Contamination Problems of the Gray Whales

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(submitted by the Russian Federation)

Samples of blubber, tongue, liver, kidney, muscle and bowels from several malodorous species and several clean species were delivered to the laboratory of organic analysis of the Moscow State University in 2005, 2008, 2009 and 2011.

The samples stored at -18°C to preserve the volatile odor compounds.

Head-space analysis.

The samples of blubber or muscles (4-5g) were grinded in porcelain mortar with anhydrous Na_2 SO_4 (5 -10g), placed into a glass bulb and triply extracted with dichloromethane (30ml) in an ultrasonic bath for 20 min. The extracts were combined and concentrated to 5 ml at rotor evaporator at 30° C. Then the concentrates were passed through the chromatographic column packed with silica gel and silica gel mixed with concentrated sulfuric acid. Dichloromethane was used as an eluent. The final extract was concentrated to 1 ml at rotor evaporator at 30° C.

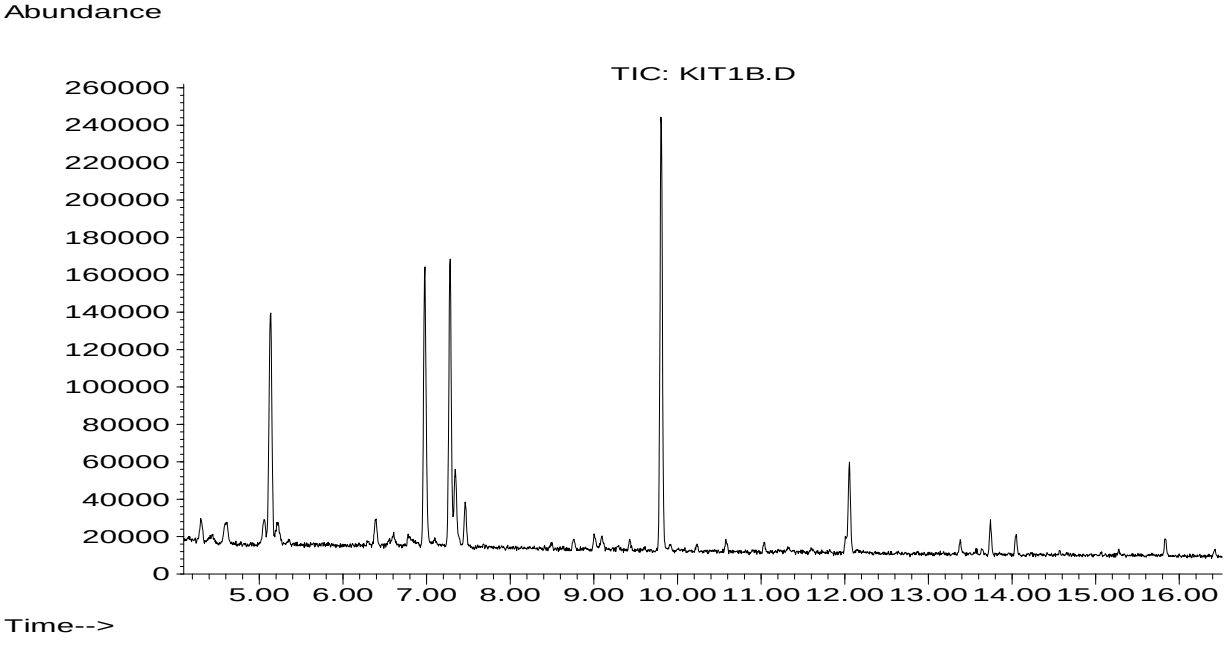
The sample vial was heated at 70° C for 5 min, 1 ml of the vapor phase was injected into GC

GC parameters: Carrier gas - He, 1 ml/min.

Column temperature program: 40° C (4 min) – 5° C/min – 100° C - 20° C/min - 280° C (5 min).

Column parameters: 30 m x 250 μm x 0,25 μm, DB-5MS.

