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Detection of Carcinogenic Polycyclic Aromatic Hydrocarbons in Stranded Caspian Seals (*Pusa caspica*)

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

*Published Version:*

Detection of Carcinogenic Polycyclic Aromatic Hydrocarbons in Stranded Caspian Seals (*Pusa caspica*) / Seyedeh Malihe Hoseini, Somayeh Namroodi, Annalisa Zaccaroni, Amir Sayad-Shirazi, Marcos Pérez-López, Francisco Soler-Rodríguez. - In: AQUATIC MAMMALS. - ISSN 0167-5427. - ELETTRONICO. - 46:1(2020), pp. 58-66. [10.1578/AM.46.1.2020.58]

*Availability:*

This version is available at: <https://hdl.handle.net/11585/713461> since: 2020-01-14

*Published:*

DOI: <http://doi.org/10.1578/AM.46.1.2020.58>

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(Article begins on next page)

1 This is the final peer-reviewed accepted  
2 manuscript of: S. M. Hoseini, S. Namroodi,  
3 A. Zaccaroni, A. Sayad-Shirazi, M. Pérez-  
4 López, and F. Soler-Rodríguez: Detection of  
5 Carcinogenic Polycyclic Aromatic  
6 Hydrocarbons in Stranded Caspian Seals  
7 (*Pusa caspica*). AQUATIC MAMMALS  
8 Volume 46 (1) Pages 58-66. The final  
9 published version is available online at:  
10 <https://doi.org/10.1578/AM.46.1.2020.58>

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14 **Detection of Carcinogenic Polycyclic Aromatic Hydrocarbons in Stranded Caspian Seals**  
15 **(*Pusa caspica*)**

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27

28 **Abstract**

29 Polycyclic aromatic hydrocarbons (PAHs), which contain many carcinogenic compounds, are a  
30 major ingredient of petroleum/oil. PAH pollution of the Caspian Sea, the world's largest lake, is  
31 rapidly occurring and may be affecting the endangered Caspian seal (*Pusa caspica*), the only  
32 marine mammal in this lake. To analyze the entrance of PAHs into the Caspian Sea food chain and  
33 the health status of Caspian seals, we measured 16 carcinogenic PAHs in the blubber, kidney, and  
34 liver tissues of 10 Caspian seal carcasses from the coastal region of northeastern Iran, using gas  
35 chromatography-mass spectrophotometry. Of the 16 PAHs investigated, anthracene,  
36 phenanthrene, and naphthalene only were identified in nine sampled Caspian seals. Concentrations  
37 of anthracene ( $\bar{x}=84.83\pm 79.86$  ppb wet weight [w.w.]), phenanthrene ( $\bar{x}=31.75\pm 52.22$  ppb w.w.),  
38 and naphthalene ( $\bar{x}=25.1\pm 31.57$  ppb w.w.) in blubber tissues were higher than in kidney and liver  
39 tissues. The concentration of PAHs in tissues was significantly higher in male than in female seals

40 and we found an inverse relationship between seal age and PAH concentration in tissues. Although  
41 no data exist concerning toxic effect concentrations of PAHs in Caspian seals, PAH detection in  
42 seal carcasses highlights a potentially stressful condition that may impact the health of Caspian  
43 seals and other sea life in this lake. Appropriate strategies for the control of PAH entrance into the  
44 Caspian Sea should be sought and studies for the determination of pathogenic and lethal doses of  
45 PAHs in Caspian seals should be pursued.

46 **Key words:** PAHs, Iran, tissues, *Pusa caspica*

47

#### 48 **Introduction**

49 The Caspian Sea, the largest closed lake in the world, is surrounded by five countries including  
50 Russia, Ghazakhstan, Azerbaijan, Turkmenistan and Iran (Shinsuke et al., 2003). This  
51 multinational access, in addition to the high density of passenger and commercial vessels and the  
52 influx of contaminated water from the numerous rivers that flow through industrial and agricultural  
53 areas, have resulted in the Caspian Sea becoming increasingly polluted (Effimoff, 2000; Kaplin,  
54 1995). Extraction of oil from the lake, especially from the central and northern parts, and the entry  
55 of petroleum-based fuels in contaminated water have also contributed to pollution of the lake  
56 (Mille, 2007; Tolosa, 2004).

