Acta Poloniae Pharmaceutica – Drug Research, Vol. 61 No. 3 pp. 233–240, 2004

ISSN 0001-6837
Polish Pharmaceutical Society

# POLYCYCLIC AROMATIC HYDROCARBONS: PHYSICOCHEMICAL PROPERTIES, ENVIRONMENTAL APPEARANCE AND IMPACT ON LIVING ORGANISMS

KATARZYNA SKUPIŃSKA, IRENA MISIEWICZ and TERESA KASPRZYCKA-GUTTMAN

Confocal Microscopy Laboratory, National Institute of Public Health,
30/34 Chełmska, 00–725 Warsaw, Poland

**Abstract:** Human exposure to environmental pollution is of great interest nowadays. Many substances present in the environment are considered as carcinogenic to humans. Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental carcinogens. In this paper, PAHs are described: their physicochemical properties, natural and anthropogenic sources, environmental, dietary and occupational exposure and impact on human health. Metabolical carcinogenic activation of PAHs and the role of metabolism products as biomarkers of PAH exposure have been discussed.

**Keywords:** polycyclic aromatic hydrocarbons; carcinogenesis; metabolism; toxicity; sulforaphane; biomarkers

The environmental pollution, in particular contamination by polycyclic aromatic hydrocarbons (PAHs) is a threat to human health. Most PAHs are considered to be carcinogenic, their metabolites show mutagenic and carcinogenic activity towards humans and animals. PAHs are widely spread in the environment as natural components (i.e. as products of humous conversion by microorganism) or as pollutants (i.e. in dust emitted by carbo– and petrochemical industry, in cigarette smoke, as a product of incomplete combustion of organic materials, in particular during waste utilization and house heating) (1).

Far in the 18$^{th}$ century, a higher rate of skin cancer was observed among roofers who were exposed to soot. In 1947, the relationship between lung cancer and working conditions of gas industry workers and those working with coal tar was established (2). It was found then that induction of cancer was caused by PAHs present in coal tar and soot (3). In 1983, the International Agency for Research on Cancer acknowledged 30 PAHs as carcinogenic to people. In 1997, the United States Environmental Protection Agency defined 16 PAHs to be highly toxic and recommended to analyze their concentration. Those 16 PAHs are: anthracene, naphthalene, benz(*a*)anthracene, benzo(*a*)pyrene, chrysene, naphthalene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, dibenzo(*a,h*)anthracene, fluoranthene, pyrene, fluorene, phenanthrene, indeno(123–*c,d*)pyrene, benzo(*g,h,i*)perylene, acenaphthelene, acenaphthene (4). Benz[*a*]pyrene (B[*a*]P) is often chosen as a surrogate for the other PAHs due to its relatively high environmental levels and large health impact. The structures and properties of selected PAHs are presented in Table 1 (5–13).

## Properties of PAHs

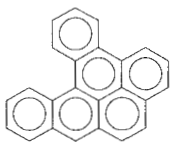
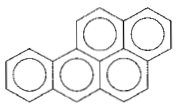
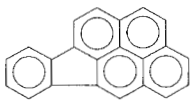
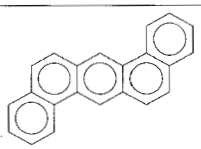
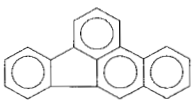
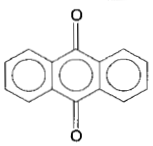
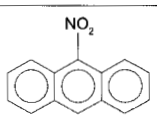
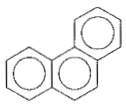
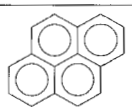
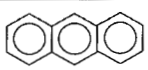
The polycyclic aromatic hydrocarbons belong to the group of organic compounds consisting of 2 to 13 aromatic rings (1). PAHs are weakly volatile and dissolve weakly in water – the solubility decreases with an increase of the number of aromatic rings. PAHs dissolve well in organic solvents and are lypophylic. All PAHs are solid and have high melting and boiling points. PAHs are chemically inactive but bond to particulate matter. When adsorbed at the surface of dust, PAH are highly thermo– and photosensitive. They can be cracked at high temperature (50$^{o}$C) and when exposed to light, especially ultraviolet light, but also visible light. Photooxidation is one of the most important ways of removing PAHs from the atmosphere. It was found that even a short time of dust irradiation (about 6 hours) results in decomposition of 15–20% adsorbed PAHs (14,15).

