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Critical Review

Internal Dynamics and Metabolism of Mercury in Biota: A Review of Insights from Mercury Stable Isotopes

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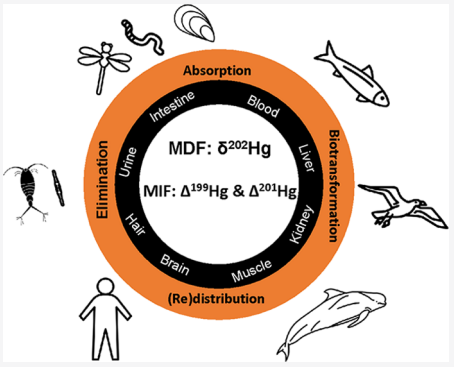
ABSTRACT: Monitoring mercury (Hg) levels in biota is considered an important objective for the effectiveness evaluation of the Minamata Convention. While many studies have characterized Hg levels in organisms at multiple spatiotemporal scales, concentration analyses alone often cannot provide sufficient information on the Hg exposure sources and internal processes occurring within biota. Here, we review the decadal scientific progress of using Hg isotopes to understand internal processes that modify the speciation, transport, and fate of Hg within biota. Mercury stable isotopes have emerged as a powerful tool for assessing Hg sources and biogeochemical processes in natural environments. A better understanding of the tissue location and internal mechanisms leading to Hg isotope change is key to assessing its use for biomonitoring. We synthesize the current understanding and uncertainties of internal processes leading to Hg isotope fractionation in a variety of biota, in a sequence of better to less studied organisms (i.e., birds, marine mammals, humans, fish, plankton, and invertebrates). This review discusses the opportunities and challenges of using certain forms of biota for Hg source monitoring and the need to further elucidate the physiological mechanisms that control the accumulation, distribution, and toxicity of Hg in biota by coupling new techniques with Hg stable isotopes.



KEYWORDS: *biota, bioaccumulation, mercury, metabolism, monitoring, stable isotope*

1. INTRODUCTION

Mercury (Hg) is a globally distributed toxic metal released to environments through diverse natural (e.g., volcanic and hydrothermal eruptions) and anthropogenic activities (e.g., coal combustion, smelting, and mining).[1,2] Mercury undergoes long-range transport and complex biogeochemical processes in natural environments.[3,4] Among various processes, microbial methylation in aquatic sediment[5] and within the water column[6−8] transforms inorganic Hg (iHg) to monomethylmercury (MeHg), a potent neurotoxicant that can bioaccumulate in food webs.[9,10] Monomethylmercury causes significant ecosystem and human health concerns, and general human populations are primarily exposed to MeHg via dietary intake, particularly in fish and rice.[11−13]

Recently, there has been rapid growth in the number of studies reporting spatiotemporal variability in Hg concentrations in various environmental media including biota.[14−17] This is in line with the international convention on Hg, the Minamata Convention on Mercury, to mitigate anthropogenic use and emissions of Hg. In particular, the convention emphasizes the need to implement existing and new monitoring approaches to distinguish between natural and anthropogenic Hg sources subject to regulation, and to evaluate the effectiveness of the convention by tracking Hg in various environmental and biological media.[18] Substantial technical and scientific challenges, however, remain in identifying sources and interpreting Hg concentration, especially in biota. This is because Hg burden in biota is largely controlled by both ecological (e.g., trophic position and feeding behavior) and geochemical factors that govern environmental methylation and demethylation, rather than the degree of Hg input into the system.[14,15,19] Internal metabolic and physiological processes are also known to further modify the chemical forms and concentration of Hg within biota.[20] In regards to Hg sources, the landscape and watershed features have been shown to determine the type of Hg sources (e.g., terrestrial runoff, atmospheric deposition, and industrial releases) introduced to the system.[15,20] Therefore, the biological Hg level alone may not be a precise indicator for Hg contamination level in the environment. In addition, Hg concentration does not provide direct information on the environmental sources of Hg.

The application of Hg stable isotopes has shown promise for deciphering environmental sources of Hg in biota[21−25] and for identifying biogeochemical processes of Hg in natural environments.[21] Growing evidence suggests that Hg speciation and

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isotope ratios vary across biological species, tissues, and life stages as a result of complex internal Hg processes. To apply Hg isotopes for biomonitoring Hg sources and environmental Hg levels, precise characterization of internal Hg isotopic fractionation processes in biota and their underlying mechanisms is needed.

The objective of this review is to illustrate the decadal scientific progress on exposure sources and metabolic processes of Hg within biota using Hg stable isotopes. We open this review with the general systematic of Hg stable isotopes. The main body of the review is divided by the types of organisms, beginning with birds, marine mammals, and humans, which we have the greatest amount of knowledge on the internal dynamics of Hg isotopes, and concluding with fish, plankton, and invertebrates, which we have the least amount of understanding. Each section is guided by a series of questions, including the following:

1) What are the environmental sources, internal dynamics, and fate of different chemical forms of Hg across tissues?
2) What internal processes lead to Hg isotope fractionation between the organism and environmental sources and across tissues?
3) What are the opportunities and challenges of using Hg isotopes in biota for source attribution and biomonitoring?

We close the review with future research directions including coupling other analytical techniques with Hg stable isotopes to further elucidate the mechanisms governing Hg accumulation and toxicity in various types of biota.

2. THE MERCURY ISOTOPE SYSTEM

Mercury has seven stable isotopes with the following natural abundances in the environment: ^{196}Hg = 0.155%, ^{198}Hg = 10.04%, ^{199}Hg = 16.94%, ^{200}Hg = 23.14%, ^{201}Hg = 13.17%, ^{202}Hg = 29.73%, ^{204}Hg = 6.83%.[26] Mercury stable isotopes undergo two different types of fractionation: mass-dependent (MDF) and mass-independent fractionation (MIF). MDF is expressed in δ^{202}Hg in permil (‰) and follows a typical fractionation in which the degree of fractionation is proportional to the mass differences between the isotopes. MDF is calculated using eq 1.

$$\delta^{202}\mathrm{Hg} = [(^{202}\mathrm{Hg}/^{198}\mathrm{Hg})_{\mathrm{sample}}/(^{202}\mathrm{Hg}/^{198}\mathrm{Hg})_{\mathrm{NIST3133}} - 1] \times 1000‰ \quad (1)$$

Most biogeochemical processes occurring in the environment including biotic and abiotic methylation-demethylation, redox reactions, sorption, and volatilization can impart measurable MDF.[21,27] Kritee et al. (2009)[28] first characterized significant MDF during microbial demethylation, and since then, measurable δ^{202}Hg changes were observed within the tissues of a variety of biota. In biological systems, Hg undergoes complicated internal processes, including absorption, partitioning, redistribution, metabolism, and excretion. All these processes have the potential to cause differences in Hg isotope ratios between exposure source and biological tissues; therefore, the MDF signature (i.e., δ^{202}Hg) in biota represents the net change in Hg isotope signatures from the summation of these processes.

