

## Relative impact of starvation and oil on digestive and kidney functions of sea birds

Impact relatif du jeûne et du mazoutage sur les fonctions digestives et rénales des oiseaux marins  
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## Introduction

Since Erika wreckage in december 1999, the wildlife centre of National Vet School of Nantes, care for oiled birds and carries studies in order to upgrade the management of oiled birds, after chronical pollution as well as oil spill (see Le Dréan-Quénez'hdu 2001, 2002, 2003, 2004).

The arguments for wildlife rehabilitation are ethical, biological, efficiency, public relation and financial criterions (Table I)

However, in order to have more convaincing arguments « for » the rehabilitation, we need to have a very efficient rehabilitation. The efficiency need triage of birds able to be rehabilitated, and it all the more that the number of oiled birds is high.

This triage is based on medical and non medical criterion (see Le Dréan-Quénez'hdu 2004).

The non-medical criteria are :

- the carrying capacity of the wildlife centre : it need to be in adequation with the number of birds and with the species
- the birds species and their age : some specie may by firtsly cared because of their patrimonial value, for example
- the time between the supposed oiling and the arrival in the wildlife centre : the longer it is the lower the survival chances are

The medical criteria are :

- the weigth: the birds need to have a minimal weigth to be washed. The their arrival weight need to be compatible with reaching this weight into few days
- the presence of irreversible lesions or of not reversible lesions in oil spill conditions (that is to say, needing care technics to expensive or to long) or in few days (needing a detention stay to long for the species)
- the presence of diseases highly contagious for other birds, for environment and for people

Table I : Rehabilitation of Oiled Wildlife: Common Arguments for and Against (after IPIECA, 2004).

<b>Arguments for</b>	<b>Arguments against</b>
<b><i>Philosophical</i></b>	
Anthropogenic- Oil spills are caused by humans and as such there is an ethical responsibility for humans to mitigate the damage to the environment and the individual animals who are impacted.	The rehabilitation of oiled wildlife life causes stress and suffering. They should be euthanised.
We have the ability: This argues that since we have the ability to stop suffering and return animals harmed by oil spills that we should.	
<b><i>Biological</i></b>	
When a significant percentage of a population of a threatened or endangered species is oiled successful rehabilitation can make a very real difference in that species survival.	Rehabilitation of oiled wildlife has no impact on the population.
The techniques developed working with oiled individuals of common species can be utilized when a spill threatens rarer species.	Rehabilitated oiled wildlife fail to reproduce.
<b><i>Legal</i></b>	
There are legal requirements for the rehabilitation of oil-affected animals in some countries.	
<b><i>Effectiveness</i></b>	
Post-release survival data are encouraging for a number of species	All the animals die anyway.
<b><i>Public Relations</i></b>	
The public may demand that oiled wildlife be cared for and may do so themselves if an organized effort is not mounted.	Rehabilitating oiled wildlife gives the impression that oil spills are not a problem.
<b><i>Financial</i></b>	
The cost of rehabilitation of oiled wildlife is a small percentage of the overall response	Rehabilitation of oiled wildlife is too expensive.

In studies on oiled birds (see for synthesis Bastien-Ventura *et al.* 2005), the impacts of oil on digestive and kidney functions are often mentioned without knowing if the observed lesions (mainly hemorrhagic enteritis) are reversible. In the other hand we don't know if the lesions are in direct relation with oil toxicity or with the prolonged starvation.

In this study, we will see the two aspects. After a presentation of the study methods, we will expose the results and then we will propose a first triage guideline of oiled birds in case of oil spill.



## I. Materials and methods

### 1. Birds collected

A total of 13 birds have been included in our study. This low number is due in one hand to the low number of oiled birds in the wildlife center because of the warm weather and perhaps because of the decrease of chronic pollution. In the other hand, the threat of an influenza breakdown in Europe have very largely contributed to decrease the number of birds cared in all the wildlife centres.

Each birds is individually noted : the card are given in annexs.

The characteristics of each birds are given in Table II.

Table II : characteristics of common Guillemots included in the study

Number	Age	weight	Behaviour	Oiling	Dropping	future
06-084	Adult	502	Very weak	Ventral 40 %	Normal	Dead arrival
05-029	Adult	526	Dead	Ventral 85 %, dorsal 10 %	Normal	Dead arrival
05-023	Adult	538	Dead	Ventral 40 %	/	Dead arrival
06-071	Adult	638	Sharp	Ventral 50 %	Normal	Dead in swimming pool
06-028	Adult	652	Sharp	Ventral 60 %	Normal	Dead before washing
06-018	Adult	490	Weak	Ventral 50 %	Normal	Dead 24 hours
05-021	Adult	859	Sharp	Ventral 95 %, dorsal 5 %	Normal	Released
05-031	Juvenil	800	Sharp	/	Normal	Released
05-030	Juvenil	779	Sharp	/	Normal	Released
05-024	Immature	516	Weak	Ventral 30 %, dorsal 5 %	Not noted	Dead 24 hours
05-013	Adult	470	Dead	Ventral 90 %	Not noted	Dead arrival
05-015	Adult	480	Dead	Ventral 30 %, dorsal 50 %		Dead arrival
05-022	Adult	616	Sharp	Ventral 60 %, dorsal 20 %	Normal	Dead 48 hours

We can see the arrival weights are very low with some time a lost of more than 50% of the total weight: this implies that these birds have starved since a long time before arriving in the wildlife centre.

In the case of death, an autopsy is performed. We noted the lesions on the different organs and particularly the presence of haemorrhagic diathesis on gut (see annex, autopsy card).

The following samples are made :

- Gut: a fragment of intestine and caecum are collected on the area with and without diathesis for histological analysis. A brushing is also made in the same place for bacteriological analysis.
- Kidney: the kidneys are collected for histological analysis
- Liver: the liver is collected for PAH analysis

## **2. Analysis performed**

### **2.1 Histological analysis**

The organs collected are fixed in formaldehyde and managed after classical histological techniques (cutting, inclusion coloration Hemalun-Eosine Safran, basic coloration and eventually specific coloration). The samples are made during autopsy.

The analysis has been made in the pathological anatomy department of National Vet School of Nantes (France).

## 2.1 Bacteriology

The aims of the analysis are to identify the bacteria and eventually serotyping in case of *E. coli* isolment.

The analysis have been performed in Diagnostic Laboratory NOIVBD de Veldhoven, Pays Bas, by Professor G. Dorrenstein.

Les analyses ont été réalisées au Diagnostic Laboratory NOIVBD de Veldhoven, Pays Bas, par le Professeur G. Dorrenstein.

After arrival of the samples they were refrigerated (+ 4° C) until processed.

Between 10.30 and 11.30 all samples were added to 5 ml buffered peptone water (BPW pH 7.2, batch 20051200187, Tritium Microbiology, Veldhoven). During the day the BPW samples were stored at +4°C and were several times during the day vigorously shaken.

Between 19.00 and 20.00 1 ose was plated to a Columbia blood agar disk in 3 line streaks (photo 1) and to a brilliant-green-agar disk (photo 2) (Col BL, pH 7.0, batch 20060400441, and BGA M pH 7.0, batch 20060400254, Tritium Microbiology Veldhoven).

To get a semi-quantitative impression of the number of microorganisms present in the BPW suspension to be cultured, 3 drops of the BPW suspension were plated to a Col BL (photo 3).

All plates were incubated at 37°C under aerobe conditions for 24 hours.

To get an impression about the number and ratio of microorganisms (rods, cocci or yeasts) in the BPW suspension 1 drop was added to a microscopic glass-slide and dried at the air and stained with Hemacolor® (Merck).

