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Original Research

Concentration of Porphyrins in Rat Liver and Kidneys after Repeated Administration of Hexabromobenzene and Tetrabromobisphenol-A

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Abstract

The aim of this study was to complete research on the porphyrogenic effect of hexabrombenzene (HBB) and tetrabromobisphenol-A (TBBP-A).

Experiments were performed on female Wistar rats. The examined compounds were administered in sunflower oil intragastrically in 3 doses. The determination of porphyrins in tissues was carried out by means of high-performance liquid chromatography.

Repeated HBB administration caused a 3-8 times increase of total concentration of high carboxylated porphyrins (octa- and heptacarboxyporphyrins). The concentrations of these porphyrins were significantly lower in the liver of rats exposed to TBBP-A. However, in the kidneys significant alterations in porphyrins concentrations concerned only HBB.

Keywords: hexabrombenzene, tetrabromobisphenol-A, porphyrins, liver, kidneys

Introduction

A number of chemical substances affecting liver cause disturbances in heme synthesis. This activity, defined as porphyrogenic, is characterized by (among other elements) excessive production and cumulation of heme precursors in liver, *e.g.* porphyrins. Heme is a prosthetic group for hemoproteins being the centre of all oxygen metabolism which takes place in hepatocytes. Heme bound in hemoproteins participates in intracellular transport of electrons (cytochromes class b and c), in activation of molecular oxygen (cytochromes P-450) as well as in the activation of hydrogen peroxide (peroxidase) and its degradation (catalase) [1]. The most excessive amounts of heme are synthetized in the erythropoietic system, whereas about 20% of total systemic heme is formed in liver.

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The investigations on the toxicity of bromine compounds applied as flame-retardants have demonstrated that these are not entirely safe compounds for humans or the environment. Epidemic of domestic animal intoxication in the USA in the 1970s with polybromobisphenyls (DL_{50}> 20 000 mg/kg b.w.) is tangible evidence of the risk of application of these compounds. Intoxication of animal food being the consequence of accidental intoxication of fodder led to the epidemic of poisoning. In subjects consuming the intoxicated food, an increase of some enzymes (ALAT, AspAT, AP and microsomal enzymes) was observed, as well as dermatosis, neurological disturbances and heme synthesis disorders (coproporphyrinuria or chronic hepatic porphyria type A). Since then, toxicologists have been interested in this group of compounds [2].

The wide use of flame-retardants has for many years led to environmental contamination. Recent studies have demonstrated significant environmental pollution

by flame-retardants, among, which bromic compounds are of marked importance. The presence of these compounds was detected in sewage sludge, bottom deposits, and aquatic animals [3, 4, 5]. Thus, consumption of the contaminated food can be, beside from occupational exposure, the source of the exposure to these compounds [3, 6]. Some authors suggest an analogy between the current risk of PBBs and the risk of PCBs several dozen years ago, when the wide use of the latter caused the manifestation of toxic effects in humans and animals [7].

Hexabrombenzene (HBB) and tetrabromobisphenol-A (TBBP-A) belong to the group of compounds determined to be flame-retardants. These agents are added to plastics, textiles, wood, and laquers to decrease their flammability. Owing to these substances, less heat and carbon monoxide is released during fire. TBBP-A, contrary to HBB, binds with the medium and thus is considered to be safer for the environment than other flame-retardants [8]. As an additive flame-retardant, it is used in the production of plastic parts of electronic and electric equipment, whereas its main application is in electrical system lamination (reactive retardant). TBBP-A also serves as an intermediate product for the production of the latest generation of flame-retardants, *e.g.*: high-molecular polymeric compounds F2400 type [9].

Earlier literature data related to porphyrogenic activity of HBB were contradictory. Evaluation of this activity was based mainly on the measurement of porphyrins concentration in rat liver. Taking this into account, Carlson [10] recognized HBB to be a weak inductor, whereas Smith and Francis [11] took it for stronger porphyrogenic than hexachlorobenzene (a model porphyrogenic compound).