In 1976 over 100 different PAHs found in atmosphere were identified, and in 1981 more than 200 PAHs were found in cigarette smoke (16,17). Many PAHs contain the same amount of rings but the differences in configuration of rings may lead to differences in the compound's properties. In the environment one can also find substituted PAHs with functional groups such as –OH, –$NO_2$, =O and –$CH_3$.

## Sources of PAHs

The compounds, like PAHs, are generated during incomplete combustion of materials con-

Table 1. Physicochemical properties of selected polycyclic aromatic hydrocarbons (5)

| Name | Formula | Structure | Molecular weight | Boiling Point [°C] | Water solubility [μg/l] |
|---|---|---|---|---|---|
| Dibenz[*a,l*]pyrene | $C_{24}H_{14}$ |  | 302 | 595 (6) | n.a |
| Benz[*a*]pyrene | $C_{20}H_{12}$ |  | 252 | 496 (7) | 3,8 |
| Indeno[1,2,3–*c,d*]pyrene | $C_{22}H_{12}$ |  | 276 | 536(8) | 62 (9) |
| Dibenz[*a,h*]anthracene | $C_{22}H_{14}$ |  | 278 | 524 (8) | 0,5 (10) |
| Benz[*b*]fluoranthene | $C_{20}H_{12}$ |  | 252 | 481 (7) | 1,2 (11) |
| Benz[*g,h,i*]perylene | $C_{22}H_{12}$ |  | 276 | 545(12) | 0,26 |
| Antraquinone | $C_{14}H_8O_2$ |   | 208 | 380 | n.a. |
| 9–Nitroanthracene | $C_{14}H_9NO_2$ |   | 223 | n.a | n.a |
| Benz[*e*]pyrene | $C_{20}H_{12}$ |  | 252 | 493 (7) | 5,1 (13) |
| Fenanthrene | $C_{14}H_{10}$ |  | 178 | 340 | 1000 |
| Pyrene | $C_{16}H_{10}$ |  | 202 | 360 | 135 |
| Anthracene | $C_{14}H_{10}$ |  | 178 | 340 | 45 |

n.a – data not available

taining carbon and hydrogen: coal fuel, crude oil, wood, gas and organic materials, as well as: combustion of polypropylene and polystyrene, communal and industrial waste and used tires (18,19). The process of combustion is incomplete when the temperature of combustion is low and when there is no access of air. Chemical synthesis of hydrocarbons in the flame is initiated by free radicals. Certain types of free radicals can bind to each other at high temperature (500–800°C), which can be obtained in an upper part of flame. It was found that methane can lead to production of PAHs particles, however the generation of large particles occurs in the presence of free radicals of higher molecular weight. The aromatic compounds and diolefins can be precursors of PAHs.

