The Xenoestrogen Effects of Polycyclic Aromatic Hydrocarbons and Organochlorines Compounds: Historical Perspective and Update

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SUMMARY
There is considerable concern about the increasing incidence of endocrine-related cancer and deteriorating reproductive health in humans. A large number of natural and man-made chemicals have the ability to mimic the action of the endogenous steroid hormone 17b-estradiol by binding to and activating the estrogen receptor. In this matter, there is no consensus regarding the role of xenoestrogens in these effects and no conclusive studies demonstrating that xenoestrogens initiate or contribute to the development of these effects. The molecular structure of exogenous natural and synthetic estrogens may be very similar to, or strikingly different from the natural hormone. Despite their structural diversity, all of the exogenous estrogens, when ingested either as natural compounds (phytoestrogens, mycoestrogens) or contaminants (xenoestrogens), have the capacity to bind to the ER at a given concentration in target cells of the body and can initiate (agonist) or inhibit (antagonist) estrogen-like actions. Assessment of the impact of xenoestrogenic compounds will require additional research on identification and quantitation of these compounds in serum, their interactions with plasma and cellular proteins, and their uptake in target tissues. Aim of this paper is indicate to the possible estrogenic effects polycyclic aromatic hydrocarbons (PAHs), organochlorines- dichlorophenyl-trichloroethane (DDT) and polychlorinated biphenyls (PCBs).

Keywords: carcinogenesis, estrogen, xenoestrogens, polycyclic aromatic hydrocarbons (PAHs), organochlorines- dichlorophenyl-trichloroethane (DDT), polychlorinated biphenyls PCBs.

1. INTRODUCTION
Breast cancer has dramatically increased during the course of the last several years internationally and such an increase can not be interpreted solely by improved and more advanced detection methods. Such observations refer to environmental factors that play a role in developing one or more ways leading to development of carcinoma. Environmental pollutant may be prevented from its impact but in the first place it is necessary to establish interconnection between development of breast carcinoma and exposition to chemical compounds from environment (1).

Epidemiological studies indicate increase of breast carcinoma with women, which is linked to various sources of exposition of endogenous and exogenous estrogens and other hormones, supporting hypothesis that hormonally active material and pollutants increase this risk. Current data that refer to the research of biological mechanism of breast carcinoma, including chemical and hormonal factors, support another hypothesis implying that hormonally active chemicals contribute to development of breast carcinoma (1). The goal of this paper is to focus on current scientific perceptions related to environmental factors in developing breast carcinoma.

Numerous data indicate that chemicals and chemical mixtures may provoke endocrine disorders with damaging effects to human health. On the other hand, there is significant increase of hormonal carcinoma incidence as well as reproductive tract abnormalities. It was suggested that many of these effects may occur due to exposition to chemicals and its mixtures, the feature of which imply their capability to activate estrogenic receptors (1). Such materials are marked as environmental estrogens, xenoestrogens or exoestrogens.

Estrogens are vital for functioning and maintaining different tissues and physiological systems of mammals. In physiological response, it is known that within specific tissues, estrogens acts through two estrogen receptors (ER), which are ERα and ERβ. Researching the distribu-
tion of ER and their spread in tissues, it turned out that
Era are more widely spread while Erβ are more focused
on particular organs, with the most concentrated distri-
bution at ovary (2). Both receptors have nearly the same
affinity for 17β-estradiol (3).

Exposition of humans to estrogen materials in the en-
vironment may be responsible for occurrence of increased
number of hormonally dependant carcinoma of breast,
ovary, endometrium, tyroidea as well as different abnor-
mal physiological and morphological forms (4).

2. IMPACT OF ESTROGEN AND
ESTROGENIC MATERIALS

Estrogens are materials that may directly provoke
stimulation of mitotic activity in the tissues of female
genital tract. The inciting mitotic activity through es-
trogen is complex and multi-level process, which may
be vulnerable in any of the stages of occurrence. Five
stages of this process have been identified: linking to
estrogen receptor, transcription, macromolecular syn-
thesis, cellular proliferation and clinical consequences.
Linking of estrogen to estrogen receptor (ER) increases
relationship of ER for estrogen-specific-response ele-
ments EREs) in DNA target gene. Complex of ER-ERE
and gene induced define the status of transcription and
initiate transcription. It is manifested through synthesis
of macromolecules, including mRNK, proteins (growth
factor, onkoprotein, estrogen, progesteron of receptor)
and DNA. Cellular proliferation occurs as transcription
manifestation. Eventually, clinical consequences that may
develop, include fail of reproductive cells, interruption of
reproductive cycle and development of abnormalities (5).
There is a number of genes that are found to be estrogen
inductive, including factor of growth, receptor growth fac-
tor, transcription factor and some other genes, proteins of
which do have function but it is still not clear what exactly
is their function. Researches prove that estrogen inducive
proteins may provoke health effects, resulting from impact
of estrogenic materials (6).