After 25 hours all plates were evaluated, photographically documented and separate colonies selected from the different birds for pure culturing. The amount of growth is noted as streak 1, 2, or 3 and the estimated number of colonies. The first streak with separate colonies is given. E.g. 2/>50 means streak 2, at least 50 colonies.

Smears were made of the different colonies and stained with Hemacolor® for morphological evaluation.

Based on morphology of the growth on Col BL and microscopic evaluation different bacterial isolates (total 17, from 9 birds) were selected and sent to Brabant Veterinary Laboratory (Diessen, The Netherlands) for determination. The isolated *E. coli* strains (n=7) were sent to the reference laboratory of the National Health Institute (RIVM, Bilthoven, The Netherlands) for serotyping.



Photo 1 : Col BL de  
l'échantillon 23 :  
3/>50 colonies



Photo 2 : BGA de  
l'échantillon 23: 2/ >100  
colonies



Photo 3 : échantillon 23,  
pousse complète (+++++)

## 2.1 PAH analysis

The samples were feathers and livers.

The method used by CEDRE have several stages :

- Extraction / purification of sample : the extraction is done with dichloromethane which is then filtered, dried then evaporated. The hydrocarbons are then weighed, just as the separate feathers of the residues. The hydrocarbons are possibly purified according to their mass. The purification is a separation of phase on silica column.
- Analysis with GC/MS : the analysis is carried out by gas chromatography coupled to a detection by mass spectrometry (GC/MS).
- Quantification of the aromatic compounds: the aromatic compounds are quantified compared to the internal standards, the factors of response being given using standard solutions.

The analyses were carried out with the CEDRE, Brest (France), after calibration for each category of sample (Table III): the response of the apparatus to the PAH to be proportioned is expressed compared to the internal standards. The ki reports/ratios then calculated are included in the worksheets of the concentrations.

Table III : resultats of calibration (A : feathers, B : liver)

A)

		Area	Ci (µg/mL)	Cf (µg/mL)	$k_{IS} = C_{IS} / A_{IS}$	
164	SRM 2269	Biphenyl d <sub>10</sub>	1,00	0,1	1,1E-06	
188		Phenanthrene d <sub>10</sub>	116694	0,1	8,6E-07	
240		Chrysene d <sub>12</sub>	109901	1,0	0,1	9,1E-07
136		Naphtalène d <sub>8</sub>	122534	1,00	0,1	8,2E-07
264		Benza[a]pyrene d <sub>12</sub>	116381	1,00	0,1	8,6E-07

	Etalon interne	PAH	Area	Ci (µg/ml)	Cf (µg/ml)	$k_i = C_f / A_i$	$K_i = k_i / k_{IS}$
128	Naphtalène d8	Naphtalene	8 420 966	20,13	8,05	9,6E-07	1,17
152		Acenaphtylene	8 429 455	15,49	6,20	7,4E-07	0,90
154		Acenaphtene	7 273 608	20,77	8,31	1,1E-06	1,40
166		Fluorene	1 669 024	4,75	1,90	1,1E-06	1,39
178	Phenanthrene d <sub>10</sub>	Phenanthrene	1 609 449	3,42	1,37	8,5E-07	0,99
178		Anthracene	325 141	0,79	0,32	9,7E-07	1,13
202		Fluoranthene	4 556 095	7,64	3,06	6,7E-07	0,78
202		Pyrene	5 541 720	8,47	3,39	6,1E-07	0,71
228	Chrysene d12	Chrysene	1 777 529	3,67	1,47	8,3E-07	0,91
228		Benz[a]anthracene	1 801 065	4,09	1,64	9,1E-07	1,00
252		Benzo[b+k]fluoranthene	4 273 535	8,89	3,56	8,3E-07	0,91
252	Benza[a]pyrene d12	Benzo[a]pyrene	2 098 251	4,91	1,96	9,4E-07	1,09
276		Indeno(1,2,3-cd)pyrene	1 772 191	4,28	1,71	9,7E-07	1,12
278		Dibenz(a,h)anthracene	1 242 864	3,54	1,42	1,1E-06	1,33
276		Benzo(g,h,i)perylene	1 298 945	3,68	1,47	1,1E-06	1,32

[b] 1757173

[k] 2516362

calibration solution: 20 µL of SRM 2269 + 80 µL of SRM 1647d  
 + 100 µL CH<sub>2</sub>Cl<sub>2</sub>

**B)**

		Area	Ci (µg/mL)	Cf (µg/mL)	$k_{IS} = C_{IS} / A_{IS}$	
164	<b>SRM 2269</b>	Biphenyl d <sub>10</sub>	1,00	0,1	5,5E-07	
188		Phenanthrene d <sub>10</sub>	133316	0,1	7,5E-07	
240		Chrysene d <sub>12</sub>	78470	1,0	1,3E-06	
136		Naphtalène d <sub>8</sub>	180169	1,00	0,1	5,6E-07
264		Benza[a]pyrene d <sub>12</sub>	49490	1,00	0,1	2,0E-06

	Intern standard	PAH	Area	Ci (µg/ml)	Cf (µg/ml)	$k_i = C_f / A_i$	$K_i = k_i / k_{IS}$
128	<b>Naphtalène d8</b>	Naphtalene	12 545 823	20,13	8,05	6,4E-07	1,16
152		Acenaphtylene	9 386 426	15,49	6,20	6,6E-07	1,19
154		Acenaphtene	9 547 068	20,77	8,31	8,7E-07	1,57
166		Fluorene	2 044 343	4,75	1,90	9,3E-07	1,67
178	<b>Phenanthrene d10</b>	Phenanthrene	1 550 696	3,42	1,37	8,8E-07	1,18
178		Anthracene	778 748	0,79	0,32	4,1E-07	0,54
202		Fluoranthene	5 440 473	7,64	3,06	5,6E-07	0,75
202		Pyrene	6 424 623	8,47	3,39	5,3E-07	0,70
228	<b>Chrysene d12</b>	Chrysene	1 413 964	3,67	1,47	1,0E-06	0,81
228		Benz[a]anthracene	882 394	4,09	1,64	1,9E-06	1,45
252		Benzo[b+k]fluoranthene	2 601 086	8,89	3,56	1,4E-06	1,07
252	<b>Benza[a]pyrene d12</b>	Benzo[a]pyrene	958 274	4,91	1,96	2,0E-06	1,01
276		Indeno(1,2,3-cd)pyrene	455 877	4,28	1,71	3,8E-06	1,86
278		Dibenz(a,h)anthracene	310 715	3,54	1,42	4,6E-06	2,26
276		Benzo(g,h,i)perylene	359 942	3,68	1,47	4,1E-06	2,02

[b]

[k]

Calibration solution : 20 µL of SRM 2269 + 80 µL of SRM 1647d + 100 µL CH<sub>2</sub>Cl<sub>2</sub>

## **II. Results**

### **1. Autopsy – histology**

A total of 11 birds was the subject of histological analyses for the digestive tract and the kidneys (Table IV).

Concerning the digestive tracts, there are no characteristic lesions: only two birds show an exfoliation of the cells of villousities and two birds of the microabscesses or cysts being able to be in connection with the presence of coccidies. It should be noted that in the case of hemorrhagic diathèse one observes lesions of autolysis and the presence of a maroon granulous material which is not hydrocarbon

Concerning the kidney and as in the preceding studies one observes modifications histological on all the analyses with in particular of the more or less marked urétérites and a metaplasie malpighienne on the level of the uretere. In two birds one notes in more of the lesions of exfoliation or erosion in relation to the presence of coccidies.

