Table 1. Approaches and criteria for assigning mode of action (MOA)

<table>
<thead>
<tr>
<th>Approach</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verhaar prediction of MOA</td>
<td>MOA derived from publication by Verhaar et al. (1992)</td>
</tr>
<tr>
<td>Russom prediction of MOA</td>
<td>MOA derived from publication by Russom et al. (1997)</td>
</tr>
<tr>
<td>3 species acute (mg/L) algae/invertebrate⁹/fish (acute factor)</td>
<td>The difference between EC₅₀ vs log Kₐw regression lines (QSARs) was determined for nonpolar and polar narcotic MOA for each species. For nonpolar narcotic (MOA1) substances, EC/LC₅₀ values for algae/invertebrates⁹/fish should be within a factor of 3 of the nonpolar narcotic QSAR. For polar narcotics, EC/LC₅₀ values should be less than or equal to the difference between the determined min and max values at the log Kₐw of the compound of the polar QSAR for MOA II.</td>
</tr>
<tr>
<td>3 species chronic (mg/L) algae/invertebrate⁹/fish (chronic factor)</td>
<td>Provisionally, NOEC values should be within an order of magnitude of each other for MOA I or II, regardless of the log Kₐw</td>
</tr>
<tr>
<td>Do measured data fit with QSAR estimate for narcotic MOA?</td>
<td>QSAR for neutral organic and polar (phenols) toxicity from ECOSAR expressed as a ratio to the measured values; MOA I or II should be within an order of magnitude of the QSAR</td>
</tr>
<tr>
<td>Acute:chronic ratio</td>
<td>Use the same taxa (or preferably the same species, if available) to determine acute to chronic ratio; provisionally, EC₅₀ to NOEC ratio should be within 1 order of magnitude for MOA I or II</td>
</tr>
<tr>
<td>Chronic body burdens ([CBB], mMol/kg ww)</td>
<td>CBB measured if possible, otherwise calculated as follows: CBB = NOEC × BCF. Provisionally, CBB &gt; 0.05 mMol/kg ww (Thompson and Stewart 2003). An estimation of LBB is not recommended; an LBB would be based on a minimal 28-d BCF and LC₅₀ or EC₅₀ of, typically, &lt;7 d; a comparison of these 2 values, due to the difference in exposure time, may not result in a meaningful estimate.</td>
</tr>
</tbody>
</table>

EC₅₀ = median effect concentration; QSARs = quantitative structure–activity relationships; LC₅₀ = median lethal concentration; NOEC = no-observed-effect concentration; ECOSAR = ECOWIN v 0.99h (USEPA 2004); BCF = bioconcentration factor; LBB = lethal body burden.

b Daphnia preferred if available.

The approach will increase confidence in the prediction of the MOA.

Conclusion

ECETOC has further developed its methodology for risk assessment for some PBT-like chemicals. All chemicals with a narcotic MOA (Types I and II) are considered suitable for risk assessment under this methodology because the toxicity and bioaccumulation of such chemicals can be predicted. This does not infer that chemicals that exhibit MOA III or IV are not able to be risk assessed, but rather that these must be reviewed on a case-by-case basis. In the first step of the ECETOC risk assessment proposal, a prediction of MOA is required. This WoE approach can be used to predict MOA with greater confidence than any single method alone.