MIF is primarily driven by photochemical reactions, and MIFs of both odd mass ($\mathrm{MIF_{odd}}$) and even mass isotopes ($\mathrm{MIF_{even}}$) exist. Unlike MDF, $\mathrm{MIF_{odd}}$ occurs independent of the masses of isotopes and is caused by the differences in the nuclear volume (the nuclear volume effect; NVE)[29] or nuclear spin of isotopes (the magnetic isotope effect; MIE).[30] $\mathrm{MIF_{odd}}$ is expressed in either Δ^{199}Hg or Δ^{201}Hg and calculated using the difference between the measured Δ^{XXX}Hg value and the predicted value based on MDF (eqs 2 and 3).[26] Significant $\mathrm{MIF_{odd}}$ via the NVE has been observed during both kinetic and equilibrium reactions of Hg reduction,[31] liquid−vapor evaporation,[32] Hg-thiol complexation,[33] and recently in the tissues of birds, which we discuss in detail in section 3.1. $\mathrm{MIF_{odd}}$ via the MIE has not been observed in internal tissues of biota and is thought to occur primarily via kinetic reactions of aqueous iHg photoreduction and MeHg photodegradation.[34] $\mathrm{MIF_{odd}}$ during Hg metabolism is thought to be negligible, and the relatively conservative nature of $\mathrm{MIF_{odd}}$ in biota has allowed source tracing and the characterization of environmental conditions (e.g., extent of sea ice and latitude) associated with the extent of MeHg photodegradation.[35−41] In particular, the Δ^{199}Hg/Δ^{201}Hg ratio reflects different photodegradation pathways of Hg before uptake and bioaccumulation into food webs (i.e., 1.36 ± 0.02 for photodemethylation of MeHg and 1.00 ± 0.02 for photoreduction of iHg).[34] Prior studies found that Δ^{199}Hg/Δ^{201}Hg is preserved during trophic transfer and metabolism.[13,25,35,39,40,42−47]

$$\Delta^{199}\mathrm{Hg} = \delta^{199}\mathrm{Hg} - (\delta^{202}\mathrm{Hg} \times 0.252) \quad (2)$$

$$\Delta^{201}\mathrm{Hg} = \delta^{201}\mathrm{Hg} - (\delta^{202}\mathrm{Hg} \times 0.752) \quad (3)$$

While the exact mechanism driving $\mathrm{MIF_{even}}$ is unclear, $\mathrm{Hg^0}$ photo-oxidation in the atmosphere is considered responsible for the positive Δ^{200}Hg and negative Δ^{204}Hg in the product $\mathrm{Hg^{2+}}$.[48] The Δ^{200}Hg in biota has been used to distinguish between the relative input or the bioavailability of atmospheric Hg deposited in lakes and oceans.[21,22,49] Given that $\mathrm{MIF_{even}}$ is unlikely to occur during internal dynamics and metabolism of Hg in biota, discussion on $\mathrm{MIF_{even}}$ is not provided in this review.

Mercury isotopes undergo isotopic mixing, in which sources and/or environmental pools of Hg that have distinct Hg isotope ratios mix to varying degrees in the environment. When different Hg sources (e.g., geogenic vs anthropogenic) and chemical forms (e.g., iHg vs MeHg) carry distinct Hg isotope signatures and these signatures are preserved in biological consumers, isotope mixing models (eqs 4 and 5) become useful tools for Hg source and species attribution across food webs that have negligible Hg isotope fractionation.[50−53] However, Hg metabolism within biological systems can cause isotopic fractionation, which complicates the use of isotope mixing models to assess Hg sources and chemical species. A wider application of the Hg isotope mixing approach needs to account for the Hg isotope dynamics within biota.

$$^{\mathrm{XXX}}\mathrm{Hg}_{\mathrm{mixture}} = {}^{\mathrm{XXX}}\mathrm{Hg}_{\mathrm{MeHg}} \times f_{\mathrm{MeHg}} + {}^{\mathrm{XXX}}\mathrm{Hg}_{\mathrm{iHg}} \times f_{\mathrm{iHg}} \quad (4)$$

$$1 = f_{\mathrm{MeHg}} + f_{\mathrm{iHg}} \quad (5)$$

where $^{\mathrm{XXX}}$Hg represents either δ^{202}Hg or Δ^{199}Hg, and f represents the fraction of either MeHg or iHg.

3. MERCURY ISOTOPE DYNAMICS IN BIOTA

3.1. Birds. Birds are primarily exposed to MeHg through dietary sources, and therefore, piscivorous and carnivorous waterbirds and seabirds exhibit the highest Hg burdens.[54] Akin to fish and mammals, MeHg from dietary sources is assimilated

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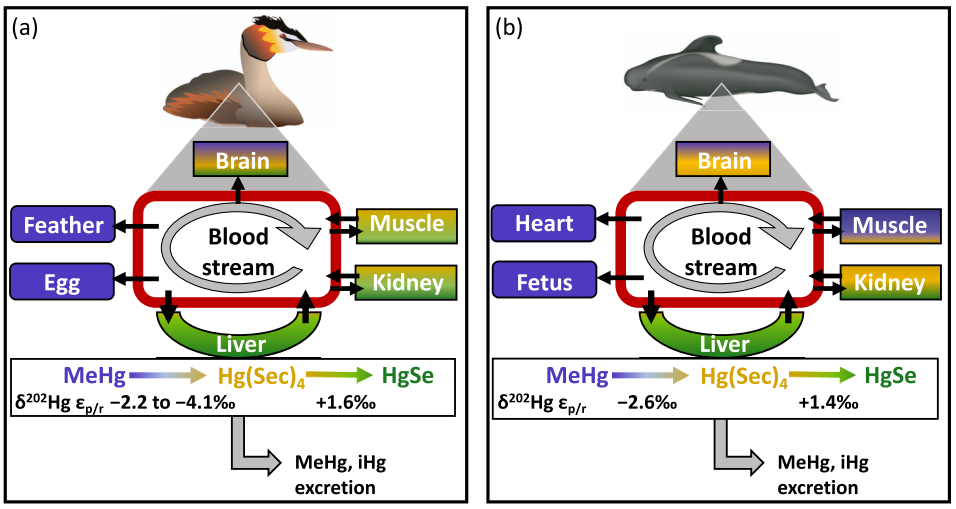

Figure 1. Schematic view of Hg distribution and transformation in (a) birds and (b) pilot whales. The color shading indicates the degree of the reaction progress between the three dominant chemical species observed, including MeHg (in purple), $Hg(Sec)_4$ (in yellow), and HgSe (in green). $\delta^{202}Hg\ \varepsilon_{p/r}$ is the magnitude of isotopic fractionation between product and reactant for each reaction and was either measured directly or determined mathematically using chemical speciation and stable Hg isotope data.[58,59,70,71] $Hg(Sec)_4$ has been identified in diverse birds[55,56,58] but not in marine mammals,[71] though the latter is supported by observed shifts in stoichiometric ratios of Hg-to-Se[72] and associated with Hg isotopic values.[70,73] We do not exclude the possibility of other intermediate Hg species documented in birds[58] and mammals,[74,75] such as Hg-dithiolate and Hg-metallothionein.

