A large fraction of mercury residing in mussel shell would support the contention that enhanced uptake of ^{203}Hg noted in smaller whole mussels may have been due to greater surface area per unit volume available for isotope adosrption.

As with mussels, viscera also contained the highest concentration of total mercury noted in any of the shrimp tissues examined. The mercury content of exoskeleton was low, with the lowest concentrations residing in molts which make up the outer layer of the exoskeleton. Bertine and Goldberg[18] found similar levels (1.3 ppm dry) in both dissected exoskeletons and the remaining tissues of shrimp and suggested a possible role of molts in the cycling of mercury in marine waters. Our data indicate that molts which account for almost 20% of the shrimps wet weight will contain only about 2% of the total mercury body burden. Results from our radiotracer experiments, which showed that less than 2% of either incorporated inorganic or organic mercury was lost with the molt, also point to a relatively minor role in mercury transport through crustacean molting. Furthermore, the relatively low mercury concentration in molts compared to other tissue would appear to rule out the possibility of surface adsorption as a principal mode of mercury uptake. Closely related studies on mercury kinetics in macroplanktonic crustaceans have led to similar conclusions (see below).

1.2 Mercury kinetics in marine zooplankton

S.W. Fowler, M. Heyraud and J. LaRosa

Mercury, like many other heavy metals, is potentially available to marine animals by uptake directly from water and/or through the organisms food. Furthermore, bioavailability, assimilation and subsequent retention in biota may be affected by the chemical species of the element in sea water. While mercury is known to exist in the inorganic form in sea water, recent work has indicated that, in certain coastal areas, a good portion of the total mercury appears to be organically bound[19]; however, the exact chemical nature of the organic fraction has yet to be determined. Methyl mercury may be one constituent of the natural organically bound fraction since microbial mechanisms for *in situ* methylation of mercury have been demonstrated in the aquatic environment[20]. Despite the fact that naturally produced methyl mercury probably comprises only a small fraction of an aquatic ecosystem, the well-documented toxic effects of this organo-mercurial, caused by man-made introductions into marine food chains, make it an important compound to study.

As part of the programme dealing with the cycling of pollutants by marine macrozooplankton, laboratory studies were designed to delineate the kinetics of different chemical forms of mercury in euphausiids. To compare the bioavailability of methyl mercury with the inorganic form, euphausiids *(Meganyctiphanes norvegica)* were exposed to $CH_3{}^{203}HgCl$ and $^{203}HgCl_2$ in sea water at a radiotracer concentration of approximately 0.1 nCi/ml for a period of 4 weeks. Differences in specific activity of the tracer solutions resulted in stable mercury additions of 0.25 and 1.6µg/ℓ in the CH_3HgCl and $HgCl_2$ experiments, respectively. The natural mercury level in the water used in these experiments was approximately 0.02µg/ℓ (Fukai, unpublished results). Loss of mercury from the sea water solutions (measured as loss of ^{203}Hg) due to physical adsorption on the container walls and volatilization necessitated changing the media every 2 days. Fresh sea water solutions were prepared so as to maintain the stable mercury concentration relatively constant throughout the experiment. During the time when the solutions were changed, the euphausiids were briefly placed in non-radioactive sea water and fed brine shrimp *(Artemia)* to ensure good health throughout the uptake period. The amount of stable mercury accumulated by the euphausiids was computed from the known specific activity of the tracer solution. For purposes of relative comparison of the bioavailability of the two chemical forms, the results are given in terms of concentration factors which are defined as the amount of stable or radioactive mercury taken up per gram wet euphausiid divided by the added stable or radioactive mercury per ml of sea water. Previous tests showed that over a stable mercury concentration range from 0.2 to 2µg/ℓ which encompasses that used in this experiment, mercury accumulation was directly proportional to the concentration in sea water; hence, any differences in concentration factors observed in the uptake experiments utilizing methyl and inorganic mercury could be interpreted as differences in bioavailability of the two forms and not to stable mercury concentration differences in the two sea water media.

As an aid in interpreting the results, aliquots of the radioactive sea water were filtered with 0.45µm membrane filters to assess the particulate fraction of mercury added to the sea water.

