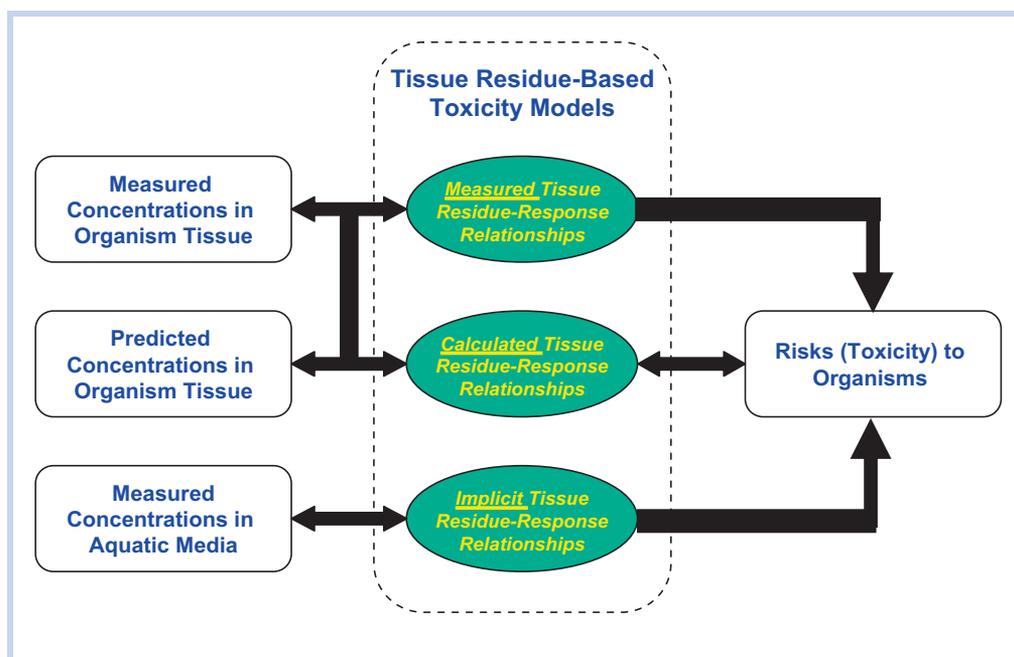
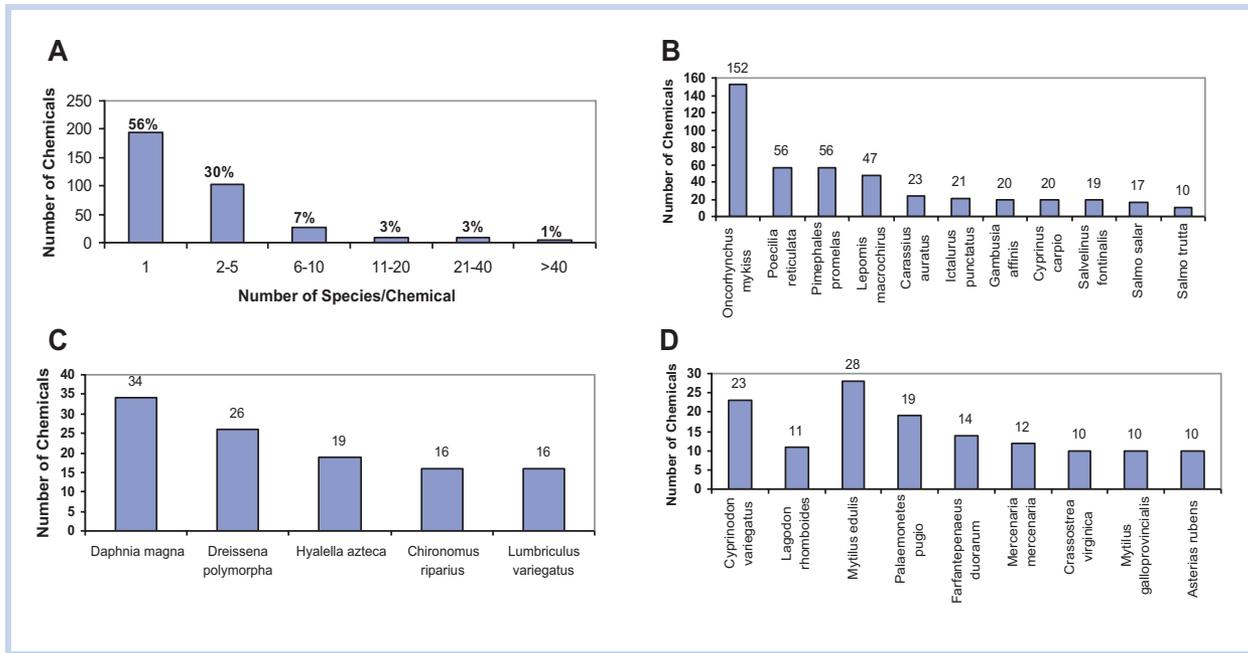


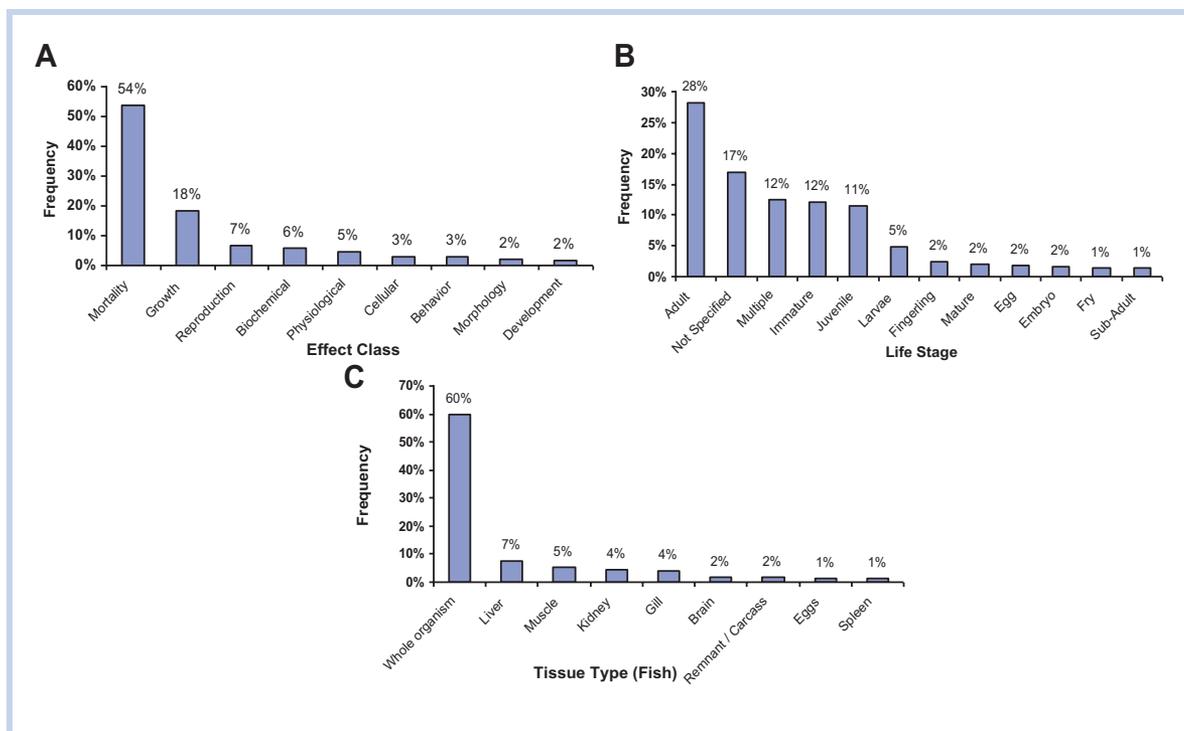
Figure 2. Illustration of different applications of TRA based on measured, calculated and implicit tissue residue-response relationships.



**Figure 3.** Distribution of critical residue data for aquatic organisms from two databases: Number of species/chemical (A); number of chemicals/freshwater fish species (B); number of chemicals /freshwater invertebrate species (C); number of chemicals/saltwater species (D). Source: Jarvenin and Ankley (1999) and ERED (Bridges and Lutz 2002).

should ideally be increased by measuring tissue residues in toxicity experiments, in addition to the analysis of water, sediment, and food. However, because of financial, practical, and ethical constraints, the gap is unlikely to be filled soon. Therefore, methods to overcome some of the aforementioned limitations in the critical residue information base will probably be necessary for broad application of the TRA in the near term. This requirement may include not only

methods for using calculated or implicit tissue residue methods (described in the next sections), but also the better use of the vast information on chemical residues in biota from the field. Examples include monitoring data on residues in organisms from pristine areas associated with no known adverse effects or internal concentrations measured in species known to suffer from contamination (e.g., Hendriks et al. 1998).



**Figure 4.** Distribution of critical residues according to endpoint class (A), life stage (B), and tissue type (C) from two databases. Source: Jarvenin and Ankley (1999) and ERED (Bridges and Lutz 2002).

### TRA based on calculated residue-effect relationships

As described in the companion papers of this series (McCarty et al. 2011; McElroy et al. 2011), bioaccumulation data can be combined with media-based effects concentrations to estimate toxic residues. We consider these to be TRA applications based on calculated residue-effect relationships. More specifically, critical body residues (CBRs) can be estimated by multiplying the media-based toxicity metrics with the bioconcentration (BCF) or bioaccumulation (BAF) factor according to (McCarty et al. 1992):

$$LR50 = BCF \times LC50. \quad (1)$$

A BCF measures chemical uptake from water only; in contrast, a BAF measures chemical uptake from all available sources (e.g., water, food, sediment). This equation can be applied to individual substances to estimate the critical body residue for a chemical. In addition, it can also be used to calculate the LR50 as a function of the same chemical properties that determine the BCF and LC50. For organic substances acting through nonspecific modes of action, quantitative structure activity relationships (QSARs) indicate that both BCF and LC50 are related to  $K_{OW}$ , according to:

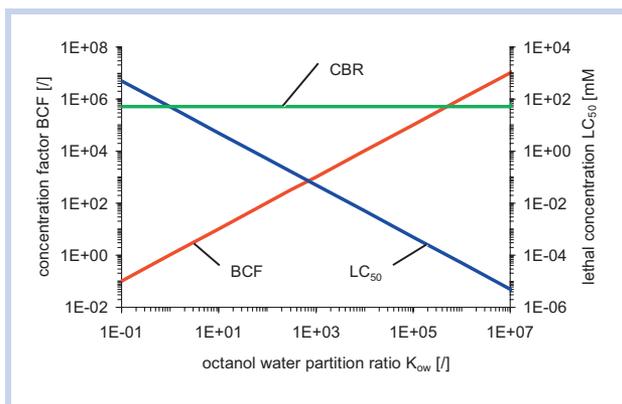
$$BCF = a_1 \times K_{OW}^{b_1} \quad (2)$$

$$LC50 = a_2 \times K_{OW}^{b_2}. \quad (3)$$

Combining these equations yields

$$LR50 = BCF \times LC50 = a_1 \times K_{OW}^{b_1} \times a_2 \times K_{OW}^{b_2} = a_1 \times a_2 \times K_{OW}^{b_1+b_2}. \quad (4)$$

