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WORKING DOCUMENT

THE SHELLFISH INDUSTRY IN THE NETHERLANDS: ORGANISATION AND REGULATION

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1. Production

1.1. Mussels

In the Netherlands mussels (*Mytilus edulis*) are cultivated in the Dutch Wadden Sea (70% of the national production) and in the Eastern Scheldt estuary (30% of the national production). The average production of the Dutch mussel industry is about 100,000 tons per year. The production fluctuates strongly, mainly due to meteorological conditions (gales). The production season runs from mid-July to April in the next year.
Cultivated mussels reach the required market size in 2 years after seeding of the spat. The efficiency of the culture, as of any form of bottom cultivation of molluscs, is low compared with off-bottom cultivation. As an average, 1,5-2 tons of mussels are harvested from 1 ton of mussel sprat, and one ton from one ton of half-grown mussels. Ships with which the mussels are dredged mostly measure 30-35 m long and 6-9 m wide, have a draught of 0.6 m and can load 120-150 tons of mussels. A shipload can be fished in 4-5 hours time with 4 dredges, each 1.9 m wide.

After harvest, all mussels are shipped to the township of Yerseke at the Eastern Scheldt (fig. 1), where the mussel auction is located. After buying a lot of mussels (mostly 50 to 120 ton), and prior to processing, the mussels are relayed for 10-14 days on rewatering plots at a short distance from the area in Yerseke where the processing plants are located.

1.2 Oysters

Cultivation of oysters is carried out on culture plots in the eastern part of the Eastern Scheldt and in the non-tidal Lake Grevelingen. The production of flat oysters (*Ostrea edulis*) is about 10 million (800 tons) pieces a year. The season peaks between Christmas and New Year and ends at Eastern. The introduction and the subsequent outbreak of a epizooty caused by the protozoarian *Bonamia ostreae* in the oyster culture areas in the Netherlands, resulting in high mortalities, makes an economical exploitation very difficult. Pacific oysters (*Crassostrea gigas*), locally called by their French name "creuses", have been cultivated in the Eastern Scheldt form the late seventies, after their introduction and successful settlement in 1965 from Japan. Per year about 100 tons of Pacific oysters are produced. This oyster is marketed throughout the year.

1.3 Cockles

Although pilot-scale trials of extensive cultivation of cockles (*Cardium edulis*) have been successful, no commercial culture of this species exists in the Netherlands. The mechanical fisheries on cockles yields a yearly average of 10,000 tons of cooked meat, equivalent to about 60,000 tons of fresh cockles. The fishing season runs from the end of August to

December. There are 37 cockle dredging vessels, licensed to fish in the inshore waters, and another 7 vessels , which are only allowed to fish in the deeper offshore area. The vessels measure about 30 x 10 m and have a draught as shallow as 0.5 m. Each vessel is equipped with two one meter wide suction dredges.

1.4 Miscellaneous

Other species, fished and/or marketed in minor quantities, comprise cut trough shells (*Spisula subtruncata*), whelks (*Beccinum undatum*) and periwinkles (*Littorina littorea*).

2. Organisation

2.1 Mussels

Most of the mussel cultivating firms are independent family enterprises, whereas most of the merchants and exporters of mussels and the mussel processors are firms.
Culture plots are rented from the Dutch Government. Each grower is charged according to his share in the total landings.
Mussel traders are often also active in mussel processing and in other shellfish trades, like cockles, oysters and even lobsters.
The mussel industry is represented toward Government in the Mussel Section of the Commodity Board of Fish. Besides, the mussel growers are organised in a cooperative Producers Organisation and, additionally, have their own local associations. The degree of organisation of the mussel industry is by far the highest in the Dutch fishing industry.

2.2 Oysters

Oyster growers export their own product. Apart from cultivation plots rented from the Dutch Government, they dispose of vessels, on-shore storage basins and cleansing sorting and packing facilities. The oyster growers operate much more individually than the mussel growers, which is illustrated by the fact that there are three separate associations of oyster growers. The oyster industry is also represented towards Government in the Commodity Board of Fish.

2.3 Cockles

The about 20 companies which own cockle-dredging licenses are organised in two associations, which through the Commodity Board of Fish, negotiate each your with the Dutch Government about the length of the fishing season and the allocation of fishing areas. Cockle meat is mostly canned (ca. 70%), IQF or block frozen.

The major share in capture, processing and marketing is in the hands of 4 big companies, who together own 21 of the 37 vessels.

3. Regulations concerning public health

With the disappearance of the internal borders between the member states of the European Union at the end of 1992, control measures are only carried out at the external borders for imports from non-member countries. Uniform regulations for all stages of harvesting, handling, storage, transport an distribution of bivalve molluscs in order to safeguard the public health of consumers within the European Union are laid down for all member states in the Council Directive 91/492/EEC of 15 July 1991. This Council Directive lays down health conditions of the production and the placing on the market of live bivalve molluscs, which are intended for immediate human consumption or for further processing before consumption.

According to the provisions of the Council Directive 91/492/EEC for Community production, placing on the market place of live bivalve molluscs in the Netherlands is subject to the following conditions:

(a) they must originate form production areas which comply with the requirements laid down in Chapter I of the Annex;

(b) they must have been harvested and transported from the production area to a dispatch centre, purification centre, relaying area or processing plant under conditions laid down in Chapter II of the Annex;

(c) where provided for in the Directive, they must have been relaid in suitable areas approved for that purpose and complying with the conditions laid down in Chapter III of the Annex;

(d) they must have been handled hygienically, and where appropriate, they must have been purified in establishments approved for that purpose and complying with the requirements of Chapter IV of the Annex;

(e) they must comply with the criteria set out in Chapter V of the Annex;

(f) heath controls must have been carried out in accordance with Chapter VI of the Annex;

(g) they must have been appropriately wrapped in accordance with Chapter VII of the Annex;

(h) they must have been stored and transported under satisfactory conditions of hygiene in accordance with Chapter VIII and IX of the Annex;

(i) they must have a health mark as provided for in Chapter X of the Annex.

Live bivalve molluscs intended for further processing must comply with the relevant requirements of paragraph 1 and be processed in accordance with the requirements of Council Directive 91/493/EEC.