A second experiment was designed to assess any differences in assimilation and subsequent retention of mercury when the two forms are administered through the food chain. *Artemia* were allowed to grow in a labelled phytoplankton suspension containing either $CH_3{}^{203}HgCl$ or $^{203}HgCl_2$ for a period of two weeks. Following labelling, the adult *Artemia* were rinsed for several minutes in clean sea water to remove any loosely

adsorbed mercury. Five euphausiids held individually in 750ml glass jars each received 15 radioactive *Artemia*, all of which were consumed after approximately 1 hour. Immediately following ingestion the euphausiids were monitored for their ^{203}Hg content, replaced in clean sea water and fed *ad libitum* non-radioactive *Artemia*. Periodically during the next 3 days, the euphausiids were counted to follow the excretion of ^{203}Hg and at the same time their sea water medium was filtered to remove any radioactive fecal pellets. Radioactive mercury on the filter paper and in the euphausiid compared to the radioactivity contained in the animal at any previous period allowed assessment of the fraction of total loss due to fecal excretion. In addition, retention of ^{203}Hg by the euphausiids was measured for several weeks in order to estimate the excretion rate of tissue incorporated mercury following a single ingestion of labelled food.

In order to measure the total turnover rate of both chemical forms of mercury, several euphausiids were uniformly labelled with ^{203}Hg via the food and water route for 17 days. The experimental design was similar to that for the uptake from the water experiment except that the euphausiids simultaneously ingested radioactive *Artemia* which had been previously labelled in a phytoplankton suspension containing the same radiotracer concentration as that in the euphausiid medium. Radioactive feedings ceased two days before the start of the excretion phase in order to allow the euphausiids to clear their gut of any radioactive residue. Following uptake the organisms were rinsed for several minutes, monitored for ^{203}Hg and replaced in clean sea water. Throughout the 6-week excretion phase, the water was changed daily and the euphausiids fed *Artemia*. As a comparison with mercury excretion kinetics in euphausiids labelled from both food and water, the individuals which had accumulated the isotope only from water were also transferred to clean sea water and their subsequent elimination of mercury followed under identical conditions.

Several studies have emphasized the importance of released zooplankton particulate products in the general cycling of certain radionuclides and heavy metals in the marine environment[21-23]. The fate of mercury in these products was examined by studying the retention times of the two forms of mercury in released radioactive feces and molts as well as in euphausiid carcasses. During the course of the experiments described above, fecal pellets, molts and dead euphausiids were kept in sea water and frequently monitored to assess loss of the radioisotope to the surrounding water. The water was changed frequently to reduce re-accumulation of the released ^{203}Hg by the material and the duration of the experiments was limited by the length of time the material remained intact. Counting techniques for measuring ^{203}Hg were similar to those described in Section 1.1.

A preliminary study was initiated to measure the levels of total mercury in euphausiids and their particulate products. Natural fecal pellets and molts were collected in the laboratory by methods described previously[24,25]. In addition, several dozen individuals were dissected into eyes, muscles, viscera and exoskeleton to study the tissue distribution of mercury in *M. norvegica*. All tissues, whole individuals, particulate products, and microplankton which serve as food for the euphausiids were freeze-dried and analyzed for total mercury by neutron activation analysis at the International Atomic Energy Agency's Seibersdorf Laboratory.

The results of the mercury bioavailability experiment are presented in Figure 4. Methyl mercury was accumulated at a nearly constant rate reaching a concentration factor of approximately 10^4 after one month. Inorganic mercury, on the other hand, was taken up much more slowly and appeared to be approaching a steady state toward the end of the 4-week period. Concentration factors for both chemical forms were considerably higher than those reported for several other metals and radionuclides in the same organism[22,26-28]. Membrane filtration of the labelled media indicated that approximately 80% of both chemical forms of mercury was present in a