For neutral substances with a narcotic mode of action, the exponents  $b_1$  and  $b_2$  more or less cancel, yielding  $K_{OW}$ -independent critical residues (Figure 5) (McCarty et al. 1992, 2011). Using more complex, but principally similar equations, the approach has been extended to polar substances, hydrophilic compounds, specific modes of action, and compartments other than lipids (Hendriks et al. 2005). The values of the coefficients  $a_1$  and  $a_2$  and the exponents  $b_1$  and  $b_2$  can be derived from BCF and LC50 QSARs. In fact, the values of the intercepts and slopes in the LC50 QSARs reflect the site of action, explaining differences between different chemical classes (e.g., neutral vs polar narcotics and organo-

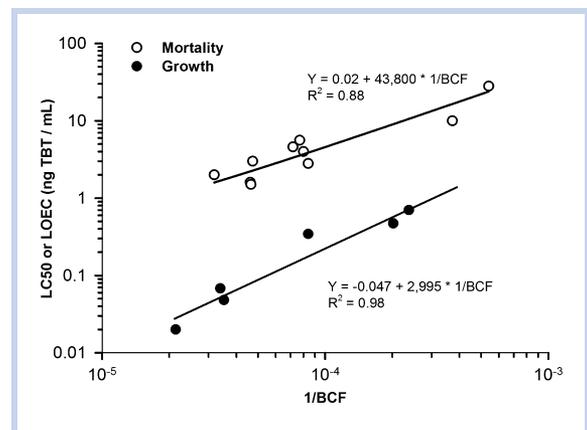


**Figure 5.** Idealized bioconcentration factor BCF, media-based lethal concentration LC50, and the critical body residue CBR as a function of  $K_{OW}$ . Modified after McCarty et al. (1992).

chlorine vs organophosphate insecticides). The BAFs and external toxicity values presented in Equation 1 can also vary by exposure time and species-specific toxicokinetics, which was explored by Meador (2006) for characterizing CBR values of tributyltin.

Keeping in mind the limitations noted in application of the TRA in the companion paper on metals (Adams et al. 2011), similar approaches are also becoming available for metals. For instance, the LC50 of different metals has been described as a function of various metal characteristics (e.g., Tatara et al. 1998). Likewise, bioaccumulation was related to several metals characteristics of which the covalent index ( $\kappa_m^2 r$ ) turned out to be the best descriptor (Van Kolck et al. 2008). In a review of the fate and effects of tributyltin, Meador (2000) demonstrated a high correlation between external exposure toxicity metrics for mortality and BCF and/or BAF values (10 unique species) and growth (6 unique species). The slope coefficients for the regression between LC50 and/or LOEC values and the inverse of the BCF values define the CBR (LR50 or LOER) for these relationships (Figure 6).

Estimating CBRs from LC50 for metals and organics has its limitations. First, in some cases, CBRs are calculated by multiplying an LC50 from an acute toxicity assay (usually 96 h) with a steady-state BAF. For both metals and hydrophobic organics, the accuracy of predicted CBRs improved when a correction for non-steady state was applied (Hendriks et al. 2005, Van Kolck et al. 2008, Meador et al. 2011). Methods for estimating the elimination rate constant needed to correct for non-steady state conditions have been described by Hendriks and colleagues (Hendriks et al. 2001; Hendriks and Heikens 2001) based on  $K_{OW}$  and the mass of the organism. Second, although the analysis so far has focused on LC50, no a priori reasons have been offered as to why the approach could not be applied on other types of toxicological endpoints (e.g., LOECs and NOECs from chronic toxicity tests). During chronic exposures, the correction for non-steady state would only be a concern for compounds with extremely slow elimination kinetics. Third, the above-mentioned estimations mainly focused on chemicals that do not undergo substantial dissociation or transformation and subsequent reduction in bioaccumulation. Dissociation may



**Figure 6.** Relationship between exposure-based toxicity metrics (LC50 for mortality with 10 species and LOEC for growth with 6 species) and dry weight BCF values. Regression equations and  $r^2$  are given for each response. The slope of the line is the tissue concentration (ng/g dry wt) for the response (i.e., LR50 or LOER). Source: Meador (2000), page 35, Fig. 7, with kind permission of Springer Science+Business Media.

be accounted for by an appropriate calculation of the bioavailable fraction, whereas transformation can sometimes be included at an order of magnitude (see example in Hendriks et al. 2005). However, labile compounds have no obvious solutions, and additional refinements are needed for application of the TRA approach.

In the future, the uncertainties associated with these approaches may be decreased by including more predictive characteristics of chemicals and species. For metals, including indicators of their affinity to bind to metallothionein in addition to the total metallothionein content of species may improve predictions based on the TRA. Likewise, indicators for covalent binding of organics to proteins may improve the prediction of the concentration at the site of action in addition to hydrophobic and H-bond interactions.

#### TRA based on implicit residue-effect relationships

Explicitly including TRs (either measured or calculated) in assessments precludes the use of a large body of data that relates toxicity directly to environmental exposures. However, many models that explicitly include residues can be reformulated to apply such toxicity data without actual knowledge of TRs, although they are still an implicit component of the model. One example is the biotic ligand model for metal bioavailability discussed by Adams et al. (2011). This model is premised on toxic effects being associated with specific levels of metal accumulation on a receptor in gills. However, this TR-based model is often implemented without actual measurement or calculation of this critical accumulation; rather, the model is used to define a relationship between toxicity and environmental conditions that can address effects without explicit values for accumulation.

Another important case of an implicit TR approach involves the various models for the relationship of toxicity to time and concentration discussed in Escher et al. (2011). Most of these models can be converted to a form in which residue is not explicitly included, although the functional form of the model still reflects a TR approach. A simple example of this is a model that assumes an organism accumulates a chemical by 1-compartment, first-order kinetics and dies when a lethal accumulation (critical body residue) is reached (the 1CFOK/CBR model, McCarty et al. 2011). This model describes the commonly observed exponential decline of lethal concentrations to an asymptotic value:

$$\begin{aligned} LC(t) &= \frac{LA}{BCF_{SS} \cdot (1 - e^{-k_e t})} \text{ (Explicit Residue Version)} \\ &= \frac{LC_{\infty}}{(1 - e^{-k_e t})} \text{ (Implicit Residue Version)} \end{aligned} \quad (5)$$

where, for an individual organism,  $LC(t)$  is the lethal concentration for a constant exposure of duration  $t$ ,  $LA$  is the lethal value for the accumulation,  $BCF_{SS} (= k_u/k_e)$  is the steady-state bioconcentration factor,  $k_u$  is the uptake rate constant,  $k_e$  is the elimination rate constant, and  $LC_{\infty}$  is the asymptotic lethal concentration. By equating  $LA/BCF_{SS}$  to  $LC_{\infty}$ , the model is converted from a form that explicitly includes accumulation to one that implicitly is based on accumulation and has 1 fewer parameter to estimate. To address sensitivity differences among individual organisms, the parameters ( $LC_{\infty}$ ,  $k_e$ ) are assigned distributions rather than single values.

The DEBtox model (Escher et al. 2011) provides another example of a model that has a version that explicitly includes

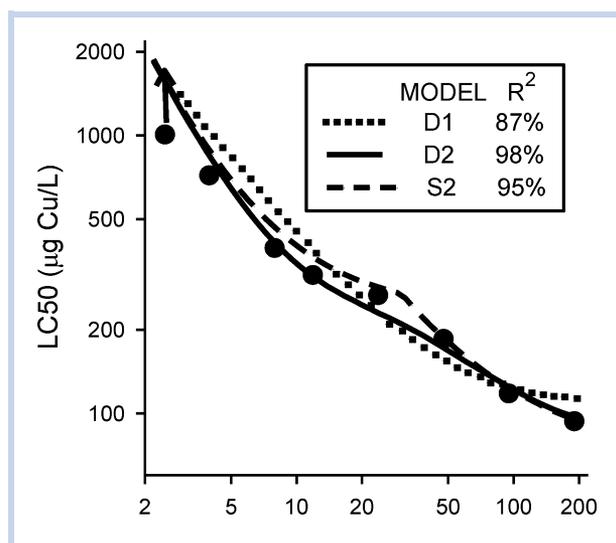
accumulation and one that relates effects directly to water concentration; for a constant water concentration  $C$ :

$$\begin{aligned} h(t) &= \max(0, d \cdot (A(t) - A_0)) \text{ (Explicit Residue Version)} \\ &= \max(0, d' \cdot (C \cdot (1 - e^{-k_e t}) - C_0)) \text{ (Implicit Residue Version)} \end{aligned} \quad (6)$$

where  $h(t)$  is the hazard rate (proportion mortality rate),  $A(t)$  is accumulation,  $d$  is a proportionality constant called the “killing rate” (Kooijman and Bedaux 1996),  $A_0$  is the accumulation threshold for effects,  $d'$  is the killing rate on a water concentration basis, and  $C_0$  is the concentration threshold for effects. This model represents a stochastic viewpoint of an individual organism’s mortality in contrast to the deterministic viewpoint of Equation 5.

The utility of these implicit TR-based models is that they can be parameterized based on standard, constant-concentration toxicity tests. As noted by Mancini (1983), once parameters are so estimated, they can be used to predict toxicity under any exposure time series. This provides more information to risk assessments than statistics such as LC50 values at 1 or a few constant-concentration exposure durations. More complicated models incorporating damage/repair, multiple mechanisms, and multiple toxicokinetic compartments can also be formulated to remove explicit reference to accumulation (e.g., Escher et al. 2011; Erickson 2007).

For the acute toxicity of Cu to larval fathead minnows (Erickson 2007), models with various formulations were parameterized using results of constant-exposure toxicity tests and were then evaluated for their fit to these tests and their predictions of the effects of fluctuating exposures. For this data set, observed LC<sub>P</sub> values versus time indicated the existence of multiple kinetic processes (Figure 7). Although the simple deterministic model of Equation 5 (model D1 in Figure 7) provided a fair approximation to the time dependence of LC50 values, a deterministic model incorporating multiple processes (model D2 in Figure 7) provided a much better fit. A 2-phase version of the stochastic model in



**Figure 7.** LC50 versus time for constant exposures of juvenile fathead minnows to Cu. Filled circles denote observed effect concentrations. Lines denote fit of various models that are conceptually based on toxic residues but parameterized entirely from the relationship of toxicity to water exposure concentration (see text for nature of models). Source: Erickson (2007).