Estrogenic effects of chemical materials

The group of chemical materials that are responsible
for estrogen effects includes polycyclic aromatic hydro-
carbons and organochlorines (Picture 1.).

3. POLYCYCLIC AROMATIC
HYDROCARBONS

Polycyclic aromatic hydrocarbons (PAHs) belong to
the group of toxic organic chemical materials, which are
very numerous and disbursed ubiquitously in the envi-
rionment and the food. They are generated and released
into environment through incomplete combustion of
fossil fuels and within industrial processes. Important
sources of PAHs imply smoking and cigarette smoke,
exhaust gases of vehicles, steel-plants, power plants using
coal and a number of other industrial and non-industrial
processes. Likewise, PAHs may be found in food or are
generated during food processing at high temperatures
(roasted meet). Additional antropoghenic sources of
PAHs (forest fires) contribute to overall quantity of PAH
in the athmosphere. Many of PAHs are known as or are
suspected carcinogens, reports on which indicate that
they do have tumor-initiating and/or tumor-promoting
features. PAHs and their metabolites were subject to the
research on their genotoxicity, as initiating agents, while
their nongenotoxic and tumor-promoting effects, that may
be linked to carcinogenesis and endocrine disorders are
less known. PAHs reveals carcinogenous features at ani-
mal models (7), and its impact to human breast carcinoma
has been registered as well (8), as well as PAHs impact on
estrogens and antiestrogen features that potentially may
lead to the increase of risk of breast carcinoma (9).

Some researches suggest that PAHs may be involved
into development of breast carcinoma (10), with unclear
way of impact to estrogens. It has been established that
they do have estrogen and anti-estrogen features in dif-
ferent experimental models.

Research on exposure to PAHs during the lifetime
and relation to carcinoma (8) was in the focus of several
earlier studies. There are evidences that PAH-DNA aducts
in tumor tissue and peripheral blood exercise high values
in cases with breast carcinoma to control group. Level of
tumor PAH-DNA aducts are the sign of recent exposure
and PAH-DNA aducts in mononuclear cells are the best
indicator of the exposure of previous years. This way they
support hypothesis implying that exposure to PAH may
be joined with the risk of breast carcinoma and it indica-
tes that exposure to these combinations in earlier part
of life may have share in ethiology of breast carcinoma,
especially in cases of postmenopausal women, while with
women at the premenopause we do not have any data on
such a combinations with the risk of breast carcinoma (11).

Case-control research (Long Island, NY), made at the
sample of women with in situ and invasive form of
breast carcinoma, with regard to measurable values of
PAH-DNA aducts, indicate that there are no consistent
data with regards to risk of carcinoma and increase of
adduct level, as well as it indicates that there is no clear
link between the level of adduct and two major sources
of PAHs, active and passive smoking or consumption of smoked or barbecued food. Data indicate that generation of PAH-DNA adducts, with potential genetic mutations, may impact development of breast carcinoma (12).

There are reports on PAH operating as estrogens, i.e. opposite, that these chemical materials operate as anti-estrogens in in vitro bosees, and this stands for their hydroxilated forms in particular. PAHs have large number of different chemical structures, but it was generally concluded that majority of natural estrogen combinations possess the structural part that is similar to at least two PAH rings (1,13,14,15).

Nowadays, PAHs are mostly considered to be antiestrogens, due to their capability to activate Ah-receptor (aryl hydrocarbon receptor, AhR), which may lead to suppression of estrogen elements (estrogen response elements ERE), controlling genetic expression through AhR-estrogen receptor-α (ERα) (16). This may include several mechanisms, like direct interaction of AhR with estrogen genes, induction of inhibiting factors, competition for usual places of co-activators of protein-agents ERα degradation (17).

