ANNEX 06 : POLLUTION ASSESMENT STUDY

Annex 06.1 Final PAH report

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March 2016

ENERGEAN OIL & GAS

PRINOS OFFSHORE DEVELOPMENT PROJECT

Final Report for Polycyclic Aromatic Hydrocarbons (PAHs)

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PRINOS OFFSHORE DEVELOPMENT PROJECT FINAL REPORT FOR POLYCYCLIC AROMATIC HYDROCARBONS (PAHs)	
Environmental Consultant:	**LDK Engineering Consultants SA**
Scientific advisor:	**National Technical University of Athens (NTUA)**

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	Name – Company	Responsibility	Signature	Date
Prepared by:	Pr. Manos Dassenakis, NTUA	Laboratory of Environmental Chemistry		
	Dr. Michael Christolis, NTUA	Civil Engineer		
Checked by:	Prof. Nikolas Markatos	Chemical Engineer, PhD		
Approved by:	Costis Nicolopoulos, LDK	Head of LDK Environment, principal, project director		
	Vassilis Tsetoglou – Energean	HSE Director		
	Dr. Steve Moore – Energean	General Technical Director		

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1 INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs), heavy metals, organic solvents, inorganic salts, industrial and urban sewage are some of the most important anthropogenic pollutants in the aquatic environment. PAHs are of special concern because of their toxic properties.

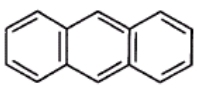
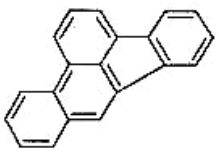
Polycyclic Aromatic Hydrocarbons (PAHs) are organic compounds containing aromatic rings in their molecules. The term PAHs refers to those containing only atoms of carbon and hydrogen. They are compounds of major environmental importance due to their possible carcinogenic and teratogenic action, their occurrence and their persistence in the environment, especially the marine environment.

PAHs derive from the incomplete combustion of organic matter, fossil fuels in particular (pyrolytic origin), loading/ unloading of petroleum and its products (petrogenic origin) and the alteration of biogenic compounds (biogenic origin)

PAHs solubility in water decreases with increasing molecular weight. In the marine environment, PAHs containing more than 4 aromatic rings are almost exclusively connected to suspended particulate matter and are finally led to being deposited in marine sediments. On the contrary, PAHs of 2-3 aromatic rings are mainly dissolved in the water. In addition, PAHs containing up to 4 rings biodegradate under aerobic conditions but the biodegradation of PAHs having more rings is very low. Biodegradation under anaerobic conditions is a very slow process for all PAHs.

1.1. PAHS PRIORITY LIST IN EUROPEAN UNION (2001)

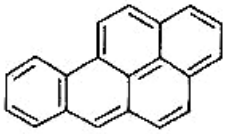
In the European Union Priority List, 8 PAHs are included, as presented on table.

(Ant) Anthracene 	**(BbF)** Benzo[b]fluoranthene 

(BaP)

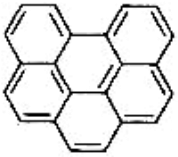
Benzo[a]pyrene *

**the most extensively studied compound*



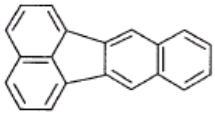
(BghiP)

Benzo[ghi]perylene



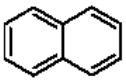
(BkF)

Benzo[k] fluroanthene



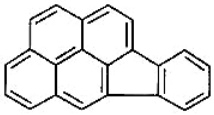
(Nap)

Naphthalene



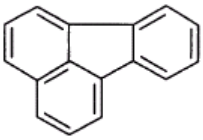
(IcdP)

Indeno[1,2,3-cd]
pyrene



(Fla)

Fluoranthene



1.2. PAHS IN THE MARINE ENVIRONMENT

PAHs enter the marine environment via the effluents of oil refining, shipping activities, oil extraction, industrial and wastewater treatment wastes, landfill drainage, highway washout. In addition, PAHs are generated due to incomplete combustion during the production stage of aluminum, iron, and steel.