The main anthropogenic sources of PAHs are power plants and house heating (51%). Incinerating plants and outdoor combustion are responsible for 28% emission to the atmosphere, industry (aluminum and steel foundries and gas engineering) for 20% and (car) transportation is responsible for 0.9% of emission (20). Among natural sources of PAHs forest fires and volcano's eruption are of great importance. Table 2 shows the influence of fuel type and method of combustion on the emission of benz[*a*]pyrene to the atmosphere. In Table 3, ratios of PAHs emission in dependence on combustor type are collected (when coal is combusted). As a result of combustion, a mixture of many PAHs in gaseous phase is emitted to the atmosphere. In the atmosphere heavier PAHs (containing more than 4 rings) are adsorbed on dust particles, whereas the lighter ones, which are not adsorbed, remain in the gas phase (21). In this form, PAHs can be carried by wind and may remain in the atmosphere until they are removed with precipitation. When washed out, PAHs are accumulated in soil and can also be absorbed by plants. PAHs can penetrate into water with precipitation or with refuse water. Due to their weak solubility, PAHs concentrations in water are low (~100 ng/l), instead they accumulate in sediments and aquatic organisms (22). Finally, almost all hydrophobic pollutants like PAHs, are accumulated in soil. As far as 89% of PAHs is accumulated in soil, 10% in sediments, 0.5% in air and water, respectively (23). There are many sources of soil contamination by PAHs: PAHs airborne dust, sludge that is used in agriculture as a fertilizer, compost and other organic fertilizers, refuse water and water that flows from asphalt roads, fuel and grease used in agriculture, and accidental contamination by oil–derivates (24).

Table 2. Influence of fuel type and method of combustion on the emmision of benz[*a*]pyrene to atmosphere (15)

| Fuel | Emission source | Benz[*a*]pyrene emmision [mg/BTU*] |
|---|---|---|
| Coal | Industrial | 0.056–0.07 |
| Coal | Domestic | 0.12–61.0 |
| Wood | Domestic | 27–6.3 |
| Oil | Domestic | 0.00026 |
| Gas | Domestic | 0.02 |
| Diesel | Transport | 0.6 |
| Gazoline | Transport | 2.3 |

* BTU – British Thermal Unit

Table 3. Indicators of PAH emmision from coal combustion in dependence on type of combustor (14)

| Combustor | Emission indicators [mg/Mg] | | |
|---|---|---|---|
| | Benz[*a*]pyrene | Carcinogenic PAH | Total PAHs |
| Fluidized bed combustors | 3 | 1 | 10 |
| Industrial Strokers > 10Gcal/h | 20 | 87 | 225 |
| Domestic fireplaces | 5 000 | 50 000 | 86 000 |

## Exposure to PAHs

Human exposure to PAHs occurs through 3 routes: respiratory tract, gastrointestinal tract and skin contact. Up to 70% of PAH exposure for non smoking person can be associated with diet. The main sources of PAH in diet are cereals, oils and vegetables. The highest contributor of PAH intake is cooked food (in particular food prepared over open flame), for example, in barbecued meat the PAH level can be as high as 10–20 μg/kg (25). Water is also a very important source of PAH. In drinking water, the most frequently present are: fluoranthene, phenanthrene, pyrene and anthracene. These compounds are not considered carcinogenic. The PAHs concentrations in drinking water vary between 1 ng/l and 11 μg/l (the highest acceptable by WHO concentration of B[*a*]P is 0.7 μg/l). It is estimated that the mean intake of PAH with water is 1% of total acceptable level (26).

A very important factor of risk is smoking habit. Having smoked one cigarette, causes intake of 20–40 ng of benz[*a*]pyrene (27,28).

The PAHs concentrations in air vary from <5 to 200 000 ng/m$^3$ (18,29): in urban areas the

Tabela 4. PAH concentrations in the atmosphere (28)

| | Low–moderate urban air pollution | High urban air pollution | Workplace (coke–ovens, foundries) | Tabacco smoke |
|---|---|---|---|---|
| Total PAHs [ng/m$^3$] | <20 | 20–200 | 100s–1000s | 1000s |
| Benz[*a*]pyrene [ng/m$^3$] | <5 | 5–50 | 100s | 1000s |

concentration can be tenfold higher than in rural areas (i.e. B[*a*]P concentration in Copenhagen was 3.9 ng/m$^3$ (29), in Athens 7.81 ng/m$^3$ (21), the mean level in European cities reaches 1–20 ng/m$^3$ (30) whereas in rural areas 0.08–0.5 ng/m$^3$) (29). It was also shown that the PAHs concentrations at main traffic junctions in Munich in Germany (1.9–5.0 ng/m$^3$) were two times higher than the concentration on the suburbs (0.8–2.9 ng/m$^3$) (31). It was noted that in highly polluted regions like Silesia, the B[*a*]P concentration can be as high as 66 ng/m$^3$ (21, 32). All mentioned levels exceed the WHO acceptable level, which is 0.01 ng/m$^3$ (for the risk of lung cancer equals $10^{-6}$ – one additional cancer case caused by exposure to B[*a*]P in a group of $10^6$ people). The mean concentrations of PAHs in air are shown in Table 4.

