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Sources and Variations of Mercury in Tuna

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While the bulk of human exposure to mercury is through the consumption of marine fish, most of what we know about mercury methylation and bioaccumulation is from studies of freshwaters. We know little of where and how mercury is methylated in the open oceans, and there is currently a debate whether methylmercury concentrations in marine fish have increased along with global anthropogenic mercury emissions. Measurements of mercury concentrations in Yellowfin tuna caught off Hawaii in 1998 show no increase compared to measurements of the same species caught in the same area in 1971. On the basis of the known increase in the global emissions of mercury over the past century and of a simple model of mercury biogeochemistry in the Equatorial and Subtropical Pacific ocean, we calculate that the methylmercury concentration in these surface waters should have increased between 9 and 26% over this 27 years span if methylation occurred in the mixed layer or in the thermocline. Such an increase is statistically inconsistent with the constant mercury concentrations measured in tuna. We conclude tentatively that mercury methylation in the oceans occurs in deep waters or in sediments.

Introduction

The biogeochemical cycle of mercury, one of the most toxic elements, has been considerably perturbed by anthropogenic activities. Human exposure to mercury, mostly through the consumption of marine fish, is cause for concern (*1*). Our understanding of the biogeochemistry of mercury comes chiefly from studies of freshwater systems, however, and mercury levels in marine fish as well as the mechanisms controlling them have been comparatively little studied.

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High mercury concentrations, sometimes exceeding the FDA recommendations of 0.5 ppm, are typically measured in carnivorous pelagic fish, even in fish caught in regions of the oceans away from any direct pollution source. It is currently a matter of debate whether these high concentrations represent background levels or are, to some degree, the result of anthropogenic mercury emissions. The current atmospheric concentration of mercury has been estimated to be two to three times higher than it was 150 years ago (*2*−*4*), and because the residence time of mercury in the atmosphere is comparable to the mixing time (∼1 year), mercury pollution is truly global (*5, 6*) resulting in elevated concentrations in the far reaches of the globe, including the open ocean. Partly on this basis, it has been argued that mercury in oceanic fish must have increased as a result of anthropogenic emissions (*7*). Nonetheless, the analysis of the mercury concentration in museum samples of tuna caught between 1878 and 1909 showed no evidence for an increase in mercury concentrations in tuna over the last century (*8*).

Methylmercury (MeHg, CH_3Hg^+) is efficiently bioaccumulated in the food chain and is the major form of mercury in fish. The accumulation of mercury in fish thus depends primarily on the concentration of methylmercury, rather than total mercury, in the water (*9*). Only a minor fraction of mercury in natural waters is in the form of methylmercury, however, and methylmercury concentrations in the surface oceans are extremely low, near the detection limit of the currently available techniques (<50 fM) (*10*−*12*). MeHg in freshwaters is believed to be synthesized from Hg(II) through the activity of sulfate reducing bacteria in anoxic or suboxic environments, and the methylation rate depends on a number of factors, such as the extent of anoxia and the activity of sulfate reducers as well as on the total concentration of mercury in the water (*13*). But the source of MeHg in the oceans and the mechanisms of its formation are still unclear, although it is generally believed to be of biological origin. It has sometimes been proposed to result from the (biotic or abiotic) demethylation of dimethylmercury ($(CH_3)_2Hg$, DMHg), also believed to derive from biological activity (*10*). In addition, some have argued, based on oceanographic data, that methylmercury (and dimethylmercury) in the oceans are formed in the oxygen minimum zone (e.g., refs *10* and *11*), but MeHg and DMHg could also have a deeper source (*14*). Even though most biological activity occurs in the euphotic zone, MeHg and DMHg are not generally thought to be formed there: the concentration of these species being lower at the surface than at depth, the euphotic zone is likely a sink (via particulate transport and photodegradation) rather than a source of methylmercury.

Here we report a new data set for Hg in Yellowfin tuna (*Thunnus albacares*) collected in the Equatorial and Subtropical Pacific in 1998 and compare it to published data for tuna collected in the same region in 1971. We then compare the changes in mercury concentrations in tuna with predicted changes in the MeHg concentrations in the mixed layer of the Equatorial and Subtropical Pacific calculated according to various model scenarios. The comparison yields information on the likely sources of MeHg in the oceans, which in turn has implications for past and future changes in mercury concentrations in oceanic fish.

Experimental Section

Yellowfin tuna were collected off Hawaii (outside the 50 miles limit) between 10°N and 30°N and 145°W and 165°W by PACMAR (Pacific Management Resources, Honolulu, HI)

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during the fall of 1998. For comparison purposes (see below), fish chosen for analysis were selected to cover a weight distribution as close as possible to that of the set of Yellowfin tuna analyzed by Thieleke (*15*). Each sample was identified by a unique code. A Chain of Custody form accompanied each sample through all steps of the sampling, transportation, and analysis.

From each fish that was selected for testing, a one-pound sample of muscle from a side of the fish slightly ahead of the caudal peduncle (base of the tail) was obtained. (The same muscle was selected for Hg analysis in Yellowfin tuna by Thieleke (*15*)). The sample was placed into a clean ziplock plastic bag, labeled, and immediately frozen. The samples were maintained frozen during storage and transport to the analyzing laboratory (The National Food Laboratory, Inc., Dublin, CA).

For each sample of frozen tuna muscle, approximately 250 g of the sample were homogenized. Any skin and bone was removed before grinding. The unhomogenized portion was retained for back-up purposes. Five-gram aliquots of the homogenized sample were transferred to whirl-Pak type sample bags and held frozen until needed for analysis.

The potential for contamination due to sampling procedures was evaluated. Five samples of frozen fish were randomly selected, and three portions of each sample, representing the exterior and interior portions of the fish, were analyzed to determine if contamination from handling or during sampling had occurred. The average ratio of the mercury concentration of the exterior portion to that of the interior portions was 1.04 ± 0.15, and contamination was thus found to be negligible.

