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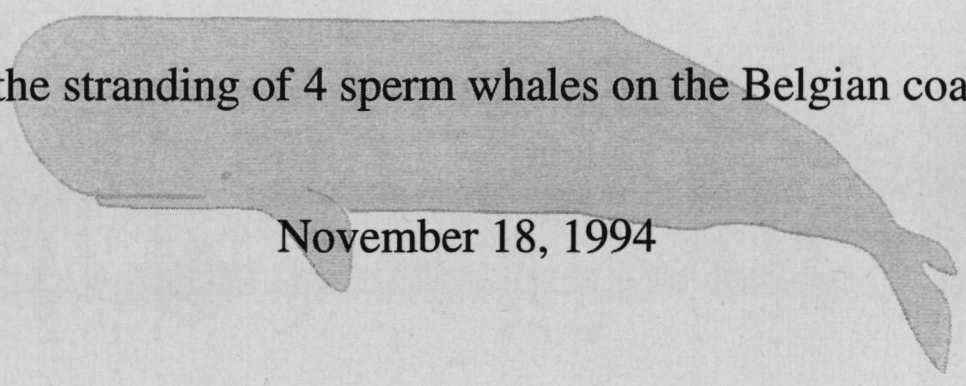
On the stranding of 4 sperm whales on the Belgian coast,



November 18, 1994

Preliminary report of
Laboratory for Ecotoxicology and Polar Biology (V.U.B.),
Oceanology department (ULg),
Pathology department (ULg).

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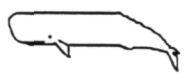

1. SUMMARY

Abstract

Male sperm whales found dead on the coast of Belgium in November 1994 had suffered a weight deficit and displayed debilitating lesions. High levels of Cd, Hg, PCB's and DDE contamination affected all four animals. A combination of these factors could explain the stranding.

Key words
Sperm whales - stranding - pathology - ecotoxicology

Résumé

Les cachalots mâles trouvés morts à la côte belge, en novembre 1994, étaient amaigris et porteurs de lésions débilitantes. Des niveaux élevés de contamination de Cd, Hg, PCB's et DDE affectaient les quatre animaux. Ces éléments combinés pourraient expliquer l'échouage.

Mots clés
Cachalots - échouage - pathologie - écotoxicologie

Samenvatting

De mannelijke potvissen die in november 1994 dood aangetroffen werden aan de Belgische kust leden aan gewichtsverlies en vertoonden verzwakkende letsels. De vier dieren waren allen aangetast door hoge niveau's van Cd, Hg, PCB en DDE verontreiniging. Een kombinatie van deze factoren zou de aanspoeling kunnen verklaren.

Sleutel worden
Potvissen - stranden - pathologie -ecotoxicologie

2. FOREWORD

The present report was designed to provide the scientific community with a first evaluation on the stranding of 4 sperm whales in Belgium in November 1994. It is clear that work is still in process at the present date in the various laboratories involved and it is understood that some of the data presented may eventually have to be re-assessed. Contact addresses are given for additional precisions or comments.

The authors are endebted to those who made this work possible. The financial support was guaranteed by three grants from the "Impulse program in Marine Sciences" of the Belgian Federal Office for Scientific, Technical, and Cultural Affairs (O.S.T.C.) (contracts MS/03/031, MS/12/032, MS/12/033). Thanks are also due to those people who helped the scientific teams during the successive steps of this evaluation.

Mr. Dewulf , mayor of the city of Koksijde and the director of the Apostroff hotel, who offered his facilities to the necropsy team, the Marine Fisheries Station of the Belgian Ministry of Agriculture, and the Service of Waterways/Coast of the Ministry of the Flemish Community, deserve our very special commends and gratitude.

Prof. BOUQUEGNEAU J.-M.
Prof. COIGNOUL F.
Prof. JOIRIS Cl.

June 1995

3.1. Necropsies and toxicology

BOUQUEGNEAU J.M.(2)	Head toxicology (Cd, Cr, Cu, Zn, Fe, Pb, Ni, Ti, Se, metallothioneins, total lipids)
BROSENS L. (3)	Necropsies, parasitology
COIGNOUL F. (3)	Head pathology (necropsies, histopathology, samplings)
DEBACKER V. (2)	Toxicology
GOBERT S. (2)	Toxicology
HOLSBEEK L. (1)	Toxicology
JACQUINET E. (3)	Necropsy sperm whale #2
JAUNIAUX T. (3)	Necropsies coordination + necropsy sperm whales #1 and 4
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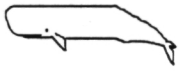
ROGGEMAN W. (5)	Head technical team
TAVERNIER J. (5)	Technical coordination



3.3. Backup laboratories

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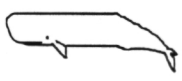
[6] and [7] Contact through [3]

3.5. Additional comments

The presence during necropsies and the valuable help from a group of 14 veterinary students interested in cetaceans (the CETO club) was most appreciated. For any additional information on this group, contacts can be made through the Department of Veterinary Pathology of the University of Liège [3].



4. CASE HISTORY

Three beached sperm whales *(Physeter macrocephalus)* were discovered on Friday November 18, 1994 between 06:00 and 07:00 local time (05:00-06:00 UT) in the tidal zone of the beach, east of Koksijde, a city located at the Belgian coast, 7 km from the French border. Stranding was considered to have occurred between 00:00 and 06:00, the same day.

