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THE PRESENCE AND TOXICITY OF PHENOL DERIVATIVES — THEIR EFFECT ON HUMAN ERYTHROCYTES

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The occurrence of phenol and its derivatives in the environment is mainly caused by human activity. However, some phenol derivatives are also produced in natural processes. These compounds accumulate in living organisms disturbing their proper function. Phenols are considered to be very harmful ecotoxins. They possess carcinogenic, cytotoxic and teratogenic properities. Phenol and its derivatives change enzyme activity and the cell metabolism. In particular, they inhibit oxidative phosphorylation process (pentachlorophenol), stimulate glycolysis (dinitrophenols; Nikonorow, 1979), influence activity of antioxidant enzymes — glutathione peroxidase, superoxide dysmutase, catalase (hydrochinon; Nimmagudda & Snyder, 1995; chlorophenols; Bukowska, Chajdys, Duda & Duchnowicz, 2000), change the cell morphology (trichlorophenol; Bukowska, 2004b), oxidize haemoglobin (Bukowska, Reszka & Duda, 1998) and provoke haemolysis of the cell (Duchnowicz, Koter & Duda, 2002). The article is a brief review on the toxicity of phenols to living organisms, particularly to human erythrocytes.

INTRODUCTION

Development of technology together with increasing demand for food products have brought about introduction of all kinds of new food products, new pharmaceuticals, chemicals and pesticides. These products contain phenol compounds and their use leads to increasing pollution of the natural environment. The phenol compounds of anthropogenic origin are harmful for many organisms, in particular for man. Phenol and its derivatives are contained in preservatives of food products, e.g. BHA, BHT, etc., pesticides e.g. 2,4-dichlorophenoxyacetate acid (2,4-D), 2,4,5-trichlorophenoxyacetate acid (2,4,5-T) or pentachlorophenol (PCP). Phenols are used in industry for production of resins, plastics, plasticine, disinfectants, paints, antioxidants, perfumes (Chapman, 1972). Hence, phenol compounds are met in everyday life. It is known that they are not neutral for living organisms and can lead to significant changes in the cell metabolism so affect the whole organisms.

THE PRESENCE OF PHENOLS IN THE NATURAL ENVIRONMENT AND IN LIVING ORGANISMS

Phenol and its derivatives are commonly met in the water environment, on the land and in the air (in the biosphere). Reports by Michałowicz (1999) and Michałowicz and Duda (2002) have confirmed the presence of these compounds in drinking water, in the water of lakes, rivers and in fish tissue (Table 1).

Phenols are harmful for living organisms, in particular those from water environment (Genoni, 1997). According to the World Health Organisation recommendations the concentration of phenol in tap water should not exceed 1-2 µg/l (WHO, 1986). The determinations in tap water in Łódź, Warsaw, Poznań, Wrocław have shown that the values close to the limit of tolerance are admissible (Table 2) (Michałowicz & Duda, 2002). From among the most toxic compounds occurring in tap

Table 1. The content of phenol derivatives in the liver of *Salmo gairdneri*, from the culture in Mylof, in the Tucholski Landscape Park), determined in the summer 1997 (Michałowicz, 1999).

Liver tissue	Summer 1.08.1997 Number of phenol fractions	Phenol concentration mg/kg
1	19	2,227
2	19	1,830
3	20	2,730
4	17	2,651
5	18	3,181
6	21	2,371
7	22	2,284
8	22	2,802
Mean	20	2,509

Compound	concentration in (µg/l)			
	Łódź	Warszawa	Poznań	Wrocław
4-chlorophenol	-	-	0,011	-
2,4-dichlorophenol	-	0,077	-	-
2,4,6-trichlorophenol	-	0,075	-	-
2,4,5-trichlorophenol	-	**0,330**	-	-
Tetrachlorophenol	-	-	0,047	**0,221**
4-chlorocatechol	0,030	-	0,050	-
4,6-dichloroguaiacol	-	0,036	-	0,132
tetrachloroguaiacol,	0,244	-	0,109	0,136
3-chlorosyringol	0,022	0,015	0,024	0,028
trichlorosyringol	0,083	0,019	0,029	0,044
5,6-dichlorovanillin	0,341	-	-	-

Table 2. The concentrations of chlorophenols, chlorocatechol and dichlorovaniline determined in tap water in Łódź, Warsaw, Poznań, Wrocław in summer 2000. The values are means for four repetitions n = 4.