In the Netherlands the Council Directive 91/492/EEC has been implemented in Dutch law. In bread terms this implementation holds in that the Commodity Board of Fish, that is the organised Dutch fishing industry, is appointed as the competent authority for the fixation of locations and boundaries of production areas, and all other requirements of the Annex. Their activities need the approval of the competent authorities of the Ministry of Agriculture, Nature Conservation and Fisheries and the Ministry of Public Health.
With regard to the requirements concerning live bivalve molluscs laid down in Chapter V of the Annex, the Commodity Board of Fish has asked the DLO-Netherlands Institute for Fishery Research (RIVO-DLO) to set up a monitoring programme, taking into account all aspects of Chapter V. This monitoring programme on the sanitary quality of live bivalve molluscs in the different approved production areas in the Netherlands includes the annual measurement of persistent contaminants (heavy metals, organohalogene compounds, PAHs and radionuclides), the weekly measurement of the microbiological quality of the bivalves (faecal coliforms, salmonella), and the weekly measurements of the (potential) presence of algal toxins produced by certain phytoplankton species (PSP, DSP, ASP).
The monitoring programme of algal toxins includes in compliance with Chapter V of the Annex, the measurement of the total Paralytic Shellfish Poison (PSP) content and the total Diarrhetic Shellfish Poison (DSP) in the edible part of the molluscs. In addition to this also the potential presence of Amnesic Shellfish Poison (ASP) is monitored. Complementary to the analysis of the edible part of molluscs also the presence of potential algal toxins producing phytoplankton species is monitored in the production areas of live bivalve molluscs.

For the measurement of algal toxins in molluscs the Council Directive stipulates the use of biological methods. Chemical methods are allowed if necessary for the detection of saxitoxin, one of the PSP-toxins.
In the case of DSP the Council Directive demands that the customary biological testing method must not give a positive result to the presence of DSP in the edible part of molluscs (the whole body or any part edible separately).
As up till now only DSP is from time to time found in the Dutch production areas of live bivalve molluscs, the control of this algal toxin will be discussed in some detail.

4. Control of DSP

4.1. Introduction

Symptoms of DSP include diarrhoea (92%), nausea (80%), vomiting (79%) and abdominal pain (53%). Onset occurs from 30 minutes to a few hours after eating toxic shellfish. The duration is usually short, with a maximum of a few days in severe cases. The disease is not life threatening (Yasumoto et al., 1978). The structures of the best known types of diarretic shellfish toxins are given in figure 2. They include okadaic acid (OA) and the related dinophysistoxin-1 (DTX-1) and dinophysistoxin-3 (DTX-3), a mixture of acylderivatives of DTX-1 (Yanagi et al., 1989). Recently the structure of yet another dinophysis toxin has been elucidated: DTX-2 (Tingmo et al., 1993) (figure 3). Some of the polyether toxins of the DTX-complex are not only diarrhetic but may also promote stomach tumours in man and thus produce chronic problems in shellfish consumers (Suganuma et al., 1988; Yanagi et al., 1989).

The minimum dose for diarrhoea in adult man is 40-48 µg okadaic acid, corresponding to 10-12 mouse units (MU). A mouse unit is the minimum quantity of toxin needed to kill 2 out of 3 mice (18-20 gram) in 24 hours after intraperitoneally injection. This corresponds to 3.2 µg DTX-1 or 4.0 µg OA (Yasumoto et al., 1980).

Since 1961 several cases of Diarrhetic Shellfish Poisoning (DSP) were observed in the Eastern Scheldt in the Netherlands (Korringa and Roskam, 1961; Kat 1983). In the Dutch Wadden Sea, several additional cases of DSP were recorded from 1976. In all cases in the Netherlands *Dinophysis spp.*, notably *Dinophysis acuminata*, were implicated as potential source of the toxin.

4.2 Bioassays

4.2.1 Rat bioassay

For the detection of DSP in the Netherlands a rat bioassay was suggested in 1961. After subsequent improvements, this bioassay became the basis of the DSP control in the Netherlands (Kat, 1983). In the rat bioassay the presence of diarrhetic shellfish toxines is determined by feeding white female Wistor random rats, type *Rattus norvegicus*, with 10 gram of the hepatopancreas of the shellfish sample to be tested. The test animals are purchased at a weight of 80-100 gram and are need up to a weight of 200 gram.
The hepatopancreas of the shellfish to be tested is used, because most (mussels) or all (scallops, oysters) of the toxin is found to be present in the hepatopancreas (Yasumoto et al., 1978).

The ratbioassay is started at 16.00 p.m. by a starvation period of the test animal of 24 hours. Water supply is ad-libidum. After 24 hours (that is again at 16.00 p.m.) 10 gram, of hepatopancreas of a shellfish sample is given to the rat and the results of the test is read off the next day at 09.00 a.m. The (partly) refusal of the hepatopancreas and the consistency of the faeces produced by the rat (diarrhoea or soft) is used in a rating system for the presence of diarrhetic toxins according to the following scheme:

Percentage hepatopancreas eaten by the rat	Consistency of the faeces	Degree of toxicity	Rating
100 - 90	normal	negative	-
100 - 90	normal / soft	very slightly toxic	±
90 - 50	soft	slightly toxic	+
90 - 50	soft / diarrhoea	moderately toxic	++
< 50	diarrhoea	seriously toxic	+++

In case of total refusal of hepatopancreas (very toxic material) an admixture is made with normal rat fodder in the appropriate ratio (e.g. 1:1 or 2:1). The text is than respected with that mixture.

Sensitivity of the rat bioassay for DSP has been reported until recently as about 1 mg/kg hepatopancreas. This detection limit has been derived from tests with pure okadaic acid, added to lyophilized mussel hepatopancreas (table 1).

Table 1: Calibration of the rat bioassay for DSP with spiked lyophylized mussel hepatopancreas

Amount of OA per rat in µg	Wake-response	Dr. Norte-response	LC Services-response
10	+/-	-	+/-
14	+/-	+	
20	+	+	+
25	++	+	
30	++	+	+
40	+	++	++
50	++	++	++
60		++	++
70		++	
84		++	
100	+++		

Hagel, 1990. Unpublished results

From table 1 it seems that for a ++ response of the rat bioassay one needs some 40 µg of okadaic acid. However, results of an intercalibration exercise performed in 1991 were not in agreement with this (Hagel, 1991). Possibly, instability of the okadaic acid under the circumstances used (peroxidation of the etherbridges?) may have lead to significant losses of this toxin.

