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Distribution Kinetics of Dietary Methylmercury in the Arctic Charr (*Salvelinus alpinus*)

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We fed immature 1+ arctic charr with a single dose of methyl[^{203}Hg]mercury (MeHg) and quantified distribution kinetics with a new and simple three-compartment caternary model having well-perfused viscera and blood as the central compartment (VB), whereas gut (*G*) and the rest of body (*R*) constituted the peripheral compartments. The model accurately described distribution kinetics of MeHg in the fish, using either data of MeHg content in compartments or blood concentration data. Despite the known fast translocation of MeHg between binding sites at the molecular level, its translocation rate between compartments was surprisingly slow, 27 days being needed to complete 95% of the transfer from gut to blood and 48 days for the subsequent transfer to compartment *R*. This probably results from a limitation of the stepwise transfer rate of MeHg from red blood cells, which contain most of blood MeHg, to plasma and then to tissues due to low plasmatic concentration of small mobile sulfhydryl ligands. The model presented is a convenient tool that could be used to compare the fate of MeHg and other organometals, such as butyltins and alkylleads, in various aquatic and terrestrial animal species.

Introduction

Pharmacokinetic modeling is an application of chemical kinetics to the study of the behavior of chemicals in biological systems which allows the mathematical characterization and the quantification of processes related to the time dependent bioaccumulation of xenobiotics in living organisms, e.g. absorption, distribution, and elimination (*1*). Despite advances in the understanding of the fate of methylmercury (MeHg) in aquatic biota (*2*−*7*), the knowledge about the pharmacokinetics of dietary MeHg in fish is still limited to a small number of published work (*8*−*11*). It is known that MeHg distribution in living organisms is ubiquitous and that elimination rate is very slow. However, the temporal evolution of MeHg concentration has been shown to differ between tissues (*8, 10*) and elimination kinetics observed were described by biexponential equations (*9, 11*). This indicates that the distribution process of dietary MeHg in fish tissues is complex and involves two or more kinetically distinct groups of tissues. To our knowledge, no attempt has been yet made to establish the identity of these tissue groups and quantify the distribution kinetics of dietary MeHg between them with a suitable model.

In a previous work on marine invertebrates, we developed and applied a three-compartment caternary model described with simple algebraic equations to quantify the distribution kinetics of dietary organometals (*12*). In the present work, we adapted this model to fish physiology to study the fate of a single dietary dose of Me^{203}Hg(II) in the body of the arctic charr (*Salvelinus alpinus*).

Material and Methods

Experimentals. Immature 1+ year old arctic charrs (48 ± 18 g) were acclimatized to experimental conditions for 15 days (dechlorinated tap water, T = 5 ± 1 °C, 12h:12h light:dark). Fish were fed ad libitum every 3 days with commercial dry pellets (Moore Clark Co.).

Radioactive Me^{203}Hg(II) was synthesized from $^{203}HgCl_2$ (Amersham Co.) (*13*). Food pellets were labeled by a 30-min immersion in an aqueous solution of Me^{203}Hg(II) and dried overnight, and their radioactivity was measured individually with a LKB Clinigamma counter (counting window 250−325 keV, counting time 10 min). On day 0, fish were force-fed with these pellets, after anesthesia with 0.02% Tricaine (MS-222). The average MeHg dose was 0.26 ± 0.05 μg Hg·g^{-1} body weight (b.w.). Radiotracer amount was 1140 Bq·μg^{-1} Hg. Fish were then fed with uncontaminated food every 3 days until the end of the experiment. A second group of fish received pellets containing inorganic ^{203}Hg(II), according to the above protocol. Groups of three fish were sampled at day 0.5, 1, 2, 3, 4, 6, 8, 11, 14, 18, 22, 26, and 30. After lethal anesthesia, blood (via caudal vein), gut, kidney, spleen, liver, gills, and brain were collected, weighted, and radioactivity measured. Remaining tissues, i.e., bones, muscles, fins, skin, and head, hereafter called "rest of body", were counted collectively.