Mercury was determined by cold vapor atomic absorption spectrophotometry as described by the AOAC Method 977.15 (*16*) using a Perkin-Elmer 3030 Atomic Absorption Spectrophotometer with MHS 10 Mercury/Hydride System. Our method differs slightly from that described in the AOAC method 977.15 in that mercury vapors are swept by argon into an open tube for detection in contrast to the closed loop equipment described in AOAC. The method detection limit, as determined from 6 replicate analysis of a spiked tuna sample, was approximately 0.01 ppm.

Each set of 10 samples contained one sample analyzed in duplicate and one spiked sample. Spiking was done by adding known volumes of a mercury standard solution to comminuted tissue. A tuna sample was randomly selected to serve as an internal reference and was repeatedly analyzed after each set of twenty samples. The average relative difference between duplicates of individual samples was 4.7%. The average mercury concentration of the reference sample was 0.128 ppm, with a standard deviation of 0.0098 on 6 samples. The spike recoveries were between 90.5% and 103% with an average of 95.2%. For comparison, in the Thieleke study (*15*), multiple analysis of a shark tissue sample (7 replicates) yielded an average of 0.17 ppm with a standard deviation of 0.0157 ppm and the recovery of Hg spikes in tissue of Yellowfin tuna ranged from 92% to 103%, with an average of 96.7%. (All the mercury concentrations reported in this study are in gram of mercury per gram of wet weight of tuna.)

Model Section

To study the changes in mercury concentrations in the Equatorial and Subtropical Pacific over time, we built a three box model to represent the Pacific ocean from 30 °S to 30 °N and from 150 °E to 75 °W. The three boxes represent the mixed layer (from the surface to 100 m depth), the permanent thermocline (from 100 to 900 m depth) and the deep ocean and sediments (below 900 m). Mercury species are transported from one box to the other by water advection and vertical particulate transport.

Total Mercury Concentrations. We assume that mercury emissions, like CO_2 emissions, have approximately followed an exponential increase since the onset of the industrial revolution (taken here as 1860). In our baseline model, we assume that the total mercury concentration in the mixed layer has also increased exponentially over time and has doubled between 1860 and 1990 (see below). The evolution over time of $[Hg]_s$ (in mol m^{-3}), the total mercury concentration in the mixed layer, follows

$$[Hg]_S = [Hg]_s^{\,0} e^{\eta \cdot t} \text{ (t in year since 1860)} \qquad (1)$$

where $[Hg]_s^{\,0}$ (in mol m^{-3}) is the total mercury concentration in the mixed layer in 1860, and η (in yr^{-1}) is the rate of the exponential increase.

The variation in the total mercury concentration in the thermocline, $[Hg]_{therm}$ (in mol m^{-3}) with time can be derived from a simple mass balance

$$\frac{d[Hg]_{therm}}{dt} = \frac{1}{h_T}(F_1[Hg]_S + P_S^{Hg}[Hg]_S - F_1[Hg]_{therm} - P_{therm}^{Hg}[Hg]_{therm} + F_2[Hg]_D - F_2[Hg]_{therm}) \qquad (2)$$

where $[Hg]_D$ (in mol m^{-3}) is the total mercury concentration in the deep ocean considered to be a constant over time; h_T (in m) is the depth of the thermocline; F_1 and F_2 (in m yr^{-1}) account for advective fluxes between the mixed layer and the thermocline and between the thermocline and the deep ocean, respectively; and P_S^{Hg} and P_{therm}^{Hg} (in m yr^{-1}) account for the particulate settling flux of mercury from the mixed layer to the thermocline and from the thermocline to the deep ocean, respectively. These factors include both the partition coefficient between the water and the particles (chiefly biotic) and the vertical particle flux.

Assuming a steady-state flow field and constant particulate fluxes from the mixed layer to the thermocline and from the thermocline to the deep ocean, we can integrate eq 2 to obtain the evolution of the mercury concentration in the thermocline over time

$$[Hg]_{therm} = C + De^{\eta t} + Ee^{\gamma t} \qquad (3)$$

with

$$\gamma = -\frac{F_{1+}F_2 + P_{therm}^{Hg}}{h_T}$$

$$C = -[Hg]_D \frac{F_2}{h_T \gamma}$$

$$D = [Hg]_s^{\,0} \frac{F_1 + P_S^{Hg}}{h_T(\eta - \gamma)}$$

$$E = [Hg]_{therm}^{\,0} - [Hg]_s^{\,0} \frac{F_1 + P_S^{Hg}}{h_T(\eta - \gamma)} + [Hg]_D \frac{F_2}{h_T \gamma}$$

Methylmercury Concentrations in the Mixed Layer. The mechanisms underlying methylmercury formation in the oceans are yet largely unknown, and MeHg may be formed (most likely through biotic processes) in the mixed layer, the thermocline, or the deep ocean and sediments. Since inorganic mercury serves as a substrate for methylation reactions, methylmercury concentrations at (pseudo) steady-state are assumed to be proportional to total mercury concentrations in the oceanic reservoir where MeHg is formed. This assumption is reasonable, because chemical transformations between mercury species are fast relative to

the time scales of the anthropogenic perturbation and physical transport. In the absence of other evidence, we also take the proportionality coefficient to be constant over time. We consider three hypotheses in turn.

Hypothesis 1: MeHg is formed in the mixed layer.

At any given time, the MeHg concentration in the mixed layer, $[MeHg]_s$ (in mol m^{-3}), is proportional to the total mercury concentration in the mixed layer. The proportionality coefficient is unknown, however, since $[MeHg]_s$ is too low to be measured.