in the digestive tract of birds and exchanges between the bloodstream and diverse tissues including the muscle, kidneys, livers, and brains as well as feathers.[55−57] Within tissues, MeHg forms a complex with cysteine residues in proteins[55,56,58] that exhibit a high degree of lability to exchange bidirectionally with the bloodstream.[58−61] The toxicological risk of MeHg to birds is ultimately governed by the intake of dietary MeHg relative to internal demethylation of MeHg and depuration of solely MeHg into feathers during molt[62] or to offspring by maternal transfer.[63] Environmental studies of birds using stable Hg isotopes provide critical toxicological information on the above-mentioned processes and highlight the need to consider internal transformations of Hg when Hg stable isotopes are used to source- or process-track Hg in the environment. As such, birds have been proposed to behave as open isotope systems with continuous inputs of MeHg (the "reactant"), several internal transformations that induce isotopic fractionation in products, and selective depuration of primarily MeHg.[64]

It is well documented that MeHg can be demethylated internally in birds.[56,60,65−67] The extent of internal demethylation in tissues is commonly ranked in the order of liver > kidneys > muscle ≈ brain, with seabirds exhibiting a greater extent of MeHg demethylation compared to estuarine and lacustrine birds. Until recently, little was known about the locations and mechanisms of the demethylation reaction. The recent application of advanced synchrotron-based X-ray analyses on tissues from waterbirds from lacustrine, estuarine, and marine environments identified that MeHg is demethylated to Hg-selenide (HgSe) via a two-step reaction.[55,58,59] First, MeHg is demethylated to primarily a $Hg(selenocysteine)_4$ complex, in short $Hg(Sec)_4$, associated with selenoprotein P (SelP),[55] a prominent selenoprotein that governs selenium (Se) homeostasis in vertebrates.[68] This was observed in the liver, kidney, and muscle tissues of birds and can even occur in the brain. Second, $Hg(Sec)_4$ associated with SelP can be biomineralized to particulate HgSe.[55,56] Both reaction steps induce a significant effect on $\delta^{202}Hg$ values of tissues and influence $\delta^{202}Hg$ of the residual MeHg in tissues and MeHg depurated in feathers and eggs (Figure 1a). Given that tissues often contain MeHg and one or more inorganic Hg species, product−reactant isotopic enrichment factors ($\varepsilon_{p/r}$) have been determined mathematically on tissues analyzed for both chemical speciation and stable Hg isotope data.[58,59] The demethylation of MeHg to $Hg(Sec)_4$ results in isotopically lighter $Hg(Sec)_4$ due to MDF from a kinetic isotope effect, with $\delta^{202}Hg$ of product $Hg(Sec)_4$ being 2.2 to 4.1‰ depleted relative to the reactant MeHg ($\delta^{202}Hg\ \varepsilon_{p/r}$ = −2.2 to −4.1‰).[58,59] Conversely, the biomineralization of HgSe from $Hg(Sec)_4$ results in a positive MDF ($\delta^{202}Hg\ \varepsilon_{p/r}$ = +1.6 ± 0.1‰) in the HgSe product relative to $Hg(Sec)_4$ as observed in the kidney and liver of seabirds (Figure 1a). A separate study by Queipo-Abad et al. that measured $\delta^{202}Hg$ of HgSe isolated from bird liver tissues largely agreed with the value of $\delta^{202}Hg$ of HgSe determined by a combination of X-ray and isotope analyses (~0.2‰).[69]

The positive shifts in $\delta^{202}Hg$ due to HgSe biomineralization are thought to be accompanied by subtle MIF (i.e., $\Delta^{199}Hg \leq 0.3‰$ and $\Delta^{201}Hg \leq 0.2‰$), which is attributed to equilibrium reactions leading to both MDF and the NVE (MIF).[59] Consequently, the $\delta^{202}Hg$ values of bird tissues often exhibit a negative trend with decreasing %MeHg due to the demethylation reaction and a positive trend in $\delta^{202}Hg$ in tissues with low % MeHg (<10%) due to HgSe biomineralization.[59,60,69] The different chemical forms of Hg exhibit unique Hg isotope signatures ($\delta^{202}Hg_{MeHg} > \delta^{202}Hg_{HgSe} > \delta^{202}Hg_{Hg(Sec)4}$) that were observed to be relatively invariant between tissues within bird species.[58,59] The directly measured $\delta^{202}Hg_{MeHg}$[76] confirms its invariance within an individual bird regardless of the degree of MeHg transformations.[58,59] This supports the notion that MeHg exhibits bidirectional exchange between tissues and the bloodstream at a time scale faster than the internal demethylation. The $Hg(Sec)_4$ formed from an isotopically uniform MeHg pool is imprinted with an invariant $\delta^{202}Hg_{Hg(Sec)4}$, and the particulate and immobile HgSe particles from biomineralization of the $Hg(Sec)_4$ pool also exhibit a stable and uniform $\delta^{202}Hg_{HgSe}$.[56] In marine birds, the measured $\delta^{202}Hg$ of total Hg in tissues was the weighted mean of compound-

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specific δ^{202}Hg values (i.e., $\delta^{202}Hg_{MeHg}$, $\delta^{202}Hg_{HgSe}$, and $\delta^{202}Hg_{Hg(Sec)_4}$) and the proportions of these chemical species.[59]

Stable Hg isotopes provide key insights into the toxicokinetics of Hg in birds. Despite the isotopic invariance of each chemical form of Hg within a bird, internal transformations can shift δ^{202}Hg of total Hg in bird tissues, feathers, and eggs by as much as 4‰,[58−60] highlighting the need to consider the chemical forms of Hg when interpreting δ^{202}Hg of bird samples. Differences in the demethylation efficiency across birds are expected to govern the δ^{202}Hg value of the residual MeHg pool across all tissues. The greater efficiency of the MeHg → $Hg(Sec)_4$ demethylation relative to dietary replenishment of MeHg and depuration will yield a heavier residual MeHg pool and a larger isotopic offset between tissue MeHg and $Hg(Sec)_4$. Indeed, the δ^{202}Hg offset between MeHg and $Hg(Sec)_4$ observed in diverse birds with modest levels of internal demethylation[58] was smaller (2.2 ± 0.1‰) than those in seabirds (4.1 ± 0.1‰) that exhibited enhanced internal demethylation.[59] The high efficiency of demethylation in polar birds likely explains the elevated δ^{202}Hg isotope values observed in Arctic bird eggs[35] and Antarctic bird feathers[23,60] relative to the δ^{202}Hg values measured in all other organisms within those respective environments. Mechanistic studies linking transformations of Hg and internal redistribution in the circulatory system are currently lacking. Stable Hg isotopes (δ^{202}Hg and Δ^{199}Hg) can improve the understanding of how MeHg depuration in feathers (i.e., molt)[62,65] and how the mobilization of MeHg from muscle tissues during migration[61] contribute to the internal cycling of Hg in birds.

The application of Hg stable isotopes in birds for source apportionment should prioritize MIF anomalies (Δ^{199}Hg, Δ^{201}Hg) rather than δ^{202}Hg because of the dramatic and bidirectional effects of internal transformations on δ^{202}Hg isotope values. Studies of seabirds across various foraging habitats[77−79] and sea-ice extent[80] show spatial MIF anomalies, interpreted to reflect the photodegradation of MeHg and photoreduction of iHg before food web assimilation. Although evidence points to subtle MIF anomalies due to HgSe biomineralization,[59] at present, the observation is limited to liver tissues, and further investigation is needed to assess the prevalence of this phenomenon in nature.