A team including 3 graduate students in veterinary pathology, 3 graduate students in oceanology, and 14 undergraduate veterinary students was dispatched. They arrived on the spot at 12:00 (UT).

Local authorities forbade any sampling, except for small skin fragments, until waste disposal equipment was available at the site of necropsy.

After further delays due to tidal immersion of bodies, necropsies started on Saturday Nov. 19 at 02:00 (UT). They were simultaneously performed on the 3 bodies and lasted for 5 hours, until the tidal movement interrupted the procedure.

A fourth sperm whale was found dead on Friday November 18, 1994 (15:00 UT)in shallow water, near the beach of the city of Nieuwpoort, 8 km east of Koksijde and was towed to the beach.

Weather conditions: Fine drizzling rain and low visibility. The air temperature was 12°C, the wind was moderate to strong, blowing from the S. W., parallel to the coastline. Tidal movements were: high tide: 12:30 (UT); low tide: 18:00(UT).

5. BODY WEIGHTS

Bodies were weighted at the process plant at the time of carcasses disposal, on November 21, after partial dissection on the beach and loss of body fluids (table 1)

Table 1 : weight of carcasses reported by the processing plant (Animalia Produkten N.V., Denderleeuw)

Viscera (animals 1+2)	6,500 kg
Body weight (animals 1+2)	54,040 kg
Body weight (animal 3)	19,080 kg
Body weight (animal 4)	34,040 kg
Lower jaws (animals 1, 3, and 4)	500 kg

Calculations on total body weight were :

Sperm whales 1 and 2 :

(54,040 + 6,500 + 330) x 1.14 = 69,392 kgs

Note : The correction factor 1,14 is used to compensate for loss of body fluids during dissection and transport (Lockyer, 1991).

Estimated individual weight (1 and 2) = 35,000 kgs

Sperm whale 3 :

(19,080 + 165) x 1.14 = 21,940 kgs (estimate)

Sperm whale 4 :

(34,040 + 165) x 1.14 = 38,994 kgs (estimate)

According to the literature, a predictive formula of normal weight can be used from measured length (Lockyer, 1991) as :

$W = 0.0218 \times L^{2,74}$

The normal weight derived for the Belgian whales is given in table 2 in comparison with actual weights.

Table 2 : Length, observed weight and predicted normal weight of sperm whales stranded on the Belgian coast, Nov. 18, 1994.

Animal #	Length (m)	Weight (kg)	Predicted weight (kg)
1 + 2	15.40 and 14.90	70,000	74,800
3	14.40	21,940	32,500
4	18.20	38,994	61,800

6. NECROPSIES

A summary description of carcasses, necropsies, and histopathology is presented bellow. Additional information for veterinary pathologists can be obtained on request [3].

A standardized necropsy procedure derived from the "ECS protocol for postmortem examination and tissue sampling of small cetaceans" was applied (Kuiken and Garcia Hartmann, 1991).

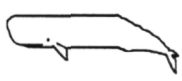
Samples were collected for additional evaluations (Appendix). For histopatholgy, organs and lesions were stored in 10% neutral buffered formalin. For bacteriology, intestinal segments were collected. For parasitology, intestinal content was sampled, parasites were collected and preserved in 70% ethanol. For toxicology, samples were collected and frozen. Skin samples were stored in 20% DMSO saturated with NaCl for DNA analyses. Two to four teeth from the middle of the lower jaw were collected for age determination. Body measurements were taken on each animal. The body condition was estimated using code from 1 (live animal) to 5 (mummified carcass). Photographs of both sides were taken, plus body openings and lesions. The blubber thickness was measured at the caudal insertion of the dorsal fin.

Skin, body openings (mouth, eyes, blow-hole, ears opening, genital slit and anus) were examined, and any lesions or discharges were characterized. Pictures were taken when needed.

For abdominal cavity opening, incisions were made throught the skin and the blubber. One, horizontally, at the mid height of the body, from the pectoral flipper to the anus level and two vertically, the first from the cranial part of the horizontal cut to the belly and the second from the caudal part to the anus. The skin and blubber flap was tied near the horizontal opening and the ropes were pulled for dissection of the abdominal blubber and exposure of abdominal muscles. A strip of tissue was removed and subcutaneous tissue and blubber were examined for lesions and parasites. With the same technique, abdominal muscle layers and peritoneum were removed.

Bacteriological samples were collected before handling abdominal organs. Organs, lesions and parasites were characterized, photographed and collected. The gastro-intestinal tract was examined *in situ*. The stomach was opened and the gastric content was stored. Various segments of intestine were opened and examined. After removing the digestive system, the kidneys and the liver were examined and sampled.

The diaphragm was incised and through the opening, the lung was examined and sampled.



6.1. Description of carcasses

The 3 whales of Koksijde were numbered 1 to 3, according to their location on the beach, starting from the west. All three were in the tidal zone at the eastern edge of the town (Figures 1 and 2). When first examined, animals were fresh, with no evidence of decay. Death probably had occurred not more than 6 to 12 hours before.