MS parameters: Ionization energy – 70 eV, mass range 29-450 Da, rate 20 spectra/sec; 1-Fluoro-4-chlorobenzene was used as internal standard.



TIC Chromatogram of the volatile compounds in the blubber of "stinky" gray whale. From the left: isobutanal, butanal, 2-mwthylbutanal, 3-methylbutanal, 2-ethylfuran, pentanal, petan-2-one, isomeric brunched hexanals (4), hexanal (base peak), isomeric brunched octanals (3), octanal, alkylketones (3), nonanal.

Semi volatiles analyses. The tissue samples (4-5g) were grinded in porcelain mortar with anhydrous $Na_2 SO_4$ (5 -10g), placed into a glass bulb and triply extracted with dichloromethane (30ml) in an ultrasonic bath for 20 min. The extracts were combined and concentrated to 5 ml at rotor evaporator at 30°C. Then the concentrates were passed through the chromatographic column packed with silica gel and silica gel mixed with concentrated sulfuric acid. Dichloromethane was used as an eluent. The final extract was concentrated to 1 ml at rotor evaporator at 30° C.

0,2 µl of CH_2Cl_2 extract was injected into GC in splitless mode.

GC parameters: Carrier gas - He, 1 ml/min.

Column temperature program: 40° C (2 min) – 20° C/min - 300 ° C (20 min).

Column parameters: 30 m x 250 µm x 0,25 µm, DB-5MS.

MS parameters: Ionization energy – 70 eV, mass range 29-450 Da, rate 20 spectra/sec; Internal standards - D8 - naphthalene, D10-phenananthrene, D12-perylene).

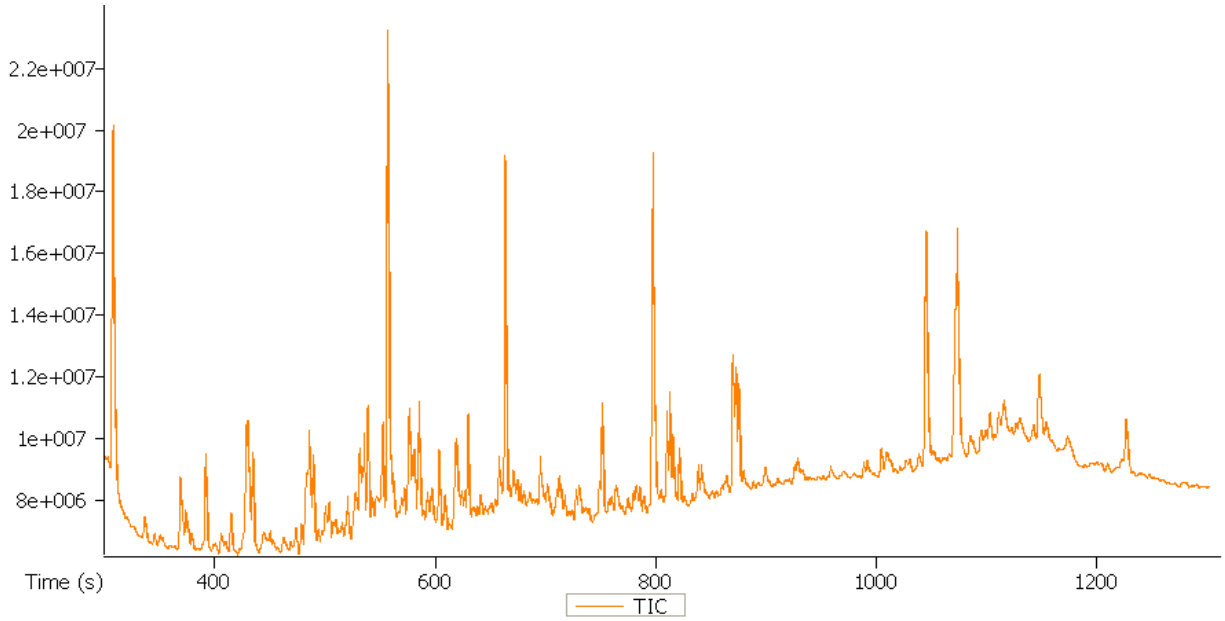
Two-dimensional GCxGC-MS analysis.0,2 µl of CH_2Cl_2 extract was injected into GC in splitless mode.