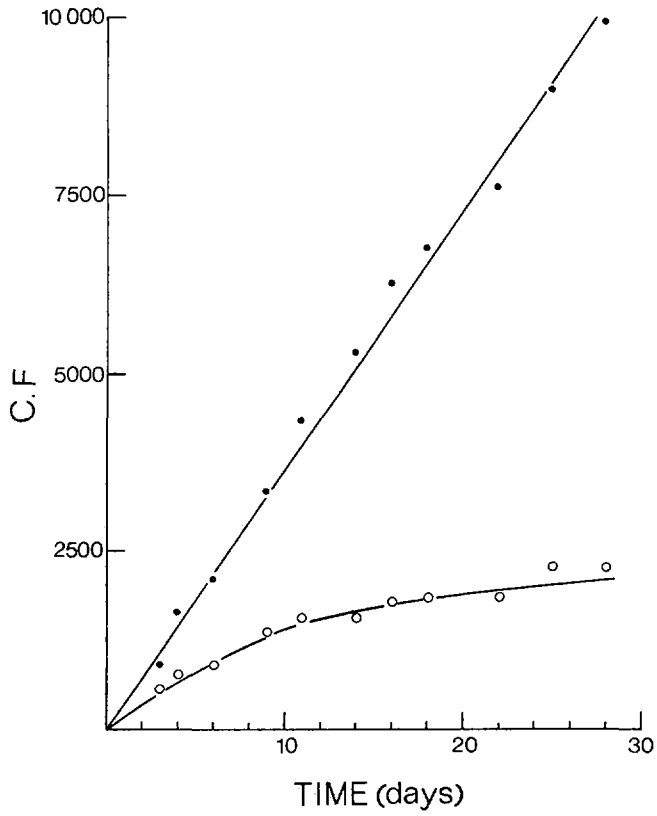

Figure 4. Accumulation of $^{203}HgCl_2$ (O) and $CH_3{}^{203}HgCl$ (●) from water by the euphausiid *Meganyctiphanes norvegica*. Points represent the mean values of 4-12 individuals. C.F. = concentration factor.

particulate form which could be retained on 0.45μm filters; hence, the observed difference in bioavailability of the two species of mercury was due most likely to the chemical form and not the physical state of this element in sea water. Enhanced uptake of methyl mercury over mercuric chloride has been described previously in other species such as molluscs[9,10].

The retention of ingested mercury in euphausiids is shown in Figure 5. Clearly methyl mercury is assimilated and retained to a much greater degree than inorganic mercury. The total amount of mercury eliminated was quite different for the two chemical species; after 2 days an average of 62% of the ingested $HgCl_2$ had been lost of which 67% was excreted with the feces, whereas after 3 days only 3% of the methyl mercury was eliminated from euphausiids with fecal pellet excretion accounting for 88% of this elimination.

The data in Table 5 indicate that with time, as the labelled food passed through the gut, fecal excretion represented an increasingly smaller fraction of total radioactive excretion of both chemical forms of mercury. Periodic monitoring of fecal pellets produced by certain individuals showed that only negligible amounts of radiotracer were associated