The solution for MeHg concentrations in the mixed layer becomes

$$[MeHg]_s = [MeHg]_s^0 e^{\eta t} \quad (4)$$

With this hypothesis, the ratio of methylmercury concentrations between two given dates (between, for example, 1971 and 1998, see below) depends only on η, the time constant for the exponential increase in total mercury concentrations in the mixed layer.

Hypothesis 2: MeHg is formed in the thermocline.

The MeHg concentration in the thermocline is proportional to the total mercury concentration in the thermocline. In this case, the proportionality coefficient K_2 can be estimated by dividing the MeHg concentration measured in the thermocline by the total Hg concentration:

$$K_2 = \frac{[MeHg]_{therm}}{[Hg]_{therm}} \quad (5)$$

The MeHg concentration in the mixed layer is the result of MeHg inputs (by advection from the thermocline) and MeHg removal (by photolysis, particulate transport and advection to the thermocline) and is related to the total mercury concentration in the thermocline through the differential equation

$$\frac{d[MeHg]_s}{dt} = \frac{F_1}{h_s} K_2 [Hg]_{therm} - \left(k_2 + \frac{P_S^{MeHg}}{h_s} + \frac{F_1}{h_s}\right)[MeHg]_s \quad (6)$$

where h_s (in m) is the depth of the mixed layer; k_2 (in yr^{-1}) is the pseudo first-order constant for the photodemethylation of MeHg in the mixed layer; and P_S^{MeHg} (in m yr^{-1}) is the particulate flux of methylmercury from the mixed layer to the thermocline.

Since the rate of MeHg elimination from the mixed layer by water advection, photolysis, and particulate transport is relatively rapid (see below), the methylmercury concentration in the mixed layer must be at pseudo steady-state on the time-scale of several years which is of interest to us (i.e., $d[MeHg]_s/dt \approx 0$), and eq 6 yields

$$[MeHg]_s = \frac{F_1 K_2}{h_s k_2 + P_S^{MeHg} + F_1} [Hg]_{therm} \quad (7)$$

This simple model (eq 7) predicts that, if methylmercury is formed in the thermocline, methylmercury concentrations in the mixed layer should have increased at the same rate as total mercury concentrations in the thermocline.

Hypothesis 3: MeHg is formed in the deep ocean or sediments.

In this scenario, the methylmercury concentration in the mixed layer depends (eventually) on the total mercury concentration in the deep ocean which cannot have been affected by anthropogenic inputs by more than a few percents (*17*).

Model Parameters. The initial concentration of total mercury in the mixed layer, $[Hg]_s^0 = 5.5 \times 10^{-10}$ mol m^{-3}, and the rate of the exponential increase in total mercury concentrations in the mixed layer over time, $\eta = 0.00533$ $year^{-1}$, may be obtained from two pieces of information: (i) a doubling in mixed layer concentrations between 1860 and 1990 ((*18*), see below) and (ii) a total mercury concentration in the mixed layer of the Equatorial and Subtropical Pacific in 1990 $[Hg]_S^{130} = 1.11 \times 10^{-9}$ mol m^{-3}, as measured by Mason and Fitzgerald (*11*).

$[Hg]_T$,[130] the mercury concentration in the thermocline in 1990, is 1.88×10^{-9} mol m^{-3} (*11*).

The water exchange rates between the mixed layer and the thermocline ($F_1 = 10$ m yr^{-1}) and between the thermocline and the deep ocean ($F_2 = 2.4$ m yr^{-1}) in the model domain are chosen to mimic the flow-field of the GFDL ocean general circulation model as described in Toggweiler et al. (*19*). Dominant transport features in the Equatorial and Subtropical Pacific are equatorial upwelling and the subtropical overturning cells. The intensity of these circulation patterns is uncertain. For example, estimates of the intensity of the subtropical overturning cell in the North Pacific vary between 10 and 18 Sv (*20*). We return to the effects of this parameter uncertainty below.

$[Hg]_D$, the mercury concentration in the deep ocean of the Equatorial and Subtropical Pacific, is considered constant over the time scale of interest (a few hundred years) and equal to 9.9×10^{-10} mol m^{-3}, estimated from the available data (*11*).

The value for P_S^{Hg} is adjusted to fit the observed gradient of total mercury concentrations between the mixed layer and the thermocline in 1990 (*11*).

The exact value of P_{therm}^{Hg} is poorly known but is likely to be small, i.e., less than 10% of P_S^{Hg} (*21*), and for simplicity, we take $P_{therm}^{Hg} = 0$.

K_2 is estimated to be about 0.1, using again the data published by Mason and Fitzgerald (*11*).

An estimate for $k_2 = 0.06-0.6$ yr^{-1} was calculated for a depth-averaged concentration of $OH^\bullet = 2 \times 10^{-19}-2 \times 10^{-18}$ M, based on the study by Chen et al. (*22*); the reaction with the $OH^\bullet$ radical is assumed to be the major mechanism for photodegration of methylmercury in seawater.

P_S^{MeHg} can be estimated as the product of P_S (in g C m^{-2} yr^{-1}), the particulate carbon flux from the mixed layer to the thermocline and α_{MeHg} (in m^3 g C^{-1}), the partition coefficient of MeHg in the particles that are exported. $\alpha_{MeHg}[MeHg]_s$ was estimated by Topping and Davies (1981) as 6 pg MeHg/mg C, which yields $\alpha_{MeHg} P_S [MeHg]_s = 6 \times 10^{-10}$ molMeHg yr^{-1} m^{-2} with $P_S = 60$ mgC m^{-2} d^{-1} (*21*). The concentration of MeHg in the mixed layer is unknown, since it is below the detection limit (*11*) and the value of $\alpha_{MeHg} P_S$ ($= P_S^{MeHg}$) cannot be estimated. A simple calculation shows that if $[MeHg]_s$ is close to 50 fM (*11*), particulate transport, photolysis and transport to the thermocline by water advection are likely to be of similar importance for the removal of MeHg from the mixed layer (see eq 6). Since we are interested in the relative increase in $[MeHg]_S$ over time, the exact value of the proportionality coefficient is unimportant.