HBB is a compound of low acute toxicity (DL_{50} about 10 g/kg b.w.). However, the studies carried out by Szymańska and Piotrowski [12] demonstrated that repeated exposure of rats to this compound caused statistically significant increase in the level of cytochrome P-450 observed in weeks 3 and 4 of the experiment, as well as in increase of δ-aminolevulinic acid (ALA-U) excretion with urine detected already 7 days after HBB administration at all the applied doses. The elongation of the exposure time to 28 days intensified this effect. HBB also increased porphyrins elimination with urine (particularly tetracarboxyporphyrins) and decreased the activity of δ-aminolevulinic acid synthase (ALA-S) in rat′s liver [12]. Thus, the change in exposure revealed the toxic action of the compound considered to be harmless.

There are significantly fewer data on TBBP-A porphyrogenic action than on HBB. Experiments carried out on rats (28-days) demonstrated that, only after 14 days administration of the highest dose of TBBP-A increased urinary excretion of ALA-U was observed. Insignificant inhibition of ALA-S activity was observed only in week 4 of the experiment [13].

The aim of this study was to complete the research on the porphyrogenic effect of HBB and TBBP-A with the use of parameters other than those so far applied – the measurement of the concentration and the profile of porphyrins in the tissues.

Materials and Methods

Animals

The experiments were performed on 144 female Wistar rats. The animals were fed on standard "Murigram" chow and tap water ad libidum. Hexabromobenzene (HBB) (CAS 87-82-1) and tetrabromobisphenol-A (TBBP-A) (CAS 79-94-7) were administered intragastrically, in 3 repeated doses (1 dose/day): HBB- 15, 75 and 375 mg/kg and TBBP-A- 10, 50, 250 mg/kg.

The animal groups consisted of 3 to 5 animals. Two kinds of control groups were used in the tests:

a) pure control, not administered any compounds,
b) oil control, where the animals were administered sunflower oil (medium in which the investigated compounds were given).

Sections were performed 24 hours following 7, 14, 21 and 28 days of administering each of the compounds. In each experiment, the results were compared with the controls.

Preparation of Material

50% homogenates in phosphorus buffer, pH=6.8, were prepared from livers and kidneys (in ice bath). Isolation of porphyrins from tissues was performed according to the method of Luo and Lim [14]. The obtained homogenate was shaken for 30 seconds with dimethyl sulfoxide (DMSO) 1:1. Subsequently, it was centrifuged for 5 minutes at 2500g, and the obtained supernatant was applied into the chromatography column (HPLC).

Measuring Porphyrins by Means of HPLC Method

The determination of porphyrins in tissues was carried out by means of high performance liquid chromatography, according to Lim and Peters [15]. Analytical parameters were as follows: ODS Hypersil column (20 cm x 4.6 mm).

Detectors:

1) UV-VIS (measurements were performed at 404 nm)
2) Spectrofluorometer LDC Analytical (measurements were carried out at Ex=404 nm; Em=618 nm, fortified 1000x).

Liquid phase:

A – 10% acetonitrile solution [v/v] in 1M solution of ammonium acetate, pH=5.16

B – 10% acetonitrile solution [v/v] in methanole.

Flow: 1ml/min

Volume of injection: 100 µl.

Calibration curves were prepared from tetra- penta-, hexa-, hepta- and octa-carboxyporphyrins (Porphyrin products, Logan, USA).

The following compounds were as porphyrin standards – the mixture of octa-, hepta-, hexa-, penta- and tetracarboxyl porphyrins (Porphyrin Acid Chromatographic Marker Kit obtained from Porphyrin products, Logan, USA). The mixture of porphyrins was dissolved in 10 ml 3M HCl, to obtain the solution of each porphyrin with the concentration of 1 nmol/ml.

Statistical Analysis

The obtained results were analyzed statistically by means of Tukey test [16].

Results

In the experiments carried out the concentration of five fractions of porphyrins (tetra-, penta-, hexa-, hepta-, octacarboxyporphyrins) in two tissues (liver and kidney) was determined. Experiments were performed after intragastric repeated exposure to HBB and TBBP-A. There were no significant differences between "oil controls" and "pure controls."