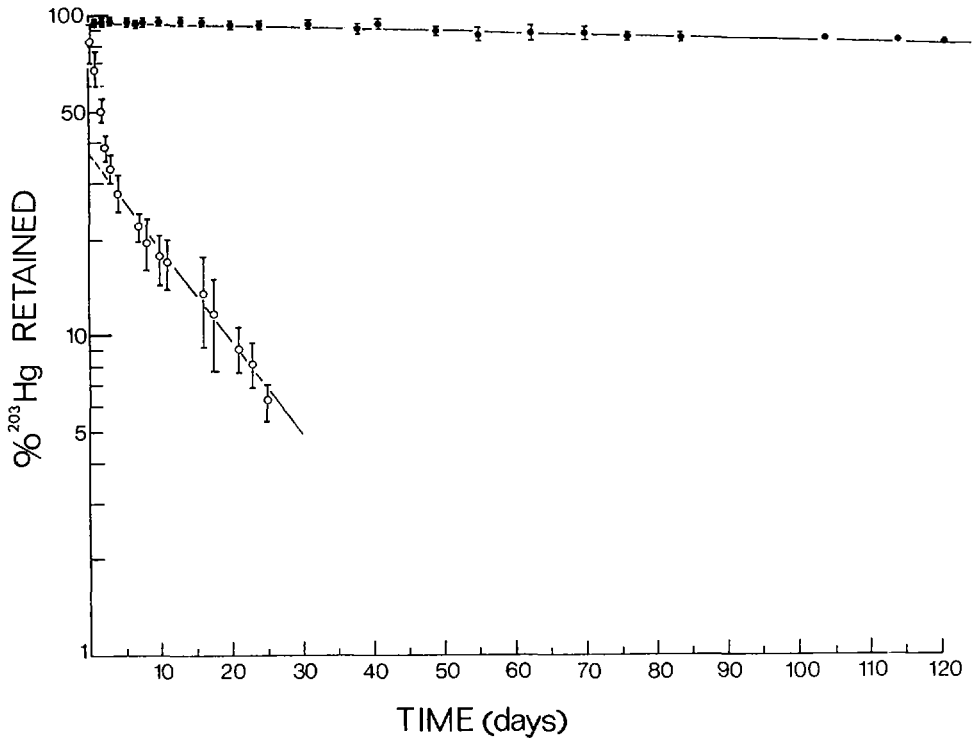

Figure 5. Excretion of ^{203}Hg from the euphausiid, *Meganyctiphanes norvegica*, after receiving a single ration of *Artemia* labelled with $^{203}HgCl_2$ (O) and $CH_3{}^{203}HgCl$ (●). Y-axis intercept of extrapolated loss curve represents fraction of ingested ^{203}Hg assimilated into tissue. Points represent mean of 1-5 individuals. Bars = ± 1σ where applicable.

Table 5. *Meganyctiphanes norvegica*. Percent of total elimination of ingested mercury represented by fecal pellet excretion during various intervals of time. Values represent mean of 4-6 individuals

Time interval	$HgCl_2$	CH_3HgCl
0 - 5 hr	77%	
5 - 23 hr	45%	
0 - 22 hr		75%
22 - 48 hr		24%
23 - 46 hr	39%	
48 - 72 hr		15%

with the feces after day 3 and that loss of radioisotope was principally due to excretion of tissue-assimilated mercury. In fact, as a first approximation, the curves in Figure 5 after day 3 can be treated as single exponentials which represent turnover of the respective mercury compound in the tissue compartment(s). Least squares analyses were performed on the exponential segment of each elimination curve and it was found that approximately 36% of the ingested $HgCl_2$ was assimilated into tissue and subsequently excreted in a soluble form with a biological half-time of about 10 days. In the case of methyl mercury, 97% of the ingested dose was assimilated from the food with subsequent excretion resulting in a half-time of approximately 450 days.

The total turnover of soluble mercury was estimated in euphausiids which had been labelled with ^{203}Hg in a manner closely approximating that which would occur with mercury in the natural environment, *viz.*, contamination from both food and water. The uptake phase closely followed the pattern displayed when mercury was accumulated directly from water (Fig. 4), i.e., relative uptake of methyl mercury and mercuric chloride differed by a factor of approximately 4.5. Although the euphausiids had probably not reached isotopic equilibrium with the ^{203}Hg in their milieu after 17 days, the animals were considered to be adequately labelled with tracer in order to obtain first approximations of soluble excretion rates from the different mercury pools within the organism.

The excretion phase of ^{203}Hg for each chemical form of mercury is shown in Figure 6. For estimating the total excretion rate of dissolved mercury, the loss curves have been resolved into major "linear"

