Phenols – Sources and Toxicity

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Abstract

Phenols and their derivatives commonly exist in the environment. These compounds are used as the components of dyes, polymers, drugs and other organic substances. The presence of phenols in the ecosystems is also related with production and degradation of numerous pesticides and the generation of industrial and municipal sewages. Some phenols are also formed during natural processes. These compounds may be substituted with chlorine atoms, may be nitrated, methylated or alkylated. Both phenols and catechols are harmful ecotoxins. Toxic action of these compounds stems from unspecified toxicity related to hydrophobicity and also to the generation of organic radicals and reactive oxygen species. Phenols and catechols reveal peroxidative capacity, they are hematotoxic and hepatotoxic, provoke mutagenesis and carcinogenesis toward humans and other living organisms.

Keywords: phenols, catechol, natural origin of phenols, anthropogenic sources of phenols, toxicity of phenols

Introduction

Phenols of anthropogenic origin exist in the environment due to the activity of the chemical, petrol, tinctoral or pharmaceutical industries. The compounds penetrate ecosystems as the result of drainage off the municipal or industrial sewage to surface water [1]. Moreover, the occurrence of phenols in the environment stems from the production and use of numerous pesticides, in particular phenoxyherbicides like 2,4-dichlorophenoxyacetic acid (2,4-D) [2] or 4-chloro-2-methylphenoxycetic acid (MCPA) and also phenolic bioicides like pentachlorophenol (PCP) [3], dinoseb or diaryl-ether pesticides [4]. Some phenols may be formed as a result of natural processes like the formation of phenol and p-cresol during decomposition of organic matter or synthesis of chlorinated phenols by fungi and plants [5].

Phenol toxicity is related with two main processes – unspecified toxicity related with hydrophobicity of the individual compound and formation of free radicals [6]. Hydrophobicity affects the solubility of phenol in a cells’ fractions and thus possibility of interaction of the compound with specified cell and tissue structures. For example, the increase of hydrophobicity of chlorophenols is related to the increasing number of chlorine atoms that enhances toxicity of the individual compound [7]. The strength of toxic influence of the compound also stems from localization of the substituent. For instance, a chlorine atom substituted in ortho position in phenol molecule decreases its toxicity and meta substitution increases toxic action of the compound. Phenols, after penetration of the cell, undergo active transformation, mainly at the participation of oxidases within cytochrome P450. Sometimes transformation processes lead to increase of toxicity of individual compounds by the formation of electrophilic metabolites that may bind and damage DNA or enzymes. The noxious influence of phenols and their derivatives concerns acute toxicity, histopathological changes, mutagenicity and carcinogenicity. The compounds presented in the review represent...
phenols most commonly present in the environment and human surroundings that reveal toxic influence towards living organisms, including human.

**Anthropogenic and Natural Sources of Phenols**

**Phenol**

Phenol (hydroxybenzene) is a colourless, crystalline substance of characteristic odour, soluble in water and organic solvents. Phenol was one of the first compounds inscribed into The List of Priority Pollutants by the US Environmental Protection Agency (US EPA). Phenol is synthesized on an industrial scale by extraction from coal tar as it is formed by high quantities of cumene present in plants that were used for tar production. Phenol is also obtained in a reaction between chlorobenzene and sodium hydroxide, toluene oxidation and synthesis from benzene and propylene. It is commonly used in different branches of industry including chemical – production of alkylphenols, cresols, xyleneols, phenolic resins, aniline and other compounds [8], oil, coal processing and metallurgic [9]. Phenol is also used in pesticides, explosives, dies and textiles production [10].

Phenol also penetrates the environment through vehicle exhaust, and it is used as a disinfectant and reagent in chemical analysis. In the United States alone, are 580,000 people occupationally exposed to phenol influence [9]. Phenol is also formed as the result of chemical reactions that occurred in the atmosphere in condensed water vapour that forms clouds. Hydroxybenzene is also formed during natural processes like biosynthesis by plants or decomposition of organic matter [11]. This compound is also formed from aminoacids contained in plants’ hemicelluloses under the influence of UV irradiation (sunlight) [12] and tyrosine transformation in mammalian (including human) digestive tract [13]. The concentrations of phenol in surface water are different. In natural waters its amounts are between 0.01 – 2.0 µg/L [14]. Relative fast degradation of phenol causes its concentration in waters exposed to strong anthropogenic pollution may be comparable. Concentration of phenol in surface water of Netherlands were of 2.6 – 5.6 µg/L. River water polluted with sewage derived from petrol processing plants contained the concentration of phenol over 40 mg/L [9]. Phenol was also found in domestic water supply in the USA at a level of 1 µg/L. Background levels of phenol in air are expected to be low, at about 1 ng/m³ [15]. In high concentrations phenol is determined near factories that impregnate wood and its value reaches 9.7µg/m³ [16]. Phenol is also present in food. Moderate quantities of this compound (5 µg/kg) were determined in honey [17], also in coffee, in which is formed from ferulic acid. In this process ferulic acid it the natural compound present in corn-undergoes conversion to vinyl-guaiacol, guaiacol and finally to phenol [18]. Phenol concentration in processed food may reach alarming concentrations. For example, in grilled sausage and pork the content of phenol was 7 and 28.6 µg/kg, respectively [19]. Other authors have found phenol in the outer layer of smoked meat in concentrations of 37–70 mg/kg.

The exposure data are inadequate to determine the degree of exposure of the general population to phenol. However, persons exposed to phenol through inhalation of air from strongly industrialized areas or with frequent consumption of smoked food with high phenol content may accept toxic doses of phenol about 4 mg and 2 mg per day, respectively. It also has been estimated that 0.3–0.4 mg of phenol per cigarette is released during its burning. Exposure to phenol may also be accidental. Delfino and Dube described the case of contamination of ground water with phenol that was then used for drinking purposes. The authors evaluated the daily exposure to be 10–240 mg of phenol per person. The result was statistically significant increases of diarrhoea, mouth sores, dark urine and burning pain in mouth [20].