The correction of δ^{202}Hg values due to internal transformations can be carried out using the published offset values between different chemical forms of Hg[58,59] and chemical speciation data, the latter preferably using synchrotron-based X-ray analyses and mass spectrometry that can differentiate biologically relevant iHg forms.[81] Identifying the underlying physiological or metabolic reason(s) why birds exhibit varying capacities for the demethylation and biomineralization transformations will assist the interpretation of the total δ^{202}Hg of bird tissues, feathers, and eggs. Lastly, birds present a unique opportunity to use nonlethal samples (e.g., feathers, blood,[23,24,77−79] and eggs)[35] that imprint Hg isotope signatures over different time scales to track spatiotemporal trends in Hg stable isotopes for monitoring and research applications. Expanding the use of stable Hg isotopes beyond seabirds to songbirds and other waterbirds has great potential to advance environmental and toxicological understanding of Hg risk, particularly if paired with measurements of Hg concentration, speciation, and isotopic composition of other matrices in these systems (e.g., sediments, water, and prey items).

3.2. Marine Mammals. Dietary intake is the dominant route of MeHg exposure in marine mammals. While the Hg speciation in some tissues (i.e., kidney, muscle, and brain) of pilot whales differs from those in birds, prior studies suggested that similar to seabirds two-step reactions of MeHg demethylation likely occur in pilot whales (Figure 1b). The exact Hg-selenocysteine complex as the major demethylation intermediate product(s) has not been directly identified in marine mammals and warrants future speciation analyses.

To date, about 10 studies have used Hg stable isotopes to identify sources of Hg exposure and/or to track metabolic processes of Hg in marine mammals. The use of Hg isotopes has greatly advanced our understanding of metabolic turnover and distribution of Hg in marine mammals. Laboratory and field studies have shown that a wide range of mammals can demethylate MeHg, resulting in heavier Hg isotope compositions in the remaining MeHg pool relative to the dietary pool.[70,82,36,70−73,83] The results from compound specific Hg stable isotope ratios of various tissues from beluga whales and seals demonstrated that the iHg fraction is consistently depleted in the heavier isotopes relative to that of MeHg (i.e., $\delta^{202}Hg_{iHg} < \delta^{202}Hg_{MeHg}$).[83,84]

Large MDF (~3‰ in δ^{202}Hg) was observed across tissues of aquatic mammals and over life stages. Several studies on pilot whale, beluga, and seals showed that the tissue δ^{202}Hg value is significantly correlated with Hg speciation, and the variability in δ^{202}Hg across multiple tissues (brain, muscle, heart, intestine, diaphragm, pancreas, and hair) can be described by the simple binary mixing of distinct MeHg and iHg endmembers.[70,73,83] The demethylation of MeHg in the liver and to a lesser extent in kidneys is considered the main driver for whole-body isotopic fractionation between demethylated product −iHg and the remaining MeHg prior to their accumulation in other tissues.[36,70−73,83] The MeHg endmember has relatively uniform δ^{202}Hg values across tissues, likely explained by the efficient exchange of MeHg between blood and tissues throughout the body.[70,71,73]

Anomalous MDF signatures that cannot be explained by the binary mixing of MeHg and iHg endmembers were observed in the liver of older pilot whales, indicating that additional mechanism(s) influences δ^{202}Hg of livers in their late life stages.[70,73] During *in vivo* demethylation of MeHg, several labile iHg compounds (e.g., Hg-cysteinates, $Hg(Sec)_4$) are produced during demethylation and are understood to undergo biomineralization to immobile and chemically inert HgSe nanoparticles.[55,85] Recent studies identified the intermediary Hg compound as $Hg(Sec)_4$ in a range of wildlife.[55,86,87] Assuming $Hg(Sec)_4$ is also the major intermediate demethylation product in marine mammals and using the Hg isotope measurements of HgSe particles and total Hg,[70,73] Manceau et al. (2021)[71] applied a three endmember mixing model (i.e., $\delta^{202}Hg_{MeHg}$, $\delta^{202}Hg_{HgSe}$, and $\delta^{202}Hg_{Hg(Sec)4}$) to estimate the chemical composition of Hg in pilot whale tissues. The results support that both the MeHg → $Hg(Sec)_4$ demethylation and the $Hg(Sec)_4$ → HgSe biomineralization processes are pronounced in the liver, while the demethylation process dominates in other tissues (Figure 1b).[71] Additional studies have identified MeHg bound to a cysteine in hemoglobin[74] and Hg(II) bound to cysteine residues of metallothionein in dolphin liver;[75] the role of these interactions on Hg transformation, redistribution, and stable isotopic composition require further study. Compared with marine birds, the biomineralization of HgSe in the brain, kidney, and muscle tissues of pilot whales is less evident (Figure 1).

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The Hg stable isotope data of pilot whales indicated that HgSe biomineralization and iHg redistribution rather than demethylation is the dominant process resulting in a variable Hg isotope ratio in the liver at late life stages.[70,73] As the whale liver accumulates higher Hg burden and more HgSe nanoparticles with age,[71,72] it exports more labile iHg with a lower δ^{202}Hg value relative to HgSe to other organs (e.g., muscles), which increases δ^{202}Hg in the residual pool of total Hg in the liver of older whales.[70,73] The redistribution of Hg is considered a protective mechanism against Hg toxicity in the liver[88] and occurs in the liver of laboratory fish, which generates similar progressive enrichment in heavier Hg isotopes.[89] Whale kidneys, another important organ for demethylation and biomineralization,[71] however, has little variation in δ^{202}Hg over the organism lifespan.[70,71,73] Li et al. (2020)[70] inferred that no substantial changes occur during the redistribution of labile Hg thiolic complexes in kidneys across different life stages. They postulated that Se availability, rather than Hg concentrations, may moderate Hg redistribution and explain the different metabolic dynamics observed in whale kidney and liver.[70]

Various tissues of marine mammals have been used to investigate Hg sources and to assess Hg exposure. Blood δ^{202}Hg is the result of MeHg input from diet and subsequent whole-body demethylation of MeHg. Despite changes in physiology and Hg body burden during growth, the whale blood exhibits almost constant Hg isotope composition, making blood a promising bioindicator of MeHg sources and environmental changes. In contrast, other tissues (e.g., brain, liver, kidneys, and muscle) could vary drastically in δ^{202}Hg across life stages due to demethylation and redistribution processes. Therefore, caution should be taken when using these tissues for biomonitoring purposes, and other factors (age, sex, and Hg speciation) need to be considered simultaneously.

Among various internal Hg processes studied in mammals thus far, Hg transfer across placenta and through lactation is rarely studied.[73] We expect additional Hg isotope measurements of maternal and fetal samples will provide insight into the early life MeHg exposure and its effects on young mammals, which is key to understanding long-lasting neurological and developmental deficits caused by MeHg exposure. In addition, large interspecies differences in tolerance to Hg toxicity and metabolism exist, particularly between terrestrial and aquatic mammals. For example, the liver is considered the primary demethylation site in marine mammals, but the kidney may be more important for terrestrial mammals or early stage aquatic mammals.[90−92] The fractionation of Hg isotopes will be useful in elucidating differences in toxicokinetic processes and toxicity of Hg across mammalian species and life stages.

