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ASSIMILATION EFFICIENCIES OF CHEMICAL CONTAMINANTS IN AQUATIC INVERTEBRATES: A SYNTHESIS

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Abstract—Assimilation efficiencies of contaminants from ingested food are critical for understanding chemical accumulation and trophic transfer in aquatic invertebrates. Assimilation efficiency is a first-order physiological parameter that can be used to systematically compare the bioavailability of different contaminants from different foods. The various techniques used to measure contaminant assimilation efficiencies are reviewed. Pulse-chase feeding techniques and the application of gamma-emitting radiotracers have been invaluable in measuring metal assimilation efficiencies in aquatic animals. Uniform radiolabeling of food is required to measure assimilation, but this can be difficult when sediments are the food source. Biological factors that influence contaminant assimilation include food quantity and quality, partitioning of contaminants in the food particles, and digestive physiology of the animals. Other factors influencing assimilation include the behavior of the chemical within the animal's gut and its associations with different geochemical fractions in food particles. Assimilation efficiency is a critical parameter to determine (and to make predictions of) bioaccumulation of chemicals from dietary exposure. Robust estimates of assimilation efficiency coupled with estimates of aqueous uptake can be used to determine the relative importance of aqueous and dietary exposures. For bioaccumulation of metals from sediments, additional studies are required to test whether metals bound to the acid-volatile sulfide fraction of sediments can be available to benthic deposit-feeding invertebrates. Most assimilation efficiency studies have focused on chemical transfer in organisms at the bottom of the food chain; additional studies are required to examine chemical transfer at higher trophic levels.

Keywords—Assimilation efficiency Metals Organic contaminants Invertebrates

INTRODUCTION

Aquatic invertebrates are exposed to chemicals from both the particulate and dissolved phases. Recently, there have been renewed interest and new revelations in the trophic transfer of contaminants in aquatic food chains as food uptake has been increasingly recognized as an important source for contaminant accumulation [1–6]. One critical parameter in understanding and modeling a contaminant's trophic transfer and accumulation in aquatic systems is the assimilation efficiency (AE) of the contaminant in animals from ingested food [7,8]. Contaminant trophic transfer can be controlled by its AE and efflux rate and by the animal's feeding and growth rates. In principle, the AE is a first-order physiological parameter that can be quantitatively compared among different chemicals, species, and food particles under various environmental conditions. Furthermore, metal bioaccumulation has been shown to be directly proportional to AE for metals, which highlights the significance of AE in understanding and predicting chemical bioaccumulation [9]. Given the difficulties in quantifying chemical bioavailability from food in previous studies involving long-term exposures, measurement of AEs is an important approach to addressing contaminant bioavailability [4].

In bioenergetic studies, absorption of an element or compound equals total ingestion of the substance minus its quantity in fecal matter and is the sum of assimilation and postdigestive soluble excretion (i.e., loss of material into the dissolved phase after postingestive metabolism). Thus, AE is the fraction of ingested elements or compounds that is incorporated into biological tissue, whereas absorption efficiency is the fraction of ingested material that is taken up across the gut lining [10]. Assimilation thus equals absorption minus excretion. These terms (assimilation efficiency and absorption efficiency) have often been used interchangeably in ecotoxicological studies.

Until recently, AE in aquatic invertebrates, particularly for metals, has been largely ignored in ecotoxicological studies for two major reasons. First, food was not considered to be an important source for metal uptake in aquatic invertebrates. Most previous studies focused on contaminant uptake from the dissolved phase (e.g., development of the free metal ion model, reviewed by Campbell [11]). Second, the techniques to measure chemical assimilation were not well developed and standardized.

In this review, we discuss the application of AE measurements in kinetic bioaccumulation models and critically examine the methodology used in AE measurements in aquatic invertebrates to evaluate the advantages and disadvantages of each method. Because AE methodology is chemical and species specific, both biological and chemical factors are considered. We summarize recent measurements of chemical (metals and organic compounds) AEs in diverse aquatic invertebrates, especially marine zooplankton, bivalves, and deposit feeders, which have been most extensively studied. Mechanisms underlying chemical assimilation and the importance of AE in understanding bioaccumulation and toxic effects of chemicals are discussed. We do not consider the assimilation of contaminants in fish, nor do we examine in detail the significance of AEs for delineating contaminant exposure pathways for invertebrates; that has been reviewed elsewhere [12].

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APPLICATION OF ASSIMILATION EFFICIENCY TO KINETIC MODELING OF CONTAMINANT BIOACCUMULATION

One approach that has recently received considerable attention for studying contaminant bioavailability and separation of the relative importance of different pathways of chemical uptake (e.g., food vs water) uses a bioenergetics-based kinetic model [8]. This model can incorporate various environmental and biological conditions that are likely to be encountered by animals in natural waters and thus has great appeal to environmental scientists. This model has been applied to study metal accumulation and to quantitatively separate the pathways of metal uptake in marine suspension- and deposit-feeding invertebrates [13–19]. According to this model, which treats chemical accumulation in terms of bioenergetics, contaminant accumulation can be described by the following first-order equation, assuming that chemicals are available from both the dissolved and particulate phases [7,8]:

$$dC_A/dt = (k_u \cdot C_w) + (AE \cdot IR \cdot C_f) - (k_e + g) \cdot C_A \quad (1)$$

where C_A is the chemical concentration in the animals (μg/g), t is the time of exposure (d), k_u is the uptake rate constant from the dissolved phase (L/g·d^{-1}), C_w is the chemical concentration in the dissolved phase (μg/L), AE is the chemical assimilation efficiency from ingested particles, IR is the ingestion rate of the animal (mg/g·d^{-1}), C_f is the chemical concentration in ingested particles (μg/mg), k_e is the efflux rate constant (per day), and g is the growth rate constant (per day).