Benzo(a)piren (BaP) is one of major representatives of the group in the large group of PAHs, which is used as prototype of chemical carcinogens and is widely present in the environment. Based on the research, it is confirmed that BaP neither has estrogen activity nor links to estrogen receptors nor it induces activity of β-galactosidase, but its metabolic derivatives – monohydroxyl derivate, do impact estrogen receptors. Among monohydroxyl derivate of BaP, 1,- 2-, 3- and 9-OH-benzo(a)piren link to estrogen receptors of both types and act as agonists, but the link is far stronger with β-estrogen receptors than it is with α. Monohydroxyl derivate 8-OH-benzo(a)piren affects receptors as antagonist, exercising antiestrogen impact by its linking to β-receptors, acting as antagonist of 17β-estradiol and by inhibiting induction of β-galactosidase. BaP monohydroxyl derivate induce transcription of β-galactosidase achieving stronger links with β-receptors than the links they have with α-receptors. Structural similarity between monohydroxyl derivate of BaP and 17β-estradiol allows manifestation of minor differences in OH group position, which actually changes interaction and makes some chemical combinations agonists, at the same time making other antagonists (3, 9,13).

Several researches documents that PAHs, including BaP and benzo(a)antracen (BaA) or their hydroxil metabolites act as estrogen materials in different ER essay (9), as well as there are opposed data suggesting that these are only hydroxil metabolites of PAHs that activate transcription of endogen estrogen genes (9).

Benzo(a)piren is metabolized through enzyme system of cytochrome P_450 (CYPs) to dihydrodiol, fenol and kinon derivate. Cytochromes CYP1A1 and CYP1B1 catalyze formation of hydroxilated and dihydroid metabolites of PAHs, where these are only hydroxilated metabolic intermediers that have structural similarity with 17β-estradiol and there is possibility of interactions with estrogen receptors. Hydroxilated metabolites of PAHs my activate ER-dependent genes or ER-regulating endrogen genes, in which process they may, hypothetically, increase stimulative effect to proliferation of estrogen sensitive cells and/or tissues, which is typical for estrogen activity (18).

There is limited number of information about proliferative effects of PAH at in vitro cultures, which were used for detection of estrogen effects of xenobiotics, as in the case of cellular line of human breast carcinoma. Proliferation of epithelial cells of a breast is possible through stimulation of alternative mechanisms, which include activation of epidermal growth factor receptors (EGFR), which again may be activated through BaP kinon by making reactive oxygen groups (10).

PAU, especially BaP may be predisposing factor in ethiology of breast carcinoma so that it changes BRCA-1 (breast-cancer-suppressor gene) transcription into ER+ cells, changing expression of BRCA-1 gene, which, as tumor suppressor gene, controls proliferation of cells (19).

PAH is potent mutagen known for production of large quantity of DNA adducts that induce apoptosis. Kinons are made by BaP metabolism and they induce oxidative stress, joined with oxidative damage of DNA (20). BaP stimulates proliferation of MCF-7 cells, including the mechanism of ER activation. Induction of cell proliferation and DNA synthesis may contribute to carcinogenicity of BaP, by increase of DNA replication (20). Capability of proliferative operations of BaP suggests that both genotoxic and non-genotoxic events may bring about proliferation in their mutual interaction, which contribute to the promotion of tumor and/or carcinogenicity in estrogen sensitive tissues. In doing so, it remains to be clarified whether this phenomenon is limited just to isolated cell cultures and in vivo relevant (20).

Epidemiological studies suggest that estradiol metabolism run through two major routes, out of which each metabolic route may be damaged by xenobiotic exposition: pathway I leads to development of 2-hydroxiestron (2-OHE1), which has minimal estrogen activity and is nongenotoxic, or, pathway II, which develop 16α-OHE1, with full estrogen potential and genotoxic features as well (21). Breast carcinoma risk is related to both of these metabolic pathay. Materials that increase pathway II or inhibit the pathway I, increase this risk, as well as inhibition of pathway II or stimulation of pathway I reduces this risk. It is known that benzo(a)piren, dimethyl benzanthracene (DMBA) inhibits pathway I and induces glandular tumor (22).

4. ORGANOCHLORINES

A group of organochlorine chemical compounds includes a number of individual materials, like organochlorine pesticides, such as dichlorophenyl-trichloroethane (DDT), lindan and hexachlorobensine, polychlorinated biphenyls PCBs) and d Exxon. In the past, large quantities of these materials were used in agriculture and forestry to protect herbs, just as they were used in the industry. Production and use of PCB and DDT has been forbidden in United States of America (USA) since 1972 and later
Organochlorine compounds degrade slowly, they dissolve in lipids, bioaccumulate in the food chain and may be revealed in human fatty tissue, blood and mother’s milk. The most frequently found organochlorine residues found in human tissue are dichlorophenyl-trichloroethane (DDT), as DDT metabolite and PCB. The level of metabolites of DDT and PCB in human tissue comes in positive correlation by years, as the result of accumulation from the environment and historically high level of exposition. After ban on use of DDT and PCB, level of DDT, DEE and PCG dected in food, human tissue and blood declines in the countries of the western world (23).