Table 3. The content of 2,4,5-trichlorophenol (2,4,5-TCP) and pentachlorophenol (PCP) in wood boxes (Diserens, 2001).

Box no.	2,4,5-TCP (mg/kg)	Pentachloro phenol (mg/kg)
1	0,19	0,05
2	5,00	0,21
3	0,05	11,0
4	0,22	1,20
5	0,17	1,10
6	0,19	0,23
7	0,45	0,11
8	0,24	0,19
9	31,0	8,00

water the concentration of 2,4,5-trichlorophenol was determined in Warsaw in the summer of 2000 to be 0.330 µg/l. The concentration of strongly toxic tetrachlorophenol in tap water samples from Wrocław, (summer 2000) was 0.221 µg/l. These values are higher than the admissible standards recommended for drinking water.

The presence of chlorophenols in river water has been reported by (Gonzalez-Toledo, Prat, Alpendurada, 2001) and in bottom sediments by (Czapicka, 2001). Chlorinated derivatives of phenols are present in wood tissue and can migrate to the food stored in wood casing (Table 3). Diserens (2001) reported the presence of 2,4,5-TCP in the wood of boxes used for fruit storage.

The same author (Diserens, 2001) has reported the content of chlorophenols in fruit and their products (Table 4).

Phenol and its derivatives are widespread in chemical industry based on processing of resins into plastics, disinfectants, pesticides, paints, antioxidants and perfumes (Chapman, 1972). Phenolic derivatives are formed in the processes of degradation of some pesticides, e.g. degradation of pentachlorophenol leads to chlorocatechols, while that of 2,4-D, with involvement of micro-organisms and plants leads to formation of phenol (Różański, 1998; (Bukowska, Reszka, Michałowicz & Duda, 1998). Phenoxyacetate herbicides are degraded under the effect of physical, chemical or biological factors into metabolites, mainly chloro- and methylphenols. They can also be formed as a result of degradation of phenoxyl herbicides by photoreaction initiated by UV from sunlight.

Chlorophenols are biosynthesised by numerous higher fungi species from the order of *Basidiomycetes* (Swarts, Verhagen, Field & Wijnberg, 1998; Verhagen, Swarts, Field & Wijnberg, 1998). Methylphenols are components of coal tar and natural oils. Of particular harmful effect for people and the natural environment are the phenol compounds present in cigarette smoke and in the smoked food products (Kjällstrand & Peterson, 2001). Some phenol compounds and their esters are isolated from the plant essential oils. The phenol compounds of natural origin (present in low concentrations) do not pose threat to living organisms. The occurrence of phenol compounds in the environment is related to the living activity of many organisms (Kad, Singh, Khurana & Singh, 1998).

THE PRESENCE OF PHENOLS IN HUMAN AND ANIMAL ORGANISMS

In the natural conditions in the human and animal organisms phenol is synthesised from tyrosine and its derivatives. It is present in the thin and large intestines in faeces and in decomposing organic matter (Tsaruta, Watanabe & Inoue, 1996).