Under steady-state conditions, Equation 1 becomes

$$C_{ss} = [(k_u \cdot C_w) + (AE \cdot IR \cdot C_f)]/(k_e + g) \quad (2)$$

where C_{ss} is the chemical concentration in the animals (μg/g) at steady state. Chemical concentrations in organisms obtained from both the dissolved phase ($C_{w,ss}$, μg/g) and food ($C_{f,ss}$, μg/g) can also be calculated as follows:

$$C_{w,ss} = (k_u \cdot C_w)/(k_{ew} + g) \quad (3)$$

$$C_{f,ss} = (AE \cdot IR \cdot C_f)/(k_{ef} + g) \quad (4)$$

where k_{ew} and k_{ef} are the efflux rate constants following chemical uptake from the dissolved phase and particulate phase, respectively. Thus, the fraction of chemical coming from the food uptake pathway (R) is

$$R = C_{f,ss}/C_{ss} \quad (5)$$

The trophic transfer factor (TTF) is the ratio of chemical concentration in trophic level n to concentration in trophic level $n - 1$, and can be calculated as follows:

$$TTF = C_{f,ss}/C_f = (AE \cdot IR)/(k_{ef} + g) \quad (6)$$

Bioaccumulation can thus be assessed on the basis of several biological and geochemical parameters. Previously, it has been considered difficult to quantify chemical bioavailability in aquatic organisms because the processes responsible for bioaccumulation and bioavailability were not well defined. The kinetic model, which requires measurements of rate constants of uptake and loss of contaminants in animals, provides a conceptual framework in which chemical bioavailability and trophic transfer are clearly defined and quantified [4,16]. In marine bivalves and copepods, the metal concentrations predicted by this model were very close to measured metal concentrations in animals collected from the field [13,15; N.S. Fisher et al., unpublished manuscript]. These studies therefore suggest that the simple kinetic model accounts for all the major factors that influence metal bioaccumulation in these animals and that the model can be used to predict metal accumulation in marine invertebrates on a site-specific basis. Furthermore, the effects of variability of each parameter described in the model under different environmental and biological conditions can be experimentally measured. With this model, metal bioavailability can be evaluated and quantified by the bioaccumulation factor, defined as the ratio of metal concentration in an organism to the metal's total ambient concentration (including particulate and dissolved phases).

METHODOLOGY IN ASSIMILATION EFFICIENCY MEASUREMENTS

Nonradiotracer approach

Assimilation efficiency is most commonly measured by mass balance and ratio methods. The mass balance method requires quantification of total ingestion, excretion, and egestion (i.e., loss of material in feces after absorption or postingestive metabolism) of the chemical in question, and AE is calculated as follows:

$$AE = (Ingestion - Excretion - Egestion)/Ingestion \quad (7)$$

For small aquatic invertebrates, however, it is difficult to measure total egestion (e.g., collecting the tiny fecal pellets), ingestion rate (particularly for deposit-feeding animals), and excretion; therefore, application of this method to these animals is limited. In the ratio method, an inert tracer (e.g., insoluble Cr_2O_3) is used to indicate the passage of food materials. Conover [20] modified this method to measure food assimilation in marine copepods by using the ash content of food particles as an inert tracer. By quantifying the ratios of ash-free dry weight to total dry weight in both food and feces, the AE of organic matter can be computed with the following equation [20]:

$$AE = [(F' - E')/(1 - E') \cdot F'] \cdot 100 \quad (8)$$

where F' is the ash-free dry weight/dry weight ratio in the ingested food, and E' is the same ratio in a representative sample of feces. Conover [20] found good agreement between assimilation measured by the mass balance and ash-ratio methods in copepods feeding on diatoms and dinoflagellates. This ratio method was then widely used to study organic matter assimilation in marine suspension feeders because it does not require complete recovery of fecal material. Instead, this method requires that the food particles contain measurable amounts of ash (e.g., siliceous tests of diatom cells), which is not assimilated by the animals and is effectively resistant to dissolution in the gut.

Radiotracer approach

Application of radiotracer techniques to the study of AE in aquatic animals has facilitated both methods. For example, C assimilation from food can be measured by the mass balance method using ^{14}C as a radiotracer. To calculate C AE, the total amount of ^{14}C ingested and egested (including excretion) must be known. Alternatively, C assimilation can be determined by the ratio method. Calow and Fletcher [21] developed the ^{14}C:^{51}Cr ratio method to measure C assimilation in aquatic invertebrates, with the assumption that Cr acts as an inert tracer during food gut passage. In their method, only ^{14}C:^{51}Cr ratios in the food and feces need be determined, and C AE is calculated by

$$AE = [1 - (^{14}C:^{51}Cr \text{ in feces})/(^{14}C:^{51}Cr \text{ in food})]\cdot 100 \quad (9)$$

Several conditions must be met for the radiotracer ratio method. The gut passage times must be identical for both elements. If gut passage times are not identical, then the $^{14}C:^{51}Cr$ ratio in the cumulative feces produced during gut evacuation should be used to calculate C AE [22,23]. The losses from feces into the dissolved phase should also be similar for both elements. It is also critical that ^{51}Cr must be truly inert to the animals. Many studies have shown that the trivalent ^{51}Cr is largely inert to animals [23,24], but a few studies also indicate that Cr(III) can be appreciably assimilated in some marine bivalves. For example, the AE of Cr(III) in the marine clam *Macoma balthica* feeding on bacteria can be as high as 80% [25]. Under such circumstances, it is inappropriate to use ^{51}Cr as an inert tracer. An important assumption implicit in this approach is that these two radioisotopes ingested by the animal must be in the same ratio as in the bulk food.