In the intercalibration exercise ++ contaminated mussel hepatopancreas was sent to 7 different laboratories in lyophilized form, to enable transport at room temperature. These laboratories analysed the intercalibration material with HPLC-techniques, more or less according to the method described by Lee et al. (1987). The results indicated an okadaic acid level of 5-6 mg/kg (dry weight) or 1-2 mg/kg (wet weight), much lower than expected. Another intercalibration, 1994/1, of contaminated material between RIVO-DLO (rat bioassay, P. Hagel) and the University of Göteborg, Sweden (HPLC-method, L. Edebo) produced the results given in table 2. These results seem to prove excellent correlation between the rat bioassay and the HPLC-method. However, the most remarkable point was that the sensitivity of the rat bioassay would be around 0,2 mg/kg hepatopancreas, 5 times less than assumed before. On the basis of this outcome yet another intercalibration was performed, 1994/2, in which Prof. Dr. B. Lucas, University of Jena, Germany) did analyse a sample of contaminated mussel hepatopancreas with the 4-bromo-methyl-7-methoxy-coumarin (Br-Mmc) HPLC-method, Prof. Dr. L. Edebo, University of Göteborg, Sweden, with the 9-anthryldiazomethane (ADAM) HPLC-method of Lee et al. (1987), Dr. S. Dyring Jacobsen, SCANTOX, Kopenhagen, Denmark, with the mouse bioassay, and Dr. P. Hagel, RIVO-DLO, with the rat bioassay. The results of the 1994/2 exercise are given in table 3 and are, together with the results of the 1994/1 exercise, graphically represented in figure 4.

Table 2: Intercomparison of the rat bioassay with HPLC-analyses -1994/1.

Sample code	Rat bioassay*	HPLC-method**	
		µg/100 shellfish	mg/kg hepatopancreas
T. Röd I	+	12,4	0,86
Nösund II	+	8,1	0,56
Näsholmen III	±	3,0	0,20
ZW/4-171	++	17,9	1,2
ZW/4-215A	++	21,1	1,5

*) RIVO-DLO (P. Hagel)

**) University of Göteborg (L. Edebo)

Table 3: Intercalibration of the rat bioassay with the mouse bioassay and different HPLC-techniques - 1994/2.

Laboratory	Method	Results
University of Jena	HPLC -Br-Mmc	0,6-0,8 mg/kg
University of Göteborg	HPLC-ADAM	1,26 mg/kg
SCANTOX	mouse bioassay	3 mice died within 24 hours
RIVO-DLO	rat bioassay	+

The "tolerance level" of 0,4 mg/kg hepatopancreas indicated in figure 4 is arrived at in the following way.

In the Council Directive 91/492/EEC it is stated, that the PSP content in the edible part of molluscs must not exceed 0,80 mg/kg saxitoxine equivalents. As the minimum dose of PSP toxin for an adult person is somewhere around 500 µg, at the tolerance level of the EU some 500 grams of molluscs may be safely consumed. In the case of DSP the minimum dose of toxin needed for the first effects in an adult person is 40-48 µg okadaic acid (Yasumoto, 1980). At a "tolerance level" of 0,4 mg/kg hepatopancreas in mussels and with the hepatopancreas taken at 20% of the meat weight in the mussels, 500 grams of mussel meat will contain 40 µg okadaic acid, exactly the level which must not be exceeded in order to prevent the first toxic effects. Put it the other way around, when the edible part of molluscs contains 80 µg/kg okadaic acid, "a tolerance level" for the whole molluscs, an adult person may eat 500 grams without significant risks, just as with PSP contamination.

Table 4: Response of the rat bioassay to okadaic acid (OA).

mg/kg OA in hepatopancreas	response rat bioassay
<0,2	-
0.2 - 0.4	±
0.4 - 0.8	+
0.8 - 1.6	++
>1.6	+++

A "tolerance level" of 0,4 mg/kg hepatopancreas corresponds to a + level of the rat bioassay (table 4). Up till the end of 1994 RIVO-DLO considered a ± result of the rat bioassay as not acceptable for human consumption. This in accordance with the Council Directive 91/492/EEC, which stipulates: "the customary biological testing methods must not give a positive result to the presence of DSP in the edible part of molluscs". However, as ±

can be considered not to be a positive result RIVO-DLO now consideres only a + response or more of the rat bioassay to be a positive result in terms of the Council Directive.
In the calculation given above there is a complication when the weight percentage of the hepatopancreas of the shellfish is significantly different from 20%. In the figure 5 a graphical representation is made of the effect of the weight percentage of the hepatopancreas on the recalculation of a result of the rat bioassay in mg/kg hepatopancreas to µg/kg in the whole shellfish meat. In case of 25% hepatopancreas (e.g. small mussels) a "tolerance level" of 0,32 mg/kg hepatopancreas will already result in the edible flesh "tolerance level" of 80 µg/kg of okadaic acid. At the other hand, in the case of 15% hepatopancreas (e.g. oysters) a "tolerance level" of 0,4 mg/kg hepatopancreas may be to strict.