Model Equations for a Linear Increase in Mercury Concentrations in the Mixed Layer. Mason et al. (*4*) assumed a linear rather than exponential increase in mercury emissions and using a different model, found a linear increase in the concentration of mercury in the mixed layer. In this case, which will be discussed later as one of the variations of the baseline model, the model's equations become (using a as the rate of linear increase)

$$[Hg]_s = [Hg]_s^0 + at \text{ (mol } m^{-3}\text{, t in year since 1860)} \quad (8)$$

$$[Hg]_{therm} = L + Mt + [[Hg]_{therm}^0 - L]e^{\gamma t} \quad (9)$$

with

$$L = -\frac{ah_T(F_1 + P_S^{Hg})}{(F_1 + F_2)^2} + \frac{(F_1 + P_S^{Hg})[Hg]_s^0 + F_2[Hg]_D}{F_1 + F_2}$$

$$M = \frac{a(F_1 + P_S^{Hg})}{F_1 + F_2}$$

$$\gamma = -\frac{F_1 + F_2 + P_{therm}^{Hg}}{h_T}$$

Results and Discussion

Mercury Concentrations in Tuna: 1998 versus 1971. Extensive data are available for the mercury concentrations in Yellowfin tuna caught off Hawaii in 1971. The data sets of Thieleke (*15*), who analyzed 100 Yellowfin samples in 1971, and Rivers et al. (*23*), who reported an additional 22 observations in this region, are combined in Figure 1. As has often been observed in fish, mercury concentrations tend to increase with the weight of tuna (*24*). The 1971 samples range from 0.09 to 1.32 ppm of mercury, for weights ranging from 10 Kg to 97.5 Kg. For reference, a 50 Kg tuna is about 4 years old (*25*), swims continuously in the top 100 m of the water column (*26*), and feeds opportunistically on a whatever preys are available (*27*). Tuna are known to migrate over thousands of miles (*28*), although individuals that have been tracked near Hawaii seem to remain in that area of the open ocean (*26*).

In this study, we focused on the same tuna species caught in the same area 27 years later and analyzed mercury concentrations in 105 Yellowfin tuna caught off Hawaii in 1998 (Figure 1). The range of tuna weights in the 1998 data set (from 27.2 Kg to 70.8 Kg) is similar, although somewhat narrower, to that of the 1971 data set. Despite some scatter in the data, the mercury concentration tends to increase with the weight of the tuna. The range of mercury concentrations is narrower in the 1998 data set (going from 0.012 to 0.68 ppm) than in the 1971 data set, probably reflecting the decreased range in tuna weights in 1998.

The average concentration of mercury in tuna in 1998 is slightly lower than in 1971, but not significantly so (0.210 ± 0.112 ppm versus 0.274 ± 0.172 ppm). A simple comparison of the average concentrations may be misleading because of the increase in Hg concentrations in tuna with weight. We thus choose from each study the subset of fish between 38.6 Kg and 52.2 Kg representing more than half of each population. The number of fish in both subsets is similar (71 in 1971 versus 66 in 1998), and the average fish weights are virtually identical (44.0 Kg in 1971 versus 43.9 Kg in 1998). The concentration distributions in the considered weight range in 1971 and 1998 are remarkably similar (see Figure 2), as are the means (the mean mercury concentrations are 0.218 ppm in 1971 versus 0.206 ppm in 1998, corresponding to a ratio of 0.95). A t-test of these observations indicates no significant change in Hg concentrations between 1971 and 1998 ($p < 0.05$). Because the data are not normally distributed, we verified this conclusion with a nonparametric bootstrap analysis, percentile method, using 10^5 simulations (*29*). Again, we found no evidence for a statistically significant change in mercury concentrations in Yellowfin tuna between 1971 and 1998. The 95% quantile of the test-statistic on the ratio of the mean mercury concentrations is estimated by the bootstrap analysis as 1.06. In other words, mercury concentrations in tuna are inconsistent, at the 95% confidence level, with an increase of more than 6% between 1971 and 1998.

This result can be compared with studies of change in mercury concentrations in fish and seabirds over the past century using museum specimens as early controls. Miller et al. (*8*) found no difference in the mercury concentrations of tuna caught between 1878 and 1909 and tuna caught in 1972. In contrast, depending on the species of seabirds, Thompson et al. (*30*) found significant increases or decreases in birds caught near the British Isles before 1930 and after 1980. In a similar study off the Azores, Monteiro and Furness (*31*) found an increase in mercury in two species of birds over the period 1890 and 1993 with a particularly significant increase between 1967 and 1993. A further report by Thompson et al. (*32*), which reports the same data and extends the study to other birds found a significant increase pre 1931 to post 1979 in all seabirds tested. The reasons for the differences among the bird studies are not known, but

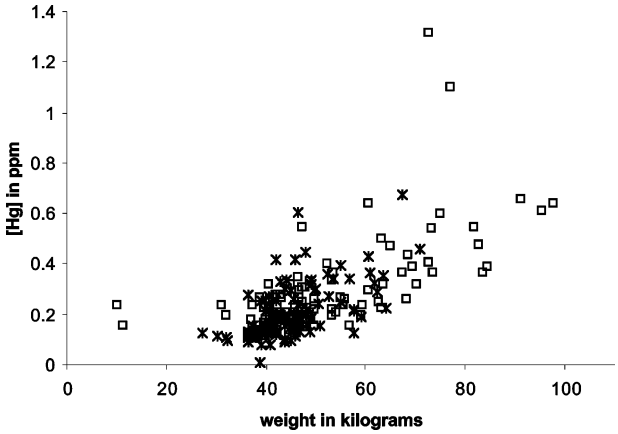

FIGURE 1. Mercury concentration in Yellowfin tuna caught off Hawaii in 1971 and 1998. The open squares are a collection of the data published by Rivers et al. (*23*) and Thieleke (*15*) and correspond to tuna caught in 1971. The stars represent tuna analyzed in this study and caught in 1998.