Table IV: results of the histological analyses (NSL : no significant lesions, SM : small intestine)

<b>birds</b>	<b>Gut</b>	<b>kidneys</b>
05-013	SM : NSL + coupled abundant maroon material granulous with the mucous membrane	Paving epithelium pluristratified on the level of the mucous membrane of uretere, heavy lymphocytary infiltration of the chorion
05-29	SM : In-depth enchased abscess of bacterial origin + coupled abundant maroon material granulous with the mucous membrane	Paving epithelium pluristratified on the level of the mucous membrane of uretere, small lymphocytary infiltration of the chorion
05-23	SM : Severe autolytic deterioration + coupled abundant maroon material granulous with the mucous membrane	Paving epithelium pluristratified on the level of the mucous membrane of uretere, heavy lymphocytary infiltration of the chorion
05-24	SM : Exfoliation attends cluster of pycnotic cells between intestinal villosities, often dilated crypts, filled with mucuses, remains cellular and of frequent elements of aspect compatible with coccidies	Paving epithelium pluristratified on the level of the mucous membrane of uretere, heavy lymphocytary infiltration of the chorion moderate erosions or ulcerations multifocales, abundant parasitic elements in the epithelium
05-15	SM : NSL	Paving epithelium pluristratified on the level of the mucous membrane of uretere, small desquamation, small lymphocytary infiltration of the chorion
05-22	SM : Severe autolytic deterioration	/
05-983	Severe autolytic deterioration	Squameuse metaplasia in uretere
06-28	SM : Marked exfoliation of necrotic enterocytic cells	Squameuse metaplasia in uretere
06-71	SM : Infestation coccidienne diffuses intense with moderate multifocaux secondary rehandlings inflammatory	Squameuse petaplasia in uretere
06-84	Duodenum : cysts similar, scattered, exfoliation attends cells necrotic in the light and frequent microabscesses of bacterial origin in the intestinal mucous membrane Jejunum & cæcum: presence of bacterial microabscesses in the crypts, erosions frequent at the tops villositaires	Paving epithelium pluristratified on the level of the mucous membrane of uretere
06-18	Autolytic deterioration	Autolytic deterioration

## 2. Bacteriology

The description of the samples and the results of the study are presented in an XLS table as appendix 1.

A total of 26 samples from 11 guillemots arrived in the diagnostic laboratory of the NOIVBD in Veldhoven, The Netherlands. The number of samples per bird ranged from 1 to 5.

Four samples (1, 3, 20 and 21) originating from 3 different birds (13, 23 and 983) showed no growth. On the blood agar (Col BL) growth was seen in 22 samples (8 birds); on the BGA growth was only seen in 12 samples (5 birds). The amount of growth varies for each colony-type, but was especially on the Col BL optimal. Almost all plated Col BL showed abundant growth (figure 3).

The results of the selected microorganisms for determination and serotyping are presented in table V.

From 4 birds only Gram-positive cocci were found. Only from the intestines of 4 birds (36%) *E. coli* (n = 7) was isolated. There were 4 different serotypes present (O8, O75, O80 and O unknown).

No obvious relation was seen between the hemorrhagic diathesis and microorganisms present.

Table V : Isolated bacteria from the intestinal tract of the different birds

Birds	description	identification BVL	Serotype <i>E.coli</i>
GT 05-13	Small intestin with haemorrhagic diathesis		
	Small intestin without haemorrhagic diathesis	<i>Enterococcus sp</i>	
GT 05-23	Small intestin with haemorrhagic diathesis		
GT 05-22	Small intestin with haemorrhagic diathesis		
	Small intestin without haemorrhagic diathesis	<i>Enterococcus sp</i>	
GT 05-15	Small intestin without haemorrhagic diathesis	<i>Escherichia vulneris</i>	
GT 05-24	Small intestin without haemorrhagic diathesis	<i>Enterococcus sp</i>	
GT 05-29	Small intestin with haemorrhagic diathesis	Levures	
GT 06- 28	Cæcum sans diathèse hémorragique	<i>E. coli</i> <i>Streptococcus group D</i>	serotype O8
	Intestin grêle sans diathèse hémorragique	<i>E. coli</i>	serotype O8
	Cloaque sans diathèse hémorragique		
GT 06-18	Cæcum sans diathèse hémorragique		
	Cloaque sans diathèse hémorragique		
	Cæcum grêle avec diathèse hémorragique	<i>E. coli</i> <i>Morganella morganii</i>	serotype O75
	Small intestin without haemorrhagic diathesis	<i>E. coli</i> Oxidase + Gram negative <i>Bacillus sp.</i>	serotype O8
	Small intestin with haemorrhagic diathesis		
GT 06-71	Cloacal without haemorrhagic diathesis	<i>Streptococcus group D</i>	
	Small intestin without haemorrhagic diathesis		
	Cæcum without haemorrhagic diathesis	<i>E. coli</i> <i>Streptococcus group D</i>	serotype O86
GT 05-983	Small intestin without haemorrhagic diathesis		
	Cloacal without haemorrhagic diathesis		
GT 06-084	Cloacal without haemorrhagic diathesis	<i>E. coli</i>	O-type inconnu
	Small intestin with haemorrhagic diathesis		
	Small intestin without haemorrhagic diathesis		
	Cæcum without haemorrhagic diathesis		
	Proventriculus with haemorrhagic diathesis	<i>E. coli</i>	O- type inconnu
GT 06-71	Cæcum with haemorrhagic diathesis	<i>E.coli</i> <i>Streptococcus spp.</i>	Non O1, O2, O78K80

### 3. PAH analysis

The summary of concentrations of the 16 PAH of the US EPA list are reported in Table VI and in figure 1.

Samples of feathers of 8 birds were analyzed and the samples of liver of 11 birds

Concerning the feathers, 5 birds show contents of HAP higher than 400 µg/g. On the other hand, only two birds show contents of HAP in the livers relatively important (563 and 328 µg/g). It also should be noted that these two birds do not present contents raised at the level of their plumage.

If we interested to the 6 HAP of list AFSSA the contents remain weak.

In detail of the HAP, for the feathers 4 HAP have contents higher than 100 µg/g, which take part to a significant degree on the whole: phenanthrene, pyrene, chrysene and the benzo[a]anthracene. To also note that the 16 HAP are found for all the feathers. On the other hand for the livers, only a HAP, the acenaphtene contribute to a significant degree on the whole measured and only 5 HAP show concentrations superiors to 10 µg/g.

There are thus no relations between the importance of the HAP on the feathers and the importance of the HAP in the bodies of the birds.