Equation 6 (model S2 in Figure 7) also provided a good description for the LC50 values, but was inferior to model D2 for describing LC10 values and effects of pulsed exposures (Erickson 2007). However, Erickson (2007) also evaluated the predictions of the various models for exposure scenarios of relevance to USEPA water quality criteria and concluded that the differences in predictions were not so great as to reject utility of the simpler models or to support a preference between the deterministic and stochastic models.

Application of these lethality models to risk assessments requires information on mortality at multiple times within a toxicity test rather than just at the end. However, the data demands are not great, with information needed for a limited number of times that encompass an adequate range of response. Full application of these models would also require addressing mortality from chronic exposures, which might require more complexity in the models. Nonlethal endpoints can be addressed via comparable modeling approaches and are already a component of the DEBtox system (Escher et al. 2011).

### RISK ASSESSMENT ISSUES ASSOCIATED WITH TRA APPLICATION

Before conducting an ecological risk assessment or developing environmental quality guidelines, there usually is a step or process by which the goals, scope, and overall methodology used in the assessment are defined and justified. Within the context of USEPA's ecological risk assessment guidelines, the decision to use the TRA in ecological risk assessments is made in the problem formulation step in the assessment (USEPA 1998). Problem formulation is basically a phase of the risk assessment where available information is gathered on the characteristics of the stressor, exposure profile, toxicological effects, and organisms and ecosystems at risk to produce a plan for conducting the assessment. This phase considers input from risk managers regarding the goals and scope of the risk assessment. Generally, the analysis plan should contain a description of the risk assessment design, measures of effect, and methods for conducting the assessment, associated data gaps, and uncertainties. It may be limited or extensive, depending on the complexity of the assessment.

It is in this problem formulation step where the potential benefits and feasibility of using the TRA are evaluated (Table 1). Once the decision is made to use a TRA-based approach for effects assessment, a number of other risk

assessment issues must usually be addressed for its successful application. Highlighted below in Table 2 are assessment questions considered important by the workgroup for defining the scope and applicability of the TRA in an ecological risk assessment.

#### Mode and mechanism of action

Understanding the mode of action (MoOA) and mechanism of action (MeOA) of the stressors of concern is important for numerous reasons (Meador et al. 2011). First, information on MoOA and/or MeOA can help in distinguishing among taxonomic groups as to their expected sensitivity, particularly when combined with information on key physiological attributes (e.g., presences and/or activity of AhR receptors for exposure to dioxin-like compounds). Second, accurate classification of the mode or mechanism can also be important for interpreting TR-based toxicity data even for a given species. For example, knowledge of the relative reversibility or irreversibility of the MeOA may aid in understanding the importance of exposure duration in affecting the potency of chemical concentrations in tissue (Legierse et al. 1999; Vehaar et al. 1999; Lee et al. 2002a, 2002b; Landrum et al. 2004, 2005). This in turn may affect how one chooses to aggregate tissue-based toxicity data (i.e., across different exposure durations). Third, MoOA/MeOA information is also important to consider when conducting assessments on the basis of chemical mixtures, as discussed in Dyer et al. (2011). We note that there can be considerable ambiguity and uncertainty in identifying the MoOA and/or MeOA for the stressor and species of concern. Although various classification schemes have been developed for both the mode and mechanism of toxic action (e.g., Russom et al. 1997), most information for aquatic organisms has been derived from acute lethality toxicity tests for fish, which may or may not be applicable to the species of concern in the assessment (see Escher et al. 2011; McElroy et al. 2011 for additional information on MoOA and/or MeOA categories). Further, MoOA and/or MeOA may also vary between different life stages of the same species (i.e., due to development or loss of biochemical pathways) and possibly even for the magnitude and duration of exposure (e.g., narcosis from acute exposures to high concentrations versus specific mechanisms of action associated with low-level, chronic exposures).

**Table 2.** Assessment questions for application of TRA in ecological risk assessments

Topic/Issue	Assessment question
1. Mode of action	What is the expected MoOA/MeOA of the chemical for the species and life stage of concern? How might MoOA/MeOA differ across species, life stages, and exposure conditions?
2. Choice of tissue	What are the most toxicologically relevant tissue(s) for deriving tissue residue-response relationships for the species of concern? What is the availability of TR-based toxicity data for the tissue(s) of concern? What is the relative strength of residue-response relationships across tissues??
3. Exposure routes	To what extent are tissue residue-response relationships likely to be affected by the route of chemical exposure to the organism (e.g., food vs. water)?
4. Toxicity data extrapolations	Are tissue residue-response data being extrapolated between species, life stages, tissues and/or exposure durations? If so, what approaches will be used and how well are their key assumptions supported?
5. Extrapolation between media	Will tissue-residue response data need to be translated to corresponding concentrations in other media (water, sediment, prey organisms)? If so, how will these extrapolations be conducted?

### Choice of tissue

Choosing tissues for expressing the effects characterization is an integral part of conducting a TR-based assessment. Some of the more common factors that come into play when making this decision include the mode, mechanism, and sites of toxic action for the chemical and species of concern; the availability of TR-based toxicity data and the assessment endpoints of concern; and the strength of the TR-response relationships across tissues and species.

From a toxicological perspective, it is desirable to select a tissue that is closest to the sites of toxic action for the species of concern. However, practical constraints on the quantity and quality of TR-based toxicity data may require that other tissues be considered in order to meet the goals of the assessment. As illustrated in Figure 4, the vast majority of TR-based toxicity data have been expressed as whole body residues, which likely reflects practical constraints associated with obtaining and conducting chemical analyses on individual tissues. Furthermore, the tissues involved in the sites of toxic action may not be known. To this end, we note that the long history of successful application of exposure-based toxicity relationships (e.g., measures of toxicity expressed as a function of concentrations in water) indicates that knowledge of the specific site(s) of toxic action is not necessarily required. Rather, what is required is a valid exposure-response relationship (or tissue residue-response relationship, in the case of TRA) for the species and endpoint of concern and the presumption that such concentrations correlate with those at the sites of toxic action.

The importance of establishing a valid tissue residue-response relationship in the application of the TRA can not be understated. The TR-based toxicity databases illustrated by Figures 3 and 4 are derived from studies that contained measurements of chemical residues in multiple tissues with little regard to whether or not these were the most toxicologically or statistically appropriate expressions of toxicity. For example, in these studies a single toxicological endpoint (e.g., LOEC for survival) may be ascribed to chemical residues measured in multiple tissues (e.g., kidney, muscle, liver, spleen). However, these associations do not necessarily mean that residues in such tissues are correlated with the toxicological response. Chemical concentrations in some tissues may have little or no correlation with toxicological effects. Therefore, the strength of the residue-response relationship for the tissues of concern should be evaluated explicitly before its use in TRA applications, in much the same way as exposure-based toxicity data are currently evaluated. Factors to consider in evaluating the strength of tissue-residue response relationship include the statistical and toxicological significance of residue-response curve, overall consistency of increasing response with increasing concentrations in tissue, the range of residue-response information, and the quantity and quality of residue-effects information.

### Exposure route

For some chemicals, an important issue for interpreting tissue concentration-based toxicity data is how one addresses the potency of a chemical concentration in a tissue that results from different exposure routes (e.g., uptake from water vs diet). As described previously, integration of chemical exposure through different routes is one of the attractive

features of TRA. If this were not the case, the utility of tissue concentration-based toxicity data would be significantly compromised because of the highly heterogeneous nature of toxicity test designs (e.g., exposures from water, sediment, food). For organic chemicals, this workgroup is not aware of evidence that TR-based toxicity values routinely vary by exposure route. Although not expected to be a major source of uncertainty with most organic chemicals, it is prudent to first evaluate whether or not exposure route affects the potency of a given residue in tissue (particularly for less common routes such as intraperitoneal injection) before integration of this information in the effects assessment. For metals, sufficient evidence indicates that the route of exposure as well as the rate of uptake can affect the toxic potential of a given metal residue in tissue. Such differences may be determined by the degree of inactivation of metal in tissue, which is related to the biologically effective dose (or biologically available metal [BAM]). These and other challenges of developing and applying the TRA for metals are discussed further by Adams et al. (2011).

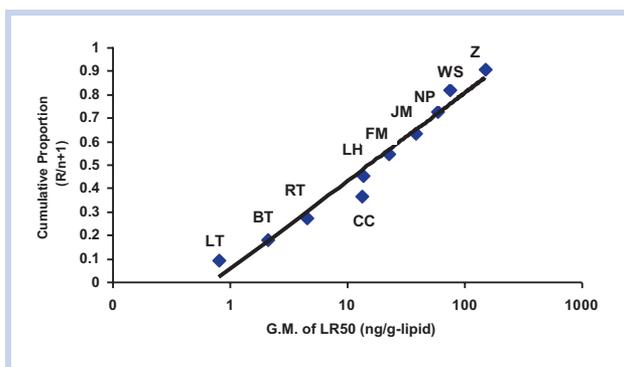
### Extrapolations involving tissue residue-based toxicity data

Similar to traditional exposure concentration-based ecological risk assessments, extrapolations of TR-based toxicity will often be necessary to address differences between species and conditions associated with the toxicity study and those of the ecological risk assessment. Common toxicity data extrapolation issues and methods are discussed in the following sections.

*Extrapolations between species.* Interspecies extrapolation of toxicity data is a common need in most ecological risk assessments because of the small number of species for which toxicity data are developed relative to those that are the focus of risk assessments. Interspecies extrapolations may be required between a tested and target species (e.g., rainbow trout to lake trout) or between tested species and a hypothetical species (e.g., the species corresponding to the 5th percentile of a species sensitivity distribution, or SSD). As with the previously described issues associated with application of TRA, understanding of the MoOA/MeOA for the species of concern is critical in defining the nature of the interspecies extrapolation. For example, if the chemical is acting through nonpolar narcosis for the effects of concern, experience has shown that a relatively narrow range of sensitivity is typically encountered when data are expressed on a lipid basis (e.g., approximately 2–8  $\mu\text{mol/g}$  lipid; McCarty and Mackay 1993; Escher and Hermans 2002). This observation and the underlying toxicological principles of narcosis theory form the basis of equilibrium partitioning-based criteria for PAHs and other narcotic chemicals (Di Toro et al. 2000a, 2000b) and the subsequently proposed target lipid model (McGrath et al. 2004, 2005). Consistent with the understood mechanism of narcosis toxicity, improvements in interspecies extrapolations of TR-based toxicity measurements for polar and nonpolar organic chemicals acting through a nonspecific MoOA have been achieved through the use of membrane-bound lipids over total or storage lipids (Escher and Schwarzenbach 2002; Escher and Hermans 2002).