To monitor the possible leaching of Me^{203}Hg from feces into water, 3-mL water samples were collected and counted daily. Control fish that had not received labeled food were also placed in experimental aquariums, and three of them were collected at day 6, 19, and 30 for dissection as above. To obtain further information on the body-distribution of Me^{203}Hg, two additional animals were sampled at day 1 and 22 and used for whole-body autoradiography (*14*). Speciation of mercury in tissues was determined in 15 arctic charrs (100−180 g b.w.) which received a single dose of nonradioactive MeHg accordingly to the above protocol. Five fish were sampled every 10 days, and inorganic Hg and MeHg concentrations in 0.5−3 g samples of liver, muscle, and red blood cells were determined (*15*).

Model Development. MeHg taken up with food is first received in the gut (*G*) of fish and then crosses the intestinal wall barrier to be distributed toward other visceral organs (*V*) and the rest of body (*R*) via the blood stream (*B*). This process can be represented with the mammillary model shown in Figure 1A. Rate constants k_1, k_2, k_3, k_4, k_5, and k_6 characterize metal exchanges between compartments. Rate constant k_e stands for the fecal elimination of MeHg (*8*). Blood perfusion rate is often an important factor in determining the relative rate of distribution of xenobiotics between organs and tissues (*16*). Exchange of MeHg between blood

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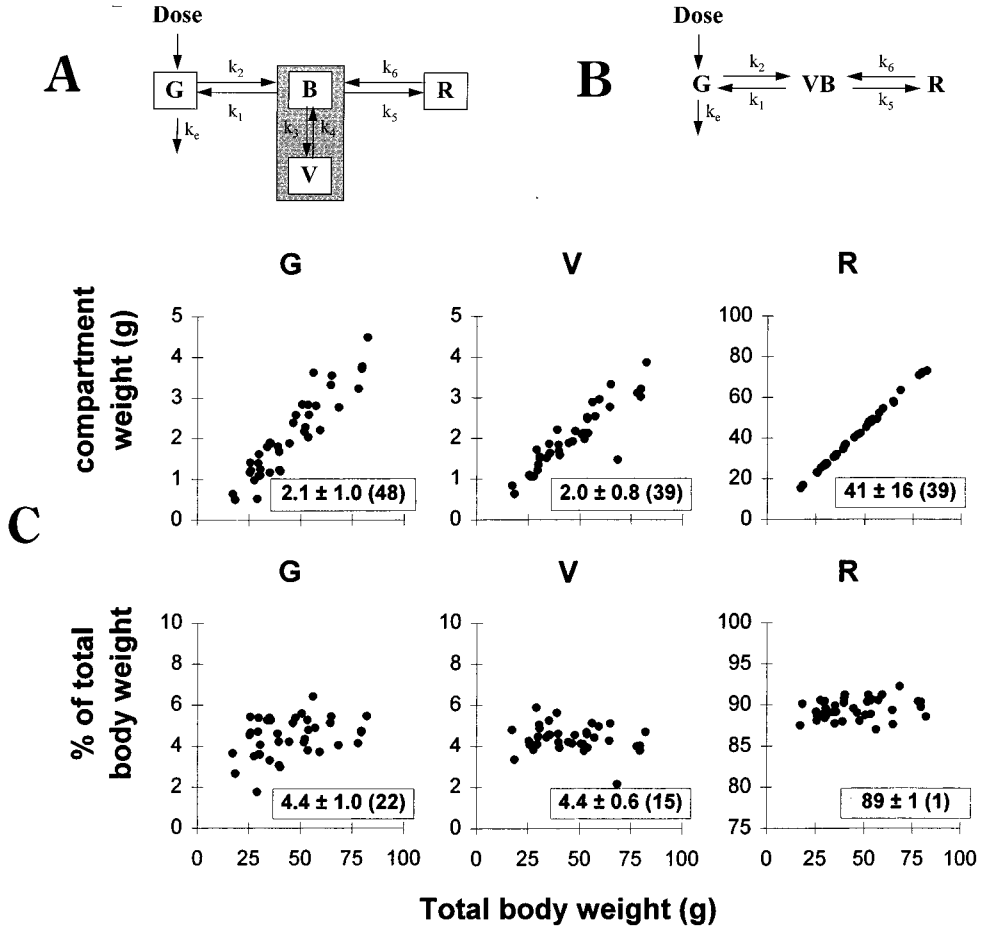