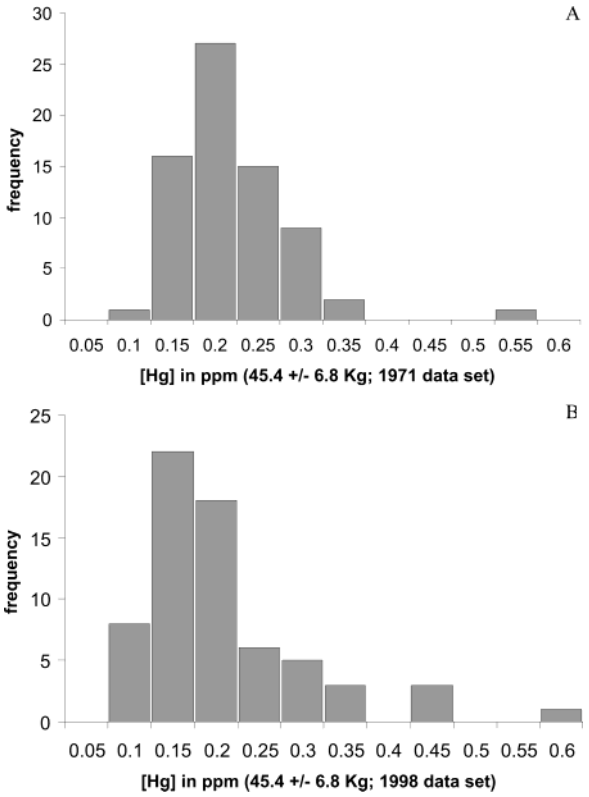

FIGURE 2. Histograms of mercury concentrations in Yellowfin tuna in 1971 and 1998. The histograms are restricted to tuna with weights ranging from 38.6 Kg to 52.2 Kg. Upper panel (A): Yellowfin tuna in 1971 (*15, 23*). Lower panel (B): Yellowfin tuna in 1998 (this study).

they may reflect different pollution patterns (e.g., coastal versus open ocean) and different food chains. The difference in trends between the Pacific tuna data and the Atlantic bird data are not likely due to the differences between the two oceans since the Pacific ocean has likely been subjected to a larger increase in Hg inputs than the Atlantic ocean between the late 1960s and the mid 1990s (see below). We note, however, that the use of museum specimens (which comprise all the bird data up to 1967 included) to provide long-term comparison is fraught with difficulties (*33*).

Model Predictions. Since Yellowfin tuna and their prey feed mostly in the mixed layer (*27*), their mercury concentrations must reflect the concentration of methylmercury at the surface. Clearly, we cannot rule out uncertainties introduced by additional factors not considered in our model, such as changes in the Yellowfin migration patterns, or changes in the functional relationship between Hg concentrations in the water and the Hg concentrations in tuna, resulting for example from a change in the food web structure or in the tuna diet. Nonetheless, in the absence of any evidence for such changes in the Equatorial and Subtropical Pacific, the most parsimonious assumption is that the mercury concentration in tuna is proportional to the methylmercury concentration in the mixed layer. We thus compare directly the changes in methylmercury concentrations in the mixed layer predicted by our model with the measured mercury concentrations in tuna.

For the sake of simplicity, we discuss first the results of the baseline model that assumes an exponential increase and a doubling in mercury concentrations in the mixed layer between 1860 and 1990. The increase by a factor of 2 of the mercury concentration of the mixed layer between preindustrial and present times is based on the results of the GRIMM model as described by Lamborg et al. (*18*). The evolution of mercury concentrations in the mixed layer over time is of course uncertain, and we examine later the consequences of making different assumptions on the time-dependence and magnitude of the increase in Hg concentrations.

As is apparent in Figure 3, our model predicts a time lag between the onset of anthropogenic mercury emissions and the increase in total mercury concentrations in the thermocline. This is mainly the result of slow exchange rates between the thermocline and the mixed layer. Between 1860 and 1990, Hg concentrations are calculated to have increased by 44% in the thermocline while they have increased by a factor of 2 in the mixed layer. These increases, calculated for the Equatorial and Subtropical Pacific, cannot be compared directly with those calculated by models calibrated for the whole oceans (*17*, *18*). Nonetheless, if we extrapolate our results to the global ocean (by simple proportionality of surface areas), we obtain an accumulation of 20 Mmol in the mixed layer and 168 Mmol in the thermocline. The corresponding accumulations calculated by Lamborg et al. (*18*) who considered similar oceanic reservoirs, are 25 Mmol and 178 Mmol, respectively.

As explained in the Model section, MeHg concentrations in the mixed layer are taken to be proportional to total Hg concentrations in the oceanic reservoir (i.e., mixed layer, thermocline, or deep ocean) where MeHg is formed. The predictions of the evolution of MeHg concentrations in the mixed layer over time thus depend on where MeHg is formed in the oceans.

Hypothesis 1: MeHg is formed in the mixed layer of the Equatorial and Subtropical Pacific.

At any given time, the MeHg concentration in the mixed layer is proportional to the total mercury concentration in the mixed layer. According to this hypothesis, MeHg concentrations in the mixed layer have increased exponentially since the beginning of industrial times and doubled between 1860 and 1990 (see eq 4 and Figure 3). Between 1971 and 1998, they have increased by 15%. But according to our statistical analysis of the mercury concentrations in Yellowfin tuna, a 15% increase in the average concentration in tuna between 1971 and 1998 can be rejected ($p < 0.05$).