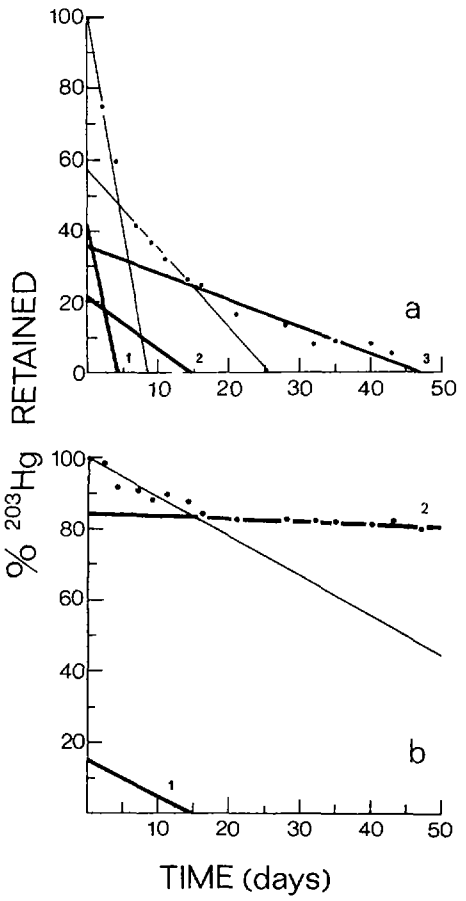

Figure 6. Excretion of a) $^{203}HgCl_2$ and b) $CH_3{}^{203}HgCl$ residues from the euphausiid, *Meganyctiphanes norvegica*, following labelling from both food and water. Loss curves have been broken into "linear" compartments each with their own characteristic loss rate. Points represent means of 2-10 individuals.

compartments, and the excretion rates of each compartment summed to give the total excretion rate after the method of Small *et al.*[21] and Benayoun *et al.*[22] Inorganic mercury was excreted relatively rapidly with the majority of the total excretion rate being governed by a compartment containing 42% of the mercury burden which displayed a very rapid turnover rate of about 9.3%/day (Fig. 6a). Summing the rates from three major compartments gave a total excretion rate of 11.6% inorganic mercury residues/day. Methyl mercury excretion followed a somewhat different pattern (Fig. 6b). Total excretion was resolved into two compartments: one containing approximately 15% of the incorporated methyl mercury which turned over at a rate of about 1%/day and another containing 85% of the compound with an insignificant excretion rate. Hence, euphausiids at steady state with methyl mercury in their environment would turn over this compound at a rate of approximately 1%/day.

The data have also been plotted on a semi-logarithmic scale to obtain information on biological half-times of the two chemical species

in zooplankton. Half-times for mercury pools displaying the slowest turnover rates were 13 days for a pool representing 57% of the body burden of inorganic mercury and 250 days for a compartment containing 92% of the incorporated methyl mercury. For purposes of comparison long-term loss from the euphausiids which had accumulated ^{203}Hg from sea water only was also monitored for two months. Elimination of the two forms of mercury followed essentially the same pattern; pools with 60% of the inorganic mercury and 93% of methyl mercury turning over with biologicalhalf-times of 30 and 223 days, respectively. The exact chemical nature of the excreted mercury in these experiments is not known. Nevertheless, regardless of the manner in which the isotope is administered, excretion rates for the two chemical forms of mercury were distinctly different and remained constant over long periods of time. This fact suggests that following uptake little biological interconversion between these forms was occurring.

The concentration of total mercury in euphausiid tissues, their particulate products and their natural food is given in Table 6. The high mercury concentration in the viscera which includes the hepatopancreas, a known storage organ for many trace metals in crustaceans[29], is consistent with the high levels of cadmium, selenium and

Table 6. Total mercury concentration in euphausiid tissues, particulate products and mixed zooplankton

Sample	**µg Hg/g dry weight
Meganyctiphanes norvegica	
Whole animal (gut empty)	0.35
Eyes	0.95
Viscera	1.67
Muscle	0.55
Exoskeleton	0.38
Particulate products	
Molts	0.17
Stomach contents	0.07
Fecal pellets	0.34
Food	
*Microplankton	<0.05

* Principally small crustaceans, phytoplankton and detritus
** Detection limit = 0.05 ppm

^{210}Po which also accumulate in these tissues[22,23,28]. High levels of zinc which have been found in the eyes of euphausiids are thought to be associated with the animals' screening pigments[30]; similarly the relatively high amount of mercury noted in this tissue may be bound in a like fashion. By far the majority of the mercury in euphausiids resides in muscle and exoskeleton which account for approximately 45 and 48% of the organism's dry weight, respectively.