Occupational exposure to PAHs is considerably higher: in aluminum production plants it is at the level of 6 ng/m$^3$ (33), in coke ovens 135–200 000 ng/m$^3$ (19), in iron foundries 6400 ng/m$^3$ (34). It is easy to estimate the total daily intake of PAHs if a person uses 20 m$^3$ air daily.

**The toxicity of PAHs**

Not all PAHs are of the same toxicity. The structure of a particle and the substituted groups determine harmful properties of PAHs. Many PAHs belong to the group of carcinogens, in particular the unsubstituted PAHs as well as the nitrated and methylated ones, and those containing the carboxylic group. Among 16 PAHs recognized by US EPA as toxic substances, one can find carcinogens (benz[*a*]pyrene, dibenz[*a,h*]anthracene, benz[*b*]fluoranthene, indeno[1,2,3–*c,d*]pyrene) and PAHs that are thought not to be carcinogenic (phenanthrene, anthracene, pyrene, benz[*g,h,i*]perylene) (25).

J.L.Durant (35) studied mutagenic activities of 67 PAHs. Human lymphoblastoids were grown in the presence of varied concentrations of the studied PAHs. After the required time of incubation, the rate of mutated cells was calculated. The minimal PAH concentration that induces mutations was determined when the amount of mutated cells in the probe was higher than in control. According to the paper, the mutagenic activities of PAHs presented in Table 1 are as follows: dibenz[*a,l*]pyrene > benz[*a*]pyrene > indeno[1,2,3–*c,d*]pyrene > dibenz[*a,h*]anthracene > benz[*b*]fluoranthene > benz [*g,h,i*]perylene > antraquinone > 9–nitroanthracene > benz[*e*]pyrene » phenanthrene and pyrene. When comparing benz[*a*]pyrene and dibenz[*a,l*]pyrene one can easily see that the change in the number of rings causes a difference in toxicity of those PAHs. Not only the number of rings, but also the shape, the dimension of particles and the presence of functional groups determine the PAH's biological activities. In the studies, it was shown that some PAHs are stronger carcinogens than B[*a*]P. Nevertheless, B[*a*]P is still used as a surrogate of PAHs (25).

Buters J (36) in his work discussed the influence of 7,12–dimethylbenz[*a*]anthracene (DMBA) on induction of cancers. It was shown that the target organs of DMBA treated mice were different for different doses: the cancers of bone marrow, skin and lung were observed after treatment with 200 μg/mouse/day, when after treatment with 20 μg/mouse/day, cancers of ovaries, uterus, skin and lung were developed.

It is crucial to establish the PAHs concentrations that are safe for humans. The highest acceptable levels of PAHs concentrations in various countries are presented in Table 5 (37–44). In Poland, since 2001, the maximum acceptable level in a workplace has been set to be 2 μg/m$^3$ (45). The Ministry of Environment in Great Britain ascertained in the Air Quality norm that it is not possible to assess the absolutely safe level of exposure to carcinogens (in particular PAH) (46). There is no such idea as safe concentration for carcinogenic and mutagenic substances. Even a small amount of such substances can enter the living organism and accumulate continuously for years, which increases the risk of neoplastic disease.