**Chlorophenols**

Chlorophenols are the most widespread and the largest group of phenols. Chlorophenols are formed in the environment by chlorination of mono and polyaromatic compounds present in soil and water. Synthesis of chlorophenols proceeds at the participation of chloroperoxidases contained in plants and microorganisms in the presence of hydrogen peroxide and inorganic chlorine [21]. The example is synthesis of chlorinated phenols by fungi from *Hypholoma* genera [5, 22]. The concentrations of chlorophenols in oceanic waters are of 5-10 ng/L. The highest concentrations are noted for river waters and are in the range of 2-2000 µg/L. Chlorophenols are also present in drinking water due to substitution of organic matter and low molecular weight compounds (present in purified water) with chlorine atoms derived from inorganic chlorine oxidants. The investigations of drinking water of Warsaw and Łódź (Poland) revealed the presence of 2,4,5-trichlorophenol and tetrachlorophenol in concentrations of 0.2 – 0.3 µg/L [23]. The highest concentrations of phenols are noted in industrial sewages and may reach (for pentachlorophenol) 0.1-10 mg/L. Atmospheric concentrations of chlorinated phenols are usually contained in low concentrations of 0.25 to 7.8 ng/m³; however, in urbanized areas of Holland the concentration may reach even 1 µg/m³ [24]. The concentrations of chlorophenols in soils that are not exposed to anthropogenic pollution are rather low. However, Garrett has reported that soil samples from the farmer site of pesticide plant in Richmond (British Columbia) contained 2 mg of TeCP and 0.18 mg of TCP per kg of soil. Soils situated within sawmills are usually heavily contaminated with chlorophenols. Kitten and co-workers determined the concentrations of chlorophenols near preserving facilities at 4 different sawmills in the range of 500 to 3500 µg/kg [25]. Both tetrachlorophenol and pentachlorophenol were identified in agricultural products like carrots, potatoes and turnips.
in concentrations of 1 to 45 µg/kg of wet weight [26]. Chlorophenols were also determined in poultry with concentrations of 2 to 3 µg/kg of chicken flesh. Usually 10 mg/kg levels of the lower chlorinated phenols are found in serum and adipose tissue of the general population. Performed analysis in Germany concerning a large group of people revealed the chronic exposure of investigated populations (both children and adults) to pentachlorophenol with its mean concentration in blood plasma of 2.48 µg/L [27]. The other investigation, performed in the Arctic area of Russia (Czukczi Peninsula, Uelen locality), revealed a mean concentration of PCP in human plasma of 0.64 µg/L [28]. Exposure of chlorophenols may occur via ingestion, inhalation or dermal absorption. The general population is thought to be exposed mainly through the ingestion of food and drinking water. However, non-occupational exposure by inhalation may be significant if chlorophenols are used for extensive treatment of the interior of houses. In Canada estimated exposure of chlorophenols for a 60-kg person was evaluated for 3.84 µg of 2,4-DCP, 1.62 µg of TeCP and 0.084 µg of TCP. Including PCP, exposure to the total concentration of chlorophenols is about 10 µg per person. Similarly, NRCC estimated that total chlorophenol exposure per day of the general population in Canada is about 10-30 µg per person [29]. Long-term exposure of people to these concentrations may in some cases lead to cancer. Chlorophenols are used or formed as a result of the activity of some branches of industry – mainly chemical, textile, pharmaceutical and metallurgic [30]. The presence of chlorophenols in the environment is also related to the use and degradation of organic compounds like growth regulators, pesticides and, in particular, phenoxyherbicides and phenolic biocides. The most popular are 2,4-dichlorophenoxyacetic acid (2,4-D) [2], 4-chloro-2-methylphenoxyacetic acid (MC24A) [31] and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) that biodegradation leads to the formation of both phenols (phenol, 2-chlorophenol and 2,4-dichlorophenol) and catechols (catechol and 4,6-dichloro-catechol).

Biotransformation of 4-chlorophenoxyacetic acid leads to the formation of 4-chlorophenol and photodegradation of dichloroprop and 2,4-D causes the formation of 2-chlorophenol, 4-chlorophenol and 2,4-dichlorophenol. Moreover, transformation of the widely used group of diaryl-ether pesticides like nitrophen or dichlorodiphenylnitrophen results in the formation of very toxic and resistant to biodegradation 2,4-dichlorophenol [4]. The other well-known pesticide used as a herbicide and insecticide is pentachlorophenol (PCP). This compound is also used to impregnate wood, textile and skin products as it has strong fungicide capacities. In the environment pentachlorophenol is usually degraded to chlorophenols of lower number of chlorine atoms [3]. The compound may also be formed from other pesticides including hexachlorocyclohexane, hexachlorobenzene, pentachlorobenzene and pentachloronitrobenzene. Other phenolic biocides are 2-chlorophenol, 2,4-dichlorophenol and 2,4,5-trichlorophenol used as herbicides and 4-chlorophenol employed as fungicide [32]. Microbiological transformation of chlorophenols, mainly PCP used in finishing materials, leads to the formation of other toxic compounds – trichloroanisole and tetrachloroanisole [33].

Catechol and Chlorocatechols

Chlorocatechols in regard to anthropogenic origin more commonly occur in polluted water. The analysis of samples of water obtained from the polluted Ner river (central Poland) revealed considerable amounts of chlorocatechols including very toxic tetrachlorocatechol in a concentration of 2 µg/L. The concentrations of chlorocatechols in municipal raw sewages that contaminated the river exceeded 5 µg/L [1]. In water of natural origin chlorocatechols may be absent or exist in small concentrations. The annual investigation in the drainage of the Dzierżązna river (central Poland) revealed lack of chlorocatechols in investigated ecosystems [14]. The presence of chlorocatechols – 4-chlorocatechol and 3,4,5-trichlorocatechol in low concentrations was noted in drinking water of the largest cities of Poland [21]. Catechol is aromatic alcohol that has hydroxyl residues on the first and the second carbon positions. It is soluble both in water and organic solvents. On an industrial scale it is formed in a process of catalytic hydrolysis of 2-chlorophenol in high temperature. It is also formed in the result of phenol and benzoic acid hydroxylation process. Catechol is used in photography [34], rubber and synthetic material production and drug synthesis [35]. It is also used in cosmetic [36], dye and insecticide production [37]. Catechols are also employed in production of 4-tert-butylycatechol, the compound that inhibits the polymerization process of synthetic materials [38]. Chlorinated derivatives of catechol are used in dichloroaniline and chlorinated biphenyls production [39]. Catechol and chlorocatechols are the main products of phenol and chlorophenols environmental transformation. The processes are mainly performed by microbes that hydroxylate phenols at the second carbon position [40]. For example, the transformation of pentachlorophenol lead to tetrachlorocatechol formation that may be further degraded to chlorinated catechols of lower number of chlorine atoms. It was also noted that microbiological transformations of chlorobenzenes, chlorinated phenoxyacetates and chlorobiphenyls result in chlorocatechols formation [41].