Transformations in living organisms

Phenol is able to get into the human organism through the alimentary system, respiratory system or through the skin (Geinoz, Rey, Boss, Bunge, Guy, Carrupt, Reist & Testa, 2002). Phenol de-

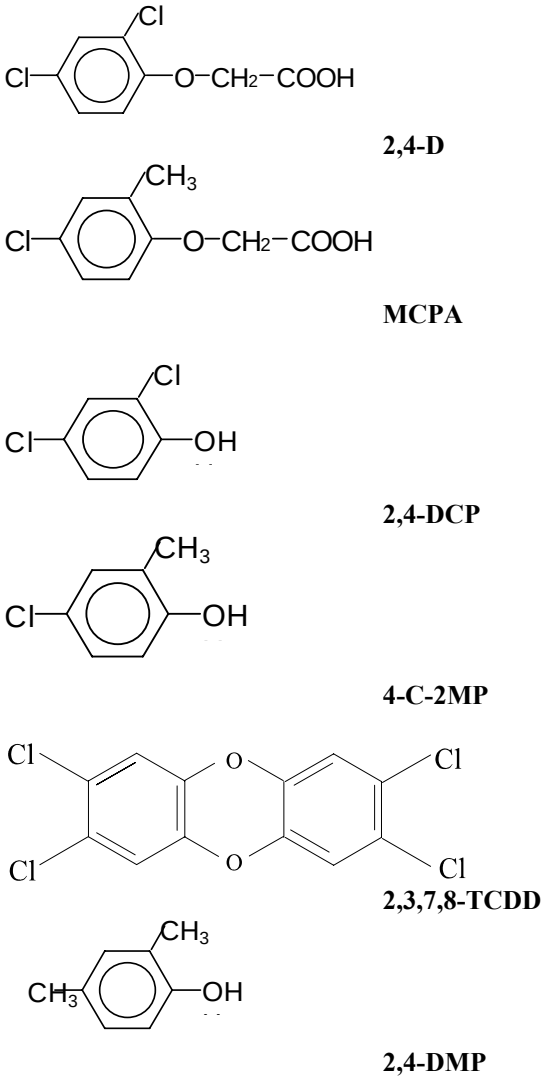


Fig. 1. The chemical formula of herbicides: 2,4-dichlorophenoxyacetate acid (2,4-D), 4-chloro-2-methylophenoxyacetate acid (MCPA) and their metabolites: 2,4-dichlorophenol (2,4-DCP), 4-chloro-2-methylophenol (4-C-2-MP), 2,3,7,8-tetrachloro-dibenzo-p-dioxin (2,3,7,8-TCDD) and 2,4-dimethylphenol (2,4-DMP)

rivatives undergo transformations in the human organism, some are accumulated in fat tissue (which is particularly dangerous and can lead to secondary poisoning on intense dieting), some are excreted as phenol (3%), phenol glucuronide (81%) and phenol sulphate (16%) and some are transformed into catechol and hydrochinone. The phenol metabolites, catechol and phenyl phosphate are eliminated through the urinary system (Mickim, Kolanczyk, Lien & Hoffman, 1999). After intravenous administration of the metabolite (2,4-DCP) in a dose of 10 mg/kg body weight, its concentrations in different tissues of rats have been determined. It has been found to accumulate mainly in the kidneys, liver, fat tissue and the brain (Somani & Khalique, 1982). At each subsequent stage of the alimentary cycle the concentration of the toxic substance increases, moreover, the fact that a given xenobiotic/pesticide has decomposed does not mean that it has become harmless, because sometimes the metabolites are more toxic than the parent substance (Bukowska *et al.*, 2000). Phenol derivatives, and in particular chlorophenols are sources of particularly toxic metabolites as e.g. dibenzo-p-dioxins. In the environment, dioxins occur in concentrations as low as 10^{-3}µg/kg and are highly toxic (Bukowska, Duda, Stefan & Michałowicz, 1998; Bukowska, 2004a).

Table 4. The content of chloro- derivatives of phenol in fruit and their products (Diserens, 2001).