Hypothesis 2: MeHg is formed in the thermocline.

The MeHg concentration in the thermocline is proportional to the total mercury concentration in the thermocline; the MeHg formed in the thermocline is then transported to the mixed layer, where it is rapidly degraded by photolysis or transported back to the thermocline by water advection or adsorption onto the sinking particulate flux. In either case, the pseudo steady-state concentration of MeHg in the mixed layer is controlled in part by the rate of supply from the thermocline and is directly proportional to the MeHg concentration in the thermocline (and thus to the total mercury concentration in the thermocline; see eqs 5 and 7 and Figure 3).

According to this hypothesis, methylmercury concentrations in the mixed layer should have increased at the same rate as total mercury concentrations in the thermocline. For the specific assumptions described above, this corresponds to an increase by roughly 45% between 1860 and 1990. If MeHg is formed in the thermocline, the model predicts that MeHg concentrations in the mixed layer have increased by 12% between 1971 and 1998. But again, the data on mercury in Yellowfin tuna (Figure 1, see above) indicate that a 12% increase in the average Hg concentration in Yellowfin tuna between 1971 and 1998 can be rejected ($p < 0.05$)

Hypothesis 3: MeHg is formed in the deep ocean.

In this case, the methylmercury concentration in the mixed layer depends on the total mercury concentration in the deep ocean, whose increase over time is difficult to estimate. But an upper limit of the potential effect of such an increase on the concentration of methylmercury at the surface is easily calculated. The maximum increase in the deep ocean inventory of mercury (and thus of methylmercury) is on the order of 0.2% per year (*17*, *18*). If it is assumed to have remained at this level for 130 years, such an increase could have led to a maximum increase in the surface concentration of methylmercury of 1.5% over the 27 years of interest, given a minimum mixing time between the deep ocean and the surface of 400 years. According to this hypothesis, there has thus been no significant increase in the methylmercury concentration of the mixed layer, consistent with the lack of a significant change in the mercury concentrations of tuna between 1971 and 1998 (Figure 1, see above).

Given our assumptions, we conclude tentatively that MeHg in the Equatorial and Subtropical Pacific is likely formed in the deep ocean rather than in the thermocline or in the mixed layer. We explore whether this conclusion is robust with respect to the estimated model parameters and boundary conditions by a model sensitivity study.

Model Sensitivity. The most important assumptions of the model are as follows: (i) the magnitude of the increase in mercury concentrations in the mixed layer since 1860; (ii) the time-dependence of the increase (e.g., whether we assume an exponential or linear increase in mixed layer concentrations); and (iii) the estimated model parameters such as F_1, the water flux between the mixed layer and the thermocline, F_2, the water flux between the thermocline and the deep ocean, P_s^{Hg}, the particulate flux, h_T, the depth of the thermocline, and $[Hg]_D$, the total mercury concentration of the deep ocean.

If the total mercury concentration in the mixed layer has increased by a factor of 3 (rather than 2), as proposed by Mason et al. (*4*), the model predicts an even higher increase of MeHg concentrations in the mixed layer between 1971 and 1998: MeHg is predicted to increase by 26% if it is formed

