

PESTICIDES WITH ENDOCRINE DISRUPTING FUNCTION – BIOMARKERS OF EXPOSURE

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Abstract

The enzyme complex P-450 cytochrom is responsible of pollutant biotransformation and neutralization in animal organism. Practically, these enzymes are present in most tissues, being peculiarly good represented in liver. They play a major role in the metabolic pathways of Ist phase reactions. P-450-dependent cytochrom enzymes catalyze oxidation, reduction and hydrolysis reactions, supposing introduction of some reactive groups with high polarity into the pollutant molecule: -OH; -SH; -COOH and -NH₂. Pollutant biotransformation processes are differently carried on, function of the hidrosoluble or liposoluble nature of the xenobiotic. Liposoluble pollutants are metabolized in the endoplasmatic reticle, while hidrosoluble pollutants are metabolized in other cell organelles. Identification and quantification of enzymes specific activity from this overfamily indicates organism exposure to contaminants, being widely used as biomarkers of environment quality and agro-feeding products.

Key words: pollutant, animal bioaccumulation, detoxification, cytochrom CYP 1A1

Action assessment of pollutants in environment on animal organisms but also on humans, can be realized as early as initial phase by biomarkers. Pollutant action on animal organism determines modifications in cell and molecules level, including genetic material modifications (DNA), but also the presence of some responsive enzymes (proteins) to pollutant action. Such a family of enzymes is represented by P450 cytochrom (CYP). To the level of February month, 2008, the cytochrom P450 (CYP) enzyme super-family was constituted of above 7000 representatives with known nucleotides sequence, identified in animals, plants, bacteria and fungus (Pelkonen, et al., 2008). Human genome has as components 57 genes of CYP type. CYP fifteen enzymes from 1, 2 and 3 families metabolize xenobiotics. Other CYP-s present a more “narrow” specificity for the substratum and principally metabolize endogen substances, as: sterols, fat acids, eicosanoides and vitamins. P450 cytochrom enzymes (CYP) practically are

found in all tissues, the best being represented in liver. CYP different representatives can be identified in intestine, lung, kidney, brain, gonads, hart, suprarenal glands, nasal and tracheal mucosa and in skin (Pelkonen, et al., 2008).

Xenobiotic conversion into metabolites supposes some enzymatic reactions, classified by Williams (1959) into I-st phase reactions and II-nd phase reactions. From the category of I-st phase reactions, oxidation, reduction and hydrolysis are belonging to, while in the category of II-nd phase reactions, conjugation and synthesis reactions are appointed. Logically, one or more reactions of I-st phase precede reactions of II-nd phase.

Metabolic path ways of I-st phase reactions, catalyzed by enzymes of oxidase, reductase and hydrolase category, supposes introduction into pollutant molecule of some reactive groups with high polarity: -OH, -SH, -COOH and -NH₂. Displaying headquarters of biodegradation processes are different

function of hydrosoluble or lyposoluble nature of pollutant. Lyposoluble pollutants are metabolized in endoplasmatic reticle, and those hydrosoluble in other cell organelle.

Oxidation is catalysed by oxidases which transform a series of exogenous substances such as nitrites, sulphides and sulphites in nitrates and sulphides less nocives.

Reduction contents couple reactions of nitro- and amino-derivates reduction with methemoglobinisant products forming and those of pentavalent arsenic reduction into a trivalent compound.

Hydrolise supposes desesterification, desamination and hydrolytic scission reactions of some amides and glycosides.

Cytochrom P-450 – dependant oxidation reactions, represent the first step in most xenobiotic and a lot of endogenous compounds metabolization, including fat acids, prostaglandins, steroids and vitamins. The system P-450 (cytochrom P-450 or CYP or CYP-450) represents the generic term for a large number of oxidative enzymes implicated into bacterial, vegetal and animal physiology. The P-450 system is connected to the cell membrane level and it is characterized by two major components:

- cytochrome c P-450, an hemoprotein, and
- P-450 cytochrome reductase, a flavoprotein which contains FMN and FAD prosthetic groups. This enzyme reduces the cytochrome c and it is known as NADPH-cytochrome c reductase. Another protein cofactor implicated in electron transfer, nearly P-450 cytochrome reductase, is b_5 cytochrome. P-450 system uses molecular oxygen in view of oxidation reactions.