Both the mass balance and the $^{14}C:^{51}Cr$ ratio methods were recently used to measure C AEs in marine copepods and mussels ingesting different algal species [23,26]. In the mass balance method, total egestion is the sum of ^{14}C radioactivity of all feces collected during the depuration period, and total ingestion is the sum of ^{14}C radioactivity in cumulative feces and tissues after depuration. Respiratory loss of C was considered a small fraction of the total C budget in these organisms and was ignored. The release of C from fecal material into the dissolved phase was minimized by collecting fecal pellets as frequently as possible. With application of the ratio method to bivalves, it was necessary to use the $^{14}C:^{51}Cr$ ratio in the cumulative feces (instead of feces collected at one time point) to calculate the overall C AE because of the difference in gut passage times of C and Cr [22]. In both copepods and mussels, C AEs calculated by the mass balance and ratio methods were comparable (e.g., in mussels feeding on diatoms, AE was 85% when determined by the mass balance method and 86% when calculated by the ratio method [23]), suggesting that both methods can be used to quantify C assimilation in marine herbivores. For copepods, the ratio method was determined using a point sample because the gut passage times of C and Cr were similar.

Other inert radiotracers have also been sought to examine chemical assimilation in invertebrates. Weeks and Rainbow [27] proposed using Co, and Fisher and Reinfelder [28] proposed using Am. Amercium shows very little assimilation in most invertebrates [15,16,28,29] but is appreciably assimilated by marine clams (e.g., *M. balthica* and *Mercenaria mercenaria*) [30]. Both Am and Cr essentially remain on algal cell surfaces and penetrate very little into the cytoplasm [23,31,32]. Assimilation of Co by the amphipod *Orchestia gammarellus* is minor [27], but Co can be highly assimilated by many other invertebrates [16,23,30], and its applicability as an inert tracer may be limited.

For many metals that have commercially available gamma-emitting radioisotopes, AE measurements can be rather straightforward and do not require an inert radiotracer to follow the food passage. The gamma radioactivity in live animals can be counted nondestructively throughout an experiment, so the total amount of radioactivity ingested and subsequent loss of radioactivity from the animals can be accurately quantified. This technique does not necessitate complete recovery of total feces egested, and it is easier to use in measuring AE than the traditional mass balance method.

Using radiotracers to measure the bioaccumulation of organic contaminants is more complicated than for metals because the isotopes used, typically ^{14}C or ^{3}H, have lower specific activities than most metal radioisotopes; consequently, higher concentrations are required, often resulting in unrealistic contaminant concentrations. Moreover, analysis of these beta emitters requires that animals need to be killed for radioactivity measurements. Therefore, many more animals are required for each experiment and biological variability is likely to be greater (especially for organisms with complex behavior) than with gamma-emitting radioisotopes, which can be measured in the same individual organisms over time.

Other approaches

Assimilation efficiences of organic contaminants are commonly measured by three methods [33]. The first method (total organic carbon [TOC] method) is a modified mass balance method in which the AE is calculated by comparing the relative concentration of chemicals in the sediments to that in the fecal material on a carbon-normalized basis. This method also calibrates for the selectivity of the animals for the organic carbon–enriched sediment fraction. Using this method, absorption of C during gut passage needs to be known. In the second method (feeding method), also a modified mass balance method, AE is determined by measuring the chemical body burden and the feeding rate of the animals. Feeding selectivity for organic carbon is used to calculate the concentrations of organic contaminants in ingested sediment. In the third method [33–36], ^{51}Cr or ^{14}C-polydimethylsiloxane is used to indicate the passage of sediment particles. Polydimethylsiloxane is a nonassimilated organic tracer that readily sorbs to the organic matter fraction of sediments [33].

Uniformly radiolabeled particles

It is presumed that radioisotopes behave identically to stable isotopes of the same atoms. Producing uniformly radiolabeled phytoplankton (or other microorganisms) is relatively easy because of the rapid growth and short generation time of these organisms. Typically, algal cells are grown for several generations (usually 3–7 d, depending on the species) while being exposed to radioisotopes and nutrients. Equilibrium between isotopes and algae can be reached within days [37]. Cells are then harvested from radioactive water and resuspended in unlabeled water, where they are grazed by herbivores. This technique has been routinely used to study chemical assimilation in diverse marine herbivores [23,29,32,38].

It is more difficult to uniformly radiolabel inorganic particles, particularly particles that have several geochemical components, such as sediments. Once in contact with the sediments, radioisotopes typically display a multiphasic sorption pattern, in which there is a rapid adsorption onto particle surfaces, followed by slower binding with the sediment matrix [39]. The radiolabeling time may therefore significantly affect chemical AEs in animals feeding on sediments. S.B. Griscom and N.S. Fisher [unpublished manuscript] recently demonstrated that metal AEs in marine bivalves ingesting sediments were two to five times lower for sediments radiolabeled for 6 months than for the same sediments labeled for 3 d. The effect of sediment aging is presumably due to the association of radioisotopes with different sediment components (easily exchanging pools, iron or manganese oxides, carbonates, labile and possibly refractory organic matter, and sulfides) as a function of labeling time. For the marine polychaete *Nereis suc-*

activity measured for whole individual animals may include already assimilated material, and AE will be overestimated.

To produce radioactive food for suspension feeders, phytoplankton (or other suspended particles) are typically radiolabeled and then resuspended (via centrifugation or resuspension off membrane filters) in unlabeled water so that the only radioactivity in the feeding suspension is associated with the suspended food particles. By doing this, the uptake from food alone can be measured without trying to discern the simultaneous uptake from the dissolved phase. However, during the radioactive feeding period, radioisotopes associated with the food particles may desorb into the dissolved phase after resuspension in the unlabeled water, and uptake from the dissolved phase may contribute to the total amount of radioactivity taken up by the animals. To minimize concentrations of desorbed metals from radiolabeled particles in feeding suspensions, the radioactive food particles can be resuspended several times before being provided to the animals to remove the weakly bound metals by desorption into unlabeled water. In addition, uptake from the dissolved phase can be monitored by removing the radiolabeled food particles (after resuspension) and then exposing control animals to the water containing the desorbed radioisotope. Any radioactivity associated with these animals due to uptake from the dissolved phase should be subtracted from the radioactivity of animals that fed on radioactive food to determine the radioactivity due to ingestion of food. For marine mussels and copepods, uptake due to metal desorption during the short pulse-feeding period is typically negligible for most metals (Ag, Cd, Co, Se, and Zn) [17,23].