Repeated administration of HBB caused an increase of the concentration of total high carboxylated (hepta- and octacarbocyporphyrins) porphyrins in liver, reaching 300-800% of control values. The increase (statistically significant) evoked by all applied doses of the investigated compound, was observed in all the measurement points. These changes depended on the dose but not on the number of times the compound was administered, which is demonstrated in Fig. 1.

The concentrations of high carboxylated porphyrins in liver observed after repeated exposure to TBBP-A were markedly lower (max 140% of the values obtained in the control groups), which is illustrated in Fig. 2.

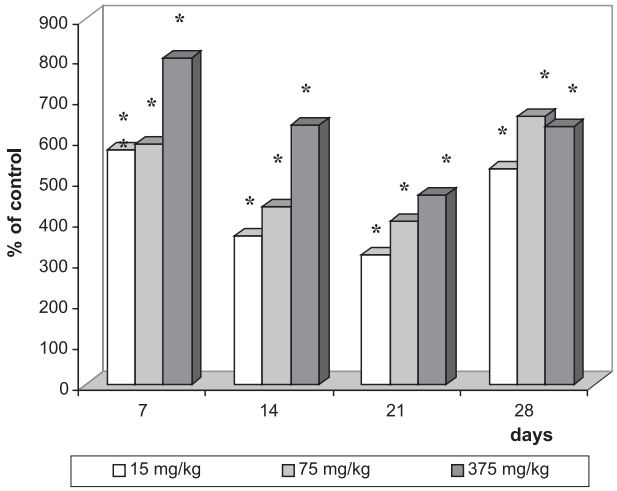

Fig. 1. Concentration of octa- and heptacarboxyporphyrins in the liver of female rats after repeated exposure to different doses of HBB [% of control]. Asterisk indicates results significantly different from control, α=0.05.

The measurement of this parameter in kidneys showed that HBB administered 7 days at doses of 15 and 375 mg/kg and 14 days at 375 mg/kg caused statistically significant increases of the concentration of total hepta- and octacarboxyporphyrins. Elongation of the exposure time did not affect the increase of these porphyrins concentration in rat kidneys, but on the contrary the return to the control values was observed (Fig. 3).

However, the increase of the concentration of high carboxylated porphyrins in kidneys (by about 4 times) was seen only in week 3 of the experiment after exposure to TBBP-A in the lowest dose (10 mg/kg). In the remaining measurement points changes of this parameter oscillated within the limit of about 50% over the values obtained for the control group, which is illustrated in Fig. 4.

The most profound cumulation of tetra-, penta- and hexacarboxyporphyrins in rat liver exposed to HBB was

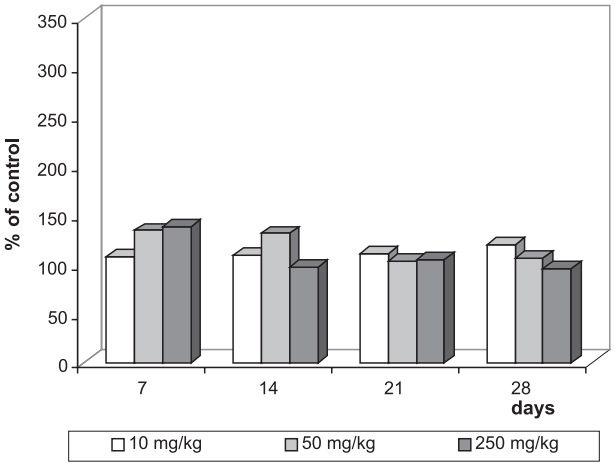

Fig. 2. Concentration of octa- and heptacarboxyporphyrins in the liver of female rats after repeated exposure to different doses of TBBP-A [% of control].

