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Evaluation of the potential toxicity of sediment and pesticides on the basin of Marennes-Oléron using a bivalve bioassay

Final project performed from the 28th of April to the 15th of October 2003

Under the direction of:

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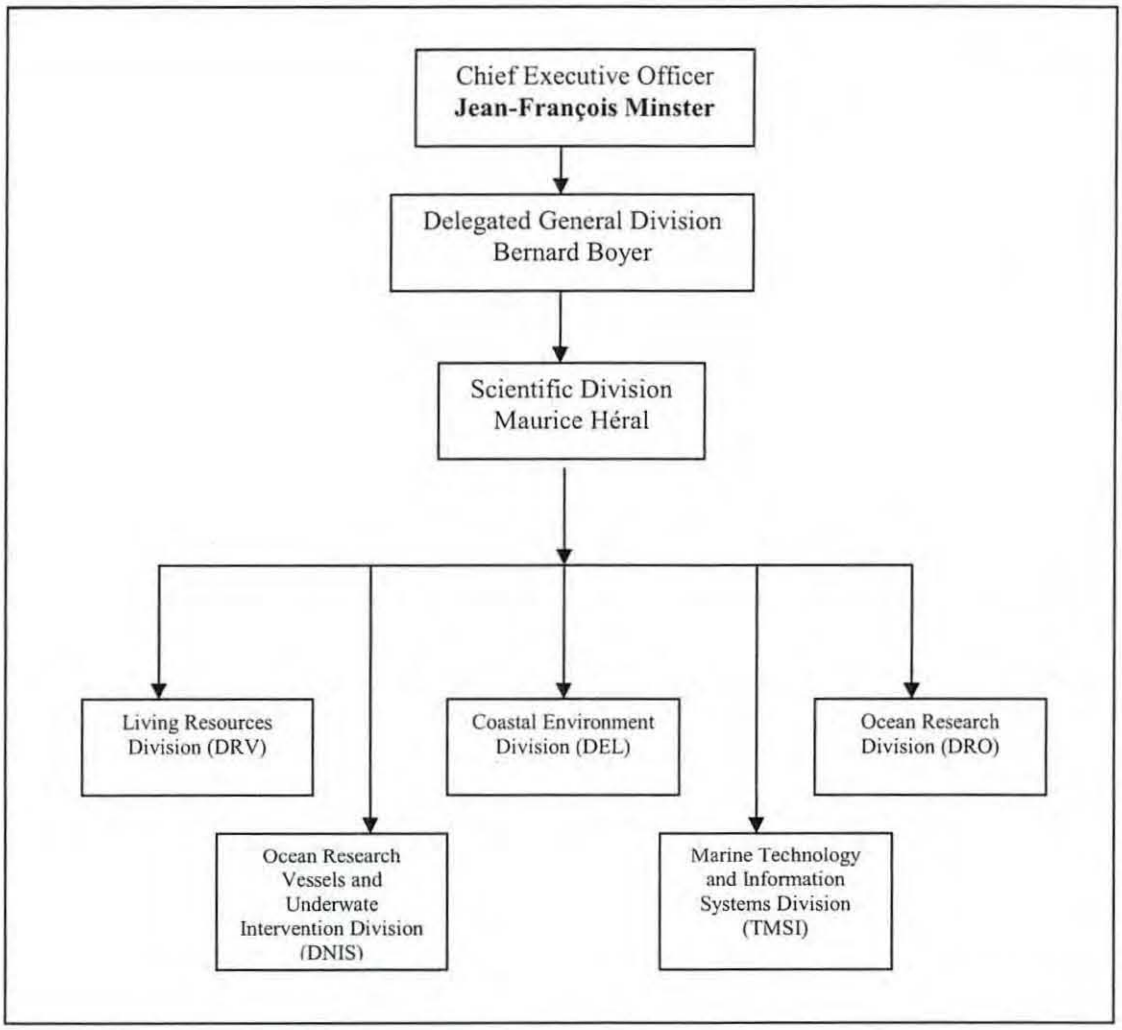
I thanks the overall staff of DEL for their cordial welcome.

ii. Presentation of IFREMER

Created by decree of 5 June 1984, modified on 18 February 1998, Ifremer means French Institute for Explotation of the Sea. It is a public institute of industrial and commercial nature. From January 2002, it will be placed under the joint supervision of four ministries: Research, Agriculture and Fisheries, Transport and Housing, Environment. Its annual budget is nearly 150 million euros. Ifremer has 1,385 employees divided into 72 laboratories or research departments over 5 centres (Boulogne-sur-mer, Brest, Nantes, Toulon, Tahiti) and 24 stations along the entire coastline of metropolitan France and in French overseas departments and territories. Moreover, 7 vessels (4 of them deep-sea vessels), 2 manned submersibles, 1 remotely-operated vehicle are available for deep sea explorations, a full set of test facilities.



Coastal Environment Division (DEL) (The division in which my final project is involved)

This division contributes to the knowledge of coastal ecosystems. Through developing methods and concepts usable by all those involved in environmental affairs, it supervises and manages networks to observe and monitor the coastal sea, undertaking research on the fate of water masses and on various biological and chemical processes.

This Division is made up of:

- 12 coastal laboratories
- 3 theme-based research departments
  - Coastal Ecology (DEL/EC)
  - Microbiology & Phycotoxins (DEL/MP)
  - **Chemical Pollutants (DEL/PC)**
- 1 studies and regional valuations unit (DEL/CR)
- 1 operational applications service (DEL/AO)

iii. SUMMARY

The department of Charentes-Maritime has a maritime facade of 440 Km and a surface area of 6,383 Km^2. The type of soil and the large maritime facade has allowed the development of two different activities: agriculture and shellfish farming. This shellfish farming takes place for a large part in the Marennes-Oléron basin. It regroups the production of oysters (50,000 tons per year) and the production of mussels. The shellfish farming creates 5,000 direct jobs. The agriculture, for its part, is divided into two main activities: cattle farming and plant production, such as the culture of cereals, oleaginous, wine and other vegetables. The intensive culture induces the farmers to use a lot of pesticides, such as insecticides and herbicides in order to comply with the required efficiency. Nevertheless, the culture on marhlands gives some constraints and draining is necessary. Thus the products spread by the farmers will partly flow away in the effluent downstream.

Thus in the department of Charentes-Maritime, four slope beds have been defined: The Charente, the Seudre, the short coastal rivers and the coastal municipality effluents. All of those feed the Marennes-Oléron basin with freshwater. In 1997, 2,700 tons of pesticides were used by agriculture and other activities in those slope beds; the main period of spreading occurs during the spring time.

Regarding to their chemical and physical parameters, pesticides will be more or less able to be diffuse in the environment and become bio-available. Eight pesticides considered to be the most frequently met in the environment have been studied: (Alachore, Atrazine, Carbaryl, Diuron, Glyphoste, Fosetyl-Al, Metolachlore and Terbuthylazine). The pesticides could be adsorbed on suspended matter or on sediment, leached in the soil and found back in the aquifer, evaporated or transported by volatilisation. Few models of the quantification and the dispersion of pesticides in the environment allow to assess the impact of it. In 2001, the concentrations analysed in the rivers of Seudre and Charente do not exceed one miligram of pesticides per litre. Until the pesticides are carried to the river estuary, they will undergo a series of transformations that affect their toxicity to the marine organisms and then they will be adsorbed on the sediment of the Marennes-Oléron basin.

Since the end of the nineties, the oyster farmers have encountered problems of mortality and especially in the Marennes-Oléron basin during the summer. A challenge « Défi MOREST » has been designed to gather laboratories of research on different themes around the oyster. It will try to find the reasons of those mortalities. An experimental study site « Dynamor » situated in the south of the Marennes-Oléron basin is the device for monitoring the oyster behaviour along the year and moreover during the mortalities. The laboratory of ecotoxicology of DEL/PC Ifremer Brest, has focused on the role of the sediment and the pesticides on the oyster mortality, using a bivalve bioassay on *Crassostrea gigas*.

The spatiotemporal study of the sediment from the Marennes-Oléron basin shows that the potential toxicity of the sediment increase at the end of May (more than 50 % of abnormal D larvae whereas it was 25 % in the middle of May). Furthermore, the experimental study site « Dynamor » is potentially more toxic regarding the sediment than the site Agnas situated in the middle of the basin.

Oysters have then been taken out from the « Dynamor » site to assess their capacity to produce normal D larvae and their sensitivity to $CuSO_4$. Thanks to those oysters, two environmental sites and two livestock have been studied. The proximity of the oyster to the sediment influences the sensitivity of its larvae to $CuSO_4$: $EC_{50\text{-}24h}$ = 2.62 µg $CuSO_4$/L at 15 cm whereas $EC_{50\text{-}24h}$ = 14.4 µg $CuSO_4$ /L at 70 cm. Moreover, oysters at 70 cm above the sediment produce more normal D larvae than the ones at 15 cm (73 % versus 57 %). Furthermore, it has to be noticed that oysters genetically selected to be resistant are less sensitive that the ones from the natural spat.

The study of the effect of pesticides on the embryonic development has shown that three of the eight pesticides studied induce a significant response of toxicity (from 12 to 32 % of abnormal D larvae for 1 µg of pesticides per litre): respectively for Carbaryl, Metolachlore and Fosetyl-Al. But the effect of the mixing of the eight substances leads to 35 % of abnormal D larvae at concentrations met in the environment.: This results indicates that it exists a synergy effect of the pesticides between themselves.

The massive spreading of pesticides during the spring could be put into relation with the sudden increase of the potential toxicity of the sediment. Moreover, pesticides are hazard for the oysters, and its potential adsorption on sediments induces different effects regarding to the proximity of the oysters to it. A hypothesis could be advanced: the energy spent to face pesticides stress during the ripeness may reduce the sum of energy allocated for the growth, the feeding and the pathogens protection.This could induce its death.

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1 INTRODUCTION

Since the 40's, a phenomenon of oyster's mortality (*Crassostrea gigas*) has been observed at an international scale, but it takes now such an extent that the oyster farming sector of activity could be financially damaged. Besides, the risk of a global downgrading of the production has to be taken into account if the origin of the mortality is not resolved in time (example of the portugueshe oyster).

Ifremer has estimated this problem as one of the most critical in the aquaculture, that is why it designed the challenge MOREST (Summer mortality of oysters *Crassostrea gigas*). This program started in 2001 for a 4 years period, associating professionals, regional structures and 8 laboratories of research. The objective is to gather information from complementary competencies and knowledge in order to find reason why motalities occur.

One of the causes of mortality which could be the potential toxicity of the sediment and the potential toxicity of pesticides discharged by rivers on oysters' farming areas, that is why the laboratory DEL/PC of ecotoxicology is involved in the project since 2002.

Ecotoxicology is the branch of ecology that combines ecology and toxicology. It deals with fate and transport of pollutants "with the study of toxic effects, caused by natural or synthetic pollutants, to the constituents of ecosystems, animals (including human), vegetable, and microbial, in an integral context" (Truhaut, 1977).

One of the orientation of the laboratory is the evaluation of the quality and the potential toxicity of middles thanks to bioassays such as the bivalve embryonic development (Quiniou *et al*, 1999 and His *et al*, 1999). This test could be applied for substances in liquid mixing, few effluents and also with sediments. In the case of the challenge "Defi MOREST", the bivalve test is used so as to evaluate the potential toxicity of sediment under growing tables of oysters. Furthermore, this bioassay allows to determine the evolution of this potential toxicity along the year, the water quality and the toxicity threshold of the main pesticides found in the oysters' concession found in Marennes-Oléron, study area in which the subject of the training period is involved.

In fact, in the aquatic middle, a large part of the xenobiotics are absorbed on suspended particles, and then accumulated at the level of the sediment. That is why the sedimentary deposit, at the link of the continent and the ocean, constitutes a real source of contamination. These coatline areas, which are habitats for a lot of species as the oysters, have to be protected in that respect (Géffard, 2001).

Along the reading of the report, a presentation of the project will be done in the chapter two, explaining how the challenge was designed and how the training period subject is linked to the overall project. The chapter three gives an overview on a potential source of pollution for the oysters and their habitat, the pesticides used in the department of Charente-Maritime. This chapter makes a non-exhaustive study of it, going from the first application by farmers to its transfer to the estuaries. The study of its behaviour in the environment will be achieved, the concentrations analysed in rivers of the department and some advice for the evaluation of its impact to the fauna. The chapter four is divided into two main parts, a technical part that discusses the materials and methods used to perform the bioassays and the results and conclusions.

Thus through out the performation of the bioassay on the the embryonic development of oysters, different axis of research will be assess:

- the evolution of the potential toxicity of the sediment sampled under growing tables.
- the potential toxicity of eight chosen pesticides tested alone and in mixed solution.
- the impact of the sediment proximity on the gamete quality of oysters growing in the Marennes-Oléron basin shellfish farming.

The last chapter of the study will end with conclusions and recommendations concerning the problem of mortality in the basin of Marennes-Oléron.

2 PRESENTATION AND STUDY GOALS

2.1 Problematic : nature of the subject

With a surface of 6,893 Km^2, the department of Charente Maritime has a maritime facade of 440 Km that include the islands of Oléron, Aix, Ré and Madame. It represents 6 % of the French coast (IFEN,1997). The particularity comes from the fact that 11 % of the department superficy are swamps, ideal for the agriculture. It is thus understandable that the two activities could have some difficulties to develop together without any problem.

2.1.1 The oysters' production in Charente-Maritime

The shellfish-farming is the main activity in Charente Maritime, the first production region in France. The production represents around the third part of the national oyster farming and a quarter of the national mussel farming.

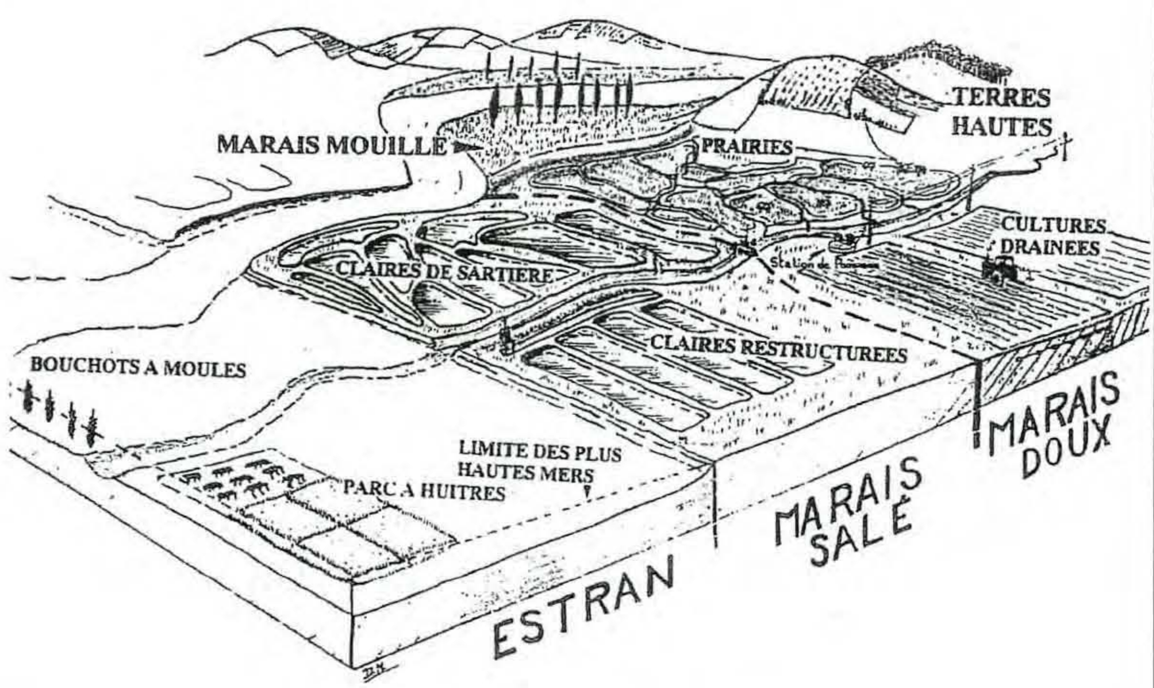


Figure 1 : schéma du marais (D. Masson)

Figure 1: Representation of the different activities by geographic situation on the Charente-Maritime coastline. (Ifremer student report: Grand'mourcel, 2000)

Table I: Superficy of the inside seas

Inside seas	Pertuis Breton	Pertuis d'Antioche	Basin of Marennes-Oléron
Superficy (ha)	36,000	35,000	26,000

The locality where the study effect is achieved is the Bassin de Marennes-Oléron. Two-thirds of the oyster farming production in Charente-Maritime is practiced there.