For chemicals with specific-acting MoOAs, a much greater range in TR-based toxicity is typically observed compared with nonspecific acting MoOAs (Escher and Hermans 2002). This presumably reflects toxicodynamic differences across species that exist for specific-acting toxicants, since use of a TR-based measure of effects presumably incorporates differences in toxicokinetics among species. For example, Steevens et al. (2005) reported over a 100-fold range in LR50 values (expressed on a ng/g-lipid basis) for 10 species of fish exposed to 2,3,7,8 TCDD and other dioxin-like compounds during the egg and embryo life stages (Figure 8). A similar range in the chronic sensitivity of freshwater fish to DDT and methylmercury was reported by Beckvar et al. (2005) based on estimated no effect residues (NER), although some of the observed variability is undoubtedly attributable to variation in lipid content among species, test designs, and endpoint measured for expressing the NER. Interspecies extrapolations of TR-based toxicity values is complicated by the lack of standardization of toxicity tests designs that support the existing TR-based toxicity database (e.g., Barron et al. 2002). As a result, careful and systematic evaluation of TR-based toxicity data is required to avoid spurious results when estimating TR-based toxicity thresholds for specific-acting MoOAs (e.g., Beckvar et al. 2005). An exception to the observed high variability for some toxicants can be found in Meador (2010) for the specific-acting organotins, which exhibit very low variability among species for a given response.

Apart from the use of TR-based SSDs when ample TR data are available, other methods for addressing species sensitivity differences on a TR-basis remain relatively unexplored compared with those methods in use for exposure-based toxicity data. For example, interspecies correlations, SSD extrapolation factors, QSAR and MoOA “read across” methods currently available for estimating the toxicity of untested species are derived from toxicity relationships expressed on an external exposure basis (e.g., Pennington et al. 2003; USEPA 2009e, 2010; OECD 2010). Methods commonly used for extrapolating between species based on external exposure concentrations reflect both toxicokinetic and toxicodynamic differences among species. Therefore, these methods are not directly applicable



**Figure 8.** Lognormal species sensitivity distribution for freshwater fish lethal residue for 50% mortality (LR50) exposed to 2,3,7,8-TCDD and other dioxin-like compounds (converted to TCDD toxicity equivalents). LT=lake trout, BT=brook trout, RT=rainbow trout, CC=channel catfish, LH=lake herring, FM=fathead minnow, JM=Japanese medaka, NP=northern pike, WS=white sucker, Z=zebrafish. Adapted from Steevens et al. (2005).

to interspecies extrapolations using TR-based toxicity values because they presumably incorporate differences in toxicokinetics.

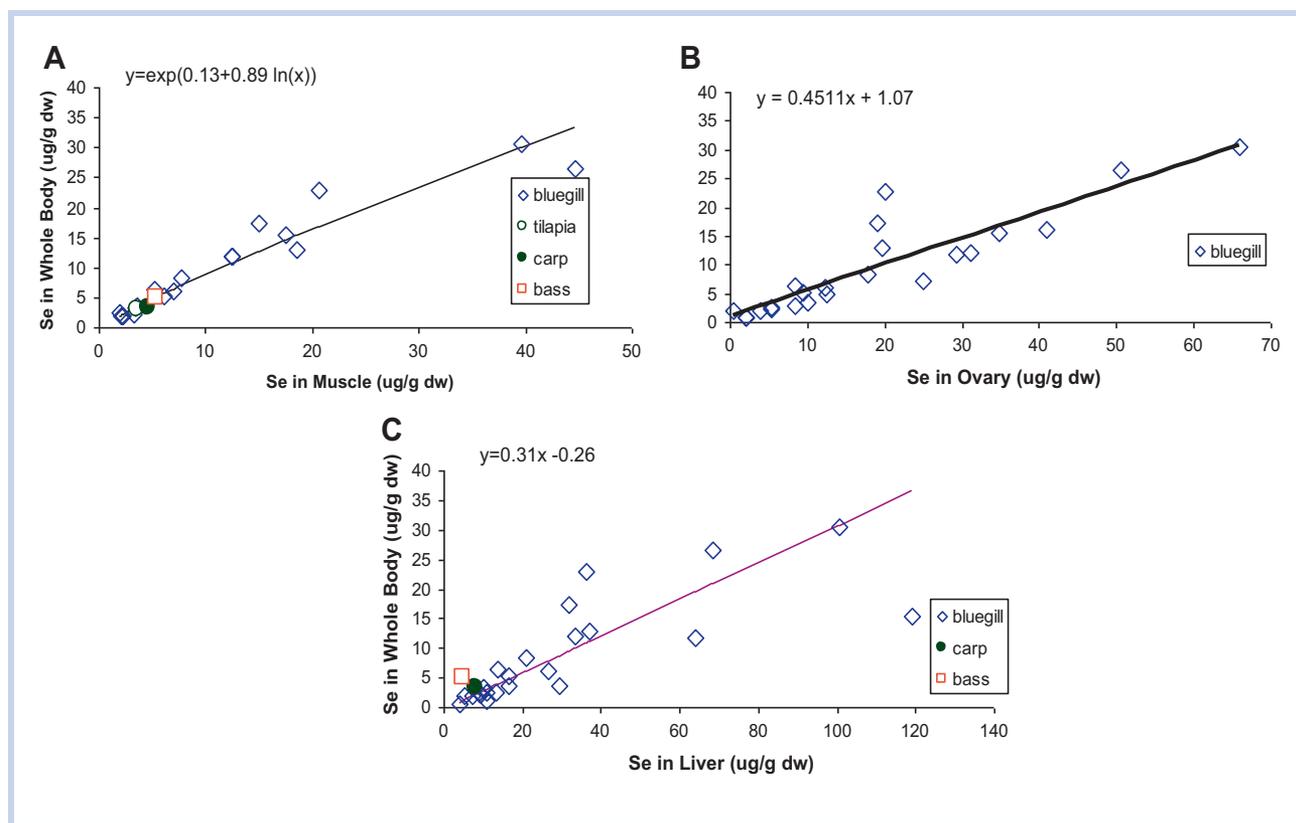
**Extrapolations between tissues.** Extrapolating TR-based toxicity data between species life stages or tissues may be required in TR-based risk assessments or criteria derivations to account for differences between measurement and assessment endpoints used in the risk assessment (e.g., residues in muscle vs whole body) or simply to standardize available data for developing a TR-based SSD. For nonpolar organic chemicals, extrapolating across life stages or tissues has been conducted on the basis of lipid normalization, which essentially assumes equilibrium partitioning among the water, lipid, and nonlipid phases within the organism. For chemicals whose critical effects are expressed through a nonpolar narcosis, extrapolation among tissues, life stages, and even species is rooted in equilibrium partitioning theory.

For chemicals to which lipid partitioning theory does not apply, empirical and mechanistically based approaches have been used to extrapolate chemical residues between tissues. In the derivation of draft TR-based criteria for Se, USEPA relied on regression relationships to extrapolate TR-based toxicity values between whole body, liver, and ovary tissues (Figure 9) (USEPA 2004).

Physiologically based pharmacokinetic models (PBPK) provide a mechanistic basis for relating chemical residues in 1 tissue to another. For example, Nichols et al. (1991, 1993, 1998, 2004) have developed PBPK models for the uptake and distribution of organic chemicals in a variety of fish, including rainbow trout, channel catfish, and brook trout. Such models are ideally suited for coupling with the TRA but require sufficient knowledge of underlying physiological processes and species-specific information for model parameterization and calibration.

**Extrapolations between tissues and external media.** Application of the TRA in ecological risk assessment or chemical criteria development commonly requires extrapolation of chemical residues in tissues to their corresponding concentrations in external media. Such extrapolations may be needed as part of a regulatory process to define and regulate chemical loads to the ecosystem (e.g., development of total maximum daily loads under the US Clean Water Act). Extrapolation between residues in organisms and concentrations in external media may also be required for estimating critical tissue residues from traditional toxicity tests whose results are expressed as external concentrations in media (Figure 2). We further note that for TRA applications involving assemblages of species (e.g., for setting TR-based criteria using SSDs), translating TR-based toxicity values to external exposure concentrations may be used to identify species that are most vulnerable to exposures to the chemical stressor. For highly bioaccumulative chemicals, for example, differences in bioaccumulation potential among different species may result in a different SSD when expressed on an external exposure concentration basis when compared with a TR basis.

A variety of methods are available to translate toxicity values expressed on a TR basis to those on an external exposure concentration basis. These span the range of simple empirically derived relationships (e.g., BCFs, BAFs, BSAFs) to single-compartment and multi-compartment



**Figure 9.** Intertissue extrapolation of Se TR-based toxicity values with fish. A = muscle to whole body prediction; B = ovary to whole body prediction; C = liver to whole body prediction. Adapted from USEPA (2004).

bioaccumulation models (e.g., Arnot and Gobas 2004; Clason et al. 2004; Meador 2006). Although a detailed review of bioaccumulation methods for organics and metals is beyond the scope of this article, it is worth noting that the translation of toxicity data to and from a TR basis to an exposure concentration basis can introduce considerable uncertainty into the assessment and should be conducted with a clear understanding of the domain, strengths, and limitations of available methods. An important consideration is the time required for achieving steady state accumulation for the chemical and organism of interest in relation to the study sampling design (USEPA 2009c). Table 3 presents a brief overview of common methods for assessing bioaccumulation potential of organic and inorganic (metals) chemicals, including some of their strengths and limitations.

To illustrate this concept, the SSD of TR-based toxicity values from Steevens et al. (2005) (Figure 10) is compared with the SSD for the same species after translation to an external exposure concentration-basis using a food web bioaccumulation model, which considers accumulation from water, food and sediment ingestion (Gobas 1993). In Figure 10B, the species abbreviations shown in red indicate a change in that species' relative position in the SSD compared with the TR-based SSD (Figure 10A). These changes in relative species sensitivity when expressed on an external exposure basis result from differences in factors expected to affect TCDD bioaccumulation (e.g., dietary composition, lipid content). The difference is most striking for Japanese medaka, which is the 7th most sensitive species when expressed on a TR-basis but is by far the least sensitive when expressed on the basis of exposure concentrations in

water. This change in relative sensitivity ranking results from the reduced bioaccumulation potential of medaka due to its assumed dietary preferences for organisms at lower trophic levels of the aquatic food web (e.g., zooplankton).

### TRA-BASED CRITERIA FOR BIOACCUMULATIVE CHEMICALS

Water quality criteria for chemicals that bioaccumulate extensively in aquatic food webs (e.g., poorly metabolized organic chemicals with  $\log K_{OW}$  values  $>5$ ) must address several important attributes in order to be sufficiently protective. These include incorporating chemical uptake through multiple exposure routes (e.g., water, diet, sediment), addressing the relatively long exposure durations that are required to achieve steady state for highly hydrophobic chemicals, and accounting for biological and environmental factors that can affect chemical bioavailability and bioaccumulation. As outlined earlier in this paper and also by McCarty et al. (2011), the TRA is well suited to address these attributes.

