

## Polycyclic aromatic hydrocarbons (PAHs) traceability in old buffalos living in south west part of Iran

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### ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are a class of complex substances that are produced during incomplete combustion of fossil fuels. PAHs are known to be persistent pollutants which can remain in nature for years. In 1991 during the first Persian Gulf War south west part of Iran was contaminated heavily. This study was undertaken to see if the PAHs can be traceable in animal tissues many years after the incidence. One hundred of buffalos aged 12±2 years old were selected from polluted regions and to compare the results, 50 camels at same range age were also selected from a control region where expected to be contaminated by local crude oil. All the animals were scarified and fat samples were collected. Sixteen PAHs were determined by HPLC. While some of low molecular weight PAHs were not detectable, in samples from polluted region significant amount of heavy molecular weight PAHs were detected in samples of same animal fat tissues. On the other hand although most of PAHs were detected in animal samples from control region but the amount of PAHs of high molecular weight were comparatively lower in control region. Results of this study reveals that south west part of I.R. of Iran was highly contaminated by pollutants emitted during burning of Kuwait oil well in 1991. This study may be helpful in finding the PAHs contamination and traceability of organic pollutants even after many years has passed from the incidence.

**Keywords:** PAHs, Persian Gulf War, Benzo[ $\alpha$ ]pyrene, Pollutants, Livestock,

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### INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are persistent organic pollutants and can remain in nature for many years. The half life for different PAHs is varied depending on their molecular weight and molecular structure. In general, polycyclic aromatic hydrocarbons and some of their metabolites

are known to react with cellular macromolecules, including DNA, which may account for both their toxicity and carcinogenicity. In 1991 during the first Persian Gulf War, more than 700 oil wells set on fire by Iraqi troops. The amount of oil burned during those days was estimated to be 2.5 millions barrels per day (Husain 1995). One of the regions that was polluted by wet and dry deposition of pollutants was south west part of Iran (Aminipuri 1998). One of the

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most hazardous chemicals that emitted to the air was polycyclic aromatic hydrocarbons (PAHs).

Animal studies have shown that polycyclic aromatic hydrocarbons in general and benzo [*a*]anthracene in particular are absorbed from the gastrointestinal tract (Rees 1971). Polyaromatic hydrocarbons as a class poisonous chemical are considered capable of crossing epithelial membranes and studies with benzo[*a*]pyrene and pyrene have shown rapid pulmonary absorption by rats (Kotin 1969, Vainio 1976, Mitchell & Tu 1979).

This study investigated old buffalos with average age of 12±2 years living in close proximity to a significant environmental point source of PAHs (south west part of Iran) during first Persian Gulf War in 1991, and compare with control region where there is a contamination of oil shield routinely. This analysis thus offers the opportunity to find out if there is possibility to detect years back exposure to organic pollutants.

## MATERIALS AND METHODS

One hundred of buffalos and camels aged 12±2 in polluted region and 50 camels at same age range in north west part of Iran as control region were selected for this study. During sampling extreme care was taken to collect samples from animals that have been born and grown in the collected sites and avoided sampling from animals transferred from other regions to sample collection sites.

The history of each animal was taken and especial sampling format containing all the characters of animal like sex, age, diseases, birth place etc. were noted.

Each animal was scarified in Slater house and 200 grams of fat were collected in glass tubes and preserved at -20 °C until the time of analysis.

**Reagents.** Acetonitrile and Toluene from purchased from Fisher Scientific and n-hexane from Acros Organics in Germany. All the chemicals used in this analysis were analytical grade.