The spawn picking up of oysters is one of the main activities (around 50 % of the french spawning). The oysters are cultivated in tables at 15 or 70 cm up from the sediment. Sometimes it can develop naturally on rocks and constitutes in that case to a natural oyster deposit. On the bank of the river Seudre the biggest centers for oysters' maturing and expedition can be found. The "affinage" is a speciality of the region, that is why other production region send their oysters to Marennes- Oléron for the maturation. It is practiced on old salt marshes: les claires. A level of 50 cm of sea-water is maintained for the development of a blue algae *Haslea osteraria*. This algae is consumed by the oysters and give to it a green colour, enjoyed by the consumers.

The annual production of Marennes-Oleron is 30,000 tons of oysters (26 % of the french production) for a selling of 50,000 tons on the market (45 % of the french production). The production of wild oysters from natural deposit is estimated to 15,000 tons. The oyster farming represents 4,500 direct jobs.

The price of the oysters production per kilo has increased a bit, it is estimated at 2 euros and has been maintained for the expedition at 3 euros. This thanks to the quality of the product and a good publicity at a regional and a national scale.

2.1.2 The agriculture in Charente-Maritime

The proximity between the agriculture and the sea seems to be paradoxal. Actually, for economical reason, PAC: Common Agricultural Politic, and the decrease of the meat price, the number of sweet swamp has increased in order to introduce the production of cereals on that land. In fact since 10 years the proportion of cereals production / cattle farming has increased up to 80/20.

Soils of salt marshes have many properties, a first slim layer of organic matter. The soil contains 40 to 50 % of clay, a lot of salt and a proportion of limestone with a pH between 7 and 9. The following table shows the proportion of each field of agriculture regrouped in two main categories, the plant and the animal production.

Table II: Value of the agriculture production per field of activity - 1998 in percentage.

Production	**1998**
Cereals	25
Oleaginous	35
Wine	7
Other vegetables	13
Total of plant production	**80**
Cattle	6
Milk	9
Other meat	5
Total of animal production	**20**

As it can be seen, a large part of the plant production concerns cereals and oleaginous, kind of agriculture that need a lot of active substances so as to increase its production efficiency by killing undesirable target plants and insects and to protect against hillness.

2.1.3 The problem of farmers

The wet areas in Charente Maritime represents 100,000 ha divided into, wet swamps, salt marshes (for the aquaculture) and the dry sweet swamps (for the agriculture)

Due to their features, soils have to be worked out in order to have the best quality and a few techniques are employed:

- Draining: it lowers the water level. This process is the cause of the agricultural effluent.
- Maintaining the water level in the ditch for the stability of the bank.
- Removing of the salinity: Thanks to ion exchange with gypsum, the salt marshes become a mix of limestone and clay, better than a mix of limestone and sodium.

Depending on the season and the culture, the farmers use chemical products. In fact the intensive culture obliges them to spread a lot of pesticides including: herbicides, fongicides, insecticides and sometimes moluscicides. Which products arrive in the marine environment later on.

2.1.4 The problem of oysters producer

The "claires", the place where the "affinage*" of oysters is done, is designed to hold back the water in order to remove the salt. In fact in summer, the water evaporates and the "claire" gets a bigger salinity. The incoming of fresh water from the river is necessary and allows to maintain a buffered salinity. However, during the autumn and the winter, this large amount of incoming river-water could have effects on the normal development of oysters:

- A too large adding of fresh water will induce problems of osmosis on the tissues of the oysters and on its food, the phytoplancton.
- The water containing substances spread by the farmers could have potential noxious effects on oysters.

For ten years, the relations between farmers and oyster farmers have been good. Thus time schedule of the hours of opening lock gate, have been implemented to prevent accidents. Nevertheless an abnormal mortality of oysters is still observed in Marennes-Oléron, thus a large scale research program has started to understand and to find the reasons ofthese mortalities. It is the challenge "Défi MOREST".

*affinage: Last step of the production of oysters. The oysters are put in basin in order that they eat blue algae, their flesh become green, a colour enjoyed by the consumers.

2.2 Presentation of the challenge "Défi MOREST"

Since few years, the end of the 90's, abnormal increase of the oyster mortality has occurred during summer time. The previous studies tend to show that not only one cause can be identified, but that this phenomenon results from a combination of parameters. A programme was started in 2001 to precise these interactions. It is the challenge "Défi MOREST", (summer mortality of *Crassostrea gigas*).

2.2.1 Localisation

The "défi MOREST" concern to three sites in France where it was observed, an abnormal increase of the oysters' mortality non explicated. This sites are:

- Bay of Veys: Normandy in the Cotentin East.
- South of Britain: Morbihan gulf, Auray's river and bay of Quiberon.
- Atlantic coast: Basin of Marennes-Oléron.

This is on this last site, the basin of Marennes-Oléron, that the subject of the training period is included. It is a part of the research among the different studies done on the variation of the physical and chemical parameters, the biologic features of the environment and their interactions with the oysters. In this way of research, a workshop site Dynamor: Mortality dynamic, has consisted in the follow-up of these parameters on oysters livestocks placed on growing tables at 15 cm and 70 cm above the sediment.

2.2.2 Explanation of the study site "Dynamor"

2.2.2.1 Definition of the experiment study site Dynamor .

The interest of the site "dynamor" is to work with oysters from the *in situ*. Hence, four livestocks of oysters have been placed at the dispbsal of the researchers. The different livestocks exposed to the twó growth conditions are 18 month old oysters:

- Resistant families (named **TOP**); diploïdic families genetically selected.
- Sensitive families (named **FLOP**); diploïdic families genetically selected.
- Diploïdic oysters from natural spat: **Capt – Nat.**
- Triploïdic oysters, produce by specific crossing so as to obtain sterile organism better for sell: **Triplo.**

Two environmental conditions corresponding to the growing place of oysters is proposed:

- One sensitive environment because at 15 cm above the sediment.
- One environment which presents less risk for the oysters (70 cm above the sediment)

Figure 2: Oysters' growing tables at 15 and 70 cm on the workshop site Perquis in the basin of Marennes-Oléron. (©Ifremer)

Table III: Representation of the different condition on the dynamor experiment site.

	Triplo	TOP	Capt-Nat	FLOP
70 cm				
15cm				

The previous table is a model representation of the waited mortality response of oysters, the arrow indicates the gradient of the mortality.

2.2.2.2 *The workpackages of Dynamor experiment*

In 2003, the principal aim is to identify the causes of mortality in relation with the model studied in the basin of Marennes-Oléron. Therefore 7 Ifremer laboratories and other associated laboratories, are involved to that task with an annual budget of 1.5 million of euros. Table 4 defines the different themes of study.

Table IV: List of the partners and their activities

Partner	Laboratory	Activities
P.Guilpain	RA-LCPC	Dynamic of mortality and biometry
F.Blouin	RA-LCPC	Indicator of maturation
T.Renault	RA-LGP	Emocytair activity
T.Renault	RA-LGP	Reserach of the pathogene agents
A.Le Roux	RA-PI	Ionic composition of the emolymphe
J.Haure	RA-LCPL	Ecophydiology comparative
T.Burgeot	DEL-PC	Biomarker for resistant and sensible oysters
T.Renault	RA-LGP	Immunotoxicity
N.Malet	CNRS-Thesis	Origin and assimilation of the trophic resources
T.Burgeot	DEL-PC	Study of the pesticides on a breeding population
F.Quiniou	DEL-PC	Embryotoxicity-comparison of the populations
J.L Nicolas	RA-PI	Study of the bacterial microflaura
F.Quiniou	DEL-PC	Embryotoxicity-comparison of the sediment
O.Le Moine	RA-LCPC	Study by « transecs » hydro
F.Blouin	RA-LCPC	Study by multi-parameter plummet
T.Burgeot	DEL-PC	Contamination of sea-water by the pesticides
T.Burgeot	DEL-PC	Sulfure and ammonia analysis
F.Leroux	RA-LGP	Bacterial challenge
F.Leroux	RA-LGP	Biodiversity pro and eucaryote of the sediment
P.Geairon	RA-LCPC	Histology quantitative

Thus, in summary, the different themes of research are divided on two main thrust, on one hand on the animal (the oyster) and on a second hand on the environment (sediment and water). The task in which the subject of the training period is involved is the embryotoxicity comparison regarding to the sediment and the livestocks.

2.2.3 The questions asked

As it has been presented in the summary of the tasks for the project MOREST, the laboratory where takes place this study, is implicated into two main themes of research. The potential toxicity of the sediment and its evolution on time, and the impact of the sediment proximity on the quality of the gamets descended from *Crassostrea gigas* exposed on 15 and 70 cm growing tables. This research are achieved using a bivalve bioassay. This leads to try to answer to those following questions:

- Is there a potential effect of the sediment on the oysters' embryonic development ?
- Is there an evolution on time of the potential toxicity of the sediment in Marennes-Oléron ?
- Does the proximity of the sediment regarding to the oyster have an influence on its capacity to produce normal D-larvae ?

On the same time, a study of embryotoxicity on 8 main pesticides has been conducted:

- To compare later on, the effect of the mixed pesticides solution on the embryonic development with the biomarkers study assesment done by another team.
- To determine the dose effect of each of these pesticides on the embryo-development of larvae.

2.3 The means implemented

2.3.1 The oyster *Crassostrea gigas*

Classification

Division: Lamellibranche
Order: Filibranche
Sub-order: *Anisomyaria*
Super-family: *Ostreidae*
Family: *Ostreidae*
Sort: *Crassostrea*
Race: *gigas*

Adult morphology

The shell of the Japanese oyster *Crassostrea gigas* is constituted of two unequal valves: theright valve has an operculum. The shape of the shell is in relation with the kind of soil and the way of culture of the cattle (His and Cantin, 1995). An elastic ligament, horny, links the two valves. The elasticity of that ligament tends to move away the left valve from the right one, whereas the adducent muscle tends to bring them near (Bousaïd, 2000).

Distribution

The Pacific or Japanese oyster (*Crassostrea gigas*), from east Asian continent (Korea, Japan and China), is relatively well distributed over the world (His *et al.*, 1999):

- North America (Pacific coast of America and Canada)
- Asian part of Russia (Okhotsk sea and the Sakaline island)
- Australia
- Europe: from the Channel to the Moroccan coast, the Mediterranean sea

2.3.2 Bioassay on the oyster embryonic development

The first bioassay on bivalves was performed by Loosanoff *et al* in 1955. In 1973, Woelke designed the test of toxicity on the embryo larvae development of oysters (His *et al.*, 1999). This acute test was chosen by the laboratory for several reasons:

- **Test sensibility**: from a general point of view, the first stages of the development of the organisms tend to show a high sensibility to pollutants. His *et al.* in 1999, build a rank of the sensibility of bivalve function of their age: embryo > veligere > pediveligere > juvenile > adult. Futhermore a study of Martin *et al.* (1981) on the sensibility of two bivalves (*C.gigas, M.edulis*) show that both tests have almost the same sensitivity.
- **The providing:** in Europe the natural oysters ripeness occurs from June to August in the environment (Lango-Reynoso *et al.*, 2000). But it is also possible to be provided whatever the month with ripe oysters thanks to hatchery. This allows the performance of the bioassay all the year along.
- **The tolerance regarding to the experiment conditions:** *Crassostrea gigas* has a large tolerance regarding to the environmental features. It could develop with a temperature going from 15 to 30 °C. Nevertheless, the temperature influences the embryos and larvae. The oyster's larvae supports and can develop under a range of salinity going from 20 to 35 ‰ (His *et al.*, 1989)
- **The materials means:** the tests based on the embryonic development *of Crassostrea gigas* only need a current material: thermostatic cupboard, microscope (x 400), one use flask and sterilized materials.

To carry out such a bioassay, the cost must include the purchase price, the one used glassware and the ripe oysters, which totally can goes up to 1,000 euros .

Nervertheless, this test presents some pecaution that have to be took:

- **The quality of the ripeness of oysters**: Due to the fact that the experiment is based on biological material, the quality of the parents could not be maximum all the year along. However, this parameter has a real important role on the managing of the test.
- **The care on all the steps of the experiment:** During the carrying out of the test, a particular care has to be done to the biological material. A stress coming from a pollutant or a problem during the manipulation could compromise the test. It is important to follow the protocole of the experiment and to check all the steps as well as the perfect quality of the glassware used.

2.4 Conformation to the project

In an environmental impact study, the aim is to understand how the pollution is introduced in the environment and how it could influence the flaura and the fauna as the humans. In this study, the target of the pollution is the oyster *Crassostrea gigas*. For a few years during the summer time, they have been victim of many parameters that induce them die. Nevertheless, the reasons of their death are not well known and that is why the Défi MOREST was designed two years ago.
Thanks to our knowledge and the means of work in embryotoxicity, thorough experiments will be performed to see the influence of pollutants on the oyster embryonic development. Thus three kind of experiment were performed:

- The potential toxicity of the water and the sediment sampled on oyster farming concession of Marennes-oléron.
- Effect on the environment condition on their capacity to produce normal D larvae.
- Assesment of the toxicity of 8 pesticides tested alone and in mixing.

Even if the reasons of these mortalities are not really known, in order to determine the potential impact of pesticides coming from agriculture in the upstream rivers, the specific focus on agriculture and moreover on pesticides will be done in the chapter three. It will explain from the start of the application to its fixation to the sediment, how pesticides could become a toxicant for the environment.

3 PESTICIDES IN CHARENTES-MARITIME

3.1 Presentation

Pesticides are chemical complexes which could come from organic or mineral origin, but which are often obtained from chemical synthesis, they are so called xenobiotics. They could be organo phosphoreus, organo chlorate or organo metalic. The sales of pesticides have increased between the 80's and the 90's up to 17 % with a mean of three kilograms used by hectare of intensive culture. In France, each year 95,000 tons are spread which makes it the third market of the world after USA and Japan. In Europe there are around 900 active substances in use.