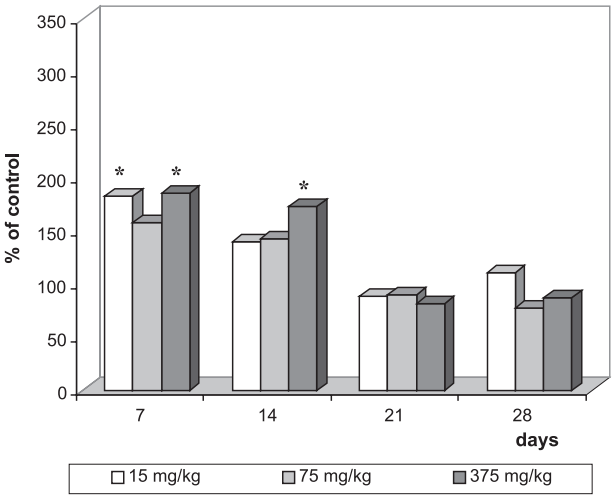

Fig. 3. Concentration of octa- and heptacarboxyporphyrins in the kidney of female rats after repeated exposure to different doses of HBB [% of control]. Asterisk indicates results significantly different from control, α=0.05.

detected on the seventh day of the experiment; however, these changes were not statistically significant. Also, in the case of TBBP-A the most effective action of the applied doses was found after 7-day exposure. Elongation of the time of the experiment did not affect significantly the concentration of these porphyrin fractions. However, in some cases a decrease of their level was observed by about 10-12% of the control values. Statistically significant increases of low-carboxylated porphyrins in kidneys was found after 7 days of exposure to HBB at the lowest dose (15 mg/kg). These changes reached 250-600% of the control values. The concentrations of these 3 forms of porphyrins in rat kidneys after TBBP-A administration changed a little. The highest values of tetra-, penta- and hexacarboxyporphyrins in this tissue reached 350% of the control values was observed after 7 days administration of TBBP-A at the dose of 50 mg/kg.

Based on the concentrations of all the determined porphyrins in the tissues, the percentage share of particular porphyrin fractions was calculated, which enabled us to establish the sequence of their occurrence. Table 1 presents the obtained data.

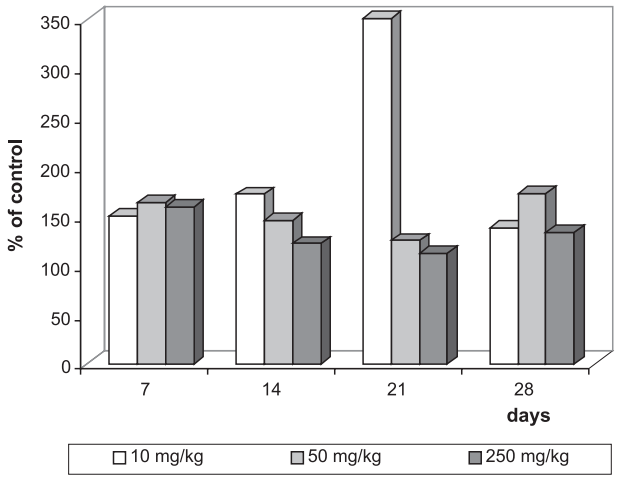

Fig. 4. Concentration of octa- and heptacarboxyporphyrins in the kidney of female rats after repeated exposure to different doses of TBBP-A [% of control].

Table 1. Sequence of occurrence of particular porphyrin fractions calculated on the basis of their percentage share in total porphyrin count.

		Control group	Exposure group
HBB	Liver	8->7-=4->>6-.5-	8->>7->4->5->6-
	Kidney	4->8->7->>6-.5-	8-=7-=4->6->5-
TBBP-A	Liver	8->>7->4->6->5-	8->>7->4->6->5-
	Kidney	8->7->>4->5->6-	8-=7->4->6->5-

Discussion

Porphyrias are a group of metabolic diseases which are characterized by deficiency of at least one enzyme participating in heme biosynthesis, different for each type of porphyria [17]. These are diseases occurring in subjects genetically conditioned by an error in heme synthesis regulation and determined as acute porphyrias [1]. Another group are the so-called chronic porphyrias associated with inborn or acquired predisposition to disturbances of heme synthesis during exposure to xenobiotics. Recent studies have proved that porphyrogenic action is demonstrated by a number of xenobiotics widely used in industry and agriculture and also by substances contaminating the environment (eg. vinyl chloride, tetrachlorodibenzodioxin) [1, 18].