PAHs in the marine environment tend to adsorb on suspended particles and sediments and become bioavailable to fish and other marine organisms through the food chain. The uptake of waterborne PAHs across the gills is the most important route depending on the exposure of these organisms in seawater. Inside the marine organisms, PAHs undergo biotransformations and are concentrated in several tissues with half-life from six to nine days

PAHs are known for their mutagenic and carcinogenic potential. Moreover, PAHs can induce oxidative stress and oxidative DNA damage through the metabolic activation and the generation of reactive oxygen species (ROS). Principal pathways of metabolic activation of PAHs are generation of diol epoxides, formation of radical cations and formation of reactive redox quinones. PAHs are capable of inducing dioxin-like responses in vitro interacting with the aryl hydrocarbon receptor. Concentrations of PAH in sea-surface microlayer, sub-surface seawater and sediments are of toxicological importance to both benthic and pelagic marine organisms. PAHs can accumulate first in fine-grained sediments and suspended particulates, which can be remobilized in the seawater and become bioavailable to indigenous aquatic organisms.

In recent decades Mytilus galloprovincialis was used by several researchers for the assessment of pollutants, including petroleum hydrocarbons, as sensitive bioindicator of environmental pollution and oxidative stress. The bioaccumulation of PAHs in M. galloprovincialis was the focus of many studies in the Mediterranean Sea.

Given the fact that PAHs concentrations in surface waters are usually at the level of ng/l, is unlikely to cause direct adverse effects on marine organisms. However, negative impacts can be induced due to bioaccumulation.

Algae, molluscs and more primitive invertebrates hardly metabolise PAHs and as a result they accumulate high concentrations. Fish and higher invertebrates (arthropods, echinoderms) are able to metabolise PAHs and hence accumulate only slight concentrations in their tissues. Biological transformation takes place in the liver, lungs, kidneys, placenta, intestines and the skin. Fish are able to metabolise PAHs to intermediate products of teratogenic, mutagenic or carcinogenic properties. Moreover, certain PAHs may influence fish physiology having effects on growth, reproduction, breathing and swimming performance.

For Good Environmental Status (GES) determination, GES thresholds were set and applied by EU. To set these thresholds many parameters have been taken into consideration; Sediment Quality Guidelines, Assessment criteria for heavy metals, Regulation of the EU No 1881/2006 for fish and seafood, Assessment criteria for heavy metals, directive 2006/118/EC, Water Framework Directive, Environmental Quality Standards, OSPAR Ecotoxicological Assessment Criteria for water, EPA National Recommended Water Quality Criteria as well as the expertise of the Laboratory of Environmental Chemistry of the University of Athens. The pristine state of open sea surface waters is used as a baseline.

Table 1: GES thresholds and Background Concentrations (BC) for PAHs in sediments and water column

	SEDIMENTS	WATER COLUMN

PAHs	GES thr	BC	GES thr	BC
total PAH	3.0 μg/g dw	0.2 μg/g dw	5 μg/l	0.1μg/l

Upper limit for human consumption (wet weight): Benzo[a]pyrene, 2 μg/kg for fishes, 10 μg/kg for bivalves (6, 30 μg/kg dry weight).

Upper limit of total PAHs for GES in mussels 700 μg/kg wet weight (20 mg/kg dry weight).

1.3. PAHS IN NORTH AEGEAN

Concerning PAHs, their concentrations range from <0.01 to 0.83 μg/l with the mean value being 0.29 μg/l. Generally, all the contaminants of this group are at low levels in the water column.

Not surprisingly, the values of all contaminants are higher in Thermaikos Gulf where there are more intense human activities compared to the rest of the assessment area (industrial and agricultural activities). Generally, although available data are from relatively polluted coastal areas all the contaminants of the group are at low levels in the water, probably due to their restricted solubility in the water. On the other hand, analyses of sediment shows that the concentrations of PAHs in the assessment area are rather high ranging from 0.8 to 7453 μg/kg. Thermaikos Gulf presents quite high values (5.6 -943 μg/kg), due to the urban agglomeration of Thessaloniki, harbour operation and the discharge of, until recently (2002), untreated domestic wastes. Another contributing factor is the pollution load carried by the river Axios. In addition, the available information about the concentrations of contaminants in biota comes from Mutilus Galoprovinciallis. PAHs are from 25-72 μg/kg; However, Thermaikos Gulf is an area presenting relatively high values; PAHs concentrations are quite high 40-640 μg/kg obviously due to effluents.

The main anthropogenic activities contributing to the input of contaminants in the marine environment are industry, shipping and agriculture.

