Bull. Environm. Contam. Toxicol. 22,813-818 (1979)

Bioaccumulation and Bioamplification of Mercury Compounds in a Second Level Consumer, *Gambusia affinis* —Temperature Effects

A. Boudou, A. Delarche, F. Ribeyre, and R. Marty

Laboratoire d'Ecologie animale, Université de Bordeaux I, Faculté des Sciences, 33405—Talence, France

The contamination of aquatic ecosystems by compounds that are only partly or not at all biodegradable is the result of a series of varied and very complex processes. Parallel to the specific physico-chemical properties of the contaminating agent, the abiotic and biotic components of the natural system play a fundamental role : temperature, pH, oxygenation..., the qualitative and quantitative compositions of the trophic systems, biocenotic dynamic...

Living creatures possess, to differing degrees, the capacity to accumulate in their tissues any substance that is only slightly biodegradable. The resulting bioaccumulation is the difference between the pollutant's capacity to penetrate the organism and its facility of excretion. The combined effects of time and food transfers cause a bioamplification of the pollution as it proceeds up the food chain. Thus, for a consumer organism, the quantities bioaccumulated correspond to the amount of toxicant ingested directly (teguments, gills) and indirectly (food). The respective roles of these two contaminations varies with the organism under study, the nature of the pollutant and the conditions of intoxication.

In our research, we have quantified the bioaccumulation of mercury compounds in a fresh-water fish, **Gambusia affinis**. The direct contamination was analysed with regard to the chemical form of the compound (mercuric chloride - $HgCl_2$ - and methyl mercurous chloride - CH_3HgCl), its concentration in the environment, duration of the intoxication and temperature. The global contamination (water and food) was achieved by setting up an experimental trophic chain : Producer (**Chlorella vulgaris**) - First level consumer (**Daphnia magna**) - First level carnivorous (**Gambusia affinis**). The bioaccumulation process was studied over a period of 30 days, starting with a quantity of 1 μg of methyl-mercury per litre of water (1 ppb), at three temperatures (10, 18 and 26°C).

METHODS

Experimental trophic chain in fresh-water environment:

The setting up of a laboratory ecotoxicological model demands careful control and regulation of experimental conditions and should yield precise results that can easily be reproduced. Much preliminary research proved necessary to define the trophic sequence between the three levels, the means of contamination and the renewal cycles of the environments (BOUDOU et al. 1977a-1977b , DELARCHE and RIBEYRE 1978).

0007-4861/79/0022-0813 $01.20

The chart schown in figure 1 summarizes the theoretical functioning of the experimental trophic chain.

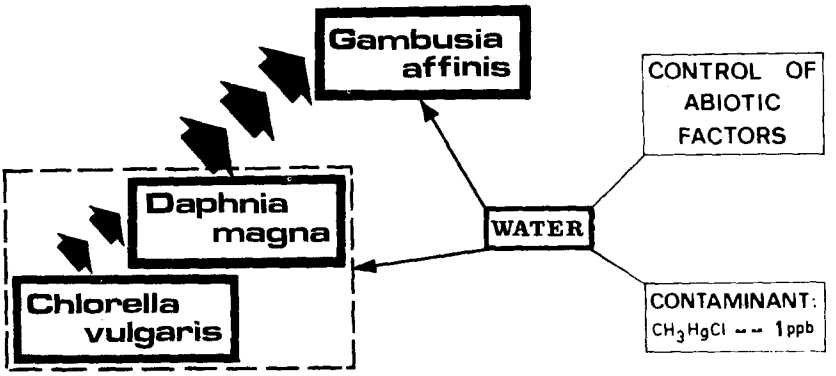

Fig. 1 - **Theoretical functioning of the experimental trophic chain.**

For each study, the gambusies were progressively acclimatised to the chosen temperature (4 weeks). Groups of ten individuals were contaminated each time, which received daily a quantity of daphnia corresponding to the maximum food intake at the temperature concerned (BOUDOU et al., 1977b).