Porphyrogenic action of the compound is determined on the basis of porphyrins concentration and their profile in the tissues, urine and faeces as well as the activity of enzymes involved in heme biosynthesis. Investigation of the porphyrins profile, that is the ratio of the concentrations of porphyrins of various grades of carboxylation, demonstrated that it is characteristic for the particular biological material. Tetracarboxyporphyrins dominate in urine, whereas in tissues porphyrins with the highest number of carboxyl groups (octa- and heptacarboxyporphyrins) have the highest concentrations [19]. Chronic hepatic porphyria is diagnosed based on high carboxylated porphyrins cumulation in liver bioptic material [20].

A majority of authors [21, 22, 23] suggest that an increase in δ-aminolevulinic acid and/or prophobilinogen excretion, an increase in urine or tissue level of porphyrins and changes in the activity of enzymes from heme biosynthesis path speak to the porphyrogenic action of the compound.

The data on porphyrogenic action of aromatic halogen derivatives in humans and animals are related to hexachlorobenzene (HCB), hexabromobenzene (HBB) and polybromobiphenyls (PBB).

Porphyrogenic action of HCB (chronic hepatic porphyria) was proved on the basis of an increase of high carboxylated porphyrins concentration (octa- and heptacarboxyporphyrins) in rat liver (female) and an increase of total porphyrins excretion [21, 24, 25]. These alterations were accompanied with an decrease of URO-D activity – the enzyme catalyzing octacarboxyporphyrins (uroporphyrins) conversion to tetracarboxyporphyrins (coproporphyrins).

The results obtained in this study confirm the theory of Smith and Francis about the strong porphyrogenic action of HBB. Repeated exposure of rats to HBB caused intensified cumulation of high carboxylated porphyrins in tissues (mainly in liver) on the 7th day of the experiment. In the investigated groups the concentrations of these porphyrins were even 8 times higher than in the control groups. Investigations on HBB carried out by Szymańska and Piotrowski [12] demonstrated that repeated administration of this compound to rats resulted

in the increase of ALA elimination with urine observed already 7 days after exposure. Elongation of the time of exposure intensified this effect. HBB being administered 28 days caused 4-times increased concentration in urine ALA in rats. ALA-S activity was significantly decreased in 7 and 14 days of the experiment. The increase in porphyrin elimination with urine (porphyrinuria) was seen throughout the whole experiment. The highest urine concentrations reaching 200-400% of the control values were detected for tetracarboxyporphyrins. Repeated exposure of rats to HBB resulted in about 2-times increased level of total cytochromes P-450 in liver.

There is little literature data on TBBP-A porphyrogenic action. The studies on TBBP-A toxicity carried out by Szymańska et al. [13] demonstrated that this compound, administered repeatedly, intensified excretion of porphyrins (mainly tetracarboxyporphyrins) with urine in rats.

In the hereby presented experiments TBBP-A administered to rats for 28 days, similarly to HBB, intensified cumulation of high carboxylated porphyrins in the tissues. However, the observed changes were significantly weaker as compared to the alterations evoked by exposure to HBB. TBBP-A increased the concentration of these porphyrins to a max of 140% in relation to the values obtained in the control group.

Clinical examinations of the cases of chronic hepatic porphyria demonstrated that appearance of clinical symptoms is preceded by changes in corresponding proportions of porphyrins of different number of carboxyl groups. In the early stage of hepatic heme synthesis disturbances, with normal amount of total elimination of porphyrins with urine, an increase in the concentration of porphyrins with 8 or 7 carboxyl groups in relation to those with 6, 5 and 4 – the so called shift in porphyrins profile is found [1]. According to some authors [26], the alterations in porphyrin profile observed both in urine and in blood may be the biomarker of exposure to various compounds, e.g. PCB.