Nitrophenols

The presence of nitrophenols in the environment is related both to natural processes and anthropogenic activity. Nitrophenols, particularly 2-nitrophenol and 4-nitrophenol, are formed in the reaction of phenol with nitrite ions in water. The reactions proceed under the influence of UV irradiation (sunlight) and in the wide range of pH values [42, 43]. Environmental reactions also lead
to the formation of nitrophenols in the atmosphere. The reaction of phenol, nitrite ions and hydroxyl radical leads to the formation of 2-nitrophenol and other nitrated compounds [44]. Nitration of phenols substituted mainly in ortho and para positions also proceeds at the participation of enzymes present in plant tissues. Peroxidases and lipooxygenases in the presence of nitric ions and hydrogen peroxide catalyze phenol nitration to form nitrophenols. It is considered that this phenomenon may considerably effect the presence of nitrophenols in soil environment [45]. Nitrophenols in the atmosphere are usually determined in low concentrations of some ng/dm³, however strong pollution of air due to industrial emissions lead to increased concentrations of nitrophenols up to 320 ng/dm³ [46]. In water nitrophenols exist in concentrations that seldom exceeded some µg/L. Analysis of the Ebro river (Spain) revealed the presence of 2-nitrophenol, 4-nitrophenol and 2,4-dinitrophenol in the range of 0.1 – 5.0 µg/L of individual compound. 2-nitrophenol and 4-nitrophenol were detected in 177 samples of river waters of Japan. The concentrations were of 0.04 to 10 µg/L. 2-nitrophenol levels in rainwater and snow are between 0.03 to 5.7 µg/L. Nitrophenols are formed by man during production and degradation of pesticides such as 2-buthyl-4,6-dinitrophenol (Dinoseb) and 4,6-dinitro-2-methylphenol (DNOC). Those compounds are also used as components and precursors in polymers and drug production [47], and employed as photographic developers and preservatives. Moreover, nitrated phenols are used in dyes, solvents, plastics and explosives production [48] and formed due to electric, electronic and metallurgic industrial activity [19]. Mononitrophenols, 3-methyl-4-nitrophenol and 4-nitro-3-phenylphenol reach the environment in regards to vehicular emissions [49]. In the United States exposure to nitrophenols related with exceeded and illegal use of methylparathion has led to the accumulation of the main metabolite of this pesticide (4-nitrophenol) in tissues. The analysis of samples obtained from 16,000 people revealed the increased concentration of 4-nitrophenol and its concentration was correlated with the amount and frequency of methylparathion usage in homes [50]. Exposure of the general population to nitrophenol isomers is mainly through ambient air and drinking water. A daily uptake by inhalation of nitrophenols was calculated to be of 0.06 µg/kg per body weight. The uptake via drinking water for 2- and 4-nitrophenols is calculated to be about 0.02 µg/kg body weight [51]. Workers are usually exposed to high (toxic) concentrations of nitrophenols via inhalation and skin contact during production and processing (mainly in the manufacturing of pesticides).

The highest concentrations of methylphenols are noted in waters situated near plants that produce coal tar (creosote) – determined concentration of 4-methylphenol in ground water exceeded 2 mg/L. There are some reports concerning atmospheric concentrations of methylated phenols. The analysis of air samples obtained from eleven areas of California (with different levels of industrial emissions) revealed the range of the concentrations of methylphenols of 0.07 to 4.6 µg/m³. The median air concentration of cresols was 1.58 µg/m³ for 32 source sites in the USA. Rainwater concentrations for o-cresol were determined at 0.24 to 2.8 µg/L. These results may lead to the conclusion that methylphenols exist in the air in higher amounts than other phenolic compounds. Discussing xenobiotics are also formed due to pesticide degradation. The environmental transformation of 4-chloro-2-methylphenoxycetic acid (MCPA) lead to the formation of 2-methylphenol [31]. Methylphenols are contained in high concentrations (up to several grams per kilogram) in coal tar used for asphalt production and wood impregnation. The commonness of creosote usage is the reason for releasing considerable concentrations of methylphenols, in particular 4-methylphenol, to the natural environment. The representatives of methylphenols are cresols that form three isomers – ortho, meta and para-cresol. Chlorinated and nitrated form of o-cresol is used as a compound of herbicide and pesticide properties. It is also used for epoxy-resins, dyes and drug production [52]. Both cresols, dimethylphenol and 2,4,6-trimethylphenol are formed during coal and gasoline combustion [53]. The presence of p-cresol is also related to the production of sewage by the petrochemical industry. The occurrence of m-cresol in the environment is mainly related to use this compound in cosmetic, fragrance, disinfectant, explosive and pesticide production [52]. The mixture of m-cresol and p-cresol is used in insecticide synthesis. The solution of cresols in potassium soap is known as lisole and is used in medicine as it reveals strong disinfecting activity. Cresols at concentrations normally found in the environment do not pose any significant risk for the general population. However, the potential for adverse health effects exists for specific subpopulations living on the industrialized regions and under conditions of exposure. For example, significant concentrations of cresols (0.01 – 0.2 mg/L) have been noted in beverages. Moreover, the incineration of one cigarette leads to inhalation of 75 µg of p-cresol.

