

## Review Article

# Polycyclic Aromatic Hydrocarbons and Esophageal Squamous Cell Carcinoma

Gholamreza Roshandel PhD<sup>1,2</sup>, Shahryar Semnani MD<sup>2</sup>, Reza Malekzadeh MD<sup>1</sup>, Sanford M. Dawsey MD<sup>3</sup>

## Abstract

Esophageal cancer (EC) is the 8<sup>th</sup> most common cancer and the 6<sup>th</sup> most frequent cause of cancer mortality worldwide. Esophageal squamous cell carcinoma (ESCC) is the most common type of EC. Exposure to polycyclic aromatic hydrocarbons (PAHs) has been suggested as a risk factor for developing ESCC. In this paper we will review different aspects of the relationship between PAH exposure and ESCC.

PAHs are a group of compounds that are formed by incomplete combustion of organic matter. Studies in humans have shown an association between PAH exposure and development of ESCC in many populations. The results of a recent case-control study in a high risk population in northeastern Iran showed a dramatic dose-response relationship between PAH content in non-tumor esophageal tissue (the target tissue for esophageal carcinogenesis) and ESCC case status, consistent with a causal role for PAH exposure in the pathogenesis of ESCC.

Identifying the main sources of exposure to PAHs may be the first and most important step in designing appropriate PAH-reduction interventions for controlling ESCC, especially in high risk areas. Coal smoke and drinking mate have been suggested as important modifiable sources of PAH exposure in China and Brazil, respectively. But the primary source of exposure to PAHs in other high risk areas for ESCC, such as northeastern Iran, has not yet been identified. Thus, environmental studies to determining important sources of PAH exposure should be considered as a high priority in future research projects in these areas.

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## Introduction

Esophageal cancer (EC) is the 8<sup>th</sup> most common cancer and the 6<sup>th</sup> most frequent cause of cancer mortality worldwide, causing over 400,000 deaths in 2008.<sup>1</sup> 80% of esophageal cancers occur in developing countries. Esophageal cancer rates vary greatly between and within countries. There are two large bands of high risk, across central Asia from the Caspian Sea to northern China (the Central Asian Esophageal Cancer Belt) and from eastern to southern Africa.<sup>2</sup> Within these bands, EC incidence rates are 50 – 100/100,000 population/year, whereas in most other areas of the world they are < 10/100,000/year.

At the eastern end of the Central Asian Esophageal Cancer Belt is Iran. The highest rates of EC in Iran occur in Golestan Province, in the northeast of the country. In the 1970s, scientists from Iran and the International Agency for Research on Cancer (IARC) recorded EC incidence rates of 165/100,000/year in men and 195/100,000/year in women,<sup>3</sup> the highest rates of EC ever recorded in the world, and they began initial studies to determine its cause, but sociopolitical events in Iran in 1979 stopped these studies. Since the 1970s, EC rates in Golestan have declined,<sup>4,5</sup> but despite this decline, EC remains the most frequent malignancy in northeastern Iran,<sup>4-6</sup> and the results of the Golestan population-

based Cancer Registry show that the truncated ASR of EC is still very high, especially in the eastern parts of Golestan Province (Gonbad and Kalaleh).<sup>4,5</sup>

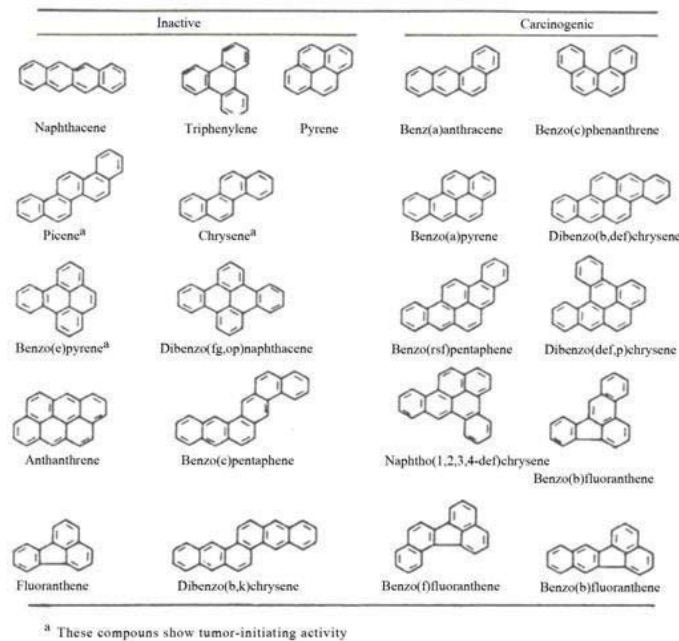
Adenocarcinoma and squamous cell carcinoma are the main histologic subtypes of EC. In the 1960s, about 90% of EC cases throughout the world were esophageal squamous cell carcinoma (ESCC).<sup>2</sup> Recent studies, however, have shown a declining trend in the rate of ESCC and a sharply increasing trend in the rate of esophageal adenocarcinoma in the United States and some European countries.<sup>7-9</sup> But the situation seems to be different in the developing world, where all of the highest risk populations are. Reports from developing countries have not shown the changing pattern of EC histologies that has been seen in western countries. For example, ESCC comprises more than 90% of EC cases in northeastern Iran.<sup>6,10</sup> So, ESCC is the most important type of EC in the developing world and a major health problem in high risk areas including northeastern Iran.