4.2.2 Mouse bioassay

The mouse bioassay for the detection of DSP has been developed in Japan (Yasumoto et al., 1978). At present the method comprises three successive extractions from 30 gram of mussel hepatopancreas with acetone. After evaporation of the solvent, the residue is resuspended in 6 ml of a Tween 60 solution. One millilitre of this extract is than injected intraperitonealy into 3 male mice (18-20 gram). Dilution of very toxic samples until approximately 24 hours lethality is observed, makes it possible to express the toxicity of these samples in terms of a mouse unit (MU), comparable to that defined by the Japanese, i.e. the minimum quantity of toxin needed to kill 2 out of 3 mice in 24 hours. In France the survival of 3 mice for more than 5 hours is considered to be a negative response in the context of a monitoring system. However, as a precautionary measure, mice are kept under observation for at least 24 hours (Marcaillou-Le Baut et al., 1990).
Samples associated with survival times "close to 24 hours" contain one mouse unit (MU) in 5 gram hepatopancreas . These samples thus contain about 0,2 MU per gram of hepatopancreas, taking 1 MU = 4 µg okadaic acid, 0,8 mg/kg okadaic acid. Determination of the relation between survival time of 3 mice and the injected amount of okadaic acid confirms that less than 4 µg of okadaic acid is necessary to observe a lethality time greater than 5 hours (Marcaillou-Le Baut et al., 1990).
Occurrence of DSP in bivalve molluscs in Spain is also detected by the mouse (16-20 gram) bioassay, in which +++ corresponds to the presence of toxins causing two mice death between 0 and 5 hours, ++ to one mouse death between 0 and 5 hours, + to one or two mice death between 5 and 24 hours and *a* to the absence of toxins (Alvito et al., 1990). A comparison of this system with HPLC-techniques shows a good correlation (Marinez et al., 1993). Taking the HPLC results of this article as being expressed in µg/100 gram edible product, one can arrive at the graphical representation of the response of the mouse bioassay to okadaic acid as given in figure 6. From this figure it also can be seen that the "tolerance level" of 0,4 mg/kg hepatopancreas , corresponding to 0,5 MU in the 5 grams of

hepatopancreas injected in the mouse bioassay, is on or just below the detection level of the assay. Included in this figure is also the result of Jacobsen from the 1994/2 intercalibration exercise mentioned earlier.

One drawback of the mouse bioassay is that the effect may be due to the genuine diarrhetic DSP toxins, but also to toxins like pectenotoxins, yessotoxin and different ichthyotoxins. The implications of the later toxins to human health are not clear. The poor correlation between the toxicity in mussels as revealed by the mouse bioassay and HPLC-analyses in Norway emphasise, that severe effects on mice of mussel extracts prepared for demonstration of diarrhoea toxins are in some cases only partly due to the presence of okadaic acid and its derivatives (Dahl et al., 1995).

A related problem is the toxic effect of free unsaturated fatty acids in the mouse bioassay of DSP by intraperitoneal injection. Fractionation of the acetone extracts from samples of hepatopancreas from poisonous scallops showed the major toxic component in the mouse bioassay of one fraction to be free unsaturated fatty acids (Tukagi et al., 1984).

Another pitfall of the mouse bioassay is the preparation of the extracts. The acetone from the extraction of the toxins from shellfish hepatopancreas has to be removed by evaporation under reduced pressure. Sometimes, in order to speed up the process, elevated temperatures are used for this evaporation, e.g. Rotavapor at 40°C (Croci et al., 1994). From our experiences with HPLC-techniques for the determination of okadaic acid, it became clear that elevated temperatures after evaporation of the solvent may significantly reduce recoveries of the toxin (figure 7).

4.2.3 Discussion

In the long experience in the Netherlands with the rat bioassay for the analysis of DSP-toxins (since 1961), this test has shown a very high degree of reliability and the absence of false positive results. The test has succeeded in preventing DSP in consumers of Dutch bivalve molluscs and has recently proven to be much more sensitive than originally assumed. Tolerance levels for DSP-toxins arrived at by a calculation analogue to the establishment of tolerance levels for PSP-toxins, are easily met by the rat bioassay. Banning of harvesting and marketing is advised when the toxicity found with the rat bioassay is + or higher.

As the rats used in the rat bioassay only suffer form some inconvenience during restricted periods, the rat bioassay can be characterised as very animal friendly. In contrast, the mouse bioassay leads to extensive suffering of the test animals, resulting in very unpleasant death. Rats which have produced positive results are put in quarantine for 2 weeks and may than be used again. Mice can be used only once. In view of their more or less comparable results, it is hard to see how one can convince society to change from an animal friendly bioassay to a very animal unfriendly bioassay with more or less the same results. The Animal Testing Law in the Netherlands will certainly not allow this.

The mouse bioassay performed in the correct way, will certainly be able to protect the consumers of molluscan shellfish. False positive results, or rather results from non-DSP toxins with unclear relevance to public health or free fatty acids, will from time to time complicate advises.

An advantage of both bioassays, rat bioassay as well as mouse bioassay, is that the effect of all DSP-toxins are detected, okadaic acid, DTX-1, DTX-2 and the DTX-3 complex. It is still difficult to envisage how physical-chemical analyses can offer comparable results in the near future. For the time being, HPLC-techniques will mainly produce additional information in association with a bioassay, e.g. about the identity of the toxins involved.

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Figure 1: Map of The Netherlands