The higher mercury concentration in the entire exoskeleton relative to the molt, which represents only the outer surface of the exoskeleton, suggests that surface adsorptive processes are not the major mode by which mercury is accumulated in euphausiids. From the data in Table 6 it can be computed that molts, which represent approximately 8% of the organism's dry weight, contain only about 4% of the euphausiid's mercury content. This fact is corroborated by our radiotracer studies in which no more than 2-3% of the euphausiid's ^{203}Hg content was lost with cast molts.

In order to assess the importance of routes of mercury flux in euphausiids, the data from the study have been inserted into a linear model developed by Small *et al.*[21] and used by others to study the flux of several heavy metals and radionuclides through the same species[22,23,28]. The model is based on the equation:

$$K_E = \mu_E + \lambda_E \qquad (1)$$

where K_E is the mercury uptake rate and is equal to the mercury elimination rate (λ_E) plus the rate at which the element is accumulated in tissues (μ_E). The μ_E term is equivalent to the product of the growth rate (ρ_g) and the concentration of mercury in whole euphausiids (Q_g). The total elimination rate (λ_E) is equal to the sum of separate elimination terms such as mercury loss due to fecal excretion ($Q_f \times \rho_f$), molting ($Q_m \times \rho_m$), and soluble excretion ($Q_g \times \rho_e$). It has been reported that most of the mercury in marine zooplankton is in the inorganic form[20]. For this reason and as a first approximation, we have used the excretion rate, ρ_e, for $HgCl_2$ residues in the model calculation. Knowledge on the distribution of inorganic and methyl mercury in euphausiids will allow eventual partitioning of ρ_e into rates for both chemical forms. Loss of mercury due to egg production occurs for only a short period during the year and will not be considered here. Uptake, K_E, in reality combines element uptake from both food and water, however, for simplification we will assume for the moment that all mercury uptake takes place through the foodchain, i.e., K_E is defined as the product of the food ingestion rate and the mercury concentration in the food. Measuring the mercury concentration in the euphausiid's

food and comparing a computed value of K_E with estimates of $\mu_E + \lambda_E$ derived from measurements irrespective of the original source of mercury can furnish information on the relative importance of the food and water route as a source of mercury to the organism.

The data necessary for the flux model calculations are given in Table 7. All notation follows that used by Small *et al.*[21] Inspection of the data in Table 7 (lines 1, 4 and 5) shows that under normal feeding conditions only 29% of the total mercury released occurs by fecal deposition. This is a far smaller fraction than in the case of selenium[28], cadmium[22] and zinc[21] in which feces accounted for 54%, 84% and 93%, respectively, of the total elimination of the element in the

Table 7. Partial fluxes (Qρ) of mercury through *Meganyctiphanes norvegica* under varying conditions of feeding and growth

Line	Hg concentration (Q) µg Hg/g dry	Fractional rates (ρ) g/g dry animal/day	Partial Hg fluxes (Qρ) µg Hg/g dry euphausiid/day	Conditions
1	$Q_f = 0.34$	$\rho_{f1} = 0.051$	0.0173	>3mg dry food,12h/day grazing
2	$Q_f = 0.34$	$\rho_{f2} = 0.038$	0.0129	2mg dry food,12h/ " "
3	$Q_f = 0.34$	$\rho_{f3} = 0.018$	0.0061	1mg dry food,12h/ " "
4	$Q_m = 0.17$	$\rho_m = 0.009$	0.0015	-
5	$Q_g = 0.35$	$*\rho_e = 0.116$	0.0406	-
6	$Q_g = 0.35$	$\rho_{g1} = 0.060$	0.0210	Maximum growth rate[31]
7	$Q_g = 0.35$	$\rho_{g2} = 0.030$	0.0105	Highest growth rate[21]
8	$Q_g = 0.35$	$\rho_{g3} = 0.015$	0.0053	Mean growth rate[21,31]
9	$Q_g = 0.35$	$\rho_{g4} = 0.007$	0.0025	Minimum growth rate[31]

* soluble inorganic mercury excretion (day^{-1})

same species. The majority of the mercury flux (68%) in this pelagic crustacean takes place via soluble excretion processes. The importance of soluble mercury excretion in euphausiids may, in part, be linked to the relatively high degree of assimilation (36%) of this metal from food.

