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Global pollution by organochlorinated endocrine disruptors – possible challenge for mankind at the onset of millennium

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Motto: "*…today, basic scientific research shows that the mechanisms of actions are much broader than originally recognized.…effects of endocrine disrupting chemicals may be transmitted via germ line epigenetic modifications from continued exposure of offsprings…*"
(from Diamanti-Kandarakis et al. 2009)

Author of this review submits a comprehensive report of his long-lasting research regarding the global pollution by endocrine disruptors (EDs), EDs and diabetes and obesity, EDs and the thyroid in highly polluted Slovakia, Ah-receptor: the central pivot responsible for such global "EDs disaster", EDs and immune system, EDs and testosterone, EDs in mothers and newborns, EDs and human genome, and EDs at the beginning of the millennium.

Short story of the global pollution by endocrine disruptors (EDs)

In the last quarter of the 19th century, during so called "golden era" of German organic chemistry, Ziedler synthesized dichlorodiphenyltrichloroethane (DDT) (1872) and later Swain (1891) polychlorinated biphenyls (PCBs), without being aware of their global impact in the future.

In 1935, Paul Muller revealed that DDT has an insecticide effect when it world widely showed up as a very effective agent in the eradication of mosquitoes. In 1942, Paul Muller received the Nobel Prize for discovering that DDT is a very effective insecticide for several arthropods. Since that time, numerous polychlorinated substances have been synthesized and widely used as insecticides, pesticides or multipurpose industrial chemicals (e.g. PCBs). Thanks to the eradication of insects, the production of foodstuffs and useful plants multiplied, and that period has been called "the green revolution"! However, after a few decades of DDT use, it appeared that its harmful effects on higher animals and humans cannot be excluded.

Nowadays, it is known that such effects of EDs are mediated by aryl hydrocarbon receptor (AhR) which occurs in the cytoplasm nearly in all animal and human cells and has been originally developed to transfer the signals of all hormones with cyclic structure (thyroid and steroid hormones, including androgens, estrogens, gestagens, and corticoids) up to the cell nucleus. By this way, the natural signals of hormones are more or less disrupted by so far not definitely elucidated "hormone/disruptor" ratio, thus resulting either in more or less beneficial (hormone-like) or harmful (toxin-like) function of post receptor cascade.

Already in the fifties of previous century, Swedish chemist Soren Jensen attempted to check for that time already suspected global pollution by DDT by analyzing the fat of eagle from Baltic Sea and, surprisingly, on his chromatograms he found several unknown peaks which were also present in hairs of his children. He was probably the first who faced to the global pollution. However, he did not realize where such substances came from and, while hesitating and rather waiting if somebody else will find some similar data, he published

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them first precautiously as an Anonymous (1966), but more or less bravely just later (Jensen 1972). Actually, in the meantime, a considerable global pollution by EDs already developed since, beginning from twenties, a total of about 1.5 million metric tons of PCBs were produced (de Voogt and Brinkman 1989) and in the meantime, large quantities of those substances were already released into the atmosphere and redistributed via long-range atmospheric transport and ocean streams on a global scale. The global atmosphere was polluted up to the altitude of about 6000 meters, and remarkable amounts of PCBs have been also found in all world oceans, in Arctic and Antarctic, in high mountains and in the middle of all deserts. There are even some heavily polluted spots that are called „PCB reservoirs", such as the Baltic See, Hudson Bay, Aral Lake, Caspic Lake, and Great Lakes in North America, etc. In Eastern Slovakia, such reservoir is the lake Sirava.

Nowadays, there are no more doubts about a considerable global pollution by a number of EDs and about their significant health effects. It appears that the progress of such pollution and of its harmful effects on human and animal health is faster than any attempts to bring such progress under control. Therefore, it should be greatly appreciated that several scientists put their knowledge together in attempting to issue at least some recommendations how to decrease human body burden by those disruptors (Thornton 2007).