The mean mercury concentrations for 60 daphnia contaminated at 10, 18 and 26°C are :

- 10°C : 0,164 $\pm$ 0,013 μg of Hg
- 18°C : 0,203 $\pm$ 0,019 μg of Hg
- 26°C : 0,238 $\pm$ 0,019 μg of Hg.

Contaminants and quantitative analysis:

Direct contamination tests were carried out, comparatively, with both mercuric chloride and methyl mercurous chloride. In the tests carried out on the trophic chain, only the organic compound was studied ; its ecotoxicological interest being much greater, in particular because of the phenomenon of biological methylation (JENSEN and JERNELOV, 1969 ; LANDNER, 1971 ; LANGLEY, 1973).

The choice of a micro-quantity of contaminant in the environment, a dose similar to those found in marine or continental ecosystems, led us to use mercury isotope 203 (Radiochemical Center, Amersham, England). This radioactive tracer makes it possible to follow precisely the progress of the toxicant in the contaminated organisms. We used a Gammatic counter, type PE 100/2. Results are given in μg of mercury/g of wet weight.

Contamination conditions:

In all studies of the chronic intoxication of aquatic organisms, over a medium or long period, it is essential to maintain the concentration of the toxic substance in the environment, in order to keep conditions as close as possible to those found incontaminated natural systems.

We chose a two-day renewal cycle of the environment, for each of the direct contamination studies (mortality tests, heavy quantities, experimental chains). This was a compromise between material constraints, the reduction of mercury content in the water (maximum 20% after 48 hours) and the evolution of the physico-chemical parameters (pH, nitrites, nitrates...).

For the quantification of the bioamplification with each sort of contamination, three food chains were used:

- **chain 1**: global contamination, direct and trophic,

- **chain 2**: direct contamination : only the environment was contaminated (1 ppb of CH_3HgCl), identical quantities of food being supplied (non-contaminated daphnia),

- **control chain**: environment and food uncontaminated.

RESULTS

Mortality tests (direct contamination):

Mortality tests were carried out with regard to time (maximum contamination period: 10 days), the chemical form of the contaminant ($HgCl_2$ and CH_3HgCl), the quantity in the environment (0,05 ppm to 2 ppm) and the temperature (10, 18 and 26°C). Results are shown in figures 2 and 3.

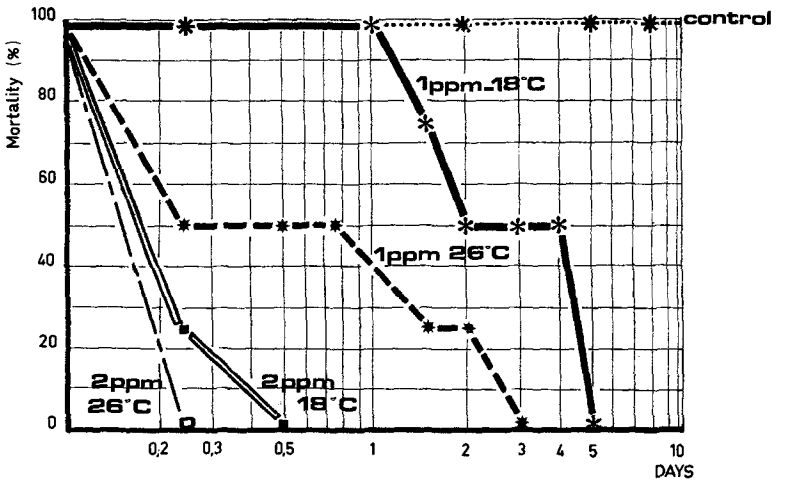

Fig. 2 - **Mortality tests with mercuric chloride** ($HgCl_2$).