57 Polycyclic aromatic hydrocarbons (PAHs) with benzene rings are a highly toxic component  
58 of oil. Sixteen compounds of PAHs from oil have been identified as highly toxic and mutagenic  
59 for animals (Freeman et al., 1990). Generally, PAHs enter the environment in two ways: (a) human  
60 activities (pyrolytic origin) such as extraction, refining, transfer and export of crude oil,  
61 anthropogenic oil spills and other products, and (b) natural entrance (petrogenic origin) of  
62 biological resources through PAH synthesis by plankton, bacteria, algae, and the decomposition

63 of plants (Nasrollahzadeh Saravi et al., 2013). Fortunately, PAHs break down quickly when  
64 exposed to light and oxygen (e.g., photo-oxidation) and some PAHs are biodegraded by naturally-  
65 occurring bacteria (Kanaly & Harayama, 2000; Mrozik et al., 2003). PAHs divided in low and  
66 high molecular weight based on number of their benzene rings. Oil spills represent one of major  
67 threats for marine mammals, both in the short and long term. However, high molecular weight  
68 (HMW) PAHs have higher tendency to accumulate in animals' tissues than low molecular weight  
69 (LMW) PAHs. In aquatic ecosystem HMW PAHs precipitate faster than LMW PAHs and have  
70 lower chance to accumulate in body of marine animals (Lawal, 2017). Limited studies have shown  
71 PAH pollution in Caspian Sea coastal areas of Baku (due to oil extraction), northern Russia and  
72 Kazakhstan, and southern Iran (Kardovani, 1995; Tolosa, 2004).

73         The Caspian seal (*Pusa caspica*), the only species of marine mammal in the Caspian Sea,  
74 is at the top of the food chain in this ecosystem. This species has migratory life style and due to  
75 various factors such as death in fishing nets and reduced reproductive rates in male and female  
76 seals because of exposure to pollutants, the health status of this species is poor (Harkonen et al.  
77 2008, ;Watanabe et al., 2002). Mass mortalities of these seals along the Caspian Sea coast during  
78 1997-2000, along with the factors cited above, have resulted in placement of the Caspian seal on  
79 the IUCN red list (Goodman & Dmitrieva, 2016). Considering the increase in Caspian Sea oil  
80 pollution in recent years and the migratory lifestyle of Caspian seals, we monitored PAH pollution  
81 in the Caspian Sea and their health status by measuring and assessing concentrations of 16  
82 mutagenic PAHs in stranded Caspian seal tissues.

83

84

## **Methods**

85 *Sampling*

86 After approval of the study by the Ethics Committee of the Deputy of Natural Environment of  
87 Golestan Province (permit number: 125/7894), 10 stranded Caspian seals along the coasts of  
88 Golestan and Mazandaran provinces, Iran, were collected during 2012-2015. Stranded Caspian  
89 seals were trapped in fishing nets and were found fresh by fisheries. Following biometric analysis  
90 and clinical examination, the seals were transferred to the laboratory and necropsied. Liver, kidney,  
91 and blubber tissues were sampled to measure PAH concentrations and a lower canine tooth was  
92 extracted to determine seal age (Amano et al., 2000).

93

#### 94 *Measurement of PAH concentration*

95 This analysis was performed at the Toxicology Lab, Faculty of Veterinary Medicine, University  
96 of Extremadura, Caceres, Spain. To measure tissue concentrations of PAHs, the standard method  
97 of Lucas and Zhao (2015) using gas chromatography-mass spectrometry (GC-MS/MS) was  
98 applied. The samples were shredded with glass slurry, freeze dried for 72 h, and homogenized  
99 using a mortar and pestle. A portion of each freeze-dried sample was weighed into 50 mL  
100 centrifuge tubes and MilliQ water was added to obtain the equivalent to 5 g of fresh sample. Ten  
101 ml of acetonitrile were added to the tube and the sample was shaken vigorously by hand for 2 min  
102 and centrifuged for 5 min at 5,000 rpm. The supernatant was transferred to a 15-ml centrifuge tube  
103 containing 1 g of Agilent Enhanced Matrix Removal-Lipid sorbent (Agilent Technologies, Santa  
104 Clara, California, USA) and placed on a vortex mixer for 60 s. The solution was then centrifuged  
105 at 5,000 rpm for 3 min. The supernatant was transferred to a second 15-ml tube containing 2 g salt  
106 (1:4 NaCl: MgSO<sub>4</sub>) and immediately vortexed and centrifuged for 3 min. The top layer containing  
107 acetonitrile was evaporated to dryness in a rotatory concentrator, reconstituted in ethyl acetate:n-  
108 hexane (1:4) and filtered (0.45 micron) into a 2-ml GC vial.

