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Comparative Study of the Trophic Transfer of Two Mercury Compounds—$HgCl_2$ and CH_3HgCl—between *Chlorella vulgaris* and *Daphnia magna*. Influence of Temperature

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The majority of "in situ" studies analysing the contamination of aquatic ecosystems by mercury indicate that the metal accumulated in fish is usually in a methylated form (KNAUER & MARTIN 1972 ; HUCKABEE & al. 1979). Since most mercury pollutants are in inorganic forms and the concentration levels of methylmercury in the water are very low, the explanation of this phenomenon must be sought in indirect ecotoxicological processes.

Among the hypotheses which have been put forward to explain this, the most important are (THELLEN & al. 1981) :

- biological methylation, bacterial in origin, which produces the organic compound in the environment, especially at the water-sediment interface,
- the ease with which methylmercury can pass through membrane barriers (gills and digestive system) due to its liposolubility,
- two processes of methylation of this element in fish: firstly by the micro-organisms associated with the branchial mucus, and secondly when anabolic reactions occur in the liver.

In an aquatic environment the contamination of a consumer organism (herbivorous or carnivorous) occurs both by direct routes (when the metal is present in the environment) and by trophic routes (when food is contaminated). The relative importance of these two ways which form the basis of the trophic chains is not as yet very well understood.

In our researches whe have used an experimental trophic chain in order to study the mechanisms of bioaccumulation and transfer of two mercury compounds - $HgCl_2$ and CH_3HgCl - in relation to the two contamination ways. In this simplified ecotoxicological approach the aim is to obtain a quantitative analysis of the processes, but taking into consideration an ever-increasing number of abiotic and biotic parameters which the heterogeneousness and the extreme complexity of naturel aquatic systems can give rise to (BOUDOU & al. 1979; RIBEYRE & al. 1979).

In this article we present a comparative study of the transfer of these two compounds between a species representative of the "producer" level - *Chlorella vulgaris* - and a primary consumer - *Daphnia magna*. The experiment was carried out at two temperatures, 10 and 18°C, and the concentration of metal in the environment was $1 \mu g.l^{-1}$ (1 ppb).

MATERIAL AND METHODS

Cultivation of the algae and production of the Daphnia:

The unicellular alga selected, *Chlorella vulgaris* - Strain 222 - came from the algothèque of the Museum of Natural History in Paris. This alga was grown in a Lefevre-Czarda medium, modified by the addition of trace elements. A considerable quantity can be produced by continuous magnetic agitation and aeration of the medium (in erlenmeyers of a capacity of 3 or 5 litres), at $22 \pm 0{,}5$°C and with an artificial photoperiod of 16 hours per day. The algal suspensions thus obtained were used directly for experimentation or as a trophic supply for the production of daphnia.

Quantification of the numbers produced was done by opacimetry, at 665 nm. Ponderal calculation - dry weight - allowed a correlation to be made between the optimal density measured and the concentration of the samples (mg - dry weight -.l^{-1} O.D.$^{-1}$ units).

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The daphnia used - *Daphnia magna Strauss* - were produced in glass flasks with a capacity of 200 litres ; these were thermoregulated at 21 ± 0,5°C, aerated and subjected to an artificial photoperiod (16/8). The production medium consisted of dechlorinated tap water, enriched daily by supplies of algae, yeast (Biomérieux 312) and meat extracts (Biomerieux 3201). A partial renewal of the medium was carried out approximately every 10 days. Four production units were developed in parallel, making it possible to cultivate large quantities of daphnia. The isolation of homogeneous lots was achieved by filtering the medium through a sieve. Young daphnia (1 day) were kept for five days in aquaria and fed daily. Next, 6-day old daphnia in lots of 100 were isolated by filtration for use in experiments. Quantification of the biomasses was achieved by measuring fresh weight and dry weight of lots of 1000 daphnia.

Procedure in transfer experiments:

The experiments were carried out in glass erlenmeyers - capacity 1 litre - placed in thermoregulated double boilers (heating or cooling systems ± 0,5°C) and lit artificially (photoperiod 16/8).

When the time factor was zero each contamination unit received a total volume of 330 ml: x ml of synthetic river water (FREAR & BOYD 1968) + y ml of algal suspension (y is the function of the optic density measured in the culture) + 0,33 ml of mercury solution ($HgCl_2$ or CH_3HgCl, 1 mg $Hg.l^{-1}$). The final concentration of chlorellae in the contaminated environment was 25 $mg.l^{-1}$ (O.D. at 665 nm = 35.10^{-3}); this biomass was defined during preliminary experiments in order to provide a slight surplus of food for the daphnia at 18°C.

The contamination period for the *Chlorella vulgaris* level is 24 hours. For each experimental condition six erlenmeyers were set up. Analysis of the bioaccumulation of mercury in the algae was achived by testing samples before and after filtration through Millipore filters, 0,22 μm (fixed and free mercury).