References


Despite a number of studies conducted on metal trophic transfer in aquatic ecosystems, there are still uncertainties in predicting metal bioavailability under field conditions. For the past decade, there has been a growing body of evidence suggesting that the subcellular compartmentalization of metals in dietary sources can influence metal trophic transfer up food chains (Reinfelder and Fisher 1991; Wallace and Luoma 2003). The relevance of metal distributions within prey to metal trophic transfer results from differential bioavailability to predators of metals sequestered among various subcellular pools (Wallace and Lopez 1997; Wallace and Luoma 2003). A series of studies have demonstrated that omnivorous decapod crustaceans readily assimilate metals partitioned to cytosolic proteins and organelles of invertebrate prey, although metal partitioned to metal-rich granules is less available for uptake (Wallace and Lopez 1997; Wallace and Luoma 2003). Due to their high trophic availability to predators, Wallace and Luoma (2003) suggested that metals associated with cytosolic proteins and organelles be considered collectively as a subcellular compartment-containing trophically available metal (TAM). Therefore, it follows that any physiological attribute of prey (i.e., prey-dependent processes) that influences the subcellular storage of accumulated metal may determine the extent of metal transfer along food chains. For example, binding of metals to metallothioneins may result in a greater overall partitioning of metal to cytosolic proteins (a component of TAM) and, therefore, may lead to an increase in metal trophic transfer (Seebaugh et al. 2005). Conversely, because metals associated with metal-rich granules generally are unavailable for uptake by a predator (i.e., non-TAM), long-term storage and detoxification via this mechanism may result in a bioreduction in metal trophic transfer (Nott and Nicolaidou 1993; Wallace and Luoma 2003).

This theorized relationship between TAM and metal assimilation efficiency (AE) has been observed in a number of studies with omnivorous decapod crustaceans ingesting a variety of Cd-contaminated prey. In these cases, a 1:1 relationship was found between metal assimilation efficiency in the predator and metal stored as TAM in prey (Wallace and Luoma 2003). However, more recent studies have found that the AE:TAM relationship may not be applicable to some predator–prey relationships (Rainbow et al. 2006). The lack of concordance between AE and TAM in these predator–prey combinations may be attributed to differences in predator digestive physiology (e.g., gut residence times, gut pH, and digestive enzyme activities) because the relationship between metal in prey and solubilization predators upon digestion is poorly understood (Wang 2002).

In order to fully understand metal trophic transfer, it is now clear that a variety of prey-dependent (e.g., internal storage of metal) and predator-dependent (e.g., digestive physiology) processes need to be considered, as it is the interaction of these processes that ultimately may determine the extent of metal assimilation by a predator. The important mechanisms involved with metal trophic transfer are summarized in Figure 1a. Steps 1 and 2 are prey-dependent (uptake and internal storage, respectively); steps 4 through 6 (digestion, gut transport, and depuration, respectively) are predator-dependent; step 3 (ingestion of prey by the predator) is at the nexus of prey and predator processes. Step 7 (metal assimilation), although ultimately a predator-dependent process, may be influenced by all previous steps. Step 2 (internal storage) is then repeated as the predator (now a prey) partitions metal among TAM and non-TAM fractions.

With this conceptualized model of the mechanisms important to trophic transfer, it is apparent that the ultimate assimilation of metal by a predator results from the interaction of prey- and predator-dependent processes. Additionally, with the systematic analysis of these various steps, it is possible to investigate which steps are most critical to the transfer of metals in aquatic ecosystems, as well as elucidate possible reasons for variable transfer among metals and among food chains.

In a recent study, we have examined how some of the above listed prey- and predator-dependent processes influence the trophic transfer of Cd and CH$_3$Hg in a typical northwestern Atlantic estuarine food chain consisting of a resident estuarine fish, the mummichog (Fundulus heteroclitus), and a variety of benthic macroinfaunal prey found in tidal salt marshes (grass shrimp [Palaemonetes pugio], juvenile sheepshead minnow [Cyprinodon variegatus], amphipods [Leptocheirus plumulosus and Macroampharides macronotus], insect larva [Chironomus dilutus], and clamworms [Nereis virens]). To conduct this work, prey were exposed to radioisotopes of the 2 metals ($^{109}$Cd and CH$_3$Hg) through solution (seawater). Radiolabelled prey were processed as described below and we then examined the relationship between/among these 3 data sets (TAM, GEM, AE).

- Prey were subjected to tissue homogenization and centrifugation techniques to obtain the TAM fraction.
- Prey were incubated in vitro with gut fluid extracted from the predator to determine the proportion of ingested metal that can be extracted by exposure to gut juice (i.e., gut juice–extractable metals [GEM]).
- Prey were fed to the predator in a typical feeding experiment to estimate the AE of Cd and CH$_3$Hg.

