MARINE ECOTOXICOLOGY : FIELD AND LABORATORY APPROACHES.

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ABSTRACT

Preliminary results on the levels of organochlorine residues and mercury in the main compartments of the North Sea are described and the advantage of presenting data on three bases (wet weight, fat weight and water volume) is emphasized.

Higher concentrations are by far found in the pelagic seabirds but no biomagnification can be detected at the first levels of the food chain (phytoplankton - zooplankton and fish). The results are discussed considering the relative importances of the uptakes by food and directly from water.

The modelling of the pollutants cycles in marine ecosystems requires notably the knowledge of the percentages of ingested pollutants assimilated, the concentration factors and the rates of excretion which all have to be determined in the laboratory.

Some physiological adaptations to pollution are described which can change their kinetics of uptake and should be taken into account in the interpretation of the ecotoxicological data.

Finally we discuss the fact that the liposolubility of all stable pollutants (organic and inorganic) would be related to their rate of uptake, suggesting that general laws describing the fate of pollutants in aquatic systems may exist.

INTRODUCTION

Ecotoxicology can be defined as the science dealing with the contamination of the ecosystems by natural and artificial pollutants as well as their mechanisms of action and effects on living organisms (RAMADE, 1977, free translation). Clearly, this means that contamination levels, rates of transfer of pollutants between atmosphere, water, sediments and biological compartments as well as their toxic effects are included in ecotoxicology.

In this frame, we are dealing with two main complementary approaches :

1. determination of the contamination levels of stable pollutants in the field (i.e. North Sea)
2. laboratory experiments needed for the understanding of their transfer mechanisms.

1. Levels of organochlorine residues and mercury in the main compartments of the North Sea

PCB's constitute by far the main organochlorine residues in marine systems and were detected in all samples. Qualitatively, their structure is much closer to a mixture of the type Aroclor 1254 than Aroclor 1260 typical for the surrounding terrestrial systems (fig.1). Such a pattern difference is not clearly understood yet but could be related to differences in biological stability and in solubility (partitioning) of the different compounds within the mixture.

A summary of the results gathered on the main compartments (fig.2a,b,c) shows that no important bioaccumulation is taking place at the first ("lower") levels of the food chain (particulate matter-mainly phytoplankton-,zooplankton, fish), but well from fish and zooplankton to seabirds (for a more detailed discussion of these results, see DELBEKE and JOIRIS, 1985). When the data are expressed on a fat weight basis, there even exists a decrease of contamination from particulate matter to zooplankton : this can be quantitatively understood by assuming that the PCB's bound to the phytoplankton lipids are transferred to zooplankton through grazing, and then diluted within the animals by newly formed, PCB free lipids.

The necessity of expressing the results in the three kinds of units is still more evident at the level of phytoplankton (= particulate matter)(see DELBEKE and JOIRIS, 1985) : the reproducibility of the results of PCB contamination per

volume unit shows that levels per weight unit directly depend on the amount of particulate matter present : the higher the phytoplankton concentrations, the lower its PCB contamination on a weight basis. Such results entirely fit the simple partition/adsorption hypothesis.
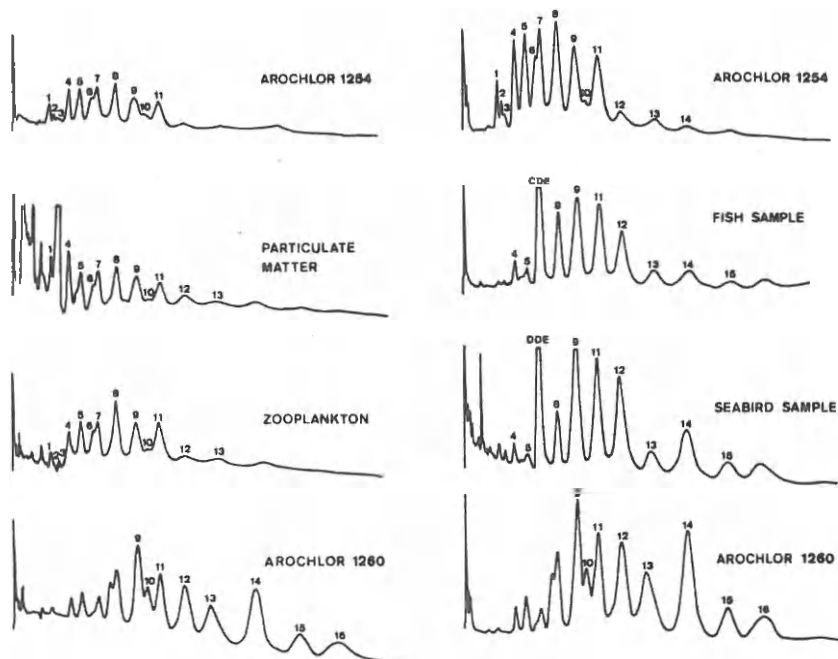