As an example, the USEPA recently proposed to its Science Advisory Board (SAB) a framework for incorporating the TRA into the derivation of numeric water quality criteria for the protection of aquatic life that is designed specifically to address the aforementioned issues with bioaccumulative chemicals (USEPA 2005, 2006). Some of the decision points and steps in the proposed TR-based criteria framework for bioaccumulative chemicals are provided in Figure 11. Whereas the scope of this proposed TR-based criteria framework involves bioaccumulative chemicals, we note the applicability of the TRA extends to a much broader suite of

**Table 3.** Overview of various methods for extrapolating between chemical residues in organisms and their surrounding media

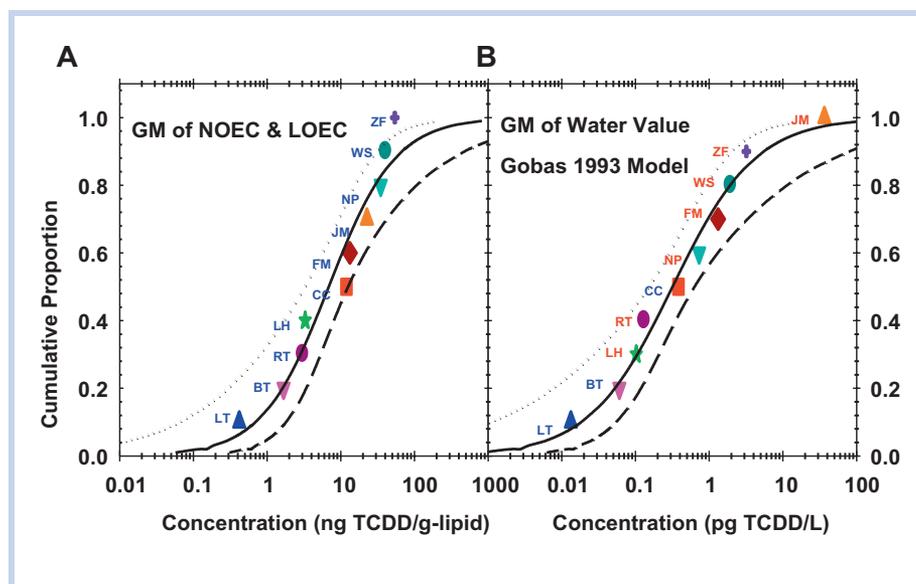
Method	Strengths	Limitations
Laboratory-derived bioconcentration factors (BCF)	<ul style="list-style-type: none"> <li>• Generally conducted using standard methods</li> <li>• BCF data are relatively plentiful</li> <li>• Incorporates chemical metabolism (biotransformation) by the accumulating organism</li> </ul>	<ul style="list-style-type: none"> <li>• Does not incorporate chemical exposure or metabolism via the food web</li> <li>• May not reflect site-specific factors that affect chemical bioavailability</li> <li>• May be subject to inaccuracies due to kinetic limitations and experimental artifacts.</li> </ul>
Field-measured bioaccumulation factors (BAF, BSAF)	<ul style="list-style-type: none"> <li>• Incorporates chemical exposure and metabolism via the food web</li> <li>• Reflects site-specific attributes that affect bioavailability and dietary exposure</li> </ul>	<ul style="list-style-type: none"> <li>• High-quality data currently limited to few sites and chemicals</li> <li>• Representative chemical concentration in water may be difficult to quantify</li> <li>• For metals, may not be predictive over wide ranges of exposure concentrations due to kinetic and other limitations.</li> </ul>
Semipermeable membrane devices (SPMD)	<ul style="list-style-type: none"> <li>• Can be readily deployed in the field for incorporating site-specific factors affecting bioavailability</li> </ul>	<ul style="list-style-type: none"> <li>• Generally limited to nonionic organic chemicals</li> <li>• Does not incorporate chemical exposure or metabolism via the food web</li> </ul>
Fugacity-based bioaccumulation models (e.g., Arnot and Gobas 2004)	<ul style="list-style-type: none"> <li>• Readily applied with minimal data</li> <li>• Can incorporate exposure via the food web</li> </ul>	<ul style="list-style-type: none"> <li>• Difficulties in incorporating accurate rates for chemical metabolism by biota</li> <li>• Applicable to nonionic organic chemicals</li> <li>• Requires assumptions for various factors that can affect bioaccumulation (e.g., organism lipid fraction, food web structure, chemical bioavailability)</li> </ul>
Single-compartment, first-order kinetic models (e.g., DYNBAM, Luoma and Rainbow 2005)	<ul style="list-style-type: none"> <li>• For metals, can be used to address dietary routes of exposure and metal- and species-specific differences in assimilation efficiency</li> </ul>	<ul style="list-style-type: none"> <li>• Requires experimental data measured under environmentally realistic conditions to derive parameters for each metal-species combination</li> </ul>
Multi-compartment (multi-order) kinetic models (e.g., Clason et al. 2004; Kahle and Zauke 2003)	<ul style="list-style-type: none"> <li>• Can be used to address saturable uptake kinetics, background concentrations (potentially important for metals)</li> </ul>	<ul style="list-style-type: none"> <li>• Requires experimental data measured under environmentally realistic conditions to derive parameters for each metal-species combination</li> </ul>

chemicals, as discussed earlier in this paper. This framework first involves the derivation of a tissue residue-based criterion and then the translation of this criterion into corresponding concentrations in environmental media. For simplicity in presentation, we have chosen to represent only some of the decision steps in the derivation process or have combined several steps into a single box. It is furthermore assumed in Figure 11 that a complete problem formulation phase would be conducted, of which only certain steps are captured in this schematic. The salient features of this proposed framework are described below.

#### *Derivation of a tissue residue criterion*

*Determining the need for a tissue-residue-based approach.* - Within the context of bioaccumulative chemicals, the relative

importance of chemical exposure via the diet is 1 of the primary factors to consider in deciding to explore a TR-based approach. Generally, the greater the importance of diet in governing chemical exposure and effects, the greater potential benefit from using a TR-based approach because of its ability to integrate aqueous and dietary exposure routes into a single expression of toxicity. Organic chemicals in this category generally have high hydrophobicity (e.g.,  $\log K_{OW} > 5$ ), long environmental persistence, and are poorly metabolized by biota. A few obvious examples include polychlorinated dioxins, furans, and biphenyls, DDT and its metabolites, and dieldrin. Selected organometallics and metalloids also fall into this category (e.g., methylmercury, Se). It should be noted, however, that the relative importance of dietary exposure can vary widely across species for a given chemical. Some groups of organisms with high food intake rates and



**Figure 10.** Comparison of SSDs expressed on a tissue residue basis (LR50 values; **A**) and exposure concentration basis (LC50 values; **B**) for 2,3,7,8-TCDD and dioxin-like compounds. LT = lake trout, BT = brook trout, RT = rainbow trout, CC = channel catfish, LH = lake herring, FM = fathead minnow, JM = Japanese medaka, NP = northern pike, WS = white sucker, ZF = zebrafish. Adapted from Steevens et al. (2005).

high chemical assimilation efficiencies may be especially susceptible to chemical exposure via the diet (e.g., high volume filter feeders). If such organisms are among the most toxicologically sensitive to the chemical in question, they may be particularly relevant in the decision to use a TR-based approach. In addition to direct evidence of the relative toxicological importance of dietary exposure, indirect evidence via bioaccumulation modeling involving multiple exposure pathways may be considered.

*Assemble, review TR-based toxicological data.* Once an initial decision to pursue a TR-based approach is made, all relevant data on the toxicity of the chemical would be assembled, with the primary focus on data that relate toxicological effects to chemical concentrations in tissues. As described earlier, this may also include the use of calculated tissue residue response relationships (Figure 2). It is at this step in problem formulation where decisions are made about the forms of the chemical of concern in tissue, the most appropriate tissues for expressing toxicological effects, the most appropriate toxicological endpoints to consider, relative sensitivity of taxa and life stages, in addition to proper screening of data for quality purposes. The latter involves evaluation of the strength of the residue-response relationships for the species, endpoints and tissues of concern. Furthermore, factors that may contribute to variability in TR-based toxicity data should be carefully evaluated (e.g., differences in life stages tested, time to equilibrium, overall study design).

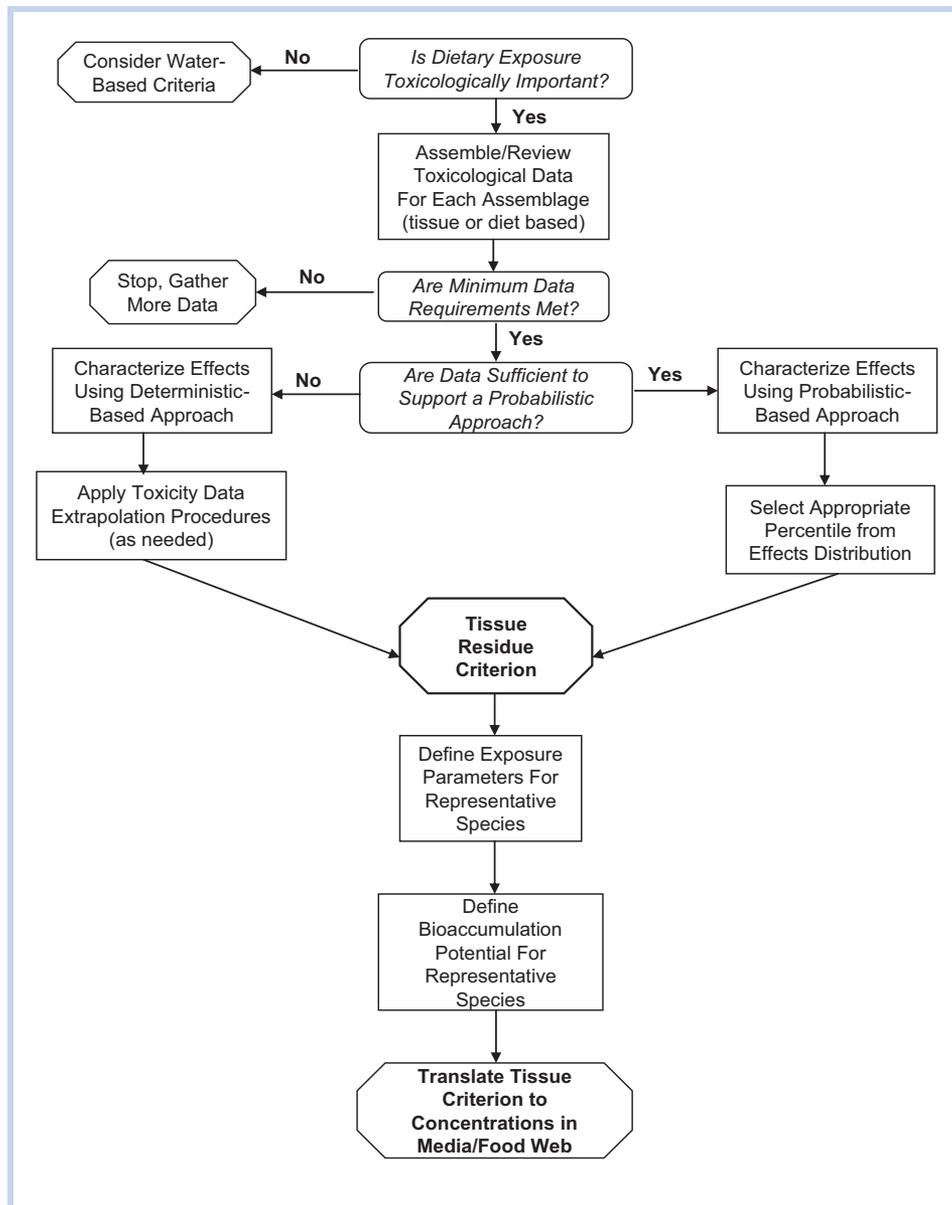
*Evaluate minimum data requirements.* Since a TR-based criterion would be designed to protect aquatic communities as a whole, TR-based toxicological data would likely need to be evaluated to ensure a diverse range of species is represented toxicologically. Because of limitations in the number of aquatic species for which TR-based toxicity data are typically available (Figures 3 and 4), consideration should also be given to use of tissue residue-response relationships that are based on calculated residues to help infer relative

species sensitivity on a TR-basis. Important to this process is an understanding of the MoOA and MeOA in relation to the expected sensitivity of different taxonomic groups (e.g., aquatic plants, invertebrates, fish, amphibians). For some specific-acting chemicals, a wide range of sensitivity can be seen even within a taxonomic group (Figure 8). In contrast, specific knowledge of the mode and mechanism of action can provide insight on which taxa may be relatively insensitive to the chemical of concern (e.g., invertebrates for chemicals with AhR-mediated toxicity).