GC parameters: Carrier gas - He, 1 ml/min.

column temperature: 40°C (2 min) - 5°C/min - 300°C (10 min).

Columns parameters: fused silica capillary column RTX-5MS (30m) and DB-17 (1m).

MS parameters: mass range 25-550 Dalton. Acquisition rate – 150 spectra/sec. Internal standards - D8 - naphthalene, D10-phenananthrene, D12-perylene).



TIC chromatogram of CH2Cl2 extract of blubber sample.

Range of PAH levels in the samples, µg/g

Naphthalene	0 - 0,05	Phenanthrene, methyl-	0,02 – 0,06
Naphthalene, 2-methyl-	0 - 0,03	Phenanthrene, methyl-	0,002 – 0,06
Naphthalene, 1-methyl-	0 - 0,06	Phenanthrene, methyl-	0,03 – 0,06
Biphenyl	0 - 0,04	Naphthalene, 2-phenyl-	0 - 0,02
Naphthalene, C2	0 - 0,01	9,10-Anthracenedione	0 - 0,01

Naphthalene, C2	0 - 0,01	Fluoranthene	0,04 – 0,10
Naphthalene, C2	0 - 0,02	Pyrene	0,003 - 0,01
Naphthalene, C2	0 - 0,01	Chrysene	0,01 – 0,48
Naphthalene, C2	0 - 0,003	Benzo[b]fluoranthene	0 - 0.07
Diphenylmethane	0 - 0,02	Benzo[k]fluoranthene	0 - 0.07
Dibenzofuran	0 - 0,07	Benzo[a]pyrene	0 - 0.001
Fluorene	0 - 0,01	Dibenz[a,h]anthracene	0 - 0.15
Phenanthrene	0,4 – 0,6	Benzo[ghi]perylene	0 - 0.01

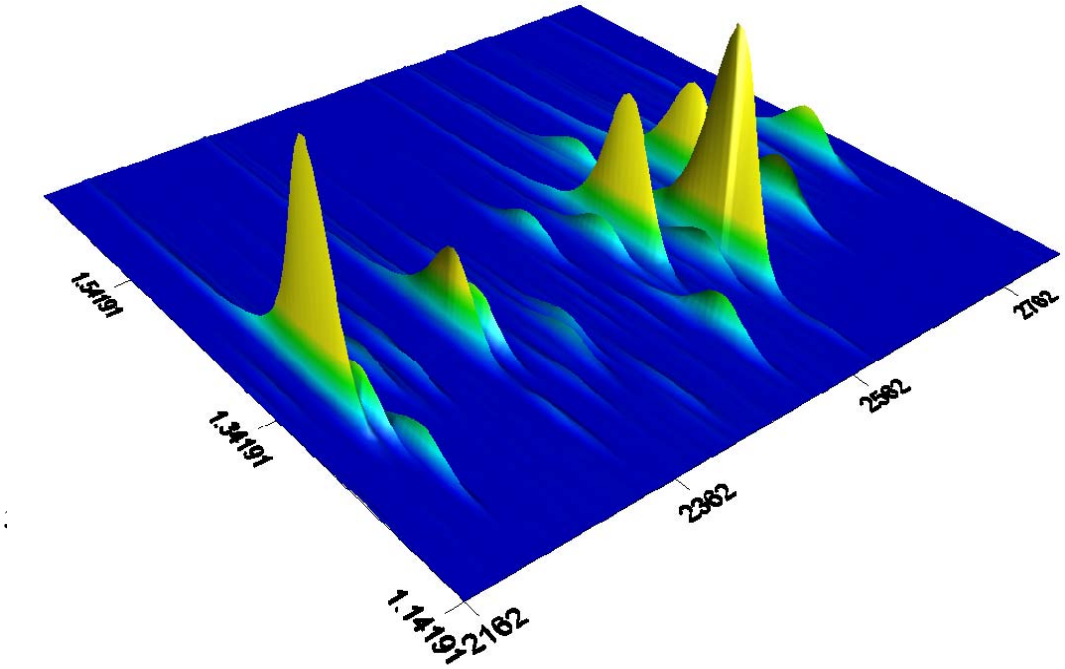
Persistent organochlorines in the tissue samples (µg/g)