FIGURE 1. (A) Schematic representation of MeHg distribution pathway in fish (adapted from ref *1*). *G* = gut, *V* = liver + kidney + spleen + gills, *B* = blood, *R* = rest of body. (B) Simplified caternary model. (C) Total wet weight and percentage of total body weight of compartments *G*, *V*, and *R*. Values in boxes are means ± SD (percent coefficient of variation). Blood samples represented 1.6 ± 0.2% of body weight.

and the well-perfused visceral organs is very fast compared to absorption from gut (*11, 17*). In such a case, the kinetics of MeHg in blood, liver, kidney, spleen, and gills are unlikely to be distinguishable. Furthermore, these well-perfused organs contains 10−20% of blood by weight (*18*). These facts justify grouping blood, liver, kidney, spleen, and gills in the same compartment renamed VB (shaded area in Figure 1A) and simplifying the model to the representation shown in Figure 1B. Such a model behaves mathematically like a caternary model rather than a mammillary model since MeHg enters the system via a peripheral compartment (*19*).

The quantity of a chemical contained in a compartment is usually expressed as a concentration or an amount, which are directly dependent upon the initial quantity of chemical administered and compartment size. When using many different animals to study kinetics, interindividual differences of both size and dose may render very difficult, if not impossible, the quantification of the temporal evolution of chemical's amount in compartments. This problem can be circumvented by expressing the amount of chemical in a compartment i as a proportion of the whole-body content. Proportional content can be related to the pharmacokinetic definition of the apparent volume of distribution V_i (ratio of the amount Q_i of chemical in compartment i and its concentration C_r in a reference tissue) with (*12*)

$$V_i/V_{wb} = (Q_i/C_r)/(Q_{wb}/C_r) = Q_i/Q_{wb} \quad (1)$$

where V_{wb} and Q_{wb} are the apparent volume of distribution and chemical's quantity in whole body, respectively. Assuming that physiological characteristics as well as partitioning and binding of the chemical to tissues are the same for individuals of a given animal species, the size of V_i depends on the anatomical volume only (*1*). As a consequence, the size of V_i/V_{wb}, and thus Q_i/Q_{wb}, depends on the relative anatomical volume of the compartment. Though weight of compartments in arctic charr is correlated to total body weight, the relative anatomical volume expressed as a percentage of the total body weight is rather constant (Figure 1C), and so will be the relative amount of chemical in them. For the caternary model shown in Figure 1B, if k_e is negligible, expressions describing the temporal evolution of MeHg content in compartments, G, VB, and R, expressed as a proportion of the dose received with food, are (*12*)

$$G = G_{SS}\,(1 - e^{-\alpha_1 t}) + D_A e^{-\alpha_1 t} \quad (2)$$

$$VB = VB_{SS}\,(1 - e^{-\alpha_1 t}) + R_{SS}\,(e^{-\alpha_2 t} - e^{-\alpha_1 t}) \quad (3)$$

$$R = R_{SS}\,(1 - e^{-\alpha_2 t}) \quad (4)$$

where G_{SS}, VB_{SS}, and R_{SS} are values of G, VB, and R when distribution is at steady-state, D_A is the overall percentage of the MeHg dose assimilated in the body ($D_A = G_{SS} + VB_{SS} + R_{SS}$), and rate constants α_1 and α_2 are equal to $(k_1 + k_2)$ and $(k_5 + k_6)$, respectively. Brain accounted for a very small proportion of the body burden and was not included in any compartment. MeHg kinetics in this organ will be treated separately.