109            Obtained extracts (1  $\mu$ l) were injected into a gas chromatograph-mass spectrometer  
110 (SCION GC-MS-Triple Quad MS/MS; Bruker Scientific Instruments, Billerica Massachusetts,  
111 USA) and PAHs were separated on a 30-m Agilent J&W DB-5ms (5%  
112 diphenyl/dimethylpolysiloxane) capillary column (250  $\mu$ m id. x 0.25  $\mu$ m film thickness). For  
113 quality control and for quantitative purposes, one sample from each matrix (blubber, liver, and  
114 kidney) was pre-spiked at 40 ng/g with a certificate standard (DE-PROM 16 EPA Priority PAHs  
115 in Toluene from LGC Standards, 100  $\mu$ g/ml) containing the 16 PAHs considered to be of primary  
116 concern by the United States Environmental Protection Agency (USEPA). Qualification and  
117 quantification ions were monitored for each compound at m/z = 102+126+127 for naphthalene,  
118 126+150+151 for acenaphthylene, 127+151+152 for acenaphthene, 139+163+164 for fluorene,  
119 152+176+177 for phenanthrene, anthracene, 200 for fluoranthene, 151+200 for pyrene,  
120 202+226+227 for benzo[a]anthracene, chrysene, 250 for benzo[b]fluoranthene,  
121 benzo[k]fluoranthene, benzo[a]pyrene, 274 for benzo[g,h,i]-perylene, indeno[1,2,3-cd]pyrene,  
122 and 276+277 for dibenzo[a,h]anthracene.

123            Results of each spiked matrix were used for quantitation of matrix-related samples. These  
124 matrix-matched samples were chosen in order to compensate for any signal  
125 suppression/enhancement compared to their relative response in pure solvent. Procedural blanks  
126 containing reagents only were screened during the analysis of each batch (one per tissue) in order  
127 to ensure that solvents and all the used material were free from PAHs residues. To evaluate the  
128 linearity of the method calibration curves were built with concentrations ranging from 4 to 400  
129  $\mu$ g/L with  $r^2 > 0.98$ . The limit of quantitation (LOQ) was determined as the lowest concentration  
130 of the compounds which can be reliably detected with the signal-to-noise (S/N) ratio higher than

131 10. In our study, the LOQ for individual PAHs in samples ranged from 0.5 to 5 ng/g, on a wet  
132 weight [w.w.] basis.

133

#### 134 *Data analysis*

135 Normality and lack of data were evaluated using Kolmogorov-Smirnov test. The result of this test  
136 was higher than 0.05, indicating that the data was normal. The effect of sex on the PAH  
137 concentration in sampled tissues was investigated using Student's t-tests. Linear regression test was  
138 used to survey impact of age on PAH concentration. Also ANOVA one way test was used to survey  
139 difference of PAH concentrations in sampled tissues.

140

### **Results**

141 Of the 16 PAHs studied, three (i.e. anthracene (Log  $K_{OW}$ =4.54, octanol-air partition coefficient  
142 ( $K_{AO}$ )=7.55, number of benzene rings (NB) = 3), phenanthrene (Log  $K_{OW}$ =4.57,  $K_{AO}$ =7.57, NB=3)  
143 and naphthalene (Log  $K_{OW}$ =3.37,  $K_{AO}$ =5.19, NB=2) were identified in the tissues of sampled  
144 Caspian seals (Table 1) (National Center for Biotechnology Information, 2019). We detected  
145 PAHs in nine of the 10 sampled seals at different concentrations and the accumulation pattern of  
146 PAHs differed among tissue types. Among blubber tissues, 70% were contaminated with  
147 anthracene, 50% with naphthalene, and 40% with phenanthrene. For liver tissues, 80% of samples  
148 also were contaminated with anthracene, 80% contained phenanthrene, and 50% contained  
149 naphthalene. For kidney tissues 90% were contaminated with anthracene and 40% with  
150 naphthalene. Phenanthrene contamination was not detected in kidney tissues (Table 1). The  
151 concentrations of anthracene ( $\bar{x}$ =84.83±79.86 ppb w.w.), phenanthrene ( $\bar{x}$ =31.75±52.22 ppb  
152 w.w.), and naphthalene ( $\bar{x}$ =25.1±31.57 ppb w.w.) in blubber tissues were significantly ( $p$ =0.001)  
153 higher than in kidney and liver (Table 2). The concentration of anthracene in all sampled tissues