Kind of fruit	Chlorophenols (w µg/kg)
Plums	18 - 27,7
apple compote	10 – 17
fruit juice	20 – 130
Infant food	1 – 27
apples	1 – 8

Cytochrome P-450. Phenols and mainly phenol are metabolised in the liver, lungs and mucous membrane of the alimentary track. Phenol is metabolised in the presence of a system of oxidases within cytochrome P-450. Of main importance in phenol metabolism is the cytochrome P450 isoenzyme CYP2E1, which metabolises about 50% of phenol concentrations present in cells (Lovern, Maris & Schlosser, 1999). Another isoenzyme CYP2B1 is involved in phenol hydroxylation leading to formation of catechol (Gut, Nedelcheva, Soucek, Stopka & Gelboin, 1996). The enzyme involved in the hydroxylation is o-diphenol oxidase (o-diphenol oxydoreductase: oxygen E.C. 1.10.3.1) oxidising o-diphenol to o-quinone by the use of O_2 with formation of water and transformation of the methylated derivatives to appropriate catechols. This oxidase acts on different o-quinone**,** and oxidizes monophenols introduced into the system. Oxygenases catalyse the oxidative cleavage of aromatic compounds, including phenols and catechols, transforming them into compounds non-toxic for the natural environment (Powlowski, Sealy & Cadieux, 1996). Cytochrome P-450 is responsible for metabolism of other phenols by the route of dehalogenation (Rietjens & Vervoort, 1992).

MAN'S EXPOSURE TO PHENOL DERIVATIVES

The most exposed to negative effect of phenols activity are the workers employed at production of phenols, their processing, packing and trade, and the persons spraying plants with pesticides. In 1992 one person was reported to have died because of the contact with 2,4-DCP in working premises. The compound penetrated through the skin and its concentration in the blood was 24.3 mg/dm^3 (Kintz, Tracqui & Mangin, 1992). The urine samples from a 1000 workers exposed to phenoxyacetate pesticides contained 2,4-DCP in 64% samples, 2,4,5-TCP in 20% samples and 2,4-D in 12% samples. These results prove that the compounds can easily penetrate inside the human organisms (Hill, Head, Baker, Gregg, Shelly, Bailey, Williams, Sampson & Needham, 1995). Other professional groups exposed to phenol are the cleaning personnel of hospitals using lisole for disinfection, workers assembling fine electronic computer parts (Noren, Weistrand & Karpe, 1999), workers involved in the production of epoxy-resin, in due-houses, in pharmaceutical industry, chemical industry, food processing, inhabitants highly industrialised areas and regions of intense farming (Michałowicz, 1999).

General society has contact with small doses of phenols mainly dissolved in drinking water or contained in food products.

TOXICITY

Phenols can cause damage to the cells of the living organisms. It has been shown that a long-time intake of phenols by experimental animals leads to changes in the skin, lungs, liver, mucous membranes, oesophagus and in the kidneys (Bruce, Santodonato & Neal, 1987). As a result of phenol penetration through the man's skin, its darkening and weakening of the muscles are observed. Lethal doses cause structural and functional changes in the brain, necrosis of the liver, and emphysema (Roy, Bernatchez & Sauver, 1998). Poisoning caused by phenol compounds provoke such symptoms as headaches, dryness of the throat, dyspnoea, nausea, vomiting, diarrhoea, (Juhl, Kim & Benitez, 2003). According to other reports phenols have cytotoxic effect on skeletal muscle and neurotoxic effect on piramidal neurones (Nikonorow, 1979). Phenol and its derivatives also show mutagenic effect by unbinding of the DNA helix, inhibition of DNA synthesis in the human Hel cells, induction of gene mutations, chromosome aberrations, and aneuploid formations (phenol, catechol) (Tsutsui, Mayashi, Maizumi, Huff & Barret, 1997). It has been established that phenol, catechol and 2-chlorophenol can induce conformational changes in the human growth hormone (Maa & Hsu, 1996). The presence of phenols has been related to such neoplasmic changes as sarcoma, lymphoma, lung cancer (chlorophenols) (Hooiveld, Heederick, Kogevinas, Boffetta, Needham, Patterson, Bas Bueno-de-Mesquita & Bas Bueno-de-Mesquita, 1998), skin cancer, (phenol), leukaemia (phenol) (Selassie, DeSoya, Rosario & Gao, 1998). A connection has been also established between the presence of chlorophenols and development of the non-Hodking lymphatic system neoplasmic changes (Buckley, Meadors, Kadin, LeBeau, Siegel & Robison, 2000). The exposure to 2,4-dichlorophenol has been reported to be related to the chromosome aberrations in the ovarian cells of Chinese hamster (Hillard, Armstrong, Bradt, Hill, Greenwald & Galloway, 1998). Phenol derivatives have been reported to cause an increase in the level of lipids peroxidation in the human blood erythrocytes — 2,4-DCP, 2,4,5-TCP, 2,4-DMP (Duchnowicz *et al.*, 2002), 3-DMAP (Bukowska & Duda, 2000) and guinea pig kidneys (chlorophenol) (Clerhata, Kovacikova, Veningerova, Lukacsova & Ginter, 1998).