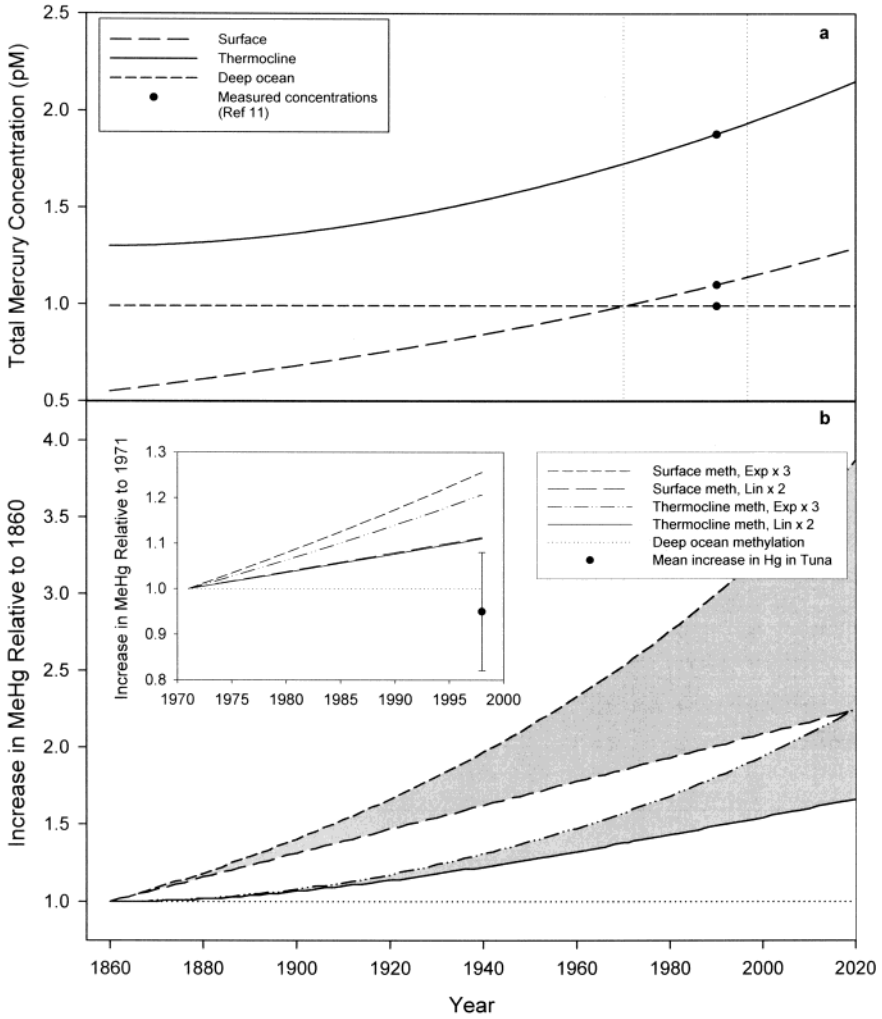

FIGURE 3. (a) Evolution with time of total mercury concentrations in the mixed layer, the thermocline, and the deep ocean as predicted by the box model. The total mercury concentration of the mixed layer is assumed to have increased exponentially over time and to have doubled between 1860 and 1990. The dots are the total mercury concentrations in the mixed layer, thermocline, and deep ocean measured by Mason and Fitzgerald in the Equatorial Pacific in 1990 (*11*). (b) Relative increase of methylmercury concentrations in the mixed layer (normalized to the concentration in 1860) over time as predicted by the box model, according to various scenario. The model predictions depend on the location of MeHg formation in the oceans (i.e., mixed layer, thermocline, or deep ocean and sediments) and on the increase in total mercury concentrations in the mixed layer over time (i.e., whether total mercury concentrations in the mixed layer have increased exponentially or linearly over time and have doubled or tripled between 1860 and 1990). The shaded areas indicate the range of uncertainty for each hypothesis. Insert: Comparison of the model predictions of the relative increase of methylmercury concentrations in the mixed layer between 1971 and 1998 (lines) with the ratio of the mean Hg concentrations in tuna (with a weight range of 45.4 Kg ± 6.8 Kg) between 1971 and 1998 (dot; the error bar represents the confidence interval at the 95% level). This time, MeHg concentrations are normalized to the MeHg concentration in 1971.

in the mixed layer and by 21% if it is formed in the thermocline. Assuming a linear rather than exponential increase of a factor of 2 yields a minimum 11% increase in the MeHg concentration of the mixed layer (see Table 1). The invariant mercury concentrations measured in tuna are inconsistent with all these scenarios and with all similar scenarios as long as the Hg concentration in the mixed layer is considered to have increased by more than 50% since 1860.

Some recently published measurements of Hg concentrations in the atmosphere between 18°29′E and 80°15′W suggest that these concentrations may have peaked in the late 1980s and then decreased and reached a plateau in the 1990s (*34*). This is unlikely to be the case over the Equatorial and Subtropical Pacific Ocean, because of the increased emissions from coal burning from China over the past 30 years (*35*−*37*). This is seen in the results of Prospero et al. (*38*) who measured a near doubling of anthropogenic sulfate aerosols (which, like mercury, originate mostly from coal combustion) over Midway Island between 1981 and the mid 1990s, followed by a possible slight decrease afterward. More directly, in a modeling study, Seigneur et al. (*39*) found a clear impact of Hg emissions from China on the annual total deposition fluxes of Hg in the Equatorial and Subtropical Pacific. Our scenario of a 15% increase between 1971 and 1998 in atmospheric inputs of Hg is thus likely conservative. Nonetheless, we tested our model using an input function based on the data of Slemr et al. (*34*) which show a peak in atmospheric Hg concentrations in 1990 (taken conservatively at 2.3 ng m^{-3}), a large increase prior to 1990 (extrapolated to 1.4 ng m^{-3} in 1971, consistent with the data of Seiler et al. (*40*)), and a decrease after 1990 (taken to reach 1.6 ng m^{-3} in 1998). We calculated that according to such a scenario, the MeHg concentration in the mixed layer has increased by 15% between 1971 and 1998 if MeHg is formed in the mixed layer and by 18% if MeHg is formed in the thermocline. Even in this case, MeHg formation in the mixed layer or in the thermocline can still be rejected with a confidence level better than 95% on the basis of the tuna data.

TABLE 1. Predicted Increases in MeHg Concentrations in the Mixed Layer between 1971 and 1998 According to Various Scenarios

	percent increase in MeHg concentrations in the mixed layer between 1971 and 1998		
	mixed layer source (hypothesis 1)[a] (%)	thermocline source (hypothesis 2)[a] (%)	deep source (hypothesis 2)[a]
exponential time-dependence; 3 x increase	25.6	20.7 (16.4−23.3)[b]	
exponential time-dependence; 2 x increase	15.5	12.3 (9.7−13.9)[b]	<1.5%
linear time-dependence; 2 x increase	11.3	11.0 (8.8−12.2)[b]	

[a] The percentage increases are calculated as follows: $[Hg]_s^{1998}/[Hg]_s^{1971} - 1$ (hypothesis 1); $[Hg]_{therm}^{1998}/[Hg]_{therm}^{1971} - 1$ (hypothesis 2); $[Hg]_D^{1998}/[Hg]_D^{1971} - 1$ (hypothesis 3). [b] The parentheses give the range of increases when the model parameters are varied to either minimize or maximize the results (see text).

The uncertainty on the value of the water fluxes F_1 and F_2 is estimated to be ±30% (see Model section), while the mercury concentration of the deep ocean, $[Hg]_D$, is estimated to be known within ±50%. If the model is run with the minimum value for F_1 and the maximum values for F_2 and $[Hg]_D$ (which all tend to minimize the rate of increase of total mercury in the thermocline), the MeHg concentration in the mixed layer is predicted to have increased by at least 9% between 1971 and 1998 (see Table 1, where the extrema obtained with various set of parameters are given in parentheses). Because the value for the depth of the thermocline, h_T, is interrelated with the magnitudes of the exchange fluxes F_1 and F_2, variations in h_T were not investigated independently.