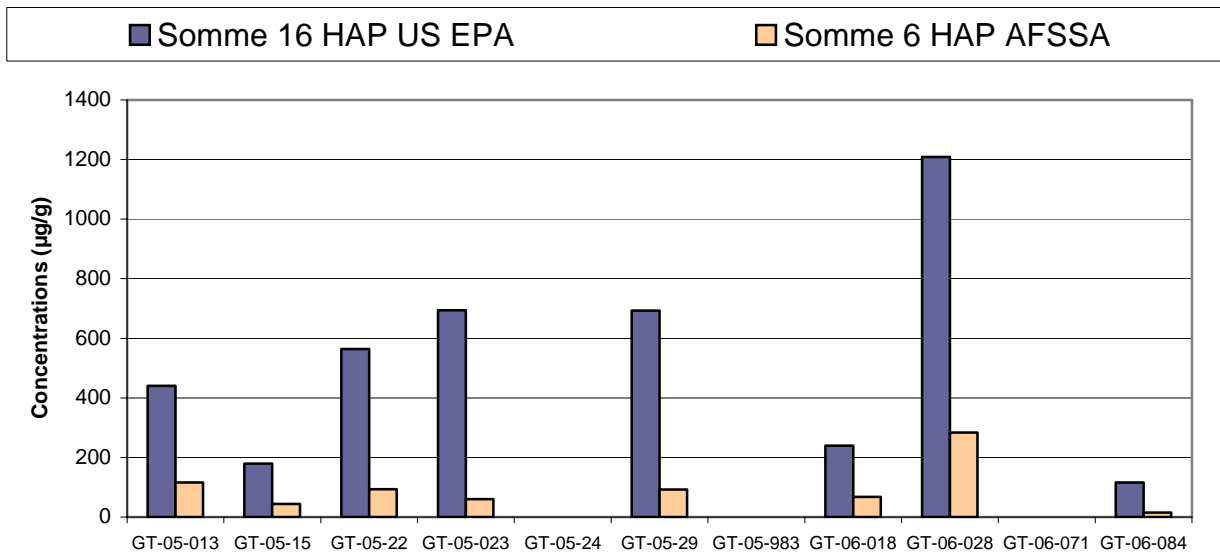
Table VI : summary of the concentrations of 16 HAP of US EPA list on feathers (A) and on livers (B)

		16 HAPs list US EPA								
		ref. <i>Cedre</i>	DV-06-22	DV-06-23	DV-06-24	DV-06-25	DV-06-26	DV-06-27	DV-06-28	DV-06-29
		ref. <i>ENVN</i>	05-013	05-015	05-22	05-023	05-29	06-018	06-028	06-084
A)	Composés (concentration en µg/g HC)	Naphtalene	10	10	10	11	13	17	17	6
		Acenaphtylene	5	3	10	5	15	1	4	0
		Acenaphtene	7	3	4	7	5	9	7	2
		Fluorene	19	7	25	19	22	21	23	9
		Phenanthrene	106	29	232	432	270	31	371	35
		Anthracene	20	5	25	66	40	3	62	21
		Fluoranthene	16	7	18	10	35	5	26	3
		Pyrene	58	33	74	40	130	32	165	15
		Chrysene	73	35	63	36	61	46	232	8
		Benzo[a]anthracene	68	22	59	30	53	30	182	2
		Benzo[b+k]fluoranthene	14	7	13	9	13	9	34	1
		Benzo[a]pyrene	24	13	16	17	19	23	53	8
		Indeno(1,2,3-cd)pyrene	4	1	3	2	3	2	5	1
		Dibenz(a,h)anthracene	6	2	4	3	4	4	9	2
		Benzo(g,h,i)perylene	12	3	8	6	10	7	20	2
		<b>Sum 16 HAP US EPA</b>		440	180	<b>564</b>	<b>694</b>	<b>693</b>	240	<b>1209</b>
<b>Sum 6 HAP AFSSA</b>		116	44	94	60	92	68	283	15	

B)

16 HAPs list US EPA												
ref. <i>Cedre</i>	DV-06-97	DV-06-98	DV-06-99	DV-06-100	DV-06-101	DV-06-102	DV-06-103	DV-06-104	DV-06-105	DV-06-106	DV-06-107	
ref. <i>ENVN</i>	GT-05-013	GT-05-15	GT-05-22	GT-05-023	GT-05-24	GT-05-29	GT-05-983	GT-06-018	GT-06-028	GT-06-071	GT-06-084	
Composés (concentration en µg/g HC)	Naphtalene	23	25	14	13	7	5	4	148	1	2	0
	Acenaphtylene	0	66	0	0	0	0	0	0	0	0	0
	Acenaphtene	16	391	5	26	10	5	4	204	2	2	0
	Fluorene	2	7	6	2	9	4	2	0	0	0	0
	Phenanthrene	19	16	2	12	5	17	2	7	2	2	0
	Anthracene	2	2	7	1	0	1	0	1	0	0	0
	Fluoranthene	2	3	0	1	0	1	0	0	0	0	0
	Pyrene	5	7	1	3	0	2	0	0	0	0	0
	Chrysene	3	1	0	1	0	0	0	5	0	0	1
	Benzo[a]anthracene	2	1	1	0	0	1	0	4	0	0	0
	Benzo[b+k]fluoranthene	1	1	0	0	0	0	0	0	0	0	0
	Benzo[a]pyrene	2	2	0	0	0	0	0	0	0	0	0
	Indeno(1,2,3-cd)pyrene	0	0	0	0	0	0	0	0	0	0	0
	Dibenz(a,h)anthracene	0	0	0	0	0	0	0	0	0	0	0
	Benzo(g,h,i)perylene	0	0	0	0	0	0	0	0	0	0	0
	<b>Somme 16 HAP US EPA</b>	<b>77</b>	<b>523</b>	<b>36</b>	<b>60</b>	<b>32</b>	<b>35</b>	<b>12</b>	<b>368</b>	<b>6</b>	<b>7</b>	<b>1</b>
<b>Somme 6 HAP AFSSA</b>	<b>6</b>	<b>4</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>4</b>	<b>0</b>	<b>0</b>	<b>0</b>	

A)



B)

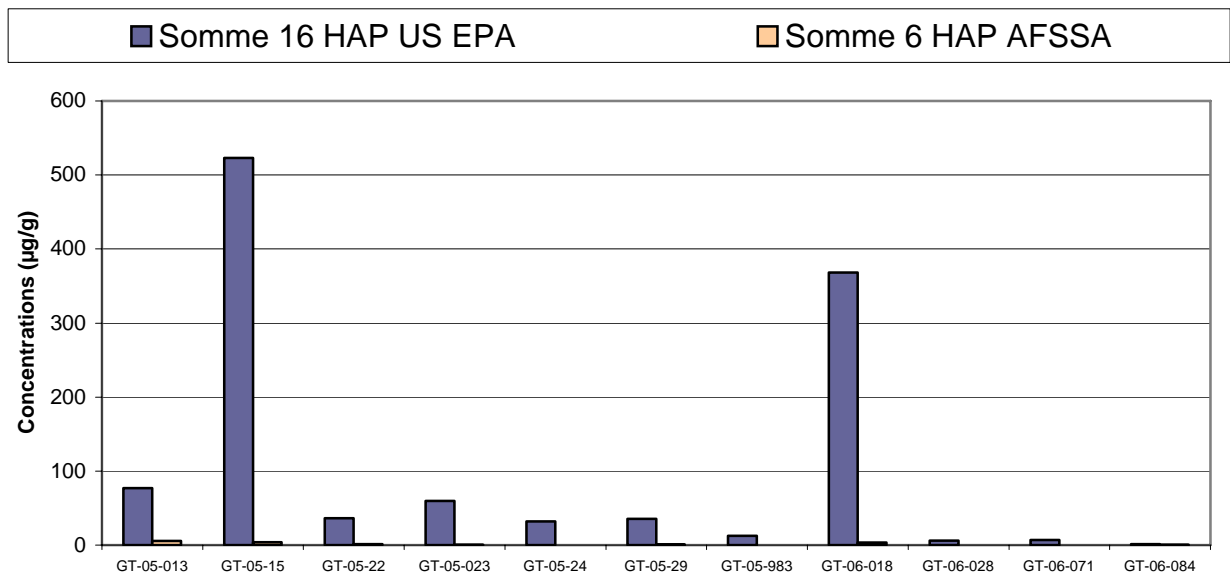


Figure 1 : graphic representation of PAH in feathers (A) and livers (B)

### **III. Discussion**

Among the causes of mortality of the mazoutés birds, first obvious is the toxicity of hydrocarbon: this cause was regarded a long time as only or at least the most important. However the appearance of secondary infections (infectious and traumatic) is a very important cause, especially at the time of massive surge, when the capacity of reception of the center of care is overflowed. From where need for having objective criteria of sorting, in particular in relation to the irreversibility of the lesions.