**HPLC.** The HPLC System was Type SCL6B LP of Shimadzu company with Auto injector, Sil 6B. Two detectors UV and Fluorescence were used for detection. The Column was CS Multospher PAH III and pre-column multospher 120 RP. Samples were eluted by acetonitril/H<sub>2</sub>O with flow rate of 0.8 ml/min at 20°C

**Sample preparation.** Two grams of fat sample rubbed with 20 g sea sand and cooked with 45ml n-hexane at the extractor. The volume was made to 50 ml with n-hexane. Sample was filtered and divided to two portions. First portion was used for quantitative determination of benzo [*α*] anthracene, chrysene, benzo [*β*] fluoranthene, benzo [*k*] fluoranthene, benzo [*α*] pyrene, benzo [*ghi*] perylene and Indol 1,2,3-cd-pyrene. Second portion was used for quantitative determination of naphthalene, acenaphthylene, acenaphthene, chrysene, phenanthrene, anthracene, Fluoranthene and pyrene (Volker 2004).

## RESULTS

Table No.1 showing the concentration of PAHs in polluted and non polluted regions. Some PAHs including Acenaphthylene, Fluorene, Anthracene, pyrene were not detectable in polluted region (Figure 2), while Acenaphthene, Fluorene, Anthracene, pyrene were detectable in control region. However naphthalene as a low molecular weight PAH was detected in higher quantity than all the PAHs in both the groups and the difference between control and polluted region was not significant. On the other hand although high molecular weight PAHs like benzo [*α*] anthracene, chrysene, benzo [*β*] fluoranthene, Benzo [*k*] fluoranthene, benzo [*α*] pyrene, dibenzo [*α*] anthracene, benzo [*ghi*] perylene, and indeno (1,2,3-cd) pyrene, were detectable in both the groups but, the amount of benzo [*α*] anthracene, Chrysene, benzo [*β*] fluoranthene, benzo [*k*] fluoranthene, benzo [*α*] pyrene, benzo [*ghi*] perylene were

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Significantly higher in polluted region when compared with control region (Table 1).

When the ratio of benzo[ $\alpha$ ]pyrene as a persistent PAH was calculated versus other PAHs in selected animals fat tissues from polluted region, it was found that the ratio was comparatively higher in low molecular weight PAHs compared to high molecular weight PAHs (Figure 2).

**Table1.** Polycyclic aromatic hydrocarbons determination in buffalos exposed to oil burn pollutants emitted during Persian Gulf war in 1991

PAHs Name	Polluted regions ( Mean $\pm$ SD) mg / kg	Control regions (Mean $\pm$ SD) mg / kg	P Value
Naphthalene	0.12 0 $\pm$ 0.090	0.110 $\pm$ 0.010	N.S
Acenaphthalene	N.D	N.D	N.D
Acenaphthene	N.D	0.080 $\pm$ 0.040	N.C
Fluorine	N.D	0.020 $\pm$ 0.010	N.C
Phenanthrene	0.012 $\pm$ 0.001	0.023 $\pm$ 0.017	P< 0.5
Anthracene	N.D	0.018 $\pm$ 0.010	N.C
Fluoranthene	0.022 $\pm$ 0.011	0.027 $\pm$ 0.030	P< 0.5
Pyrene	N.D	0.020 $\pm$ 0.025	N.C
Benzo[ $\alpha$ ]anthracene	0.041 $\pm$ 0.021	0.013 $\pm$ 0.003	P< 0.1
Chrysene	0.060 $\pm$ 0.050	0.0110 $\pm$ 0.010	P< 0.1
Benzo[b]fluoranthene	0.032 $\pm$ 0.005	0.022 $\pm$ 0.002	P< 0.1
Benzo[k]fluoranthene	0.042 $\pm$ 0.020	0.037 $\pm$ 0.008	P< 0.1
Benzo[ $\alpha$ ]pyrene	0.042 $\pm$ 0.020	0.022 $\pm$ 0.022	P< 0.5
Benzo[a,h]anthracene	0.051 $\pm$ 0.030	0.045 $\pm$ 0.015	N.S
Benzo[ghi]pyrene	0.031 $\pm$ 0.012	0.042 $\pm$ 0.005	P< 0.5
Indo(1,2,3 cd) pyrene	0.040 $\pm$ 0.035	0.040 $\pm$ 0.021	N.S

N.D: Not Detected, N.C: Not Calculate, N.S: Non Significant, Total No of samples for polluted regions were 100 samples and Total No of samples for control regions were 50 samples.