3.3. Humans. Dietary intake is the major route of MeHg exposure for human populations, who regularly consume fisheries products, rice, and vegetables grown in Hg contaminated regions.[11−13,93] Human populations with dental amalgams or those working and living in Hg contaminated areas can also obtain a considerable amount of iHg.[44,47,94,95] While little information is available for the whole-body Hg metabolic process in humans, existing evidence from human studies indicates that the metabolism of Hg in humans likely resembles marine mammals. For instance, Korbas et al. (2010)[96] identified HgSe granules in the human brain following MeHg poisoning using synchrotron X-ray absorption spectroscopy. Several *in vitro* studies found evidence of MeHg transformation to iHg in human brain and liver cells.[97,98]

The application of Hg isotopes in human biomarkers (i.e., hair and urine) in 11 studies to date demonstrated its utility in assessing exposure sources and metabolic processes of Hg. As observed in other biological systems, MIF is explained by MeHg photodegradation in ecosystems prior to trophic transfer and is absent during metabolic processes in humans.[25] The MIF signature of human hair is a robust tracer for tracking exposure sources and is often used to identify chemical forms of Hg exposure, for instance, among those that use elemental Hg and between the dietary intake of iHg and MeHg. Figure 2 illustrates

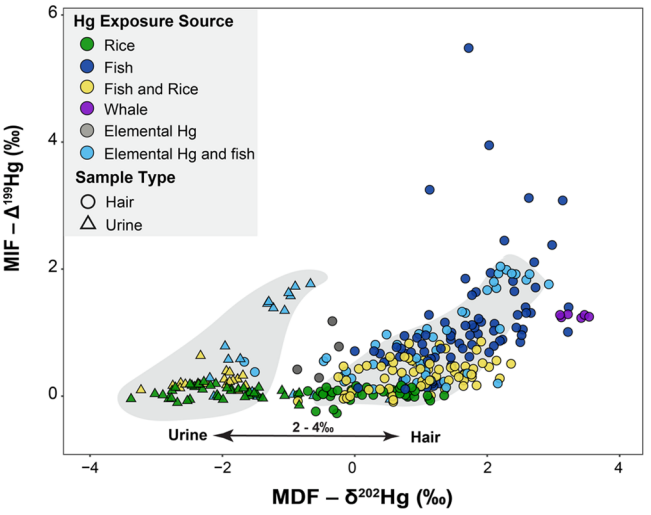

Figure 2. Mercury isotope ratios of hair and urine samples from human populations exposed to different Hg sources based on the compiled data of previous studies (see the SI for the data and references). The gray shades signify the approximate Hg isotope range of urine and hair samples of individuals obtaining Hg from rice, fish and rice, and elemental Hg and fish. Note: The Hg exposure source category "Rice" includes local rice, vegetables, and soil.

the Hg isotopic compositions in the hair of human populations exposed to different Hg sources. Several studies used the MIF signature of human hair to distinguish Hg intake from fish versus rice.[13,47,99,100] Rice often has a much lower fraction of MeHg as total Hg and a lower Δ^{199}Hg value than those in fish. Human populations that seldom eat fish and consume rice grown in Hg contaminated areas have Δ^{199}Hg close to zero, whereas fish consumers have significantly positive Δ^{199}Hg in hair samples (Figure 3).[13,99] The differences in Δ^{199}Hg values among commonly consumed fish species by the Gulf of Mexico recreational fishers[101] allowed for identifying the dietary MeHg sources of these fishers (i.e., coastal fish, pelagic fish, and a mix of both from the Gulf of Mexico).[25] In short, Δ^{199}Hg measurements of human hair are a conservative tracer for a wide variety of Hg exposure sources.

Urine is the main excretion pathway for iHg in the human body.[102,103] Measurements of Hg isotope ratios in human urine revealed that Hg in the urine reflects a mixture of direct iHg intake from dental amalgams or rice consumption and the iHg originated from demethylation of dietary MeHg (Figure 3).[44,47] Akin to hair, the distinct Δ^{199}Hg values from MeHg and iHg are shown in the urine samples, in which higher Δ^{199}Hg values are found in the fish consumers, and near zero Δ^{199}Hg appears in individuals exclusively exposed to iHg (Figure 2).[44,47] For the general population exposed to a wide range of Hg sources (e.g., fish and rice consumption, liquid Hg handling, amalgam, soil,

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Environ. Sci. Technol. 2022, 56, 9182−9195

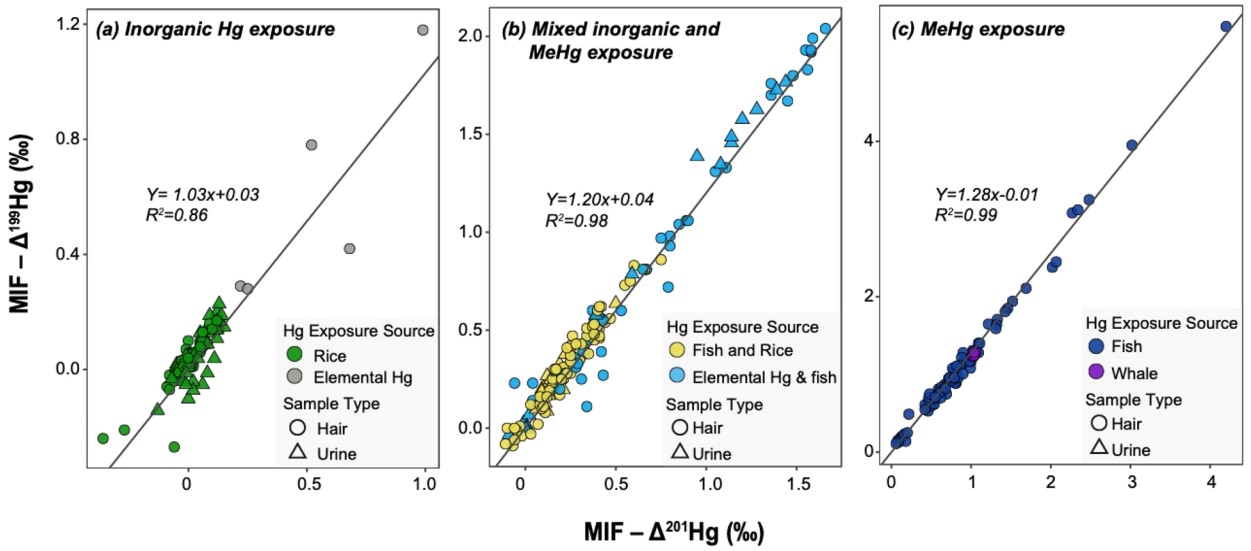

Figure 3. Correlation between two mass-independent fractionation signatures (Δ^{199}Hg and Δ^{201}Hg) of hair and urine samples across human populations primarily exposed to (a) inorganic Hg exposure, (b) mixed inorganic and MeHg exposure, and (c) MeHg exposure. The lines and statistics are calculated from the linear regression.

and vegetables), several studies applied an isotope mixing model using the MIF signatures of biomarkers and potential sources to estimate the respective Hg contribution from each source in the biomarkers.[13,25,47,94]