Sperm whale #1 (Figure 1)

The whale was a young adult male, 15.4 meter long, laying on its right side, parallel to the coastline, back to the sea, at the upper level of the tidal zone. No vestigial tooth was visible at the upper jaw. At the time of necropsy, the carcass was moderately decomposed and the penis could be extended (ECS condition code: 3) (Kuiken and Garcia Hartmann, 1991).

Sperm whale #2 (Figure 1)

The whale was a young adult male, 14.9 meter long, laying on its left side, parallel to the coastline, back to the beach, 3-4 meter lower than whale #1 in the tidal zone. Distance between animals 1 and 2 was about 50 meters. In the upper jaw, vestigial teeth were visible, 3 on the right, 5 on the left. At the time of necropsy the carcass decomposition was moderate, the penis could be extended (ECS condition code: 3) (Kuiken and Garcia Hartmann, 1991). Small round ulcers of the hard palate were observed on the mid-line.

Sperm whale #3 (Figure 2)

The whale was a young adult male, 14.4 meter long, laying on its right side, perpendicular to the coastline, tail fluke to the sea, at the middle level of the tidal zone. The distance between animals 2 and 3 was about 200 meters. No vestigial tooth was visible in the upper jaw. At the time of necropsy, the carcass was moderately decomposed and the penis could be extended (ECS condition code: 3) (Kuiken and Garcia Hartmann, 1991). Large acute ulcers of the hard palate, involving the entire cranial half of the mucosa, were observed and sampled for histopathology.

Figure 1 : Sperm whales #1 (lower left) and #2 on the beach of Koksijde, Nov. 18, 1994 (photograph E. Donnay, MUMM).

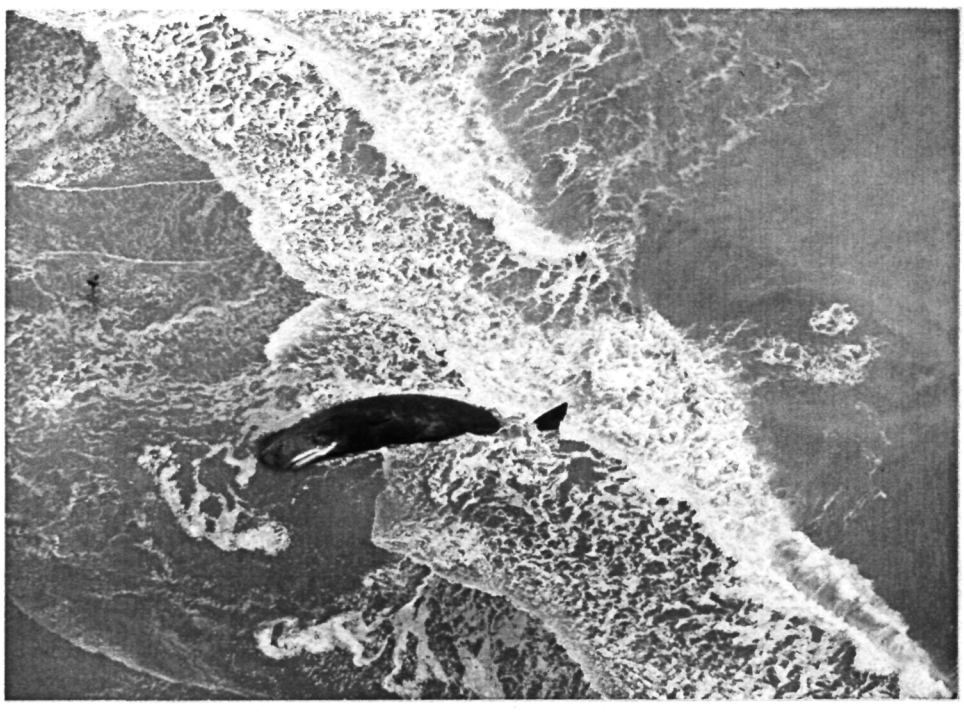


Figure 2 : Sperm whale #3 on the beach of Koksijde, Nov. 18, 1994 (photograph E. Donnay, MUMM).

Figure 3 : Sperm whale #2. Note the round white scars on the upper jaw, resulting from squid tentacles, and evenly spaced, parallel, elongated scars on the lateral aspect of the head, resulting from fights, Nov. 18, 1994 (Photograph E. Donnay, MUMM).