The partitioning of CH$_3$Hg to the TAM compartment of prey was reasonably consistent (CH$_3$Hg:TAM ~51%–61%), although Cd-TAM was variable (~39% [Cyprinodon]–83% [Nereis]; Fig. 1b). The proportion of metals extracted from prey using in vitro digestion (i.e., GEM) varied considerably among prey species (CH$_3$Hg-GEM: ~39%–82%; Cd-GEM: ~68%–94%). A comparison of these data sets, however, shows that, for 7 out of the 12 prey × metal combinations (6 prey; 2 metals), TAM and GEM were similar (Tukey’s honestly significant difference, p > 0.05; Fig. 1b). In cases where TAM and GEM differed, GEM was consistently higher (~12%–21%). These data suggest that $^{109}$Cd and CH$_3$Hg stored as TAM in prey are available for trophic transfer as these metals are released into the gut fluid upon digestion by the predator (i.e., TAM = GEM for both $^{109}$Cd and CH$_3$Hg).

This conclusion is in general agreement for the trophic transfer of CH$_3$Hg because mummichog AEs for this metal ranged from ~55% to 81% and were found to be either 1) similar to TAM (2 cases [Palaemonetes, Cyprinodon]; Tukey’s honestly significant difference, p > 0.05), 2) bracketed by TAM and GEM (1 case [Nereis]), or 3) slightly higher (~15%–Tukey’s honestly significant difference, p < 0.05) than the 2 assessments of bioavailability (Fig. 1b). These data suggest that CH$_3$Hg associated with TAM of prey is released into the gut fluid (GEM) of fish and subsequently is absorbed and assimilated. Because GEM is controlled by the storage of CH$_3$Hg as TAM in prey (i.e., TAM = GEM), the trophic transfer of CH$_3$Hg (with this predator and
these prey) was driven by a prey-dependent process (i.e., storage of CH$_3$Hg as TAM). This, however, was not the case for $^{109}$Cd where mummichogs assimilated very little of the ingested metal (3%-11%), with no overall agreement being found between AE and TAM or AE and GEM (i.e., the lowest Cd-TAM [-40%] and lowest Cd-GEM [-32%], both from Cyprindodon, were matched with the highest Cd-AE [-11%]; Fig. 1b). This indicates that, although Cd associated with TAM of prey was trophically available for uptake (i.e., released into the gut juice; TAM = GEM), it was not transported across the mummichog’s gut lining and therefore not assimilated to any appreciable extent. This greatly reduced Cd assimilation (as compared with TAM or GEM) perhaps is due to exclusion of Cd at the gut membrane by mucous (Farmanfarmaian 1985), suggesting that, unlike CH$_3$Hg, $^{109}$Cd trophic transfer (with this predator and these prey) was driven by a predator-dependent process.

The results from this study suggest that, in cases when metals are transferred readily across the gut lining of the predator, internal partitioning by prey (i.e., a prey-dependent process) may be the dominant factor controlling metal trophic transfer. Alternatively, in cases where metals can be excluded at the gut lining, these predator-dependent processes supersede any influence on metal trophic transfer resulting from the subcellular partitioning of metal by prey. In conclusion, although the partitioning of TAM in prey may be a useful 1st principle for some predator–prey–metal combinations, metal trophic transfer is complex and both prey- and predator-dependent processes need to be considered.

References


APPLICATIONS OF DIFFUSIVE GRADIENTS IN THIN FILMS (DGT) FOR METALS-RELATED ENVIRONMENTAL ASSESSMENTS

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The now widely accepted free-ion activity model stipulates that bioavailability and, hence, toxicity of metals in aqueous systems are controlled by the free ionic activity of metals rather than by their total (dissolved + particulate) or dissolved metal fractions. This is due to the fact that free metal ions (e.g., Cu$^{2+}$) are readily taken up by aquatic organisms, whereas particulate and strongly complexed metals are not. In natural and human-influenced waters, trace metals may exist as a suite of compounds that exhibit marked differences with respect to their bioavailability and hence toxicity. Such compounds include free