The pesticides allows the modern agriculture to put down certain constraints of the field in order to improve the efficiency and to produce in relation to the market demand (aspect, homogeneity, sanitary quality). The culture and the harvesting are facing lots of enemies. In the world, there are around 15,000 species of pathogenes mushrooms, 10,000 species of harmful plants. The harvesting losses each year are estimated at 200 millions of corn and 140 millions of wheat around the world.(Van der Werf, 1997)

3.2 The case of the Marennes-Oléron basin

3.2.1 Localisation of the slope beds

The public opinion has been alerted at the early 80's by the responsability of the agriculture concerning the water quality. The attention went first to nitrates, but secondly the usage of fertilizers, which has multiplied by eight between 1959 and 1990 on the national territories (Marchand and Kantin, 1995), has alarmed the departement of Charente-Maritime.

In 1997, the regional service for the crops protection has started a big survey in the region Poitou-Charentes to evaluate the fertilizers used and their quantity.
Five coastal bassins near the oyster farming area have been chosen, they are:

- La Charente
- The short coastal river
- La Seudre
- The coastal municipalities

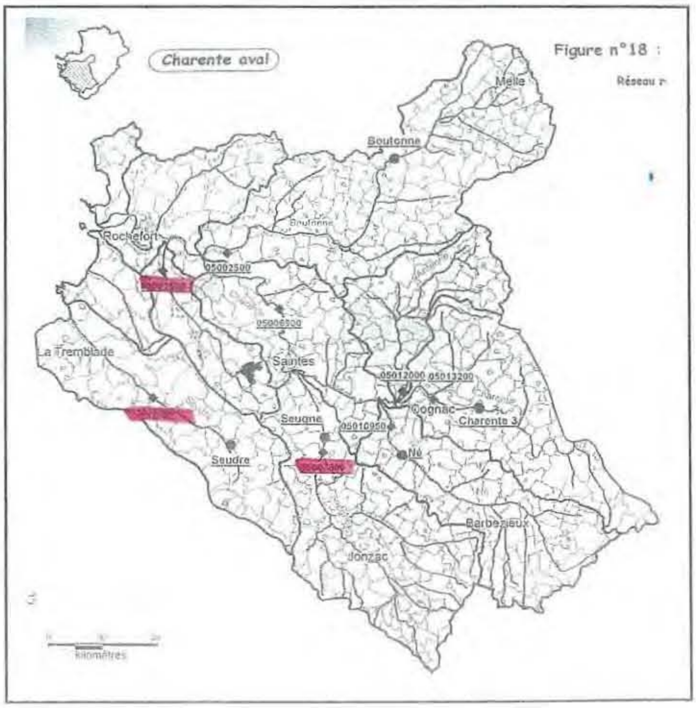


Figure 3 : Map of the department of Charente-Maritime, representation of the different tributaries and rivers. (Picture RNO,1997)

Three main use of the soils could be established by looking the figure 3. They are mainly composed of vegetal production, from the east to the west:

- The vineyard of Cognac.
- The valley of Boutonne, slope bed with a lot of cereals production.
- The coastline, market gardening and breeding.

3.2.2 Agricultural use and others

The pesticides are nowadays used commonly in many fields. Two of them could be established, the agricultural sector and the non agricultural fied. The first one regroup the culture of wheat, sweetcorn, barley, sunflower, pea, melon, vineyard, fallow, arboriculture, grassland and forest. The second field, non agricultural, used also pesticides in order to clean areas, it regroup the municipalities roads, the highways (DDE), the railway (SNCF) and the people that use pesticides at home for the garden. (GRAP Poitou-Charentes, 2002)

3.2.3 Spreading quantities

In this survey, for the three slope beds and the coastal municipalities of the downstream side of the department Charente-Maritime, a list and a quantification of the products spread have been established, it regroup 142 products. Thus in 1997, the based year for this study, the mass and the number of pesticides spread in the different slope bed are:

- 1,450 tons from 123 compounds spread in the Charente.
- 91 tons from 60 molecules used in the Seudre slope bed.
- 86 tons from 57 substances discharged near the short coastal river.
- 119 tons from 121 products used in the coastal municipalities.

For example in our following study we have tested 8 pesticides, 6 of them have been analysed in this survey and are being considered as a priority in the list of active substances established by the collaboration of Ifremer, SRPV and INRA. (GRAP Poitou-Charentes, 2002)

Table V gives an overview of the quantity of each pesticide used in 1997 in the department of Charente-Maritime:

Table V: Pesticides quantities spread in the slope bed for 6 of the 8 pesticides studied in the project, and percentage of them on the 142 substances used.

	Quantity used on the coastal slope bed (Kg)				
Pesticides studied	CHARENTE	SEUDRE	SHORT COASTAL RIVERS	COASTLINE MUNICIPALITIES	**Total**
Alachlore	83,393	8,022	3,517	8,477	**103,409**
Atrazine	103,832	9,963	4,365	10,602	**128,762**
Fosetyl – Al	81,826	5,270	5,047	5,798	**97,941**
Glyphosate	102,062	6,768	6,159	9,414	**124,403**
Metolachlore	61,471	5,864	2,583	6,199	**76,117**
Terbuthylazine	44,578	2,952	2,832	3,056	**53,417**
Total	**477,162**	**38,839**	**24,503**	**43,546**	**584,049**
Total of the 142 substances used	2,204,178	140,633	124,040	208,772	**2,677,623**
Percentage on the 142 substances	**21.6%**	**27.6%**	**19.7%**	**20.8%**	**21.4%**

In 1997, around 2,700 tons of pesticides have been spread in the slope bed of the basin of Marennes-Oléron. Among this 142 substances, one fifth is represented by six of the eight pesticides followed. The river Charente is the influent the most responsible of the feeding of pesticides in the basin. The estuarie of the Seudre, where is situated nearby the experimental study site « Dynamor », could have brought 140 tons of pesticides from its slope bed.
The datas on Diuron and Carbaryl have not been count at the time of the survey.

3.2.4 Application's Period

Table VI gives for seven of the eight pesticides chosen for the study, the period of application for the agricultural field of activity. These pesticides are considered as the most common met in the environment.

Table VI: Spraying period of the pesticides studied on the current year 2001.

	Jan	Fev	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Alachlore												
Atrazine												
Carbaryl												
Diuron												
Fosetyl-Al												
Glyphosate												
Metolachlore												

As it could be seen in the previous table, the main period of spreading is the spring time, corresponding on the field to the first period of cereals' growth. Furthermore, it corresponds in the sellfish farming concession of the basin of Marennes-Oléron to the previous period of maturation of oysters.

Carbaryl is an insecticide, Fosetyl-Al is a fungicide whereas the others pesticides are all weedkiller. Terbuthylazine, diuron and glyphosate are substances used for the weeding of vineyard whereas Métolachlore, Alachlore and Atrazine are essentially spread for the weeding of corn. Atrazine is a pesticides is now forbidden since 1997.

3.3 Physical and chemical features

It is possible to assess the behaviour of a molecule in the environment, with a minimum of parameters. But these data have to be completed to evaluate the risk in a particular situation. In fact, a lot of hydrological and sedimental processes occur in space and time. It influences the transfer and the evolution of the pollutant in the soil, in the surface water and on the aquifere to the coast. Modelling is then the only device able to integrate all these features (Andral, 1996).

In appendix I, the physical and chemical parameters of 8 pesticides studied are compiled in tables. They inform about the different features, influencing the behaviour of each substance in the environment, as following:

- **Hydrosolubility**: Gives information on the behaviour of a substance in a environment, particulary on its ability to be metabolite by living organism, or to be train in the water (presence in the dissolved phase)
- **Henry's constant (H):** Calculated from the vapour pressure, it allows to evaluate the tendency of a product to go from the dissolved phase to the gas one.
- **Log P:** Coefficient of partition between the octanol and the water, expressed K_{ow}, it represents the potential of molecules to bioaccumulate in tissues.
- **K_{oc}**: Coefficient of partition between carbone organic and the water. This value indicates the faculty of a molecule to be adsorbed on a particle, then it allows to estimate its average mobility in the soil, when it is an organic matter soil.
- **DT_{50}:** Half life in the soil. It shows the evolution of the concentration in the soil by biotic degradation and by evaporation.
- **EC_{50}** Effective concentration able to induce 50 % of effect on the organism. This biological measure gives important information for the prevention of risks and for the establishment of recommendations or restriction regarding their usage.

Table VII: Parameters and treshold values used to evaluate the behaviour of pesticides in the environment and their effects on non target species in fresh water(Andral,1996)

Hydrosolubility (mg/l)	Low hydrosolubility	10	hydrosoluble	200	High hydrosolubility
Henry's constant ($Pa.m^3.mol^{-1}$)			volatile	1,00E+06	Low volatility
Log P			Bioaccumulable	3	Low bioaccumulation
Koc (g/cm^3)	Low mobility	1	Mobile	100	High mobility
DT_{50} (days)	Instable	8	Not very	30	Stable
CE_{50} (acute in mg/l)	Low ecotoxicity	1	Ecotoxicity	0.1	High ecotoxicity

The degrade from the blue to the orange corresponds to a gradient of risk for the environment.

Table VIII : Potential behaviour of the eight pesticides in the environment regarding to their physical and chemical parameters

	Hydrosolubility	Henry's constant	Log P	Koc	DT50	EC50
Alachlore	240		1.59	170	16,5	0,0064
Atrazine	33	2.6 10-4	2.75	100	86	6.9
Carbaryl	40		1.59	300	16.5	0,0056
Diuron	42	5.1 10-5	2.85	480	500	1.4
Fosetyl-Al	109800		2.11	20	30	40
Glyphosate	10500		3.2	24000	30	37
Métolachlore	530	9.1 10-4	3.45	1700	90	25.1
Terbuthylazine	8.5	4.1 10-3	3.2	828	70	3,8

The risk assessment done thanks to the physical, chemical and ecotoxicological parameters that come from annex I allow to do some hypothesis :

- Alachlore, Fosetyl-Al, Glyphoste and Metolachlore are supposed to be diffuse easily in the environment regarding to their hydrosolubility. They could become toxics in the aquatic surroundings.
- Alachlore, Carbaryl and Terbuthylazine present high risk to organisms because their ecotoxicity is considered as elevated.
- Alachlore is thus a pesticide very harmful to the environment.
- Atrazine and Terbuthylazine do not present risk for the marine closed environment due to the fact that they could evaporate easily and be transport in another far place.
- Fosetyl-Al, thanks to its hight hydrosolubility and its high mobility will become easily bio-available in the environment. Moreover that its half life is long.

The chapter 4.2.3. gives the result of the effect, of those eight pesticides alone and in mixing, on the embryonic development of *Crassostrea gigas.*

3.4 Behaviour in the environment

It has been estimated that 2.5 millions tons of pesticides are applied on soils and cultures on the earth. The part having a direct contact with the target organism is 0.3 % whereas 99.7 % of these substances is going « away » (Pimentel, 1995).

3.4.1 Migration mecanism

As soon as the pesticide is in contact with plant or soil, it starts to disappear, it is dissipating. The active substances could volatilize, leach down in the soil or stream in the river to reach the surface water or the aquifere. It could also be absorbed by plants or organisms in the soil or simply remain in the soil.

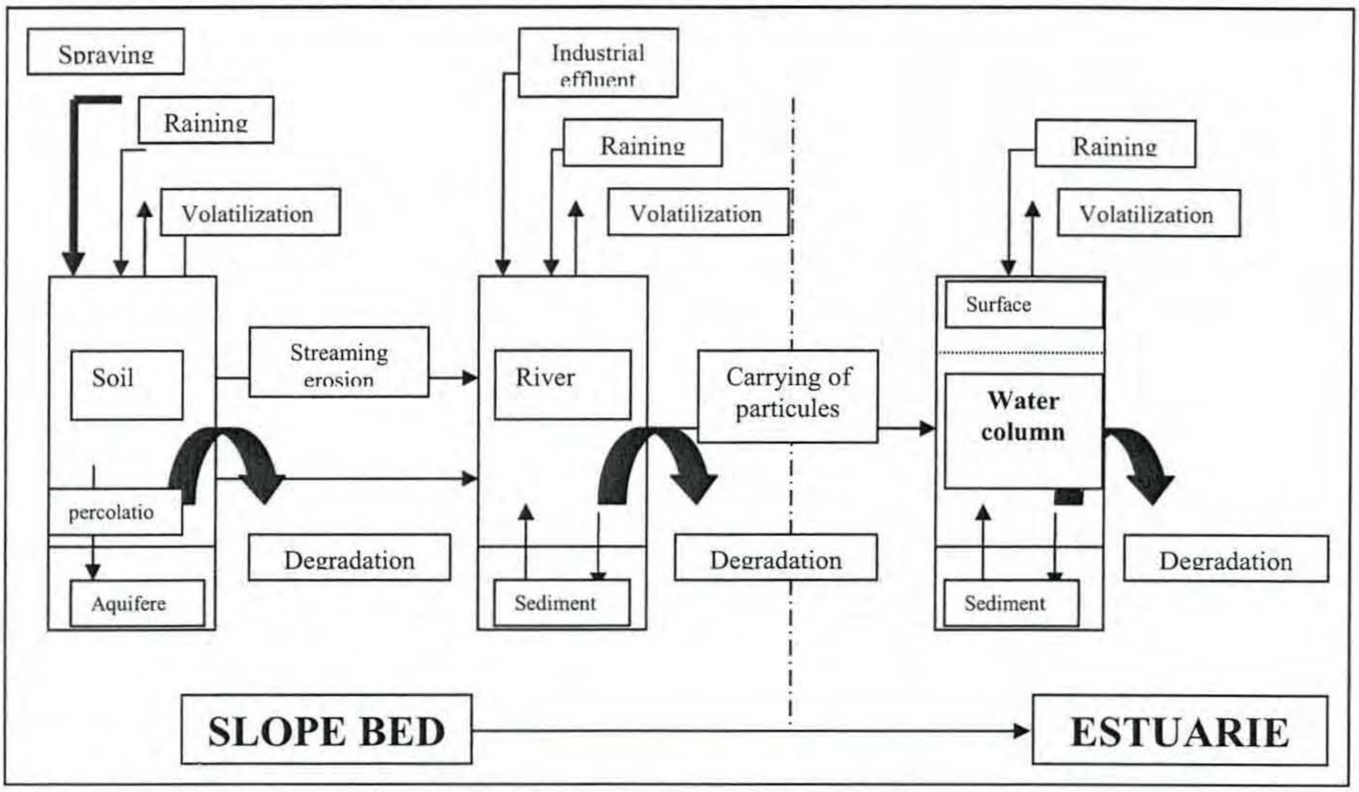


Figure 4: Pesticides transfer mode from spreading areas to estuaries (Munschy, 1995)

3.4.1.1 Becoming in the soil

The dissipation of pesticides in the soil is due to the fact that a chemical reaction occurs in it and mico-organisms act for it. The rate of degradation increases generally with the temperature and with the concentration of water in the soil (Walker, 1976). The speed of degradation is determined with the half life (DT_{50}). It also has to be considered that, the products of degradation incoming from the active substance are the metabolites. In fact they could have some toxic impact and their half life is sometimes longer than the pesticides themselves.
The mobility of the active substance is often slowed down by its adsorption on soil particles, due to some physical and chemical properties of the soil and the molecular features of the substance. It is the organic matter that essentially keeps the pesticide non ionic.
The coefficient K_{oc} is defined to measure the degree of adsorption of the pesticide by the soil. (Leonard, 1990)

$$K_{oc} = \frac{K}{\%OM}$$

With:

- **K_{oc} = Coefficient of soil/water partition**
- **%OM = Percentage of organic matter in the soil**

The adsorption of pesticides from the soil by the plants is probably one of the main ways that induces the bio-accumulation of it along the different trophic level .