Benzene, hexachloro-	0 - 0,05
Lindane	0 - 0.02
Nonachlor	0 - 0.003
o,p'-DDE	0 - 0.01
α-Chlordene	0 - 0.01
Σ PCB	0,02 – 0,04

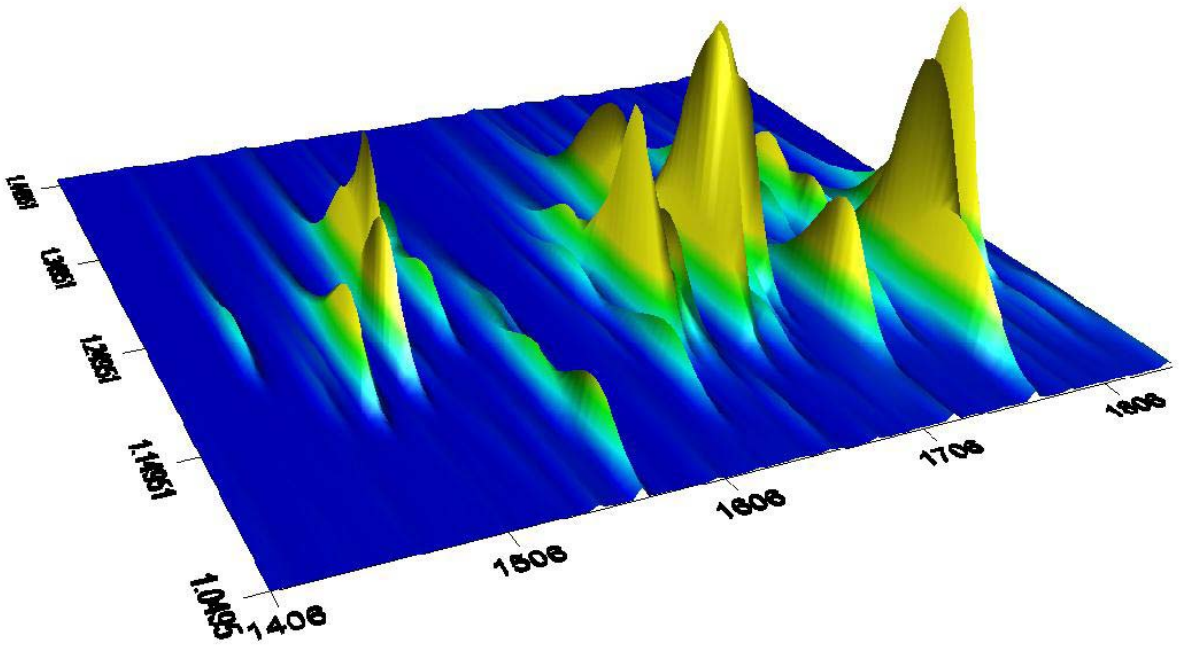
Benzene derivatives in the tissue samples (µg/g)