Alkylphenols

Alkylphenols of low molecular weight commonly exist in rock-oil and shale oils. The sources of these compounds in particular substituted in para position are geochemical processes like methylation, butylation and alklylation that proceed in geological structures [54]. These compounds are also produced in some technological processes. For example, nonylphenols are derived from nonylphenol ethoxylates – the surfactants produced for industrial and
farming purposes [55]. They are also used as emulsifiers, wetting agents and dispersing agents. Nonylphenol polyethoxylate are used in many sectors including textile processing, pulp and paper processing, paints, resins and oil production and steel manufacturing. Alkylphenols are also formed as a result of pesticide degradation, agriculture and industrial sewage production [56]. Analysis of drinking water purified by plants situated nearby textile and wood processing factories (Quebec, Canada) revealed the presence of alkylphenols in the range of mean concentrations of 0.02 to 2.8µg/L with the highest amount of 43.3µg/L [57]. The analysis of rain water collected in Germany and Belgium showed the common occurrence of 4-nonylphenol in the concentrations of 0.253 to 0.534µg/L. The concentrations of alkylphenols in surface water are different and concern the range of 0.7 to 21,000µg/L. However, the most common of these compounds exist in low concentrations. Analysis of more representative sites in the USA indicate nonylphenol levels less than 0.1 to 0.6µg/L in rivers and 0.003 to 3.0µg/g in sediments. In Canadian rivers inconsiderable levels (0.01 to 0.9µg/L) of nonylphenol were determined. Analysis of samples of water obtained from the Elba river (Germany) were between 0.028 to 1.22µg/L [58, 59]. The concentration of nonylphenol in soil may be high. In a field that received municipal sewage sludge application, the concentration of 4-nonylphenol was 2.7 mg/kg of soil. Alkylphenols and alkylphenol ethoxylates are able to accumulate in tissue of living organisms. The analysis of fish tissue collected from the Kalamazoo river (Michigan, USA) revealed the presence of alkylphenol with the highest concentration of 3.4µg/L of body weight [60]. Similar investigations performed in rivers of Germany and the Baltic Sea showed accumulation in tissues of molluscs – Dreissena polymorpha and Mytilus edulis considerable concentrations of nonylphenol up to 112µg/kg of body weight and lower concentrations of octylphenol – 5.5µg/kg [61]. The presence of p-buthylphenol and p-octylphenol was also noted in rubber products that are in contact with food. The range of high concentrations of octylphenol was of 2.6 to 513µg/kg [62]. The study of 60 food products on the market in Germany illustrated the widespread nature of alkylphenol contamination. Nonylphenol was detected in every sample within the range of 0.1-19.4µg/kg of food. The highest (toxic) concentration was noted in samples collected from apples [63]. Those compounds have also been identified in dust samples of offices and houses. Moreover, they have been found in bottles, toys, paints, cosmetics, air fresheners, T-shirts, sport shoes, mobile phones and computers [64]. The common occurrence of these compounds in food and industrial products leads to exposure of the general population to these compounds. Greenpeace analyzed the presence of 4-nonylphenol and bisphenol A in blood samples from representative group of Dutch people including 48 males and 43 females. The concentration of the compounds were in the range of 1.1-3.0 ng and 1.4-1.8 ng per gram of serum for 4-nonylphenol and bisphenol A respectively [65].

Bisphenols

Bisphenols, in particular bisphenols A and F are used as the components or are formed as by-products in lubricants, epoxy-resins, rubber and other synthetic production [66]. Brominated bisphenols like tetrachlorobisphenol in considerable concentrations are present in ashes produced during aluminium processing [67]. The mean concentrations of BPA from 21 European and 13 States was 0.016 and 0.5µg/L respectively. In the USA the median concentration of Bisphenol A in several streams was 0.14µg/L, only two streams were reported to contain BPA at levels above 1µg/L. The investigations performed in Germany revealed the presence of bisphenol A in water of the Elba river – its estuaries (4-92µg/L) and sediments (10 – 380µg/kg) [68]. The atmospheric content of bisphenols is different. Performed analysis of air samples collected over an estuary of the Hudson river did not detect bisphenol A [69]; however, the presence of the compound in high concentrations of µg – mg/dm³ were noted in the air of urbanized areas of Germany [70]. The commonness of bisphenols appearance in plastic packages and varnishes used in internal sides of tins causes penetration of these compounds to food [71]. This compound also migrates from through rubber products and plastic stretch film used in food contact applications. For example, in honey high concentrations of bisphenol A and F (2.0-33.3 mg/kg) were noted as the result of contact of this product with package materials [72]. Considerable concentrations of bisphenol A were also noted in fluids that were in contact with polycarbonate bottles intended for infants. Multiple usage of bottles (often contact with warm fluids, washing, scrubbing) caused polymer degradation and more intensive releasing of bisphenol A to water, and the determined concentration was about 6.7µg/dm³ [73]. Human infants ingest bisphenol A in a formula at an estimated rate of 1.6µg/kg/day, which is comparable with doses (about 2.0µg/kg/day) that cause toxic effects in animals [74]. In some instances, contamination has even been reported to arise from water filters [75]. Furthermore, patients on kidney dialysis may receive elevated exposures to Bisphenol A as a result of the use of polycarbonate components in the equipment. Bisphenol A was also isolated from phenol red, the preparation commonly used to investigate physiological processes among animals and human [76].