ESCC develops by progression from dysplastic lesions within the squamous epithelium of the esophagus.<sup>2</sup> In studies in a high-risk population in China, esophageal squamous dysplasia (ESD) was shown to be the only clinically important precursor lesion for ESCC, and different histologic grades of ESD identified different levels of ESCC risk.<sup>11,12</sup>

Various factors have been shown to increase the risk of ESCC,<sup>13</sup> including tobacco smoking,<sup>14,15</sup> heavy alcohol drinking,<sup>14</sup> opium consumption,<sup>15</sup> nass chewing,<sup>15</sup> hot tea consumption,<sup>16</sup> mate drinking,<sup>17</sup> low intake of fruits and vegetables,<sup>18</sup> nutrient deficiencies,<sup>19-21</sup> tooth loss,<sup>22</sup> and low socioeconomic status.<sup>23</sup> Alcohol and tobacco are the most important risk factors for ESCC in low-risk populations,<sup>24</sup> but its etiology in high risk areas is less clear. Exposure to polycyclic aromatic hydrocarbons (PAHs) has been suggested as an important risk factor for developing ESCC in many

**Authors' affiliations:** <sup>1</sup>Digestive Diseases Research Center, Tehran University of Medical Sciences, Tehran, Iran, <sup>2</sup>Golestan Research Center of Gastroenterology and Hepatology, Golestan University of Medical Sciences, Gorgan, Iran, <sup>3</sup>Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland, USA.

**Corresponding author and reprints:** Sanford M. Dawsey MD, Division of Cancer Epidemiology and Genetics, National Cancer Institute, 6120 Executive Boulevard, Suite 320, Bethesda, Maryland, 20895, United States. Tel: +1-301-594-2930, Fax: +1-301-496-6829, E-mail: dawseys@mail.nih.gov. Accepted for publication: 10 July 2012



**Figure 1.** Structures of carcinogenic and noncarcinogenic unsubstituted polycyclic aromatic hydrocarbons (Adopted from: Harvey, 1991).

populations.<sup>13</sup> The aim of this paper is to discuss different aspects of the relationship between PAH exposure and ESCC.

### Characteristics of PAHs

PAHs are a group of compounds that are formed by incomplete combustion of organic matter, including vegetation, fossil fuels and oil products.<sup>25,26</sup> They are composed of two or more condensed aromatic rings<sup>27</sup> (figure 1), and they are distributed across gaseous and particulate phases.<sup>28</sup> In the environment, they are found in soil, water, air and food.<sup>29</sup> Exposure to PAHs may occur through inhalation, ingestion or percutaneous penetration.<sup>28,29</sup> They distribute to various organs, especially the liver.<sup>29</sup> PAHs themselves are chemically inert and hydrophobic, but they are metabolized within cells to their active forms, the diol-epoxides. This change results in PAHs binding to cellular macromolecules such as DNA,<sup>27,30</sup> causing the formation of PAH-DNA adducts, which lead to mutations in proto-oncogenes and tumour-suppressor genes.<sup>28</sup> Generating other reactive intermediates by a one-electron oxidation process is another carcinogenic mechanism of PAHs. This process may cause chemical alkylation of DNA and result in potentially mutagenic depurination.<sup>31</sup>