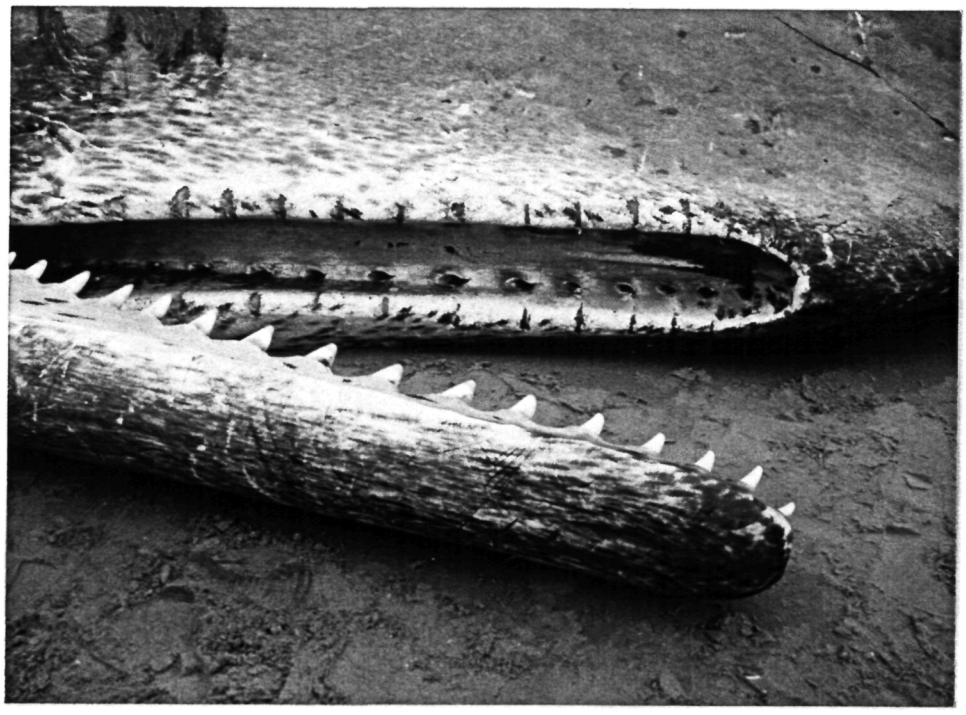


Figure 4 : Sperm whale #2. Post mortem wounds of the upper lip due to rythmic closure of the lower jaw, Nov. 18, 1994 (Photograph E. Donnay, MUMM).

Sperm whale #4

This animal, found dead at sea, off the Belgian city of Nieuwpoort, was towed on the beach of the military base of Lombardsijde. It was first examined on Saturday Nov. 19, 1994 at 03:00 PM (UT). At that time, decay was severe.

It was an adult male, 18.2 meter long. On the right upper jaw, 4 vestigial teeth were visible. The carcass was in advanced decay and the penis was extended (ECS condition code: 4) (Kuiken and Garcia Hartmann, 1991). Body openings were examined and hemorrhagic fluid dripped from the blow-hole and from the mouth. The animal was bloated.

Chronic skin lesions (parallel and round scars on the head, round scars on the tail stock) were observed on all four animals.

Skin lesions were examined on the 3 whales of Koksijde. Round scars, 4 to 5 cm diameter, that were observed on the upper jaws (Figure 3), probably resulted from the attachment of squid tentacles (Evans, 1987), squids being a normal prey of sperm whales. Longitudinal parallel scars on the head (Figure 3), probably resulted from fights between males (Evans, 1987). Those marks were separated by a more or less even distance of 10 cm, compatible with the space between adjacent teeth. A 20 cm long vertical groove on the head of whale #2 was also, most probably, a wound scar. Evenly spaced upper lip lacerations were typically post-mortem, since no bleeding was associated with the wounds (Figure 4). They resulted from the rythmic closure of the lower jaws due to water movement on the dead bodies.

Erosions were observed on all 3 animals on the lower belly, around genitalia and on the fluke (Figure 5). They probably were mechanical abrasions due to the rubbing of the stranded animals on the sand during agony. Conversely, round ulcerative lesions on the dorsal side of the tail stock resembled similar descriptions of attachement sites of sharks such as *Isistius brasiliensis* or lampreys (Evans, 1987).

Figure 5 : Sperm whale #2. Erosions around genitalia and lower belly associated with rubbing on the sand, Nov. 18, 1994 (Photograph F. Migeotte).

6.2. Dissection

Internal lesions observed on necropsy in animals #1, 2 and 3 included severe passive congestion of liver and kidneys, segmental congestion of intestine, mild lung passive congestion, and disseminated hemorrhages of the intestinal serosa.

On sperm whale #3, there were lesions in the outer ear canal : epithelial thickening, cells desquamation, and exudate. Ears were not examined on the other whales.

Body decay, rated as #3 body condition according to the ECS necropsy protocol, was somewhat different in the 3 sperm whales. Animal #1 appeared to be slightly fresher than #2, itself in slightly better condition than #3. This information might indicate a different time of death, #3 dying first, then #2, then #1.

No valid conclusion could be drawn from animal #4 that sat on another beach for an additional 15 hrs, was bloated at the time of sampling, and was not necropsied.

The lack of lung edema, at least in the small fragment of lung available from one whale (#1), suggested a circulatory failure rather than asphyxia. However, the lack of information on the heart and most of the thoracic cavity organs preclude any conclusive opinion on this topic.

The presence of a vestigial blow-hole in sperm whale #1 was reported before and appears to be a rare, but existing congenital anomaly in cetaceans. Also, the presence of vestigial teeth in the upper jaw of male sperm whales is described as an usual finding. It occured in 2 animals, namely whales #2 and 4.

6.3. Conclusions based on pathology

Animals #1, 2, and 3 were alive at the time of stranding, as suggested by hemorrhagic ventral abrasions.

Death occured less than 12 hours before discovery, therefore during the night of Nov. 17-18, 1994, as suggested by the lack of body decay when first examined.

The 3 animals died either simultaneously or during a short period of time, possibly #3 dying first, then #2, then #1.

Animals #3 and 4 had a severe weight deficit, compared to normal reference values, namely 32% for animal #3, and 37% for animal #4. Weight deficit was only 6,5% for sperm whales #1 and 2.