The effect of phenols on human blood erythrocytes

The human blood plays a very important role in the organism, first of all because it distributes oxygen and nutrients, but also toxic compounds. They can thus reach different organs and damage them, however, first of all the damage is done to the transporting cells, that is the blood cells and their components. Therefore, recognition of the mechanisms of the activity of xenobiotics, pesticides on the cellular level is of fundamental importance for protection against chemical attack.

Haemolysis

Phenols reveal hematotoxic behaviour, for instance they induce the process of haemolysis, e.g. catechols in 70% to 100% (Boge & Roche, 1996) and dinitrophenols up to 95%. They damage thiol groups (-SH) in cysteine, decrease the level of adenosinotriphosphate (ATP) (Nikonorow, 1979). On incubation with erythrocytes phenols are responsible for altering their shape as a result of interaction with the membrane. The human erythrocyte („discocyte") has a characteristic biconcave disc shape that gives it a higher surface to volume ratio than a sphere, creates better conditions for gas exchanges and increases the cell's deformability. One-hour incubation of erythrocytes with

100 ppm 2,4,5-TCP changed the shapes of most of the cells: echinocytes were observed. Therefore, the morphological changes in erythrocytes incubated with 2,4,5-TCP might have resulted from either the decrease of ATP level or the inclusion of compounds into the outer membrane monolayer (Bukowska, 2004b).

Studies on the degree of haemolysis of the erythrocytes have shown that the phenolic products of decomposition of MCPA, 2,4-D and 2,4,5-T such as 2,4-DMP, 2,4-DCP, 2,4,5-TCP dissolved in PBS provoked 22-25% haemolysis, while in the presence of the herbicides, haemolysis was within a few percent (Duchnowicz *et al.*, 2002). Phenols are oxidised to free radicals inside the erythrocytes and they induce haemolysis. As a result of haemolysis the haemoglobin is released, leading to such toxic effects as narrowing of the blood vessels, formation of Hb dimers, formation of Hb cross-bonds, induction of phagocytose, increased risk of bacterial infection (Everse & Hsia, 1997). The iron released from the haemoglobin permits the Haber-Weiss reaction in which free radicals are formed.

Oxidation of oxyhaemoglobin to methaemoglobin

It has been shown that incubation of erythrocytes with some xenobiotics, phenol included, induces formation of met-Hb (Eyer, Hertle, Kiese & Klein, 1975; Riley, 1984), so that leads to oxidation of oxy-Hb and a decrease in the level of thiol groups. In the physiological conditions a small percent of Hb (about 0.5%) undergoes oxidation giving met-Hb and superoxide anion (Jaffe, 1964). However, the presence of antioxidants: glutathione, SOD, catalase nullifies the effects of the presence of free radicals, responsible for the most cell damages. Reductase met-Hb transforms the excessive met-Hb(Fe^{3+}) into Hb(Fe^{2+}) (Hirota, Itano & Vedvick, 1978). As a result of the activity of a large amount of xenobiotics, reductase is not able to transform all met-Hb into Hb(Fe^{2+}). Met-Hb is not capable of active transportation and distribution of oxygen to tissues. Eyer *et al.*, (1975) and Riley (1984) reported that reduced xenobiotics such as phenolic compounds are able to oxidize oxyhaemoglobin to met-Hb in a so-called cooxidation reaction in which the heme oxygen serves as the active oxidant that oxidizes both the ferrous heme centre of haemoglobin and the reducing xenobiotic (R-H):

$$Hb^{2+}O_2 + R\text{-}OH \rightarrow [met\text{-}Hb^{3+}\text{-}O_2] + OH^- + R$$