### Determination of exposure to PAHs

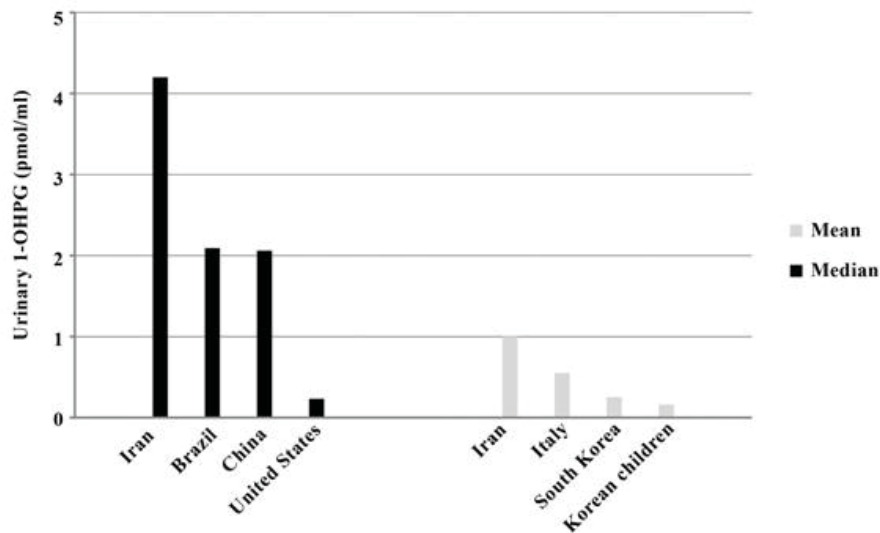
Two ways to assess the level of exposure to PAHs are measurement of the external dose and measurement of the internal dose. The external dose can be quantified by monitoring the concentration of PAHs in the air and food. The internal dose, reflecting the total uptake of PAHs, may be assessed by monitoring of PAHs or their metabolites in body fluids or tissues.<sup>32</sup> Therefore, biological monitoring, making an accurate estimation of the individual internal dose, has been considered as useful indicator for human expo-

sure to PAHs.<sup>33</sup> Urinary 1-hydroxypyrene concentration is a valid marker for assessing the level of recent exposure to PAHs.<sup>32,34-36</sup> It has also been reported to be a sensitive biomarker for low dose PAH exposure.<sup>36,37</sup> Zhao et al. reported that the maximum concentration of 1-hydroxypyrene in urine samples was found in the evening (21:00 – 23:00), and recommended this as a suitable time for sample collection for evaluation of PAHs.<sup>38</sup> Urinary concentration of 1-hydroxypyrene glucuronide (1-OHPG) has also been shown to be significantly higher in individuals exposed to high levels of PAH.<sup>39,40</sup> In a study of 10 healthy non-smoking men, 24 hours after starting a high-PAH diet (charbroiled beef), the level of urinary 1-OHPG increased 10 – 80 fold above baseline levels. Urinary concentration of this marker decreased to near baseline levels by 1 – 3 days after ending the diet.<sup>40</sup>

Seidel et al. have suggested hydroxylated metabolites of naphthalene, fluorene, phenanthrene, fluoranthene and pyrene as valid biomarkers for monitoring PAH exposure.<sup>41-45</sup> They were also reported to be valid biomarkers of occupational exposure to PAHs,<sup>46</sup> especially lowly condensed volatile PAHs.<sup>43</sup> It has been suggested that measuring hydroxyphenanthrene in addition to 1-hydroxypyrene may provide more complete information about PAH exposure in individuals.<sup>43</sup>

Urinary levels of unmetabolised naphthalene and phenanthrene have also been shown to be promising surrogates for assessing PAH exposure.<sup>45</sup> Naphthalene was suggested as a good biomarker for assessing occupational exposure to PAHs.<sup>28</sup>

PAH-DNA adducts are more persistent indicators of PAH exposure than are urinary markers.<sup>36</sup> The levels of PAH-DNA adducts in easily accessible WBCs have been suggested as valid biomarkers in exposure assessment as well as intervention studies.<sup>47</sup> The level of this marker in peripheral WBCs has been reported to reflect both occupational and dietary exposure to PAHs.<sup>48-50</sup> There was a positive association between the level of PAH-DNA ad-



**Figure 2.** Urinary concentrations of 1-hydroxy pyrene glucuronide (1-OHPG) in non-smoking individuals from Golestan, Iran;<sup>84</sup> Rio Grande do Sul, Brazil;<sup>80</sup> Linxian, China;<sup>83</sup> Maryland, United States;<sup>40</sup> Turin, Italy;<sup>116</sup> Pohang, South Korea;<sup>39</sup> and Korean children.<sup>118</sup>

ducts in WBCs and recent exposure to PAHs from consumption of charbroiled food<sup>51</sup> or smoking.<sup>52</sup> But, as Gyorffy et al. showed, the levels of PAH-DNA adducts in WBCs may not appropriately reflect its levels in target tissues.<sup>53</sup> So, tissue levels of PAH-DNA adducts are considered a more valid indicator of exposure to PAH in recent studies. van Gijssel et al. detected PAH-DNA adducts in old paraffin-embedded endoscopic biopsies of the esophagus from Linxian, China.<sup>54</sup> They observed nuclear PAH-DNA adduct staining in four out of five samples, and concluded that old biopsy samples are appropriate for assessing past exposure to PAHs.<sup>54</sup> There are some limitations in using PAH-DNA adducts in epidemiologic studies, including non-linearity at high exposures, lack of sensitivity to low exposures, the large amounts of DNA needed for PAH-DNA adduct measurements, the problem of inter- and intra-lab variability in results using the 32P-postlabeling measurement technique, and the use of surrogate tissues (e.g. WBCs) instead of the target tissues.<sup>13</sup> Nevertheless, these markers have been successfully used in establishing links between PAH exposure and risk of several cancers.