Passive congestion, observed on all 3 animals stranded at Koksijde, even in tissues located at the upper part of dead bodies confirmed an acute circulatory disturbance as the cause of death. The most likely process appeared to be cardio-vascular failure, no lesion being indicative of shock or asphyxia. This conclusion is a mere proposal and could not be confirmed due to the lack of complete dissection of the cardio-pulmonary system.

Relevant lesions were observed on animals #2 and 3, both having acute ante-mortem ulcers of the hard palate. The origin of those lesions is presently under investigation. A report will be published separately.

Ear canal lesions on whale #3 were confirmed on histopathology as being a subacute to chronic otitis. The potential extension of such lesions to the middle ear and inner ear is reported in domestic animals, with corresponding clinical signs. There was no possibility to investigate a potential extension of the lesions to the skull.

Postmortem findings were confirmed on histopathology in regard to a more severe decay in whale #3 than #1 and 2.

Parasitology and bacteriology conclusions were not significant.

7. TOXICOLOGICAL ANALYSES

7.1. Heavy metals

In order to detect possible toxic concentrations, nine heavy metals were analysed in the liver, muscle and kidney of the stranded sperm whales (table 3). Such an assessment is not easy since, on one hand, a wide range of "natural" concentrations can be encountered in a single species (in relation *e.g.* with age, sex and season) and, on the other hand, a more or less important part of toxicant may be found in the tissues bound to some ligands under a detoxified form. This is well known *e.g.* for zinc, cadmium, copper and mercury which can bind to metallothioneins (cytosolic low-molecular weight proteins with high cystein content). The speciation of the four metals has therefore been studied with respect to their binding to these proteins (tables 4, 5, 6 & 7 for zinc, cadmium, copper and inorganic mercury respectively). Inorganic mercury is moreover known to bind to selenium as non-toxic thiemannite in the lysosomes of liver and kidneys of some seabird and marine mammal species. The selenium content of both organs of the sperm whales has therefore been analysed in order to assess to which extend mercury could be stored under the thiemannite form (table 8).

Zinc, lead, nickel, cadmium, iron, chromium, copper and titanium were analysed by I.C.P.S., total mercury and selenium by flameless atomic absorption spectrophotometry and cathodic stripping voltammetry respectively. Methylmercury was determined by gas chromatography. Total lipids were estimated following the sulphophosphovanillin method. Polar lipids were extracted with HGRAMG in a 12 H Soxhlet extractor.

Tissues were homogenized and then centrifuged at 26,000 g to separate "soluble" and "insoluble" fractions. The supernatant was filtered on a LKB Ultrogel AcA 54 column. Copper, zinc , cadmium and mercury were analysed in the chromatographic fractions by atomic absorption spectrophotometry.

The fact that analyses of metallothioneins were performed on tissues deep-frozen several hours after the death of the animals requires some comments, since our technical approach implies that these proteins have not been hydrolysed and that the binding of the metals has been maintained. Fortunately metallothioneins are thermostable proteins and according to both the sharpness of the chromatographic peaks and previous observations on stranded guillemots' metallothioneins (see Bouquegneau et al, 1995), it appears that the tissues were fresh enough to assess with sufficient accuracy the actual amount of heavy metals bound to the metallothioneins.

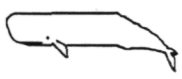


Table 3: Heavy metal content of liver, muscle and kidney (mg/kg dw) of the sperm whales stranded on the Belgian coast, Nov. 18, 1994.

	Animal #	**Liver**	**Muscle**	**Kidney**
Zn	1	90	184	84
	2	100	237	140
	3	95	140	
	4		148	
Pb	1	2.2	0.8	1.0
	2	1.1	2.8	3.6
	3	0.9	1.6	
	4		1.4	
Ni	1	0.2	0.4	0.4
	2	0.2	0.6	0.7
	3	0.3	0.4	
	4		0.4	
Cd	1	103	1.4	225
	2	71	1.7	316
	3	64	1.0	
	4		1.9	
Fe	1	2560	393	1190
	2	2110	552	1130
	3	1990	430	
	4		590	
Cr	1	0.1	0.9	0.4
	2	0.1	0.7	0.7
	3	0.3	0.3	
	4		1.2	
Cu	1	5.3	1.9	13.4
	2	7.9	1.6	43.5
	3	6.5	1.3	
	4		2.5	
Ti	1	0.2	<0.05	0.3
	2	<0.05	1.3	1.2
	3	<0.05	<0.05	
	4		<0.05	
Hg	1	8.7	3.1	2.0
	2	60.8	4.5	1.2
	3	43.6	4.1	
	4		3.9	
MeHg	1	0.7	2.4	0.4
	2	2.4	3.6	0.2
	3	3.0		
	4		2.8	

Table 4: Zinc speciation in liver and kidney of the sperm whales stranded on the Belgian coast, Nov. 18, 1994.