Aminophenols

Para-aminophenol is used in oil, lubricants and as photographic developer. As N-acetylated form it is used as the main component of paracetamol, a drug of anti-inflammatory and analgesic capacities [77]. 3-aminophenol is used as the marker in analysis of antibacterial drugs – sulphonamides [78] and 2-aminophenol is used as the precursor for indols synthesis. All isomers of aminophenols and 2.4-diaminophenol are used in dyes used in colouring of hair [79]. The presence of p-aminophenol in
The concentrations of phenols in surface water are different. Investigations in the water of the drainage of the Dzierżązna river (Central Poland) revealed concentrations of this compound between 0.01 and 2.0 µg/L [14]. The investigations of strongly polluted water of the Ner river (Central Poland) revealed the concentrations of phenol only for 1.7 µg/L; however, considerable concentrations of chlorophenols (above 2 µg/L) were determined in this ecosystem [9]. The concentrations of chlorophenols in water are related to its pollution. In lakes and rivers of the Tucholski Landscape Park the total concentrations of chlorophenols were low and did not exceed 1 µg/L. On the other hand, polluted water of the Vistula River contained about 6 µg/L of 2,4-dichlorophenol [84]. The similar total concentration of chlorophenols (0.1-6.0 µg/L) were determined in water of the Gulf of Gdańsk. Chlorocatechols in regard to anthropogenic origin more commonly occur in polluted waters. The analysis of samples of water obtained from the polluted Ner River (Central Poland) revealed considerable amounts of chlorocatechols, including very toxic tetrachlorocatechol in concentrations of 2 µg/L. The investigation performed after four years revealed that the quality of water of the discussed river has improved as only trace concentrations of these compounds were detected. Analysis of samples of water obtained from the Odra River (Poland) showed considerable concentrations of nonylphenol estimated for 0.028 to 1.22 µg/L [57]. The concentrations of chlorophenols in soils that are not exposed to anthropogenic pollution are rather low. The investigations that concerned the presence of chlorophenols in forest soils of Tucholski Landscape Park (northwest Poland) did not exceed several µg/kg of soil. Generally, phenols occur in low concentrations in air in Poland; however, high concentrations of phenol are related to urban areas. Ambient air levels of phenol were investigated in strongly industrialized and urbanized upper Silesia region of Poland were from 3.8 to 26.6 µg/m³.

Exposure of Population

Occupational exposure to phenols is related to production of phenolic resins that belong to plastic materials used in Poland to produce glue, vitreous fibre, dyes and products of common applications. During processing (induration) of resins at high temperatures, some phenols (like phenol and m-cresol) are emitted. Exposure of workers to benzene is also related to the influence of phenol on their organisms as it is formed as the main metabolite during benzene metabolism. It has been determined that about 8,000 workers in Poland are chronically exposed to benzene influence. In regard to the presence of benzene in gasoline and vehicle exhausts and also in cigarette fumes exposure of the general population to phenol is considerable [85]. Exposure to biocide – o-phenylphenol mainly concerns health service workers, and in particular assistant personnel. This compound is also used in disinfections of medical equipment and hospital waste. Other examples are trichlorophenol and p-chloro-m-cresol used as the impregnants of leather and textiles to protect them from microbes [86]. Exposure of the general population in Poland is also related to strongly industrialized areas, for example Silesia, which is characterized by high emissions of toxic compounds, including phenols.

Noxious Activity of Phenols

Toxic influence of organic compounds depends on many factors. Penetration of phenol to organisms is related with diffusion of the compound across a cell’s membrane. The factor that strongly affects diffusion is hydrophobicity of the individual compound. The increase of hydrophobicity affects the more effective penetration of a cell’s membrane by phenol and thus enhances the toxicity of xenobiotics. When comparing toxic effects of phenols one cannot omit such important parameters as pKₐ (where Kₐ is the compound dissociation constant) and log P (where P is the octanol-water partition coefficient of the undissociated acids). The increase of hydrophobicity and the value of log P, and the decrease of pKₐ value result in more effective membrane penetration by xenobiotics and, thus, enhance their toxicity [86]. The example is 2,4-chlorophenol which has (in comparison to other phenols) the highest value of log P and the lowest pKₐ value.
Hydrophobocity may be the ultimate factor when pKa values of the compounds are similar. The example are phenols that have similar pKa values and whose transport rate depends on the length of side aliphatic chain. The order of diffusion velocity of these phenols is given below:

methylphenol > ethylphenol > propylphenol > butylphenol [87].

The essential factor that determines phenol toxicity is the reactivity of the compound with a cell’s biomolecules and is related with easiness of donation of free electrons by phenol from oxidized substrate. One-electron reactions in cells are usually catalyzed by oxidative enzymes like peroxidases present in liver, lungs and other organs, prosta-glandins and myeloperoxidases contained in bone marrow [88]. The effect of their action is the formation of phenoxy radicals and intermediate metabolites – semiquinones and quinone methides that interact with biomolecules in the cell. In these reactions reactive oxygen species like superoxide radicals or hydrogen peroxide also are formed. The effect of these forms on specified cell structures depends on phenol reactivity. Phenols that exert higher reactivity quickly undergo radical reactions and provoke lipid peroxidation of a cell’s membrane. The forms of lower activity penetrate internal spaces of the cell and damage membranes of endoplasmatic reticulum, mitochondria and nucleus and also their components like enzymes and nucleic acids [89].

Interaction of phenols (nitrophenols, nitrocatechols and pentachlorophenol) or its radical metabolites with mitochondrion also leads to coupling between oxidative phosphorylation and electron transport in respiratory chain. Toxic influence of phenols is also related to the kind of substrate that comes into reaction, also its localization in cell and phase of cell proliferation. An important factor is also tissue type (cell) exposed to phenol activity. For example, diffusion of phenol to hepatocytes leads to its conjugation with glucuronides, sulphates, aminoacids and other substrates that protect cells from electrophilic metabolite influences.

Most phenols including phenol, chlorophenols, nitrophenols and aminophenols are characterized by toxic activity. Toxic influence is also exerted by catechol [90], chlorocatechols [91], methylphenols and other phenolic compounds [92].