154 was significantly ( $p=0.001$ ) higher than the concentrations of phenanthrene and naphthalene (Table  
155 2). Concentrations of PAHs in liver, kidney, and blubber were significantly higher in male than in  
156 female seals (Table 3 & Figure 1). Linear regression test indicated a negative relationship between  
157 Caspian seal age (using body length as a surrogate for age) and the concentration of PAHs in the  
158 tissues (Figure 2).

159 Place table 1, 2, and 3 here, Place figures 1&2 here

160

161

### Discussion

162 Detection of PAHs in sampled Caspian seals indicated that PAHs entered the Caspian Sea food  
163 chain. Also, previous studies have indicated pollution with just anthracene, phenanthrene, Dibenz  
164 (a,h) anthracene, fluoranthene, fluorene, and naphthalene in the Caspian Sea (Eskandarpour et al.,  
165 2014; Nasrallahzadeh Saravi et al., 2012). Absence of other 13 surveyed PAHs in Caspian seals'  
166 tissues can be due to elimination of them in Caspian seals by metabolization or absence of them in  
167 Caspian seals' habitat (Meador et al., 1995b). Nasrallahzadeh Saravi et al. (2012) studied the  
168 sediment and muscle tissue of fish in the southern Caspian Sea near Astara, White River,  
169 Tonekabon, and Amir Abad, Iran. They measured an anthracene concentration of  $7.6\pm 4$  ppb w.w  
170 and dibenzene concentration of  $66.6\pm 75$  ppb w.w in sediment. Benzo fluoranthene, and benzo  
171 pyrene were detected in muscle tissues of several Caspian Sea fish species, including the Caspian  
172 kutum (*Rutilus frisii kutum*) with concentrations of 530 ppb w.w. and 96.6 ppb w.w. and the  
173 leaping mullet (*Chelon saliens*) at 80 ppb w.w. and 176 ppb w.w., respectively. In another study  
174 on fish flour (fish meal) of Caspian Sea sprat (*Clupeonella cultriventris caspia*), naphthalene,  
175 fluorine, and anthracene concentrations were measured at  $24.66\pm 15.52$  ppb w.w.,  $1.32\pm 1.54$  ppb  
176 w.w., and  $1.1\pm 1.92$  ppb w.w., respectively (Eskandarpour et al., 2014). Absence of the other

177 studied PAHs in sampled Caspian seals can be due to short time presence of Caspian seal in sea  
178 shore of Iran (Harkonen et al. 2008). As surveyed tissues in previous mentioned studies on fish are  
179 not similar with tested tissues in our study, we cannot compare obtained results. However,  
180 Compared to the PAH concentration in some of these fish species, the higher concentrations in  
181 sampled Caspian seals may be due to higher concentrations of fat in Caspian seal tissue and their  
182 greater longevity, allowing for longer bioaccumulation.