Toriba et al. identified PAHs in human hair samples by HPLC with fluorescence detection. They reported that PAH analysis in hair samples is a good biomarker for assessing PAH exposure.<sup>55</sup> Beskid et al. used fluorescence in situ hybridization (FISH) with whole chromosome painting to assess the levels of exposure to carcinogenic PAHs.<sup>56</sup>

In a recent study from Iran, immunohistochemical staining of target tissue with an anti-PAH antibody (8E11) was successfully used for assessing PAH exposure.<sup>57</sup> It would be interesting to know if such measurements are correlated with the prevalence of genetic mutations or levels of PAH-DNA adducts in the same tissue or in a more accessible tissue such as buccal epithelium.<sup>58</sup> Answers to these questions would be helpful for conducting future epidemiological studies and surveillance programs in high risk areas.

## PAHs and cancers

Some PAH compounds, including benzo-a-anthracene, benzo-b-fluoranthene, benzo-j-fluoranthene, benzo-k-fluoranthene, ben-

zo-a-pyrene, dibenz-a,h-anthracene, and indeno-1,2,3-c,d-pyrene, are considered to be human carcinogens.<sup>36,59</sup> PAHs have been shown to induce carcinogenic changes in a number of human tissues. The report of Percivall Pott in 1775 was the first evidence of an association between PAH (soot) exposure and cancer (scrotal cancer) in chimney sweeps.<sup>60</sup> PAH exposure has now been documented to increase the risk of cancers of the lung,<sup>61,62</sup> bladder,<sup>61</sup> breast,<sup>63</sup> pancreas<sup>64,65</sup> and prostate.<sup>65</sup> PAH mutagenesis was also found in human epidermal keratinocytes in culture.<sup>66</sup> Benzo-a-pyrene, the most common PAH to cause cancer in human, has been classified as a group 1 carcinogen by IARC.<sup>59</sup> Recently, IARC also reported that diesel engine exhaust (an important source of exposure to PAHs) has a carcinogenic effect in humans and classified it as a group 1 carcinogen.<sup>67</sup> So PAHs have been shown to have carcinogenic effects in a number of human organs.

## PAH-induced P53 mutations

P53 is a tumor suppressor gene which is frequently mutated in human cancers. P53 mutations occur in about 40% of lung cancers.<sup>68</sup> Smoking tobacco, which contains many carcinogens, including PAHs, has been shown to cause lung cancer. So, PAH-induced P53 mutation was suggested as a possible mechanism in the pathogenesis of lung cancer. PAH-induced P53 mutations are mostly G:C to T:A transversions,<sup>69</sup> and they are poorly repaired. Puisieux et al. showed the direct role of PAHs in the induction of mutations in the P53 gene in lung cancer for the first time.<sup>69</sup> Recently, Gao et al. suggested that PAHs may have the potential to induce P53 mutations in the cervix of mice.<sup>70</sup> It has been proposed that the most common locations of P53 mutations, known as mutational hotspots, are preferred targets for PAHs.<sup>69</sup> Other studies similarly reported that PAH-DNA adducts preferentially occur at p53 mutational hotspots.<sup>71</sup> Shen et al. reported P53 mutation due to formation of PAH o-quinones, one of the ultimate carcinogens of PAHs, in cigarette smokers.<sup>72</sup> It was suggested that PAH o-quinones cause P53 inactivation by generating endogenous reactive oxygen species.<sup>73</sup> P53 mutation was reported to be attributed to direct PAH-induced DNA damage rather than selection of

pre-existing endogenous mutations.<sup>68</sup> Because of the clear causal relationship between tobacco smoking and lung cancer, most previous investigations about PAH-induced P53 mutations have been conducted on lung cancer. But other malignancies, including ESCC, are also known to have a strong relationship with tobacco smoke carcinogens, especially PAHs. Brennan et al. reported a fourfold increase in the prevalence of P53 mutations in heavy smoking EC patients as compared to nonsmokers.<sup>74</sup> The results of a recent study in northeastern Iran also suggested PAH-induced P53 mutations in ESCC cases. In this study, G:C to A:T transversions were the most common type of mutation, followed by G:C to T:A transversions.<sup>75</sup> Further investigations are needed to clarify if PAH-induced P53 mutations play a major role in the pathogenesis of ESCC, as they do in the case of lung cancer.