There are a lot of P-450 iso enzymes, that explain the capacity of this system to oxidize numerous substratum. P-450 iso-enzymes present substratum specificity and specific answer to enzymatic inductors such as Phenobarbital or 3-methylcolantren.

In animals there are described two kind of P-450 cytochrome-dependant mono-oxygenasic systems. The most important, major implicated in xenobiotic biotransformation is expressed in microsomal fraction of endoplasmatic reticle from hepatocytes and extra-hepatic cells (lung, kidney, placenta, tegument intestines, suprarenal, testicular, ocular, pancreatic, mammary, aorta wall, brain, nasal epithelium, colon, salivary glands, prostate, cardiac, lymph ganglions, splenic, thymus and tiroida).

The second mono-oxygenasic system is recovered to the mitochondria level, in steroidal cells (suprarenal, ovarian, testicular) and contains a flavoprotein-FAD and a sulphide-iron-protein which make easier the electron transfer from NADP to P-450. The mitochondrial P-450 system is implicated in steroid structure endogenous compounds metabolization, such as cholesterol colecalciferol (D_3 25-hydrovitamin), deoxycorticosteron (suprarenal hormone with mineralocorticoide activity) and it is characterized by significant high specificity comparatively to microsome mono-oxygenatic system (Estabrook, 1984).

In low concentrations, P-450 cytochrome system also is expressed to nuclear membrane, Golgi apparatus and plasma membrane level.

Also, there is a P-450 independent mono-oxygenatic system, placed to the endoplasmatic reticle level from liver and extrahepatic tissues virtually present in all nucleate cells. Flavin-dependent mono-oxygenase initially was isolated from pig liver (Ziegler and Mitchell, 1972), later on also in other species being identified (rat, mouse, rabbit). The system contains dinucleotid-adenin-flavine co-enzyme (FAD), needs NADPH as co-factor and as physiologic substratum cysteamine which is oxydated to cystamine.

Many organic chemical substances are oxydated during arahydronic acid H-prostaglandine-syntase conversion into prostaglandine. Xenobiotic oxidation is

catalysed by hydroperoxidase of H-prostaglandin-synthase, reaction being defined as co-oxidation. G₂ prostaglandine reduction by H-prostaglandine-synthase needs electron donation, usually derived from co-oxidated substratum. Co-oxidation is implicated in biotransformation of some xenobiotics such as benzopyren, diethylstilbestrol or acetaminofen. Some of them, such as benzopyren are converted in strong mutagens as a result of this reaction (Krauss and Eling, 1984).

H-prostaglandine synthase activity is strong expressed in extrahepatic tissues with reduced P-450 mono-oxygenase activity. These tissues include tegument, renal medullary, lung and endothelium cells from vascular walls.

Xenobiotic metabolism by peroxidation leads to metabolic compounds which can mediate toxic effects.

One of main metabolic pathway for alcohols and aldehydes biotransformation is oxidation by aldehydes and ketones, respectively to carboxylic acids. Aldehyde dehydrogenase is wide distributed in mammal tissues, but being most represented to hepatic level. Aldehyde dehydrogenase uses NAD(P)⁺ as co-factor, and aliphatic and aromatic aldehydes are quickly oxidated to carboxylic acids.

Mono-oxidases are disposed at the mitochondria external membrane level, being wide distributed in mammal tissues, excepting erythrocytes and plasma. This enzymatic system catalyses oxidative deamination of many exogen or endogen origin monoamines (ex, amines resulted following intestinal microflora action, amines with neuro-transmission role, etc).