It is evident that when working with radioisotopes to trace the uptake and loss of a chemical, it is necessary to have adequate amounts of radioactivity in all samples to allow statistically accurate and precise radioactive measurements in reasonably short counting times (typically 1–2 min). For marine suspension-feeding bivalves (e.g., mussels), which generally have high feeding rates, producing sufficiently radioactive animals during a 20-min pulse feeding (shorter than the gut transit time) is easily accomplished [44]. For animals that have a lower feeding rate, the food particles can be labeled with greater radioactivity or the animals can be fed for a longer period of time. However, if the feeding period is longer than the gut passage time, fecal pellets egested during the pulse-feeding period should be collected and assayed. In marine calanoid copepods, the fecal pellets can account for 20 to 50% of the radioactivity retained in copepods after 1 h of feeding on radiolabeled phytoplankton. Assimilation efficiencies would be overestimated by a factor of 1.3 to 1.5 if this pool was not considered in calculating the total amount of radioactivity ingested [17,26]. Inclusion of the fecal pellet pool is based on the assumption that there is negligible desorption of radioisotope from the fecal pellets during the initial egestion period. Such an assumption has not been tested rigorously for most animals. Among copepods, the initial metal loss into the dissolved phase from fecal pellets is negligible for *Acartia tonsa* and *Temora longicornis* [26,45] but not for *Anomalocera patersoni* [38].

Conditions of depuration

The length of the depuration period should be sufficient for complete assimilation and digestion of food. A shorter depuration period will result in an overestimation of assimilation. For example, depuration must be able to encompass the biphasic (i.e., extracellular and intracellular) digestion in marine bivalves, which lasts for several days. When the ^{14}C:^{51}Cr ratio method was first developed, it was presumed that it was not necessary to recover total feces [21]. More recent studies have shown that the gut passage time of Cr in mussels is shorter than that of C (see Bricelj et al. [22], Wang and Fisher [23], and above). It is therefore necessary to fully recover the feces when C assimilation is measured by the ratio method. Alternatively, AEs can be calculated as a function of depuration time and will decrease as depuration proceeds due to the faster egestion of ^{51}Cr.

It has been consistently shown that the time for complete assimilation of trace metals into the organisms, not just into the gut epithelium, is much longer than the time for gut passage of food in many marine invertebrates, including mussels [23,44], copepods [17], and polychaetes [18]. The gut passage time of food in marine mussels is less than 3 h (indicated by Cr passage because Cr is mostly associated with algal cell wall and inert to the mussels), but metal retention time in mussel gut can be as long as 3 d for Co [23]. Gut retention time of metals in marine mussels is highly dependent on the type of ingested food and is significantly correlated with metal assimilation [23]. In marine copepods, the gut retention time of metals is 4 to 15 h [17], whereas the gut transit time of food is less than 30 min [46]. In contrast to metal assimilation in marine mussels, there is little evidence that metal assimilation in copepods is related to retention times. Two methods have been used to calculate the metal retention time in animal guts. The first method defines the time at which 90% of cumulative feces is recovered as the gut retention time of metals [23,44,47]. In the second method, AE is first computed as the y intercept of the log percentage of contaminant retained in the physiologically exchanging (slowest) compartment with time, and then the gut retention time is determined when contaminant assimilation is complete [17].

During the depuration period, it is critical to regularly remove the fecal pellets and change the water to minimize fecal pellet release of radioisotope into the dissolved phase and recycling of chemicals by the animals. In addition, a constant food supply, ideally identical to the feeding conditions during the hot feeding, should be maintained throughout the depuration period to ensure steady feeding and egestion rates [44].

Calculation of AE

Two methods are commonly used to calculate metal AE using gamma-emitting radioisotopes, both of which monitor the proportion of metals retained in the animals during the depuration period (gut evacuation). The first method directly calculates metal AE as the fraction of total ingested metal retained in the animals at the time that metal digestion and assimilation are complete. To do this, it is necessary to determine the time at which digestion is complete, usually in preliminary experiments in which fecal material is continuously measured after the radioactive feeding; digestion is considered complete when there is no detectable radioactivity in the feces. For example, Decho and Luoma [25] followed the depuration of Cr in two clams (*M. balthica* and *Potamocorbula amurensis*) after pulse feeding of radiolabeled bacteria. Fecal pellets were collected at short time intervals to minimize desorption of Cr from the egested pellets into the dissolved phase. These investigators were able to show a biphasic egestion pattern of Cr in these clams that would be impossible to identify if the fecal pellets were collected less frequently. Decho and Luoma [25] concluded that the time required for com-

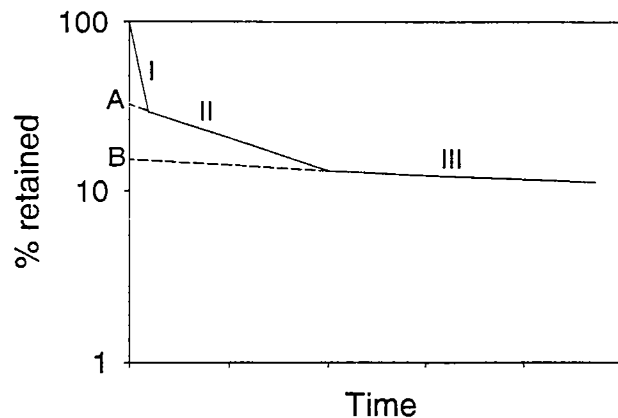

Fig. 2. Representative depuration patterns of contaminants in aquatic invertebrates after pulse feeding on radioactive food. I, II, and III represent different compartments of loss (see text for discussion). The percentage of total contaminant load in compartments I, II, and III is 100 − A, A − B, and B, respectively.

plete digestion of Cr was about 3 d for *M. balthica*, a facultative deposit-feeding bivalve, and 1 d for *P. amurensis*, a suspension-feeding bivalve. Similarly, a biphasic pattern of metal egestion was reported for marine mussels, and the time required for complete digestion of ingested trace metals was about 3 d [44].