To assess to what degree Eq. 1 can be balanced, mercury concentrations in natural euphausiid food can be compared with computed

values of this parameter derived from estimates of $\mu_E + \lambda_E$ coupled with food ingestion rates for this species. Maximum elimination and tissue accumulation needed to balance mercury intake can be computed by summing lines 1, 4, 5 and 6, i.e. $\mu_E + \lambda_E = 0.0173 + 0.0015 + 0.0406 + 0.0210 = 0.0804$ μgHg/g dry euphausiid/day. By similar reasoning the minimum mercury intake requirement is computed as the sum of lines 3, 4, 5 and 9 which gives 0.0507 μgHg/g dry euphausiid/day. Assuming that these rates represent upper and lower limits for mercury intake in nature, we can divide these values by the maximum and minimum specific food ingestion rates (0.320 and 0.113g food/g dry euphausiid/day, respectively) calculated by Small *et al.*[21] to arrive at a range of estimates of the mercury concentration in food needed to balance Eq.1. Computation indicates that a mercury concentration in food falling within the range 0.25 - 0.45μgHg/g dry food would adequately balance the amounts accumulated and eliminated by *M. norvegica* under the conditions described above.

Microplankton, the normal food of *M. norvegica*, were collected at the same time as the euphausiids and analyzed for mercury along with the euphausiids and their particulate products (Table 6). Unfortunately, the mercury in the microplankton sample was below the detection limit; however, the very low concentration of mercury in this population of microplankton is corroborated by the low level measured in euphausiid stomach contents. Assuming a maximum value of 0.05 ppm in the microplankton, it is evident that the mercury concentration in food falls far short of what is necessary to balance accumulation and loss of the element in *M. norvegica*. Considering both the model calculations and the results of the uptake experiment (Fig. 4) it seems clear that mercury uptake from water is of considerable importance in achieving mercury body burdens in euphausiids.

If water is a major route for mercury uptake by plankton it is difficult to explain why microplankton should contain lower levels of mercury than macroplankton such as euphausiids. In general, for many metals and radionuclides, smaller organisms with larger surface area to volume ratios reach higher concentration factors when the element is accumulated from water[32,33]. The data in Table 6 indicate the reverse situation may be true. The fact that so little mercury resides in the outer surface of the euphausiids (molts) suggests that adsorptive processes are of minor importance; hence, mercury concentration in these species would not be strongly influenced by variations in surface area to volume ratios between plankton of different sizes. If this is the case, and our mercury values are typical for these organisms, the possibility exists that the higher levels in euphausiids compared to their food may be due to a biomagnification

of mercury, or some chemical form of mercury, as the element passes from microplankton to euphausiids following normal food chain processes.

To our knowledge, no published data on the methyl mercury content of marine zooplankton exist, however, measurements in certain species of phytoplankton and fish[34], which form part of the marine pelagic food chain, indicate high percentages of methyl mercury may be typical in pelagic species. Our data on methyl mercury assimilation in euphausiids suggest that a build-up of this compound through the food chain could occur due to the extremely high degree of assimilation coupled with long retention times; nevertheless, it is difficult to envisage much of this compound rapidly being incorporated into euphausiid tissue without eventually showing adverse effects. Clearly, data on methyl mercury content in both euphausiids and microplankton which serve as their food are needed and samples are now being collected in order to help resolve this question.