Fig.1 : PCB patterns present in the main biological compartments of the North Sea and compared to PCB standard mixtures (Aroclor 1254 and Aroclor 1260).

As far as fish samples are concerned, both edible and by catch fish show comparable levels of contamination but the relative roles of direct and indirect contaminations are however difficult to be evaluated now (see DELBEKE and JOIRIS, 1985, for further discussion) : laboratory experiments are clearly needed in order to solve this aspect.

Schematically, the main rules for understanding PCB contamination can be summarized as follows :

- most PCB's are present in the particulate matter compartment
- partitioning between water and phytoplankton is directly dependent on the lipid fraction of the last
- contamination of zooplankton mainly occurs through food intake
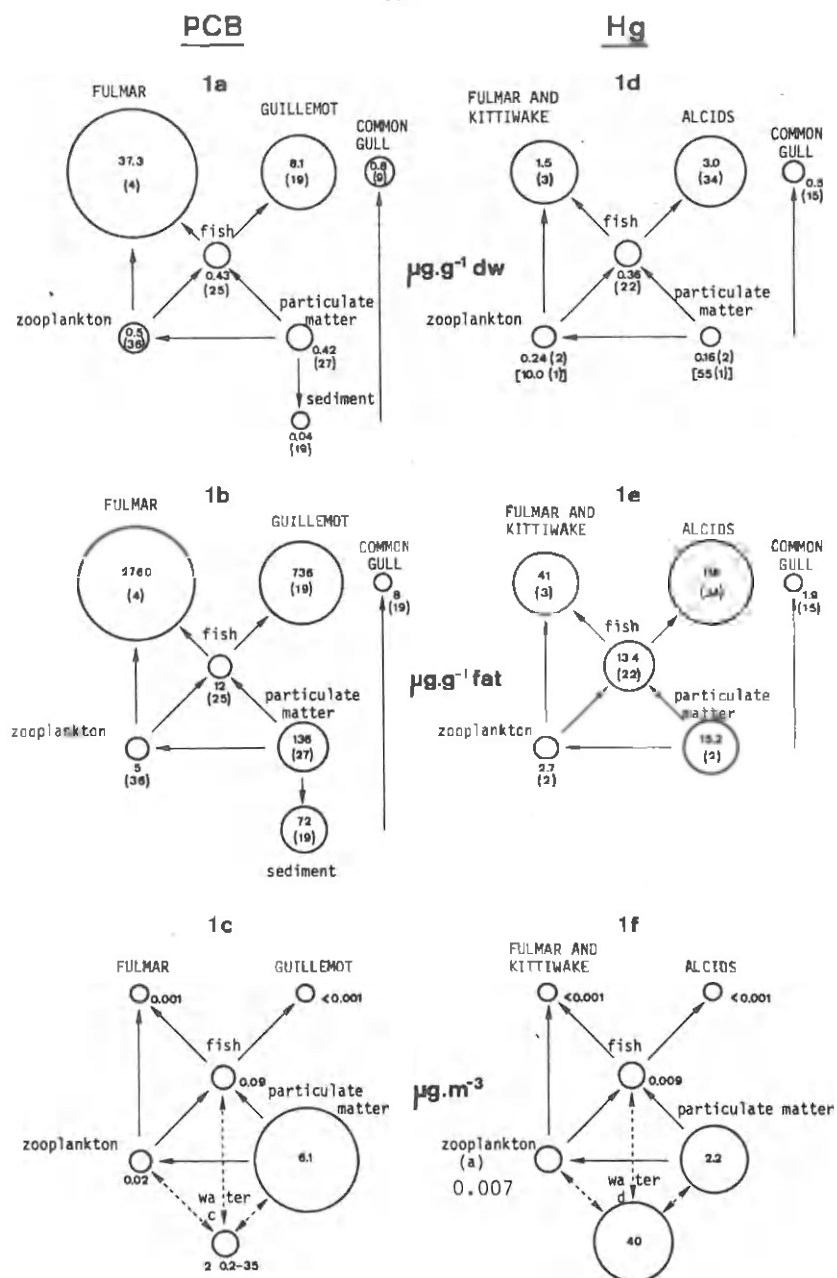- the contamination mechanisms of fish remain unclear, with arguments favou-