**Metabolism of PAHs**

PAHs present in the environment are not active and are unable to cause carcinogenesis. Only

Table 5. The maximal permissible concentration (MPC) of PAHs in selected coutries

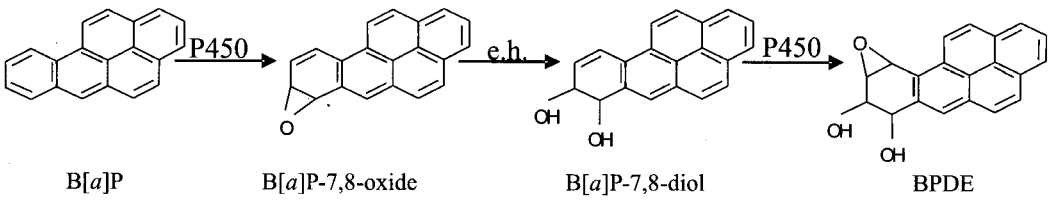
| Country Year | Occurrence | Compound | MPC |
|---|---|---|---|
| Italy 1999 | Ambient air | benz[*a*]pyrene | 1 ng/m$^3$ (3) |
| Former USSR 1985 | Ambient air | benz[*a*]pyrene | 1 ng/m$^3$ (38) |
| EEC* 1980 | Ambient water | Sum of fluoranthene, benzo[*b*]fluoranthene, enzo[*k*]fluoranthene, benzo[*a*]pyrene, benzo[*g,h,i*]perylene, indeno[1,2,3–*c,d*]pyrene | 1,2 µg/litr (39) |
| WHO 1995 | Drinking water | benz[*a*]pyrene | 0,7 µg/litr (40) |
| EEC 1980 | Drinking water | Sum of fluoranthene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, benzo[*g,h,i*]perylene, indeno[1,2,3–*c,d*]pyrene | 0,2 µg/litr (41) |
| Germany 1989 | Oven area | benz[*a*]pyrene | 2 µg/m$^3$ (42) |
| Sweden 1993 | workplaces | benz[*a*]pyrene | 2 µg/m$^3$ (43) |
| USA 1993 | workplaces | pyrene | 0,1 mg/m$^3$ (44) |

*EEC – European Economic Community

after entering the organism, PAHs are metabolically transformed to carcinogenic forms. The mechanism of this activation is known and it was found that it is similar for all carcinogenic PAHs. The absorbed PAH is metabolized in order to increase its solubility and also the excretion from the system. The first phase of metabolic transformation are reactions of oxidation catalyzed by enzymes of cytochrome P–450 family (CYP1A1, CYP1A2, CYP1B1) and hydroxylation catalyzed by epoxide hydrolase. These enzymes are responsible for PAHs metabolism (47, 48). The electrophilic products of this transformation, epoxy diols are hydrophilic and are, therefore, more easily water–soluble. The hydroxylation of PAH causes an increase of activity towards DNA and proteins – it bounds easily to nucleophilic parts of macroparticles forming adducts (19, 49). Such an adduct can be a cause of protein damage or DNA mutation, which can lead to carcinogenesis (25, 34). In the second phase, further PAH transformation to more water–soluble compounds is continued. One of such reactions could be conjugation with glutathione catalyzed by glutathione–S–transferase. The effect of this reaction is glutathione–PAH conjugate. The conjugated PAH is not able to bind to DNA or protein. Hence, glutathione has two functions – not only to facilitate excretion, but also to protect cells from mutation (50).

The process described above was well documented for benz[*a*]pyrene (15, 49). Benz[*a*]pyrene is inactive in the environment. When absorbed, B[*a*]P is metabolized by cytochrome P–450. The metabolism is complex, because the B[*a*]P particle has many reactive sites. Oxygen can be substituted in every site but not carbon–11 forming epoxides. Next, in the reaction of hydrolysis those compounds could be transformed to trans–diol or can conjugate with glutathione. The occurrence of these reactions is limited by epoxides stability or enzyme access to the reaction site. The products of these reactions could be transformed to epoxides again. The carcinogenic activation of benz[*a*]pyrene occurs as presented in Figure 1.