3.4.1.2 Volatility

It is one of the main ways of pesticides' dipersal out of the target area, especially when the treatment aims the soil surface or the plants. The loss by volatility could go up to 80 % of the applied product, few days after the treatment. (Glotfely *et al.*, 1984 ;Taylor and Spencer, 1990). The constant of Henry (H), the ratio of the vapour pressure on the water solubility, gives a good idea of the rate of volatilization of the sustance. The products getting an H over 2.5×10^{-5} are volatile but their volatility decreases with time. Whereas the products with an H with a value largely less than that, are less volatile but their volatility increases with time.

3.4.1.3 Streaming and leaching

The streaming is defined as the movement, at the surface of the soil, of the water flow that contains dissolved and suspended matter (Leonard, 1990). This streaming could carry away pesticides in solution, suspended or adsorbed in the sediment. The substances that are highly adsorbed and resist to the degradation and to the volatilization, remain at the soil surface for a long time and are induced to be swept along the water streaming. However, the importance of the pesticides migration is determined by three parameters:

- The type of soil and the culture
- The delay between the spread
- The first following rain and its intensity of it.

The leaching is a kind of percolation, it depends on the nature of the soil and the physical and chemical properties of the pesticides. These ones will lay down the transfer condition of the molecule in the gas phase (diffusion) and especially in the liquid phase (diffusion and leaching). The way of the water is determined by the soil porosity, the mechanic dispersion, the adsorption and desorption phenomena on coloidal (Hascoet and Jamet, 1987).

3.4.1.4 Models to simulate its dispersion

In the previous part, the different ways of the dispersion of pesticides have been shown. In this part, a short summary of the quantification for the environmental effects of pesticides will be done. Levitan *et al.* (1995) have drawn up a large inventory of it, by establishing a list of 38 processes of parameter integration going from the economical conditions to estimations based on only one parameter as a descriptor of the environment. A short presentation of three methods will be enough to have an overview. These methods are designed to help the farmer in the choice of products in order to improve the ratio between the amount used and the amount needed.

Economical and environmental treshold of nuisance: SNEE.

SNEE: This treshold is defined as the strength reached by an undesirable population corresponding to the equality between the cost of the treatment of it and the benefits desired. It is calculated by taking into account the physical and chemical properties of a substance and its toxicity (EC_{50}) and by asking farmers about the amount of money they accept to pay per hectare to avoid pollution induced by the treatment. (Highley and Wintersteen, 1992)

QIE: Environmental Impact quotient: it is a value to describe the harmful effects of pesticides at three levels: farmers, consumers and the environment. Each of them has the same weight on the calculation of the QIE. The risk assesment is calculated for each category, from the toxicity of the pesticides multiplied by its potential contamination which is:

- For the farmer: Time of exposure
- For the consumer: Chronic toxicity
- For the environment: Acute toxicity multiplied by the half life

This index allows to make a comparison between the environmental impacts of the pesticides and the the remediation processes. (Kovach *et al.*, 1992)

The exposure of the environment to the pesticides: EEP.

EEP: The process is to calculate the score of environment exposure to the pesticides to help the farmers to reduce it. This score is calculated for the air, the soil and the water. The user can choose the weight of each score depending on its localisation. The formula is:

- $EEP_{air} = m.p$
- $EEP_{soil} = m.DT_{50}$
- $EEP_{water} = m.DT_{50}.K_{om}^{-1}$

Where
Kom = partition coefficient between organic matter and water
m = masse of applied pesticides
p = vapour pressure of the pesticides
DT_{50} = half life of the pesticides in the soil

This calculation does not use the toxic effects, in fact the authors do think that these effects represent a small parts of the real effects. They prefer a process which focuses on the reduction of the exposure of pesticides. (Verheijken *et al.*, 1995)

Thus the process of Highley and Verheijken (1995) is principally based on the assesment of the environmental impact whereas the one of Kovatch *et al.* (1992) takes into account the effects on the farmers and on the consumers rather than the effects on the environment.

3.4.2 Adsorption of pesticides on the sediment in the aquatic surroundings (bassin of Marennes-Oléron)

According to the proposed following representation, pollutants enter the environment by diffuse input from the atmosphere or by point introduction from streams at their upper, lower and lateral boundaries of the local area. The major source of particles is detritic material, coming either from rivers or shoreline erosion, which could contain pesticides. Atmospheric deposition is a secondary, but potentially important, source of pollution in the coastal ocean.
Any pollutants introduced into the water column will undergo a series of chemical reactions or transformations that will not only govern its transport through the water column but also affect its toxicity to marine organisms.

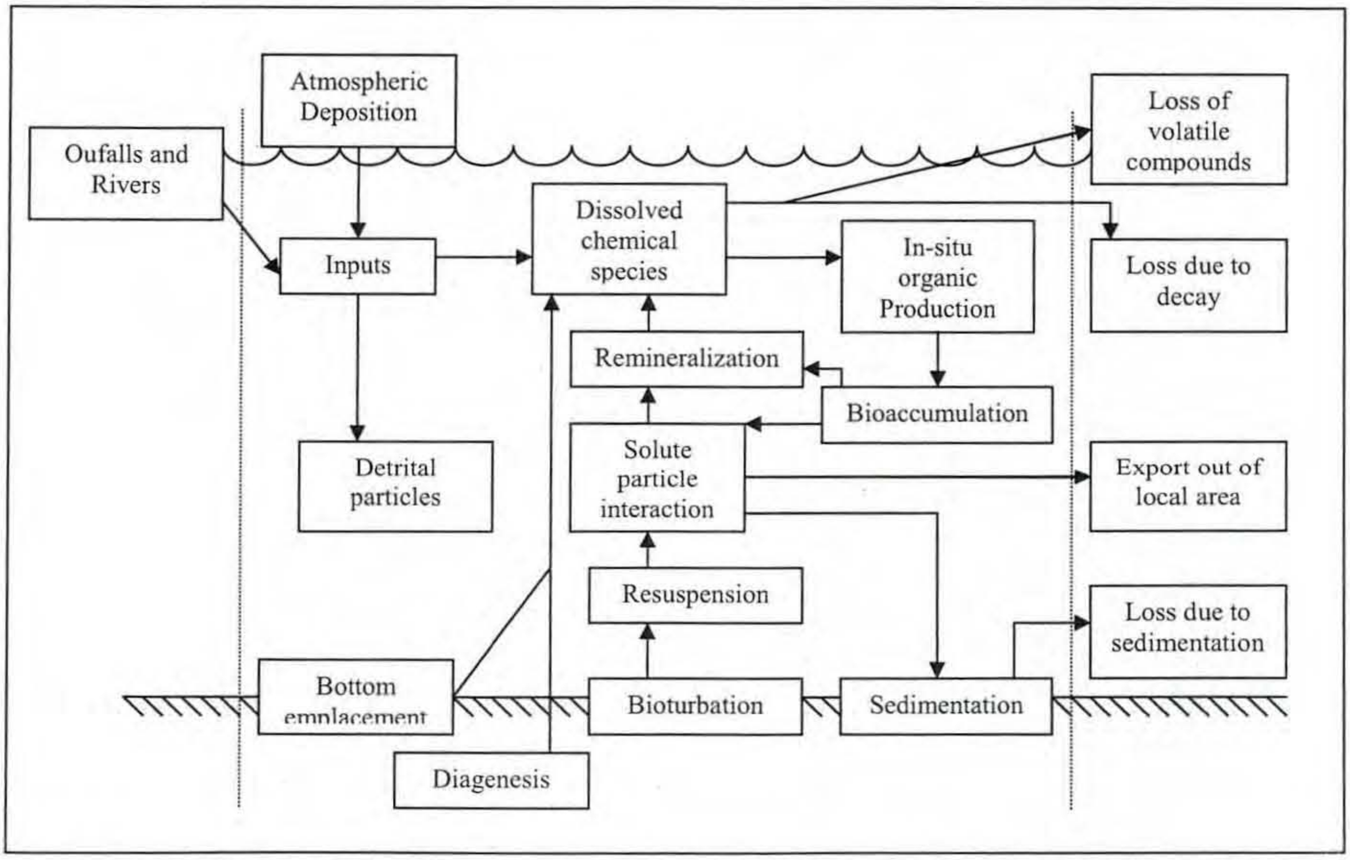


Figure 5: Possible transformation of pollutants in the water column. (Assimilative Capacity of U.S. Coastal Waters for pollutants, 1979.)

Figure 5 summarizes the possible chemical and related processes or transformations in which a pesticide may be involved from its time of introduction until is removed from the system.

3.5 Water quality

Only about a hundred of substances and its derivates are practically analysable. Also the measured concentrations given are the representation of a situation at that time (e.g. hydrological and raining conditions). These concentrations have to be associated with the notion of water flow, indeed then the effect of each slope bed on the pollution in the estuary is known. The notion of concentration allows to evaluate the potential of ecotoxicological risk at this point. Due to the fact that the sampling is performed in the rivers, it would not give the information of the real concentration in the Marennes-Oléron basin. Nevertheless, these concentrations at different stations give an idea of the pesticides feeding.

Table IX: Highest concentration (µg/L) of six of the eight pesticides found in four rivers of Charente-Maritime in 2001. (GRAP Poitou-Charente, 2002)

River Station Date	**Seudre** **n°0502500** **May 2nd**	**Arnoult** **n°05001600** **May 2nd**	**Seugne** **n°05007600** **May 2nd**	**Charente** **n°05006900** **May 9th**
Alachlore	0.06	0.02		0.03
Atrazine	0.24	0.07	0.13	0.07
Diuron	0.32	0.14		
Glyphosate	0.15	0.11		
Métolachlore	0.24	0.07	0.05	0.06
Terbuthylazine	0.43	0.14	0.05	0.02

The highest concentrations of pesticides found in the different rivers are all situated in the start of May, they do not goes over the concentration of one µg/L. In fact the period where the most of spreading take place is the month of April and May, to avoid the development of weed predators at the spring time.

Although in 1997, The Charente slope bed had a consumption of pesticides 18 times bigger than the slope bed of Seudre (Chapter 3.2.3.), in the start of May 2001, the Seudre seems to be more polluated than the Charente. Furthermore, it is interesting to notice that the estuarie of the Seudre is situated at the level of the experimental study site whereas the one of the Charente is situated on the north of the basin of Marennes-Oléron. Thus, the site « Dynamor » is more liable to receive the effluent concentration of Seudre than the one of Charente. The next part will focus on the potential effect of this pesticides for humans and to the marine environment, the flaura and the fauna of the basin of Marennes-Oléron.

3.6 The effect on the environment

3.6.1 Toxicity for humans

It has been estimated to around a million of intoxication per year due to ingestion of pesticides by accident and around 20,000 deaths (Who-Unep, 1989). Most of the time the toxicant is ingested from residues present in the food; but the absorption could be done by drinking water, by inhalating air or by skin contact to the product (Spear, 1991). The farmers and workers who prepare the mixing and perform the spreading are supposed to be more exposed and so to be injured by the product by contact or inhalation.

3.6.2 Effect on the marine environment

Despite two decades of research, the extent and importance of pesticide pollution in estuaries is poorly understood. Laboratory studies of their acute and chronic toxicity indicate that pesticides may be the cause of ill-defined but significant mortality, loss of production, and perhaps, changes in the direction of natural selection in the fauna of estuaries (Butler, Philip A., 1966). Preliminary investigations show the need for a monitoring program to identify the spatiotempoaral distribution of pesticide pollution in estuaries.

A continous study has been achieved by Mason *et al.*, (1999-2002). It was a four year follow up of the potential toxicity of short coastal rivers of the basin of Marennes-Oléron. The toxicity have been determined thanks to the bioassay on the embryonic development of oyster *Crassostrea gigas*. A bibliographic study of the results of the student report allows to do some conclusions.

- There a possible harmful effect of these water effluent, from the agricultural area, to the marine life that could be due to:
  - Fertilisers or its products of degradation.
  - Degradation of the water quality, e.g. eutrophication
- The rainfall plays a important role in the carrying of active substances and has to be taken into account in the discussion of the results.
- By good weather, the volatilisation could act as a transport of xenobiotics to the areas situated at proximity from the agricultural ones.
- The coastal areas are protected from potential pollution when the rainfall is absent.
- For almost each year of study, the maximum of effects observed on the embryo-development of oysters corresponds to samples performed in the start of June.

3.7 The way of the prevention

The aquifere and the slope bed for the surface water must be protected. Thus a simultaneous action is needed so as to treat the problem and the research of solutions. The idea that has to be transmitted, is the one of a better perception of the environment and its constraints, in order to construct an agriculture that respects the environment and the consumers on the one hand, and one of cooperation between all the users of water, which by maintaining the water quality take into account the economical aspects on the other hand.

Thus a label exists for the spreading of nitrates and could be reproduced easily in the case of pesticides, its name is "Ferti-Mieux" (Sobilote, 1999). It is based on a voluntary action of farmers to change their way of practice. That is why the advices given to farmers, aims at changing their mind along the year. Then by increasing the requirements and by doing a regular assesment of the practical modification thanks to a specific methodology, will allow to modify their practice few by few. This operation brings together in the same time the farmers and all the actors of the water quality (administration, public institution and the water office etc.)

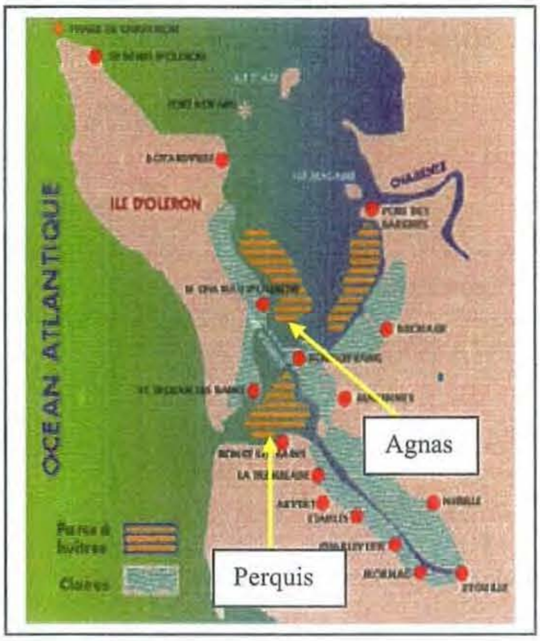
4 ASSESMENT OF TOXICITY USING BIVALVE BIOASSAY

The impact of pesticides on the marine environment can be assessed by monitoring their occurrence in the marine environment and by assessing their toxic effects using *in vitro* bioassays. Acute bioassays have been generally used to set marine water quality criteria, but bioassay techniques now can determine effects of one or more toxicants on the survival of oysters. This part will explain how the bioassay are performed and how the tested solutions are prepared.

4.1 Material and method

4.1.1 Sampling of the tested medium

4.1.1.1 Geographic localisation and date of sampling



Thanks to a "chalan", a flat-bottomed boat or a barge, the sampling could be performed in the middle of the bassin, in the two different sites of sampling. "Dynamor" is the experimental study site for the monitoring network (chapter 2.2.2.) to follow the different parameters so as to assess the effect of the sediment proximity (15 and 70 cm above the sediment) on oysters. This site named also as Perquis is an area where the oysters' mortalities occur often. It is situated on the south of the basin and is dierectly exposed to the effluent of the river Seudre. Indeed, the site dynamor is in the estuarie of this river.