183         Unfortunately, there are few similar studies on carcinogenic PAH contamination in marine  
184 mammals. Marsili et al. (2001) measured total PAHs in blubber tissues of fin whales  
185 (*Balaenoptera physalus*) and striped dolphins (*Stenella coeruleoalba*) and found high levels of  
186 contamination in these Mediterranean cetaceans. Marsili et al. (1997) surveyed PAH concentration  
187 in liver tissues of South American sea lions (*Otaria flavescens*) in the Plata Sea, Argentina. They  
188 detected naphthalene ( $\bar{x}=194\pm54.07$  ppb w.w), anthracene ( $\bar{x}= 0.30\pm2.270$  ppb w.w.), and  
189 phenanthrene ( $\bar{x}=27.1\pm21.75$  ppb w.w.) in sampled tissues. Many factors such as level and time  
190 of animal PAHs exposure, sex and age of sampled animals, species ability to metabolize PAHs  
191 and difference in volume of PAHs entrance in animals' habitat can result in detection of higher  
192 concentrations of naphthalene and phenanthrene and lower concentration of anthracene in sampled  
193 sea lions than sampled Caspian seals (Meador et al., 1995b). Also, Hellou et al. (1991) examined  
194 PAH concentration in muscle tissue of harp seals (*Phoca groenlandica*) in the northwestern  
195 Atlantic Ocean. Those specimens were contaminated with phenanthrene, anthracene, and fluorene,  
196 and PAH concentration was measured between 10 and 31 ppb lipid w.w. As we did not examine  
197 muscle tissues in sampled Caspian seals, we cannot compare our results with results of Hellou et  
198 al. (1991) on harp seals.

199           Of the three PAHs detected (naphthalene, phenanthrene, and anthracene), anthracene  
200 occurred at the highest concentrations in sampled tissues while phenanthrene had the lowest  
201 concentrations. A different trend in PAH concentrations has been documented in fish species of  
202 the Caspian Sea; for example, Eskandarpour et al. (2014) reported higher concentrations of  
203 naphthalene than anthracene in Caspian Sea sprat (*Clupeonella cultriventris caspia*). Similarly,  
204 Kannan & Perrotta (2008) detected naphthalene in kidney tissues in higher concentrations  
205 compared to other PAHs in 81 adult female sea otters (*Enhydra lutris*) from the California coast.  
206 Many factors, such as differences in the source of PAHs in sampling areas, chemical condition of  
207 the sampling area, number of PAH benzene rings, and tendency for bioaccumulation of PAHs in  
208 different species could lead to these differences in findings (Meador et al., 1995b).

209           As mentioned, tissue accumulation patterns of detected PAHs differed among Caspian seal  
210 tissue types. The number of PAH benzene rings is an important variable associated with  
211 bioaccumulation of PAHs in Caspian seal tissues. With increases in the number of rings, the  
212 hydrophobic and lipophilic properties of PAHs increase. When PAHs enter Caspian seal bodies,  
213 they can accumulate in several tissues, but especially in blubber. Microbial degradation of PAHs  
214 decreases with increases in the number of PAH benzene rings, so PAHs with more benzene rings  
215 can accumulate to higher concentrations in tissues (Landrum, 1989; Landrum & Robbins, 1990;  
216 Meador et al., 1995a; Meador, 2003). It follows that higher tissue concentration of anthracene than  
217 naphthalene could be explained by the higher number benzene rings of anthracene. Higher  
218 anthracene tissue concentration than phenanthrene, which has the same number of benzene rings,  
219 might be explained by a higher rate of entry of anthracene into the Caspian Sea.

220           The higher PAH concentrations in blubber tissues may be explained by the hydrophobic  
221 and lipophilic characteristics of PAHs. Eskandarpour et al. (2014) found that bioaccumulation of

222 PAHs in Caspian Sea sprat was directly related to the concentration of fat in sampled tissues, with  
223 higher concentrations detected in tissues with higher levels of fats (Eskandarpour et al., 2014). In  
224 contrast, there was no correlation between tissue PAH concentrations and the amount of fat in  
225 tissues of Harp seals (Hellou et al., 1991).

226 We found the lowest PAH concentrations in liver tissues. Most of the PAHs are absorbed  
227 through the digestive system and transferred to the liver for detoxification by enzymes, including  
228 the P450 family and microsomal enzymes (Engelhardt, 1982; Addison & Brodie, 1984; Marsili et  
229 al., 1997; Lee et al., 2005). This detoxification process could lead to lower concentrations of PAHs  
230 in liver tissues than blubber or kidney.