### Role of PAHs in the pathogenesis of ESCC

More than half of cancers occur in surface epithelia (such as the esophageal epithelium) that are in direct contact with the external environment.<sup>76</sup> Direct exposure to PAHs has been suggested to contribute significantly to carcinogenesis in these tissues.<sup>66</sup> A dose-response relationship was found between PAH exposure and development of EC in mice.<sup>77</sup> Gustavsson et al. reported a significant excess of esophageal cancer in chimney sweeps, and thought that the most probable explanation was a direct action of PAHs on the esophageal mucosa.<sup>61</sup> The results of studies from Canada,<sup>65</sup> Germany,<sup>78</sup> Sweden<sup>79</sup> and Brazil<sup>80</sup> have also shown an excess risk of esophageal cancer in PAH-exposed individuals. Barbecuing and grilling, important sources of exposure to PAHs, have also been associated with an elevated risk of esophageal cancer.<sup>81</sup> Roth et al. found high levels of PAHs in food samples obtained from a high risk area for ESCC in China,<sup>82</sup> and high levels of urine 1-OHPG in the local inhabitants,<sup>83</sup> suggesting a possible role for PAH exposure in ESCC pathogenesis there. Exposure to widespread and very high levels of PAHs was also reported from a high risk area for ESCC in the northeast of Iran<sup>84</sup> (Figure 2). Marjani et al. reported significantly higher levels of PAH-DNA adducts in ESCC tumor tissue than in normal esophageal tissues from these same cases or from control individuals, and concluded that PAHs are risk factors for ESCC in this same population in Golestan Province, Iran.<sup>85</sup> And in another recent study from this area, immunohistochemical staining with an anti-PAH antibody (8E11) was performed on non-tumoral esophageal epithelial tissues obtained from ESCC patients and normal controls.<sup>57</sup> There was a striking dose-response relationship between the intensity of 8E11 and ESCC case status, suggesting a causal role for PAH exposure in the pathogenesis of ESCC<sup>57</sup> (Table 1). The effects of inherited genetic variation on the levels of such PAH adducts may also be important in this context,<sup>86</sup> and need to be further investigated in future studies.

### Sources of exposure to PAHs

#### 1. Dietary sources

Foods had been identified as important sources of human exposure to PAHs.<sup>87</sup> Diet is usually the main source of PAH exposure in non-smoking individuals who are not exposed to occupational PAHs.<sup>25,26</sup> Contamination of foods by PAHs may occur during food processing, such as smoking or cooking, or as a result of accidental environmental contamination, such as atmospheric pollu-

tion of vegetables.<sup>88,89</sup>

Agricultural crops may be an important source of exposure to PAHs.<sup>90</sup> By accumulating on the surface of plants exposed to polluted air, PAHs may be present in most crops.<sup>91</sup> Because of the hydrophobic nature of PAHs, it is believed that the contamination of agricultural crops by these compounds comes mainly from the air rather than from the soil.<sup>92</sup>

The Total Human Environmental Exposure Study from the US showed that foods account for more than 96% of the daily intake of carcinogenic PAHs.<sup>93</sup> Vyskocil et al. reported no relationship between PAH levels in air and urinary concentration of 1-hydroxypyrene, suggesting that other factors (e.g. dietary intake of PAHs) may be potentially more important sources of internal dose.<sup>94</sup> An analysis of UK food samples reported that cereals and oils/fats were the most important source of PAHs in the UK diet, followed by fruits, sugar and vegetables.<sup>89</sup> De Vos et al. assessed Dutch total diet samples and reported that sugar and sweets, cereals, oils, fats and nuts were the major sources of PAHs.<sup>95</sup> The results of a study from Italy also showed that food was the main source of PAH exposure, suggesting cereals, milk products, meat, vegetables and fruits as the major contributors to total PAH intake.<sup>96</sup> Falco et al. assessed the dietary intake of PAHs in Catalonia, Spain. They found the highest levels of PAHs in cereals, meat and meat products.<sup>97</sup> Chemical analysis of foodstuffs in northeastern Iran in 1970s did not show high levels of PAHs in the major dietary items.<sup>98</sup> But in another study from this region, Hakami et al. suggested that diet is a major source of exposure to PAH<sup>99</sup> (Table 2).