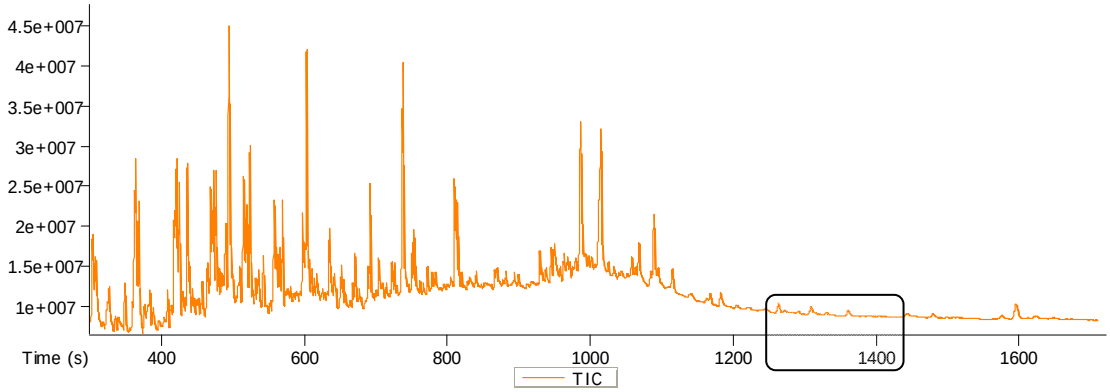
Benzene, tert-butyl-	0,01 – 0,07
Benzene, butyl-	0,20 – 0,38
Benzene, (3-methylbutyl)-	0,19 – 0,40
Benzene, hexyl-	0,30 – 0,86
Benzene, methoxy-	0 - 0,03
Benzene, nitro-	0 - 0,04
Benzene, 1-chloro-4-methoxy-	0 - 0,03
Benzene, 1-methyl-2-nitro-	0 - 0,06

3D mass chromatogram of m/z 290 ion current (tetrachlorobiphenyls).



3D mass chromatogram of m/z 155 ion current (alkylnaphthalenes)

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4e+007
3.5e+007
3e+007
2.5e+007
2e+007
1.5e+007
1e+007
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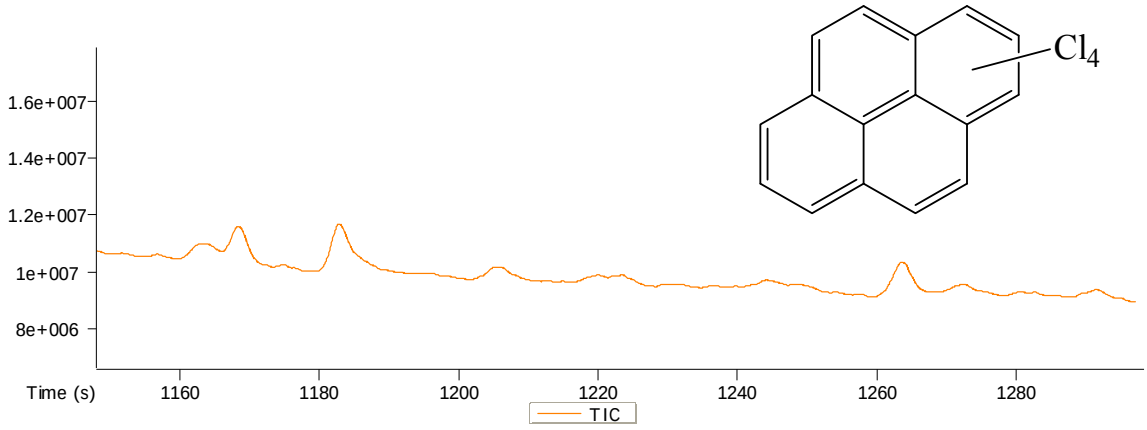
400
600
800
1000
1200
1400
1600
TIC


1.6e+007
1.4e+007
1.2e+007
1e+007
8e+006
Time (s)
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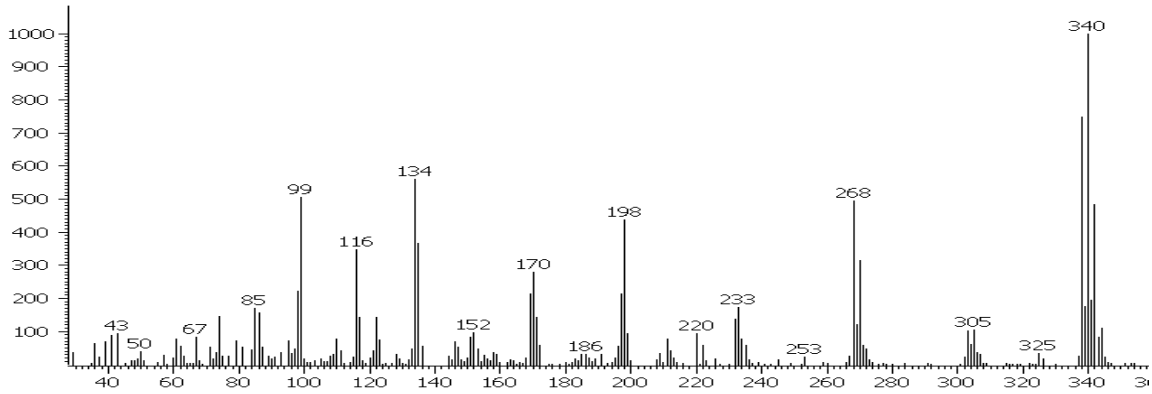
1180
1200
1220
1240
1260
1280
TIC
Cl4