231 A negative correlation between PAH concentration in tissues with the age of sampled Caspian  
232 seals was found, indicating the influence of age as a confounding factor on PAH concentrations in  
233 our seals. Similarly, studies of Northern pike (*Esox lucius*) and Caspian Sea sprat revealed that  
234 animal age can affect PAH bioaccumulation rate with similar trends (Ghorbani et al., 2010;  
235 Eskandarpour et al., 2014). As age increases, PAH concentration in tissues can decrease due to an  
236 increase in PAH metabolism. Hellou et al. (1991) showed that PAH concentration was higher in  
237 younger Harp seals than older seals. They also found that the activity of the metabolizing enzymes,  
238 including P450 family enzymes, on PAHs increases with age and can lead to a decrease in PAH  
239 concentrations in tissues by age (Hellou et al., 1991). However, Harris et al., (2011) who surveyed  
240 Hydrocarbon concentrations and patterns in blood samples of 29 live-captured sea otters (*Enhydra*  
241 *lutris*) from British Columbia, Canada, reported similar hydrocarbon concentrations among  
242 different age classes (Harris et al., 2011).

243           The higher average age sampled females (18.2 y old) than males (14.5 y old), may be one  
244 explanation for our finding showing lower PAH concentrations in tissues of female Caspian seals,  
245 highlighting the putative biotransformation of PAHs by P450 enzymes. This result supports  
246 findings of Addison et al. (1973) and Muyer et al. (1988) in harp seals and ringed seals (*Phoca*  
247 *hispida*), respectively. Those authors suggested that this difference could be due to higher activity  
248 of P450 family enzymes in females resulting in a lower PAH concentration in female tissues.  
249 Eskandarpour et al. (2014) explained that such results can occur due to maternal transfer of PAHs  
250 in fish through egg laying. However, studies by Hellou et al. (1991) and Marsili et al. (2001) on  
251 harp seals and cetaceans (fin whales and striped dolphins), respectively, showed no relation  
252 between sex and PAH tissue concentrations.

253           Elimination of organochlorine pesticides in female Caspian seals through giving birth has  
254 been described, but there is no information about offloading of PAHs in seals via that mechanism  
255 (i.e. maternal transfer) (Tanabe et al., 1978; Subramanian et al., 1987; Nakata et al., 1995). Thus,  
256 additional studies are needed on this topic.

257           Molecular ratios of PAHs have been used to identify their sources in the environment.  
258 According to Hajizadeh et al. (2010), if the ratio of phenanthrene to anthracene is >10, the origin  
259 of these pollutants is considered petrogenic, and but if the ratio is <10, their origin is considered  
260 pyrolytic. Nasrollahzadeh Saravi et al. (2012), who detected PAHs in leaping mullet and Caspian  
261 kutum in the southern part of the Caspian Sea (i.e. Mazandaran and Golestan), reported that the  
262 origin of PAHs in that region of the Caspian Sea is more pyrolytic. Nemati Varnosfaderany et al.  
263 (2014) and Baniemam et al. (2017) reported both pyrolitic and petrogenic origins of PAHs in the  
264 south Caspian Sea (Iranian coastal regions). In the present study, the ratio of PAHs with low  
265 molecular weight (phenanthrene) to those with a high molecular weight (anthracene) also indicated

266 that the PAH compounds in sampled Caspian seals may have pyrolytic sources. Nevertheless,  
267 Kannan & Perrotta (2008) concluded that the detection of PAHs with a predominance of di- and  
268 tri-cyclic PAHs over tetra- and penta-cyclic PAHs (as we also detected in Caspian seals) suggest  
269 petrogenic sources.

270 In conclusion, it appears that Caspian seals are exposed to PAHs of both petrogenic and  
271 pyrolytic origins in the Caspian Sea. Our results show that PAHs eventually accumulate in the  
272 lake's food chain and enter Caspian seal tissues. Because PAHs retention rate time are low in the  
273 environment (i.e. high elimination rate), their accumulation in Caspian seals indicates that the  
274 emission or inflow rate of PAH entering into the Caspian Sea ecosystem is much higher than their  
275 rate of removal (e.g., burial rate). Of course, given the migratory lifestyle of Caspian seals, it is  
276 not possible to identify the exact route and location of PAH contamination of Caspian seals  
277 (Anyakora et al., 2005).

278 No ecotoxicological risk assessments have been conducted to derive the toxic effect  
279 concentration and/or safe level thresholds of PAHs on Caspian seals. PAH accumulation in tissues  
280 could have negative effects on the health status of this species, even at very low concentrations.  
281 Appropriate strategies for limitation of PAH entrance into the Caspian Sea should be considered  
282 in countries bordering the Caspian Sea. Further studies are needed to determine pathogenic and  
283 lethal doses of PAHs in Caspian seals.