After 24 hours a lot of 100 six-days old daphnia was introduced into each flask. Contamination for this level occurs within 4 days; during this period these daphnia have a minimal moulting frequency and the first parthenogenetic embryos have not appeared. The amount of mercury in the system was then calculated by analysing the daphnia and the non-filtered environment.

Mercury dosage:

The mercury dosage was done by atomic absorption without flame (VARIAN AA 475). Samples of the environment (50 ml) were treated after bromination in an acid medium(FAREY & al. 1978). After filtering and rinsing the organisms were mineralised by acid attack (3 ml concentrated HNO_3) in a pressurised environment at 95°C for 3 hours.

This dosage technique usually produces a detection limit of 5 ng. The results thus obtained were expressed as a concentration (ng of Hg/ml of environment or / 100 daphnia) or as a factor of concentration (concentration of mercury in the organism / concentration in the environment).

RESULTS AND DISCUSSION

A recent publication (RIBEYRE & BOUDOU 1981) analysed the dynamics of accumulation of the two mercury compounds by *Chlorella vulgaris* at 10 and 18°C, so we will here present only the amounts of metal accumulated by the algae after 24 hours of exposure (Table 1).

These results show the potential mercury transfer when daphnia are introduced into the contamination flasks. In three of the experimental conditions - $HgCl_2$, 18°C; CH_3HgCl, 10 and 18°C - contamination of the primary consumer occurs by the trophic route only. Indeed 96 to 100% of metal initially introduced was accumulated by the algae. Complementary results obtained in identical conditions but after 48 and 96 hours of exposure demonstrate the stability of these accumulation rates.

However, at 10°C, 14% of mercuric chloride remained in the environment; in this case, a small amount of the metal bioaccumulated by the daphnia after 4 days could be of a direct origin.

Temperature (°)	$HgCl_2$	CH_3HgCl
10	284 ± 21 ng (86% of initial Hg)	330 ± 18 ng (100% of initial Hg)
18	317 ± 19 ng (96% of initial Hg)	323 ± 22 ng (98% of initial Hg)

Table 1: Quantities of mercury accumulated by *Chlorella vulgaris* after 24 hours exposure at 10 and 18°C (where time is zero: 330 ng of Hg-$HgCl_2$ and CH_3HgCl). Confidence limits: P=0,05.

The results of mercury dosage in the daphnia for all experimental conditions are given in Table 2. The mortality rate was very low and does not significantly affect these results.

Temperature (°C)	$HgCl_2$	CH_3HgCl
10	20,8 ± 11,7 ng (6% of initial Hg)	68,9 ± 10,2 ng (21% of initial Hg)
18	18,8 ± 8,9 ng (6% of initial Hg)	190,7 ± 12,9 ng (58% of initial Hg)

Table 2: Quantities of mercury accumulated by *Daphnia magna*, (ng/100D) after 4 days contamination. Confidence limits: P=0,05.

Ponderal measurements of 40 lots of 1000 daphnia aged 10-11 days made it possible to calculate the fresh weight (after surface water had been eliminated) and the dry weight (after drying in an oven):

- fresh weight: 346 ± 22 mg (P=0,05),
- dry weight: 27 ± 4 mg (P=0,05).

Thus, after contamination by methylmercury at 18°C, the concentration factors for the consumer level can be estimated at 5500 (fresh weight) and 70000 (dry weight).

Figure 1 shows the transfert of mercury between *Chlorella vulgaris* and *Daphnia magna* at 10 and 18°C, for the two compounds; the initial amount of metal introduced in all experimental conditions was 330 ng (100%).