A typical metal depuration pattern during the nonradioactive gut chase period is shown in Figure 2. The depuration pattern is typically characterized by a two-compartment (e.g., marine copepods [17]) or three-compartment loss (e.g., marine bivalves [23,30]), depending on the metal and the length of the depuration period. Rapid loss in the first compartment represents the gut passage of food materials, during which some unavailable metals (e.g., those bound to refractory pools of food particles) are purged from the gut. In bivalves, this loss is presumably due to extracellular digestion, in which the digestive enzymes released from the crystalline styles attack the ingested food particles. The second compartment of loss is presumably dominated by intracellular digestion, in which the cells in the digestive glands phagocytize the fine materials channeled from the stomach through small ducts between the stomach and the digestive diverticula [25]. Loss in the third compartment, which may represent a different pool within the organism, may simply reflect the physiological turnover from the slowest exchanging compartment after metal assimilation (i.e., metabolic loss of assimilated metals). In animals that do not have biphasic digestion (e.g., copepods), depuration will be represented by an initial rapid loss (dominated by extracellular digestion) and then a physiological loss from the slower exchanging compartment (or metabolic loss of assimilated metals) [17].

In the second method, metal AE is quantified by finding the y intercept of the slowly exchanging pool after compartmental analysis. This technique has been extensively used in radioecological studies [48]. In this method, metal loss from the animal body is modeled as follows:

$$A = A_0 e^{(-bt)} \quad (10)$$

where A is the percentage of ingested radioactivity that is retained in the animals during the physiological loss from the slowest exchanging pool, A_0 is the AE, b is the depuration rate constant from the slowest compartment, and t is the time of depuration. This method requires that the animals be depurated for a sufficiently long period of time so that the slope of the slowest exchanging compartment can be accurately determined. It is therefore not conducive for rapid assessment of metal assimilation in animals, especially for animals such as bivalves, which have biphasic digestion and which require at least 1 to 2 weeks for depuration of metals. However, the y intercept method is useful for animals in which the time for complete digestion and assimilation is difficult to measure accurately, perhaps due to difficulty in collection of fecal pellets. Assimilation efficiencies were previously measured by depurating the copepods for 4 [26,49] or 18 h [32], assuming that digestion was complete within 4 or 18 h. Wang and Fisher [17] used the y intercept method to determine metal AEs in the copepod *Temora longicornis*. By extrapolating the y intercepts, they determined that the gut passage times of metals in these animals were 4 to 15 h.

ASSIMILATION EFFICIENCIES OF CONTAMINANTS IN AQUATIC INVERTEBRATES

Table 1 summarizes recent measurements of metal AEs in diverse aquatic invertebrates. Earlier measurements of metal assimilation in marine invertebrates are summarized elsewhere [3]. In general, interelemental differences in assimilation are greater than intraelemental differences. Essential elements (Se and Zn) are generally assimilated with a higher efficiency than nonessential elements, although exceptions can be found. For example, methylmercury (CH_3Hg) is assimilated by marine animals with a much higher efficiency than most trace elements, including essential elements [42,50,51], possibly reflecting its lipophilic nature. Among different animal species, there are no major differences in metal AEs, even though animals have distinct digestive strategies (e.g., copepods and bivalves). Assimilation efficiencies are also dependent on the chemical species of metal. For example, Cr(VI) and CH_3Hg(II) are assimilated at a much higher efficiency than Cr(III) and Hg(II) by marine mussels [24,51].

Assimilation efficiencies have been measured for only a few organic compounds, including benzo[*a*]pyrene (B*a*P), hexachlorobiphenyl, hexachlorobenzene, and fluoranthene (Table 2). Kukkonen and Landrum [33] compared three methods (TOC, feeding, and dual-tracer methods; see above) in measuring organic contaminant AEs. Assimilation of B*a*P measured by both the TOC and dual-tracer methods was comparable in the oligochaete *Lumbriculus variegatus*, a general deposit feeder that processes the heterogeneous sediment quickly. Assimilation efficiencies of B*a*P in the amphipod *Diporeia* sp. measured by the TOC and dual-tracer methods, however, were much higher than the AE determined by the feeding method. Because of the differential distribution of B*a*P among the sediment particles and the selective feeding by *Diporeia*, Kukkonen and Landrum [33] concluded that both the TOC and dual-tracer methods failed to trace B*a*P accurately.