Porphyrogenic action of the compound may also be associated with an increase of cytochrome P-450 CYP1A activity. An increase of this cytochrome isoform is an indicator of receptor Ah induction [27, 28], which forms active complexes with aromatic polycyclic hydrocarbons [29]. Owing to the fact that CYP 1A2, which takes part in uroporphyrinogen oxidation to uroporphyrins and is engaged in porphyrins metabolism, it may be thought that the observed changes in their level are strictly connected with the induction of these isoforms [30]. The studies carried out by Bruchajzer et al., [31] on rats exposed to HBB seem to confirm this theory. Repeated exposure of rats to HBB caused 6-10-times increase of CYP1A activity as compared to the control group. The observed alterations in the activity of this parameter were comparable to those obtained in the positive control group on methylcholanthrene – a known CYP1A inductor.

References

1. LUTZ W., STANKIEWICZ A. Heme biosynthesis disorders as an early indicator of the lesions to the liver caused by chronic exposition to xenobiotics. Pol. Tyg. Lek. **42**, 419, **1987**
2. EHC (Environmental Health Criteria). Polybrominated biphenyls. International Programme on Chemical Safety (IPCS), WHO, Geneva. **152, 1994.**
3. EHC (Environmental Health Criteria). Brominated diphenyl ethers. International Programme on Chemical Safety (IPCS), WHO, Geneva. **162, 1994.**
4. MOON H. B., CHOI H. G., KIM S. S., JEONG S. R., LEE P. Y., OK. G. Contaminations of polybrominated diphenyl ethers in marine sediments from the southeastern coastal areas of Korea. Organohalogen Compounds **58**, 217, **2002.**
5. OLIAEI F., KING P., PHILLIPS L. Occurrence and concentrations of polybrominated diphenyl ethers (PBDEs) in Minnesota enviroment. Organohalogen Compounds **58**, 185, **2002.**
6. SJÖDIN A., THURESSON K., HAGMAR L., KLASSON-WEHLER E., BERGMAN A. Occupational exposure to polybrominated diphenyl ethers at dismantling of electronics – Ambient air and human serum analysis. Organohalogen Compounds **43**, 447, **1999.**
7. KEMI Raport. Risk assessment of polybrominated diphenyl ethers. No 9/94. The Swedish National Chemicals inspectorate. **1994.**
8. BERGMAN A. Brominated flame retardants in global environmental perspective. Proceedings, Workshop on Brominated Aromatic Flame Retardants. Skoklester, Sweden, 13, **1989.**
9. BSEF (Bromine Science and Environmental Forum) An introduction to bromine flame retardants**. 2000**.
10. CARLSON G. P. Brominated benzene induction of hepatic porphyria. Experientia **35**, 513, **1979.**
11. SMITH A.G., FRANCIS J.E. Relative abilities on a molar basis of hexafluoro-, hexachloro- and hexabromobenzenes to decrease liver uroporphyrinogen decarboxylase activity and cause porphyria in female rats. Res. Comm. Chem. Pathol. Pharmacol. **28,** 377, **1980.**
12. SZYMAŃSKA J. A., PIOTROWSKI J. K. Hepatotoxicity of monobromobenzene and hexabromobenzene: effects of repeated dosage in rats. Chemosphere **41**, 1689, **2000.**
13. SZYMAŃSKA J. A., PIOTROWSKI J. K., FRYDRYCH B. Hepatotoxicity of tetrabromobisphenol-A: effects of repeated dosage in rats. Toxicology **142**, 87, **2000.**
14. LUO J., LIM C. K. Isolation and characterisation of new porphyrin metabolites in human porphyria Cutanea tarda and in rats treated with hexachlorobenzene by HPTLC, HPLC and Liquid Secondary Ion Mass Spectrometry. Biom. Chromatog., **9**, 113, **1995.**
15. LIM C. K., PETERS T. Urine and faecal porphyrin by reversed phase high-performace liquid chromatography in the porphyrias. Clin. Chim. Acta **139,** 55, **1984.**
16. WILKINSON. L. SYSTAT: The system for ststistics. Evanston, IL: Systat, Inc. **1990.**
17. GREGOR A., KOSTRZEWSKA E., TARCZYŃSKA-NOSAL S., STACHURSKA A. Porphyrin fluorescence in plasma of various types of porphyria. Pol Tyg Lek. **49**, 12, **1994**