The product of activation of B[*a*]P by cytochrome P–450 is B[*a*]P–7,8 –oxide, which can be transformed by epoxide hydrolase to three B[*a*]P–transdiols, among them 7β is the main product. The last stage of activation is the formation of (7R,8S)–dihydroxy–(9S,10R)–epoxy–




**B[a]P** – benz[*a*]pyrene, **BPDE**–(7R,8S)–dihydroxy–(9S,10R)–epoxy–7,8,9,10–tetrahydrobenz[*a*]pyrene, **P450**–cytochrome **P450**, *e.h.* – epoxide hydrolase.

Figure 1. Carcinogenic activation of benz[*a*]pyrene (60).

7,8,9,10–tetrahydrobenzo[*a*]pyrene (BPDE) – the most carcinogenic isomer of four possible isomers (51). BPDE is a carcinogenic form of benz[*a*] pyrene – its half–life is 8 min and is long enough to bind covalently to nucleophilic parts of proteins or DNA. BPDE binds to oxygen or nitrogen atoms of purine bases (guanine and adenine) or pirymidine bases (cytosine and thymidine) (49, 52). The half–life of other forms is too short to bind to DNA or protein. In Figure 2, adducts of oxidized forms of B[*a*]P with guanine and adenine are shown. It is thought that the formation of such adducts can initiate carcinogenesis (Figure 3) (47, 49, 53). During the repair process, the PAH–DNA adduct is cut out from the DNA strand and replaced by the complementary base. When the repairing system makes a mistake, the sequence of nucleotides can be altered, and in place of the damaged DNA a new helix with incorrect order of bases is created. Such mutation can initiate carcinogenesis and further a neoplastic disease. In cancer prevention, it is very important to stop the described processes at the earliest possible stage. It was found that some substances can prevent the formation of carcinogenic PAH–DNA adducts. The mechanism is similar to that of antioxidants that stop oxidative DNA changes leading to carcinogenesis. Such compounds are sulforaphane and coumarin, which were shown to be efficient in chemoprevention of developing cancer (54–56).

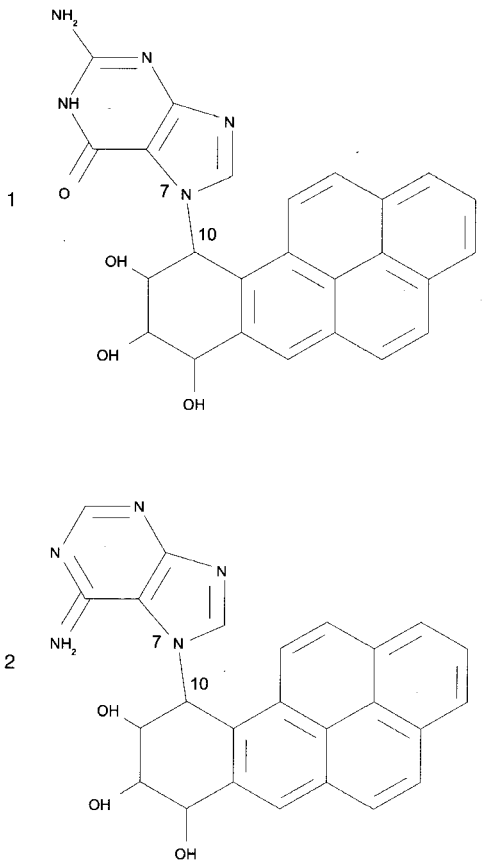
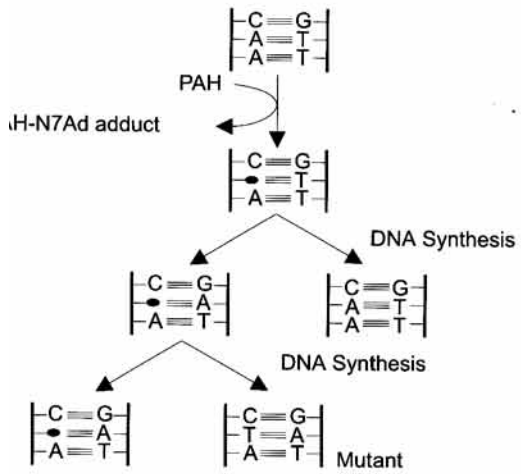



Figure 2. Structures of benz[*a*]pyrene adducts with 1. guanine; 2. adenine.