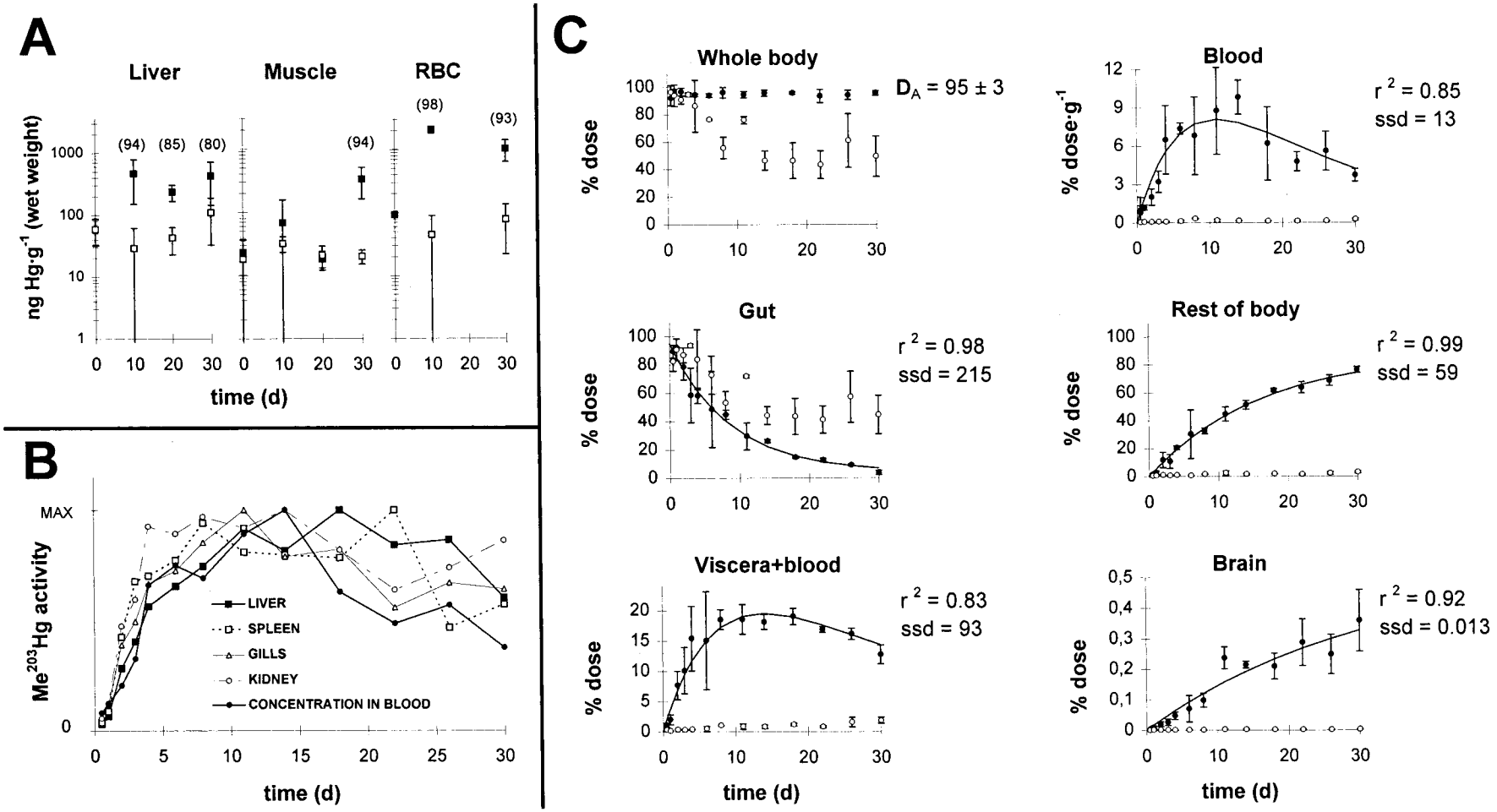

FIGURE 2. (A) MeHg (■) and inorganic Hg (□) concentrations (mean ± SD) in fish fed with nonradioactive MeHg. Data for $t = 0$ are from control fish ($n = 3$). Values within parentheses are percent of total Hg represented by MeHg. RBC = red blood cells. (B) Temporal evolution of $Me^{203}Hg$ activity in well-perfused visceral organs and blood relative to the maximum activity recorded for each of them. Data points are mean of three fish. (C) Distribution kinetics of dietary $Me^{203}Hg$ (●) in arctic charr. Data points are mean ± SD of three fish. Fitted curves were obtained by nonlinear regression analysis. ssd = sum of squared deviations. Data from fish fed with inorganic $^{203}Hg(II)$ are also shown (○).

TABLE 1. Values ± SE of G_{SS}, VB_{SS}, R_{SS}, Br_{SS}, B_{SS}/b_v, R_{SS}/b_v, α_1, and α_2 Calculated by Nonlinear Regression Analysis with Eqs 2−6

	G_{SS} (% dose)	VB_{SS} (% dose)	R_{SS} (% dose)	Br_{SS} (% dose)	B_{SS}/b_v (% dose·g^{-1})	R_{SS}/b_v (% dose·g^{-1})	α_1 (d^{-1})	α_2 (d^{-1})
gut	4.3 ± 3.5 [0.25][a,b]						0.116 ± 0.012	
viscera + blood		4.7 ± 2.7 [0.20]	89 ± 19				0.100 ± 0.007	0.062 ± 0.016
rest of body			88 ± 4 [0.26]					0.062 ± 0.006
brain				0.5 ± 0.2				0.033 ± 0.018
blood					0.7 ± 0.9	54 ± 26	0.113 ± 0.005	0.077 ± 0.014

[a] Values within brackets are calculated concentrations (μg·g^{-1} wet weight). [b] Conversion of (% dose) to concentration: (% dose in compartment (this table)/(compartment's % of body weight (Figure 1C)) × dose (0.26 μg·g^{-1} body weight).