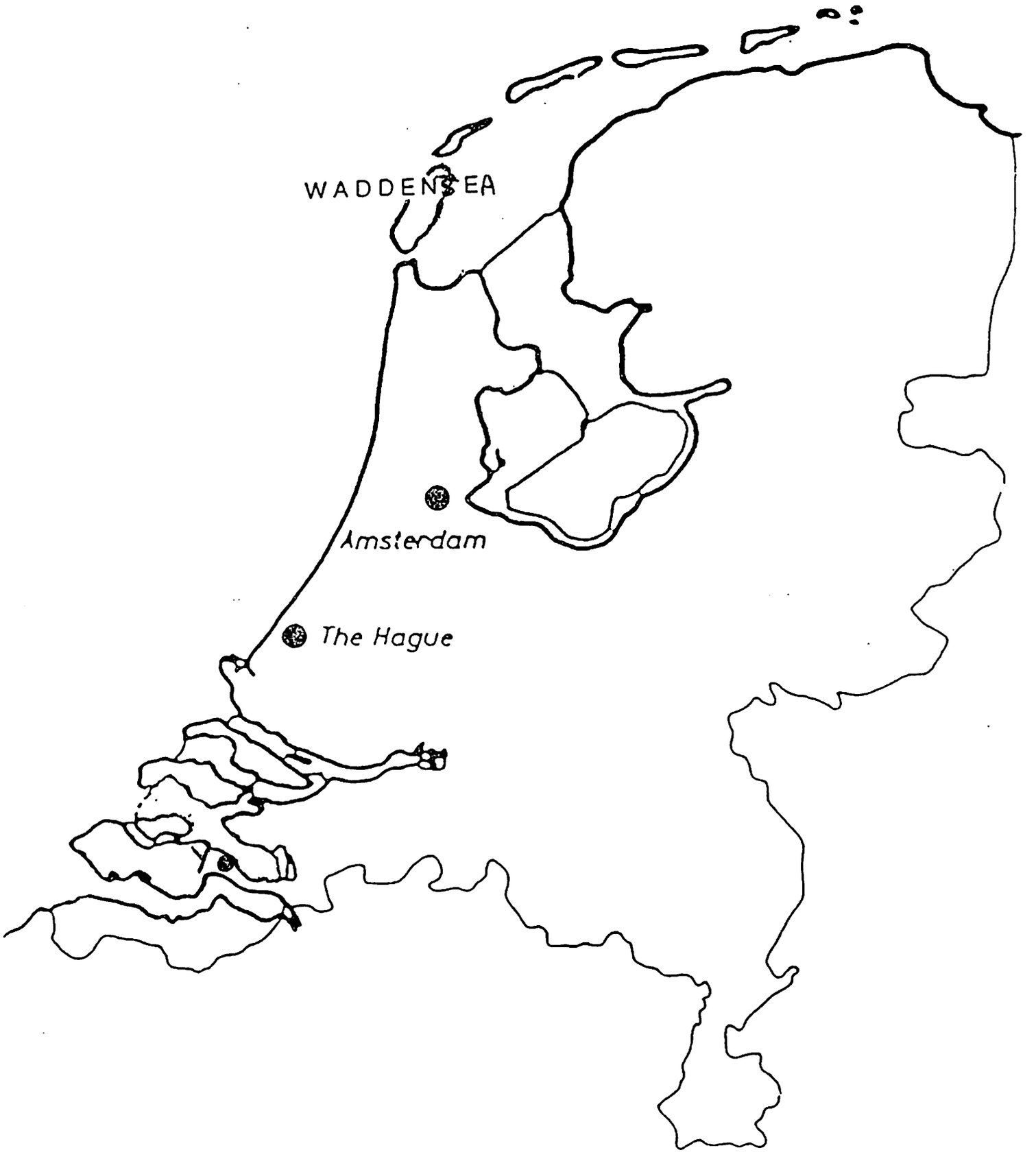
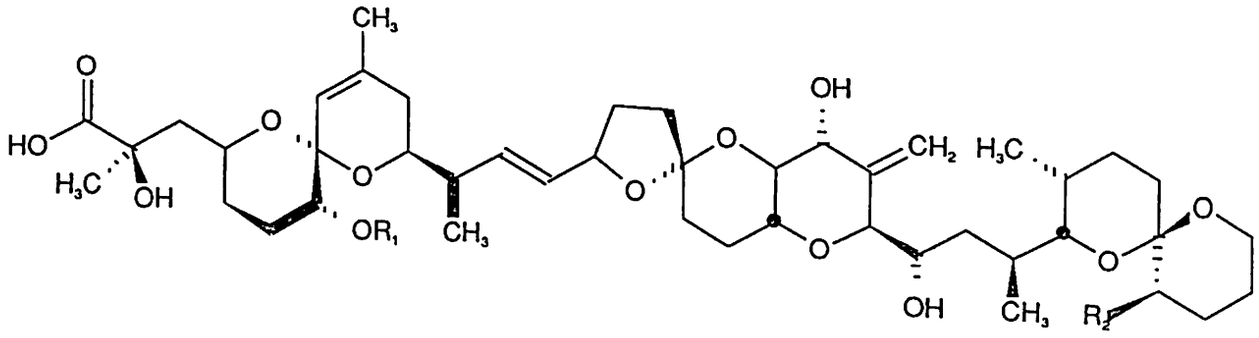
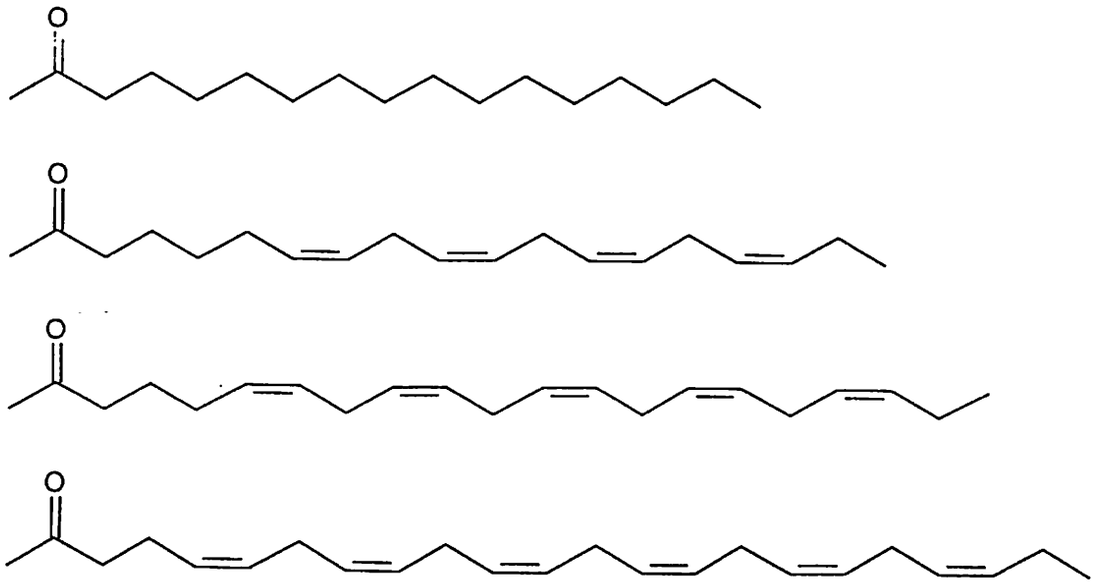


Figure 2:



OA : $R_1 = H, R_2 = H$
DTX1 : $R_1 = H, R_2 = CH_3$
DTX3 : $R_2 = CH_3$, R_1 =

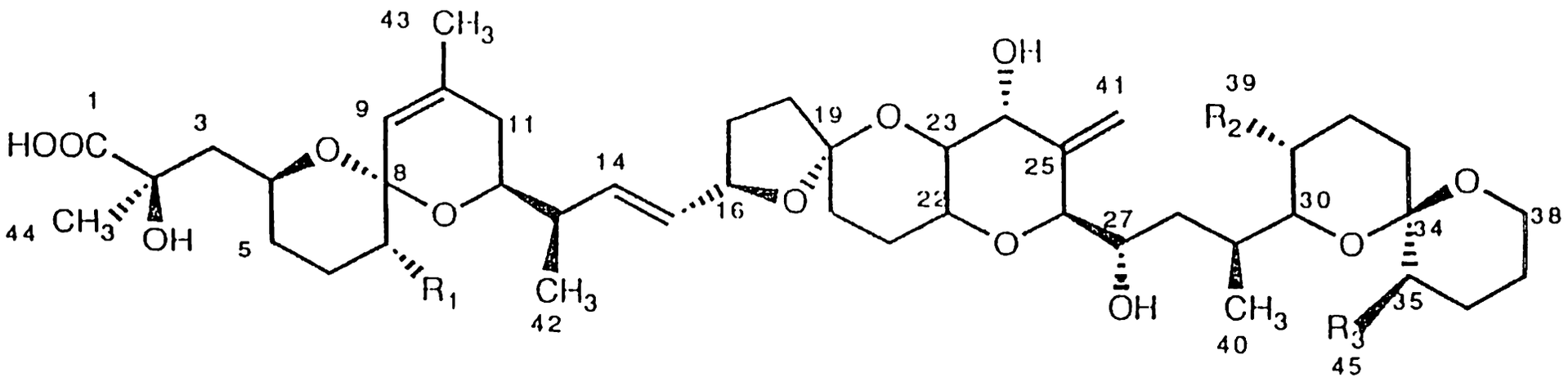


OA : okadaic acid; DTX : dinophysistoxin

Structures of okadaic acid and dinophysistoxin derivatives implicated in diarrhetic shellfish poisoning
[T. Yanagi *et al.* : Agric. Biol. Chem. 53, 525 (1989)]

Figure 3: Structure of DTX-2

(Tingmo et al., 1993)



COMPOUND	R1	R2	R3
Okadaic Acid (**1**)	OH	CH_3	H
DTX-1 (**2**)	OH	CH_3	CH_3
DTX-3 (**3**)	O-acyl	CH_3	CH_3
DTX-2 (**4**)	OH	H	CH_3

Figure 4: Response of the rat bioassay to okadaic acid (OA).

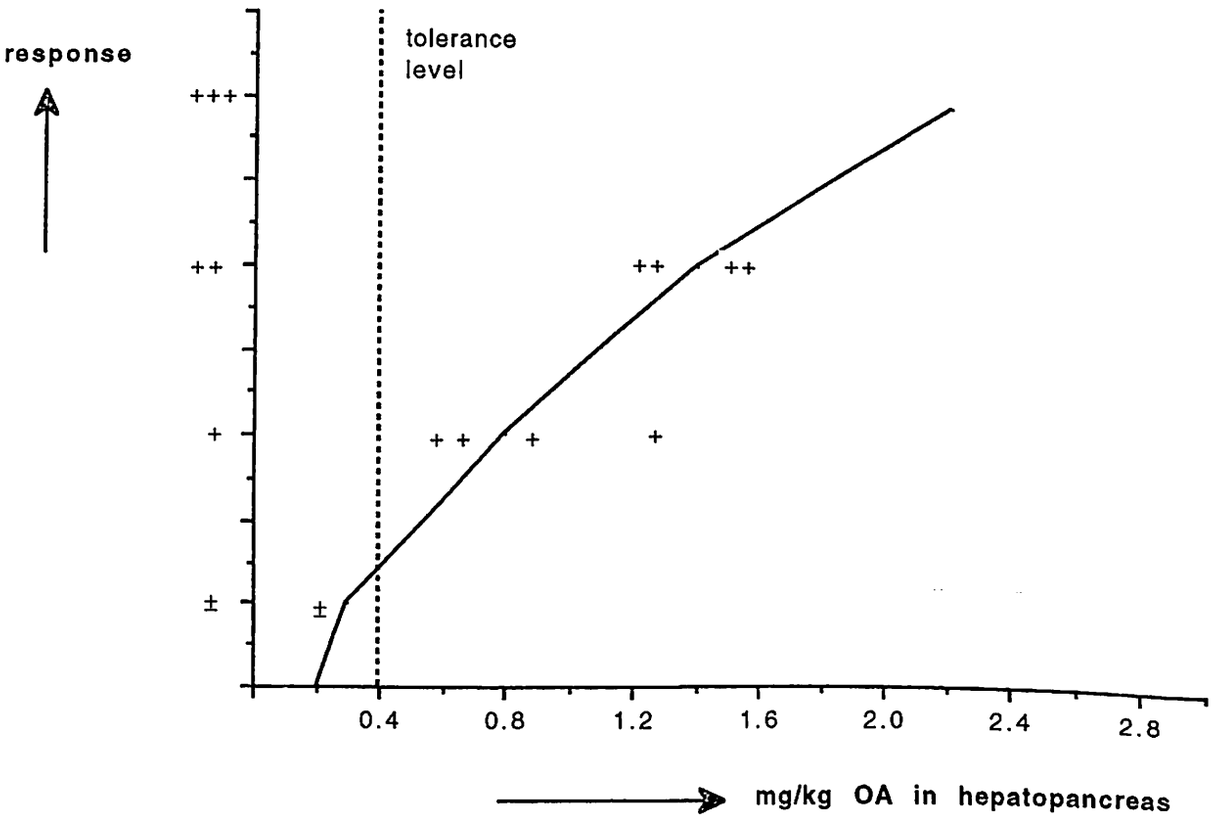


Figure 5: Content of okadaic acid (OA) in shellfish flesh as a function of the percentage hepatopancreas weight.