These materials are capable of changing hormonally regulated processes and may stimulate changes in growth factor, which correlates immunotoxic, neurotoxic, teratogenic and carcinogenic effects. These chemical pollutants operate by imitating or inhibition of endogen hormons, changing the production of endogen hormons or chang- ing hormonal receptors. Major mechanism of endocrine disorder is bonding to estrogen receptors, inhibition or stimulation of hormone metabolism, change of serum level and transport of hormons (24). These features of organochlorine materials mark them as “endocrine disruptors” (23,25,26). These features, combined with prior common use and intake and increased breast carcinoma incidence, support hypothesis that exposure to these materials may contribute to breast carcinoma development.

The researches focus on estrogen organochlorine pollutant, polychlorinated biphenyls PCBs and pesticide dichloro-diphenyl-trichloroethane (DDT), e.i. its meta-bolic product of dichloro-diphenyl-ethylene (DDE). The presence of organochlorine xenogens correlates with in- crease breast carcinoma incidence with women, disorder of spermgenesis with men and reduction of reproductive capacity with women as well as development defects of neural tube with children. Exposure to these materials is present in professional exposition in a high level, i.e. in low concentration in environmental exposition, which generate difficulties in estimation, taking into consider- ation additional or double exposure from professional and environmental space (31).

Many earlier performed epidemiological studies, which evaluate union of concentration of organochlorine xeno-estrogens in fatty tissue of a breast and risk of breast carcinoma, indicate that women with breast carcinoma have high level of organochlorine materials, including DDT and PCB.

The researches focused on serum level of DDE and PCB in the sample of women with developed breast carcinoma. It was found out that medium value of DDE and PCB level was 11.0±9.1 and 8.0±4.1 ng per milliliter, in patients with breast carcinoma, compared to 7.7±6.8 and 6.7±2.9 ng per milliliter, in control group. The authors conclude that there is fourfold increase of breast carcinoma risk when DDE level is increased from 2.0 ng per milliliter to 19.1 ng per milliliter and suggest that organochlorine residues may be important etiologic factor in development of breast carcinoma and that it implies major changes in public health (27).

Determining the same parameters of DDE and PCB in large samp of 121.700 women, other authors have es- tablished that the medium value of DDE was 6.01±4.56 ng per milliliter, in 236 women with breast carcinoma and 6.97±2.51 ng per milliliter in control group, as well as they have established that medium value of PCB was 5.08±ng per milliliter in 230 women with carcinoma, compared to 5.16±2.26 ng per milliliter in control group. After performing exhausting analysis, authors concluded that data obtained do not support hypothesis that exposure to DDT and PCB increases breast carcinoma risk (28).

Operations of organochlorine xenosterogens is based on induction of cytochrom enzymes, thus on the disorder of hydroxilation route of metabolism of endoten estrogen. It was established that organochlorine xenosterogens reduce the quantity of 2-hydroxiestrons, resulting in increase of 16α-hydroxiestron in ER+ in cultures of cells of human breast carcinoma. 16α-hydroxiestron has full capacity of estrogen, increases cell proliferation and affects development of genotoxic and tumorgenetic effects. Clinical researches suggest that this mechanism may be the first event in multi-grade process of chemical carcinomagenesis (21,29).

Quated data make you conclude that exposure to organochlorine materials, possessing estrogen features, may increase breast carcinoma risk, together with other risk factors (years of age, first pregnancy, lactation period, menopausal status) (30).

European studies reveals the similar results in the re- search on the same topic and are performed at the sample of 341 women with breast carcinoma in Germany, Nether- land, Northern Irland and Spain, where value of DDE level was 1.35 μg per gram of the tissue of the patient with breast carcinoma, compared to 1.5 μg per gram in control group. It is stated in the conclusion that aforementioned researches do not support hypothesis that DDE increases breast carcinoma risk in postmenopausal women in Eu- rope (32).

Lack of bond between level of persistent organochlo- rine components, especially DDE and PCB, has been confirmed through a number case-control researches (33,34,35) (in prospective follow-up researches (36,37), in serum/plazma researches (38), and in fatty tissue re- researches (39).