18. TARCZYŃSKA-NOSAL S., EKIERT M., KOSTRZEWSKA E. Factors inducing clinical manifestations of acute hepatic porphyria in the years 1986-1990. Acta Haemat. Pol. **32**, 100, **1991.**
19. KOSTRZEWSKA E., GREGOR A. Acute hepatic porphyrias. Detection, prophylaxis and treatment. Mater. Med. Pol. **28**, 5, **1996.**
20. STANKIEWICZ A., LUTZ W. Usefulness of determinations of porphyrins and their precursors for the evaluation of heme synthesis disturbances in liver due to xenobiotics. Postępy Hig. Med. Dośw., **42**, 574, **1988**.
21. WAINSTOK D. E., CALMANOVICI R., BILLI D. E., CATABBI S. C., ALDONATTI C. A., SAN MARTIN DE VIALE L. C. Influence of the Strain of rats on the induction of hexachlorobenzene induced porphyria. Int. J Biochem. **21**, 377, **1989.**
22. SCHOENFELD N., MAMET R., MEVASSER R., ATSMON A. Porphyria and porphyrinuria. J. Isr. Med. Ass. **125**, 449, **1993.**
23. SASSA S., KAPPAS A. Molecular aspects of the inherited porphyrias. J Inter. Med. **247,** 169, **2000.**
24. SAN MARTIN DE VIALE L.C., DEL C., RIOS DE MOLINA M., WAINSTOK DE CALMANOVICI R., TOMIO J.M. Porphyrins and porphyrinogen carboxylyase in hexachlorobenzene – induced porphyria. Biochem. J **168**, 693, **1977.**
25. VAN OMMEN B., BESSEMS J.G.M., GEESINK G., MÜLLER F., VAN BLADEREM P.J. The relation between the oxidative biotransformation of hexachlorobenzene and its porphyrogenic activity. Toxicol. Appl. Pharmacol. **100**, 517, **1989.**
26. CHO J. H., JEONG S. H., YUN H. I. Changes of urinary and blood porphyrin profiles by exposure to PCBs, lead or diazinon in rats. Vet. Hum. Toxicol., **45**, 193, **2003.**
27. CIKRYT P., GOTTLICHER M., NEUMANN H. G. Competitive binding affinity of carcinogenic aromatic amines to the rat hepatic aromatic hydrocarbon (Ah) receptor in vitro and potency to induce monooxygenase activity in vivo. Carcinogenesis, **11**, 1359, **1990.**
28. CRAFT E. S., DEVITO M. J,. CROFTON K. M. Comparative responsiveness of hypothyroxinemia and hepatoc enzyme induction in Long-Evans rats versus C57BL/6L mice exposed to TCDD-like and phenobarbital-like polychlorinated biphenyl congeners. Toxicol Sci **68,** 372, **2002.**
29. PROCOPIO M., LAHM A., TRAMONTANO A., BONATI L., PITEA D. A model for recognition of polychlorinated dibenzo-p-dioxins by the aryl hydrocarbon receptor. Eur J Biochem **261**, 13, **2002.**
30. HAHN M.E., CHANDRAM K. Uroporphyrin accumulation associated with cytochrome P4501A induction in fish hepatoma cells exposed to aryl hydrocarbon receptor agonists, including 2.3.7.8-tetrachlorodibenzo-p-dioxin and planar chlorobiphenyls. Arch. Biochem. Biophys. **329,** 163, **1996.**
31. BRUCHAJZER E., FRYDRYCH B, SZYMAŃSKA J. A. Effect of repeated administration of hexabromobenzene and 1.2.4.5-tetrabromobenzene on the levels of selected cytochromes in rat liver. Int. J Occup. Med. Environ. Health. **17**, 347, **2004.**