Pharmacokinetics are often determined by sequential sampling of blood because of its ease of collection and ubiquitous contact with other tissues (*1*). We thus wanted to compare the values of model parameters calculated from blood concentration data to those obtained with eqs 2−4. Blood belongs to the central compartment, and the algebraic expression of MeHg in blood versus time has a form similar to eq 3. As concentration data are used instead of quantity, eq 3 was modified to give

$$B/b_v = B_{SS}/b_v\,(1 - e^{-\alpha_1 t}) + R_{SS}/b_v\,(e^{-\alpha_2 t} - e^{-\alpha_1 t}) \quad (5)$$

where b_v is the vascular volume and B is the proportion of the MeHg dose in blood. Assuming that 1 g blood ≈ 1 mL blood, B_{SS}/b_v is the steady-state concentration of metal in blood, expressed as a percentage of the metal dose per gram of blood, whereas R_{SS}/b_v represents the MeHg quantity transiting per gram of blood before it reaches compartment R.

Values of G_{SS}, VB_{SS}, R_{SS}, B_{SS}/b_v, R_{SS}/b_v, α_1, and α_2 were calculated by nonlinear regression analysis (STATGRAPH) from the experimental values of G, VB, R, and B/b_v plotted as a function of time. D_A was estimated from MeHg whole body content.

Results and Discussion

Levels of $Me^{203}Hg$ in water and in control fish remained below the detection limit throughout the experiment (<0.8 Bq or 0.006% of dose in sample). In fish fed with nonradioactive MeHg (Figure 2A), increase of total Hg concentration in muscle and red blood cells was almost entirely related to the accumulation of MeHg. In the case of liver, inorganic Hg concentration was slightly higher at day 30, but MeHg always represented >80% of the total Hg accumulated in this organ.