Fig.2 : Biomagnification of PCB's (1a,b,c) and mercury (1d,e,f) through the tro-
phic chain of the North Sea. Contamination levels figured by circles :
1a,d : µg/g^{-1} dry weight ; 1b,e : µg/g^{-1} fat weight ; 1c,f : µg/m^{-3}. For
seabirds and fish, the contamination levels of the muscles are considered.
(a) : DECADT *et al.* (unpublished results) ; (b) : BAEYENS *et al.* (1979) ;
(c) : GEYER *et al.* (1984).

ring direct or indirect contamination (to be solved by laboratory experiments)
- "real" pelagic seabirds are highly contaminated by PCB's, evidently accumulated through the food web.

Mercury contamination was investigated on the same samples. The results were expressed in the three kinds of units as well (fig. 2d,e,f) which allows a similar discussion.

When considering the concentrations (dry weight and fat weight), the comments are rather similar to those concerning PCB's :

- little or no magnification at the first levels of the food chain
- decrease of contamination from particulate matter to zooplankton and fish on a fat weight basis, which suggests that part of the mercury would be under an organic liposoluble form (methylmercury for example)
- more important magnification from fish and zooplankton to seabirds.

Monitoring : a possible method for determining the evolution of contamination in function of time could be provided by the analysis of seabirds" feathers. An example (fig.3) concerns mercury levels in feathers of the Guillemot (*Uria aalge*). One can discern a period of increasing contamination with maximal values in the period 1920-1970, followed by a period of decreasing levels. Such preliminary results are confirmed by the analysis of stabilized sediment cores in the Rhine estuary (SALOMONS, 1983), but are not yet confirmed by the Hg levels in fish, where no clear decrease is to be detected (VYNCKE *et al.*, 1981).
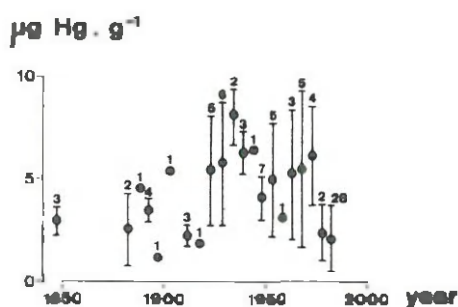

Fig.3 : Evolution of the mercury pollution from 1945 till 1983 as reflected in feathers of Guillemots, *Uria aalge* (mean, standard deviation, number of samples).

2. Laboratory approach of the study of transfer mechanisms

Modelling the distribution of toxic substances in marine ecosystems requires the knowledge of several aspects related not only to the ecology of the described systems but also to the physiological mechanisms implied in the bioaccumulation of pollutants by marine organisms.