The level of PAH in food may be affected by methods of food processing and preparation. Some studies reported no change in the PAH content of flour during the bread baking process. For example, Dennis et al. reported similar amounts of PAHs in wheat flour and bread made from this flour.<sup>100</sup> And high levels of PAHs were reported in both raw and cooked food samples from a high risk area of ESCC in China.<sup>82</sup> But many other studies showed that some methods of food processing have documented effects on PAH levels in food. High levels of PAHs have been reported in smoked foods.<sup>101</sup> The concentration of PAHs in smoked meat products was shown to be more dangerous when an uncontrolled smoking process (without appropriate technology) is used.<sup>29</sup> Charbroiled foods have been shown to contain some of the highest PAH levels among frequently used foods, suggesting them as a major source of dietary exposure to PAHs in populations which commonly charbroil their food.<sup>51</sup> The results of a study on Italian foods showed the highest levels of PAHs in pizza prepared in a wood-burning oven and in barbecued meat.<sup>96</sup> Eating meat prepared by grilling, roasting or broiling was also reported as an important source of exposure to PAHs.<sup>102</sup> The type of heat source (direct versus indirect), the geometry of the grill, grilling duration, the use of marinating sauces, and the fat content of the food were some contributors to PAH formation in grilled and smoked foods.<sup>103</sup> In a recent study from northeastern Iran (unpublished data), there was a significant relationship between cooking method and the level of PAH-DNA adducts in peripheral lymphocytes. The results showed that PAH exposure is significantly higher in individuals using deep frying methods for cooking foods. Meat tikka, whole grilled chicken, meat burgers and grilled vegetables were major dietary sources of exposure to PAHs in Kuwait.<sup>103</sup>

Drinking mate, a water infusion of the herb yerba mate (*Ilex paraguayensis*), is a common habit in southern South Ameri-

**Table 1.** Adjusted ORs and 95% CIs for the association between staining intensity of monoclonal antibody 8E11 and esophageal squamous cell carcinoma in the Golestan Case-Control Study<sup>1</sup>

Quintile	Controls (n)	Cases (n)	Adjusted OR (95% CI) <sup>2</sup>
1	20	2	Reference
2	21	6	2.49 (0.41 – 15.3)
3	21	14	5.59 (1.03 – 30.2)
4	21	19	10.9 (2.07 – 57.4)
5	20	50	26.8 (5.28 – 136)

<sup>1</sup>From Abedi-Ardekani B et al. *Gut*. 2010;**59**:1178–1183; <sup>2</sup>Adjusted for age, sex, education, ethnicity, tobacco use and opium use

**Table 2.** Benzo[a]pyrene (BaP) intake from bread, rice and water in Golestan and Fars provinces, Iran<sup>1</sup>

Food item	Daily Benzo(a)pyrene intake (ng/day)		
	Cases (n=40)	Golestan controls (n=40)	Fars controls (n=40)
Bread+rice+water BaP	99.0±39.3 <sup>a</sup>	91.4±31.6	70.6±27.1 <sup>c</sup>
Bread BaP	60.9±31.8 <sup>a</sup>	48.1±20.4	28.4±14.9 <sup>d</sup>
Rice BaP	24.7±16.8 <sup>a</sup>	33.5±21.2	34.6±18.9
Water BaP (water+tea)	13.4±6.7 <sup>b</sup>	9.7±4.3	7.5±2.9 <sup>c</sup>

<sup>1</sup>From Hakami R et al., *Nutrition and Cancer* 2008;**60**:216–221; <sup>a</sup>mean±SD; <sup>b</sup>P<0.05, <sup>c</sup>P<0.001 between cases and Golestan controls; <sup>d</sup>P<0.01, <sup>e</sup>P<0.001, <sup>c</sup>P<0.05 between the two control groups.

**Table 3.** The main and other sources of PAH exposure in the West and in different populations with a high risk of ESCC

Population	Main sources	Other sources
West	Tobacco smoke	Car exhaust, Coal smoke
China	Coal smoke	Tobacco smoke, Car exhaust
Iran	Unknown	Tobacco smoke, Car exhaust
Brazil	Tobacco smoke, Mate	Barbequed meat, Car exhaust

ca.<sup>104</sup> In previous studies, both the temperature and the quantity of mate consumption have been associated with increased risk of ESCC.<sup>17,105</sup> Mate drinking has also been associated with increased risk of other smoking-related cancers, including cancers of the lung,<sup>106</sup> larynx,<sup>107</sup> oral cavity and oropharynx,<sup>108</sup> kidney<sup>109</sup> and bladder,<sup>110,111</sup> after adjustment for smoking. A significant positive relationship was reported between drinking mate and urinary level of 1-OHPG in southern Brazil, suggesting that this factor is an important source of PAH exposure in this area.<sup>80</sup> Laboratory measurement of PAHs in commercial yerba mate leaves and in both cold and hot water infusions of these leaves showed very high levels of benzo-a-pyrene and other carcinogenic PAHs, and suggested that drinking a gourd of mate in the traditional manner can expose an individual to as much benzo-a-pyrene as smoking a typical pack of 20 cigarettes.<sup>112</sup> So the PAH content of food and the method of food processing and preparation are major contributors to human exposure to PAHs from dietary sources.