*Selecting a derivation approach.* Assuming that specified minimum data requirements are met, the next step involves deciding on the overall derivation approach for the tissue residue criterion. Ideally, sufficient data would be available from which to construct a TR-based SSD analogous to those commonly used with water concentration-based criteria (e.g., Steevens et al. 2005). An SSD approach is particularly useful because the national tissue criterion could be set using a specified percentile of species sensitivity (e.g., 5th percentile). However, development of statistically robust SSDs generally requires data for a relatively large number of species (e.g., 8 or more species in different families for deriving USEPA aquatic life criteria) which may not be available. In these cases, a deterministically based criterion may be considered (e.g., application of the TRA for deriving a threshold tissue residue concentration for pentachlorophenol; Meador 2006). Such a criterion might involve application of some type of “uncertainty” or extrapolation factors to account for the potential greater sensitivity of untested taxa. As discussed earlier, the technical basis of such extrapolation factors remains relatively unexplored within the context of TR-based toxicity data.

#### *Translating a tissue residue criterion to concentrations in media, food web*

For regulatory application, it is expected that TR-based criteria will be translated into corresponding concentrations in



**Figure 11.** Proposed decision tree for deriving TRA-based water quality criteria for bioaccumulative chemicals. Source: USEPA (2005, 2006).

environmental media (e.g., water) and relevant components of the aquatic food web. Such translations would facilitate the implementation of TR-based criteria in regulatory programs (e.g., setting a chemical discharge permit limit to water bodies) and for monitoring compliance with the criterion. For example, in some cases, monitoring chemical concentrations in the diet of fish may be more practical than direct monitoring of fish tissue. Translating from a TR-based criterion to media- or food-web based concentrations would likely involve the use bioaccumulation factors or bioaccumulation models as discussed earlier in the present work.

For bioaccumulative chemicals, a challenge in this translation is that the identity of species corresponding to the tissue criterion (summarized above) may not be known. This situation is likely to occur because both deterministic and probabilistic approaches for characterizing effects would probably involve some type of extrapolation or interpolation of toxicity values among species (e.g., selecting a percentile from a TR-based SSD or applying uncertainty factors) in

order to derive a tissue criterion that is considered sufficiently protective of the overall assemblage of species. For example, the identity of a hypothetical species corresponding to the 5th percentile from a SSD would likely be unknown as would the components of its diet. Because a species' physiology and its diet are major determinants of exposure to bioaccumulative chemicals, it remains unclear how one would translate this tissue residue criterion to corresponding concentrations in environmental media without knowledge of the feeding preferences and physiology of species corresponding to the tissue residue criterion. Furthermore, no obvious way has been found to predict the exposure potential of the hypothetical species corresponding to the tissue criterion because inherent sensitivity on a TR-basis and a species exposure potential (e.g., degree of herbivory vs piscivory) are not necessarily correlated.

To address this uncertainty, a set of representative species could be used to translate national tissue residue criteria into corresponding concentrations in environmental media.

Representative species have been used previously for setting water quality criteria to protect piscivorous wildlife (USEPA 1995). Use of representative species in this case presumes that the identity of the species associated with the tissue criterion (derived via extrapolation or interpolation as described above) is not known. A set of representative species could be used to reflect different feeding guilds (e.g., carnivory, piscivory, omnivory, herbivory), habitat preferences (e.g., benthic, pelagic), and taxonomic groups within each assemblage. This would help ensure that the range of exposures is represented for sensitive aquatic taxa whose actual identity is unknown (e.g., the hypothetical 5th percentile species of the TR-based SSD). For translating tissue criteria at a national scale, a set of representative species could be defined a priori. For translations at a regional or site-specific scale, the representative species could be defined using information specific to the region or site. Using regional or local information to define the representative species may be particularly useful, for example, if certain feeding guilds of fish (e.g., large piscivores) are not found at a particular location.

Once representative species have been defined, the next step in translating a tissue criterion to media concentrations would involve estimating bioaccumulation potential of the chemical in relation to the representative species. Bioaccumulation methods range from empirical approaches (e.g., BAFs, BSAFs) to mechanistically based methods (e.g., food web bioaccumulation model) (Arnot and Gobas 2004), as described earlier in the present work. Translation to water would be accomplished by dividing the tissue criterion by the appropriate bioaccumulation factor for each representative species (*i*). For nonionic organic chemicals:

$$\text{Water Criterion}_{ij} \text{ (mg/L)} = \frac{\text{Tissue Criterion (mg/kg-lipid)}}{\text{BAF}_i \text{ (L/kg-lipid)}} \quad (7)$$

and

$$\begin{aligned} \text{Sediment Criterion}_i, \text{ (mg/kg-soc)} \\ = \frac{\text{Tissue Criterion}_j \text{ (mg/kg-lipid)}}{\text{BSAF}_i \text{ (kg-soc/kg-lipid)}}. \end{aligned} \quad (8)$$

Conversion of a tissue criterion to corresponding concentrations in the aquatic food web (e.g., macroinvertebrates, zooplankton, algae) could be conducted using trophic transfer factors (TTFs) defined separately for each representative

species:

$$\begin{aligned} \text{Concentration in food web component}_{i,j} \text{ (mg/kg-lipid)} \\ = \frac{\text{Tissue Criterion (mg/kg-lipid)}}{\text{TTF}_{i,j,k} \text{ (unitless)}}, \end{aligned} \quad (9)$$

where  $i, j$  = the “*i*th” food web component of the “*j*th” representative species.

According to this scheme, the end result would be a table of criterion values in environmental media (water, sediment) and applicable components of the aquatic food web (e.g., trophic levels 1, 2, 3, 4) that would vary according to each representative species defined for the aquatic assemblage. Importantly, these criterion values would be internally consistent across media since they would be derived using a common tissue residue criterion (e.g., a hypothetical species at the 5th percentile of the TR-based SSD) and methods for translating between environmental compartments. This in turn would enable evaluation of whether or not a tissue residue criterion is met or exceeded using monitoring data from multiple environmental compartments (water, sediment, or components of the aquatic food web). An example of this approach might look something like Table 4, with actual chemical criteria concentrations defined in each of the checked boxes.

#### APPLICATION OF THE TRA WITH TRIBUTYLTIN

This case study for tributyltin (TBT) illustrates the use of the TRA to enhance both ERA and criteria development and the interaction between all 3 approaches, as shown in Figure 1. We will provide several cases in which tissue residue toxicity metrics have been used to assess potentially harmful impacts of TBT to biota, including 3 USEPA Region 10 Superfund sites, an application for endangered species protection, and ecological assessments for the US Navy. Furthermore, we will explore briefly some of the reasons that it took so long to identify TBT as the causative agent for nearly catastrophic biological effects.

Of the many chemicals examined in light of their utility for the TRA and reduction in variability among species, tributyltin appears to be one of the best examples supporting this approach (Meador 2000, 2006; Meador et al. 2002). The tissue concentrations associated with mortality, growth, and sterilization due to the imposex response in snails are remarkably consistent among several species. The coefficient

**Table 4.** Translation of a hypothetical national tissue residue criterion into media-based criteria that correspond to 3 representative species with varying exposure potential as determined by feeding guild<sup>a</sup>

Translated tissue criterion concentration	Representative Sp. A (piscivore)	Representative Sp. B (benthic feeding carnivore)	Representative Sp. C (herbivore)
Water	✓	✓	✓
Sediment	✓	✓	✓
Algae/Macrophytes	✓	✓	✓
Zooplankton	✓	✓	—
Macroinvertebrates	✓	✓	—
Forage fish	✓	—	—

<sup>a</sup>Check marks indicate where translation of a tissue criterion to a criterion in a specific environmental medium or food web component could occur.

of variation for each of these biological endpoints is generally less than 60% (Meador 2006), which is considered very low in light of the orders of magnitude variability observed for ambient exposure-based toxicity values (e.g., LC50 or EC50) (Meador 2000).

Two of the most dramatic field observations for TBT include shell thickening in oysters (Waldock et al. 1996) and imposex in dogwhelks, which are well documented and highly correlated with elevated tissue concentrations in field and lab studies. It is well established that imposex will likely be severe in stenoglossan snails that exhibit whole-body TBT tissue concentrations of  $>100$  ng/g wet wt (Meador 2010). Another important observation concerns the sediment-dwelling bivalve, *Scrobicularia plana*. This species was not found in the field when tissue TBT concentrations increased to  $>12$   $\mu$ g/g (wet wt), which was likely attributable to excessive mortality. The imposex phenomenon is important because it had catastrophic effects on populations of these snails in Europe (Stebbing 1985, 1996). This was also important from the TRA perspective because this provided the most compelling evidence that TBT caused imposex.

#### *TRA could have facilitated TBT causality identification*

Tributyltin was first used in the marine environment during the late 1960s, but adverse effects from exposure to TBT were not recognized until the mid-1970s (Stebbing 1985, 1996). The importance of using tissue residues to reduce uncertainty in the assessment of TBT in the field was implied in a number of early scientific papers but was not widely acknowledged. Stebbing (1985) for example, comments that “The response of organisms in toxicity experiments is not so much the levels in the water, but to the levels that accumulate in the tissues.”