Some researches are focused on determining connec- tion between detectible organochlorine compounds and individual representatives of PCBs. The results of these researches provides inconsistent data. Increased risk re- lated to the high level of organochlorine dieldrin in the body, observed in the researches with Danish women, has not been confirmed in other research performed on the same population (40).

To find out whether exposure to organochlorine comp-ounds increases the risk of breast carcinoma, the re- searchers checked the hypothesis on subgroups of women defined according to race, menopausal status, history of child-birth and lactation, body mass and gene factors. Re-
results of the researches suggest that positive link between organochlorine materials and breast carcinoma may be evident with Afro-American women, but the researches support neither the importance of racial differences nor differences of subgroups of women based on lactation, menopausal status or use of hormonal therapy (23).

Women with breast carcinoma were grouped based on tumor characteristics and estrogen status (ER) and progesteron (PR) receptors, tumor size, with the research being focused to establishment of possibility of organochlorine compounds level being related to the type and aggressiveness of the tumor. It was only one research that established positive link between the level of organochlorine materials and ER-negative breast carcinoma, while the majority of other researches provide negative correlation (23,39).

Interesting researches have been made in the countries where DDT was used until recently or is still used, therefore majority of women was exposed to direct impact of DDT and its metabolite DDE in food products. This difference may be important for the observation taking into account different estrogen potential of DDT compared to preformed ingested DDE. The results of the research are conflicted, where by one of the researches the link between DDE serum level and breast carcinoma is not established (41) while majority of recent researches suggests significantly higher level of DDE and number of breast carcinoma in control group (30). Women with the largest level of DDE had three times as high risk of breast carcinoma.

Restriction to epidemiologic data is exposure of general population, which took place years before biological measures are performed. Important issue is whether current measurement of body load with organochlorines may reflect exposition in the past adequately. If not, the difference between exposed and control group might be incorrect, hiding the unity between exposition and the disease. Organochlorines persist in environment and human body, whit DDE possessing long lifespan of semi-life (7-11 years), and PBC (5-25 years), which allows measurement of DDE and PCD in different groups during the course of a number of years. The results of the researches of collected tissue in the past do not support unity between DDE and PCB to breast carcinoma (42).

Majority of researches is directed to the evaluation of the exposure of general population, while there are insufficient data about professional exposure of women to organochlorines, which may be very high. Researches on professional exposure of women to PCB and dioxine do not support hypothesis on the relation to a high risk of breast carcinoma (43).

Researches on animal and laboratory models suggests carcinogen activity for some organochlorines. Genotoxic tests of PCB in vitro performed at bacteria systems and human cells in general have negative results (44). Epidemiological researches do not confirm positive link between total body load with persistent organochlorides, especially DDE and PCB, and development of breast carcinoma. The strength of epidemiological data is in consistency of findings in multiple researches and different subgroups as well as in preferring measurements of the level of organochlorines, taking into account that measuring body load reflects actual exposition of biologically important materials.

5. CONCLUSIONS

PAHs, as important pollutant of environment, may be included into breast carcinogenesis, as one of risk factors, i.e. factor contributing to tumor promotion in estrogen-sensitive tissue (5, 20). The hypothesis is based on capability of PAHs to induce cell proliferation of breast epithelium, which contributes to tumor promotion. PAHs and their hydroxilled metabolites operate as estrogen in different estrogen tests (15).

To make the conclusion on organochlorines, there are sufficient epidemiologically-logical data with respect to possible unity between organochlorines (measurable in blood and fatty tissue) and breast risk carcinoma. The data do not support such a unity. Such a conclusion refers to DDT and its major metabolite of DDE as well as PCB.

To make conclusion on actual carcinogen potential of xenoestrogen materials requires establishment of link between level of exposure and size of response, harmonized results of several researches, convincing results of the researches performed at laboratory animals, and confirmation of epidemiological data on humans. Interpretation of epidemiological data on humans is particularly limited with the unknown of exposition doses, i.e. unawareness on response-dose (23).

It is necessary to emphasize that beside operations and evaluation of the risk of individual xenoestrogens, what needs to be taken into account is total contribution of other chemical materials present in human environment and food, which may modify or damage endocrine metabolism (25), i.e. what is needed is estimate of all risk factors in development of breast carcinoma.

If xenoestrogens play vital role in development of breast carcinoma, reduction of exposition to such materials provides opportunity for primary prevention from this disease.

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