The following differential equation for concentration of a toxic substance in the biomass of a nth trophic level has been described by JORGENSEN (1983) :

$$\frac{d\ TOX(n)}{dt} = BIO(n)\ MY(n)\ YT(n)\ \gamma(n-1) - MORT(n)\ \gamma(n)$$
$$-EXC(n).\gamma(n) - MY(n+1).\gamma(n) + UT(n).TOX(o)$$

BIO(n) : concentration of biomass, nth trophic level

EXC(n) : rate of excretion, rated to $\gamma(n)$

MORT(n): mortality, nth trophic level

n   : trophic level (n=o, water)

TOX(n) : concentration of toxicant, nth trophic level (mg/l water)

TOX(o) : concentration of toxicant in water (mg/l)

UT   : uptake rate of pollutant

$\gamma(n)$: concentration of toxicant, nth trophic level : TOX(n)/BIO(n)

YT(n)   : yield factor of toxicant, nth trophic level

MY(n)   : growth rate of nth trophic level

The use of such a model requires :

- ecological data (biomasses and fluxes),
- pollutant concentrations of the different compartments in the field,
- ecotoxicological data which are to be determined in the laboratory :
  the assimilation rate of the toxicant from food (TY(n)),
  the direct uptake rate of the toxicant from water (UT),
  the rate of excretion of the toxicant (EXC(n)).

The percentage of ingested pollutants assimilated from food by aquatic organisms can be determined by means of a laboratory set up which allows to simultaneously follow the pollutant concentration in animals fed with contaminated preys and that of animals living in the same water, taking so into account the direct contamination from the water (BOUQUEGNEAU *et al.*, 1979).

Table 1 shows the percentage, determined by that way, of ingested heavy metals assimilated by some aquatic organisms.

	Cd (CdCl$_2$)	Hg (HgCl$_2$)	Hg (CH$_3$HgCl)
ZOOPLANKTON			
Artemia salina (1)	4	6	29
Praunus sp. (2)	7	20	43
Leptomysis spp. (2)	8	19	26
FISH			
Lebistes reticulata (1)	-	0-2	37-53
Serranus cabrilla (1)	1	-	-

Table 1 : Percentages of ingested heavy metals assimilated by some aquatic organisms.
(1) : from BOUQUEGNEAU *et al.* (1979)
(2) : BOUQUEGNEAU *et al.*, to be published
- : not determined

It appears that the assimilation rates are much lower for inorganic pollutants than for organic ones (methylmercury in this case). This results most probably from the high lipid solubility of the organic complexes. In all these experiments however, the fact that only a part of the ingested pollutant was assimilated led to an important secondary direct contamination of the animals. Those experiments (BOUQUEGNEAU *et al.*, 1979) clearly show the importance of the direct contamination of aquatic animals as far as inorganic pollutants are concerned.

However, when considering the uptake rate of toxicants, only few data are available in the literature and JORGENSEN (1983) suggests the use of the concentration factor. Indeed, at steady-state, $\frac{dBIO}{dt}$, $\frac{dTOX(n)}{dt}$ and $\frac{d\gamma(n)}{fy}$ are all equal to zero and in the experimental conditions needed in the laboratory determination of the concentration factors, MORT(n), MY(n+1) and γ(n-1) are also equal to zero, so that

$$MY(n) \ YF(n) - RESP(n) = 0 \ , \ and$$

$$\frac{\gamma(n)}{TOX(o)} = \frac{UT(n)}{EXC(n)} = C.F.$$

From this equation, UT(n) can be found if EXC(n) and C.F. are known

(JORGENSEN, 1983); EXC(n) is relatively easily determined by placing contamina-
ted animals in unpolluted water and following the decrease of the contamination
in function of time.

The use of laboratory determined C.F. for the modelling of the distribution
of toxic substances in marine ecosystems requires however some comments.

The concentration factor is not constant even for a single species. As a
rule, it seems to decrease when the concentration of the pollutant [TOX(o)] in-
creases in the medium (see for example BOUQUEGNEAU *et al.*, 1985). This probably
results from the fact that when TOX(o) increases, there is generally (in the
case of active uptake) a saturation of the sites of entry of the pollutant so
that the initial rate of uptake is not always proportional to the concentration
in the medium. So, in the laboratory, it is necessary to determine the C.F.
in a concentration range close to that of the studied environment.