During the second half of previous century a considerable global environmental pollution developed due to the tremendous use of several persistent organohalogenated pollutants (POPs), some of them being used as pesticides or fungicides (hexachlorobenzene [HCB], hexachlorocyclohexane [HCH], nonachlor, etc.), insecticides (DDT), defoliants (e.g. dioxin containing Agent Orange in Vietnam war), multipurpose industrial chemicals (PCBs), excellent fire retardants (polybrominated biphenyls [PBBs]) or plasticizers (octylphenol, bisphenol A [BPA]) and finally, some of them originating as unwanted byproducts of large scale chemical industrial production (polychlorinated dibenzodioxins [PCDDs] and dibenzofurans [PCDFs]). Among important members of POPs family are also metabolites of primary substances, like dichloro-diphenoxy-dichloroethylene (DDE - metabolite of DDT) or pentachlorophenol (PTP - metabolite of HCB). All these substances are called EDs, since they considerably alter hormonal regulations via genomic and non-genomic mechanisms.

EDs, diabetes, and obesity

In general, obesity and diabetes have joined the list of adverse effects that have been associated with developmental exposure to environmental estrogens and other endocrine-disrupting chemicals (EDCs) (Newbold 2011). As shown in the International Diabetes Federation Atlas (IDF Atlas, Brussels, 2012), as much as 382 million people had diabetes in 2012, while such figure was predicted to rise up to 472 million in 2030. At the same time, Hu et al. (2011) have denoted type 2 diabetes as a global public health crisis that threatens the economies of all nations, particularly of developing countries. Actually, such numbers are not surprising, since great attention is being paid to the prevalence of diabetes, obesity, hyperlipidemia, and hypertension since about 2-3 decades ago. In highly polluted Slovakia, the survey on diabetes and pre-diabetes in the population exposed to 5th quintile of the sum of five organic chemical substances order (PCBs, DDE, DDT, HCB and HCH) had a more than tripled prevalence of prediabetes and more than six times higher prevalence of diabetes when compared to the 1st quintile (Ukropec et al. 2010). According to Slovak National Center for Health Statistics, the prevalence of diabetes per 100 000 adults increased from 4032 (1996) to 6272 (2010), while at the same time, for instance, the total number of diabetic foot cases with lesion increased from 4568 to 7663. At the end of 2010, about 350 thousand diabetic patients (about 7% of total population) were treated for diabetes by outpatient clinics.

When considering such worldwide increasing rate, Baillie-Hamilton (2002) has suggested a link between post World War 2 increase in organic chemical production and obesity, while Neel and Sargis (2011) have raised concern about the participation of environmental pollutants in this process, which has been strongly supported by our recent data on highly significant correlations of increasing blood levels of DDE, HCB, and PCBs with these of obesity markers (cholesterol, triglycerides, total lipids and body mass index [BMI] value) and diabetes markers (fasting glucose and fasting insulin) in large groups of males and females from highly polluted Eastern Slovakia (Langer et al. 2014).

The role of the plasticizer BPA leaking from epoxy resin lining from metal food and beverages cans and also from plastic beverages flasks - has been recently reviewed by Mirmira and Evans-Molina (2014) using a great number of NHANES data from 2003-2008 as obtained from great number of US citizens and showing

positive correlations between urinary BPA concentration and prevalence of diabetes (Shankar and Teppala 2011), obesity and increased waist circumference (Melzer et al. 2010; Carwile and Michels 2011; Bhandari et al. 2013; Li et al. 2013), while the findings obtained by LaKind et al. (2012) did not show any significant association of urinary BPA with diabetes. Obesogenic effects of EDs have been recently reviewed (de Cock and van de Bor 2014); while Wang and Lobstein (2006) have concluded that increasing numbers of children have to deal with the health consequences and social stigma of being obese. Recent experimental studies have also shown that female rats exposed to PCB 153 had accelerated growth (Sitarek and Gralewicz 2009) or that body weight of mice was increased after in utero exposure to a mixture containing DDT, although the effect of higher doses sometimes appeared opposite from that of lower doses (Palanza et al. 2008).

From the survey shown in this section, the worldwide increased prevalence of diabetes and obesity as positively related to increasing intake of various EDs appears plausibly established.