In natural waters, animals are constantly exposed to a variety of food conditions, and it is likely that chemical assimilation varies considerably with these conditions. Metal assimilation has been shown to be influenced by both abiotic and biological factors, especially characteristics of the food supply (e.g., quantity and quality) [16]. In marine bivalves, food quality has the greatest effect on metal assimilation [16,23,25,52]. Assimilation efficiencies can vary by up to fivefold for Se, ninefold for Ag, and 10-fold for Cr(VI) in mussels feeding on different food particles. In the clam *M. balthica*, AEs of Cr(III)

Table 1. Assimilation efficiencies of metals reported for aquatic invertebrates

Animal	Food	Assimilation efficiency (%)							
		Ag	Cd	Co	Cr	Hg(II)	CH_3Hg	Se	Zn
Ciliate									
Fabrea salina	Prymnesiophyte	22 [72]							
Copepod									
Acartia tonsa	Diatom	17 [32]	30 [32]			15 [50]	62 [50]	97 [32]	27–47 [32]
Temora longicornus	2 diatoms	8–19 [17]	33–37 [17]	14 [17]				59 [17]	58–64 [17]
	Natural seston	15 [17]	53 [17]	20 [17]				50 [17]	52 [17]
Polychaete									
Nereis succinea	Oxic sediments	16–30 [18]	17–29 [18]	38–85 [18]		7–22 [42]	66–75 [42]	29–52 [18]	21–53 [18]
Mussel									
Mytilus edulis	2 diatoms, 2 chlorophytes, 2 dinoflagellates, 1 prasinophyte	4–34 [23]	11–40 [23]	14–43 [23]	0–1 [24]			15–72 [23]	16–48 [23]
	Oxic sediments	4–15 [65][a]	15–25 [65][a]	15–30 [65][a]	1.2 [24]	2 [51]	55 [51]		
Oyster									
Crassostrea virginica	Prymnesiophyte	44 [30]	69 [30]	34 [30]				70 [30]	73 [30]
Clam									
Macoma balthica	1 diatom, 1 prymnesiophyte	38–49 [30]	69–88 [30]	45–53 [30]	3 [61]			74–78 [13,30]	50–64 [30]
	Natural seston		13–21 [19]		4–5 [19]				47–70 [19]
Mercenaria mercenaria	1 diatom, 1 prymnesiophyte	22–35 [30]	66–83 [30]	29–34 [30]				92 [30]	86 [30]
Potamocorbula amurensis	Natural seston		21–44 [19]		2–5 [19]				35–41 [19]
Zebra mussel									
Dreissena polymorpha	2 diatoms, 1 chlorophyte, 1 cyanophyte, natural seston	4–16 [73]	19–72 [73]		5–6 [73]			18–46 [73]	
Barnacle									
Balanus amphitrite	2 diatoms		35–86[b]		22–26[b]			79[b]	76–88[b]
	2 copepods		72–88[b]		32–36[b]			63–66[b]	93[b]
Seastar									
Marthasterias glacialis	Mussels	69 [74]	73 [74]	73 [74]					78 [74]

[a] Also S.B. Griscom and N.S. Fisher, unpublished data.
[b] W.-X. Wang, J.W. Qiu, and P.Y. Qian, unpublished data.

Table 2. Assimilation efficiencies of organic contaminants reported for aquatic invertebrates

Species	Food	Compound[a]	Method[b]	AE[c] (%)
Copepod				
Acartia tonsa	Diatom	TCB	Mass balance	37–41 [75]
Temora longicornus	Diatom	TCB	Mass balance	41–45 [75]
Amphipod				
Diporeia sp.	Sediments	B*a*P	TOC	46–60 [35]
	Sediments	B*a*P	Feeding	7–33 [76]
	Sediments	B*a*P	TOC	45–57 [33]
			Feeding	11–15 [33]
			Dual tracer	56 [33]
	Sediments	HCBP	TOC	46–58 [33]
			Feeding	36–52 [33]
Oligochaete				
Limnodrilus hoffmeisteri	Sediments	HCBP	C:Cr ratio	15–37 [34]
Lumbriculus variegatus	Sediments	B*a*P	TOC	0–26 [33]
			Dual tracer	10–26 [33]
Clam				
Macoma nasuta	Sediments	HCB	TOC	39–57 [77]
Zebra mussel				
Dreissena polymorpha	Algae	B*a*P	Mass balance	53–92 [58,59]
		HCBP		69–98 [58,59]
		DDT		45 [58,59]
		TCB		78 [58,59]
	Sediments	B*a*P		21–46 [58,59]
		HCBP		30 [58,59]
		DDT		23 [58,59]
		TCB		1 [58,59]
Snail				
Potamopyrgus antipodarum	Sediments	Fluoranthene	C:Cr ratio	36–42 [36]

[a] B*a*P = benzo[*a*]pyrene; HCB = hexachlorobenzene; HCBP = hexachlorobiphenyl; TCB = tetrachlorobiphenyl.
[b] The total organic carbon (TOC) method used organic carbon as a tracer based on feeding selectivity index and the relative concentrations in the sediment and fecal material. The feeding method calculated assimilation from ingestion based on feeding rate and the selectivity index for organic carbon. One dual-tracer method used ^{14}C-polydimethylsiloxane as an inert tracer; another used ^{51}Cr as an inert tracer.
[c] AE = assimilation efficiency.

vary by 20-fold for clams feeding on bacteria and phytoplankton [25,52]. During the spring phytoplankton bloom in San Francisco Bay, a shift in seston composition to phytoplankton resulted in a notable increase in Cd, Zn, and Cr AEs in two clam species [19]. Availability of sediment-bound metals to deposit-feeding clams is, likewise, highly dependent on sediment type [53]. When sediment particles are coated with bacterial and extracellular polymers, the bioavailability of Cd, Zn, and Ag is enhanced considerably [54]. Several studies have found that hydrous iron oxides tend to decrease the bioavailability of sediment-associated Ag, As, Co, Cu, Pb, and Zn [55,56]. In addition, assimilation of metals in mussels is inversely related to food quantity [57], but this effect is small compared with the effect of food quality [44]. Food quality has also been shown to significantly affect assimilation of PCB congeners, DDT, and B*a*P in zebra mussels [58,59].

In marine copepods, there is no substantial effect of food quantity or quality on metal assimilation. Metal AEs are comparable in copepods ingesting different phytoplankton diets and different food concentrations [17,26]. Similarly, in the polychaete *N. succinea*, Wang et al. [18] recently found that the type of sediments (different organic carbon content and sediment grain size) had relatively little influence on trace metal assimilation. The only significant effect of sediment characteristics was found for anoxic sediments, in which Cd, Co, Zn, and Ag assimilation decreased considerably compared with metals associated with oxic sediments.