This decrease of the C.F. when TOX(o) increases is of value as far as no
storage mechanism is initialized from a threshold concentration in the organism.
When such storage mechanisms are concerned, at high levels of exposure, there
is clearly no tendency for equilibrium to be approached. This is clearly de-
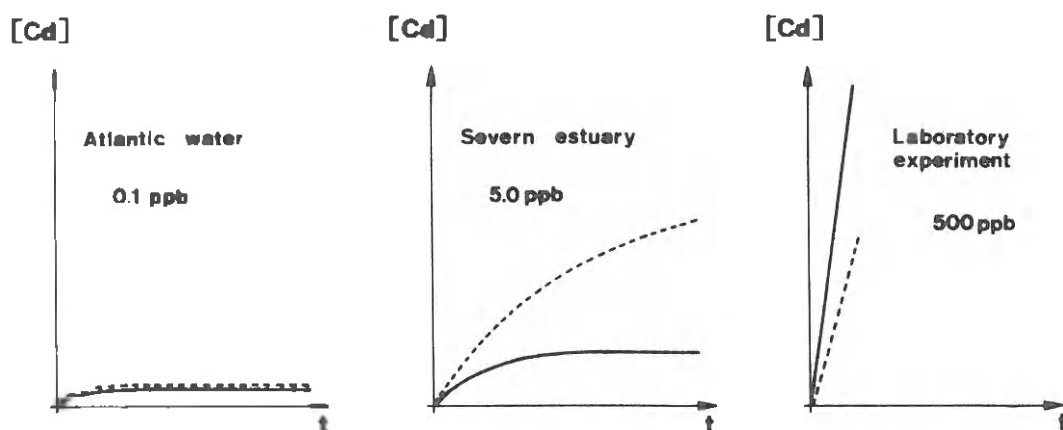monstrated in fig.4.

Fig.4 : Theoretical kinetics of accumulation of cadmium by *Patella vulgata*
(---) and *Littorina littorea* (——) in three different environments
(from data by NOËL-LAMBOT *et al.*, 1978).

In unpolluted natural seawater, low levels of cadmium are observed in *Patella vulgata* and in *Littorina littorea*. In the polluted waters of the Severn Estuary, the accumulation of Cd is much more rapid in limpets than in periwinkles. In these conditions, a high rate of synthesis of metallothioneins occurs in limpets while that protein is not detectable in periwinkles. In the laboratory however, when the cadmium concentration of the medium is sufficient to induce the synthesis of metallothioneins in both species, the accumulation of the metal is high and higher in periwinkles than in limpets. These kinetics show that no steady state can be approached once the threshold of concentration causing the synthesis of metallothioneins has been reached.

So the storage of pollutants appears as a handicap to the modelling of their transfer in ecosystems. Heavy metals can be stored and detoxified by marine organisms either by a compartmentation process within membrane-limited vesicles (lysosomes and spherocrystals) or by binding to specific proteins (metallothioneins). For a general review of the physiological role of these mechanisms, see BOUQUEGNEAU *et al.* (1984). It seems clear that those storage mechanisms, although generally considered as a protective mechanism, may be reponsible for high metal loads in some organisms living in polluted seawater. This does not necessarily mean that high contamination loads are toxic for the high levels of the trophic chain.

Another example of the diversity of the responses of aquatic organisms to the presence of pollutants in the environment is the comparison of the accumulation of mercury by three species of fish contaminated in the laboratory by the same concentration of pollutant in the medium.

Fig.5 shows the differences in the kinetics of accumulation of mercury (100 ppb in the water) by *Anguilla anguilla*, *Serranus cabrilla* and *Crenilabrus ocellatus*.