EDs and the thyroid in highly polluted Slovakia

Thyroid examinations by ultrasound in East Slovakia, but still without any respect to EDs, were started by the evaluation of thyroid volume in schoolchildren after 40 years of iodized salt administration (Tajtakova et al. 1988), with respect to the onset of puberty (Tajtakova et al. 1990; Langer et al. 1994), to possible effect of radioiodine pollution after Chernobyl nuclear accident (Tajtakova and Langer 1993) or to possible influence of high nitrate in drinking water (Tajtakova et al. 2006; Radikova et al. 2008). Attention has been also paid to children with rapidly growing thyroid (Tajtakova et al. 1998).

High pollution of East Slovakia by EDs started about 1950 by a high use of pesticides in agriculture and by a very high amount of waste release to the rivers from three large and newly built chemical factories (one producing PCBs, the other one wooden materials prepared by pentachlorophenol and the last one various plastics). Since all toxic waste was simply released to the adjacent rivers, high pollution of waters, air, landscape, agricultural products and local foods occurred resulting in very high blood levels of EDs in East Slovakian residents.

Since 1993, repeated surveys of EDs effects on humans and the environment were carried out in the great number of subjects living in the area of high pollution ("high-poll") and of low pollution ("low-poll") by the group of analytical chemists headed by Anton Kocan (Kocan et al. 1994a, 1994b; Kocan et al. 2001; Jursa et al. 2006; Petrik et al. 2006), all of them being from the former Institute of Clinical and Preventive Medicine in Bratislava (headed by Tomas Trnovec), who estimated the level of 15 prevalent PCB congeners, HCB, *p,p'*-DDE [2,2'-*bis*(4-chlorophenyl)-1,1-dichloroethylene] and *p,p'*-DDT [2,2'-*bis*(4-chlorophenyl)-1,1,1-trichloroethane], in several thousands of inhabitants by gas chromatography-mass spectrometry as described by Kocan et al. (1994a, 1994b, 2001).

Within one of several surveys (Langer et al. 2003a), a total of 101 adults from "high-poll" area (PCBs level 7300±871 ng/g lipid) and 360 adults from "low-poll" area (PCBs level 2045±147 ng/g lipid) were examined. At the same time, it appeared that in 23 subjects (17 males and six females with PCBs level >10 000 ng/g lipid) from "high-poll" area the highest thyroid volume (ThV) was 18.7±2.32 ml, while in remaining 461 pooled subjects from both areas the ThV was only 14.2±0.29 ml ($p<0.001$).

Within another survey (Langer et al. 1998), significantly higher ThVs ($p<0.001$) were found in 238 adult employees of "Chemko" exposed to PCBs (mean±SE = 18.85±0.69 ml; median = 17.3 ml, upper quartile = 22.9 ml) than in 486 randomly selected adults from "low-poll" area (mean±SE = 13.47±0.48 ml; median = 11.5 ml, upper quartile = 15.3). At the same time, significantly higher ThVs were found ($p<0.001$) in 454 gender-matched 17-year-old adolescents from "high-poll" area (mean±SE = 9.41±0.15 ml; median = 9.0 ml, upper quartile = 11.0 ml) than in 965 of those from "low- poll" area (mean±SE = 8.33±0.09 ml; median = 7.7 ml, upper quartile = 9.8 ml).

When evaluating the role of fish food from highly polluted river Laborec and lake Sirava (Langer et al. 2007a) to which the waste water from those three factories has been directly flowing for several decades via an opened channel, the values of ThV and blood levels of 15PCBs, DDE, HCB, thyroperoxidase antibodies (TPO-ab), free thyroxine (FT4) and impaired fasting glucose (IFG) were first sorted in quintiles. It was found that, in general, all mean values obtained in individual quintiles in "high-poll" area were approximately by 10-30% higher than these obtained in corresponding quintiles from "low-poll" area. Thus, in addition to the level of PCB, DDT and HCB, in all quintiles from "high-poll" area a significant increase ($p<0.01$ - 0.001) has been found in ThV, increased prevalence of posi-

tive TPO-ab as well as that of high values of IFG and FT4.These findings are also in agreement with the data obtained in the same survey showing that among 32 adults representing 16 marital pairs a total of 8 subjects with subclinical hypothyroidism were found (TSH level >3.0 mU/l in 9 cases and >4.5 mU/l in 3 cases) and 16 of those with dysglycemia (fasting glycemia >6 mmol/l). Thus, it appeared that fish from highly polluted local waters belongs to important sources of EDs resulting in thyroid disorders, the latter resulting in increasing TSH level being compatible with subclinical thyroid hypofunction.