A mechanistic understanding of the factors controlling chemical assimilation is available for only a few aquatic organisms, particularly bivalves and copepods. Feeding physiology of the animals (e.g., gut volume, gut passage time, and intracellular and extracellular digestive partitioning) and cellular partitioning and biochemical composition of food particles can all affect chemical assimilation [16]. Reinfelder and Fisher [32,60] demonstrated that in marine copepods and bivalve larvae, both of which have short gut passage times, metal assimilation is directly proportional to the partitioning of the metals in the algal cytoplasm. A 1:1 relationship between metal assimilation and metal distribution in algal cytoplasm was found for these animals [32,49,60]. These studies indicated that metal assimilation in animals characterized by a simple gut structure and digestive process can be reasonably well predicted on the basis of the cytoplasmic distribution of metals in algal cells. In mussels, a significant relationship between metal assimilation and metal distribution in algal cytoplasm has been observed for Am, Co, and Se, whereas for other metals that tend to associate with proteins (Ag, Cd, and Zn), no simple relationship between AE and algal cytoplasmic distribution was evident [23]. Gut retention time [23,44] and the relative importance of intra- and extracellular digestion [44,52,61] can both affect metal assimilation in bivalves. Decho and Luoma [52,61] showed a positive relationship between Cr assimilation in clams from ingested bacteria and the percentage of Cr channeled into intracellular digestion. In mussels, higher assimilation of metals at lower food concentrations resulted from more metals being processed by intracellular digestion [44]. Thus, the pliancy of digestive strategies in these animals can have a substantial influence on metal assimilation.

In addition to these biological factors, geochemical factors (e.g., metal desorption and speciation) can greatly affect metal

assimilation. Because some invertebrates have acidic guts containing a high surfactant concentration (pH = 5–6 for bivalves [62] and 6 for deposit-feeding polychaetes [63]; M. Ahrens and G. Lopez, unpublished manuscript), metal desorption from ingested particles within the gut may affect metal assimilation. Other invertebrates, however, have guts with a neutral pH. Mayer et al. [64] measured chemical desorption rates in vivo by incubating marine sediments with "gut juices" extracted from the deposit-feeding polychaete *Arenicola marina.* This approach to estimating contaminant bioavailability from sediments assumes that only desorbed contaminants are biologically available to the organisms. In mussels, Gagnon and Fisher [65] found that metal AEs (Cd, Co, and Ag) from sediments were positively related to their initial desorption rates from fulvic acid–coated particles in acidic seawater, indicating that desorption may control metal assimilation from ingested sediments. Wang and Fisher [66] suggested that Cd desorption controlled its assimilation in mussels feeding on diatom cells with different Cd concentrations. A higher desorption rate from food particles containing higher Cd content resulted in a higher Cd assimilation in mussels.

Mayer et al. [64] indicated that there was a linear relationship between metal desorption in seawater and in gut juices; thus, metal desorption measured in seawater should indicate desorption within the animal gut. Desorption of metals in seawater at pH 8.0 and 6.0 had no consistent relationship with metal assimilation in the polychaete *N. succinea,* indicating that desorption was not solely responsible for the variation of metal AEs in these worms [18]. However, Weston and Mayer [67] showed a significant correlation between desorption and bioaccumulation for PAHs in polychaetes.

IMPORTANCE OF CONTAMINANT ASSIMILATION EFFICIENCIES

Sensitivity analysis has shown that the overall bioaccumulation of metals in aquatic invertebrates is strongly related to AEs, particularly for metals that are accumulated predominantly from ingested food particles [9,12,15,17]. For these metals, any change in AE will result in a change in their accumulation in the organisms. For example, Cr accumulation in marine mussels is very sensitive to a change in Cr(III) assimilation from ingested particles. Even a slight change in Cr(III) assimilation from 0.2 to 1.0% (within the range of Cr(III) assimilation measured for various food particles) would lead to a predicted increase in Cr concentration in San Francisco Bay mussels from 1.9 to 5.6 μg/g (the measured Cr concentrations in San Francisco Bay mussels are 3.0 to 5.1 μg/g), and the fraction of Cr body burden coming from the dissolved phase as Cr(VI) would decrease from 52 to 18% [24]. In mussels, only Cr(III) from ingested food particles and Cr(VI) from the dissolved phase are shown to contribute to the overall Cr accumulation.

The relative importance of metal uptake from dissolved and food phases is also dependent on the metal AE (except for Se, for which nearly all the bioaccumulation is from food). As a rule, the dietary exposure pathway increases in importance for any animal as AE values increase, as shown for mussels [16], worms [18,42], and copepods [17]. For example, in copepods, the predicted percentage of total uptake of Ag and Co from the dissolved phase decreases from 90 to 50% with an increase in metal AE over the range typically observed for these two metals. For Se and Zn, which are predominantly accumulated from food, the relative importance of different uptake routes is less dependent on variations in AE [17].

Differences in metal AEs may affect trophic transfer and biomagnification. Contaminants with low AEs in animals near the bottom of the food chain (e.g., zooplankton) are unlikely to be transferred up the food chain. A particle-reactive transuranic element like Am, for example, is negligibly assimilated by zooplankton and is unlikely to pass on appreciable quantities up the food chain. For $CH_3Hg(II)$, Mason et al. [50] suggested that its biomagnification in marine systems was mainly due to its high assimilation by marine animals. Assimilation efficiencies of $CH_3Hg(II)$ in a variety of invertebrates are much higher than AEs of Hg(II) and may contribute to the increasing importance of $CH_3Hg(II)$ at higher trophic levels [42,50,51], although lower efflux rate constants of $CH_3Hg(II)$ than of Hg(II) from upper level carnivores cannot be ruled out.