## DISCUSSION

Shortly after Iraqi armed forces invaded Kuwait on August 2, 1990, coalition troops on January 16, 1991, started air attacks against the Iraqi army and opened the phase of operations known as Desert Storm Operation. The first oil well fires were started in Kuwait by the Iraqis on January 20, 1991 and the majority of oil well fires has been started by February 19, 1991. It is estimated that about 37% of

oil-related pollutants have been translocated by air from Kuwait oil well to the territories of Iran (Aminipuri 1998). The most important organic pollutants that could contaminate vegetation and soil were PAHs. At the time of incidence the chemical analysis of pollutants in polluted regions were limited. However 12 years later when we required specific data to prove contamination of livestock during the incidence, we tried to check the traceable organic chemicals in the region.

In this study we took extreme care to include only the animals which were expected to be exposed to the pollutants during the incidence and as control samples we selected the animals from Khorasan province (North West region of Iran). In Khorasan province most of our samples were from a region called Nehbandan a city close to Afghanistan border. We have given special attention to this region because there we could find out the camels that were exposed to petroleum products through feeding the dessert grasses. Because the area is full of oil shield and camels feed on dessert grasses. Hence the comparison between the recent exposed animals (animals from Nehbandan) and previously exposed animals (Polluted provinces due to pollution from the 1991 incidence) could help us to draw our conclusion.

The results of this study shows that the level of PAHs determined were detectable at levels less than 0.01 mg/Kg in 54% of animal in polluted province. In Control provinces we could see the detectable PAHs only in animals from Nehbandan region.

In polluted region, the level of low molecular weight PAHs (Acenaphthylene, Fluorene, Anthracene and Pyrene) were undetectable in fat sample tissues and other low molecular weight PAHs which were detected were very low. On the other hand 63% of samples that were showing detectable amount of PAHs were related to Benzo[ $\alpha$ ]pyrene (a high molecular weight PAH). When we compared the levels of high molecular weight PAHs with low molecular weight PAHs, we found that the level of

high molecular weight PAHs were comparatively at higher level in polluted region. In Nehbandan region (control region) although the animals showing PAHs in their fat tissue but the levels of low molecular PAHs were comparatively higher and high molecular weight PAHs were at lower level. When ratio of benzo[ $\alpha$ ] pyrene to other PAHs were calculated interestingly in polluted regions the ratio of benzo[ $\alpha$ ]pyrene was shown to be much higher while in control region it was opposite. Persistence of the PAHs varies with their molecular weight. The low molecular weight PAHs are most easily degraded. The reported half-lives of naphthalene, anthracene and benzo [ $\alpha$ ] pyrene in sediment are 9, 43 and 83 hours, respectively, whereas for higher molecular weight PAHs, their half-lives are up to several years (Neff 1979). The 95% clean-up times of the PAH compounds was suggested to be for Naphthalene 8 months, for Phenanthrene 30 months and for Anthracene 53 months, but for B[a]P after 84 years, there was no loss of the initial chemical burden. Therefore, there is little chance to achieve total clean-up (Howard *et al* 1991, Poupar *et al* 2005).

All the selected animals from polluted region were in the range 12 $\pm$ 2 years old and that means they were either born during the time of incidence or they were at fast growth stage at the time of incidence in the year 1991.

Level of naphthalene was high in both the groups. Since naphthalene is among low molecular weight PAHs and has a high rate of degradation in nature, there is possibility that the source of naphthalene contamination is local in the polluted region and can not be attributed to the pollutants from 1991 Persian Gulf War. On the other hand all high molecular PAHs including benzo [  $\alpha$ ] anthracene, chrysene, benzo [  $\beta$ ] fluoranthene, benzo [k] fluoranthene, benzo [  $\alpha$ ] pyrene, dibenzo [  $\alpha$ ] anthracene, benzo [ghi] perylene, and indenol [1,2,3-cd] pyrene, were clearly detectable (Figure 2).