A third cause and undoubtedly the major cause of mortality of the mazoutés birds is dehydration and the absence of food of the birds, which when they are limed, not only can only feed and hydrate themselves with difficulty but also exhaust their energy reserves to maintain their temperature body and their floating, seriously damaged by the loss of the cushion insulating formed by their feathers. This is shown in other oil slick, as at the time of Prestige (Balseiro *et al.*, 2005).

In this study one thus sought to know if the lesions observed at the renal and digestive level in the preceding studies could comparison with the toxicity of hydrocarbons or the phase of fast induced by engluement and in which measurements these lesions could be irreversible.

#### **1. Study results**

The malpighienne metaplasie malpighienne meets mainly in epithelial fabrics. It is a cellular adaptation to the aggressions of the medium by modifying cellular differentiation. The metaplasie malpighienne relates to the layer known as of Malpighi.

It is reversible when the cause disappears. As in the preceding studies, this lesion is frequent and present at both guillemots presenting contents of HAP raised at the hepatic level.

Concerning the digestive function, one notes that the small intestines are often injured but in a nonspecific way. At the time of the fast, 3 reversible phases are described: in phase I or phase of adaptation, the loss of weight is considerable and very rapid especially at the beginning. In phase II or phase of economy, the loss of weight is constant and weaker. Phase III intervenes when 80 to 90 % of the lipids were used: the proteins then are also used as energy substrate

(see Geiger, 2004). In phase III, there are then phenomena of modification of the cellular permeability and defects of absorption. Being given the weights of the birds of our study (less than 650 grams for all the dead birds), one could be located in phase II or III of the phase of fast.

In addition, Smirnov *et al.* (2004) show that the fast in chickens involves a reduction in the layer of mucus of the small intestine due to a weaker secretion of this mucus or a faded turnover. In all the cases the consequences are according to these authors, this deterioration would have consequences on the digestive function and defenses of the digestive tract.

It does not seem on the other hand that one can allot to a bacterial proliferation in particular by *E coli* the lesions observed. On the other hand, it is possible that the HAP play a part. Indeed, naphthalene, benzo[a]pyrene, and the anthracene are known like irritating agents primary education of the skin and the mucous membranes, even if in our study they are not the compounds mainly found. It is however interesting to note that the guillemot 06-28, which arrives in a "sharp" state "of form" noted, with a rather weak weight (652 grams), dies a few days after its arrival: its digestive tract presents a marked exfoliation of necrotic enterocytes and *E coli* serotype O8 and *Streptococcus sp.* are insulated. In addition, the sum of the three HAP quoted previously is higher than 100 µg/g, which one of strongest is measured. It also presents the strongest content for the 16 HAP (1209 µg/g) at the level of the feathers but very weak on the level of the liver. The guillemot 06-71 which also presents a "sharp" state and which takes sufficient the weight to be washed dies in swimming pool without apparent clinical signs. However it presents at the digestive level signs of ignition due to a coccidiose.

**The digestive lesions due on the one hand to the phase of fast and on the other hand possibly to the irritation of certain HAP thus seem not very reversible and in all the cases weaken the birds and expose them to the secondary infections of coccidies or bacterial diseases.** It thus seems illusory to treat birds in phase III of fast, even in phase II when these birds were exposed to hydrocarbons rich in irritating HAP.

**On the other hand concerning the renal lesions, those seem reversible and in all the cases without relationship with the concentrations of HAP measured in the liver.** It also should be noted that at the marine birds, the digestive tract and the kidneys interact in the displacement control of Na, in connection with the operation of salt gland (Hughes, 2003). If the digestive tract is injured, particularly on the level cloacal, the excretion can be disturbed

and homeostasis also. There thus remains preferable to keep fresh water the birds during the process of rehabilitation, the operation of gland with salt showing a great plasticity (Hughes, 2003).

There remains however an interrogation on the impact of composed like the naphthalene found in considerable quantity in the liver of two guillemots, compound known for its embryotoxicity. Thus, Zugerogoitia *et al.* (2006) explain the reduction of fertility of peregrine falcons *Falco peregrinus* of Bizkaia in the north of Spain by the contamination by the PAH at the time of the oil spill of Prestige. The contents measured in the not hatched close to those measured for both Guillemots "are contaminated the most" of our study.

It also should be noted that the acetanaphthalene presents a cumulative character, which could explain the contents measured in the liver without relationship with the contents of the feathers

The real toxicity of the HAP remains very difficult to show in all the cases on complex organizations like the marine birds (Lee and Anderson, 2005).

## **2. Proposition of triage guideline**

The objective of the rehabilitation is that all the animals arriving alive in center of care remain a minimum time there to be able to optimize their chances of survival in nature.

The sorting is the first stage of the rehabilitation and it is thus necessary to wonder whether the tri chances carried out will make it possible to realize at better the following stages.

In addition, it is also important to insist on the quality of the stages preceding the arrival in center by care; in particular, the duration between the capture on the beach and the arrival in center of care must be shortest possible.

The stages of the process of rehabilitation are:

- Reception, identification, sorting of the animals: it is a question of identifying well the animals arriving in term of biological characteristics (trophic species, ages, requirements, statutes...) and in term of "health" and in particular of capacity "to undergo" the process of rehabilitation

- Stabilization of the animals: it is a question of stopping the degradation of the vital functions and of restoring homeostasis.
- Hospitalization: the objective is to bring the birds able to support the process of washing-rinsing-drying in a time not exceeding 7 days. The birds will be gavés. They should not thus present disease requiring a hospitalization of more than 7 days.
- Washing - rinsing - drying: the birds "to wash" must be able to support the stress of this stage. With the end of this stage the birds must be tight.
- Rehabilitation in swimming pool: the objective of this stage is to have birds completely tight, able to remain on water permanently and to feed in a completely autonomous way.
- Release: the birds must be able to live in their natural environment. They should not present diseases or lesions which would limit their chances of survival in nature (for example blindness).

The sort criteria are detailed in Le Dréan-Québec'hdu 2004.

These are :

- Non medical criteria :
  - o Capacity of reception of the site (see Rigaudeau *et al.* 2002). By capacity of reception, one understands not only the material necessary (a many boxes, of swimming pool, number of station of washing), but also the number and the availability of qualified people (competence is well on function of the occupied station) and the existence of a plan, i.e. the possibility of providing itself in material necessary in a short time (for example, it is necessary to be ensured of the availability of the usual suppliers). According to this capacity of reception and multitude envisaged of birds, the sort criteria will be drastic, intermediate or flexible.
  - o Species :
    - Statute: the character of priority can be based on the national list of the species threatened in France (Rocamora & Yeatman-Berthelot 1999). It can well on being adapted according to local constraints (for example a species can have a patrimonial value at the local level without being on the red or orange list).
    - Adaptation to the captivity: certain species support the captivity better than others.