If, on the other hand, further analyses show the levels measured in this study to be atypical and, in fact, concentrations in euphausiids and their food are typically similar, then the interpretation of mercury kinetics proposed above will have to be revised. In fact, several studies have found similar or slightly lower mercury concentrations in herbivorous zooplankton compared to those in phytoplankton upon which the zooplankton feed[34-36]. Their results suggest that food chain amplification of mercury does not occur at the lowest levels of the marine food chain and in this respect Knauer and Martin[34] have hypothesized the existence of an efficient excretion mechanism in zooplankton which would keep the organisms from accumulating large amounts of mercury from their food. Two aspects of our data lend support to this hypothesis. First, fecal pellets contain much higher concentrations of mercury than the stomach contents which produce them. This fact implies some sort of mercury concentrating process as the food passes down the gut, one of which may be the reabsorption of mercury across the gut wall and into the feces before release. During the excretion phase of our mercury assimilation experiment, ^{203}Hg was not found in the feces produced after day 3; however, this may have been due to the fact that certain mercury pools from which mercury is excreted into the feces had not been tagged with the radiotracer after a single ingestion of radioactive food. It is planned to examine the possibility of reabsorption of mercury by feces in a future experiment which will employ euphausiids thoroughly labelled with ^{203}Hg tracer.

Second, efficient excretion of mercury may be enhanced by the rapid turnover of incorporated mercury via soluble excretion processes (Fig. 6a). This mechanism will only be operative if the majority of the

mercury taken up is in the inorganic form; hence, the relative importance of its role in total excretion can not be precisely determined until more information is available on the chemical forms of mercury taken up and accumulated by plankton.

The release of particulate products from a plankton population, *viz.* molts, fecal pellets and carcasses, have been shown to be an important parameter in the downward vertical transport of several radionuclides and heavy metals[21-23,27]. In the case of the vertical transport of mercury by plankton, transport via particulate products will be of lesser importance when compared with other elements which have been studied previously. The degree to which mercury associated with particulate products reaches bottom sediments will depend on the retention time of the element in these products.

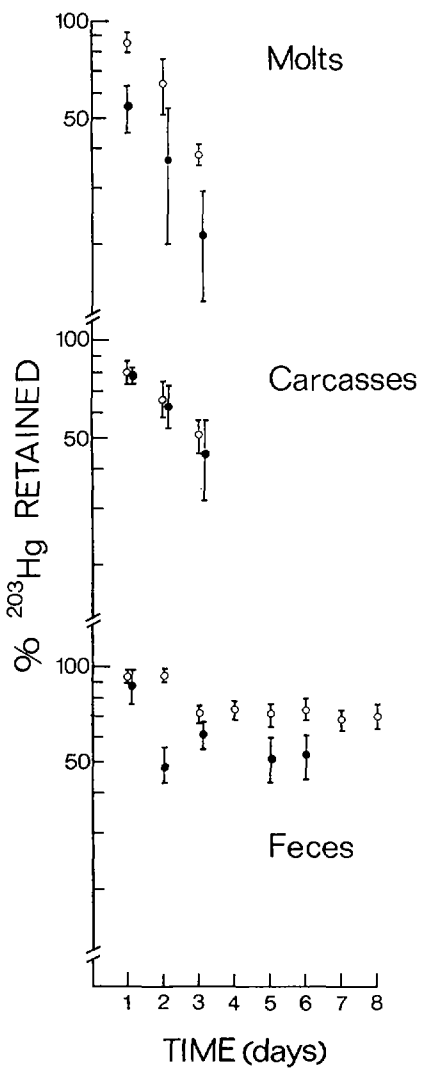

Figure 7. Loss of $^{203}HgCl_2$ (O) and $CH_3{}^{203}HgCl$ (●) from euphausiid molts, carcasses and fecal pellets. Points represent ± 1σ standard deviation of several determinations.

The loss of mercury from particulate products and dead euphausiids is shown in Figure 7. Mercury-203 is released from these products relatively rapidly with half-times ranging roughly from 2 to 13 days. Fecal pellets lose ^{203}Hg more slowly than either molts or carcasses. If ^{203}Hg loss is assumed to represent a net efflux of mercury, i.e., no mercury is reabsorbed by the pellet, then fecal pellets which sink at rates between 126 and 862 m/day[25] and decompose only very slowly will have the potential to transport a large fraction of their mercury content to the bottom in relatively shallow areas of the sea. In deeper waters, much of the element will be released to the water before the material disintegrates or reaches the bottom.