Prior studies found that Δ^{199}Hg/Δ^{201}Hg is conservative in humans and reflects Hg exposure sources.[13,25,43−47] We compiled published values of Δ^{199}Hg and Δ^{201}Hg in hair and urine samples of three populations based on their primary forms of Hg exposure (i.e., iHg, MeHg, and a mixture of iHg and MeHg). As illustrated in Figure 3, the Δ^{199}Hg/Δ^{201}Hg ratio in populations primarily exposed to MeHg through fish and whale consumption is 1.28, compatible with Δ^{199}Hg/Δ^{201}Hg ratios displayed in marine and freshwater fish (1.20−1.28).[27] In contrast, populations exposed exclusively to iHg exhibit the Δ^{199}Hg/Δ^{201}Hg ratio close to 1.0, similar to the ratios observed in Hg ore, vegetation, and soil that contain mostly iHg.[27] Populations exposed to mixed iHg and MeHg exposures should have Δ^{199}Hg/Δ^{201}Hg ratios between 1.00 and 1.28, and the Δ^{199}Hg/Δ^{201}Hg ratio can be a useful metric for evaluating the extent of the MeHg vs iHg exposure. Several recent studies have applied urine and hair Δ^{199}Hg/Δ^{201}Hg ratios to distinguish between MeHg from seafood and iHg from inhalation of polluted air and ingestion of rice, vegetables, and soil.[47,104] Based on the Δ^{199}Hg/Δ^{201}Hg slope derived from studied populations exposed to mixed Hg exposures (Figure 3b), the iHg originated from demethylation of dietary MeHg accounts for a dominant fraction of total iHg excreted in urine in these populations.

MDF between human biomarkers and MeHg exposure sources has been widely observed and is explained by the *in vivo* demethylation process. Like marine mammals and birds, the human body preferentially demethylates MeHg with lighter Hg isotopes, resulting in lower δ^{202}Hg values in urine (triangles in Figure 2) and higher δ^{202}Hg values in the residual MeHg pool in the human body, as reflected in hair (circles in Figure 2). Several studies reported that human metabolism enriches hair δ^{202}Hg by about 2‰ relative to the muscle tissues of consumed freshwater and marine products (e.g., shellfish, fish, and whales).[25,43−46] Sherman et al.[44] estimated an offset of ∼1‰ in δ^{202}Hg between iHg from demethylation in human urine and MeHg in consumed seafood. In contrast to MeHg metabolism, the hair and urine samples of gold miners whose Hg exposures are almost exclusively from liquid Hg handling show no apparent MDF from the ore-derived Hg sources (e.g, liquid Hg and local soil).[94,105] This implies the potential of Hg isotope ratios in human biomarkers as a conservative tracer for tracking various types of iHg exposure sources. Additional effort is needed to accurately characterize MDF between iHg sources and human biomarkers for better applying Hg isotopes as biomonitoring tools for iHg exposure.

Human populations exposed to both iHg and MeHg display a wider range of δ^{202}Hg in hair relative to populations that had limiting exposure sources, and the proportion of iHg exposure (i.e., iHg%) can largely explain the variability of δ^{202}Hg in biomarkers.[94,99] Compared with MDF between fish and the hair of fish consumers, the δ^{202}Hg offset (2 to 3‰) can be larger between rice and the hair of rice consumers due to the %MeHg variation in rice.[13,99,100] Li et al. (2017)[100] reported that $\delta^{202}Hg_{MeHg}$ in rice is enriched by 1.5‰ compared to $\delta^{202}Hg_{iHg}$; therefore, the larger offsets observed in consumers who have a higher fraction of iHg in their rice result from the combination of the preferential uptake of MeHg with heavier δ^{202}Hg than that of total Hg during digestion and subsequent *in vivo* demethylation of MeHg with lighter δ^{202}Hg to iHg. The larger fraction of iHg or the smaller %MeHg in the consumed food, the greater the MDF offsets could be.[13,99] This highlights the importance of incorporating Hg speciation of both human biomarkers and exposure sources when interpreting δ^{202}Hg in human biomarkers.

3.4. Fish. Over 90% of iHg and MeHg exposure in fish originates from dietary uptake, and the contribution from water via gill uptake is minimal (∼10%).[106] Past toxicokinetic studies of fish revealed distinct internal behaviors of iHg and MeHg upon exposure. Like other organisms, MeHg is efficiently assimilated and transported to various fish tissues including the muscle, liver, kidney, and brain by crossing blood barriers.[107,108] The muscle serves as the final storage organ of MeHg bound to cysteine,[55,109] where over 90% of MeHg in the body accumulates.[107,108] Prior studies have shown that a large fraction

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Environ. Sci. Technol. 2022, 56, 9182−9195

of iHg from dietary uptake accumulates in the intestinal villi and epithelial surfaces of the intestine, which is then excreted as feces and urine.[110−113] The preferential bioaccumulation and slow excretion of MeHg lead to much more efficient trophic transfer and biomagnification of MeHg than iHg in aquatic food webs. The potential methylation of iHg and demethylation of MeHg and the main locations for these processes in fish are still under debate.[87,114−117]

Feeding experiments using different fish species, chemical forms of Hg (iHg vs MeHg), and tissue types have been conducted to investigate MDF and MIF during Hg bioaccumulation, redistribution, and trophic transfer.[89,113,118−120] Kwon et al. (2012)[118] carried out the first fish feeding experiment and discovered the absence of both MDF and MIF between the fish muscle and MeHg spiked food pellets. This indicates that the measurement of Hg isotope ratios in the fish muscle can be used to track environmental sources of MeHg in the ecosystem. Subsequent fish feeding experiments with complex tissue types and fine time resolution demonstrated that the behavior of Hg isotopes in fish tissues depends on the chemical forms of Hg. Fish muscle, liver, kidneys, brain, and blood all showed isotopic equilibration to that of the MeHg diet in feeding experiments using either the natural or synthetic MeHg diet.[89,113,119,120] The time to reach isotopic equilibration, however, varied with the magnitude of MeHg exposure and the growth rate of fish. For instance, much slower isotopic equilibration (∼1000 days) was observed in the muscle tissues of adult Pacific Bluefin Tuna fed natural squid and sardine diets (∼0.1 μg/g) compared to rapidly growing juvenile fish (∼60 days; zebrafish and flatfish) exposed to MeHg spiked food pellets (0.4 to 1.6 μg/g).[89,113,120] Therefore, tissues of fish with a faster growth rate (e.g., small fish, during early life stages) or/and high levels of MeHg exposure would reflect their short-term MeHg sources better than those of large-body fish or/and low levels of MeHg exposure.

In contrast to MeHg, fish exposed to a diet containing considerable proportions of iHg (30−100%) exhibited much slower isotopic turnover and incomplete equilibration to the iHg diet, due to the efficient excretion of iHg in most tissues.[89,113,119] Intestine was the only tissue type that showed complete isotopic equilibration to the diet because iHg cannot cross the intestinal barrier and thus accumulate in the epithelial surface and intestinal villi.[110] This suggests the potential utility of the intestine for characterizing the isotopic compositions of iHg sources in the environment.[113]

Another notable finding from the dietary iHg exposure is the measurable MDF in fish liver. Lee et al. (2020)[113] recently showed positive shifts in δ^{202}Hg in the liver from the isotopic mixing line between the tissues without synthetic Hg exposure (control) and the iHg diet, whereas the isotopic compositions of the muscle and kidney fell on the mixing line.[113] A similar tissue-specific pattern has been reported from other fish feeding experiments with a high iHg diet.[89,119] Because the fish were exposed to an iHg diet and little MeHg exists in the body to be demethylated, Lee et al. (2020)[113] refuted the possibility that the observed MDF in the fish liver was caused by *in vivo* demethylation.