In addition, in age and gender matched subjects with increased ThV and organochlorine levels also the hypoechogenicity of thyroid image by ultrasound as well as increased level of TPO-ab, antibodies against thyroglobulin (Tg-ab) and thyrotropin receptor (TSHR-ab), the level and prevalence of which has been significantly higher in "high-poll" area than that in "low-poll" area (Langer et al. 2007b, 2007c),while positive prevalence of TPO-ab in females has been several times higher than that in males (Radikova et al. 2008).

As mentioned above, in most of thyroid studies the prevalence of hypoechogenicity of thyroid ultrasound image as related to increased levels of EDs has been evaluated. When attempting to explain this phenomenon, it should be noted that actually, such hypoechogenicity results from the number of impaired cell-colloid interfaces in the thyroid tissue which represent a dominant factor in sound reflection. In the normal thyroid, a large proportion of sound waves hits against the cell-colloid interface at right angles and is reflected back to the transducer (Fig. 1A). The tissue appears echonormal and its echogenicity is higher than that of surrounding muscles. In contrast, in thyroid tissue with increasing number of damaged follicles, only a small proportion of sound waves hits against the cell-colloid interface and the tissue thus appears hypoechogenic (Fig. 1B).

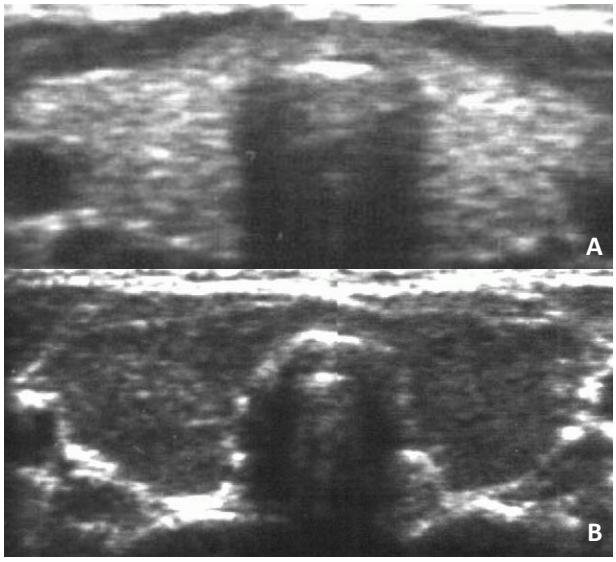

Fig. 1. Ultrasound images of normal thyroid (A) and thyroid with increasing number of damaged follicles (B).

Interrelations between hypoechogenicity of thyroid ultrasound image, as influenced by thyroid antibodies, may be further supported by increased thyroid antibodies prevalence in "high-poll" area vs. those in "low-poll" area. The prevalence of TPO-ab was significantly higher in all women from "high-poll" area vs. those from "low-poll" area (28.4% vs. 20.4%; $p<0.05$), while the respective values of Tg-ab in women aged 31-60 years were 21.3% vs. 14.6% ($p<0.05$) and these of TSHR-ab in 238 age matched men and women were 10.5% vs. 2.5% ($p<0/001$).

The thyroid by ultrasound, TPO-ab and TSH levels in blood were also estimated (Langer et al. 2003b) in 1083 adolescents 13 years old and 1089 of those 17 years old (about 40% boys and 60% girls). It was found that in each of those four groups (as divided by age first, by gender second and then stratified in terms of ThV) the mean values for ThV in the 10th decile of PCBs level were about three times higher than these in the 1st decile and similar significant difference has been obtained also in 13 years old group. Since such differences in ThV and these in hypoechogenicity and TPO-ab prevalence showed a parallel pattern, it was concluded that autoimmune thyroiditis results from increasing PCBs level and thus very likely appears one of the diseases resulting from the effect of EDs on immune system.