## 2. Tobacco smoking and opium consumption

Tobacco smoking has been identified as a major source of exposure to PAHs.<sup>113–115</sup> A significant positive relationship has been reported between urinary level of 1-OHPG and smoking.<sup>39,80,116</sup> Van Rooij et al. reported that active smoking and PAH containing food products account for 99% of urinary excretion of PAH biomarkers in individuals who are not occupationally exposed to PAHs.<sup>117</sup> In a study from the US, individuals with recent smoking had 1-OHPG concentrations 10-fold that of non-smokers.<sup>102</sup> Second hand smoke exposure was also correlated with high 1-OHPG concentration.<sup>102</sup> Lee et al. reported significantly higher urinary 1-OHPG levels in South Korean children whose parents smoke at home.<sup>118</sup> The results of studies conducted in the 1970s in northeastern Iran showed high levels of opium consumption in this region and indicated that PAHs are a major active component responsible for its mutagenicity.<sup>98,119</sup> In a recent study, smoking,

nass consumption and opium consumption were all found to be significant sources of PAH exposure in Golestan Province, Iran,<sup>84</sup> and high exposure to PAH may be one explanation for the reported association between opium or tobacco use and higher risk of ESCC in northeastern Iran.<sup>15</sup> The proportion of the excess risk of ESCC due to PAH exposure from smoking and opium consumption has not yet been clearly identified. This is an interesting issue for future studies in this area and other similar populations such as those in central China.

## 3. Occupational exposure

Occupation is also an important source of exposure to PAHs. The level of exposure to PAHs varies between different occupations.<sup>120</sup> High levels of PAH exposure have been reported in foundry workers,<sup>37</sup> chimney sweeps,<sup>61</sup> blast furnace and coke-oven workers,<sup>39</sup> vendors of broiled food,<sup>121</sup> and steel plant and waste incineration workers.<sup>39,122,123</sup> Coke-oven exposure is a major predictor of urinary 1-OHP levels.<sup>115</sup> The results of a study from the UK showed that coke-oven workers had the highest levels of exposure to benzo[a]pyrene.<sup>124</sup> The highest levels of urinary 1-OHPG were reported in timber impregnators using creosote and workers using coal tar. Both dermal and inhalation exposure were suggested as possible explanations for high PAH exposure in the above mentioned occupations.<sup>124</sup> In a study from the US, the highest levels of exposure to PAHs were detected in coke-oven workers, asphalt workers and diesel-exposed workers.<sup>45</sup> Job is an important indicator of exposure to PAH, and should be considered in future related studies.

## 4. Indoor air pollution

Combustion of solid fuels for cooking and heating is the major source of indoor air levels of PAHs.<sup>125</sup> China is the largest producer and consumer of coal throughout the world. A substantial portion of domestic energy needs in China are supplied by coal, a

known source of PAH.<sup>125</sup> Thus, domestic coal, especially “smoky coal”, combustion is known as a main source of high PAH exposure in China.<sup>126</sup> Mumford et al. showed very high PAH levels in indoor air samples from Chinese homes, especially ones which used smoky coal in unvented stoves for heating and cooking.<sup>127</sup> Similar findings were reported in other studies from China.<sup>125</sup> High PAH concentrations were also reported in soot deposits from coal-burning stoves in Chinese homes.<sup>128</sup> Gevaio et al. also reported high levels of indoor PAHs in indoor air and dust in Kuwait.<sup>129</sup>

Housewives and children usually spend a lot of time at home. This may lead to high levels of exposure to PAHs from indoor PAH sources in these groups. Therefore, housewives, children and all individuals spending a lot of time in homes with significant indoor air pollution may be considered as high risk groups in studies related to the effects of PAH exposure on human health.