- Specific needs: a center of care cannot be equipped to receive all the species of marine birds and water.
- Individuals: generally, the birds of sea are longévives species. They reproduce tardily and the "large one" of mortality takes place in the first 3 years of life. One will thus privilege always the reproductive adults with the detriment of youthful and even more of the chicks (case of the oil slicks in period of reproduction).
- medical parameters:
  - State physiological
    - Weight: this parameter is to be taken into account because the bird must reach a weight "sufficient" for washing in 5 days (time counted starting from the capture). Moreover, it acts of an indicating parameter of the stage of fast and thus of the state of the digestive function.
    - Behavior and state of hydration: an animal dehydrated with more than 10 % is in a state of shock and could not be rehydrated by oral way. In context of oil spill, it could not thus be neat. In the same way a bird requiring an oxygen treatment.
  - Irreversible lesions: in fact the lesions do not allow one to slacken animal in their natural environment such as for example a blindness one or bilateral, an amputation of a member.
  - Reversible lesions or diseases: lesions or diseases which could be neat in the case of birds supporting the captivity (for example psittacidés) or if it would be all alone, for example fractures, moderate or severe respiratory affection.
  - Diseases regulated for domestic fauna and major zoonosis.

The indicators of sorting are to be considered with the various partners of the care to the mazoutés birds. Certain countries propose guides, like the United States (see for example Berg, 2003): in all the cases they result from dialogue between the various speakers and the administrations.

The grid proposed here is a stage which supplements that proposed in 2004 but which still requires to be supplemented (Table VII).

In this grid one will put a cross in the corresponding column for each criterion (for = for the rehabilitation, against = against the rehabilitation). A column in a red line implies the euthanasia. Then, according to the number of crosses per report/ratio to the capacity of reception, one will decide or not euthanasia.

**It should be noted that compared to the grid of 2004 the renal affections were removed, initially because they do not seem irreversible once the exposure to the pollutant finished and in addition because one does not have simple means to evaluate it. The digestive affections were confirmed by the present study like a criterion "against" the rehabilitation.**

One could also add the proportioning of the irritating HAP on the feathers to consolidate the choice of "against" in the digestive affections, insofar as these proportionings are likely to be able to be done in routine. Currently fast tests of detection of hydrocarbons were developed in the United States (see Fritcher *et al.* 2002), but without distinction of the various HAP.

In table VIII, I explain the parameters, if it is necessary to refine them and how to choose.

Table VII: Example of grid of sorting (see the corresponding paragraphs) and criterion remaining to deepen (in red crippling criteria, blue patrimonial criteria; MRC = disease considered contagious, regulated disease).

<b>Critère</b>	<b>FOR</b>	<b>AGAINST</b>
Capacity of reception reached		
State of shock		
Fractures, eyes lesions		
Lesion of the central nervous system or peripheral		
Contagious affection for other birds		
Reglemented contagious disease		
Priority species		
Adult		
Time between arrival in wildlife center and oiling		
Weight		
State of hydration		
Infected wounds		
Respiratory affection		
<b>Digestive affection</b>		
Zoonosis		
<b>TOTAL</b>		
<b>DECISION</b>		

Table VIII: explanation of the grid of sorting (see the corresponding paragraphs) and criterion remaining to deepen (in red crippling criteria).

Criterion	Explanations	To detail	How ?
Euthanasie Strict criteria: blue parameters: 1 crosses, parameters black: 1 crosses Intermediate criteria: blue parameters: 1 crosses, parameters black: 2 crosses Flexible criteria: blue parameters: 2 crosses, black parameters: 4 crosses			To validate
Carrying capacited reached	So reached between 60% and 100%: strict criteria So reached between 40 and 60 % intermediate criteria So reached of less than 40 % flexible criteria	YES	
Priority species	See list	YES	Dialogue with the biologists, associations of nature conservancy, the administrations of the environment
Adult		YES	
State of shock	Presence : AGAINST		
Fractures, eyes lesions	Presence : AGAINST		
Lesion of the central nervous system or peripheral	Presence : AGAINST		
Contagious affection for other birds	Presence : AGAINST		
Reglemented contagious disease	Presence : AGAINST		
Time between arrival in wildlife center and oiling	If higher than 3 days: AGAINST		
Weight	If loss of 40 % of the weight: AGAINST	YESI (see Table IX)	Dialogue with the biologists, associations of nature conservancy (validation of the principal species and sub-species)
State of hydration	Si supérieur à 10 % : CONTRE		
Infected wounds	Presence : AGAINST		
Respiratory affection	Presence : AGAINST		
Digestive affection	Presence : AGAINST	PAH ?	

Zoonosis	Presence : AGAINST		
TOTAL			
DECISION			

Table IX: weight of the 10 species most touched during the oil spill of Erika (after Rigaudeau *et al.*, 2002)

<b>Species</b>	<b>Weigth</b>
Common Guillemot	700-1100
Common scoter	1200-1500
Razorbill	550-920
Eider duck	1200-2800
Gannet	3500
Kittiwake	340-500
Shag	1700
Red-throated diver	800-1750
Great crested grebe	700-1150
Black-necked grebe	300-400

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## **ANNEXS**

**OISEAUX MAZOUTES  
FICHE D'ENTREE**

**N° ENTREE :**

**N° BAGUE INTERNE :**

**DATE DE DECOUVERTE + LIEU :**

**DATE D'ENTREE :**

**ESPECE :**

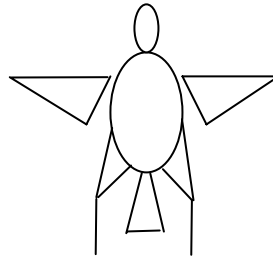
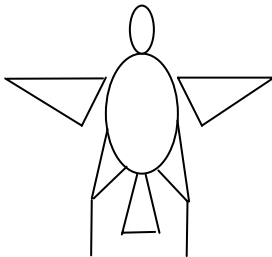
**AGE :**

**POIDS :**

**TEMPERATURE :**

**COMPORTEMENT:**

**MAZOUTE :**



Ventral : %

Dorsal : %

**FIENTES :**  Normal  Diarrhée  Diathèse hémorragique  Autre :

**AUTRES SIGNES CLINIQUES :**

**1ers SOINS :**

Nettoyage tête :

Nettoyage corps :

Alimentation spontanée :

Charbon :

Réhydratation PO :

Gavage :

**OISEAUX MAZOUTES  
 FICHE D'HOSPITALISATION**

**N° ENTREE :**

**N° BAGUE INTERNE :**

**Date de découverte (=J0) :**

**Date d'entrée :**

**EVOLUTION**

Date	Poids	Gavage (G), ou Spontané (S) ou (G+S)	Activité Faible (F), Actif (A)	Signes cliniques
J0=découverte				
J1				
J2				
J3				
J4				
<b>J5</b>				
<b>J6</b>				
<b>J7</b>				
J8				

**DEVENIR**

Date	Lavage-séchage	Piscine	Relâché	Mort

**OISEAUX MAZOUTES  
 FICHE D'AUTOPSIE**

<b>Date :</b> <b>Espèce :</b> <b>Photos :</b> <b>Age :</b>	<b>N° ENTREE :</b>  <b>N° BAGUE INTERNE :</b>  <b>N° PRELEVEMENT : étiquettes</b>
---------------------------------------------------------------------	-----------------------------------------------------------------------------------------------

**MACROSCOPIE :**

Sexe :

Bague muséum :

**EXTERIEUR**

Plumes, ailes, pattes :

Tête, yeux, oreilles :

**INTERIEUR** :

Peau, tissus sous-cutané :

Muscles, graisse :

Thyroïdes, parathyroïdes :

Péricarde :

Cœur, vaisseaux :