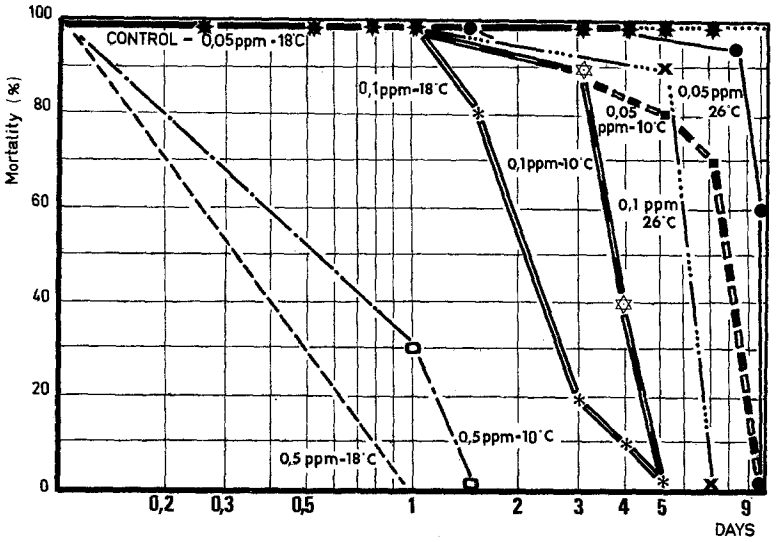

Fig. 3 - **Mortality tests with methyl-mercurous chloride** (CH_3HgCl).

The results show:

- the organic form is far more toxic: for 0,5 ppm of mercury in the environment, the lethal duration 50% has not been reached after 10 days contamination with $HgCl_2$ whereas it is reached in less than 24 hours with the methylated form.
- mortality increases as the water temperature rises, which demonstrates a synergy between this abiotic factor and the toxic effect of the contaminant.

Bioaccumulation by direct contamination (large quantities):

The facility with which an organism accumulates mercury depends on the lethal concentrations of the contaminant in the environment. The average time necessary to cause the death of the **Gambusia affinis** is not proportional to concentration factors in the fish.

With methyl mercury, the capacity for direct accumulation of the metal in the organism is higher when the quantity in the environment is low. Raising the temperature, between 10°C and 18°C, amplifies this phenomenon.

In the case of direct intoxications, using sub-lethal quantities, global metal levels in the fish are very variable, according to the duration of the contamination, the quantity used, the chemical form of the compound and the temperature. This shows how difficult it is to interpret the results, since each abiotic parameter influences the absorption and bioaccumulation of the toxic substance.

The interface between environment and organism is also of great importance in the process of direct contamination. We found, when studying the correlation between individual weight and quantity of mercury bioaccumulated, that, in identical conditions of contamination, tissular concentrations were higher in fish weighing less than the mean average weight of the fish tested (DELARCHE and RIBEYRE, 1978).

Experimental trophic chain: bioaccumulation and bioamplification of methyl-mercury:

For each type of contamination, the evolution of the bioaccumulation and bioamplification over different periods of time was studied at 10, 18 and 26°C (fig. 4).

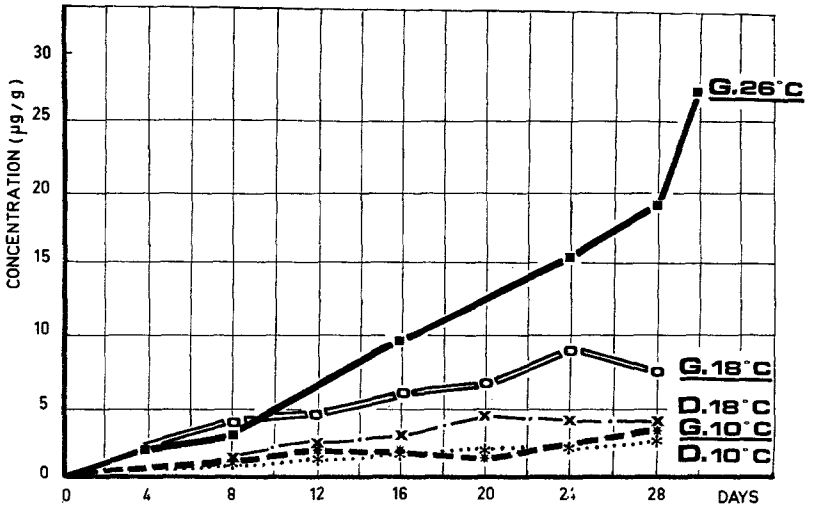

Fig. 4 - **Dynamic of the accumulation of methyl-mercury in Gambusia affinis** - Global G (water and food) and direct D contaminations.