<table>
<thead>
<tr>
<th>The compound</th>
<th>Log P</th>
<th>pKa</th>
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<tbody>
<tr>
<td>Catechol</td>
<td>0.7</td>
<td>9.30</td>
</tr>
<tr>
<td>Phenol</td>
<td>1.47</td>
<td>9.98</td>
</tr>
<tr>
<td>2,4-dimethylphenol</td>
<td>1.99</td>
<td>10.26</td>
</tr>
<tr>
<td>2,4-dichlorophenol</td>
<td>3.23</td>
<td>7.81</td>
</tr>
</tbody>
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Table 1. The values of log P and pKa.
Acute Toxicity

Phenol irritates skin and causes its necrosis, it damages kidneys, liver, muscle and eyes. Damage to skin is caused by its coagulation related to reaction to phenol with aminoacids contained in keratin of epidermis and collagen in inner skin [93]. In a dose of 1 g phenol may be lethal for an adult man, but individual tolerance for this compound can be high. Some reports reveal that a man can survive even after administration of 30 g of this compound (60 ml of 50% solution). In regard to fast absorption by skin (from 60%-90%) even contact of hand or forearm with phenol solution may cause death [94]. Acute poison with phenol is characterized by dryness in throat and mouth, dark-coloured urine and strong irritation of mucous membranes. The investigations showed that chronic administration of phenol by animals leads to pathological changes in skin, esophagus, lungs, liver, kidneys and also urogenital tract. Described changes are mainly induced by lipid peroxidation that is responsible for damage and finally degradation of a cell’s membrane. Chronic exposure of workers to phenol vapours causes anorexia, lost of body weight, weakness, headache, muscles pain and icterus [95]. Phenol is mainly accumulated in brain, kidneys, liver and muscles. Two days after phenol administration it is mainly excreted in unchanged form and also conjugated with sulphates and glucuronides. Catechol is also considered a strong toxin. Doses of 50 to 500 mg/kg of body weight usually cause death. For mice after oral administration of catechol LD₅₀ is 260 mg/kg of body weight.

Acute poison with chlorophenols is characterized by burning pain in mouth and throat, white necrotic lesions in mouth, esophagus and stomach, vomiting, headache, irregular pulse, decrease of temperature and muscle weakness, convulsions and death [96]. Chronic exposure to chlorophenols cause hypotension, fall of body temperature, weakness and abdominal pain. Poisoning by chlorophenols results in damage to lungs, liver, kidneys, skin and digestive tract. Strong toxicity of chlorophenols is expressed by very low, acceptable daily intake (ADI) for pentachlorophenol that was established for 16 µg for a man of 70 kg of bodyweight. LD₅₀ for male and female of rats after oral administration of PCP is 14 mg and 3.85 mg/kg of bodyweight respectively [97]. For 2,4,5-trichlorophenol LD₅₀ is much higher and is of 820 mg/kg of body weight. [19]. Air pollution with a mixture that contained 2-chloro-6-fluorophenol is the result of an accident in a chemical factory (New York, USA) that caused symptoms like dryness in mouth and throat, coughs, headaches and abdominal pain [98]. Chlorophenols undergo fast absorption by skin and mucous membrane of respiratory system. Pentachlorophenol and tetrachlorophenol dissolved in fats are adsorbed by skin in 62% and 63% respectively. Chlorophenol accumulation proceeds in kidneys, spleen, liver, heart, brain and fat tissue.

Clinical symptoms related to poisons with nitrophenols are similar to that exerted by chlorophenols. 2,4-dinitrophenol has been used as a slimming drug and as an additive in food at the beginning of the last century. Numerous cases of chronic heat, depression and deaths led to this compound being removed from the market [99]. It is considered that a lethal dose of 2,4-dinitrophenol for a man is of 14 to 35 mg/kg of bodyweight [100]. Lethal doses (LD₅₀) of nitrophenol orally administrated to rats and mice are 450-850 mg/kg and 380 mg/kg of body weight, respectively, and for dinitrophenol (rats) only 30 mg/kg of bodyweight [101]. 2,4-dinitrophenol undergoes fast absorption by skin and respiratory system, it is also quickly absorbed from the digestive tract. The compound is accumulated in blood plasma, kidneys, lungs and liver [102]. In work, acute poison as the result of one intake of 2,4-dinitrophenol has been described. In the first hour after poisoning a high increase of temperature and intense perspiration was observed. In the next hour, in spite of antidotes being applied, contact with the patient was broken and circulatory and cardiac failure caused death.

The highest occupational exposure is noted for methylphenols. It has been estimated that in world exposure to 4-methylphenol concerns some 600 to 1,200 thousands of workers. This mainly refers to workers who produce antioxidants, disinfectants, dyes, plastics, explosives, epoxy-resins, coal tar and steel [9]. Acute poison with methylphenols cause burning pain in mouth and throat, abdominal pain, headache, weak irregular pulse, hypotension, fall of body temperature, stentorous breathing, dark-colored urine, shock, paralysis of nervous system, coma and death. The incident of poison related with intentional administration of 140 ml of 50% of 4-methylphenol solution by a man has led to an increase of plasma aminotransferases activity and then degradation of hepatocytes. In spite of intensive detoxification, the sufferer died after 14 days [103]. It is considered that a lethal dose of 4-methylphenol for man is of 30-60 g [104]. Lethal doses for animals are different in regards to the type of chemical structure of methylphenols. For example, LD₅₀ for rats that were orally administrated of 2,4-dimethylphenol was estimated for 207 mg/kg of body weight [105]. Para-cresol is absorbed by skin, mucous membrane of digestive tract and respiratory system. It is excreted in urine and in a low concentration with bile and expired air [95]. 30 minutes after administration, 2,4-dimethylphenol is metabolized and excreted 94% conjugated with glucuronides and other conjugates.

Considerable toxicity exerts 4-aminophenol. This compound causes skin and eye irritation, eczemas, asthma and anoxia [95]. Aminophenol toxicity is related with generation of semiquinones and superoxide radicals that damage a cell’s biomolecules. P-aminophenol by formation quinonoimines damages cell membranes and in particular (in doses of 200 mg/kg of body weight) is characterized by nephrotoxic influence [106]. Lethal doses of p-aminophenol for a man are estimated at 50 to 500 mg/kg of body weight. LD₅₀ for rat after oral administration is much higher and is of 1580 mg/kg of body weight. The investigations revealed that butylhydroxytoluene
and buthylhydroxyanisole reveal histopathological activity. Those compounds cause damage of adrenal gland and increase brain and liver weight [81]. The results of clinical investigation also describe mass poison with chlorophenols. The example is pollution of water and fish in reservoir in Jarrela locality in south Finland with a mixture of 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol and pentachlorophenol derived from a wood processing plant. As the result of poison of about 2000 people – the consummates of water and fish increase morbidity on the side of digestive tract. Also, the increase of infections of respiratory system, strong exhaustion, headaches and depression were observed [107].