Foie :

Rate :

Trachée :

Poumons :

Sacs aériens :

Séreuses :

Langue, jabot, œsophage :

Proventricule, gésier :

Petit intestin (% diathèse) :

Pancréas :

Caecum, colon, cloaque (% diathèse) :

Reins, surrénales :

Appareil reproducteur :

Tissu nerveux :

Os, moelle osseuse :

**(entourer les prélèvements faits)**

**PRELEVEMENTS ETUDE (préciser TD : Ig ou colon):**

Histologie (formol) : rein / TD diathèse / TD normal / caecum diathèse / caecum normal

Toxicologie : foie (pot sec congelé)

Plume mazoutée (pot sec)

5 écouvillons : TD diathèse / TD normal / caecum diathèse / caecum normal / cloaque

## Bacteriological examination of selected intestinal sample

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### Introduction

For getting an impression of the aerobic bacteria present in the intestines of the guillemots samples were taken. From all necropsied birds samples for bacteriological examination were collected from different locations and stored at  $-20^{\circ}\text{C}$  until processing. When present, samples were collected from areas with and without a hemorrhagic diathesis. Isolated *E. coli* strains were serotyped. For culturing the samples were sent to the Diagnostic Laboratory of the NOIVBD in Veldhoven, The Netherlands.

### Material and Methods

After arrival of the samples they were refrigerated ( $+4^{\circ}\text{C}$ ) until processed (26-04-2006). Between 10.30 and 11.30 all samples were added to 5 ml buffered peptone water (BPW pH 7.2, batch 20051200187, Tritium Microbiology, Veldhoven). During the day the BPW samples were stored at  $+4^{\circ}\text{C}$  and were several times during the day vigorously shaken. Between 19.00 and 20.00 1 ose was plated to a Columbia blood agar disk in 3 line streaks (figure 1) and to a brilliant-green-agar disk (figure 2) (Col BL, pH 7.0, batch 20060400441, and BGA M pH 7.0, batch 20060400254, Tritium Microbiology Veldhoven). To get a semi-quantitative impression of the number of microorganisms present in the BPW suspension to be cultured, 3 drops of the BPW suspension were plated to a Col BL (figure 3). All plates were incubated at  $37^{\circ}\text{C}$  under aerobe conditions for 24 hours. To get an impression about the number and ratio of microorganisms (rods, cocci or yeasts) in the BPW suspension 1 drop was added to a microscopic glass-slide and dried at the air and stained with Hemacolor<sup>®</sup> (Merck). After 25 hours all plates were evaluated, photographically documented and separate colonies selected from the different birds for pure culturing. The amount of growth is noted as streak 1, 2, or 3 and the estimated number of colonies. The first streak with separate colonies is given. E.g. 2/ $>50$  means streak 2, at least 50 colonies. Smears were made of the different colonies and stained with Hemacolor<sup>®</sup> for morphological evaluation. Based on morphology of the growth on Col BL and microscopic evaluation different bacterial isolates (total 17, from 9 birds) were selected and sent to Brabant Veterinary Laboratory (Diessen, The Netherlands) for determination. The isolated *E. coli* strains ( $n=7$ ) were sent to the reference laboratory of the National Health Institute (RIVM, Bilthoven, The Netherlands) for serotyping.

### Results

The description of the samples and the results of the study are presented in an XLS table as appendix 1.

A total of 26 samples from 11 guillemots arrived in the diagnostic laboratory of the NOIVBD in Veldhoven, The Netherlands. The number of samples per bird ranged from 1 to 5.

Four samples (1, 3, 20 and 21) originating from 3 different birds (13, 23 and 983) showed no growth. On the blood agar (Col BL) growth was seen in 22 samples (8 birds); on the BGA growth was only seen in 12 samples (5 birds). The amount of growth varies for each colony-type, but was especially on the Col BL optimal. Almost all plated Col BL showed abundant growth (figure 3).

The results of the selected microorganisms for determination and serotyping are presented in table 1.

From 4 birds only Gram-positive cocci were found. Only from the intestines of 4 birds (36%) *E. coli* (n = 7) was isolated. There were 4 different serotypes present (O8, O75, O80 and O unknown).

No obvious relation was seen between the hemorrhagic diathesis and microorganisms present.

Table 1 Isolated bacteria from the intestinal tract of the different birds

Bird	Sample #	BVL	Growth	RIVM
05-13	2	<i>Enterococcus</i> sp	Col BL	n.d.
05-22	5	<i>Enterococcus</i> sp	Col BL	n.d.
05-15	6	<i>Escherichia vulneris</i>	Col BL + BGA	n.d.
05-24	7	<i>Enterococcus</i> sp	Col BL	n.d.
05-29	8	Yeasts	Col BL	n.d.
05-28	9a	<i>E. coli</i>	Col BL + BGA	O8
Idem	9b	<i>Streptococcus</i> group D	Col BL	n.d.
Idem	10	<i>E. coli</i>	Col BL	O8
06-18	14a	<i>E. coli</i>	Col BL + BGA	O75
Idem	14b	<i>Morganella morganii</i>	Col BL + BGA	n.d.
Idem	15a	<i>E. coli</i>	Col BL + BGA	O8
Idem	15b	Oxidase + G- <i>Bacillus</i> sp	Col BL	n.d.
06-71	17	<i>Streptococcus</i> group D	Col BL	n.d.
Idem	19a	<i>E. coli</i>	Col BL + BGA	O86
Idem	19b	<i>Streptococcus</i> group D	Col BL	n.d.
06-84	22	<i>E. coli</i>	Col BL + BGA	Unknown O-type
Idem	26	<i>E. coli</i>	Col BL + BGA	Unknown O-type

BVL = Brabants Veterinary Laboratory, Diessen, The Netherlands;

RIVM = National Health Institute, Bilthoven, The Netherlands

Col BL = Columbia blood agar

BGA = brilliant-green-agar

n.d. not done.

## Discussion

The number and strains of bacteria isolated from the intestines of Guillemots is very limited. Although from some individual birds no microorganisms could be cultured from the intestines (05-23 and 05-983), one of them (05-23) showed many cocci in the stained drop. Most birds (at least 9) harboured gram-positive cocci (*Enterococcus* sp, *Streptococcus* group D). Gram-negative rods were only cultured from a few birds (n=5) and the numbers in the stained drop was very low. *E. coli* was the most frequent isolated enterobacteriaceae. Based on these results there is an indication that Gram-positive cocci might belong to the normal intestinal flora, but enterobacteriaceae are limited and possible secondary invaders. There was no indication that

microorganisms, especially bacteria, play an important role as a cause for enteritis or hemorrhagic diathesis.