Other complicating factors were present during the mid-1970s when the biological effects of TBT were first observed. One important factor was that the sophisticated chemical methods necessary to quantify TBT speciation had not been adequately developed (Stebbing 1985, 1996). Adding to this impairment were the following complicating factors: 1) the observed shell thickening (chambering) in mariculture oysters from France and England coincided with the introduction of the Pacific Oyster *Crassostrea gigas*, 2) high suspended sediment loads (first associated with shell thickening) were often collocated with marinas and large numbers of boats, 3) the oyster culture areas existed long before the marinas and antifouling paints were not suspected, and 4) the chemical methods necessary to adequately quantify TBT in all matrices had not yet been developed.

It was not until the 1990s that tissue residue toxicity metrics for TBT were observed to be relatively consistent among species and could be used to characterize dose-response relationships. Moore et al. (1991) was among the first to comment on the relatively narrow range of tissue concentrations associated with adverse effects in such diverse species as polychaetes and bivalves. Meador (1997, 2000) and Meador et al. (1993) would later develop a much more comprehensive list and make an even more compelling case for utilizing the TRA to assess TBT toxicity. It was not until researchers observed the relationship between tissue concentrations and effects did the potency of organotins become fully appreciated. Given this sequence of events, we suggest that more emphasis on the TRA would have reduced

the time necessary to establish causality and enact protective legislation. For a summary of tissue residue toxicity metrics for organotins, see McElroy et al. (2011).

#### *Tissue Residue Guidelines for TBT—USEPA Region 10*

*Harbor Island Superfund site.* As part of its Remedial Investigation for the Harbor Island Superfund site (near downtown Seattle, WA), the USEPA (Region 10) developed “tissue trigger levels” for tributyltin, a contaminant of concern, to provide guidance for potential remedial action. Based on the available literature, a tissue concentration of 600 ng/g (wet wt) was selected and recommended as the tissue toxicity reference value (TRV) for this site (USEPA 2003e). This tissue TRV for remediation of sediments at the Harbor Island Superfund site has been correlated with reduced growth in several species (Meador et al. 2002). Imposex in marine stenoglossan neogastropod snails was not considered a relevant response at this site, because it was assumed that little or no habitat was available for these gastropod species.

*Lower Duwamish Superfund site.* The Lower Duwamish Waterway (LDW) is the final section of the Green–Duwamish River that terminates in Puget Sound close to downtown Seattle. The superfund site comprises approximately 9 km of the LDW. As part of the remedial investigation (RI) for this site, the USEPA required the responsible parties to assess impacts from TBT exposure. The Lower Duwamish Waterway Group (LDWG), which represented the Port of Seattle, King County, the Boeing Company, and the city of Seattle, conducted various studies at this site to determine impacts from TBT and developed a tissue residue TRV for mortality and growth (LDWG 2007). At this site the imposex response was considered; however, it was assessed by direct analysis of imposex occurrence rather than relying just on interpretation of tissue concentrations. Three species of neogastropods (*Astyris gausapata*, *Olivella baetica*, and *Nassarius mendicus*) were examined for imposex to evaluate potential relationships with sediment TBT concentrations. Imposex was not observed in *A. gausapata* or *O. baetica*; however, low-level responses (stage 2) were observed in *N. mendicus*. Tissue concentrations were not measured in these snails, so it was not possible to compare levels with the concentrations known to cause imposex in other gastropods (Meador et al. 2002). Considering that the observed sediment concentrations for most of the study sites were relatively high 170–2300 ng/g dry wt, it is surprising that these neogastropods did not exhibit imposex. The possibilities for these negative results include highly heterogeneous sediment concentrations, reduced bioavailability, less than expected bioaccumulation, elimination of sensitive neogastropods species from the estuary years ago, and that the species found and examined were less sensitive to TBT for this response.

For this remedial investigation, an invertebrate tissue residue TRV was developed for growth and mortality (LDWG 2007). This value ( $\sim 500$  ng/g wet wt) was selected from 1 study of 3 that provided data on nonsignificant (NOER) and significant ( $\alpha = 0.05$ ) (LOER) results compared with the control. The lowest LOER value was determined for growth in *Armandia brevis* (Meador and Rice 2001) and represented a 25% reduction in biomass. The NOER was also

selected from this study because it was the lowest value of the 3 studies. This single study was used to establish the NOER and LOER TRVs, which was also cited by LDWG as the largest source of uncertainty because these values were based on the results for a single species (LDWG 2007). These TRVs were then used to determine risks to the benthic community by means of a hazard quotient (HQ). The HQ is the ratio between observed tissue concentrations from field collected organisms and the tissue TRV. These HQs were determined for the NOER and LOER TRVs. Instead of comparing the tissue concentrations for individual species with the tissue TRV, all collected invertebrate species were analyzed together to generate 1 TBT tissue concentration for a given location within the waterway. All HQs were below a value of 1.0; it was concluded that TBT would not result in adverse growth effects to benthic invertebrates.

A separate TBT tissue TRV was determined for fish species at this site (LDWG 2007). Of the 3 published papers on fish considered acceptable by the LDWG for this TRV, 1 was selected and reported as having the lowest NOER (18 ng/g wet wt) and LOER (159 ng/g wet wt) (LDWG 2007). Upon review of this study, it was found that the authors actually reported a statistically significant reduction in body weight and length and an increase in sex-reversed fish (25% vs 2% for the control) for the 18-ng/g treatment; therefore, this concentration should have been selected as the LOER. If this lower whole-body value (18 ng/g wet wt) was selected as the tissue LOER TRV, most values for the HQ analysis would be >1.0, indicating a high probability of adverse effects in fish at this site.

**Portland Harbor Superfund site.** Tissue TRVs were developed for a large number of chemicals for the Portland Harbor (Oregon, USA) Superfund site that were used to assess potential impacts to biota. The literature was comprehensively examined and LOER values based on a variety of sublethal effects along with chronic mortality (LR<sub>p</sub>) values were assembled in the database and used to construct species sensitivity distributions (SSDs). Once the SSDs were developed, the 5th and 10th percentiles were determined with the software package BurliOZ. For TBT, a TR-based SSD was generated based on all available tissue residue toxicity data for invertebrates that passed a quality assurance screen. The resulting 5th and 10th percentile LOERs were 40 and 90 ng/g wet wt, respectively. These values include the imposex endpoint and are consistent with several studies that determined the threshold value for this response to be in the 10–30 ng/g range (Meador 2010). A TBT TRV was not determined for fish.

**Endangered Species Act.** One of the goals of the Endangered Species Act is to protect the habitat of the listed species, which includes their prey. The intent of this study was to develop a TBT sediment concentration that would not diminish prey availability for listed salmonids. Because of the low variability in tissue-based LOERs for growth among several species, Meador et al. (2002) were able to translate a tissue TRV (600 ng TBT/g wet wt) to a sediment concentration using sediment bioaccumulation factors. By rearranging the BSAF equation, the tissue TRV was used to solve the equation for the organic carbon (OC) normalized sediment concentration ( $sed_{OC}$ ) that would likely result in the selected tissue TRV (Meador et al. 2002). Based on the 75th

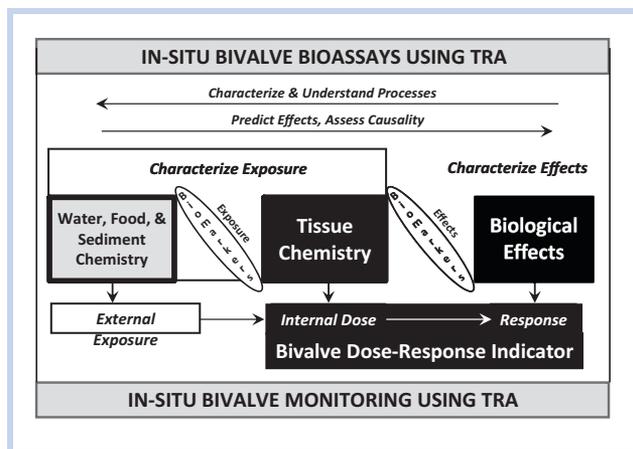
percentile for several BSAF values determined for invertebrates, the  $sed_{OC}$  for TBT was determined to be 6000 ng/g organic carbon. For a sediment containing 2% OC, this would equate to a bulk sediment concentration of 120 ng TBT/g sediment (dry wt). The intent of this value was to protect salmonid prey availability, which was set at a threshold value equal to a 25% reduction in biomass. Although this sediment guideline was developed for salmonids, it is applicable to other species of fish or as a protective value for infaunal invertebrates.

**US Navy—San Diego Bay and Hawaiian Harbors.** As part of their ERA for TBT, the US Navy measured the concentrations of TBT in water, sediment, and biota. TBT tissue concentrations in *Mytilus galloprovincialis* from San Diego Bay ranged from 32 to 2100 ng/g (wet wt) with bioaccumulation factors ranging from 560 to 13 200. The data indicated that BAFs decreased rapidly as TBT concentrations in surface water increased and approached or exceeded 50 ng/L. Variability in the BCF as a function of exposure concentration is an important observation. It can add substantial error when back-calculating water concentrations from tissue concentrations using BCFs (Meador 2006). The same is also likely true when high sediment concentrations are encountered. This phenomena was observed by Meador and Rice (2001) for the polychaete *Armandia brevis* exposed to high concentrations of TBT in sediment indicating a change in feeding behavior or ventilation rate. These results highlight the importance of including the external exposure concentrations for any assessment of adverse effects or when back-calculating with bioaccumulation factors.

**Conclusions with TBT.** This TBT case study highlights several important points that support the application of the TRA. Perhaps the most compelling is the use of tissue TRVs to enhance ecological risk assessments. They also provide another line of evidence for toxicity assessments and facilitate the important connection between laboratory toxicity results and field evaluations. Collectively, these results show the importance of measuring chemicals in organisms, but not to the exclusion of other matrices (water, sediment, or diet), which are needed for a complete assessment. These field observations are very consistent with the laboratory toxicity values and the derived tissue TRVs used in the aforementioned regulatory applications. Because the variability for TBT tissue-residue toxicity metrics is relatively low, tissue TRVs can be selected for various specific adverse responses that may reasonably protect a large number of species. While the specific MoOA and MeOA for TBT facilitated the verification of causal TR-based effect relationships from laboratory and field studies, the utility of the TRA demonstrated with TBT is considered applicable to all chemicals for which scientifically robust TR-based effect relationships can be established.

## IN SITU APPLICATIONS OF THE TRA USING BIVALVES

The generic context of using bivalves for in situ tissue residue applications is described here in support of ecological risk assessment. This approach has been described previously for caged marine bivalves and a conceptual framework presented for an exposure-dose-response (EDR) triad (Figure 12) (Salazar and Salazar 1998). The 3 elements of



**Figure 12.** The relationship exposure, dose, and response (EDR) emphasizing the importance of both water and sediment chemistry and bioaccumulation.

the EDR triad (Figure 12) are numbered for convenience, but this section will focus on relationships established between the internal dose and associated biological responses. This ecotoxicological emphasis using bivalves is not only effective in characterizing exposure and effects to support the TRA and ERA, but can be used to help characterize and understand processes, predict effects, and assess causality. This is also the area where biomarkers can be used to help elucidate mechanisms that are key to both the TRA and ERA. A more complete discussion of bivalve biomarkers as a practical approach can be found elsewhere (Garrigues et al. 2001).