1000
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67
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99
116
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198
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233
253
268
305
325
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260
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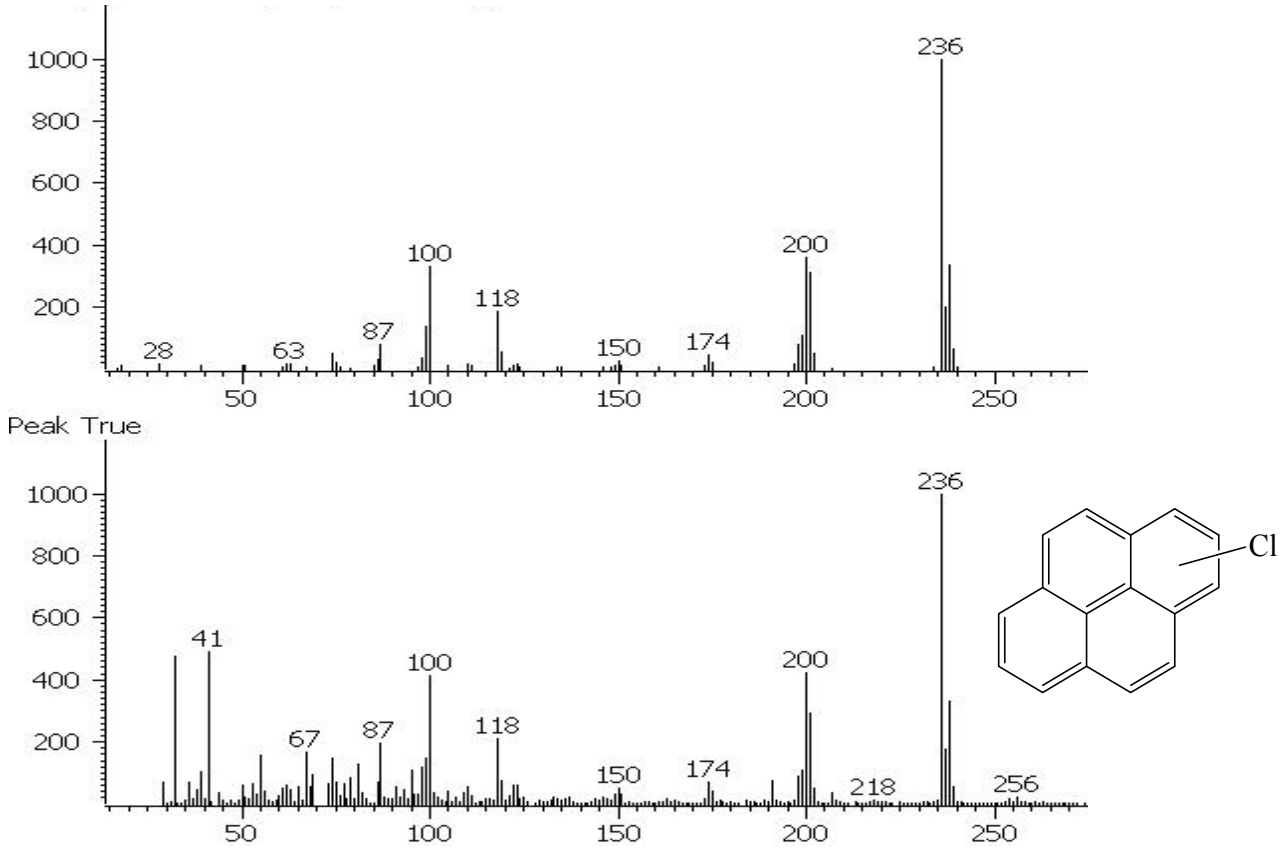
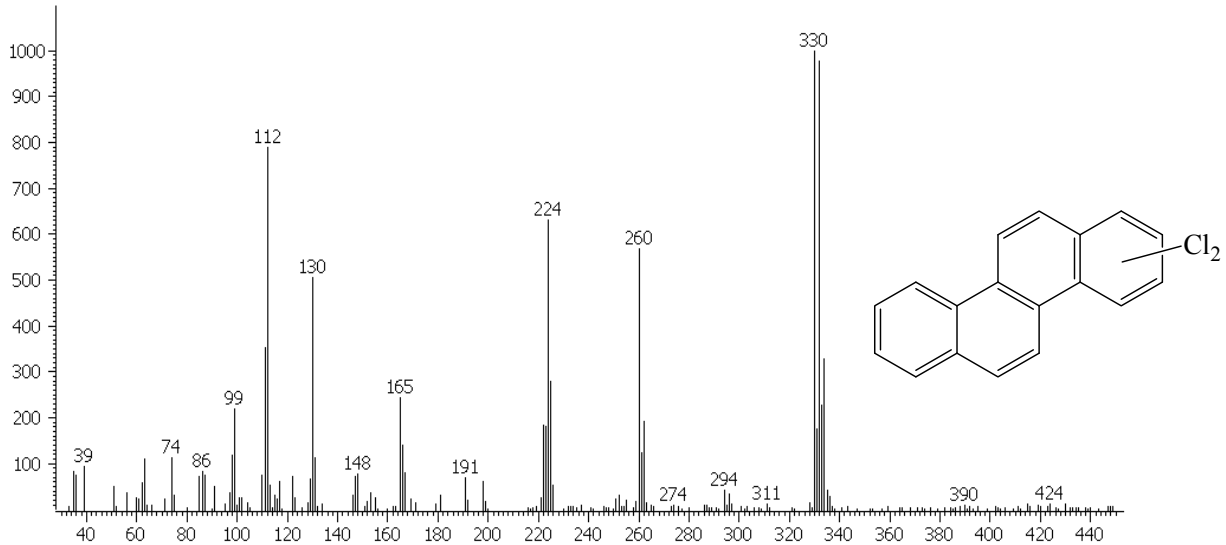
300
320
340