Although the data set published by Mason and Fitzgerald (*11*) for the Equatorial Pacific is the most extensive one, mercury gradient between the mixed layer and the thermocline may not be as important as suggested by Mason and Fitzgerald's data (*41*). In this case, particulate transport must be lower than what we estimated in the model, and water advection (i.e., F_1 flux) is responsible for most of the transport of anthropogenic mercury from the surface to the thermocline. If we assume no particulate transport ($P_s^{Hg} = 0$) as an extreme case, the model predicts (for the exponential doubling case) that the MeHg concentration in the mixed layer would have increased by 10.6% between 1971 and 1998 if MeHg is formed in the thermocline.

Overall, the model predicts that if methylmercury is formed in the thermocline, its concentration in the mixed layer should have increased by at least 9% (for a linear increase of a factor of 2 in mercury concentrations in the mixed layer) and up to 21% (for an exponential increase of a factor of 3) between 1971 and 1998 (see Table 1). If methylmercury is formed in the mixed layer, the model predicts that its concentration should have increased by at least 11% between 1971 and 1998. These are large increases since the data of Figure 1 are incompatible with an increase of mercury in tuna by more than 6% ($p < 0.05$).

Role of Dimethylmercury (DMHg). It has sometimes been proposed that MeHg in the oceans originates from the demethylation of dimethylmercury (DMHg, $Hg(CH_3)_2$) rather than from the direct methylation of inorganic mercury (*11*). For the sake of clarity, we have not considered DMHg in our discussion, but our conclusions remain the same if DMHg, rather than MeHg, is the main product of mercury methylation. In the oceanic reservoir where inorganic mercury is methylated, DMHg would be proportional to total mercury. At pseudo steady-state, the MeHg concentration in the mixed layer would be proportional to the concentration of DMHg in the reservoir where it is formed and thus to the total mercury concentration in this reservoir, as in the three hypotheses examined in our model. The evolution of MeHg concentrations with time predicted by the model thus does not depend on whether MeHg originates from inorganic mercury or from DMHg.

A Deep Source of MeHg in the Oceans? The combined analysis of the 1971 and 1998 data sets of mercury concentrations in Yellowfin tuna and a simple box model for mercury cycling in the Equatorial and Subtropical Pacific indicate that methylmercury is likely formed neither in the mixed layer, nor in the thermocline. Deposition of MeHg from the atmosphere is not significant in this part of the ocean (*42*). In addition, the export of primary production from coastal areas is practically negligible (as shown by ^{13}C analysis of organic matter, (*43*)), and since tuna spend most of their time in the open ocean ((*26*, *28*)), MeHg is not likely to originate from coastal waters, either. It thus appears that the deep oceans or sediments are the likely source of MeHg.

The current prevalent hypothesis is that mercury is methylated at the depth of the oxygen minimum in the oceans (e.g, refs *10*, *11*, and *44*). The "oxygen minimum" hypothesis is based on three lines of evidences: (i) some depth profiles of methyl and dimethylmercury in the Pacific and Atlantic oceans as well as in the Mediterranean sea seem to indicate a concentration maximum at the oxygen minimum (*10*, *11*, *14*, *44*, *45*); (ii) the only known source for methyl and dimethyl mercury is biological, and the oxygen minimum is a source of relatively intense bacterial activity; (iii) in lakes, methylmercury is produced by sulfate reducing bacteria and originates in anoxic waters and sediments (*46*, *47*).

Depth profiles of MeHg and DMHg in the oceans are few owing to the difficulty of measuring extremely low concentrations and do not necessarily provide a strong indication for a maximum at the oxygen minimum. In addition, although the oxygen concentration decreases at the oxygen minimum, anoxia is never reached, and since there is no evidence that sulfate reducers are active, the actual mechanism for the postulated methylation remains obscure. Alternatively, MeHg and DMHg could originate in the deep ocean. A number of their depth profiles could be interpreted as indicating a deep source (*10*, *11*, *14*), and sulfate reduction (often associated with methane oxidation) is known to occur in some oceanic margins and deep sea sediments, which could provide a favorable environment for the production of MeHg and DMHg (*48*). A particularly intriguing possibility is the formation of MeHg and DMHg in hydrothermal vents, where both high concentrations of mercury (particularly in the form of cinnabar and elemental Hg) and sulfate-reducing bacteria have been found (*49*−*51*). Given that sulfate-reducing bacteria are the primary mercury methylators in freshwater ecosystems and coastal areas, it is possible that significant amounts of methylmercury are produced at these locations. Although it is clear that MeHg and DMHg degrade relatively rapidly in the presence of light (*22*, *52*−*54*), there are no reliable data on the degradation rates of MeHg and DMHg in conditions resembling those of the deep ocean, and it is

not impossible that the methylated species of Hg may be stable in the deep ocean over time scales of several hundred years.

On the basis of constant mercury concentrations in tuna between 1971 and 1998 (and with help of a simple model of the mercury cycle in the oceans), we hypothesize that methylmercury is formed in the deep sea or in sediments, where mercury concentrations have been little affected by human activities. It may seem roundabout to use a study of mercury concentration in tuna over time to infer where mercury methylation occurs in the open oceans. This indirect approach reflects the much easier measurement of mercury species in fish than in seawater: the data on methylmercury in the oceans are too few and too variable to provide a sound basis for assessing its biogeochemical sources and sinks directly. Nonetheless, an elucidation of the actual origin of methylmercury in surface seawater and in pelagic fish will have to come from a direct mechanistic understanding of mercury methylation in the oceans. Our conclusion rests ultimately on the simple idea that if mercury in tuna originated wholly or partly in the atmosphere, the increase in atmospheric mercury should have been reflected to a measurable extent in the fish. The bare fact that the concentrations of Hg in tuna were identical in 1971 and 1998 either reflects a remarkable coincidence or indicate that, regardless of mechanisms, these concentrations are not responding to atmospheric pollution.

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Supporting Information Available

Mercury concentrations in Yellowfin tuna caught off Hawaii in 1971 and 1998 (Tables A1 and A2). This material is available free of charge via the Internet at http://pubs.acs.org.

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