Table 2: PAHs in sea water of various Greek areas

AREAS	Substances	Concentrations
NW Saronicos gulf	PAHs (16)	4-68.2 ng/L
Saronicos gulf 2004	(Total PAHs)	

AREAS	Substances	Concentrations
		100-1560 ng/L
Saronicos gulf 2005	(Total PAHs)	70-3010 ng/L
Elefsis gulf	PAHs (17)	425–459 ng/L
South Euvoikos gulf	PAHs (17)	12-224 ng/L (2003-4)
Maliakos gulf	PAHs (17)	12-214 ng/L (2003-4)

Table 3: PAHs in sediments of various Greek areas

Area	Substances	Concentrations
NW Saronicos	PAHs (16)	72.2-184.9 ng/g
Saronicos gulf 2004	Total PAHs	108.5-8416.9 μg/g
West Saronicos gulf	PAHs (21)	109-224 ng/g (2005-2008)
Elefsis Gulf	Total PAHs	1615.6-3569.3 ng/g (2004)
Korinthiakos gulf	Total PAHs	283-4248 ng/g (2004)
Pagasitikos gulf	Total PAHs	103-5158 ng/g (2004)
Thessaloniki gulf	PAHs (21)	145-943 ng/g (2005-2008)
North Euvoikos gulf	PAHs (21)	133-6776 ng/g (2005-2008)

1.4. DETERMINATION OF PAHS IN SEA WATER, MUSSELS, FISHES AND SEDIMENTS

The determination of PAHs for this study were carried out in the Laboratory of Environmental Analyses of the NCSR Demokritos, which is accreditate for such measurements.

- Each seawater sample was filtered and was pre-concentrated by solid phase extraction (SPE) in IST-ISOLUTE C18 cartridges. Extraction cartridges were preconditioned with methanol and then with distilled water. PAHs were eluted with a

solution of 1:1 dichloromethane and n-hexane. Extracts were preconcentrated by a rotary vacuum evaporator and then to near dryness by gently passing of dry nitrogen and then reconstituted to 250 μL of acetonitrile.

- Immediately after collection, mussels, fishes and sediments were transferred fresh inside an ice cool box to the laboratory. Samples were lyophilized during 3 days and afterwards smashed in a mortar until obtaining fine sand. For the extraction and purification procedure of PAHs a Soxhlet system was used. The extract collected and preconcentrated in a rotary vacuum evaporator, evaporated at room temperature with a gently stream of nitrogen and reconstituted in 250 μL of acetonitrile.
- 20μL of the final samples were inserted three times into a high performance liquid chromatography (HPLC) system with Fluorescence detector or a Liquid Chromatography – Mass Spectroscopy system. Individual PAHs were identified on the basis of retention time by using a processing method and library, while their concentrations were determined with the use of calibration curves (external standards of PAHs, Dr Ehrenstorfer, PAH Mix 18) and peak area ratios of the analyte peak to the area of the internal standard peak.

1.5. RESULTS OF ANALYSES OF SEAWATER SAMPLES (MG/L)

	NAPH	ANTH	FLUO	B[b]F	B[k]F	B[a]P	B[ghi]P	IP
11 Bottom/ EPA 398	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
13 Bottom/ EPA 399	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
1/EPA 445	<LOQ	N.D	N.D	N.D	N.D	N.D	N.D	N.D
2/ EPA 446	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D

ND: not detected

		LOD(μg/L)	LOQ(μg/L)
Naphthalene	NAPH	0.0302	0.1
Anthracene	ANTH	0.0017	0.01
Fluoranthene	FLUO	0.0573	0.1
Benzo(b)Fluoranthene	B[b]F	0.0027	0.01
Benzo(k)Fluoranthene	B[k]F	0.0015	0.01
Benzo(a)Pyrene	B[a]P	0.0016	0.01
Benzo(g,h,i)Perylene	B[ghi]P	0.0022	0.01
Indeno(1,2,3-cd)Pyrene	IP	0.0029	0.01