Three quite different shapes can be observed. The accumulation of Hg by the eel is quite linear, that of *Serranus cabrilla* approaches rapidly an equilibrium, and that of *Crenilabrus ocellatus*, first slow, increases significantly from the fourth to the seventh day and after reaches a steady-state. The mechanism we propose to explain this diversity is the following :

- the initial rate of uptake of mercury by the eel's gills is fast, the threshold of synthesis of metallothioneins is quickly reached and a very slow excretion of the pollutant occurs (BOUQUEGNEAU, 1975).
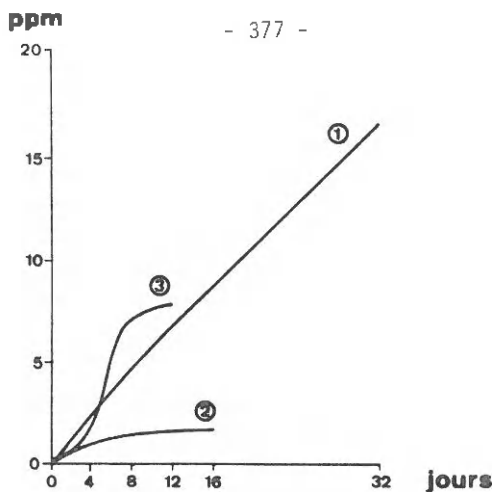
Fig.5 : Accumulation of mercury from seawater containing 100 ppb Hg (as $HgCl_2$)
1. *Anguilla anguilla* (BOUQUEGNEAU, 1975)
2. *Serranus cabrilla* (RADOUX and BOUQUEGNEAU, 1979)
3. *Crenilabrus ocellatus* (BOUQUEGNEAU and VELISSARIDES, unpublished results)

- the initial rate of uptake by *Serranus cabrilla* is fast too, but a mechanism of rapid excretion by the gills occurs (i.e. intense mucus production) which limits the entry of the pollutant (RADOUX and BOUQUEGNEAU, 1979). No metallothionein can be detected (BOUQUEGNEAU, unpublished results).

- the initial rate of uptake by *Crenilabrus ocellatus* is slow, but a synthesis of metallothionein occurs from the fourth to the seventh day of contamination (BOUQUEGNEAU and VELISSARIDES, unpublished results).

A third example of the diversity of responses to pollutants is presented in another chapter of these proceedings (BOUQUEGNEAU *et al.*, 1985) about the accumulation of technetium by marine molluscs. Finally, it is worth noticing that other physiological mechanisms can also interfere with the metal loads of the aquatic organisms such as seasonal variations (see for example BOUQUEGNEAU *et al.*, 1984).

GENERAL DISCUSSION

No general rule can, at first sight, be used to describe the transfer of stable pollutants in marine ecosystems :

  - the routes of entry of pollutants in the food chain are to be determined:

direct accumulation seems to be predominant for "inorganic" heavy metals but either both routes of entry or mainly indirect contamination occur in the case of organic pollutants.

- the adaptation mechanisms evolved by some marine organisms are to be carefully taken into account in polluted areas because they can completely modify the fluxes of pollutants between the different compartments of the ecosystems. This has been clearly demonstrated in the case of the storage of heavy metals. In the case of organic pollutants (PCB's for example), no specific mechanism of storage has been described till now. Whether lipids should be considered as PCB's storage structures is worth to be investigated. It seems clear however that their liposolubility and their level in the PCB's content of the prey determine the pollutant loads. For example, VEITH *et al.* (1980) have shown a close correlation between the bioconcentration factors of organic chemicals and the n-octanol-water partition coefficients. When considering organochlorine residues, it has been established that their partition coefficient between water and particulate matter as well as their liposolubility directly influences their bioaccumulation in marine food web (DELBEKE and JOIRIS, in preparation).

When considering heavy metals however, SIMKISS (1984) suggests that, at variance with "class a" hard acid metals (Na,K,Ca,Mg) which dissociate in water to form highly charged and hydrated particles, the "class b" metals (e.g. Cu,Zn, Cd,Hg), present the phenomenon of ion pair formation which confers to heavy metal a property of lipid solubility which could explain the high rates of penetration of these pollutants across cell membranes.

Much further research is needed in these directions to try and find general laws that would allow to integrate the actual data within a general explanation and to predict the fate of pollutants in aquatic environments.

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