The unstable $MetHb^{3+}$-OO^{2-} complex immediately stabilises the secondary products that are dependent on the nature of the respective xenobiotic. For phenolic compounds, transient formation of compound 1 of the type of ferryl hemoglobin has been postulated (Stolze, Dadak, Liu & Nohl, 1996);

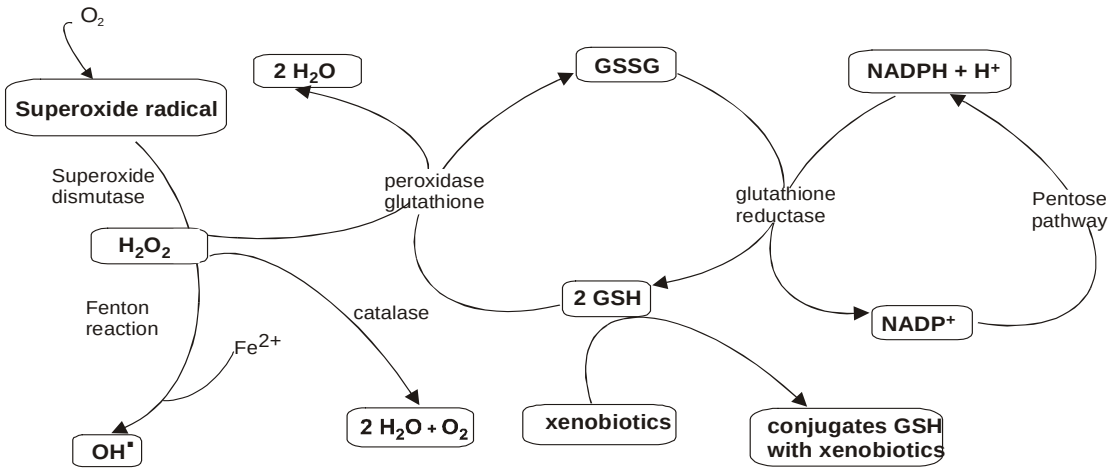
$$[TyrH\ Met\text{-}Hb^{3+}\text{-}O\text{-}O^{2-}]+H^+ \rightarrow [Tyr^{\bullet}\ Hb^{4+}{=}O^{2-}]+H_2O$$

1-feryl-Hb has Fe^{4+} and is a free radical on the α-42 tyrosine residue (MacArthur & Davies, 1993). The destructive effect of dichlorophenols and dimethylphenols on the structure of oxyhaemoglobin of the erythrocytes of the ox, carp and human blood has been experimentally evidenced (Bukowskai, Reszka & Duda, 1998). By far the greatest toxicity towards haemoglobin has shown 2,4-DMP, leading to its fast an intense oxidation. Strong oxidation properties of 3-dimethylaminophenol have been also indicated (Bukowska & Duda, 2000). It has been shown that the presence of phenol and catechol lead to an increase in the met-Hb concentration (Bukowska & Kowalska, 2004). A much stronger oxidant is catechol, whose presence in the concentration of 1000 ppm resulted in the appearance of a high concentration of met-Hb 55% already after one hour of incubation.

The antioxidant system

The oxidation stress is a disturbance of equilibrium between the rate of producing the reactive oxygen species (RFT) and the concentration of low-molecular antioxidants and the activity of protective enzymes leading to a increase in the concentration of reactive oxygen species. Increasing exposure of the cells to reactive oxygen species brings about many consequences, among others, a decrease in the level of ATP caused by inhibition of glycolysis or damage to mitochondria.