In the international project PCBRISK (supported by 5th Framework Program of European Commission under the acronym PCBRISK) a total of 2048 adults (834 men and 1212 women) aged 21-75 years were examined in 2001-2005, among them 1008 subjects from "high-poll" area 1038 subjects from "low-poll" area (that with background PCBs pollution, but with a considerable pollution by pesticides, namely of DDE and HCB.

Main thyroid and metabolic findings obtained in those 2048 adults mentioned above are summarized in Table 1, in which the numbers of several variables is indicating the thyroid and metabolic functions are expressed in terms of serum PCBs level quintiles. Highly significant increase was found in the number of thyroid volumes which were higher than arbitrary limits for individual genders and age groups ($p<0.0000$) as well

Table 1

Prevalence of parameters indicating thyroid and metabolic functions expressed in terms of serum PCBs level quintiles in 2048 adults (834 men and 1212 women) aged 21-75 years examined in 2001-2005

Parameter	Serum PCB level quintiles					Chi-square	p
	1 (n=409)	2 (n=409)	3 (n=410)	4 (n=410)	5 (n=408)		
	Range of level of 15 PCBs in individual quintile (ng/ng lipids)						
	149-627	628-906	906-1341	1347-2343	2350-101413		
	Number of positive values in quintile						
↑ thyroid volume[1]	79	79	84	122	124	29.30	0.000
positive TPOab[2]	83	98	88	126	116	17.56	0.0015
↑ free T4[3]	70	84	114	104	128	28.58	0.0000
↑ total T3[4]	100	105	116	139	132	13.44	0.0093
↑ TSH[5]	4	5	12	7	20	18.56	0.0010
↑ TSH[6]	55	65	48	56	45	5.17	0.2701
↑ fasting glucose[7]	171	190	235	272	295	110.55	0.0000
↑ fasting insulin[8]	88	86	94	105	122	11.89	0.0182
↑ cholesterol[9]	99	121	100	126	124	8.76	0.0673
↑ triglycerides[10]	89	119	127	142	134	19.36	0.0007

Cutoff values for individual parameters: 1-increased (↑) thyroid volume: values for 75th percentile for individual gender and age, for women ≥ 35 years 9.0 ml, < 35 years 11.0 ml; for men ≥ 35 years 11.0 ml, < 35 years 13.5 ml; **2-positiveTPOab:** > 35 U/ml; **3-increased (↑) free T4:** > 19 pmol/l; **4-increased (↑) total T3:** > 2.1 nmol/l; **5-decreased (↑) TSH:** 0.5 mU/l; **6-increased (↑) TSH:** > 4.0 mU/l; **7-increased (↑) fasting glucose:** range 5.6-6.9 mmol/l, diabetes > 6.9 mmol/l (according American diabetes association); **8-increased (↑) fasting insulin:** > 10mUI/l; **9-increased (↑) cholesterol:** > 6.0 mmol/l; **10- increased (↑) triglycerides:** > 2.0 mmol/l

as for following serum variables: the number of positive TPO-ab (p<0.0015), increased serum FT4 (p<0.0000) and serum total T3 (p<0.0093), decreased serum TSH (p<0.0010), increased serum TSH (p<0.2701), increased fasting glucose (p<0.0000), increased fasting insulin (p<0.0182), increased cholesterol (p<0.0673) and increased triglycerides (p<0.0007).

In summary, the data obtained during several surveys from great number of subjects showed that significant increase of ThV was positively related to increased serum level of EDs and also to hypoechogenicity of thyroid ultrasound image which strongly supports the view on autoimmune origin of that ThV increase. In addition, such signs were more frequent in females than in males (Langer 2008; Langer et al. 2008; Langer et al. 2009; Langer 2010; Radikova et al. 2008).