284

### 285 **Acknowledgments**

286 This study was supported by Gorgan University of Agricultural Sciences and Natural Resources.

287 The author thanks Dr. James Norman Milles for edition of the earlier manuscript that greatly

288 improved it. We also show our gratitude to Seyyed Reza Kahlegi for her help in obtaining some  
289 sampled Caspian seals.

290

291

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*PAHs exposure in Caspian seals*

407 **Table 1:** PAHs concentration (ppb w.w.) in the tissues of Caspian seals based on age and sex.

Sample number	1	2	3	4	5	6	7	8	9	10	Average
Sex	F	F	M	F	F	M	M	M	M	M	
Age	26	17	16	11	19	9	10	20	6	26	
A(BT)	0	56.93	82.44	95.86	44.22	194.55	174.69	0	199.68	0	84.83±79.86
A(LT)	0	6.68	7.34	8.56	6.35	10.42	10.05	7.15	14.27	0	7.08±4.40
A(KT)	0	12.04	12.49	12.24	9.33	17.93	13.06	12.01	27.30	10.24	11.64±7.91
PH(BT)	0	0	0	47.93	0	87.34	27.48	0	154.75	0	31.75±52.22
PH(LT)	0	5.83	7.13	8.64	5.71	9.56	8.31	5.72	12.68	0	6.35±3.96
PH(KT)	0	0	0	0	0	0	0	0	0	0	0
N(BT)	0	26.53	0	47.93	0	58.23	28.46	0	89.85	0	25.1±31.57
N(LT)	0	0	0	7.42	0	8.88	0	0	10.49	0	2.67±4.37
N(KT)	0	0	0	4.08	3.90	8.16	3.58	0	8.53	0	2.82±3.40

408 Anthracene: A, Phenanthrene: PH, Naphthalene: N, L: Liver, K: Kidney, B: Blubber, F: Female, M: Male

*PAHs exposure in Caspian seals*

409 **Table 2:** Results of ANOVA test on concentration of PAHs (ppb w.w.) in different tissue samples

	Blubber	P	Liver	Kidney
411 Anthracene	84.83±79.86**	0.001	7.08±4.40*	11.64±7.91*
412 Phenanthrene	31.75±52.22**	0.001	6.35±3.96*	0 <sup>b</sup> *
413 Naphthalene	25.1±31.57**	0.01	2.67±4.37*	2.82±3.40*

414 \* Unsignificant difference, \*\* Significant difference (p<0.05)

415

416 **Table 3:** Results of Student's t-tests between sex of sampled seals and concentration of PAHs (ppb w.w.) in tissue samples

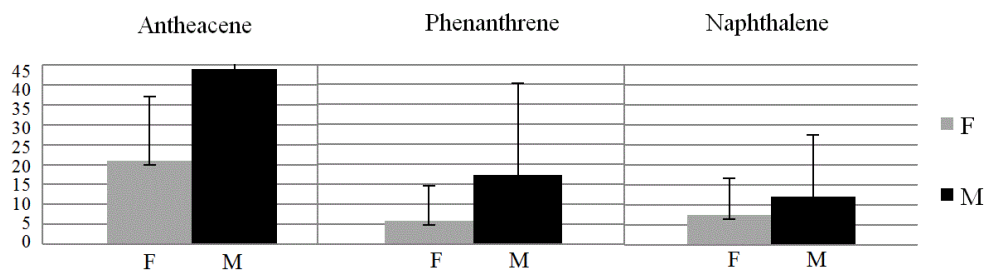
PAHs concentration in liver, kidney and blubber tissues	P	t-value
Concentration of anthracene in blubber tissue	0.00*	0.46
Concentration of anthracene in liver tissue	0.03*	0.08
Concentration of anthracene in kidney tissue	0.04*	3.70
Concentration of phenanthrene in blubber tissue	0.00*	3.58
Concentration of phenanthrene in liver tissue	0.04*	2.28
Concentration of naphthalene in blubber tissue	0.00*	5.95
Concentration of naphthalene in liver tissue	0.01*	3.65
Concentration of naphthalene in kidney tissue	0.03*	8.83