Under the effect of the reactive oxygen species, the ratio of the concentrations GSH/GSSG and the total concentration of glutathione, being a low-molecular antioxidant, can decrease. The oxidative stress also affects the plasmic membrane by increasing its permeability and by its depolarisation. The exposure of cells to the reactive oxygen species can lead to a damage of DNA, and eventually death of the cell (Bartosz, 1995). The majority of organisms have been equipped with the mechanisms. These mechanisms in mammals and hence in man, make use of antioxidation enzymes and low-molecular antioxidants. The first line of defence makes the antioxidation enzymes, mainly glutathione peroxidase (GSH-Px), glutathione reductase (GR), superoxide dismutase (SOD) and cata-



Scheme 1. Enzymatic and non-enzymatic antioxidants in human erythrocytes.

lase (CAT) (Scheme 1). The presence of phenol derivatives, that is chloro- and methylphenols, change the activity of the antioxidation enzymes. According to Bukowska *et al.*, (2000) the presence of 2,4-DCP and 2,4,5-TCP decreases the activity of catalase but increases the activity of glutathione peroxidase (Bukowska, 2003) and significantly decreases the level of reduced glutathione.

2,4-DMP and 2,4,5-TCP affect the energy relations in the erythrocyte cell. In the presence of 2,4-DMP and 2,4,5-TCP a decrease in the amount of ATP, accompanied by a simultaneous increase in ADP and AMP in the erythrocytes has been observed, which consequently causes a decrease in the energy (ACE) of the erythrocytes (Bukowska, Goszczyńska & Duda, 2003; Bukowska, 2004b). Nimmagudda & Snyder (1995) and Ovaski & Yliniemela (1998) confirmed the negative effect of hydroquinone, benzoquinone, nitrophenol and catechol on the activity of antioxidant enzymes. Boge and Roche (1996) reported that the presence of pirocatechol decreases the activity of manganese superoxide dismutase (MnSOD) and gultathione peroxidase simultaneously increasing the activity of catalase. The activity of SOD has been reported to decrease significantly in the presence of chlorophenols and catechols (Bukowska, 2003; Bukowska & Kowalska 2004). 2,4-Dimethylphenol and phenol have not shown a negative effect on SOD. Nimmagudda and Snyder, (1995) have reported changes in the activity of antioxidant enzymes glutathione peroxidase, superoxide dismutase and catalase in the HL-60 cells incubated with hydrochinone. A particularly strong negative effect has been assigned to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Already at very low concentrations (0.2-1.6 ppm) it reduces the activity of catalase and glutathione peroxidase and strongly enhances the process of lipid peroxidation in the human erythrocytes *in vitro* (Bukowska, 2004a).

Comparative analysis of the phenol derivatives toxicity

Selassie (1998) suggests that toxic properties of phenols are related to the two following processes: one being the formation of free radicals from them, and the second being a direct attack of these phenoxylradicals on biochemical processes in a number of sensitive pathways of metabolism. The chlorine substituents in phenols increase the toxicity of a given derivative towards the erythrocyte membrane (haemolysis and peroxidation) and decrease the enzymes activity, while the methyl substituted derivatives cause mainly oxidation of haemoglobin (Duchnowicz *et al.*, 2002). The experiment of the effect of mono-, di- and trichlorophenol on the bacteria *Burkholderia sp*., *Rasc c2* and *Pseudomonas fluorescens* has proved the greatest toxic influence of trichlorofenol (Boyd, Killham & Meharg, 2001). The results obtained by Boyd *et al.*, (2001) and Bukowska *et al.* (2000) suggest that an increase in the number of sites substituted with chlorine withing the phenol ring causes an increase in the compound toxicity. However, it should be noted that in some systems (lipid peroxidation and haemolysis of erythrocytes) 2,4-DCP is more toxic than trichlorophenols (Duchnowicz *et al.*, 2002).