**Mutagenicity**

The investigations of hamster fibroblasts revealed mutagenic activity of phenol. This compound also inhibited synthesis and replication of DNA in HeLa cells [108]. Moreover, phenol stopped repair of DNA in diploid human fibroblasts. Hydroquinone (1,4-dihydroxyphenol) induced damages of chromosomes in human lymphocytes, increasing deletion ratio in 7. chromosome, which may lead to leukemia development [109]. In another experiment phenol, catechol and hydroquinone induced morphological changes in cells of hamster embryos. In another experiment catechol and hydroquinone inhibited ribonucleotide reductase activity (the enzyme that participates in DNA synthesis) and thus stopped activation and proliferation of T lymphocytes. Those compounds also inhibited the proliferation cycle of lymphocytes in G1 phase [110]. Catechol in the presence of NADPH and Cu²⁺ was able to modify guanine and thymine residues and induce gene mutations and chromosome aberrations. Catechol and hydroquinone damaged chromatides and induced incorrect DNA synthesis. The similar changes were provoked by pyrogallol, which induced the strongest (among hydroxybenzenes) chromosome aberrations. Pyrogallol and hydroquinone expressed their toxicity by forming a reactive oxygen species that included a hydroxy radical that caused deprotonation of the substrates and thus degraded deoxyribose [111]. It was also observed that semiquinone and quinone radicals are involved in damage of DNA structure by discussed xenobiotics. Chromosome aberrations and other structural changes within chromosomes were also induced by pentachlorophenol and proceeded even at low concentrations of PCP [112]. Damage of DNA was provoked by the formation of the PCP product – tetrachlorohydroquinone and also harmful intermediate form – tetrachlorosemiquinone radical (TCSQ) that degraded DNA and handicaped the mechanisms responsible for its repair [113].

Mutagenic influence was also exerted by nitrophenols and nitrated aminophenols. In the test with the use of *Salmonella typhimurium* mutagenic activity was observed for 2,3-dinitrophenol, 2,5-dinitrophenol, 3,4-dinitrophenol, 2,4,6-trinitrophenol and 2-nitro-5-aminophenol. In another experiment performed on *Salmonella typhimurium* and *Eschericha coli*, mutagenic activity was noted for bisphenol F. This compound induced the increase of frequency of sister chromatide exchange and decreased the number of micronucleus in human lymphocytes [114]. 4-aminophenol is capable of interacting with genetic material at the presence of Fe³⁺ and thus damages DNA contained in mouse and human lymphocytes. The process was related with action of free radicals that were formed in the reaction of iron ions and hydrogen peroxide [115]. Some BHA and BHT metabolites also reveal genotoxic capacity toward DNA. Tert-butylhydroquinone (TBHQ) is formed in cells from butylhydroxyanisole in oxidative demethylation reaction and reveals genotoxic, cytotoxic, clastogenic and mutagenic capacities. 2,5-di-tert-buthylhydroquinone (DTBHQ) is formed from 2,5-di-tert-buthylhydroxyanisole (DBTHA), the compound that contaminates commercial preparations of BHA. In performed experiment both DTBHQ and DTBHA unplaited DNA helix by cleavage of single and double hydrogen bonds. TBHQ revealed stronger activity – 92.5% of DNA structure was damaged. As free radical scavengers like glutathione were activated in this process, it was considered that DNA cleavage was induced by free radicals generated by describing metabolites [116]. BHT metabolism is related with hydroxylation of alkyl substituents, and also with oxidation of aromatic ring. In the experiment some butylhydroxyanisole metabolites like 2,6-diterthethyl-4-hydroxyl-4-methyl-2 cyclohexadienone (BHT-OH) and 2,6-diterthethyl-4-benzoquinone (BHT-quinone) caused damage to DNA in the presence of Cu²⁺ by cleavage of

![Fig. 9. Pentachlorophenol (PCP) oxidation yields tetrachlorosemiquinone (TCHQ) radical and tetrachlorohydroquinone (TCHQ) formation.](image-url)
hydrogen bounds. These compounds also induced characteristics of apoptosis endonucleosomal DNA fragmentation. The mechanism of action of both metabolites was different: BHT-OOH indirectly damaged genetic material and BHT-quinone interacted by the formation of hydrogen peroxide [117].

Carcinogenicity

Clinical data have shown that people exposed to chlorophenols influence fall ill with of tumours, sarcoma and lung cancer. According to literature data the mixture of chlorophenols or sodium salts of these compounds is probably carcinogenic for animals [35]. An admissible daily dose of individual chlorophenol that may be taken by a man that does not induce carcinogenic changes is 5µg/kg of body weight for 2-chlorophenol, and 3µg/kg of body weight for 2,4-dichlorophenol, 2,4,6-trichlorophenol and pentachlorophenol [118]. Catechol also reveals carcinogenic activity. The U.S. Environmental Protection Agency classified this compound as a carcinogen and the World Health Organization classified catechol in 2B group as a compound of possible carcinogenicity [35]. Para-cresol was classified as probable carcinogenic for human [119] and 2,4-dimethylphenol was considered as the compound responsible for carcinogenic influence [120]. Chronic exposure of skin rats to 2,4-dimethylphenol caused the formation of skin tumours (31% towards control). In the experiment an additional application of 3% dimethyl-benzanthracene caused the formation of skin tumours (50% towards control) and 18% of skin cancer. These changes were induced by o-quinones, in particular quinones methide that revealed high toxicity and additionally generated reactive oxygen species [121]. Occupational exposure of workers to phenoxyherbicides is related to an increase of death incidents. The observed increase of mortality was linked to morbidity on cancer of respiratory system, lymphoma and myocardial ischaema [122]. The positive correlation was also noted between non-Hodgekins lymphoma appearance among children and documented frequency of using pesticides and their effect on the organism of birth child [123]. The investigations of 10,000 workers employed in vinyl chloride production factories revealed that they suffered from liver and lung cancer [124]. Chlorophenols are the main by-products that are formed during vinyl chloride production. The exposure of people to chlorophenol influence appears also in factories that produce chloroorganic pesticides, mainly phenolic biocides. The main compound that is formed in this process is pentachlorophenol that was classified by the U.S. EPA as a probable carcinogen. The workers that are employed in pesticides production suffer from non-Hodgekins lymphoma and sarcoma [125]. Carcinogenic properties are also characteristic for 4-methylcatechol and 4-methoxyphenol that are responsible for skin cancer and epithelium cancer development. In an experiment catechol, 4-methoxyphenol and butylhydroxyanisole individually and particularly in mixture induced papillomas in stomach of rats [126]. Carcinogenic activities of catechol were also confirmed in investigations of mice, the compound given in a dose of 85 µg/kg of body weight in a few weeks caused skin cancer development. In other investigations 4-nonylphenol in concentrations of 25 and 250 ppm given in food to rats by 28 weeks provoked proliferation of cancer cells in lungs. In the experiment 8-hydroxy-2’-deoksysguanosine as a marker of DNA damage was determined [127].