P-450 cytochrome dependent reduction reactions take place in aerobic or anaerobic conditions, having as co-factor NADH or NADPH. They intervene in polyhalogenate alkanes catalysation. By P-450 cytochrome dependent reduction reactions, are converted pollutants such as DDT, halothantrichlorinefluormethan, hexachlorinethan and pentachlorinethan.

Other classes of xenobiotics metabolized by the same pathway are insaturated hydrocarbons (epoxides), primary amines (hydroxylamines, hydrazo-derived) and tertiary amines .

Hydrolyse reactions are mostly implicated in ester and amide metabolism, but also of compounds converted in epoxides by P-450 system. Hydrolyse reactions are integrated in I-st phase reactions because they generate RCOOH, RNH₂ and ROH groups, representing sites to whom level conjugation reactions take place (II-nd phase reactions). These catalyse epoxydes hydratation to dihydrodiols and represent important enzymes in toxifying-detoxifying processes. Aliphatic and aromatic insaturated hydrocarbons are converted into epoxydes by P-450 mono-oxygenases . To their turn, some epoxydes are tied covalent to proteins and DNA, inducing nocives effects such as cell necrosis, acute and chronic toxicity, mutagenesis, carcinogenesis and teratogenesis. Diols resulted following epoxyde hydrolase action are less toxic than substratum, although in the case of some aromatic polycycling hydrocarbons, diols are forerunners of some carcinogenic and mutagenic compounds. There are described two distinct epoxyde hydrolase with their place at the level of endoplasmatic reticle, respectively in cytosol with substratum specificity.

As a result of different enzyme groups and intimate mechanisms of P-450 complex enzymes action identification, it appears the possibility of extension studies on a lot of species. Last years researches put in evidence significant data on different organism reactivity from varied terrestrial and aquatic ecosystems, as a result of exposure to pollutants, from the perspective of CYP stress responsive enzymes, which are validated as significant biomarkers for environment quality assessment. So ,series of researches done on different fish species (Jonsson et al,

2008; Jaksic et al, 2008; Marhon et al, 2008; Ahmad et al, 2004; Corsi et al, 2003; Marrottini et al, 2003) put in evidence the presence and an increasing activity of P-450 cytochrome to the level of different organs (liver, kidney, heart, gills, brain, eye etc) as a result of exposure to different pollutants (organochlorinated) and make conclusions that these enzymes can be used as biomarkers of aquatic environment pollution. So, Jonsson et al., (2007) identify in zebra fish (*Danio rerio*) CYP 1A1; CYP 1B1 and CYP 1C1 enzyme induction, to the level of many organs (gills, heart, kidney, brain, eyes, intestines, gonads) as a result of exposure to PCB-126 (polychlorine biphenyl 126). In another fish species, respectively in anguilla (*Anghilla anghilla*), Marhon et al (2008) identify CYP 1A1 expression (by RT-PCR method) in gills, as a result of exposure to PCB-77. Also in anguilla, Mariottini et al, (2003) propose use of EROD activity (in muscles) as exposure biomarker to different PCB combinations (polychlorine biphenils). In trout, EROD activity (in gills) can be used as biomarker of water pollution with PCB – 126.

Corsi et al (2003) use 2 fish species, *Zosterisessor ophicephalus* and *Mugil cephalus* to determine PCB and PAH action to liver level by following somatic index of liver (SLI); EROD activity and acetyl-colin-esterase (AChE) activity, these one could be also used as biomarkers of water pollution.

Organochlorinated pollutants (lyndan) can be monitored by biologic indicator species *Gambusia affinis* through EROD activity quantification to liver level (Jaksic et al., 2008). The presence of atrazin can induce P-450 1A1 cytochrome activation to liver and carp blood level (*Cyprinus carpio*), (Chang et al., 2005).