LOD: level of detection, LOQ: level of quantification

1.6. RESULTS OF SEDIMENTS ANALYSES (MG/G – DRY WEIGHT)

		NAPH	ANTH	FLUO	B[b]F	B[k]F	B[a]P	B[ghi]P	IP
1	D1/EPA 372	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
2	D3/EPA 373	N.D	<LOQ	N.D	**0.005**	<LOQ	<LOQ	<LOQ	N.D
3	D4/EPA 374	N.D	N.D	N.D	<LOQ	N.D	N.D	N.D	N.D
4	D6/EPA 375	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
5	D7/EPA 376	N.D	<LOQ	N.D	**0.004**	<LOQ	<LOQ	<LOQ	N.D
6	D8/EPA377	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
7	D10/EPA 378	N.D	N.D	N.D	<LOQ	N.D	N.D	<LOQ	N.D
8	D11/EPA 379	N.D	<LOQ	<LOQ	**0.008**	**0.004**	**0.006**	**0.005**	<LOQ
9	D12/EPA 380	N.D	N.D	N.D	<LOQ	N.D	N.D	<LOQ	N.D
10	D13/EPA 381	N.D	N.D	N.D	<LOQ	N.D	N.D	N.D	N.D

		NAPH	ANTH	FLUO	B[b]F	B[k]F	B[a]P	B[ghi]P	IP
11	D20/EPA 382	N.D	N.D	N.D	<LOQ	N.D	N.D	<LOQ	N.D
12	PRINOS 1/EPA 447	N.D	N.D	N.D	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
12	PRINOS2/ EPA 448	N.D	N.D	N.D	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

ND: not detected, LOD: level of detection, LOQ: level of quantification

		LOD(μg/g)	LOQ(μg/g)
Naphthalene	NAPH	0.01	0.03
Anthracene	ANTH	0.001	0.003
Fluoranthene	FLUO	0.01	0.03
Benzo(b)Fluoranthene	B[b]F	0.001	0.003
Benzo(k)Fluoranthene	B[k]F	0.001	0.003
Benzo(a)Pyrene	B[a]P	0.001	0.003
Benzo(g,h,i)Perylene	B[ghi]P	0.001	0.003
Inden0(1,2,3-cd)Pyrene	IP	0.001	0.003

1.7. RESULTS OF MUSSELS , FISHES ANALYSES (MG/KG – DRY WEIGHT)

		NAPH	ANTH	FLUO	B[b]F	B[k]F	B[a]P	B[ghi]P	IP
1	FISH1/EPA 368	18.32	0.69	<LOQ	N.D	N.D	N.D	N.D	N.D
2	FISH2/EPA 369	27.45	1.03	0.98	N.D	N.D	N.D	2.04	N.D
3	FISHES 3+4/EPA 370	17.66	0.65	1.13	N.D	<LOQ	N.D	0.93	N.D
4	MUSSELS/EPA 371	4.17	<LOQ	<LOQ	N.D	N.D	N.D	0.35	N.D

ND: not detected, LOD: level of detection, LOQ: level of quantification

		LOD(μg/kg) dw	LOQ(μg/kg) dw
Naphthalene	NAPH	1.012	3.035
Anthracene	ANTH	0.076	0.228
Fluoranthene	FLUO	0.304	0.911
Benzo(b)Fluoranthene	B[b]F	0.247	0.74
Benzo(k)Fluoranthene	B[k]F	0.052	0.156
Benzo(a)Pyrene	B[a]P	0.079	0.238
Benzo(g,h,i)Perynene	B[ghi]P	0.108	0.325
Inden0(1,2,3-cd)Pyrene	IP	0.194	0.583

1.8. CONCLUSIONS

The results of the determination of the main PAHs in seawater, sediments and marine organisms of the studied area indicate the non-existence of pollution problems concerning this type of pollution.

As it concern seawater almost all values were below detection limit, and probably below of the EU thresholds for Good Environmental Status of marine environment. Taking into account the LOD and LOQ values the concentrations of PAHs in the area should be in levels similar to the lower values that have been measured in various Greek Gulfs (see relative table).

A similar situation was observed in marine sediments. The concentrations were below the EU thresholds for Good Environmental Status of marine environment and of the values that have been reported in other Gulfs.

As it concern marine organisms the values were lower from the upper limits both for human consumption and for Good Environmental Status.

The general conclusion of the results of the analysis of these samples is that although the area is near to an oil drilling activity, the concentrations of poly aromatic hydrocarbons are in low levels. It is an encouraging result that must be followed by the establishment of a permanent monitoring system in order to avoid any deterioration of marine environment.

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