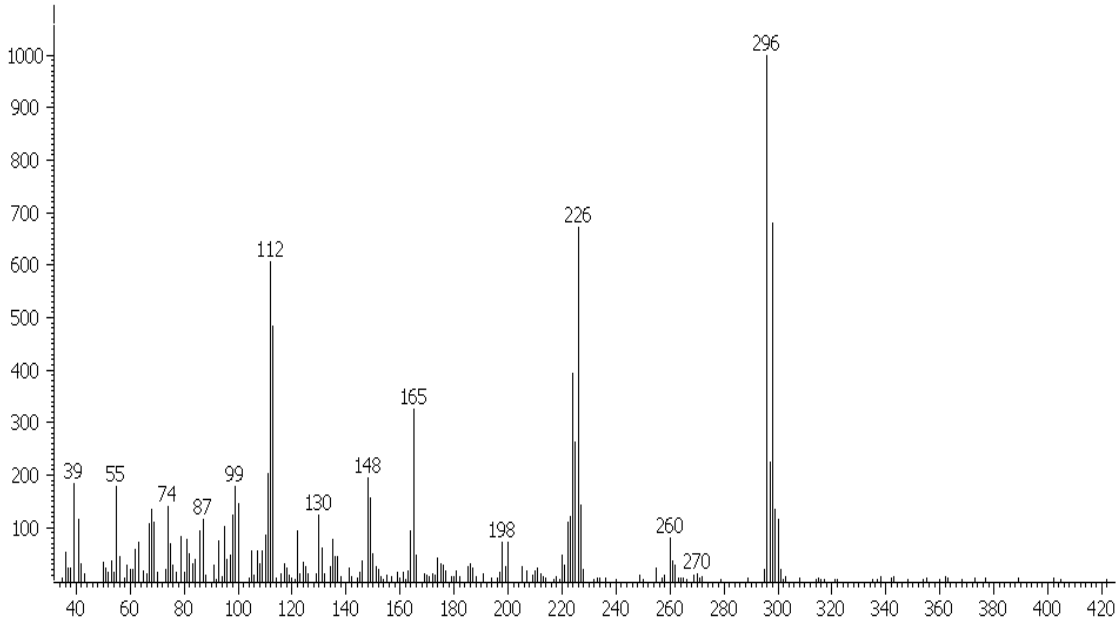
Chloropyrene



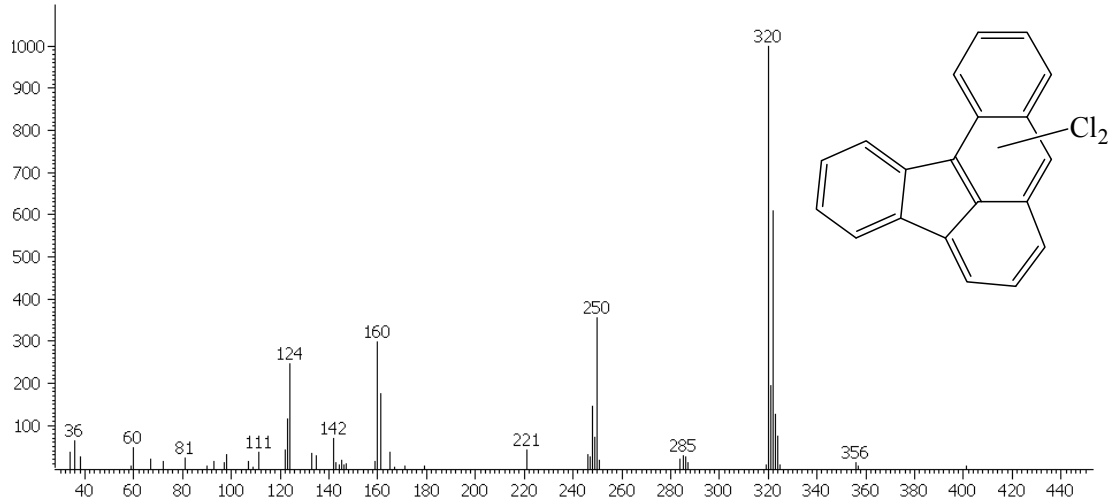
Dichlorochrysene



Trichlorochrysene



Dichlorobenzofluoranthene



Cl-PAH found in the sample of whale's blubber

	Cl-PAH	Concentration ng/g
1	Chloropyrene	2
2	Trichloropyrene	9
3	Trichloropyrene	57
7	Tetrachloropyrene	36
4	Dichlorochrizene	10
5	Dichlorochrizene	33
6	Dichlorochrizene	10
9	Trichlorochrizene	10
8	Chlorobenzofluorantene	18
10	Dichlorobenzofluorantene	11
11	Dichlorobenzofluorantene	3
12	Dichlorobenzofluorantene	1
13	Dichlorobenzofluorantene	4

Conclusions

Contamination of the Pacific Ocean with petroleum hydrocarbons is the most probable reason of the "stinky whales" as slow metabolization of the aliphatic chains of these hydrocarbons in the whales organisms bring to the formation of odorous carbonyl compounds.

The levels of persistent organochlorines are not very high. The absence of DDT in all the samples is a good sign. This dangerous compound is not a problem anymore for the North Pacific region.
The source of the chlorinated PAH remains unknown.