Fig. 10. Pentachlorophenol transformation by hepatocytes of rat and mouse.
The cancer development in people exposed to phenols is related with microsomal activation of cytochrome P450. The oxidation reactions lead to conversion of some xenobiotics to electrophilic forms that actively interact with a cell’s structures. For example, pentachlorophenol activation leads to the formation of tetrachloro-1,4-benzoquinone and tetrachloro-1,2-benzoquinone by intermediate steps with formation of respective semiquinone radicals. Formation of the above-mentioned compounds is also related to liver cancer development in mice. The essential is that cancer development is also correlated with the level (strength) of microsomal activation of cytochrome P450 of hepatocytes. Much lower activation of this cytochrome by PCP in rats does not lead to cancer development in spite of the identical pentachlorophenol metabolism in this species [128].

Other Toxic Influence of Phenols

4-octylphenol and 4-nonylphenol induce immunotoxicity by inhibition of lymphocytes proliferation. The second compound revealed stronger toxic activity and induced this process even in a concentration of 1 µM/kg of body weight [129]. Administration of 4-nonylphenol to rats in doses of 125-375 mg/kg of body weight caused changes in the activity of the immunological system. The mechanism of action was related to modulation of genes expression that are responsible for mRNA synthesis in tymocytes. Decrease of mRNA synthesis led to apoptosis and finally inhibited thymocyte proliferation [130].

Phenols also affect the function of the hormonal system. Some phenols are capable of disturbing sexual hormones function, which finally may lead to sterility of animals and humans. The examples are alkylphenols, bisphenol A, 2,4-dichlorophenol and pentachlorophenol [131, 132]. Those compounds express their activity by binding with ER receptors. There are some places within a receptor that may bind not only 17-β-hydroxy groups of hormones but also hydroxyl residues of phenols as well. Moreover, it is considered that core of alkylphenols imitates a ring A in E2 estrogens and thus reveal estrogenic activity [133]. In another experiment bisphenol A caused protein expressions in TM4 cells in mice, which play a key role in spermatogenesis. It was noted that viability of cells decreased 10 to 70% after exposure to doses of 50-250 µM/kg of body weight over 16 hours. Obtained results showed that bisphenol A may induce infertility in mice.

Phenols also modulate the activity of ion channels in the nervous system. It was noted that simple phenols and in particular trichlorophenols, trijodophenols and butylphenol may block ion channels in a micromolar concentrations range. The conclusion of investigation was that phenol and hydrophobic residues – alkyl chains or additional phenyl rings substituted in third, fourth and fifth positions are responsible for the above-described kind of toxic activity [134].

Some phenols like phenol and p-cresole may be formed from non-toxic compounds like tyrosine in digestive tract of mammals, including humans. P-cresol is also a marker of organism exposure to toluene. This compound in the presence of hydrogen peroxide caused DNA adducts formation in HL-60 cells. Researchers revealed that DNA damages were induced by a metabolite of 4-methylphenol – quinone methide of p-cresol (PCQM) that also may be used as biomarker of organism exposure to toluene influence [135]. Damages caused by aminophenols are related to fast oxidation of these compounds in physiological conditions to benzosemiquinonimines that
are finally transformed to p-benzoquinonimines. The second metabolite generates a superoxide radical that in a dismutation reaction forms hydrogen peroxide converted in the presence of Fe$^{3+}$ to a highly reactive oxygen form – hydroxyl radical. In an experiment damage of epithelium cells of colon was induced by catechol and p-aminophenol. As the authors suggest, the above process may lead to chronic inflammation of large intestine [136].

The investigations led by Bukowska, Duchnowicz and co-workers have revealed numerous toxic effects caused by phenols on human erythrocytes. The authors observed lipid peroxidation in erythrocytes incubated with 2,4-dichlorophenol, 2,4,5-trichlorophenol, 2,4-dimethylphenol, and 3-(dimethylamino)-phenol [137-140]. Chlorophenols and catechol decreased human membrane erythrocytes acetylcholinesterase activity [141]. Chlorophenol and dimethylphenol changed ATPase activity and membrane fluidity and also damaged membrane proteins [137, 141]. All investigated phenols oxidized haemoglobin, and the highest activity was revealed by 3-(dimethylamino)-phenol, catechol and 2,4-dimethylphenol (2,4-DMP) [137, 142]. 2,4-dichlorophenol (2,4-DCP), 2,4,5-trichlorophenol (2,4,5-TCP) and catechol decreased the activity of catalase [143]. Moreover, catechol decreased superoxide dismutase activity [144]. In the presence of 2,4-DMP and 2,4,5-TCP, a decrease in the amount of ATP that coincided with a simultaneous increase in ADP and AMP content was observed, which in the consequence caused a decrease of the energy charge of erythrocytes [145]. The changes in the above parameters provoked haemolysis of the cells. The level of haemolysis was the highest in the presence of catechol and the lowest in the presence of phenol. In the light of obtained results the most toxic compounds towards erythrocytes were 3-(dimethylamino)-phenol and catechol.

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