Figure 1 Col BL of sample 23: 3/>50 colonies, grey with navel, 3 mm



Figure 1 BGA of sample 23: 2/ >100 yellow green 2 mm



Figure 3 Col BL spatel of sample 23, 3 drops of BPW. Fully grown (+++++)

Oiled birds bacteriological samples

PA 06-0121 Animal # Evaluation of growth after 25 hours

	Col BL	BGA	Col BL spatel
1	GT 05-13 NG (No Growth)	NG	5 separate colonies, grey
2	2/30 1 mm grey (In red are the selected colonies)	NG	++++ grey, watery small
3	GT 05-23 NG	NG	NG
4	GT 05-22 1/25 3 types most grey 1mm with halo	NG	+++
5	5 col, mixed	NG	++ 2 diff col
6	GT 05-15 2/100 grey, navel, 3 mm and small grey halo	1/100 yellow	++++ grey and + light grey
7	GT 05-24 2/30 grey halo 2 mm and 1 bacillus-like	NG	++++ grey
8	GT 05 29 1/100 very tiny clear col and 1/100 white 1 mm swabs	NG	++++ very tiny clear col
9	GT 05 28 3/25 grey, smooth 5 mm and 3/20 small grey halo and 3/10 very tiny clear	2/50 yellow smooth	+++++
10	3/7 large grey smooth and 2/>100 5mm and a few very tiny clear	NG	+++++
11	3/>100 grey smooth 2 mm and a few tiny col (overgrowth with Proteus sp.)	3/>50 yellow-green	+++++
12	GT 06-18 2/>100 grey smooth 3 mm	NG	+++++
13	2/>100 white beige 2mm and few grey 3-4 mm	1/>100 small, clear 1mm	+++++ plus 5 hemolytic col
14	2/>50 grey smooth 3 mm and 2/>75 tiny clear 1 mm and some with halo	1/>50 yellow smooth 3 mm	+++++ mixed
15	2/40 grey smooth 4 mm and 2/50 tiny clear 1 mm and few hemolytic	NG	+++++
16	3/50 grey creamy 4 mm and 3/10 small grey 1 mm	3/50 yellow green smooth 4 mm	+++++
17	GT 06-71 2/>100 very small < 1mm hemolytic	NG	+++++ very tiny (plus 10 col grey
18	2/100 tiny grey < 1mm and few grey < 1 mm	NG	+++++ plus 20 grey
19	3/20 grey 4 mm and 3/40 grey 1 mm and 3/ +/- clear <1 mm	1/25 pale rose 1mm	+++++
20	GT 05-983 NG	NG	
21	NG	NG	
22	GT 06-084 3/>100 grey dark 4 mm navel and >10 clear tiny droplets 1 mm	3/>100 pale tiny rose 1 mm	+++++

23	3/>50 grey navel 3 mm	2/yellow green 2 mm	+++++
24	2/>100 grey navel 3 mm	2/>100 yellow green 2 mm	+++++
25	2/>100 grey halo 3 mm and few small grey 1 mm	1/>100 tiny rose < 1mm	+++++
26	3/50 grey navel 3 mm and few clear grey 1 mm	2/>50 yellow green thick 2 mm and few < 1mm	+++++

PA 06-0121	Animal #	stained drop	selected colonies	results BVL	RIVM serotyping E.coli K1 PCR for all isolates negative
	1	GT 05-13 cocci ++			
	2	cocci ++, and many epithelial cells	2	Enterococcus sp	
	3	GT 05-23 cocci +++, hemorrhagic background, few rods			
	4	GT 05-22 rod-shaped bacteria + and few cocci			
	5	very thin drop, +/- rods and cocci	5a and 5b	Enterococcus sp	
	6	GT 05-15 yeasts ++, cocci + and few rods	6	Escherichia vulneris	
	7	GT 05-24 mixed rods and few rows cocci ++ some yeasts	7	Enterococcus sp	
	8	GT 05 29 yeasts +, cocci ++, hem background swabs	8a and 8b	Yeasts	
	9	GT 05 28 very tiny cocci in rows ++ few rods	9a and 9b	<b>E. coli</b> Streptococcus group D	serotype O8
	10	very tiny cocci in rows ++ few rods	10	<b>E. coli</b>	serotype O8
	11	thin drop, very tiny cocci in rows ++ few rods			
	12	GT 06-18 some lost rods, sometimes in plucks			
	13	Short, thick rounded rods ++, +/- long thin rods			
	14	thick cocci +, long thin rods + and some rods with spores	14a and 14b	<b>E. coli</b> Monganella morganii	serotype O75
	15	thick cocci +, long thin rods + and some rods with spores	15a and 15 b	<b>E. coli</b> Oxidase + Gram negative Bacillus sp.	serotype O8
	16	irregular short rods ++, some tiny cocci and yeast +/-			
	17	GT 06-71 cocci +, thin short rods and +/- yeast	17	Streptococcus group D	
	18	thin drop, occasionally a rod and coc			
	19	thin drop, very tiny cocci in rows +	19a and 19b	<b>E. coli</b> Streptococcus group D	serotype O86

20	GT 05-983	thin drop, no bacteria			
21		no bacteria			
22	GT 06-084	short thin and short thick rods (++) and occasionally a coc	22a	<b>E. coli</b>	Unknown O-type
23		short thin and short thick rods (+++) and occasionally a coc			
24		short thin and short thick rods (+) and occasionally a coc			
25		thin drop with some rods		<b>E. coli</b>	Unknown O-type
26		mixed rods and few rows cocci ++	26		

05-013

	IS	A <sub>IS</sub>	C <sub>IS</sub> (µg/mL)
164	Biphenyl d <sub>10</sub>	943 358	1,0
188	Phenanthrene d <sub>10</sub>	1 219 275	1,0
240	Chrysene d <sub>12</sub>	1 095 375	1,0
136	Naphtalène d <sub>8</sub>	1 747 652	1,0
264	Benza[a]pyrene d <sub>12</sub>	449 412	1,0

masse foie (g)	7,9492
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	composés	abréviation	A <sub>i</sub>	K <sub>i</sub>	C <sub>i</sub> = K <sub>i</sub> x (A <sub>i</sub> / A <sub>IS</sub> ) x C <sub>IS</sub>	C <sub>i</sub> (ng/g)
128	<b>Naphtalène d8</b>	Naphtalene	1 369 464	1,16	0,9	23
152		Acenaphtylene	0	1,19	0,0	0,0
154		Acenaphtene	696 391	1,57	0,6	16
166		Fluorene	84 105	1,67	0,1	2,0
178	<b>Phenanthrene d<sub>10</sub></b>	Phenanthrene	786 935	1,18	0,8	19
178		Anthracene	170 928	0,54	0,1	1,9
202		Fluoranthene	110 907	0,75	0,1	1,7
202		Pyrene	362 541	0,70	0,2	5,3
228	<b>Chrysene d12</b>	Chrysene	153 737	0,81	0,1	2,9
228		Benzo[a]anthracene	70 154	1,45	0,1	2,3
252		Benzo[b+k]fluoranthene	50 359	1,07	0,0	1,2
252	<b>Benza[a]pyrene d12</b>	Benzo[a]pyrene	36 353	1,01	0,1	2,1
276		Indeno(1,2,3-cd)pyrene	0	1,86	0,0	0,0
278		Dibenz(a,h)anthracene	0	2,26	0,0	0,0
276		Benzo(g,h,i)perylene	0	2,02	0,0	0,0

**16 HAPs liste US EPA**

**6 HAPs liste AFSSA**

<b>composés</b>	<b>Concentration (ppb)</b>	<b>composés</b>	<b>Concentration (ppb)</b>
Naphtalene	23	Benzo[a]anthracene	2,3
Acenaphtylene	0,0	Benzo[b+k]fluoranthene	1,2
Acenaphtene	16	Benzo[a]pyrene	2,1
Fluorene	2,0	Indeno(1,2,3-cd)pyrene	0,0
Phenanthrene	19	Dibenz(a,h)anthracene	0,0
Anthracene	1,9		
Fluoranthene	1,7		
Pyrene	5,3		
Chrysene	2,9		
Benzo[a]anthracene	2,3		
Benzo[b+k]fluoranthene	1,2		
Benzo[a]pyrene	2,1		
Indeno(1,2,3-cd)pyrene	0,0		
Dibenz(a,h)anthracene	0,0		
Benzo(g,h,i)perylene	0,0		