Evolution of $Me^{203}Hg$ level in blood, liver, spleen, kidney, and gills was similar (Figure 2B), showing that they constituted a kinetically homogeneous unit. Values of D_A, constant and close to 100%, are indicative of the negligible losses of MeHg from the body (Figure 2C). The fitting of model equations with experimental data was excellent, as shown by high r^2 and low sums of squared deviations, and it is noteworthy that values of R_{SS}, α_1, and α_2 calculated from data of different compartments agreed quite well (Figure 2C and Table 1). Calculation of the time needed to reach 95% of the steady-state distribution from the values of α_1 and α_2 ($t_{0.95} = -(\ln 0.05)$/rate constant) shows that the initial transfer of MeHg from G to VB was faster than transfer from VB toward R (27 days versus 48 days). This result also reveals that the distribution of MeHg was a slow process that extended beyond our 30-day experimental period. Values of G_{SS}, VB_{SS}, and R_{SS} indicate that almost 90% of the MeHg initial dose would be found in R at steady-state. Nevertheless, conversion of percent dose to concentrations (Table 1) shows that MeHg is uniformly distributed at steady-state.

Values of B_{SS}/b_v and R_{SS}/b_v calculated for blood with eq 5 were 0.7%·g^{-1} and 54%·g^{-1}, respectively, and values of α_1 and α_2 were similar to those obtained from compartments G, VB, and R. Assuming that all MeHg transported to compartment R transited through blood and that partitioning of MeHg between blood plasma and red blood cells (rbc) is fast (*20, 21*) and at equilibrium at any time, the value of R_{SS} found with data of compartment R divided by the total volume of blood in fish (mL·100 g^{-1} b.w. × mean fish b.w. of 48 g/100) should give a result similar to the R_{SS}/b_v value found with blood concentration data (54 ± 26%·g^{-1} blood). Calculation using estimate of blood volume in salmonids of 3.5 mL·100 g^{-1} b.w. (*22, 23*) yielded a value of 52%·g^{-1} blood.

In fish fed with inorganic ^{203}Hg, radioactivity in gut decreased by 50% 4−7 days after feeding as a result of the elimination of nonretained metal with feces. Most of the retained Hg was trapped in the gut (Figure 2C). Only 4% of the Hg dose was distributed to VB and R, and metal amounts in these compartments, which were 10−40 times lower than in MeHg-fed fish, remained constant once fecal elimination of nonretained metal had occurred. These data and those on the speciation of nonradioactive MeHg (Figure 2A) confirm that the high assimilation and progressive transfer of radioactivity from G to VB and R observed in $Me^{203}Hg$-dosed fish are representative of MeHg actual behavior.

The time trend of MeHg accumulation in brain (Br) was comparable to compartment R (Figure 2C) and can be quantified with an expression similar to eq 4

$$Br = Br_{SS}\,(1 - e^{-\alpha_2 t}) \quad (6)$$

Values of Br_{SS} and α_2 (Table 1) show that 0.5% of the MeHg dose would be found in the brain at steady-state, though it would take more time to reach steady state than in other tissues ($t_{0.95} = 91$ days). The slower kinetics of MeHg in brain may be attributed to the particular properties of the blood brain barrier (*8*). Accumulation of inorganic Hg in brain was 200 times lower than for MeHg.

Autoradiograms from contaminated fish are shown in Figure 3. After 1 day (Figure 3A,B), the gut was the only structure labeled. Content and wall of stomach were highly labeled. However, much less radioactivity was present in the pyloric caeca and intestine. To compare semiquantitatively the labeling of gut, autoradiograms were digitized as black and white images with 256 values of gray and average value of gray of the various areas was determined (Table 2). Labeling decreased in the order stomach > pyloric caeca wall > intestine wall, whereas that of intestinal lumen was similar to background values. This shows that the complete assimilation of the MeHg dose was related to its efficient absorption in the first part of the gut. After 22 days (Figure