Agnas is another followed site in order to achieve other measures, an area where the mortalities are lower. Agnas is situated between the Oléron's island and the continent. It is on the middle of the basin as far from the estuary of the Charente than from the estuary of the Seudre.

Figure 6: Geographic situation of the basin of Marennes-Oléron: experimental study site "Dynamor" on Perquis and the other followed site Agnas.

It has been planned for the project MOREST, to assess the potential toxicity of sediment and sea-water using bivalve bioassay. Different period of time for the sampling have been planned:

1. Before the start of the oysters' mortalities (14^{th} & 26^{th} of May 2003)
2. During the oysters mortalities (16^{th} & 23^{rd} of June 2003)
3. After the oysters mortalities (9^{th} of September 2003)

4.1.1.2 The sediment

At each sampling period, sediment from two sites, Perquis and Agnas are sampled.

The sediment is sampled a low tidal (coef 70-90) on the two sites under the growing oysters' tables. The first centimeter is sampled and put into a sterile flask. It is preserved at low temperature in a cool box for the transport at 7-8°C.

Figure 7: Sampling of the first cm sediment, Basin of Marennes-Oléron, June 2003.
© IFREMER

4.1.1.3 *The seawater*

The sampling of the sea has to be performed while respecting a few rules; the water cannot be sampled directly on the site. In fact, when tidal is low, it is possible to find some puddle pool of stagnant water; nevertheless it is not interesting to sample it due to the fact that this sea-water does not represent the feeding of the water flow.

The seawater is sampled in poly-propilene flask at 100 m from the tables in the sea-current.. It is preserved at low temperature in a cool box for the transport at 7-8 °C and then put in the fridge in the laboratory.

Figure 8: Sampling of the seawater on the current, Basin of Marennes-Oléron, June 2003.
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4.1.2 The tested medium

4.1.2.1 *The reference seawater: RSW*

The reference seawater used for the experiment is provided by an experimental hatchery Ifremer (station of Argenton). Thus the seawater has a standard quality of oceanic sampling that is filtrated at 1 µm. It is stored in the laboratory at low temperature (15 °C) without any light.The day before the test, the reference sea-water is adjusted to the salinity 30 ‰ thanks to demineralized fresh water. It is then one more time filtrated through a 0.2 µm sterile membrane.

4.1.2.2 *The reference toxic*

In order to check the sensitivity of oysters parents, copper sulfate is used as a reference toxic from 0 to 80 µg/L. The $EC_{50\text{-}24h}$ determined must be 34.88 µg of $CuSO_4$ /Litre (+/- 6.07) (Quiniou *et al.*,1999) to valid the test. All the bioassay performed with this reference toxic comply with that interval.

4.1.2.3 *The sediments*

The two sediments sampled in the basin of Marennes-Oléron have grain sizes that define them between clay and sand. In order to remove broken shells and other big particles, a 2 mm sieve is used. Tests were performed in contact with sediment at concentration of 0.1-0.5-1-5-10 wet grams per litre. During the shacking, the solution is distributed into several 30 ml pots. The dry weight is determined on three replicats dried 48 hours at 60 °C in order to remove all the water from it.

Figure 9: Solution distribution in 30 mL pots.

4.1.2.4 *The seawater*

The seawater water is used in few bioassays with and without filtration. Filtration is used in order to remove suspended matter, and by consequence to remove the pollutants adsorbed on it. It allows thus to obtain as a result the only effect of the seawater on the development of the oysters larvae.

4.1.2.5 *The pesticides*

The pesticides are coming from the same laboratory maker, ANOPOL WARSAW POLAND. Regarding to their physical properties and technical features. Thus the solubility, the coefficient of partition water solvent and the $EC_{50\text{-}96h}$ on algae have been studied to determine the possible concentration feasible for the experiment.

Because of their low hydrosolubility, six of the eight pesticides are preparedin ethanol as solvent for the mother solution. Glyphosate and Fosetyl Al are directly dissolved in demineralized fresh water. All the mother solution are shacked by vortex and passed into a sonificator to increase the dilution to the maximum. Nevertheless, it happens, like e.g. the glyphosate, that the pesticide does not solubilized completly. The range of concentration is obtained by the dilution of the mother solution (pesticides-ethanol) into reference sea-water.
The nominale concentrations tested for a pesticide alone are 0-1-10-100-1,000 µg/L.
Besides, a mixing of the eight pesticides together has been tested at concentrations met in the environment :

- Carbaryl : 0.2 µg/L
- Alachlore, Fosetyl-Al, Glyphosate and Metolachlore : 2 µg/L
- Diuron : 4 µg/L
- Atrazine and Terbuthylazine : 6 µg/L

An undersample of each concentrations tested has been done at the start and the end of the test in order to determine the effective concentration subsequently.

4.1.3 Origin of the parents

Oysters used for assessing the potential toxicity of the seawater and the sediment come from Satmar hatchery (Barfleur, Normandy). They are conditionned to be mature and ready to spaw in the laboratory. While the natural oysters exposed in « Dynamor » growing tables are used to find the potential effect of sediment proximity in Marennes-Oléron oysters farming. Thus, three living oyster livestock samples were provided by the station of la Tremblade:

- Oysters from the natural spat living on 70 cm growing table: $\mathbf{NAT_{70}}$
- Oysters from the natural spat living on 15 cm growing table : $\mathbf{NAT_{15}}$
- Oysters genetically selectionned, considered as resistant and living on 70cm growing table :$\mathbf{TOP_{70}}$

4.1.4 Bioassay, the experimental protocol

4.1.4.1 The reception of the oysters

As soon as oysters' parents arrive in the laboratory, they are put into sea-water to help them to recover from the transport. During two or three hours they can eliminate a major part of their feces. In the thermostatic room, they are brushed to remove the sludge and to excitate it. (Annex II)

4.1.4.2 The emission of the gamets

The oysters spawn is induced thanks to a thermal shock in natural sea-water vats from 15°C to 29 °C as described in Quiniou and Alzieu (1999). Standard protocole (ASTM E 724 – 94) modified.

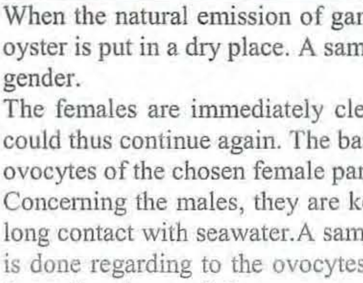

Figure 10: Induction of the oysters spawning in the laboratory by thermal stimulation.

If the natural emission of gamet does not occur after several cycles of thermal schock (3-4 times), the adding of sperm from a sacrified male is done, in order to act as a stimulus. Furthermore, if the emission does not occur yet, oysters are opened and the gonads are stripped so as to recover the gamets.
When the natural emission of gamet has occurred (appearance of a white flow that goes out of the oysters), the oyster is put in a dry place. A sampling of the emission is examined through a microscope (x 400) to indentify its gender.
The females are immediately cleaned to remove eventual sperm and put into a beaker. The natural emission could thus continue again. The bath are changed from 2 to 3 times in order to rinse the shell and to keep only the ovocytes of the chosen female parents. (Annex II)
Concerning the males, they are kept in a dry place to keep their fertilisation power that would be damaged in a long contact with seawater.A sampling is done to choose the females with the most mature ovocytes. The choice is done regarding to the ovocytes quality: the way of the emission (continual flow, fluid, without clusters) and from the shape of the ovocytes (piryform and with a homogeneous contents). The male selection is done regarding to the density and the mobility of the sperm.

4.1.4.3 The fertilisation

After selection of the best female, the ovocytes are filtrated through a 100 µm sieve in order to eliminate deposits and oystersfeces or detrital. The density of ovocytes is determined by the counting on a binocular of 3 sampling of 20 µL. The sperm, after emission by the male in the seawater, are immediately recovered on a 32 µm sieve and filtrated. The fertilisation is performed by adding sperm on the solution of ovocytes, so as to obtain 5 to 10 sperm around each ovocyte. In the 15 following minutes, the fertilized eggs are inoculated in 30 mL tested solution, in order to have around 20,000 eggs/L. One couple is performed by bioassay, but the test is duplicated.

4.1.4.4 Hatching and larvae fixing

The larvae are kept in the dark, during 24 hours at 24 °C (± 1 °C) in a thermostatic cupboard. At the end of the hatching, the larvae are fixed thanks to neutral formol (8 %) with 0.5 mL for 30 mL test medium. (Annex II)

4.1.5 Examination of the results

4.1.5.1 The effect criteria measured on the larvae

Results are expressed in percentage of abnormal D larvae obtained after 24 hours incubated at 24 °C.
Definition of abnormalities: Woelke (1972) states, "normal larvae as referred to here, are those which are fully shelled, even though many may be misshapen or undersized". Woelke himself admitted that he adopted these criteria in order to simplify the evaluation, but his definition greatly diminishes the quality of the bioassay. The abnormalities found by looking at the larvae through the microscope are really different (Quiniou *et al*, 1999) :

- Abnormality of segmentation: Embryo-genesis block.
- Shell abnormality: Hinge of the shell, concave or convex shape.
- Mantle abnormality: hypertrophy of the mantle.

D-larvae (N) | Mantle hypertrophy (M) | Hinge abnormaliy of the shell (C) | Development blocked at embryo step(E)
(Larvae's lenght 50 to 60 µm)
© *Ifremer – Françoise QUINIOU.*

- Size abnormality: The larvae have got a smaller size than the others.

Figure 11: The different abnormalities observed in D-larvae of oyster, *Crassostrea gigas*.

4.1.5.2 Expression of results

After examination through a microscope of 100 larvae by replicat, results are expressed as PBA and PNA:

- PBA (Percentage of abnormal larvae bruto)

$$PBA = \frac{Number.assay.abnormal}{Number.of.total.larvae} \times 100$$

These results could be transformed in Percentage of Abnormal larvae Netto (PNA) thanks to the Abott formula (Anonymous, 1980), so as to compare the results between experiments.

- PNA (Percentage of abnormal larvae netto)

$$PBA = \frac{Number.assay.abnormal - Number.control.abnormal}{100 - Number.control.abnormal} \times 100$$

4.2 Results

4.2.1 Sediments from the basin of Marennes-Oléron

The complexity of all the parameters that play a role in the mortality of oysters in the Basin of Marennes-Oléron does not seem to answer the problems easily. One of them could be the sediment, on which xenobiotics have the particularity yo adsorb on it (chapter 3.4.2.) It is the reason why a specific study was planned to assess their potential toxicity, looking at the spatiotemporal evolution. The effect, or the potential toxicity is defined by the the rate of normal D larvae obtained by the embryonic development in contact with sediment gradient

4.2.1.1 Spatiotemporal evolution of the sediments' potential toxicity

So as to see if there is an interaction between the sediment, its potential action, and the mortality, continuous sampling of sediment were done in May and June. End of June corresponding to the start of mortalities. By the way, in the laboratory sediment contact bioassays were performed to show the effects on the embryonic development of oysters. Graph 12 and 13 give the toxic effect of sediment coming from two sites in the Marennes-Oléron basin at three dates.

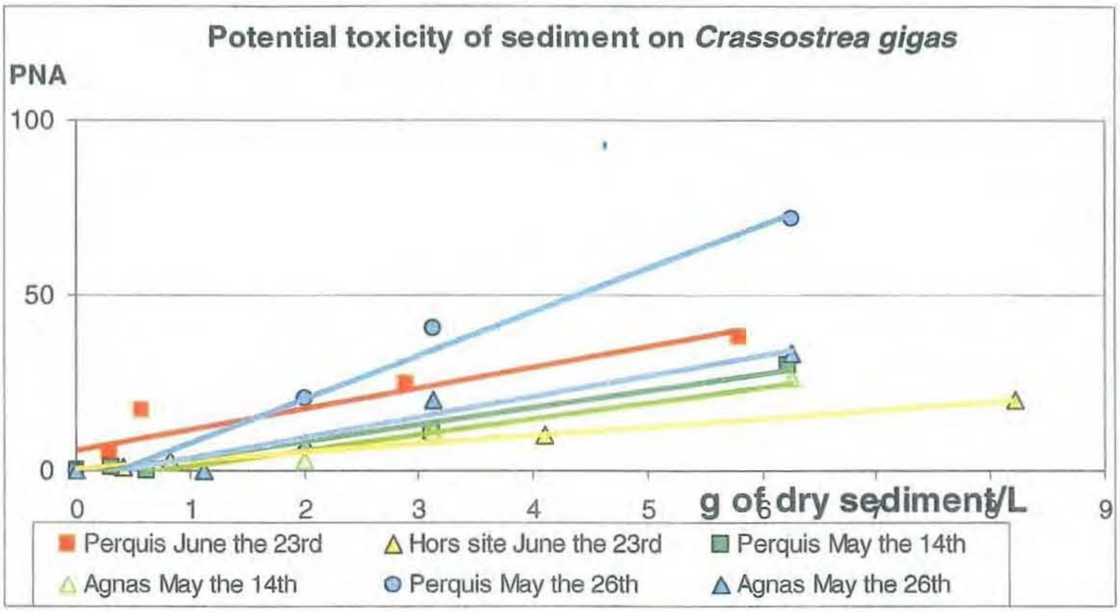

Figure 12: Toxic effect of sediment tested in direct contact on the embryonic development of *Crassostrea gigas*. Sediment sampled in Marennes-Oléron basin (Perquis & Agnas) the 14[th] and 26[th] of May and the 23[rd] of June.

From graph 12, thanks to the equation of the evolution of the potential toxicity for each condition (linear regression), an **index** could be determined: the percentage of mortality for a sediment contact solution with a concentration of 5 dry grams of sediment per litre, concentration recommanded by Quiniou et Alzieu (1999). Actually, *in situ*, it corresponds to the natural average concentration of suspended matter during tidal in the seawater.

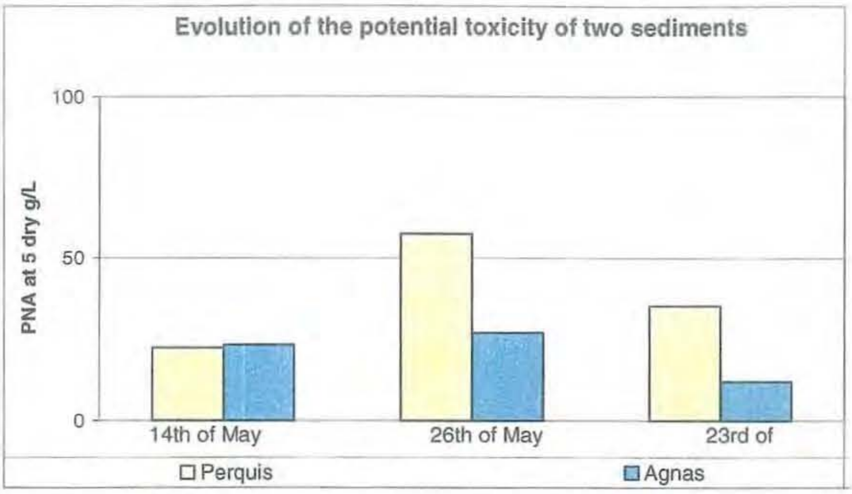

Figure 13: Spatiotemporal evolution of the potential toxicity of the sediment in the Marennes-Oléron basin.