The AE should also be critical for determining the toxicity of a contaminant to a specific animal, although we are unaware of any studies that have directly related a contaminant's AE with its toxicity. Clearly, a contaminant cannot be toxic to an animal if it is not accumulated in its tissues, and there is increasing interest in expressing toxic effects of contaminants to aquatic organisms on a tissue residue basis. The AE essentially measures the efficiency with which an ingested contaminant crosses an animal's gut lining and is available to interfere with some metabolic process (i.e., toxic effect). Thus, a contaminant that is ingested in contaminated food but has an AE of zero in an animal should pose no danger to that animal (or animals higher up the food chain), although the contaminant may be toxic if it is accumulated in tissue from the dissolved phase.

In addition to its toxicological relevance, AE can be important in affecting the residence times of metals in oceanic surface waters [3]. Metals that have low assimilation may be egested as fecal pellets and rapidly sink to deeper waters, leading to a shorter residence time in surface waters. In contrast, metals that have high assimilation will be retained by the animals and should recycle many times before being transported from surface waters. Furthermore, once metals are assimilated by copepods, they are excreted at a high rate (0.07–0.3/d) into the dissolved phase; consequently, the residence times of these metals can be lengthened considerably [68]. Regeneration should play a less important role in lengthening residence time of those metals that are mostly unassimilated by zooplankton.

Even though chemical AE is a first-order physiological parameter, chemical bioavailability from ingested food particles cannot be fully predicted if the feeding rates of animals, chemical concentration in the food particles, and metal efflux rates are not known. Particle selectivity in marine invertebrates may greatly affect chemical uptake from ingested particles. A preferential ingestion of nutritionally rich particles coupled with a high assimilation can considerably increase the overall metal influx from food. One critical parameter is the total suspended solids load in the water column, which can affect the feeding rate of the animals and the chemical concentration in both the dissolved and particulate phases [15]. Wang et al. [15] showed that the total suspended solids load can significantly affect the bioavailability of particle-reactive metals (e.g., Ag and Am) to mussels, whereas particle loads have a relatively minor effect on metals that are less particle reactive.

In summary, substantial progress has been made in recent

years in quantifying chemical assimilation in aquatic invertebrates, largely because of the refinement of pulse-chase feeding techniques and the application of gamma-emitting radiotracers in these studies. Assimilation efficiency and feeding rate are both required to predict the trophic transfer of chemicals in aquatic food chains, but current knowledge of contaminant AEs and feeding rates are limited to only a few important aquatic invertebrates. In addition, many assessments of chemical AEs are based on a single diet, whereas in natural waters, food quality and quantity can vary greatly over temporal and spatial scales. It is not known whether the presence of other food materials (inorganic or organic) can affect chemical assimilation, although evidence of metal assimilation in mussels feeding on mixed particle assemblages showed similar, but slightly lower, AEs compared with pure algal cultures [15].

Many organic compounds of environmental concern are very lipophilic, displaying high octanol/water partition coefficients (K_{ow}). These compounds typically associate primarily with the lipid fraction of organisms; consequently, organisms richer in lipids may be expected to accumulate more of these compounds. This has been particularly evident in arctic organisms, which are rich in lipids and which display elevated concentrations of diverse organic contaminants [69]. For organic contaminants, experimental measurements of AE in aquatic invertebrates are limited to a few benthic animals, but few studies have evaluated the factors that affect AEs of organic contaminants [58,59]. Recent work by Gossiaux et al. [59] indicated a positive correlation between AE in zebra mussels and log K_{ow} of various hydrocarbons. In addition, as with metals, particle selectivity may present a challenge for assessing appropriate AE values in animals that feed selectively. In contrast with metals, however, some organic contaminants are appreciably metabolized by some animals, and this metabolic breakdown of a parent compound in the tissues of animals greatly complicates evaluations of AE of such compounds [70].

The controls of sediment geochemistry on metal assimilation are not well known. Many studies have shown that the ratio of acid-volatile sulfide (AVS) to simultaneously extracted metal in sediments has a direct impact on metal bioavailability and toxicity to deposit-feeding animals [71]. Additional studies are required to measure metal assimilation from different geochemical components of sediments, such as metals associated with the AVS fraction. The chemical behavior of metals in an animal's gut is clearly different from that in anoxic sediments, and metals that are strongly bound to AVS in anoxic sediments may undergo chemical reactions in the gut that may affect assimilation, but this has not been considered in previous studies. Recently, we found measurable assimilation of metals (Ag, Cd, Co, Se, and Zn) in deposit-feeding polychaetes (*N. succinea*) ingesting anoxic sediments [18]. Appreciable assimilation of metals from anoxic sediment was also found in mussels and clams [S.B. Griscom and N.S. Fisher, unpublished manuscript]. In these experimental studies, anoxic sediments were radiolabeled with radiotracers for days (as with most AVS toxicity experiments), but binding of the radioisotopes to AVS was not examined. To test whether AVS can be bioavailable to animals, one could experimentally prepare AVS radiolabeled with metals and then measure AEs in these organisms.

It is presumed that chemicals are available to cross an organism's gut lining primarily when they are in solution (either from the ambient environment or within the gut). It is also possible that organic contaminants are assimilated through coassimilation with lipids and need not dissolve within the gut before assimilation. One experimental approach in quantifying bioavailability includes measuring the chemical extraction efficiency from particles using gut juices prepared in vivo from the animals [64]. However, conflicting observations were found for the relationship between chemical AE and extraction efficiency. Assimilation determines the bioavailable fraction of a desorbed chemical pool within the gut and thus has more toxicological relevance than extraction efficiency. Studies should be conducted to examine the relationship between AE and extraction efficiency measured in gut juices for a variety of invertebrates [67].

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