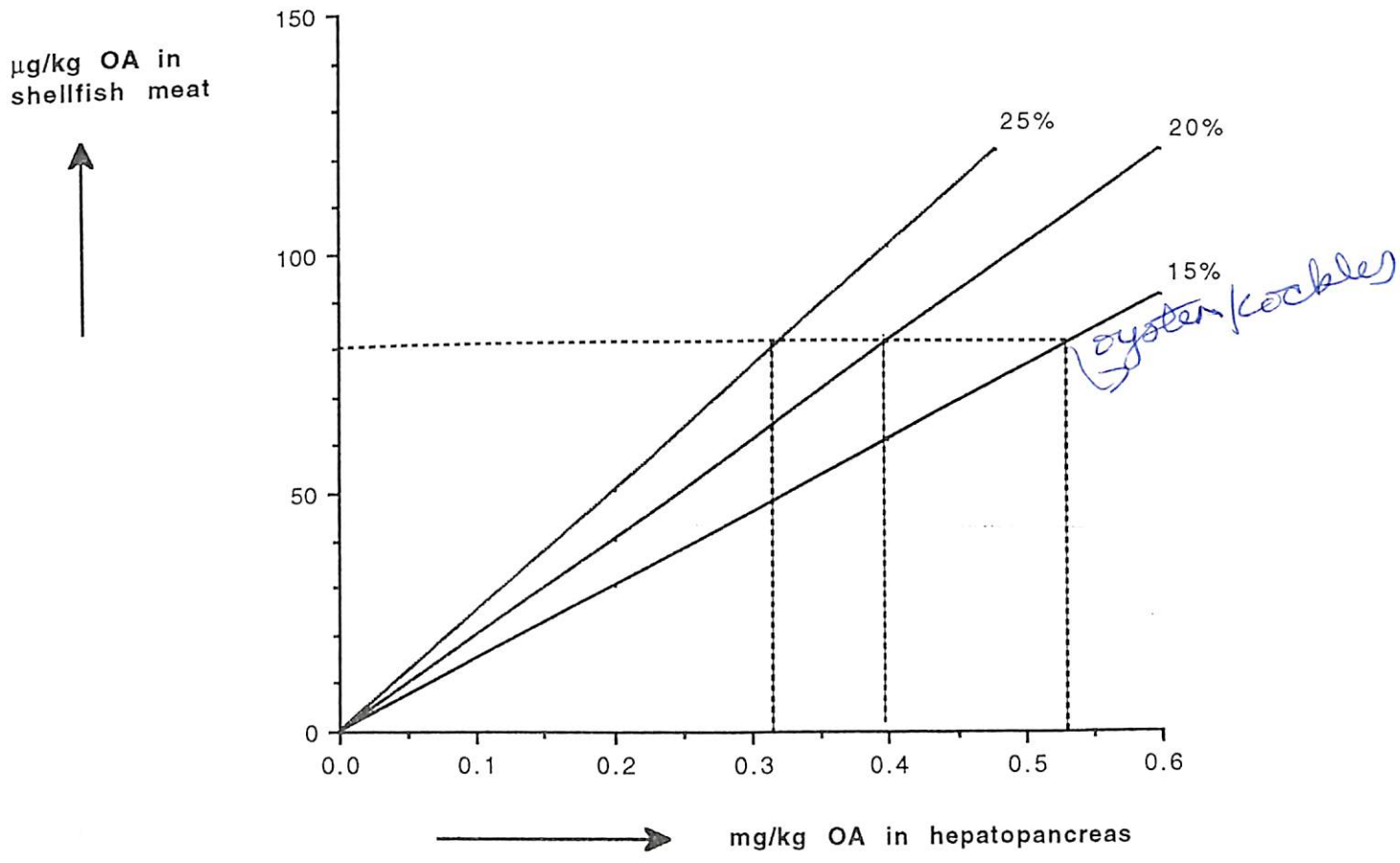


Figure 6: Response of the mouse bioassay to okadaic acid (OA).