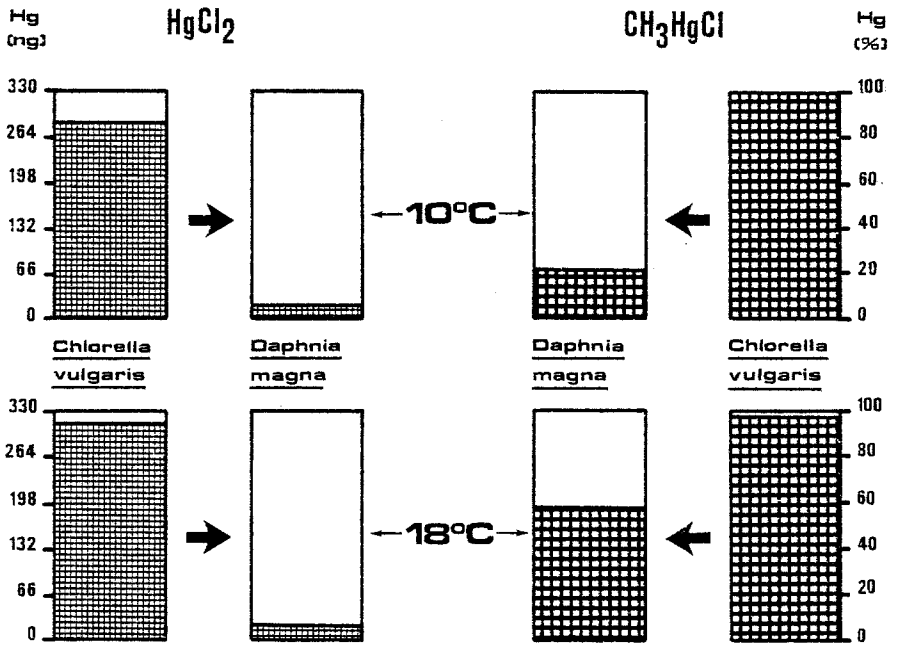

Figure 1: Results of the transfer of mercury between the two trophic levels, *Chlorella vulgaris* and *Daphnia magna*, at 10 and 18°C and for the two compounds, $HgCl_2$ and CH_3HgCl.

These results bring to light a considerable difference between the transfer rates of the two mercury compounds, a difference which becomes even more marked when the temperature of the environment is increased.

There are, nevertheless, several factors which ensure that the quantities of algae consumed by the crutaceans at any one temperature - 10 or 18°C - can be considered equal: the homogeneity of the daphnia used (age, size and sex) the rigorous control of the experimental conditions, and the small quantities of mercury introduced (contamination which was well below a lethal dose).

However, the alimentary demand, like behavioural and metabolic activities, is very strongly influenced by temperature factors; in this case the supply of algae remained excessive, after 4 days of experimentation.

In the case of methylmercury, at 10°C, 21% of the metal initially introduced accumulated in the daphnia; at 18°C the transfer rate was 58%. Since the contaminant enters the organism by trophic routes only, the differences observed are linked with an increase in the amount of food ingested when the temperature of the environment is increased. In earlier experiments using marked CH_3HgCl (Hg^{203}), supplies of algae were very similar in quantity to the alimentary demands of the daphnia, and in these cases transfer rates were of the order of 100% (RIBEYRE & al. 1979).

In the case of mercuric chloride, 6% of the metal initially introduced was transferred to the consumer level at 10 and 18°C. At 10°C, when consumption of algae was less than that observed at 18°C, an identical mercury transfer rate can be explained by contamination by direct route (14% of free mercury at the time when the daphnia were introduced) in addition to the trophic transfer which was less than at 18°C. Indeed, experiments into contamination by direct way show at 10°C an important capacity for accumulation of the inorganic compound (DELARCHE & RIBEYRE 1978).

At 18°C, with contamination conditions identical for the two compounds (96 and 98% of mercury accumulated by the algae from $HgCl_2$ and CH_3HgCl respectively) amounts retained by the daphnia were ten times greater in the case of the methylated form.

These results seem to indicate that the two contaminants, which are first introduced into the environment and then fixed by the unicellular algae, retain their specific property of crossing the digestive barrier of the consumer link. Indeed, experiments in which $HgCl_2$ or CH_3HgCl was administered orally to mammals in aqueous or lipoid solutions show that the amounts of mercury which cross the digestive barrier and are retained within the organism are 80 to 90% of the dosage administered in the organic form and 2 to 20% in the inorganic form (SELL & DAVISON 1975).

CONCLUSION

If we try to transfer these experimental results to phenomena which occur in the natural environment the phytoplanktonic biomasses, which have a high turnover rate during periods of growth, would show a very considerable facility to accumulate mercury, since the capacity to accumulate is similar for both compounds. But the first trophic transfers between these producers and the herbivores (zooplankton for example) would show a marked inbalance in accumulation levels, in favour of methylmercury.

However, in this study we must take into consideration the very great diversity of biocoenoses and the interspecific differences which can manifest themselves with regard to processes of bioaccumulation and transfer. There is, moreover, continuous competition between the inert particles in suspension and the living organisms to absorb the mercury compounds which are in a free state in the aquatic environment. According to HAVLIK & al. (1979), certain phytoplanktonic species have a tendency to transform the bioaccumulated organic compounds: in this case the efficacity of the transfer processes would depend on the extent to which the phenomenon occurs and the speed with which it begins.

With the results we have so far collected it is possible to suppose that the large quantities of the methylated form of mercury to be found in the terminal aquatic consumers - especially carnivorous fish II or III - can be accounted for by the mechanisms of bioaccumulation and transfer of the mercury compounds at the lower end of the trophic chains.

At this stage in our research we are interested in knowing how transfers between the primary consumer level and the secondary consumer lever occur. Is it the same process as that observed between the first two links in the chain, or is it slightly modified ? By using our experimental trophic chain - *Chlorella vulgaris* → *Daphnia magna* → *Salmo gairdneri* (newly-hatched) we should be able to answer this question, provided that it proves possible to quantify the transfer and bioaccumulation processes according to the two contamination routes.

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