More recently, this framework was modified to link bioaccumulation and biological effects to chemicals in water and sediment for caged freshwater bivalves (Salazar and Salazar 2007). As part of this development, consensus-based protocols were developed for conducting in situ field bioassays with marine, estuarine, and freshwater bivalves (ASTM 2001). Integration of the in situ bivalve bioassays with monitoring natural populations of indigenous organisms is also considered an important evolution of this framework. Furthermore, while the EDR triad remains as the cornerstone for the approach, bivalves as dose-response indicators will be emphasized here. Use of bivalves in application of the TRA is described below within the context of mussel watch monitoring programs, in situ bioassays and selected case studies involving Cu.

### Mussel watch monitoring

In concept, mussel watch monitoring is a sentinel network to monitor the status and trends (and sometimes the effects) in resident organisms. As the name implies, mussels are the most frequent organism of choice for the reasons previously mentioned. Mussel watch monitoring programs from various parts of the world are discussed below with an emphasis on those that involve linkage of tissue residues to biological effects.

*Mussel watch monitoring in the United States.* As discussed previously, measurement and interpretation of tissue residues of aquatic organisms has probably seen its widest application in a variety of environmental monitoring programs such as the NOAA Status and Trends Mussel Watch marine

monitoring program (Kimbrough et al. 2008), the USGS National Water-Quality Assessment (NAWQA) Program, which monitors a variety of biota including freshwater clams (*Corbicula fluminea*), and various regional monitoring programs on the West Coast. The stated purpose for most of these programs, as the name implies, is to establish the status and trends of chemicals accumulated in bivalve tissues over space and time. Although some effects and biomarkers are routinely measured as part of these programs, it is more difficult to use the dose-response indicator feature in passive monitoring, because the approach is more observational than experimental and adequate gradients of exposure, accumulation, and effects may not always be achieved.

The California State Mussel Watch Program (Martin and Severeid 1984) and the Regional Monitoring Program for Trace Substances (Gunther 1999) have conducted biomonitoring for trace elements and organic contaminants in the San Francisco Estuary, using both resident and transplanted bivalves. These programs have linked tissue residues with biological effects (Figure 12) by measuring survival and body condition synoptically with bioaccumulation. Their results demonstrate that the integration of resident and transplanted bivalves enhances the assessment, when part of a consistently supported long-term program (Gunther 1999).

*Mussel Watch monitoring in the United Kingdom.* Mussel Watch monitoring in the United Kingdom differs significantly from that in the United States in a variety of ways. Specifically, the UK monitoring program involves a much longer time record, includes other species besides bivalves, incorporates both passive and active dose-response monitoring, and is more integrated with laboratory toxicity testing and bivalve aquaculture. All of these factors are distinct advantages with respect to characterizing and understanding processes and interpreting residue-response data.

Bryan et al. (1987) summarize the effects of Cu, Zn, and organotin as long-term factors governing the distribution of organisms in the United Kingdom. Evidence of long-term metal pollution in the Fal Estuary is provided by analyses of Cu in oyster (*Ostrea edulis*) communities extending over more than 120 y and by analyses of Cu and Zn in sediments dating back to 1921. In one of the first examples of bivalves as dose-response indicators, the greenness of oysters has been related to their Cu tissue burdens, and green oysters have been observed in the vicinity of Restronguet Creek for at least 123 y (Bryan et al. 1987). Given this long-term record, scientists at the Plymouth Marine Laboratory have established relationships between tissue burdens and the presence/absence of bivalves and other species and tissue burdens of metals such as Cu, Zn, and organotin. As an example, it became clear that the existence of oysters (*Ostrea edulis*) in the lower reaches of Restronguet Creek is due in large part to their ability to detoxify accumulated Cu and Zn. In addition, scientists at the Plymouth Marine Laboratory have conducted passive and active dose-response monitoring with bivalves, algae, and worms (Bryan et al. 1987). All of these studies have also been supported by an extensive laboratory testing program to help interpret the dose-response data.

### In situ bioassays

In contrast to passive Mussel Watch monitoring, in situ bioassays represent active biomonitoring or translocation where

individuals, populations, or communities are transplanted to selected aquatic environments for various time periods. This approach has several advantages. These include a well-defined exposure period for comparing exposure and effects at the beginning and end of the test, control over the size, age, and genetic and environmental history of the deployed organisms.

*In situ bioassays in the United Kingdom.* Between 1970 and 1990, methods were developed to integrate ecophysiology and scope for growth (SFG). This included adaptation of mussels to natural environmental stressors (constant and fluctuating temperature, salinity, suspended particulate matter, food quality, and anoxia and/or hypoxia). This was followed by ecotoxicological assessments, including specific toxicants on mussel SFG (establishing relationships for tissue concentration versus SFG and QSARs). Between 1980 and 1995, *in situ* TRA assessments were made by combining effects measurements (SFG) and tissue burdens along pollution gradients (e.g., Widdows and Donkin 1992).

*In situ bioassays in the United States.* In the United States, Salazar and Salazar applied the ASTM Standard Guide (2001) and conducted over 70 bivalve transplant studies in marine, estuarine, and freshwater environments. These studies included the use of 20 different species and more than 70 000 test animals. In each study, survival, bioaccumulation, and growth were measured as part of the TRA and ERA.

*In situ bioassays in Canada.* The work of Bonneris, Giguère, Perceval, et al. (2005), Bonneris, Perceval, and Masson (2005), and Campbell et al. (2005) is important because of their mechanistic implications for freshwater bivalves and TRA. These investigators have combined monitoring indigenous and transplanted freshwater mussels (*Pyganodon grandis*), measurement of biochemical changes (biomarkers), bioaccumulation, subcellular distribution, and comparisons with other species (*Perca flavescens*). They studied the partitioning of metals (Cd, Cu, and Zn) in the gills of *Pyganodon grandis* and evaluated metal sequestration in both indigenous and caged animals (Bonneris, Giguère, Perceval, et al. 2005; Bonneris, Perceval, and Masson 2005; Campbell et al. 2005). They concluded that in nature, metals in *P. grandis* are bound differently in the gills and in the digestive gland, and that metal detoxification in the former organ may be less effective than in the latter. They further suggest that differences in laboratory and field responses support the need to study metal exposure-dose-response relationships in natural populations or in transplant studies. Related work using *Pyganodon* transplants along a metal contamination gradient integrated biomarker responses (metallothionein) to Cd and Zn exposure, cytotoxicity, and effects at higher levels of biological organization (Couillard et al. 1993, 1995a, 1995b).

#### The Cu TRA example

Three different effects endpoints (survival, growth, and reproductive effects) associated with threshold body residues for Cu in marine bivalves are used as examples to demonstrate the ability of field studies to establish meaningful TRA relationships. Of these relationships, 2 were developed from *in situ* bioassays using caged mussels and 1 from field-collected clams (Salazar and Salazar 2007).

The first *in situ* bioassay used caged *Mytilus edulis* to study the effects of acid mine drainage from an abandoned copper mine in Howe Sound, British Columbia, Canada (Grout and Levings 2001). This bioassay estimated a threshold body residue for survival of 40 µg/g dry wt. Their work is particularly important because they were able to link Cu concentrations above a specific threshold with declines in survival, weight growth, length growth, and condition index. The second example is from a series of transplant experiments conducted in San Diego Bay between 1987 and 1990 (Salazar and Salazar 1995). Two threshold concentrations were estimated from these studies: an effects concentration for growth of 75 µg/g dry wt and a no observable effects concentration (NOEC) of 25 µg/g dry wt. A series of multiple regression analyses were used to determine where the relationship between Cu residues and effects began to change. The third example is from a long-term study in San Francisco Bay where *Macoma balthica* were collected from a mudflat with elevated Cu exposures (Hornberger et al. 2000). This long-term study estimated the threshold body residue for reproduction to range between 100 to 200 µg/g dry wt. The 3 field studies are nearly unique in that they all linked multiple effects endpoints to tissue residues. Whereas the Grout and Levings (2001) study demonstrated the high availability of Cu associated with acid mine drainage and the Salazar and Salazar (1995) study included many sites studied over several years, the Hornberger et al. (2000) study is of importance because of a single site studied over a 25-y period.

## SUMMARY AND CONCLUSIONS

The aim of this review has been to describe recent progress in the application of the TRA and to promote a framework for its application in a regulatory context. Currently, application of the TRA has progressed furthest for those chemicals acting by a nonspecific MoOA (narcosis). It is also particularly well suited for addressing the challenges of assessing bioaccumulative chemicals and for assessing exposure-residue-response relationships using *in situ* bioassays. Although the TRA has been widely applied for chemicals with nonspecific MoOA, a number of well-developed examples are available for its application to specific acting toxicants (e.g., TBT, selenium, dioxin-like chemicals).

Importantly, we suggest that the TRA not be viewed as a replacement for traditional media-based or dose-based toxicological assessments; rather it should be used to complement these approaches to the extent that data and resources allow. We further suggest that application of the TRA not be restricted to its use with measured residue-effect relationships, which have limited availability compared with media- and dose-based toxicity relationships. Viable alternatives include the use of calculated and implicit tissue residue-response relationships, as illustrated in Figure 2. Although the potential advantages of the TRA in ecological assessments and chemical guidelines development are many, we recognize that the TRA is not a panacea and must be applied with a clear understanding of its underlying assumptions and limitations in available data. For example, a number of challenges exist with application of the TRA with metals due to the existence of physiological mechanisms for adapting to varying exposures in certain metals and/or species combinations (Adams et al. 2011). Conceptually, acclimation or adaptation of a population of organisms to a chemical stressor could influence its TRA-based toxicity. These phenomena have been explored

extensively using media-based expressions of toxicity. However, the effect of acclimation or adaptation on media-based expressions of toxicity may reflect changes in chemical uptake, metabolism, and elimination rather than alterations of TR-based toxicity. To date, we are not aware of studies that have documented alterations of TR-based toxicity caused by acclimation or adaptation. Furthermore, our review of the available data on measured residue-effects relationships reveals substantial limitations in the scope of these data. Although techniques are available to address some of these limitations (e.g., for extrapolating TR-based toxicity data across species, tissues, and life stages), broader application of the TRA will undoubtedly require the generation of additional data linking tissue residues to toxicological effects. In this regard, it is critically important that such toxicological linkages be established based on toxicologically relevant residue-response relationships, and that they be made within the context of available information on the MoOA and MeOA for the chemical and species of interest. For advancement of environmental quality guidelines, application of the TRA will likely require the translation of numeric TR-based guidelines into corresponding concentrations in environmental media (water, sediment, soil) to accommodate the practicalities of implementation. Methods for translating between environmental compartments (biota, water, sediment, and soil) are well established for organic chemicals but are somewhat more limited for most metals (Fairbrother et al. 2007).

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