Two mechanisms may lead to the observed MDF in the liver. First, the fish in the study by Kwon et al. (2013)[119] experienced prolonged starvation, and all tissues including the muscle, liver, kidneys, and brain exhibited positive δ^{202}Hg shifts from the mixing line, suggesting that the widespread fat and protein remobilization during starvation may cause the excretion of lighter δ^{202}Hg. Second, and the most plausible explanation is the preferential redistribution and/or excretion of Hg with lighter δ^{202}Hg from the liver to other tissues, corroborated by the experimental results by Xu and Wang (2015).[121] It is important to note that while the liver serves as the main organ for redistributing Hg to other regions, laboratory studies suggest that the redistribution of this lighter δ^{202}Hg is not enough to alter Hg isotope ratios in other tissues. This contrasts with mammals and birds, which have profound demethylation capabilities in the liver. Further studies are needed to identify internal processes leading to the differences between fish and mammals and birds.

Many previous studies have already employed fish Hg isotopes to monitor sources and to quantify the extent of environmental MeHg photodegradation in a wide range of ecosystems.[36−41,122,123] The aforementioned tissue-specific behaviors of Hg isotopes highlight ways in which fish tissues can be selected more appropriately for certain types of the environment. For instance, owing to the rapid turnover and redistribution of Hg in fish liver, prior studies have used the fish liver to muscle Hg concentration ratio ($[Hg]_{liver}/[Hg]_{muscle} > 1$) to identify aquatic systems subject to severe anthropogenic iHg pollution.[124−126] Given the measurable MDF in fish liver, we suggest that the liver Hg isotope is not suitable for biomonitoring and instead the intestine is promising for identifying sources of iHg at polluted sites.[126] The absence of MDF and MIF in the fish muscle makes it an effective tracer for environmental sources of MeHg. In the future, the estimation of MeHg half-life or the rate of isotopic turnover in the fish muscle would be important for understanding species-specific sensitivity to changes in Hg inputs. Given that fish is widely consumed by humans, the monitoring of Hg isotopes in the fish muscle may also be a useful and reliable tool for identifying health-relevant MeHg sources. We, however, note that certain fish species, such as billfish, have nearly all Hg as iHg in the muscle,[87,127] in contrast to high %MeHg (>90%) commonly observed in other fish species.[128] To improve the utility of fish Hg isotopes as a biomonitoring tool, additional research efforts are also needed to understand how metabolic processes and ecological factors (i.e., habitat, dietary sources, and feeding behavior) affect the variation in Hg isotope signature across biological species and tissues.

3.5. Plankton. The uptake of dissolved MeHg by phytoplankton is the initial entry of Hg accumulation in the aquatic food webs and presents the largest bioaccumulation step along aquatic food chains, resulting in 10^2 to 10^6 times higher MeHg concentration in phytoplankton than water.[129] Despite the importance of plankton in the biogeochemical cycling of Hg, little is known about the abiotic or biotic transformations of Hg within plankton due to the analytical difficulties associated with the very low Hg concentrations in plankton and ambient waters.[130,131] Most plankton studies have focused on measuring total Hg and MeHg concentrations in the field or in laboratory experiments to estimate the fluxes of Hg uptake and bioaccumulation,[130,132−135] which provide little insight into the mechanisms that govern Hg transformation within plankton.

The application of Hg stable isotopes in experimental studies can help elucidate Hg uptake and intracellular transformation in phytoplankton. Experimental Hg isotope enrichment studies with freshwater phytoplankton[136] and three marine phytoplankton strains[137] indicate that iHg and MeHg are preferentially bound to high- and low-molecular-weight dissolved organic matter (DOM), respectively, which offers clues to the disparate

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Environ. Sci. Technol. 2022, 56, 9182−9195

bioaccumulation of MeHg over iHg in phytoplankton. Several experimental studies have suggested that various phytoplankton and phototrophic bacteria strains can reduce intracellular iHg and MeHg to volatile forms of Hg, which may significantly reduce the pool of Hg available for bioaccumulation.[135,138−140] Seminal Hg stable isotope experiments with marine phytoplankton *Isochrysis galbana* confirmed that phytoplankton can effectively reduce the bioavailability of iHg and MeHg and provide new insights into the potential degradation pathways of intracellular Hg.[139] Kritee et al. (2018)[139] estimated that MeHg photodegradation by phytoplankton accounts for 20 to 55% of the total photochemically driven MeHg degradation in the open ocean and transparent freshwater ecosystems with deep euphotic zones. Additional field studies in aquatic ecosystems with a range of light intensities are required to examine if the Hg isotope fractionation associated with Hg intracellular transformation in phytoplankton also occurs in natural environments and the controlling factors, which determine the fractionation magnitude.

Motta et al. (2019)[141] recently characterized Hg concentrations and Hg isotope ratios in marine zooplankton, which illustrated spatiotemporal differences in Δ^{199}Hg. Zooplankton collected across the water column (0−1400 m) from the North Pacific Subtropical Gyre at Station ALOHA exhibited elevated Δ^{199}Hg values in shallow depths and small size fractions. Significant diurnal variations were also observed, with greater Δ^{199}Hg values during the day than at night by up to 1.4‰.[141] As Hg within zooplankton is thought to originate exclusively from grazing on phytoplankton, the Δ^{199}Hg variation in marine zooplankton likely originates from marine phytoplankton and light permeable bacterioplankton that can photochemically degrade MeHg in natural waters.[139] The Hg isotopic compositions of zooplankton and particles demonstrated a tight link,[141] consistent with recently reported zooplankton diets using compound-specific isotope analyses of amino acids.[142,143] Collectively, these recent findings show that Hg stable isotopes are a powerful tool for inferring Hg transformations at the base of the food web.

3.6. Invertebrates. Invertebrates comprise the most diverse groups of animals in the world[144] and play an important yet often overlooked role in transferring and biomagnifying highly toxic MeHg from the lower trophic levels (e.g., phytoplankton, bacteria, detritus, foliage, etc.) to the consumers in the upper trophic levels (e.g., fish, birds, and mammals).[145,146] The exact ecological roles of many invertebrates remain unclear (e.g., herbivore, detritivore, suspension feeder, omnivore, or predator), which makes it difficult to interpret their relative positions in food webs and source attribution of Hg exposures.

Similar to phytoplankton, measurements of Hg isotope ratios and the fractionation occurring in invertebrates are not widely studied in comparison to animal consumers of higher trophic levels. This can be attributed to the difficulty in collecting enough biomass and amount of Hg to perform high-precision Hg isotope analysis.[147] Further, the highly variable fraction of MeHg present as total Hg (%MeHg between 0.7 and 100% in forest floor food webs[146]) makes it difficult to interpret the Hg isotope data of total Hg because the isotope ratios of iHg and MeHg could be drastically different even in a single region or ecosystem. For example, in a study on stream and forest food webs in a northern California watershed, Tsui et al. (2012)[42] demonstrated that %MeHg is significantly correlated with δ^{202}Hg and Δ^{199}Hg in each food web. The Δ^{199}Hg of invertebrate groups with low %MeHg (e.g., <10%) showed slightly negative values, resembling those found in foliage and litter of the surrounding landscape. In contrast, the Δ^{199}Hg were higher in predatory invertebrate groups with high %MeHg (>50%), which was attributed to MeHg photodegradation in surface waters or aqueous compartments (e.g., moisture associated with soil or litter) prior to the uptake.