The environmental profile agrees reasonably well with data reported by researchers, that 99% of environmental benzo [  $\alpha$ ] pyrene partitions into soil. (Holly *et al* 1991)

Few researchers reported that the half-life of BaP in water is about 2.3 years and a degradation rate coefficient of BaP in soil was estimated from the half-life value of 15 years (Holly *et al* 1991). Terrestrial plants can take up PAHs through their roots and/or leaves and translocate them to various plant parts (Edwards, 1983). Animals can receive the PAHs through grass ingestion. In animals, the mixed-function oxygenase (or oxidase) (MFOs) enzyme systems are responsible for the biotransformation of PAHs.

When the ratio of all 16 PAHs by benzo[ $\alpha$ ] pyrene were calculated, the ratio of benzo[ $\alpha$ ]pyrene to low molecular weight PAHs were significantly high as compared to heavy molecular weight PAHs (Figure 1), while it was opposite in control region. This fact can be another reason that the source of PAHs contamination in old animals from polluted region was not local.

Hence it can be concluded that old animals, sampled in several sites in south west region of Iran were polluted by wet and dry deposition of pollutants during the 1991 Persian Gulf War. On the other hand this study may help to show that PAHs can be traced in live stocks even many years after the incidence.

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### References

- Aminipouri, B. (1998). Tracking of oil spills and smoke plumes of Kuwait's oil well fires to the coast and territory of I.R. of Iran as a result of the 1991 Persian Gulf War. *A Report Submitted to UNCC for Claim of I.R. Iran* 375-392.

- Edwards, N.T. (1983). Polycyclic aromatic hydrocarbons (PAHs) in the terrestrial environment. *Journal of Environmental Quality* 12: 427-441.
- Howard, P.H., (1991). Handbook of environmental degradation rates, Pp: 725-730.
- Holly, A., Hattemer-Frey and Curtis, C.T. (1991), Benzo-A-Pyrene: Environmental Partitioning and Human Exposure. *Toxicology and Industrial Health* 7 (3): 41-152.
- Husain, T. (1995). Kuwait Oil Fires: *Regional Environmental perspectives*, Pp: 125-157. Pergamon Press.
- Kotin, P. (1969). Distribution, retention, and elimination of <sup>14</sup>C-3, 4-benzopyrene after administration to mice and rats. *Journal of Netherland Cancer Institute* 23: 541-548.
- Law, F.C.P., Sit, D. and Coppock, R.W. (2002). Development of physiologically based toxicokinetic models for risk assessment of cattle and wildlife exposed to crude oils or refined petroleum products. *Annual Symposium*, Pp: 122-141. CNTC, Canada.
- Mitchell, C.E. and Tu, K.W. (1979). Distribution, retention and elimination of pyrene in rats after inhalation. *Journal of Toxicology and Environmental Health* 5: 1171-1179.
- Poupar, N. Meude, A., Ponce, F. and Dussaue, J. (2005). Polycyclic aromatic hydrocarbons monitoring in terrestrial plant biota after Erika oil spill, *Symposium on marin accidental oil spills*, Pp: 122. Vertimar
- Neff, J.M. (1979). Polycyclic Aromatic Hydrocarbons in the Aquatic Environment: Sources, Fates and Biological Effects. Pp: 262-271. Applied Science Publishers Ltd., Essex, England.
- Rees, E.O. (1971). A study of the mechanism of intestinal absorption of benzo[α]pyrene. *Biochemistry Biophysics. Acta* 225: 296.
- Vainio, H. (1976). The fate of intratracheally installed benzo[α]pyrene in the isolated perfused rat lung of both control and 20-methylcholanthrene pretreated rats. *Research Communications in Chemical Pathology and Pharmacology* 13: 259-267.
- Volker, L. (2004). PAH- Analysis of buffalo fat, Orga Lab, Zirndorf. Edited in Report To UNCC, by Abbas Z.M. *Biostatistical monitoring of livestock diseases incidence in damaged provinces of Iran from burning Kuwait Oil-wells in 1991*, Pp: 761-769.

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