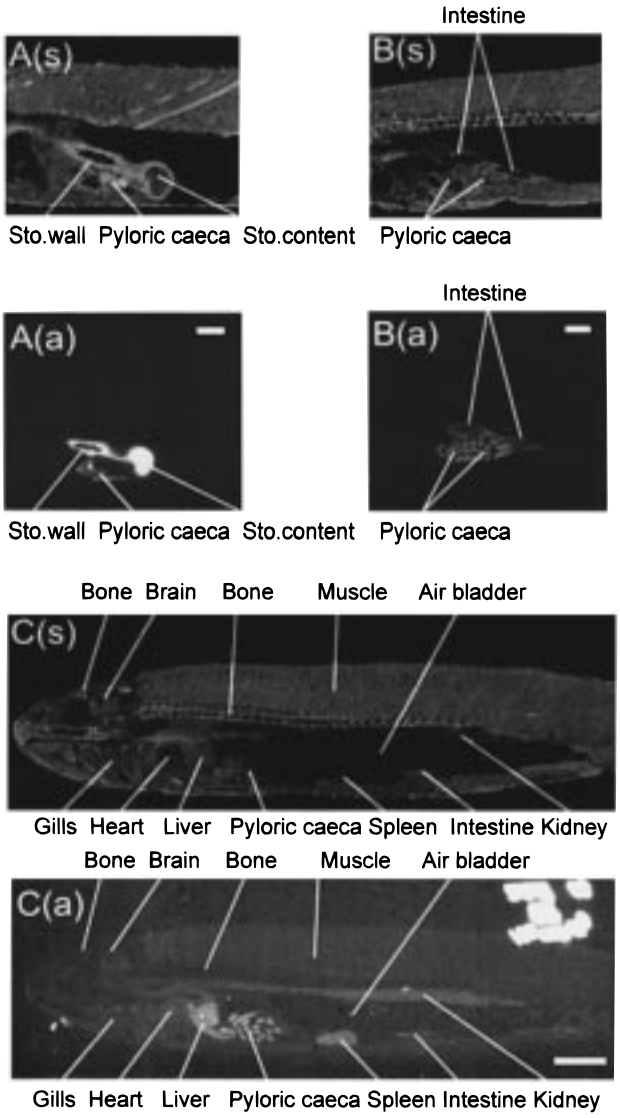

FIGURE 3. (A, B) Autoradiograms from 20-μm-thick whole-body saggital sections of *Salvelinus alpinus* 1 day (bar = 0.5 cm) and (C) 22 days (bar = 1 cm) after feeding with $Me^{203}Hg$. (A) and (B) are from the same fish, but sections were taken at different levels: (s) = tissue section, (a) = autoradiogram. Intensity of labeling is related to $Me^{203}Hg$ concentration.

3C), radioactivity can be seen in all tissues. Labeling of gut, liver, spleen, and kidney was higher than in the rest of the body (Table 2). Though 60% of the MeHg dose has been already transferred to compartment *R* at that time (Figure 2C), concentration is still lower compared to visceral organs because of the much bigger size of *R*. Nevertheless, autoradiographic data support kinetic data which indicated that MeHg was progressively distributed from well-perfused organs toward compartment *R*. In the latter, distribution of MeHg appeared uniform but in bony tissues. As bones account for only 10−15% of compartment *R* (*18*), quantitative data obtained for this compartment can be considered as representative of the soft tissues, of which 85−90% are muscle tissues (*18*).

This simple model accurately described and quantified the distribution kinetics of MeHg within the body of arctic charr. Kinetics observed are probably representative of the distribution process of MeHg within the body of wild fish since experiments were done at a temperature representative of the cold freshwater environment where arctic charr lives. The approach used in the present work could be extended to similar studies with other aquatic and terrestrial species (fish, amphibians, birds, and mammals) and with other organometals found in the aquatic environment, such as butyltins and alkylleads. Alternatively, α_1, α_2, and R_{SS} could be determined by using only blood samples and fitting MeHg blood concentration data to eq 5. MeHg accumulation in edible flesh of fish, which constitutes most of compartment *R*, is the major concern for human health.