The toxicity of phenols depends on the type and positions of the substituents in the aromatic ring. The additional (the second) OH group in phenol in fourth position - hydrochinon – (Bartosz, 1995); and in second position - catechol (Bukowska & Kowalska, 2004) enhances the radical activity of the compound leading to toxic effects from the dose of 10 ppm. Catechol incubated with erythrocytes for one hour has been reported to decrease

the activity of S-glutathione transferase (starting dose from 500 ppm), glutathione reductase, glucose-6-phosphate dehydrogenase (starting dose from 1000 ppm) and decrease of the level of GSH (starting from 10 ppm) and total glutathione (starting from 50 ppm), a decrease in the activity of catalase (starting from 100 ppm) and SOD (starting from 250 ppm) with a simultaneous increase of the level of met-Hb and a significant haemolysis.

Lipid peroxidation was observed in erythrocytes incubated with 2,4-dichlorophenol, 2,4-dimethylphenol, 3-(dimethylamino-)phenol. Lipid peroxidation in erythrocytes incubated with phenol and catechol was not observed. The decreasing effect of phenolic compounds towards lipid peroxidation is: 3-(dimethylamino-)phenol > 2,4-dichlorophenol > 2,4-dimethylphenol (Bukowska 2003; Bukowska & Kowalska, 2004; Bukowska & Duda, 2000).

All phenolic compounds oxidized hemoglobin, the highest activity was provoked by 3-(dimethylamino-)phenol (presence of amino residues within this compound) and catechol (the compound able to generate semiquinone radicals) and the lowest by phenol and 2,4-dichlorophenol. According to the intensity of hemoglobin oxidation by phenolic derivatives the compounds are ordered as follows: 3-(dimethylamino-) phenol> catechol > 2,4-dimethylophenol > 2,4-dichlorophenol > phenol (Bukowska, Reszka & Duda, 1998; Duchnowicz *et al.*, 2002; Bukowska, 2004).

The changes in the above parameters (lipid peroxidation; the level of met-Hb) have resulted in the haemolysis of the cell. The process of haemolysis was the strongest in the presence of catechol and 3-(dimethylamino-)phenol and the weakest in the presence of phenol. From among the phenol derivatives studied, the most toxic towards human blood erythrocytes proved 3-(dimethylamino-)phenol and catechol, while the least toxic was phenol.

Mechanism of the toxic effect of phenols

The toxic effect of phenols and their derivatives is directly related to the formation of free radicals, known as semiquinone, in the reaction of oxidation and reduction. The fundamental precursors of semiquinones are quinones, catechols and catecholamines (Głębska & Gwoździński, 1998). It is known that in physiological conditions semiquinone radicals quickly react with oxygen generating superoxide anion radicals being precursors of other toxic species of oxygen (Lown, 1985). Phenols, undergoing biotransformation to catechols, can be precursors of semiquinone (Zhang, Robertson, Kolachana, Davison & Smith, 1993, Hiraku & Kawanishi, 1996). Some aromatic xenobiotics, e.g. benzene, 4-bromobenzene, acetaminophenon undergo hydroxylation processes catalyzed by cytochrome P450 which result in catechol formation (Gut, Nedelcheva, Soucek, Stopka & Tichavska, 1996). Semiquinones are able to bind to nucleophilic residues like -SH or -NH_2 of proteins and nucleic acids, respectively (Takahashi, Schreiber, Fischer & Mason, 1987). As a result of binding, macromolecules may undergo inactivation (Segura-Aguilar, Baez, Widersten, Welch & Mannervik, 1997). The toxicity of catechol is manifested first of all in formation of semiquinones and their effect on the spatial structure of proteins, while no changes in lipids have been reported.

The toxic effect of such compounds as 3-(dimethylamino-)phenol or 2,4-dimethyphenol first of all involves oxidation of haemoglobin but they also enhance the process of lipid peroxidation leading to the cell death. Therefore, 3-(dimethylamino-)phenol and 2,4-dimetylophenol damage proteins as well as lipids.

Chlorophenols do not show strong oxidizing potential towards iron ion in haemoglobin, but they can participate in free radicals generation and indirectly lead to the cell death.

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