417 \* Significant difference (p<0.05)

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419

420 **Fig 1:** Mean (SD) PAHs concentration (ppb w.w.) in male and female Caspian seals



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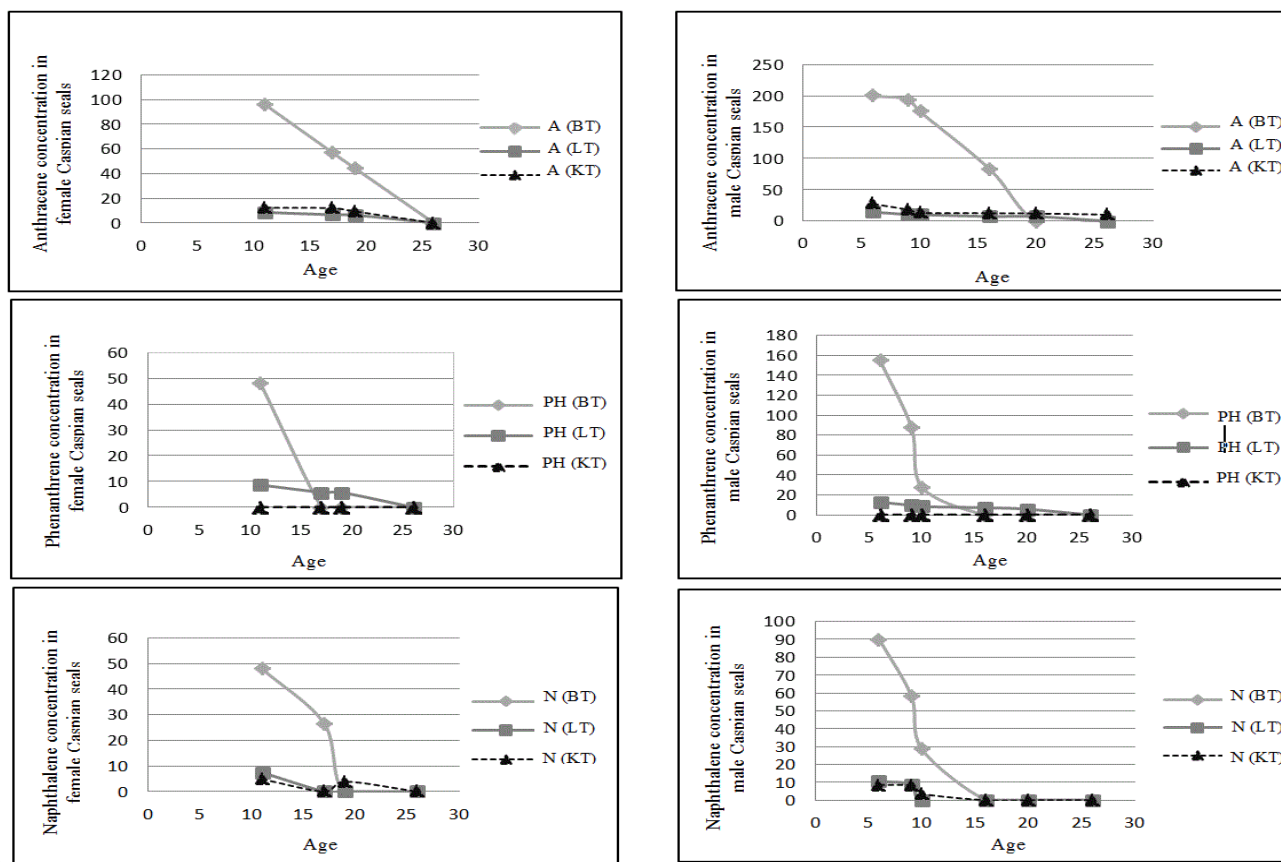
422 F: female, M: male

423 **Fig 2:** Results of liner regression test on relationship between PAHs concentration (ppb w.w.) and age (year) of male and female

424 Caspian seals

425

PAHs exposure in Caspian seals



426

a

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A: anthracene, N: naphthalene, PH: phenanthrene, BT: blubber tissue, LT: liver tissue, KT: kidney tissue