The main finding of this study is the slow release of MeHg within fish body, a phenomenon we have also recently reported for the American plaice ($t_{0.95} = 35 \pm 4$ days) (*24*). The distribution of MeHg in living organism is generally considered a fast process based on chemical calculations (*25, 26*) showing that MeHg is quickly exchanged between sulfhydryl sites of macromolecules. The slow transfer rates of MeHg from compartments *G* to VB and from VB to *R* indicate a clear discrepancy in the behavior of MeHg between the molecular and the systemic levels of biological organization.

At the molecular level, the exchange mechanism of MeHg between sulfhydryl-containing ligands (SL) in biological system proceed through bimolecular nucleophilic displacement, an associative mechanism that presumably involves an intermediate of the type $MeHg(SL)_2$ (*25, 26*). The formation of this intermediate is likely to be facilitated if one of the ligands is a small and mobile molecule. Actually, glutathione (GSH), a small sulfhydryl-containing tripeptide, appears to play a major role in the mobility of MeHg in living organism (*25, 27, 28*).

At the systemic level, autoradiograms showed that dietary MeHg was efficiently and rapidly taken up in the intestinal wall of arctic charr. MeHg transferred to blood thereafter is partitioned between rbc and plasma. The rbc/plasma ratio, though species dependent, is generally ≥10 because sulfhydryl concentration in rbc is higher than in plasma (*29*). In arctic charr, MeHg concentration in rbc was very high (Figure 2A) whereas that of plasma was below the detection limit.

TABLE 2. Average Gray Value ± SD (1 = Black, 256 = White) for Various Areas of Digitized Whole-Body Autoradiograms Shown in Figure 3

	t = 1 day			t = 22 days	
background	40 ± 5	[171 600][a]	background	55 ± 13	[136 100]
stomach content	241 ± 4[b]	[1960]	liver	137 ± 17[b]	[1780]
stomach wall	224 ± 26[b]	[4420]	spleen	116 ± 12[b]	[1650]
pyloric caeca wall	111 ± 24[b]	[387]	pyloric caeca wall	148 ± 20[b]	[365]
intestine wall	72 ± 16[b]	[1010]	intestine wall	86 ± 12[b]	[640]
intestine lumen	≈ background		kidney	95 ± 14[b]	[9640]
liver, kidney, gills, spleen	≈ background		gills	72 ± 13[b]	[4150]
brain	≈ background		brain	72 ± 12[b]	[1767]
muscle	≈ background		muscle	76 ± 11[b]	[54 400]
bones	≈ background		bones	≈ background	

[a] Values within brackets are number of pixels quantified. [b] Significatively different from background (two-tailed t-test; $p < 0.001$).

Thus, the passage of MeHg from rbc to tissues belonging to compartments VB and *R* likely implies transfer through plasma as an intermediate step. The transport of MeHg out of rbc's cytoplasm proceeds by successive transfers, from cytoplasmic proteins to membrane proteins to plasma, a multistep mechanism mediated by exchanges involving intracellular GSH and small plasmatic sulfhydryl ligands (*30*). However, the latter constitutes a very small proportion of the sulfhydryl pool in plasma (*17*, *29*). Furthermore, low extracellular concentrations (10^{-4} M) of GSH and cysteine where shown to be unable to remove MeHg from fish rbc in vitro (*20*). Hence, despite fast exchange at the molecular level, MeHg transfer between rbc and tissues is probably rate limited by the low concentration of small mobile sulfhydryl ligands in plasma. It is noteworthy that Schultz and Newman (*17*) found that kinetics of MeHg injected intravenously in two fish species were faster for the one that had a higher percentage of plasmatic MeHg bound to low molecular weight ligands. This further supports the hypothesis that these ligands are essential for the translocation of MeHg in living organisms. By the same reasoning, the faster transfer rate of MeHg from gut to blood might be related to a greater abundance and the transfer of such small thiol ligands resulting from digestion process.

Acknowledgments

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