## Biomarkers

For many years, carcinogenic potency of PAHs was assessed on the basis of disease symptoms. Nowadays, researchers are searching for indicators of the PAHs influence on the human organism even if the PAH concentrations are below the level of detection. Such indicators are named biomarkers. Biomarkers are defined as biological particles that undergo detectable transformation when the organism is exposed to harmful substances (56, 58). The ideal biomarker should be a biological particle that can be easily isolated from the tissue, should also be sensitive and specific for the studied toxic substances. The PAH–DNA and PAH–protein (hemoglobin, albumin) adducts as well as cytochrome P–450 enzymes (CYP1A1, CYP1B1) are used as biomarkers of exposure to PAH (59, 60).




re 3. Process of mutation caused by PAH–DNA adduct tion.

For the last 20 years, intensive studies over ous biomarkers have been conducted. Biomarkers e been used in risk assessment concerning people ng in industrial areas, or in occupational exposure ssment. Biomarkers help to detect disease at the y stage (47). It is hard to overestimate the role of narkers, therefore intensive research on the im- of environmental factors on structural elements ells has been carried out. It is also very important nvent sensitive detection methods in order to use narkers widely in diagnostics.

## FERENCES


Bojakowska I., Sokołowska G.: Bulletin of Polish Institute of Geology Warsaw 1995.

Kennaway E.: Br. Med. J. 2, 749 (1995).

Kjaerheim K.: Environ. Health Perspect. 107, 233 (1999).

Mastral A. M., Sallen M. S.: Envir. Sci. Tech. 34, 3051 (2000).

Agency for Toxic Substances and Disease Registry 1990.

Bjorseth A., Olufsen S.: Long transport of polycyclic aromatic hydrocarbons. 507, New York, Marcel Dekker 1983.

Grimmer G.: Chemistry. 27, Boca Raton Florida CRC Press 1983.

National Toxicology Programme. 1993.

Davis W.W., Krahl M.E., Clowes G.H.A.: J. Am. Chem. Soc. 64, 108 (1942).

Sims R.C., Overcash M.R.: Res. Rev. 88, 1 (1983).

White C.M.: J. Chem. Eng. Data 31, 198 (1986).

12. Schwarz F.P.: J. Chem. Eng. Data 22, 273 (1977).
13. Solomons G.T.W.: Fundamentals of Organic Chemistry, J. Wiley, Ed. NY 1986.
14. Masłowski J.S.: Doctoral Thesis, IKŚ Katowice 1992.
15. Zakrzewski S.F.: Principles of environmental toxicology, PWN, Warsaw, 1995.
16. Lee M.L., Novotny M., Bartle K.D.: Anal. Chem. 48, 1566 (1976).
17. Lee M.L., Novotny M., Bartle K.D.: 35, 78, 156, Academic Press, New York 1981.
18. Cherng S.H., Lin S.T., Lee H.: Mut. Res. 367, 177 (1996).
19. Lewitas J., Walsh D., Williams R., Dobias L.: Mut. Res. 378, 51 (1997).
20. Suess H.J.: Sci. Total Env. 6, 239 (1976).
21. Ruchirawat M. et al.: Sci. Total Envir. 287, 121 (2002).
22. Klimaszewska K.: Doctoral Thesis. Warsaw 1998.
23. Miron M.: IGO, Katowice1995.
24. Maliszewska–Kordybach B.: The organic compounds in environment and methods of detection (Związki organiczne w środowisku i metody ich oznaczania), BMŚ, Warsaw, 1994.
25. Phillips D.H.: Mut. Res. 443, 139 (1999).
26. Guidelines for drinking–water quality. 123, WHO Geneva 1998.
27. Phillips D.H.: Environ. Health Persp. 104, 453 (1996).
28. O'Neill P.: Chemia środowiska, PWN, Warsaw 1997.
29. Georgiadis P., Kyrtopoulos S.A.: Mut. Res. 428, 91 (1999).
30. Nielsen P.S. et al.: Int. Arch. Occup. Environ. Health 68, 170 (1996).
31. Schauer C., Niessner R., Poschl U.: Envir Sci Tech 372861 (2003)
32. Perera F.P., Hemminki K., Gryzbowska E., Motykiewicz G., Michalska J., Santella R.M., Young T.L., Dickey C., Brandt–Rauf P., De Vivo I.: Nature 360, 256 (1992).
33. Autrup H., Daneshvar B., Dragsted L.O., Gamborg M., Hansen M., Loft S., Okkels H., Nielsen F., Nielsen P.S., Raffn E., Wallin H., Knudsen L.E.: Environ. Health Persp. 107, 233 (1999).
34. Sherson D., Sabro P., Sigsgaard T., Johansen F., Autrup H.: Brit. J. Ind. Med. 47, 448 (1990).
35. Durant J.L., Busby W.F., Lafleur A.L., Penman B.W., Crespi C.L.W.F.: Mut. Res. 370, 123 (1996).
36. Buters J., Quintanilla–Martinez L., Schober W., Soballa V.J., Hintermair J., Wolff T.,