The results show that for a concentration of 5 dry grams of sediment per litre, the two sediments are weakly toxic on the 14th of May (25 % of abnormal D larvae). This toxicity increase with values of more than 50% of abnormal D larvae in the site of Perquis on the 26th of May. In the 23rd of June, the potential toxicity of the site Perquis has decreased but remains potentially more toxic than in the 14th of May (35 % of abnormal D larvae).

On a second hand, the graph 13 which shows the potential toxicity of the sediment gives some new information. Although on the 14th of May, the two sites induced the same effect on the embryonic development of oysters, in the following weeks, whatever the period of time, the sediment from the experimental study site "Dynamor" of Perquis leads to a bigger effect on the embryonic development of oysters. This sediment is potentially more toxic than the sediment of Agnas along June.
Graph 12 show as well the temporal evolution of the potential toxicity of the sediment from Perquis as its determined EC_{50} on embryonic development of oyster.

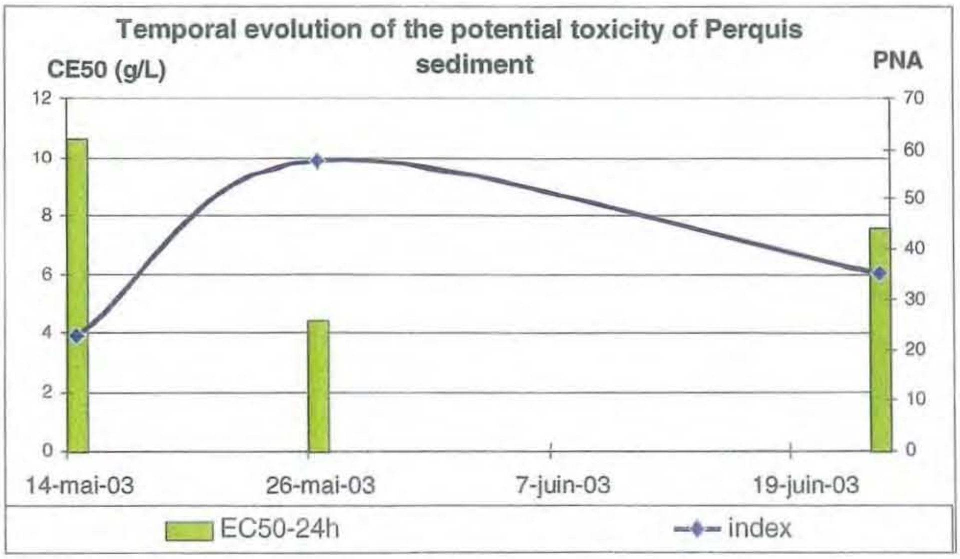

Figure 14: Abnormalities (PNA) observed thanks to the bioassay of embryo-toxicity on oysters Crassostrea gigas, with a solution of sediment from the bassin of Marennes-Oléron (Perquis) sampled at three dates (14th and 26th May & 23rd June). The index corresponds to a concentration of 5 dry g of sediment per litre. The EC_{50} is obtained by linear regression.

From figure 14, it is possible to read in another way the informations. Firstly concerning the temporal evolution of the potential toxicity of the sediment. In the end of May, in less than 15 days, the EC_{50} of the sediment Perquis decreased from 10 to 4 dry gram per litre, the sediment became twice more toxic. Secondly in June its EC_{50} increased but kept lower than in the middle of May. By looking at the index, the same observation is made, the PNA reach its maximum in the end of May following a big increase on the previous week .

4.2.1.2 *Element of the mortality explanation.*

On the Défi MOREST, a continual following of the mortality of the oysters *in situ* is performed on the experimental study site Perquis (see the following graphics of the different livestock mortality per day during a 4 months period).

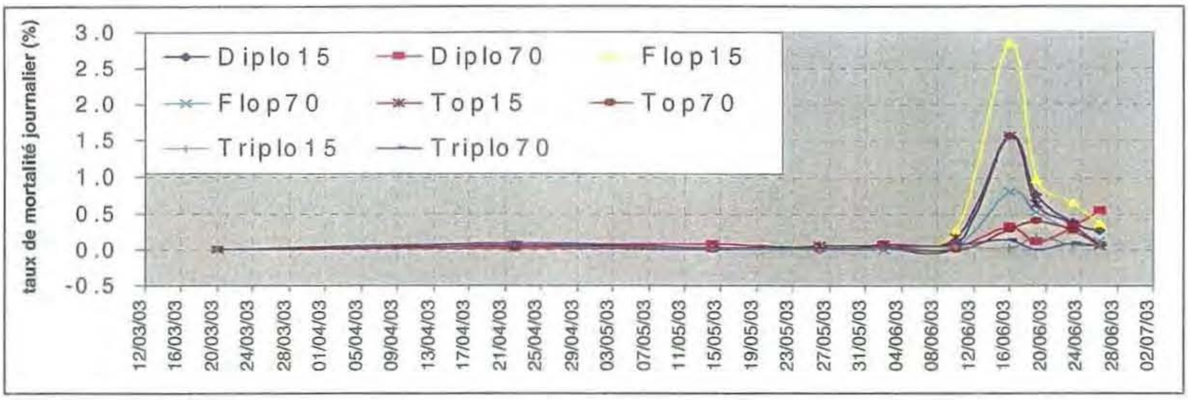

Figure 15 : Representation of the percentage of mortality per day on the period going from 12th March to the 2nd July in the basin of Marennes-Oléron. (4 oysters livestock and 2 environmental conditions). (Soletchnick, personal communication - MOREST data laboratory LCPC La Tremblade, 2003)

Among all the causes that play a role in the mortality of the oyster *Crassostrea gigas*, the toxicity of the sediment could appear as a possible actor of disturbance. In the end of May, a sudden increase of the potential toxicity of the sediment was observed.

The facts are the following:

- During the spraying in April and May (chapter 3.2.2.), thanks to the rain and the runoff, by leaching and lixiviation, the pesticides and the other pollutants are back in the downstream after few weeks, in May and June..
- As described in chapter 3.6., the results of bioassays performed with river waters show that abnormalities are higher in the end of May and the beginning of June, than in April.
- An increase of the potential toxicity of the sediment occurs in the end of May to finally drop in the current month of June (figure 15).
- The maximum of mortality occurs just after the pesticides sprreading, when freshwater and sediment show the maximum potential toxicity.

As soon as the pesticides are adsorbed on the sediments or on suspended matter, they are carried to the estuary. They become bioavailable and will cause trouble to oysters altough they are low bioaccumulable (chapter 3.3.). The energy spent to face pesticides stress during the ripeness may reduce the sum of energy allocated for the growth, the feeding and the pathogens protection.This could induce its death.

Thus without making a direct correlation between the two following events, pesticides pollution in April and the oysters mortality in June, it could be supposed that the sediment in suspension that may adsorbed a lot of xenobiotics from the upstream, is a potential source of toxicity for oysters and be a possible cause of oyster mortalities in the Marennes-Oléron basin.

4.2.2 *In situ* oysters from the bassin of Marennes-Oléron.

On this experiment, the principal objective is to observe the difference in offspring quality from oysters production growing at 15 and 70 cm above the sediment of the Marennes-Oléron basin. The effect is determined by its capacity to produce normal D larvae by the measurement of their sensitivity to $CuSO_4$. Thus, three living oyster livestock samples are tested: NAT_{15}, NAT_{70} and TOP_{70} (chapter 3.3.)

With the results, three parameters will try to explain the difference of effect, the proximity of the sediment on oysters and then the livestock feature. These parameters are:

- The capacity of oysters to produce normal D larvae in reference seawater.
- The sensitivity to $CuSO_4$ to the embryonic development.
- The difference of EC_{50} between the two oysters living conditions or two livestock.

The tests with gradient concentration of $CuSO_4$ on the embryonic development are performed either using a fertilisation on different couples of oysters, or by testing the effect on pools of few males and females.

4.2.2.1 Effect of the sediment proximity on the capacity of oysters to produce normal D larvae.

The year 2003 being a particulary warm year for the seawater, a lot of the oysters intend to the bioassays have started to spaw before the experiment week. Thus the bioassay performed on oysters for the comparison NAT_{15} and NAT_{70} is only done on pools of a small number of animals. For the performance of the pools, the following number of parents are:

- NAT_{15} livestock (2 females and 3 males)
- NAT_{70} livestock (2 females and 2 males)

Pools are the result of the *in vitro* fertilisation of gametes provided by a few males and females. The interest of pools is to compare, for the two environmental living condition, not only the response of larvae fertlised by one couple, but the larvae produced by a few males and females. It corresponds to the closest response of what happens really *in situ*.

Figure 14 is a representation of the larval sensitivity to the reference toxic, $CuSO_4$. It will allow comparing the offspring quality of each livestock.

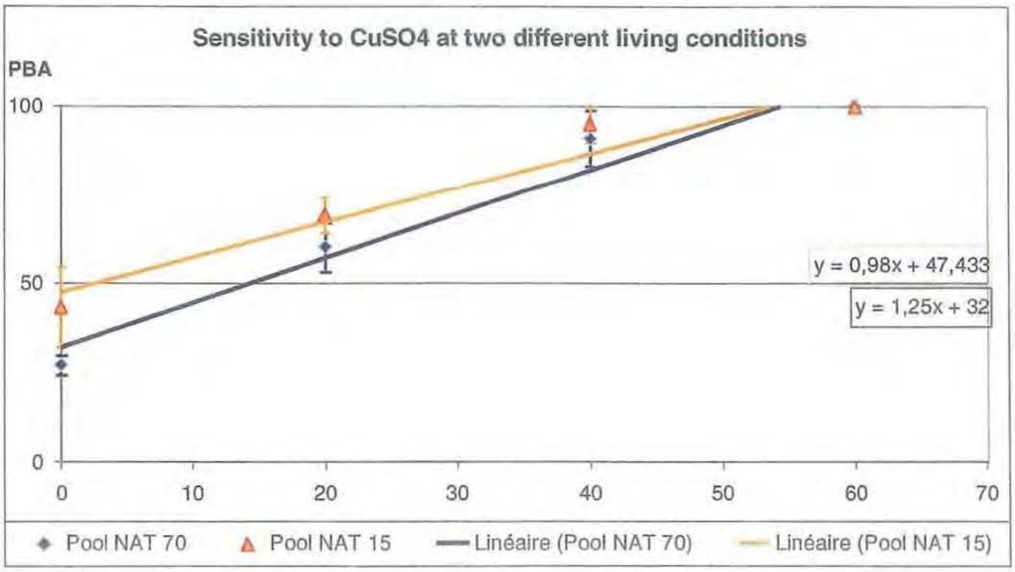

Figure 16: Representation of the sensitivity of larvae to $CuSO_4$ Bioassays on the oyster embryonic development larvae *Crassostrea gigas* provided from the *in situ* (Marennes-Oléron basin). Livestock of 18 months old is studied: NAT: oysters from the natural spat, diploidic. Two environmental conditions are represented: 15 and 70 cm above the sediment. Results expressed in PBA.

From the previous graphic and thanks to the equation of the response on the sensitivity of larvae to a gradient of $CuSO_4$ concentration, the EC_{50} are determined. They are shown in the following table.

Table X: Response of the control and EC_{50} (µg $CuSO_4$ /L) obtained thanks to the bio-assay of embryo-toxicity on oyster *Crassostrea gigas*, from Marennes-Oléron (Perquis) sampled on the 15th of July 2003.

Living conditions	*Pool NAT15*	*Pool NAT70*
EC_{50-24h} (µg $CuSO_4$/L)	2.62	14.4
PBA in the RSW	43	27
% normal D larvae	57	73

First, the interesting parameter that could be focused on is the capacity of the oysters to produce normal D-larvae in reference seawater. The livestock NAT_{15} is only able to produce 57 % of normal D-larvae whereas the livestock NAT_{70} produces 73 %.

The response to $CuSO_4$, the second parameter observed, does not show big difference of behaviour, apart from the fact that when the concentration increases, the deviation between the two conditions has a tendency to be reduced.
The EC_{50-24h} for the pool NAT $_{70}$ is 14.4 µg $CuSO_4$/L whereas the EC_{50} for the pool NAT_{15} is 2.6 µg $CuSO_4$ /L. By looking at the EC_{50} of the two livestock to $CuSO_4$, it shows that the livestock living on the growing tables 70 cm above the sediment is less sensitive than the one at 15 cm above the sediment. Nevertheless, the two livestock have a EC_{50} largely lower than a normal response of oyster to $CuSO_4$ (see chapter 4.3.3.)

To conclude, by looking at the three parameters studied: the closest the oysters are to the sediment, the most sensitive to $CuSO_4$ are their D larvae. This result again seems to underline the fact that the sediment is potentially toxic. By conclusion the proximity of the organisms to the sediment plays a role on the quality of gamet and on the capacity of oysters to produce normal D larvae.

4.2.2.2 Comparison of the capacity of oysters NAT and TOP to produce normal D larvae.

For this comparison, the best choice is to compare the results from the couples NAT_{70} and TOP_{70}. They have both grown on a table at 70 cm above the sediment. During the performing of the experiment, the livestock TOP was already ripe for a while, so that the oysters had almost all spawn. This induces that only one female and three males were used. Thus, it has not been possible to performed pools as it had been done with the NAT livestock. That is why in this comparison, the response of embryonic development to $CuSO_4$ is made on couples, the PBA is the average of the whole response of the couples for each livestock.

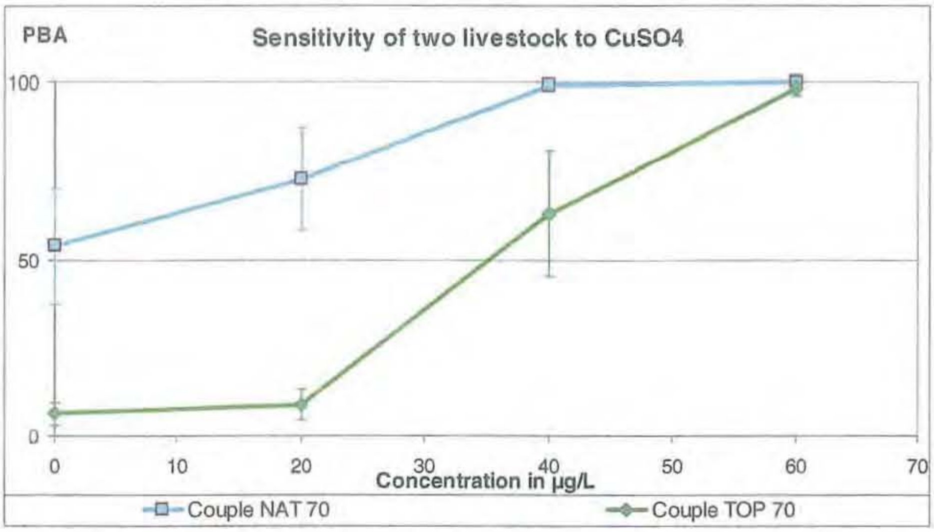

Figure 17: Representation of the sensitivity of larvae to $CuSO_4$. Bioassays on the oyster embryonic development larvae *Crassostrea gigas* growing on "Dynamor" experimental study site (Marennes-Oléron Basin). Livestock of 18 months old is studied: NAT: oysters from natural spat, diploidic. TOP: oysters genetically selected, considered as resistant. Results expressed as PBA.