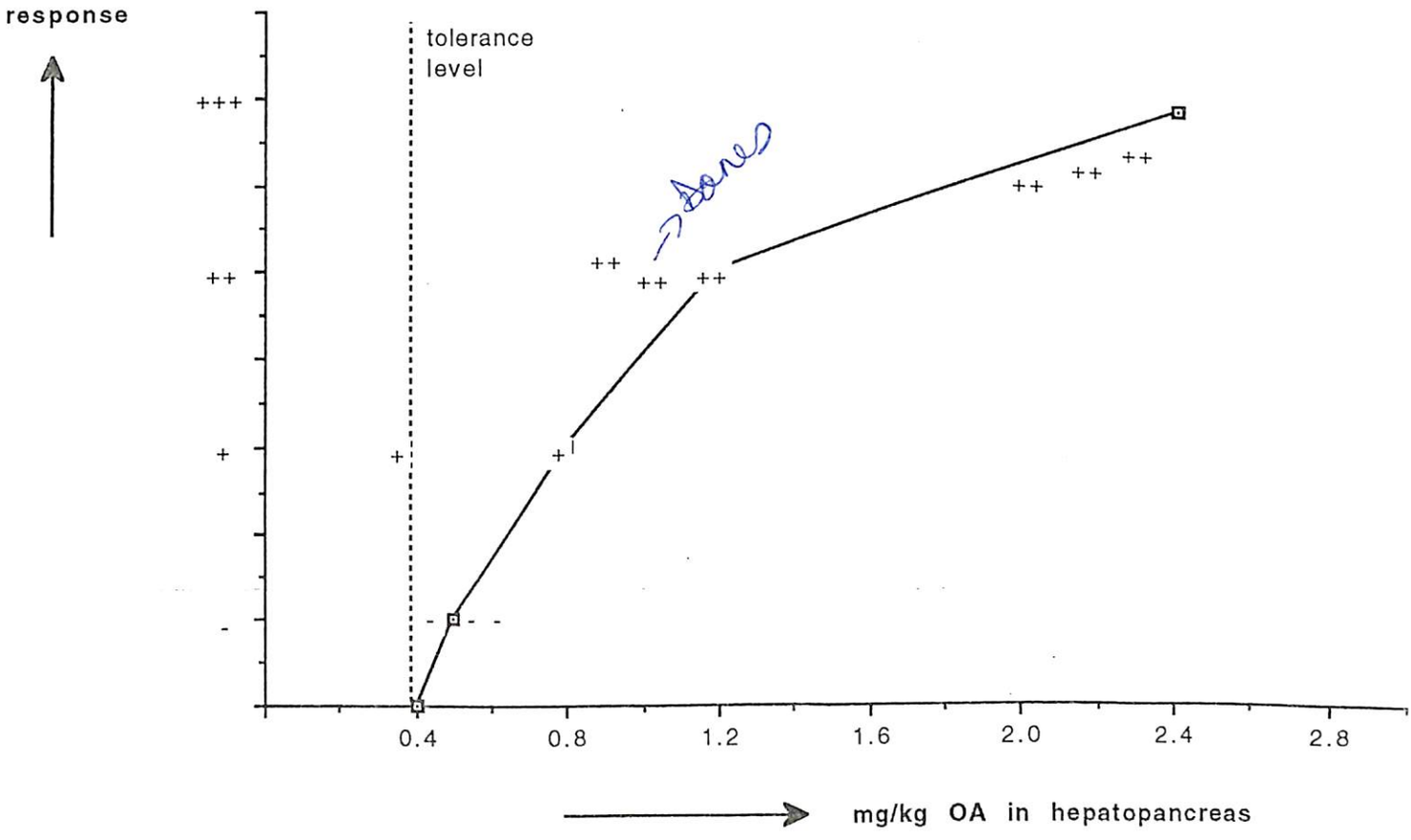


Figure 7: Losses of okadaic acid (OA) after evaporation of solvent during 30 minutes at different temperatures in air.

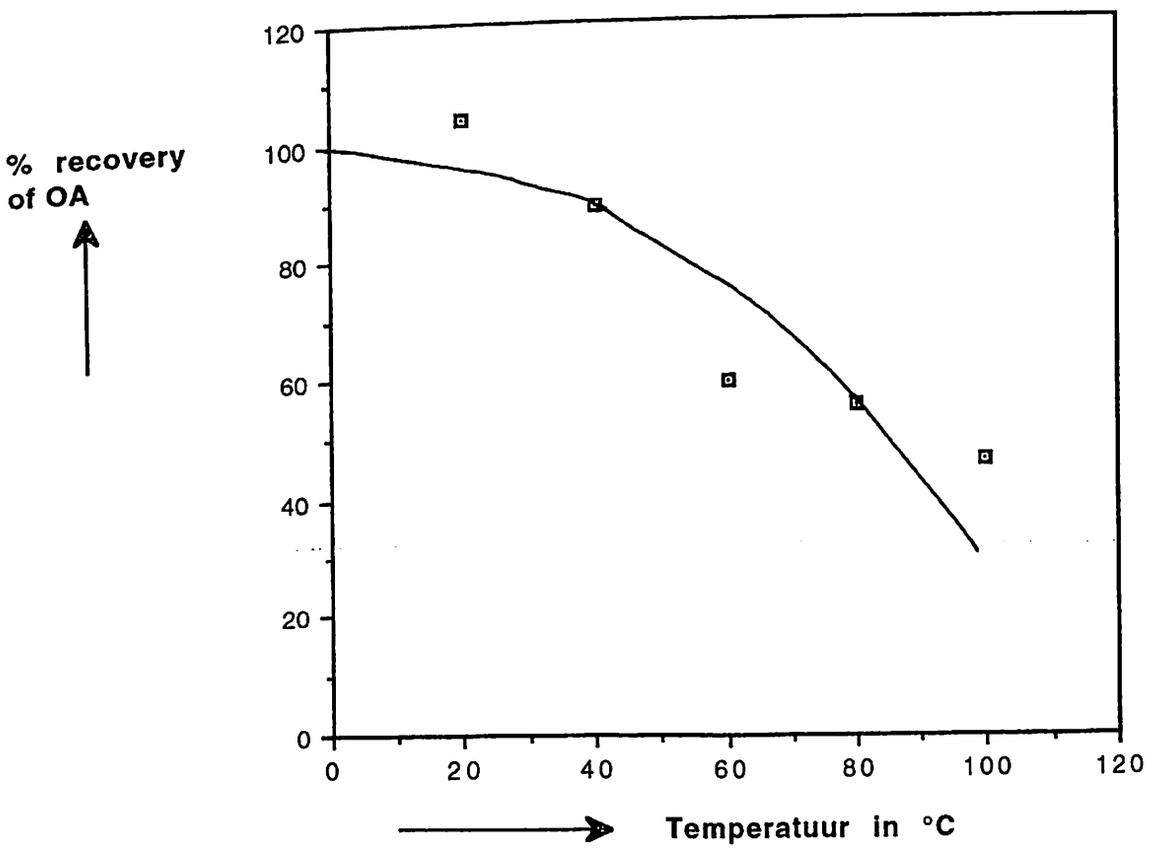


Table 1: Calibration of the rat bioassay for DSP with spiked lyophylized mussel hepatopancreas

Amount of OA per rat in µg	Wake-response	Dr. Norte-response	LC Services-response
10	+/-	-	+/-
14	+/-	+	
20	+	+	+
25	++	+	
30	++	+	+
40	+	++	++
50	++	++	++
60		++	++
70		++	
84		++	
100	+++		

Hagel, 1990. Unpublished results

Table 2: Intercomparison of the rat bioassay with HPLC-analyses -1994/1

Sample code	Rat bioassay*	HPLC-method**	
		µg/100 shellfish	mg/kg hepatopancreas
T. Röd I	+	12,4	0,86
Nösund II	+	8,1	0,56
Näsholmen III	±	3,0	0,20
ZW/4-171	++	17,9	1,2
ZW/4-215A	++	21,1	1,5

*) RIVO-DLO (P. Hagel)
**) University of Göteborg (L. Edebo)

Table 3: Intercalibration of the rat bioassay with the mouse bioassay and different HPLC-techniques - 1994/2.

Laboratory	Method	Results
University of Jena	HPLC -Br-Mmc	0,6-0,8 mg/kg
University of Göteborg	HPLC-ADAM	1,26 mg/kg
SCANTOX	mouse bioassay	3 mice died within 24 hours
RIVO-DLO	rat bioassay	+

Table 4: Response of the rat bioassay to okadaic acid (OA)

mg/kg OA in hepatopancreas	response rat bioassay
<0,2	-
0.2 - 0.4	±
0.4 - 0.8	+
0.8 - 1.6	++
>1.6	+++