To the best of our knowledge, there is no internal Hg isotope fractionation study in invertebrate species. This would be an important topic to pursue in the near future as internal Hg isotope fractionation by invertebrates would alter the Hg isotope signatures of the upper food web consumers (e.g., fish, songbirds, and mammals). We anticipate that Hg isotopes can be useful for assessing the extent and pathways of *in vivo* demethylation across invertebrate species. Sarica et al. (2005)[148] revealed strong efflux of Hg happens during the pupal stage of blowflies or just right at the emergence. It is worthwhile to use Hg stable isotopes to further investigate internal Hg dynamics during several key physiological processes in invertebrates, such as metamorphosis and autotomy.

4. COMPLEMENTARY TOOLS FOR ELUCIDATING MERCURY TOXICOKINETICS

As iHg and MeHg often have very different isotopic compositions across all types of biota, it is challenging to use the Hg isotope data of total Hg to accurately interpret Hg sources and internal processes in samples with high fractions of iHg, such as low trophic organisms (e.g., plankton and invertebrates) and certain animal tissues (e.g., brain, liver, and kidneys). We emphasize the importance of measuring the Hg chemical speciation and/or Hg compound specific isotope ratios of these samples. In addition, ample evidence indicates that the metabolic processes of Hg and Se are closely intertwined in birds, mammals, and humans. We therefore encourage future studies to include measurements of both Hg and Se speciation across tissues to gain atomic-, molecular-, and protein-level insights into how Se affects the demethylation and redistribution of Hg species. Here, we identify complementary analytical approaches that could further the toxicological and environmental applications of Hg stable isotopes. These include, but are not limited to, the use of the following:

(1) Compound specific isotope measurements of Hg, which generate Hg speciated isotope ratios and differentiate Hg isotopic composition of MeHg from various forms of iHg:[76,84,100,104,149] This technique has proven suitable for identifying MeHg and iHg sources in rice[100] and in human populations that have complex dietary patterns (e.g., rice, freshwater fish, and marine fish).[104] We expect this technique will enable the quantification of isotope fractionation of iHg and MeHg during different internal processes and further expand the utility of Hg isotopes in studying Hg toxicokinetic processes within populations and across organisms.

(2) Advanced techniques in X-ray absorption spectroscopy, namely high-energy resolution fluorescence detection X-ray absorption near-edge structure (HERFD-XANES) spectroscopy, which is capable of identifying and quantifying mixtures of biologically relevant Hg species at subparts-per-million concentration:[55,56,150−152] Similarly, HERFD-XANES spectroscopy was recently shown to have an enhanced resolution of Se species relative to normal-resolution XANES.[153] HERFD-XANES spectroscopy can resolve prominent chemical forms of Hg

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Environ. Sci. Technol. 2022, 56, 9182−9195

pertinent to identifying the various reactions involved in the two-step MeHg → HgSe reaction.[55,56,58] Future research using these methods will advance the understanding of Hg cycling across the scale of food webs to biomolecules.

(3) Molecular and protein-level measurements of Hg and Se and S biomolecules,[154] including targeted mass spectrometry of selenoamino acids[72] and low-molecular weight biomolecules (e.g., selenoneine and cysteine)[74,81,155] and hyphenated elemental mass spectrometry speciation,[156] will be essential to addressing scientific needs on the interactions of Hg with Se and S biomolecules. For example, hyphenated elemental mass spectrometry speciation can distinguish five major classes of Se biomolecules, including low-molecular-weight Se speciation (e.g., free selenocysteine), glutathione peroxidase, SelP, thioredoxin reductase, and Se-albumin. This method was successfully used to screen for the association of Hg with various SelP in bird tissues, to complement Hg speciation by X-ray absorption spectroscopy[55] and Hg isotope measurements.[58]

5. FUTURE RESEARCH DIRECTIONS

This review synthesizes the observations and underlying mechanisms of Hg isotope fractionation across species, tissues, and life stages, which provides essential information for choosing appropriate biomarkers for specific biomonitoring purposes. Based on the research progress and challenges to date, we identify several research priorities for expanding the use of Hg isotopes in ecotoxicological and environmental applications in the future (not listed in order of priority).

(1) There is a large knowledge gap and great potential of using Hg stable isotopes to understand internal Hg transformation and elimination in low trophic level organisms (i.e., plankton and invertebrates). This is crucial for interpreting Hg isotope signatures and speciated Hg concentrations in upper food web consumers. For example, many aquatic invertebrate species can gain tolerance to Hg by facilitating Hg binding to metallothionein (-like) proteins through the thiol group[157] and inorganic and insoluble granules (e.g., pyrophosphate granules in barnacles[158]) or by autotomy of Hg concentrated tissues.[159] Different pathways of Hg binding and removal may lead to distinct Hg isotope signatures in the remaining tissues. Of particular interest is evaluating whether the autotomized segment is isotopically distinct from the remaining body segments, as this would have a widespread impact on the interpretation of Hg isotope signatures in upper food web consumers.

(2) An increasing number of studies showed that gastrointestinal microorganisms in some organisms can methylate and demethylate Hg, but the exact microbial communities that participate in these processes remain unclear.[115,117,160] The wide variation in the elimination rate of MeHg in humans has been attributed to the gut microbiome diversity as well as genetic and/or dietary factors.[43,161] Future studies that apply metagenomics to characterize microbial communities responsible for (de)-methylation within biota may help explain the observed variability in MeHg burden, speciation, and isotope ratios in biota.

(3) Prior studies showed a range associated with δ^{202}Hg $\varepsilon_{p/r}$ = −2.2 to −4.1‰ from MeHg to Hg(Sec)$_4$ across bird species but not within the pilot whales (Figure 1). It is still unclear whether the variability of δ^{202}Hg $\varepsilon_{p/r}$ directly relates to different capacities of *in vivo* demethylation within and across species. To the best of our knowledge, no study has directly addressed how metabolic and physiological factors and life traits affect the magnitude of Hg isotope fractionation of internal MeHg demethylation across and within organisms.

(4) The dietary offset of δ^{202}Hg between food and consumers is the consequence of internal processes, primarily demethylation, in high trophic level organisms, which explains why seabirds, marine mammals, and humans who have advanced demethylation capacity generally have a higher dietary offset of δ^{202}Hg than organisms that cannot efficiently demethylate, such as fish. Li et al. (2016).[43] revealed the challenges of using the δ^{202}Hg offset between human hair and consumed fish as a tracer for differentiating demethylation capacity among individuals. Additional dietary exposure−response experiments on human populations that likely have different demethylation capacities are required to characterize the relationship between the dietary δ^{202}Hg offset and internal demethylation efficiency. This research effort, if successful, can help identify susceptible human populations to MeHg exposure.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.1c08631.

Table of human isotope data compilation (XLSX)

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Notes

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