From the previous graphic and thanks to the data of the response on the sensitivity of larvae to a gradient of $CuSO_4$ concentration, the EC_{50} were determined using the probit method (Software EPA Probit Analysis Program-version 1.5).

Table XI: Response of the control and EC_{50} (µg $CuSO_4$/L) obtained thanks to the bioassay of embryo-toxicity on oysters *Crassostrea gigas*, from Marennes-Oléron (Perquis) sampled in 15th July 2003.

Living conditions	*Couple TOP_{70}*		*Pool NAT_{70}*	
EC_{50} (µg $CuSO_4$/L)	38	[34.8-40.1]	-	
PBA in the RSW	6	[3-9]	54	[48-60]
% of normal D larvae	94	[91-97]	46	[40-52]

Regarding to the percentage of normal D larvae, the livestock TOP is able to produce in reference seawater 94 % of D larvae whereas the livestock NAT only produced 46 % of it.
On a second hand, the behaviour of the response to $CuSO_4$ is completly different between the two livestock. Indeed, the TOP response is almost stable up to a concentration of 20 µg/L whereas at that same concentration, the NAT response has started to increase. Furthermore, the threshold of the whole abnormal effect is reached only at 80 µg/L for the TOP, whereas for the NAT this level is obtained at a concentration much lower, 60µg/L.
Finally the EC_{50} for the couple TOP 70 is 38 µg $CuSO_4$ /L which corresponds to a normal sensivity to $CuSO_4$ (EC_{50} = 34.88 µg of $CuSO_4$ per litre (+/- 6.07) (Quiniou *et al.*,1999). By contrary, the EC_{50} for the couple NAT is not possible to determine due to the fact that the control itself goes over the threshold of 50% of abnormality.
On conclusion, the response to $CuSO_4$ of TOP is comparable to a waited response, obtained with ripe oysters from hatchery. On contrary, NAT are really sensitive to $CuSO_4$ and a fertilisation in reference seawater does provide less than 50 % of efficiency.
TOP are more resistant to $CuSO_4$ than oysters from the natural spat and the effects on the embryo-larvae development check the hypothesis advanced in chapter 2.2.1., the model of mortality gradient. Finally, in a particular environment TOP will produce more normal D larvae than NAT. This implies that TOP is less sensitive to environmental features. Experimentally, it is confirmed, 5% of the total livestock TOP70 is died whereas 10% of NAT70 is died (see annex 3). (Soletchnick, personal communication - Data MOREST)

4.2.3 Effect of pesticides on oysters.

In the previous part, two main fields of research have been focused on. At first, the potential toxicity of the sediments on the embryonic development of oyster has been demonstrated. This has induced on a second part to see if the oysters from the experimental study site "Dynamor", in permanent contact with suspended sediment, were reached by contaminants: a study of their capacity to produce viable offspring, and response of the embryonic development in a toxicant reference solution. At least, the last experiment turned on the study effect of several pesticides on *Crassostrea gigas* bioassays.
The eight chosen pesticides studied in the chapter 3 were the most observed substances in the rivers of Charente-Maritime. That is why, in this experiment, although the effect-response of each pesticide on oysters' larvae was studied, the effect of a mixing of all of those pesticides is also an axis of research. Therefore, a mixing of the eight pesticides together has been tested at concentrations met in the environment :

- Carbaryl : 0.2 µg/L
- Alachlore, Fosétyl-Al, Glyphosate and Métolachlore : 2 µg/L
- Diuron : 4 µg/L
- Atrazine and Terbuthylazine : 6 µg/L

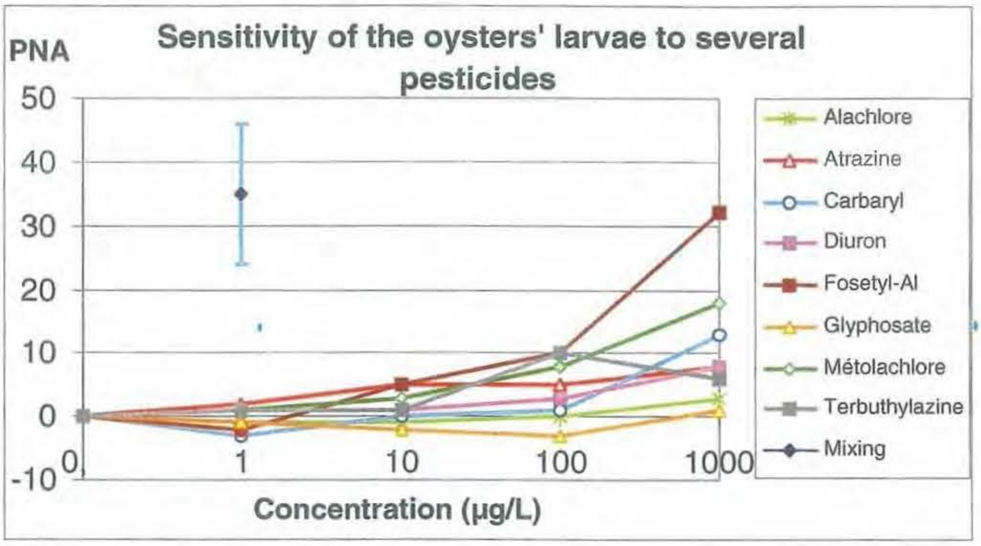

Figure 18: Representation of the sensitivity of larvae to pesticides concentration gradient. Bioassays on the oyster embryonic development *Crassostrea gigas*. The mixing is composed of the eight pesticides at different concentration: terbuthylazine and atrazine (6 µg/l), diuron (4µg/l), metolachlore (0.2µg/l), the others (2µg/l). Nominal concentrations. Results expresses as PNA.

It has to be noticed that all the concentrations which appear in the previous graphics are nominal concentrations. Only a quantity determination will allow later on, to know the real concentrations tested.

4.2.3.1 Toxic effect of the eight pesticides

The results obtained thanks to the bioassay of the embryonic development could be compared with the bibliography datas on the $EC_{50\text{-}48h}$ of those same pesticides on the fresh water crustacean *Daphnia magna*.

Table XII: Comparison for the eight pesticides studied of the $CE_{50\text{-}48h}$ *Daphnia magna* and the PNA at 1 mg/L obtained thanks to the bioassay of the embryonic development of the oysters' larvae *Crassostrea gigas*.

	$EC_{50\text{-}48h}$ *D.m.* (mg/L)	$EC_{50\text{-}24h}$ *C.g.*. (mg/L)	PNA on C.g. at 1mg/L
Alachlore	0.05	>1	8
Atrazine	3.6	>1	3
Carbaryl	0.0064	>1	13
Diuron	1.4	>1	8
Fosetyl-Al	37	>1	35
Glyphosate	40	>1	1
Métolachlore	25.1	>1	18
Terbuthylazine	-	>1	6

The PNA observed in graph 18 show that at 1 μg/L, few substances as Alachlore, Carbaryl, Fosetyl-Al and Glyphosate, have more D-larvae than in the control group. At a concentration of 10 μg/L, Atrazine, Fosetyl-Al and Métolachlore induce the most of effect (5 % of abnormal D larvae) among all the pesticides. At 100 μg/L, it exists a start of significant effect, 10 % of abnormal larvae, for two pesticides, Fosetyl-Al and terbuthylazine. At 1,000 μg/L, three pesticides are noticeable, Carbaryl and Métolachlore with around 15 % of abnormal D larvae and Fosetyl-Al with a higher effect of 35 % of abnormal D-Larvae.
Table XII makes a comparison of the EC_{50} on the crustacean *Daphnia magna* and the EC_{50} of *Crassostrea gigas*. The EC_{50} measured on *Daphnia magna* are upper than one miligram of pesticides per litre except for Alachlore (50 μg/L) and Carbaryl (6.4 μg/L).
On conclusion, only three of the eight pesticides induce a toxic effect at the highest concentration tested: Carbaryl (13 %), Metolachlore (18 %) and Fosetyl-Al (32%) at 1 mg/L Not any effect is observed for each pesticides at concentrations met in the environment, these concentrations are all less than 6 μg/L.

Finally, after being adsorbed on sediment or on suspended matter, pesticides are carried in the run-off, the concentrations go down due to the dilution in the estuary area. Either it is transformed in another underproducts or it is bioaccumulated.

Thus not any conclusion could be drawn of the only effect of each pesticide on the embryonic development, moreover as the concentrations of 1 mg/L of a pesticides has never been measured in the environment (see table 8, chapter 3.2.). It is obvious that is more interesting to focus on the effect of the mixing of pesticides.

4.2.3.2 Toxic effect of the mixing of pesticides.

The interest of this experiment is to measured the effect of a soap of pesticides: this will allow the best representation of what happens in the environment. In fact pesticides and others pollutants are never back in the river alone, their behaviour and action depend of the other xenobiotics that coexist with them in the aquatic environment.

Figure 18 show that the mixing of pesticides induce 35 % of abnormalities, whereas the concentrations of each pesticides are all lower than 6 μg/L. This percentage of abnormalities corresponds to the same rate of abnormality induce by Fosetyl-Al tested alone at a concentration of 1 mg/L, whereas in the mixing, its concentration is only 2 μg/L.

Regarding to the effect of each pesticide tested alone at the concentrations used in the mixing, there is not any effect observed, thus there is a synergism of the pesticides when there are together in solution.

In other words, the addition of the substances could act as a pollution for the oyster, there is a synergism between the different pesticides, and even at low concentration, it could affect the oysters and their offspring. Although, pesticides leads to effect on oysters, the concentration determination of it on the water and the sediment in the basin of Marennes-Oléron is not yet done. So it is thus not possible to conclude on the direct responsibility of those xenobiotics to the oysters' mortality. Analysis of the sediment and the seawater have to be performed, to confirm or not the presence of pesticides.

5 REMEDIATIONS

The pesticides are toxic substances that present more risks when they are not used with care, not only for the environment but also for the user. The respect of few rules contributes to reducing their impact on the environment

1. Have a good reading of the label and the indications for the pesticides' use. The label is really important and provides all the information necessary for the environment protection.
2. To know the possible impact of pesticides on fauna, fish, birds, and in the flora of rivers and lakes.
3. To lower the dispersion of pesticides and to respect the designated buffer areas.
4. To avoid the mixing and the manipulation of pesticides close to the water point.
5. To not clean equipment used for the applications of pesticides near wells or water points.
6. To clean the small areas where pesticides were accidentally discharged.
7. To ensure a non-delivery mechanism installed on the pump that takes the water directly on the device used for the mixing or the application of pesticides.
8. To choose a pesticide or a preparation that presents the least risk as possible for fish, crustacean and animals, especially when the application should take place near a habitat.
9. To choose a pesticide or a preparation that presents the least risk to be leached to the aquifer.

In that respect, a Best Management Practice (BMP's) like those previous rules must be designed to remediate diffuse pollution in agricultural storm events. Not only must total storm pesticides loading to aquatic ecosystems be reduced, but also the transient pesticides peaks occurring within the rain storm.

Until now, in the rural environment the ban of chlorinated hydrocarbons for use as agricultural pesticides and their replacement by products that are degradable in the soil has been a major improvement. However, in France the list of the authorised products is composed of no more than 900 pesticides or its derivatives, whereas in comparison, in the Netherlands this same list of authorised products does not go over the threshold of 250 pesticides.

New way of thinking have to be communicated as it was the case with the exemple of "ferti mieux" for nitrates and phosphates. The organic farming should be prefered than the intensive agriculture and the farmer have to be more conscient of the impact to the environment of the active substances that they use

6 CONCLUSIONS AND RECOMMENDATIONS

The implementation of a continuous follow-up of the toxicity of the rivers from the north of the Marennes-Oléron basin has allowed understanding one of the sources and the frequency of pollution. It has shown that during the past four years there has been a tendency of an increase of the potential toxicity of water-rivers in the end of May and the beginning of June. The bioassay performed thanks to the oyster *Crassostrea gigas* confirm it. On 11^{th} of June 2001, the points of sampled site Brouage, Montportail and Beaugeay localised on small canals which discharge directly in the basin of Marennes-Oléron, were toxic. Indeed, the sampling of river water leads to 100% of abnormality for those three sites. It is interesting to see that there is a source of potential toxicity coming from those small canals, moreover that those waters have an effect on the oysters embryonic development. The oyster farmers must be informed that when the rain is important, there is risk of carrying of xenobiotics to their oyster parks by water influent or due to atmospheric transport by good weather.

In the study it has been demonstrated that the sediment plays a role in oysters mortality. During its carrying from the rivers to the coastline, the sediment has adsorbed xenobiotics and arrives in the Marennes-Oléron basin near the oyster farming site. Among the experiment performed in the laboratory, it has been shown that the sediment reveals a potential toxicity which increases in the end of May, 57.5 % of abnormality on the bioassay of oyster development. The following month corresponds to the beginning of mortality. Thus the stress induced by the pollutants on the oysters just before the beginning of the reproduction period, draws on its energetic reserve. Then the least xenobiotics added by the sediment in suspension during tide make theoyster becoming more and more sensitive inducing death.

The fundamental objective of the study was to perform a comparison of the behaviour of oysters living in two different environments and from two different livestock. It has been demonstrated that the sensitivity of the oysters from two different livestock is not equal. Genetic parameters have to be taken into account. Thus, in order to help oyster farmers to fight against mortality, livestock more resistant could be introduced so as to defend themselves from pollution. Like this the farmers would have less losses, but the bioaccumulation of pollutants into oysters would still have to be followed for the protection of the consumers.

Finally, it has been shown that the oysters living on growing tables at 15 cm above the sediment are more sensitive when the sediment is in suspension, than the oysters at 70 cm above the sediment. Furthermore, the follow-up of the mortality on the experimental study site "Dynamor" has shown the fact that the mortality is higher on growing tables at 15 cm above the sediment. It is obvious that the oyster farmers have to be informed of that fact so as to reduce their losses. The sediment is a source of pollution, carries xenobiotics due to adsorption but could especially remove it to the environment.

One of the possible xenobiotics that could influence the mortality of oysters are pesticides. The period of maximale spreading, from March to May for almost all the substances, corresponds to the problem of pollution encountered by the oyster farmers and indeed the mortality appears in June. Although, the concentrations found downstream in the rivers have been diluted since their spreading, 3,200 Kg used in the department per year, it has been analysed in laboratory concentrations of pesticides not going up to 0.43 µg/L. At this concentration, the impact of the eight pesticides tested alone on *Crassostrea gigas* is not harmful. The problem is the addition of substances in the same environment which leads to a synergism of the effect. Thus on the oysters bioassay performed with a mixing of pesticides, 35 % of abnormality is observed for concentrations of pesticides that looks like of the reality of the rivers environment. This seems to be upsetting, because 142 active substances as pesticides were found in the rivers of Charente-Maritime in 1997. Furthermore, in October 2003 l'IFEN published its annual report concerning the observation of the rivers in France and declared that 73 % of the followed rivers are contaminated by pesticides. The European Union has just decided to forbid the sales of 250 molecules in the coming years, but still 900 of them are used in France in the agriculture and by others activities. Actions like the Best Management practices and the respect of the elementary rules of pesticides manipulation could allow a reduction of that pollution.

The problem of the coexistence of the agriculture and the oyster farming in the same area has allowed to alarm the principal socio-economic actors of the department of Charente-Maritime. The implementation of the challenge MOREST is the first step that will allow finding answers concerning the problem of oysters mortality. It is to be expected now that it will be followed by regulations and actions for all the farmers of the coastline area.