Ah-receptor: the central pivot responsible for such global "EDs disaster"

Since the chemical structure of several cyclic EDs closely resembles that of thyroid and steroid hormones (such as estrogens, androgens and corticoids), at the cellular level they can cross-react with non specific AhR and thus effectively disrupt all actions of those natural hormones at the level of cellular nuclei, as repeatedly summarized by innumerable reports (Hinson and Raven 2006; Craig et al. 2011; Hampl et al. 2014). Thus, at the cellular level, they can act via aryl-hydrocarbon receptor and thus interfere with all hormonal functions of androgens, estrogens, corticoids and thyroid hormones.

Possible multiple interrelations between EDs and several hormones appear a special category of endocrine disruption which has been so far treated predominantly with respect to adrenal steroids.

EDs and immune system

Numerous findings showed that increasing prevalence of several diseases associated with immune response (e.g. asthma, certain autoimmune diseases and cancer) can be hardly related to improved diagnostics alone. As reviewed by Corsini et al. (2008), this trend could be at least partially attributable to some newly widespread chemicals such as pesticides, although some further studies are needed.

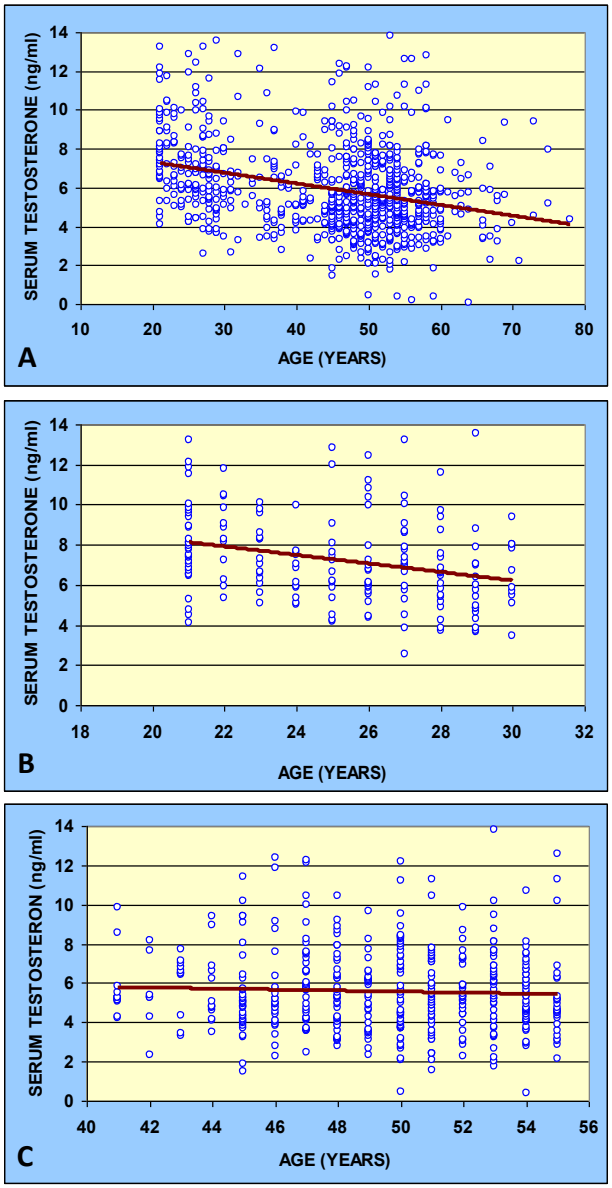

Fig. 2. Serum testosterone levels (ng/ml) in males (A) aged 20-78 years (n=834; p<0.0000), (B) aged 21-30 years (n=175; p<0.001) or (C) aged 41-55 years (n=444; not significant).