	Sperm whale #1	Sperm whale #2	Sperm whale #3	Ridlington et al (1981)
Liver				
Total content (mgZn/kg fw)	28	30	33	39.8
I.F.	15.1 (54%)	15.0 (50%)	15.8 (48%)	10.6 (27%)
S.F.	12.9 (46%)	15.0 (50%)	17.2 (52%)	29.2 (73%)
MT	4.5 (16%)	4.8 (16%)	6.6 (20%)	14.0 (35%)
Kidney				
Total content (mgZn/kg fw)	26.7	45		
I.F.	6.2 (23%)	10.4 (23%)		
S.F.	20.5 (77%)	34.6 (77%)		
MT	8.9 (33%)	21.1 (47%)		

I.F.= insoluble fraction / S.F. = soluble fraction (cytosol)
MT = fraction bound to metallothioneins.

Table 5: Cadmium speciation in liver and kidney of the sperm whales stranded on the Belgian coast, Nov. 18, 1994.

	Sperm whale #1	Sperm whale #2	Sperm whale #3	Ridlington et al (1981)
Liver				
Total content (mgCd/kg fw)	32	21	22	12
I.F.	19.5 (61%)	9.5 (45%)	10.3 (47%)	1 (8%)
S.F.	12.5 (39%)	11.5 (55%)	11.7 (53%)	11 (92%)
MT	1.9 (6%)	2.1 (10%)	2.6 (12%)	11 (92%)
Kidney				
Total content (mgCd/kg fw)	83	101		
I.F.	15.8 (19%)	15.2 (15%)		
S.F.	67.2 (81%)	85.8 (85%)		
MT	21.6 (26%)	54.9 (54%)		

I.F.= insoluble fraction / S.F. = soluble fraction (cytosol)
MT = fraction bound to metallothioneins.

Table 6: Copper speciation in liver and kidney of the sperm whales stranded on the Belgian coast, Nov. 18, 1994.

	Sperm whale #1	Sperm whale #2	Sperm whale #3	Ridlington et al (1981)
Liver				
Total content (mgCu/kg fw)	1.6	2.4	2.3	3.0
I.F.	1.2 (75%)	1.8 (74%)	1.8 (78%)	1.1 (37%)
S.F.	0.4 (25%)	0.6 (26%)	0.5 (22%)	1.9 (63%)
MT	0.1 (8%)	0 (0%)	0.1 (4%)	0 (0%)
Kidney				
Total content (mgCu/kg fw)	5.1	13.9		
I.F.	2.5 (49%)	9.0 (65%)		
S.F.	2.6 (51%)	4.9 (35%)		
MT	1.6 (32%)	2.4 (17%)		

I.F.= insoluble fraction / S.F. = soluble fraction (cytosol)
MT = fraction bound to metallothioneins.

Table 7: Mercury speciation in liver, kidney and muscle of the sperm whales stranded on the Belgian coast, Nov. 18, 1994.

	Sperm whale #1	Sperm whale #2	Sperm whale #3
Liver			
Total content (mgHg/kg fw)	3.2	19.8	14.7
MeHg	8%	4%	7%
I.F.	85%	95%	84%
S.F.	15%	5%	16%
MT	2%	<1%	<1%
Kidney			
Total content (mgHg/kg fw)	0.5	0.6	
MeHg	21%	21%	
I.F.	72%	70%	
S.F.	28%	30%	
MT	1 %	14%	
Muscle			
Total content (mg/kg fw)	0.9	1.3	1.1
MeHg	76%	79%	

I.F. = insoluble fraction / S.F. = soluble fraction (cytosol)
MT = fraction bound to metallothioneins

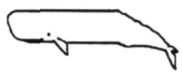


Table 8: Molar ratio between total Hg and Se contents in the liver, kidney and muscle of the sperm whales stranded on the Belgian coast, Nov. 18, 1994.

	Sperm whale #	Hg/Se
Liver	1	0.59
	2	0.55
	3	0.87
Kidney	1	0.09
	2	0.08
Muscle	1	0.54
	2	0.67
	3	0.49
	4	0.67

Table 9: Heavy metal content (mg/kg dw) of liver and muscle of sperm whales stranded on Belgian and North Pacific American coasts.

	Liver				**Muscle**
	Zn	Cd	Cu	Hg	Hg
Belgian coast (individual data)					
1994 : 1	90	103	5.3	8.7	3.1
2	100	71	7.9	60.8	4.5
3	95	64	6.5	43.6	4.1
4					3.9
1989 [1]				50.0	2.7
North Pacific coast mean values	124[2]	39[2]	9.4[2]		
range					(4.0 - 5.6)[3]

[1] male adult stranded alive on 12.02.89 (Joiris et al., 1991)

[2] from Nagakura et al (1974)

[3] from Ridlington et al (1981)

Few data are available in the literature which allow to assess the potential toxicity of metals contained in the sperm whales stranded at Koksijde on November 18th 1994. From table 1 and literature data about cetaceans (see Thompson, 1990) and sperm whales in particular (Ridlington et al, 1981; Nagakura et al, 1974; Joiris et al, 1991); zinc, lead, nickel, chromium and copper concentrations are to be considered low (see table 9). Mercury content of muscle was high, but in both the range of sperm whales from the North Pacific (table 9) and the sperm whale which was found stranded in 1989 along the Belgian coast. However, sperm whale #1, probably the youngest individual among the four, displaid a lower mercury concentration.