Gonzalez F.J., Greim H.: Carcnogenesis 24, 327 (2003).
37. Guidelines for air quality, European Economic Community 1994.
38. Khesina A.Y.: Environ. Health Persp. 102, 49 (1994).
39. Slooff W., Janus J.A., Matthijsen A.J.C.M., Montizaan G.K., Ros J.P.M.: Bulletin of National Institute of Public Health and Environmental Protection, 15, Bilthoven 1989.
40. Guidelines for drinking–water quality. 495, WHO Geneva 1996.
41. Guidelines for drinking–water quality, European Economic Community 1980.
42. Disposition of German Federal Department for Worker Safety 1989.
43. 41.Disposition of Swedish National Board of Occupational Safety & Health 1994.
44. American Conference of Govermental Industrial Hygienists 1995.
45. Corpus of Polish Law (Dziennik Ustaw) 2001.
46. UK Department for Environment, Food & Rural Affairs (1999).
47. Bentsen – Farmen R.K. et al.: Biomarkers 4, 37 (1999).
48. Surh Y.J., Shlyankevich M., Lee J.W., Yoo J.K.: Mut. Res. 367, 219 (1996).
49. Decaprio A.P.: Envir. Sci. Tech. 31, 1837 (1997).
50. Autrup H., Vestergard A.B., Okkles H.: Carcinogenesis 16, 1305 (1995).
51. Boysen G., Hecht SS.: Mut. Res. 543, 17 (2003).
52. Shu–Xin Qu, Cheng–Long Bai, Stacey NH.: Biomarkers 2, 3 (1997).
53. Jankowiak R., Small G.: J. Envir. Chem. 119, Springer 1998.
54. Misiewicz I., Skupińska K., Kasprzycka–Guttman T.: Oncol. Rep. 10, 2045 (2003).
55. Zhang Y. and Talalay P.: Cancer Res 58, 4632 (1998).
56. Kleiner H.E., Reed M.J., DiGiovanni J.: Chem. Res. Tox. 16, 415 (2003).
57. Bennett D.A., Waters M.D.: Envir. Health Persp. 108, 907 (2000).
58. Niyogi S.: Sci. Total Envir. 281, 237 (2001).
59. Oh Seung Min, Ham Byung Woo, Kim JH, Chung KH.: J. Health Sci. 49, 59 (2003).
60. RABB t, Nylund L., Vaavanriuta R., Savela K., Mutanen P., Veidebaum T., Sorsa M., Rannug Am., Peltonen K.: J. Expo. Anal. Envir. Epid. 12, 81 (2000).

*Received: 8.12.2003*