Jonsson et al (2003) use many fish species (*Salmo salar*, *Salvelinus alpinus*, *Gadus mohua*, *Pollachius vireus* and *Anarhichas minor*) and following EROD activity to gills level as a result of dioxin

action exposure, make conclusions that this biomarker can be used as useful instrument in water pollution monitoring processes.

Studies and researches were also realized on bird species to determine the presence of P-450 cytochrome in different organs level as a consequence of pollutant action. Dubois et al., (1996) use quail (*Conturnix conturnix*) in studies that have in view exposure assessment in different pollutants and identify the presence of CYP 2B increase activity as a result of exposure to lyndan and pentachlorinephenol.

Head et al.(2007), as a consequence of embryohepatocyte culture exposure obtained from hen (*Gallus gallus*) and silver sea-gull (*Larus argentatus*) to TCDD (dioxin) observe CYP 1A5 induction which can be used as biomarker of exposure to dioxin.

Also it were used aquatic mammals (otter and seal) to determine P-450 cytochrome to the cell level of different types. Thus, Hook et al. (2008) use sea otter (*Enhydra lutris*) and identify CYP 1A1 and CYP 1B1 as a result of exposure to PCB and PAH.

Tilley et al (2002) determine in two seal species (*Phoca groenlandica* and *Halichoerus grypus*) CYP 1A induction as biomarker of exposure to different contaminants in environment.

Induction of CYP 1A increased activity (in epidermal tissue) as a result of PCB, dioxin and furans exposure in seal species (*Phoca vitulina*) has permitted validation of this one as exposure biomarker to these pollutants.

Dubois et al. (1996) identify CYP 1A1 in rat, in the hepatocytes culture as a consequence to lyndan and pentachlorinephenol exposure. Also in rat, Nims et al. (1998) and Ngui et al. (1999) identify induction of a high CYP 2B activity to the hepatic cell level as a result of DDT, DDE and DDD exposure, respectively to PCB and Arochlorine.

Nearly effected studies on wild animal species, also were realized studies concerning P-450 cytochrome induction, as a result of exposure to different pollutants in environment, to human species. Researches principally were realized on hepatic cells (Hep G₂ line) and somatic cells (epithelial) obtained from maternal milk. So, Dehn et al. (2005) expose human hepatic cells (Hep G₂) to different organochlorined pollutants (o, p'-DDT, dieldrin, endosulphan, kepone, metoxychlorine and toxaphen), obtaining induction of CYP 1A and CYP 1B activation.

It was obtained CYP 1A1 expression by human hepatic cells exposure (HepG₂) to PCB-77.

Some organophosphorous pollutant action (azynphos-metylchlorinepyriphos, diazynon, parathion) on hepatic cells determine induction of CYP 1A2 and CYP 2B6, and these can be used as biomarkers of exposure .

In human, it was succeed to determine P-450 cytochrome expression (CYP 1A1) from hepatic cells (epithelial)isolated from maternal milk and establishment of a positive correlation between CYP 1A1 high activity and exposure to dioxin (Diehl-Jones et al., 2000; Yonemoto et al., 2004). Yonemoto et al., (2004) collect human milk (1 week after birth) and establish positive correlations between presence of dioxin in maternal organism and the quantity of CYP 1A1 from somatic cell level in maternal milk (epithelial), doing conclusion that CYP 1A1 expression in somatic cell level from milk can be used as biomarker of maternal organism exposure to dioxin.

Identification and quantification of P-450 cytochrome (CYP) enzymatic complex activity as stress-responsive reaction of organisms exposure to different environmental pollutants make a circumscription of an important series of methods for the active monitoring process of environment quality and of agro-

alimentary products by these biomarkers. Species which express most pregnant the exposure to a specific pollutant are considered biologic indicator species, early signaling the presence of pollutant in environment or in agro-system. These techniques can be extended also in general assessment process of animal origin alimentary products quality, establishing possible exposure and consequently the presence of pollutant in specific bioaccumulation medium of these products.

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