On the contrary, the cadmium content of the liver was very high, twice the figures given for the livers of the North Pacific sperm whales described by Ridlington et al (1981), but however in the range of the liver cadmium concentration of mammal species which are feeding on cephalopods, as shown in table 10.

This suggests a potential toxicity of cadmium which is strengthened by the study of the metal speciation (table 5): only a small part of the cadmium appeared to be detoxified through binding to metallothioneins (10 %) against 92 % in the livers of North Pacific sperm whales described by Ridlington. Cadmium, on the opposite to zinc and copper which were normally bound to metallothioneins (see tables 2 & 4), was potentially highly toxic for the animals.

Table 10: Mean cadmium concentration in the liver of cetaceans.

Species	(mg/kg fw)	Main diet
Harbour porpoise	0.2[(1)]	fish
Beluga	0.9[(1)]	fish
Bowhead whale	1.5[(1)]	crustacea/pteropods
Bottlenose whale	5.6[(1)]	**cephalopods**
Striped dolphin	6.3[(1)]	fish/**cephalopods**
Sperm whale	12.0[(2)]	**cephalopods**
Sperm whale	25.0[(3)]	**cephalopods**
Narwhal	32.0[(1)]	**cephalopods**
Ziphius	50.5[(1)]	**cephalopods**
Pilot whale	69.4[(1)]	**cephalopods**/fish

[(1)] compiled from Thompson (1990)
[(2)] from Ridlington et al (1981)
[(3)] sperm whales stranded at Koksijde on November 18th 1994.

Considering mercury (the other potentially toxic metal found in high amounts in tissues), hepatic concentration was low in sperm whale #1 (table 9) whilst it was high in the other three animals. The metal stored in the liver and kidney was mainly inorganic (less than 10 % was found under the methylated form in liver, 21 % in kidney) but, however, was not significantly bound to metallothioneins (table 7). Mercury could be mainly detoxified under the thiemannite form in the liver and kidneys of the 3 sperm whales, since the molar ratio of mercury and selenium was lower than 1 (table 8). We concluded that cadmium was potentially highly toxic for all the sperm whales. That metal is known to induce debilitation in mammals. Such a debilitation, previously quoted, is confirmed by the relatively low lipid content of liver and muscle, compared with other available data (table 11).

Table 11: Total lipid content of liver and muscle (% dw) of cetaceans.

	Liver	**Muscle**
Sperm whales stranded along the Belgian coast, Nov.18,1994		
n° 1	11	6
n° 2	12	7
n° 3	11	7
n° 4		7
mean	11	7
White-beaked dolphins stranded along the Belgian coast in		
92-93	13	11
93-94	13	5
mean	13	8
Harbour porpoise stranded along the Belgian coast in 92-93	13	15
Sei whale Bottino, 1978	22	10

7.2. Organic xenobionts

PCBs concentrations were determined by ECD-gas chromatography using a capillary CP-Sil 4 column, N_2 as carrier gas, a temperature programme from 60 to 270°, detector T300° after an hexane extraction and a florisil clean-up.

Data for Total PCBs (T PCBs) ranged from 1 mg/kg dw in muscle up to 17 mg/kg dw for adipose tissue. Expressed on a lipid weight basis, all tissues had concentrations exceeding 10 mg/kg. DDE concentrations up to 6 mg/kg were found.

Individual T PCBs and DDE results for muscle (M), liver (L), kidney (K) and blubber tissue (Bl), expressed respectively on a dry weight and on a lipid weight (lw) basis, are reported in table 12. DDT, Aldrine, Heptaclor were never found (detection limits on average 0.2 mg/kg lw).

Table 12: Total PCBs and DDE content in muscle (M), liver (L), kidney (K) and blubber of stranded sperm whales on the Belgian coast, Nov. 18, 1994.

Sperm whale #	tissue	mg/kg dw tot PCBs (1260)	mg/kg dw DDE	mg/kg lw tot PCBs (1260)	mg/kg lw DDE
1	M	2.6	0.3	14.2	1.9
	L	3.1	0.6	13.5	2.4
	K	29.1	14.9		
	Bl	17.5	2.4	21.0	2.9
2	M	1.6	0.2	24.0	3.2
	L	1.8	0.2	16.1	1.9
	K	7.4	0.8		
	Bl	10.9	6.3	11.7	6.8
3	M	1.0	0.1	14.3	1.6
	L	1.1	0.1	10.8	0.8
4	M	2.4	0.3	3.0	2.6
	Bl	12.2	4.8	14.6	5.8

Our results not only showed high concentrations, they also indicated a very high total load if we consider that *e.g.* 30 to 40 % of the body weight consist of adipose tissue, an equal amount of muscle tissue. A quick calculation thus leads us to total burden of 200 g organochlorine compounds for each of the sperm whales.

Reference data are not at all abundant. Because of differences in the units used, comparisons are sometimes difficult to make. We made a first selection of the literature (table 13).