The importance of direct contamination as against trophic contamination can be seen in the ratio between the levels measured in the gambusies intoxicated by water and those noted after global contamination. After 30 days, this rate is 83% at 10°C, 40% at 18°C and 11% at 26°C.

Concentration factors obtained after 30 days were, according to the temperature, between 2500 and 4300 by direct contamination and 3000 and **27000** by global contamination. The parameters "duration of contamination" and "temperature of the environment" play a vital part in the accumulation of mercury from trophic sources.

DISCUSSION

Comparative study of the two mercury compounds (mortality tests, direct contamination) show the methylated form to be considerably more toxic and more easily bioaccumulated. These results are due to two processes:

- easier penetration of membrane barriers (liposolubility particularly), facilating absorption of the contaminant in the organism, its transport and fixation in the different tissues.
- higher capacity for intracellular stockage, thus increasing the "biological half-life" of the toxicant in the organism.

Global contamination, using an experimental trophic chain, illustrates perfectly the phenomenon of **bioamplification**. With a quantity of 1 ppb of methyl-mercury in the environment, the trophic level **Gambusia affinis** yields a concentration of toxicant mass of 27000 after 30 days of contamination at 26°C. Raising the temperature of the environment has a **synergic effect** on the quantities of mercury bioaccumulated by the carnivorous fish. Levels observed at 26°C reflect an increased capacity for accumulation in **Daphnia magna** at this temperature and also a far higher food intake. Conversely, the concentrations observed at 10°C, which approach those obtained by direct contamination, can be explained by the fact that the trophic supply of mercury is much reduced.

Study of the evolution of bioaccumulation of methyl-mercury by direct contamination (1 ppb in the water) shows no proportional relation of the process to the time factor. A "levelling-off" is particularly pronounced at 18°C,showing the appearance of a "threshold value" in the conditions particular to our study. This phenomenon can be related to a saturation of the mechanisms of direct absorption of the contaminant or to an increase in its excretion.

CONCLUSION

These results underline the importance of setting up **experimental models in ecotoxicology**. The use of trophic chains, in conditions that are perfectly controlled and quantified, makes it possible to determine, from a fundamental and applied point of view, the mechanisms responsible for the contamination of natural systems. Nonetheless, such models should be progressively complexified in order to approach the conditions found "in situ".

We are pursuing our research at the moment on two higher trophic levels, represented by a second level carnivore **Salmo gairdneri** and a terrestrial mammal at the end of the chain, **Rattus norvegicus**. The ecotoxicological effects on the level "trout" are being investigated through research on the tissular distribution of the contaminant and modifications caused to the liver (optic and electronic microscopies, quantitative enzyme and protein analysis) and blood (circulating proteins and enzymes). Using the rat as terminal consumer enables us to simulate the trophic position of man and to measure precisely the degree of contamination in conditions close to those found in the natural environment.

Acknowledgements

Support of this research was partially derived from funds provides by the French Ministry of the Environment (trophic chains contamination research programmes) and the Commission of the European Communities (environment and raw materials research programmes).

Bibliography

BOUDOU, A., A. DELARCHE, F. RIBEYRE and R. MARTY : Symbioses **9**(1), 57 (1977a).

BOUDOU, A., A. DELARCHE, F. RIBEYRE and R. MARTY : Bulletin d'Ecologie **8**(4), 401(1977b).

DELARCHE, A. and F. RIBEYRE : Thèse n°1436, Bordeaux I University, 265 p. (1978).

JENSEN, S. and A. JERNELOV : Nature **223**, 753 (1969).

LANDNER, L. : Nature **230**, 452 (1971).

LANGLEY, D. : Journal Water Pollution Control Federation **45**(1), 44 (1973).