#### 5. Environmental air pollution

There are many sources of PAHs in environmental air. Unknown sources of environmental PAHs make it difficult to assess the exact level of PAH exposure from the environment. Various methods may be used to estimate the concentration of PAHs in the environment. For example, the level of PAHs in the environment is reflected by their presence in some uncooked foods or fruits.<sup>25</sup> PAH levels in olive fruits have been considered one measure of air pollution.<sup>91</sup>

It has been suggested that constituents of car exhaust, industrial emissions and smoke are the main sources of outdoor air PAHs. In a study from the US, outdoor concentrations of PAHs were higher in highly urbanized areas (containing many cars and industries) than in the Great Lakes region.<sup>93</sup> The results of another study from an area near a large city bus terminal showed higher air levels of PAHs on weekdays and during morning rush hours, probably related to road traffic.<sup>130</sup> They also reported significantly higher PAH levels in areas closer to the bus terminal.<sup>130</sup> Marr et al. reported high levels of PAHs along Mexico City’s roadways, suggesting vehicle traffic as a source of human exposure to PAHs.<sup>131</sup> Significantly higher 1-OHPG concentrations have also been reported in individuals who recently traveled in automobiles for more than 3 hours.<sup>102</sup> The results of a study from Shenzhen, China, showed significantly higher levels of PAHs in areas with many roads than in forested land.<sup>132</sup> Individuals living in the vicinity of an aluminum plant in Canada were exposed to high levels of PAHs.<sup>133</sup> Sediments from certain commercial and residential ponds have also been suggested as potential sources of exposure to PAHs.<sup>134</sup>

Identifying the nature and characteristics of environmental sources of exposure to PAHs as well as the levels of PAH exposure from these sources are interesting issues for future studies. These investigations are especially important in high risk areas of PAH-related cancers such as northeastern Iran.

#### Effects of genetic changes on an individual’s response to PAH exposure

Genetic factors may affect an individual’s response to PAH exposure.<sup>86</sup> Usually, p450 enzymes (CYP1A1, CYP1A2, CYP1B1) metabolize PAHs (such as benzo-a-pyrene) to biologically active intermediates (such as benzo-a-pyrene diol epoxide) and detoxification enzymes, including glutathione S-transferases (GSTM1, GSTP1, GSTT1), UDP-glucuronosyltransferases, and sulphotransferases, conjugate these PAH metabolites and de-

toxify them.<sup>26</sup> But genetic polymorphisms may affect the activity of these enzymes and make individuals more or less susceptible to the carcinogenic effects the same PAH exposure.<sup>86</sup> Roth et al. showed an association between the inactive polymorphism of the GSTM1 gene and the presence of esophageal squamous dysplasia, the precursor of ESCC, in a PAH-exposed population in China.<sup>135</sup> Also, a significantly higher expression of the aryl hydrocarbon receptor (AhR) gene, which codes for the cell membrane receptor for PAHs, was reported in subjects from the same population who had a positive family history of upper gastrointestinal cancers, suggesting that these individuals might be more susceptible to PAH-related carcinogenesis.<sup>136</sup> And Abnet et al. reported a strong association between CYP1B1 and urinary 1-OHPG concentrations in a Brazilian population with a high risk of ESCC.<sup>137</sup> So, in addition to sources of exposure, genetic susceptibility to PAH-related carcinogenesis may also influence the relationship between PAH exposure and ESCC pathogenesis.

#### Identifying the main sources of PAH exposure

PAH exposure has now been documented to be an important risk factor for developing ESCC. Thus, reducing the levels of exposure to PAHs may be an effective strategy for controlling ESCC, especially in high risk areas. Appropriate PAH-reduction interventions may differ, depending on the main sources of PAH exposure in each area. For example, Li et al. showed that replacement of open pit stoves by closed stoves equipped with a chimney significantly reduced PAH exposure in Peru.<sup>138</sup> Identifying the main sources of exposure to PAHs will result in maximum effectiveness of such PAH-reduction interventions. The major sources of PAH exposure have been identified in some populations with high ESCC rates (Table 3). But the situations in other high risk populations such as the northeast of Iran remain unclear. Previous studies conducted in the 1970s in northeastern Iran showed low levels of PAHs in food samples,<sup>139,140</sup> and the investigations by the joint Iran-IARC study group on major dietary items in northern Iran showed no unusual levels of carcinogens such as PAHs in food samples.<sup>141</sup> These studies suggested that food is not the main source of PAH exposure in this area. On the other hand, Hakami et al. reported that food may be an important source of exposure to PAHs in Golestan Province.<sup>99</sup> As reported by Kamangar et al. smoking and opium consumption are probably not the main sources of exposure to PAHs in this area.<sup>84</sup> Further evaluations, including environmental studies, are needed to identify the major sources of PAH exposure in this region as well as other high risk populations.

#### Conclusion

An association has been found between PAH exposure and development of ESCC in many populations. Identifying the main sources of exposure to PAHs is the first and most important step for conducting appropriate PAH-reduction interventions to control ESCC, especially in high risk areas. Coal smoke and drinking mate have been suggested as important modifiable sources of PAH exposure in China and Brazil, respectively. But the primary source of exposure to PAHs in other high risk areas for ESCC, such as northeastern Iran, has not yet been identified. Thus, environmental studies to determine important sources of PAH exposure should be considered as a high priority in future research projects in these areas.

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