Table 13: sperm whales T PCBs and DDE values in muscle (M), liver (L), kidney (K) and blubber (Bl) reported in the literature.

	n	M	L	K	Bl	unit	land
DDE	4-8 males	2.3	4.0	3.3	2.9	µg/g lw [1]	Spain
	4-6 females	2.9	6.5	2.9	4.0	µg/g lw [1]	id.
DDE	10				4.2	µg/g lw [2]	Iceland
DDE	6				3.6	µg/g fw [3]	California
DDE	12				0.22	µg/g fw [4]	Antarctic
∑PCBs	4-8 males	24.1	30.1	9.4	9.9	µg/g lw [1]	Spain
	4-6 females	30.7	18.6	9.2	15.6	µg/g lw [1]	id.
∑PCBs	2				39.1	µg/g lw [5]	France
∑PCBs	10				10.5	µg/g lw [2]	Iceland

[1] Aguilar, 1983
[2] Borrel, 1993
[3] Wolman &Wilson, 1970
[4] Henry & Best, 1983
[5] Alzieu & Duguy, 1979

Any comparison with literature data without the exact ages is hard to make. Age data are usually not available but perhaps more important, our own data for the age of the animals are not yet available.

The only conclusion that can be drawn at this stage is that our data correspond very well with the levels previously mentioned (table 13). Expressed on a lipid weight basis, all data clearly exceed 10 ppm, and this for almost all individual tissues. However, the fact that the levels of organochlorine compounds found did not exceed median levels when compared with literature data does not imply that no effect would evolve from these concentrations. Organochlorine levels by far lower were found to have severe effects (teratogenic, immunodeficiency) in cetaceans and pinnipeds.

Some of our data still need to be completed; *e.g.* those on the lipid content of kidney tissue or the analysis for adipose tissue of sperm whale #3.

7.3. Conclusions based on toxicology

The four sperm whales stranded on the Belgian coast in November 1994 exhibited high levels of contamination by cadmium, mercury, PCBs and DDE.

Cadmium, which is known to induce debilitation in mammals, was found in very high concentrations, twice those previously described in the literature for sperm whales. It was not found, as it is generally the case, to be detoxified by metallothioneins. Concentrations of PCBs, DDE and mercury were in the range of literature data, but high enough to also induce severe effects such as debilitation and immunodeficiency. Mercury was not under the Hg-thionein, but could be detoxified under the thiemannite form, which should be confirmed later.

8. GENERAL CONCLUSIONS

For the present, available data on the stranding of sperm whales on Nov. 18, 1994 in Belgium, indicate that 3 animals, namely #1, 2 and 3, stranded alive at Koksijde. The 4th animal, the largest of the group, was found dead at sea and was towed to the shore during the night of 18-19 Nov.. There was no evidence of live stranding for that animal.

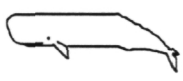
Severe debilitation, with weight loss of 32-37%, was evident in whales 3 and 4. The slight deficit in weight of sperm whales 1 and 2 (around 6% together) could have been shared by both animals, or represent a 12% loss in one animal. No definitive answer can be provided, since carcasses were weighted together. Blubber thickness was similar in both whales, but the validity of this parameter as an indication of weight loss in sperm whales is debatable (Lockyer, 1991).

On necropsy, there was no clear evidence of chronic lesions compatible with severe weight loss. A chronic exposure to debilitating toxics, such as xenobionts and heavy metals, is therefore a strong possibility, that is sustained by toxicology data. Total loads in cadmium, mercury, PCBs and DDE were high. Among those, it is noteworthly that mercury levels were low in sperm whale #1 only, an animal with a weight close to the normal range.

An acute disease could have prompted the stranding of the group. The most likely cause would have to be found in most severely exposed animals, namely severely debilitated #3 and 4.

Number 4, the largest, and possible leader of the group is a likely candidate. Unfortunately, it could not be necropsied. In the 3 smaller whales, #3 is to be singled out as severely debilitated, affected with large acute ulcers in the mouth and with external ear canal lesions.

However incomplete the conclusions might appear at the present time, it is coherent to imagine that the group leader (#4) died at sea and caused considerable stress to its companions, particularly one of the younger males (#3), chronically debilitated for unknown reasons, most probably stable pollutants, a condition possibly compounded by an acute severe disease. This situation may have been responsible for the stranding of the group: the shallow waters of the Belgian coastline, particularly around Koksijde, may have been fatal to the whales, animals #1 and 2 becoming disoriented by unfamiliar environment, the loss of their leader and the erratic behavior of their diseased companion (#3).



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10. APPENDIX

SAMPLES	Sperm whale #1	Sperm whale #2	Sperm whale #3	Sperm whale #4
Histopathology (1)				
diaphragm	x			
spleen	x	x		
lung	x			
kidney	x	x		
liver	x	x	x	
colon	x	x	x	
ileum	x	x	x	
gastric wall	x		x	
intestinal node	x			
palate ulcer		x	x	
blubber node		x	x	
ear duct			x	
skin			x	
Toxicology (2) (3) (4) (5)				
kidney	x	x		
muscle	x	x	x	x
liver	x	x	x	
blubber	x	x	x	x
bone (rib)	x			
ileum	x	x	x	
Bacteriology (6)				
intestine	x	x	x	
Parasitology (7)				
intestine	x	x	x	
parasites		x	x	
DNA analysis (8)				
skin	x	x	x	x
Ophthalmology (9)				
eye	x	x	x	
Age determination (10)				
lower jaw	x	x	x	x
Prey study (11)				
food remain	x	x	x	x

Samples collection on the 4 sperm whales stranded on the Belgian coast (November 18, 1994).

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(11) collection in (1)