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8 ABBREVIATION LIST

CNRS = Centre National de la Recherche Scientifique

DDE = Direction Départementale de l'Equipement

DEL-PC = Département Environnement Littoral – Polluants Chimiques

DYNAMOR = DYNamique de MORtalité : Mortality dynamic

IFREMER = Institut Français de Recherche pour l'Exploitation de la MER

IFEN = Institut Français de l'Environnement

INRA = Institut National de la Recherche Agronomique

PAC = Common Agricultural Politic

PBA = Percentage of Abnormality Bruto

PNA = Percentage of Abnormality Netto

MOREST = MORtalité ESTivale : Summer MORtality

RNO = Réseau National d'Observation

SATMAR = Société Atlantique de MARiculture

SNCF = Société Nationale des Chemins de Fer

SRPV = Service Régional de la Protection des Végétaux

ANNEXES

ANNEX 1 : Physical, chemical and ecotoxicological parameters and of the studied pesticides.

Alachlore		**Références**
Biologic activity	herbicide	Agritox INRA
CAS number	15972-60-8	Agritox INRA
Formula	$C_{14}H_{20}ClNO_2$	Agritox INRA
Synonymes	sevin	Agritox INRA
Molecular mass	269.77 g.mol^{-1}	Agritox INRA
Physical and chemical parameters		
Boiling point		
K_{oc}	170 cm^3/g	Andral, 1996
Henry's constant		PM 96
Hydrosolubility at 20°C	240 mg/L	Andral, 1996
Coefficient of partition K_{ow}	1.59	Agritox INRA
Half life	16.5 Days at 25 °C pH = 7	Agritox INRA
Being in the environment		
Spreading period	March to May	FREDON Poitou-Charentes
Concentration max find	0.76 µg/L (05/01, le Né)	FREDON Poitou-Charentes
Ecotoxicology		
CE_{50} *Daphnia magna* 48H	6.4 µg/l	Agritox INRA
CE_{b50} : *Selenastrum capricornutum*	0.06 mg/l	Agritox INRA
CE_{b50} : *Scenedesmus subspicatus*	0.4 mg/l	Agritox INRA
Acceptable daily intake	0.008mg/kg/d	OMS

Atrazine		**Sources**
Biologic activity	herbicide	Novartis Agro SA
CAS number	1912-24-9	Novartis Agro SA
Formula	$C_8H_{14}ClN_5$	Novartis Agro SA
Synonymes		Novartis Agro SA
Molecular mass	215.69 g.mol^{-1}	Novartis Agro SA
Physical and chemical parameters		
Boiling point		
K_{oc}	100 cm^3.g^{-1}	Andral, 1996
Henry's constant	2.6 10^{-4} Pa.m^3.mol^{-1}	Ciba Geigy AG
Hydrosolubility at 20°C	33 mg/l	Ciba Geigy AG
Coefficient of partition K_{ow}	2.75	Ciba Geigy AG
Half life	86 days at pH=5	Ciba Geigy AG
Being in the environment		
Spreading period	Forbidden since 97	FREDON Poitou-Charentes
Concentration max find	0.64 µg/L (05/01, le Né)	FREDON Poitou-Charentes
Ecotoxicology		
CE_{50} *Daphnia magna* 48H	6.9 mg/l exp : 48 h	Ciba Geigy AG
CE_{b50} : *Selenastrum capricornutum*	0.13 mg/l exp : 96H	Ciba Geigy AG
CE_{b50} : *Scenedesmus subspicatus*	0.043 mg/l exp : 72 h	Ciba Geigy AG
Acceptable daily intake	0.04mg/kg/d	Commission des Toxiques

ANNEX 1 : Continuation

Carbaryl		**Sources**
Biologic activity	insecticide	Rhone Poulenc Agrochimie
CAS number	63-25-2	Rhone Poulenc Agrochimie
Formula	$C_{12} H_{11} N O_2$	Rhone Poulenc Agrochimie
Synonymes	Cristalised solid	Rhone Poulenc Agrochimie
Molecular mass	201.23	Rhone Poulenc Agrochimie
Physical and chemical parameters		
Boiling point	142°C	Sevin
K_{oc}	300 cm^3/g	PM 96
Henry's constant		
Hydrosolubility at 20°C	40 mg/L	Rhone Poulenc Agrochimie
Coefficient of partition K_{ow}	1.59	Rhone Poulenc Agrochimie
Half life	16.5 days at 25 °C and pH 7	Rhone Poulenc Agrochimie
Being in the environment		
Spreading period	April and May	FREDON Poitou-Charentes
Concentration max find		
Ecotoxicology		
CE_{50} *Daphnia magna* 48H	5.6 µg/l	Rhone Poulenc Agrochimie
CE_{b50} : *Selenastrum capricornutum*	0.008mg/kg/j	Comission des toxiques
CE_{b50} : *Scenedesmus subspicatus*		
Acceptable daily intake		

Diuron		**Sources**
Biologic activity	herbicide	Du Pont De Nemours France
CAS number	330-54-1	Du Pont De Nemours France
Formula	$C_9H_{10}Cl_2N_2O$	Du Pont De Nemours France
Synonymes	Cristalised solid	
Molecular mass	233.1 $g.mol^{-1}$	Du Pont De Nemours France
Physical and chemical parameters		
Boiling point	180-190°C	Krovar*I
K_{oc}	480 cm^3/g	Andral, 1996
Henry's constant	$5.1 * 10^{-5}$ $Pa.m^3.mol^{-1}$	Andral, 1996
Hydrosolubility at 20°C	42 mg/l	Andral, 1996
Coefficient of partition K_{ow}	Log P = 2.85	Du Pont De Nemours France
Half life	>500 days stable at pH from 5 to 9	Du Pont De Nemours France
Being in the environment		
Spreading period	January to July	FREDON Poitou-Charentes
Concentration max find	0.66 µg/L (05/01, Charente aval)	FREDON Poitou-Charentes
Ecotoxicology		
CE_{50} *Daphnia magna* 48H	1.4 mg/l exp : 48 h	Du Pont De Nemours France
CE_{b50} : *Selenastrum capricornutum*		
CE_{b50} : *Scenedesmus subspicatus*	3.3 µg/l exp : 96 h	Du Pont De Nemours France
Acceptable daily intake	0.0015 mg/kg/d	Commission des Toxiques

ANNEX 1 : Continuation

Glyphosate		**Sources**
Biologic activity	herbicide	Agritox INRA (UE)
CAS number	1071-83-6	Agritox INRA (UE)
Formula	$C_3 H_8 N O_5 P$	Agritox INRA (UE)
Synonymes		
Molecular mass	169.08 g.mol^{-1}	Agritox INRA (UE)
Physical and chemical parameters		
Boiling point		
K_{oc}	24000 cm^3/g	Agritox INRA (UE)
Henry's constant		
Hydrosolubility at 20°C	10.5 g/l au pH de 2	Agritox INRA (UE)
Coefficient of partition K_{ow}	3.2	Agritox INRA (UE)
Half life	**>30 jours au pH de 5 à 9**	Agritox INRA (UE)
Being in the environment		Agritox INRA (UE)
Spreading period	March to december	FREDON Poitou-Charentes
Concentration max find		FREDON Poitou-Charentes
Ecotoxicology	**0.65 µg/L (05/01, le Né)**	
CE_{50} *Daphnia magna* 48H	37 mg/l	Agritox INRA
CE_{b50} : *Selenastrum capricornutum*	0.64 mg/l	Agritox INRA
CE_{b50} : *Scenedesmus subspicatus*	0.4 mg/l	Agritox INRA
Acceptable daily intake	0.3mg/kg/d	UE

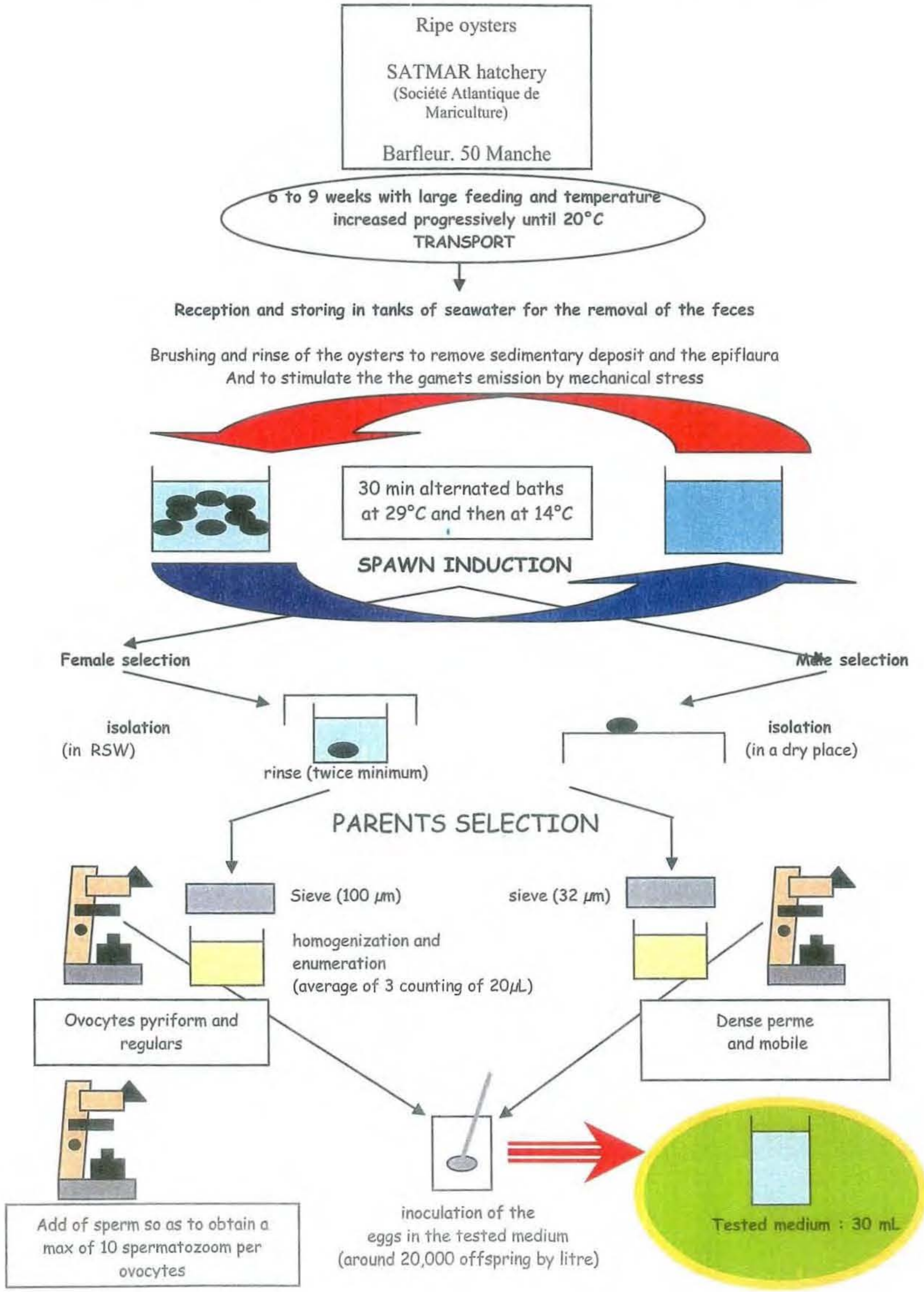
fosetyl-al		**Sources**
Biologic activity	fungicide	Agritox INRA (Rhône poulenc)
CAS number	39148-24-8	Agritox INRA (Rhône poulenc)
Formula	$C_6 H_{18} Al O_9 P_3$	Agritox INRA (Rhône poulenc)
Synonymes		
Molecular mass	354.1 g.mol^{-1}	Agritox INRA (Rhône poulenc)
Physical and chemical parameters		
Boiling point		
K_{oc}	20 cm^3/g	Agritox INRA (Rhône poulenc)
Henry's constant		
Hydrosolubility at 20°C	109.8 g/l at pH 8.6	Agritox INRA (Rhône poulenc)
Coefficient of partition K_{ow}	2.11 à 22 °C at pH 6	Agritox INRA (Rhône poulenc)
Half life	>30 days at pH from 5 to 9	
Being in the environment		Agritox INRA (Rhône poulenc)
Spreading period	February to september	Agritox INRA (Rhône poulenc)
Concentration max find		
Ecotoxicology		FREDON Poitou-Charentes
CE_{50} *Daphnia magna* 48H	40 mg/l	Agritox INRA
CE_{b50} : *Selenastrum capricornutum*	0.64 mg/l	Agritox INRA
CE_{b50} : *Scenedesmus subspicatus*	0.4 mg/l	Agritox INRA
Acceptable daily intake	2.98mg/kg/j	Comission des toxiques

ANNEX 1 : Continuation

Métolachlore		**Sources**
Biologic activity	Herbicide	Agritox INRA
CAS number	51218-45-2	Agritox INRA
Formula	$C_{15}H_{22}ClNO_2$	Agritox INRA
Synonymes	metetilachlore	Agritox INRA
Molecular mass	283.8 g.mol^{-1}	Agritox INRA
Physical and chemical parameters		
Boiling point		
K_{oc}	1700 cm^3/g	
Henry's constant	9.1* 10^{-4} Pa.m^3.mol^{-1}	Andral,1996
Hydrosolubility at 20°C	530 mg/L	
Coefficient of partition K_{ow}	3.45	Andral,1996
Half life	90 days	Andral,1996
Being in the environment		
Spreading period	March to june	FREDON Poitou-Charentes
Concentration max find	0.64 µg/L (05/01, la Seugne)	FREDON Poitou-Charentes
Ecotoxicology		
CE_{50} *Daphnia magna* 48H	25.1 mg/l	Agritox INRA
CE_{b50} : *Selenastrum capricornutum*	0.06 mg/l	Agritox INRA
CE_{b50} : *Scenedesmus subspicatus*	0.4 mg/l	Agritox INRA
Acceptable daily intake	0.026 mg/kg/d	OMS

Terbuthylazine		**Sources**
Biologic activity	herbicide	Ciba Geigy AG
CAS number	5915-41-3	Ciba Geigy AG
Formula	$C_9H_{16}ClN_5$	Ciba Geigy AG
Synonymes		
Molecular mass	229.72 g.mol^{-1}	Ciba Geigy AG
Physical and chemical parameters		
Boiling point		
K_{oc}	828 cm^3/g	Ciba Geigy AG
Henry's constant	4.1* 10^{-3} Pa.m^3.mol^{-1}	Andral, 1996
Hydrosolubility at 20°C	8.5 mg/l at 20 °C	
Coefficient of partition K_{ow}	3.2	Ciba Geigy AG
Half life	70 days	Andral, 1996
Being in the environment		
Spreading period		FREDON Poitou-Charentes
Concentration max find	0.53 µg/L (05/01, la Charente)	FREDON Poitou-Charentes
Ecotoxicology		
CE_{50} *Daphnia magna* 48H	3.8 mg/l	Agritox INRA
CE_{b50} : *Selenastrum capricornutum*	0.06 mg/l	Agritox INRA
CE_{b50} : *Scenedesmus subspicatus*	0.4 mg/l	Agritox INRA
Acceptable daily intake	0.008mg/kg/j	OMS

ANNEX 2 : BIVALVE EMBRYONIC DEVELOPMENT



Annex 3 : Cumulative mortalities of oysters between the 20^{th} of March and the 26^{th} of June