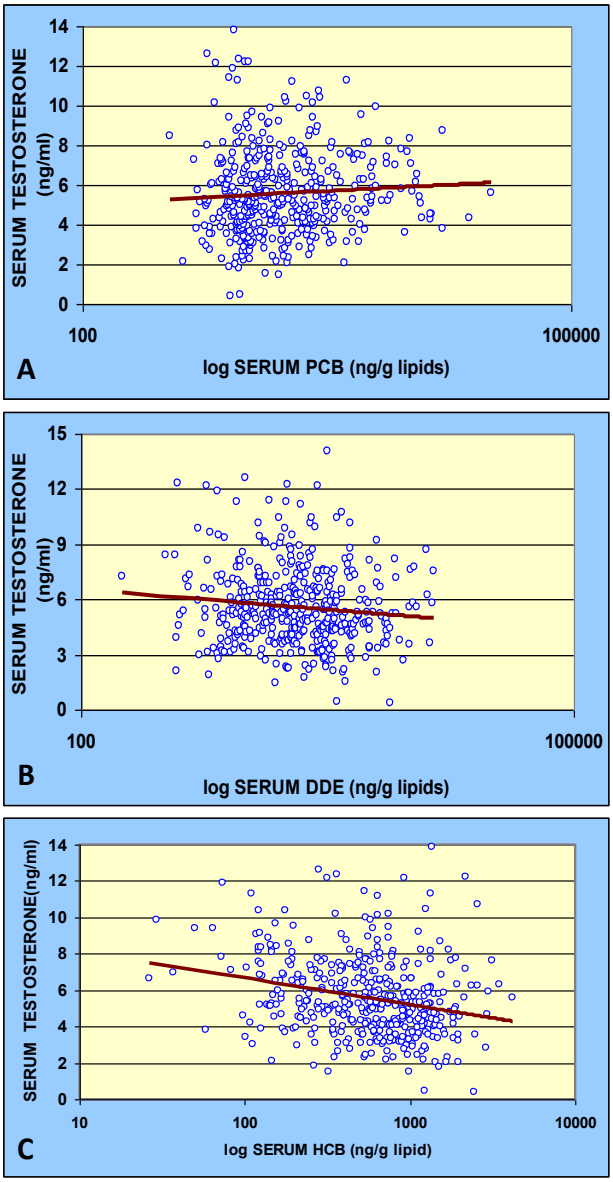

Fig. 3. Correlation between serum levels of testosterone (ng/ml) and serum levels (ng/g lipids) of PCB (A), DDE (B) or HCB (C; p<0.001) in males aged 41-55 years (n=444).

In general, two main effects of EDs on immune system are: 1. decreased capacity to neutralize external organisms, which may result in repeated or prolonged infections as well as in the development of cancer; 2. immunoenhancement, an exaggerated expression of immune response, which adversely leads to immune-mediated diseases (hypersensitivity reactions or autoimmune diseases); 3. inflammatory response, in which immune cells and mediators actively participate.

Some properties make the immune system vulnerable to chemical or physical insults: 1. immune system develops rather late in life (thymus development continues at least until puberty), and bone marrow-derived immune components are continuously being renewed; 2. each response to the pathogen attack, including immune surveillance, demands a delicate control of the balance between activation, silencing and regulation of immune reactivity.

It has been hypothesized that altered immune function may be an early indicator of immunotoxicity, eventually having an effect on immunologically based diseases, such as cancer, hypersensitivity, and autoimmunity. Industrialized countries have been facing a significant increase in some of these conditions over the past few decades, although the rate of increase has recently slowed down.

Although the levels of evidence differ, these diseases can be attributed to environmentally related alteration of immune system. As for instance, about 35 million people in USA suffer from allergic diseases, 2 - 5% of which occurred due to occupational exposure.

EDs and testosterone

In one of the previous surveys (unpublished) also the interrelations between EDs and blood testosterone (TE) level in 834 males aged 20-78 years (Fig. 2A) was found significantly decreasing with age from the average of about 7 to about 4 ng/ml ($p<0.000$). Nevertheless, it should be underlined that the most rapid decrease of TE (from about 6 to 4 ng/ml) has been observed between ages of 21 to 30 years (Fig. 2B), while in 444 males aged 41-55 years the level of TE remained relatively stable (Fig. 2C) in spite of that in all age groups the range of TE level remained about the same, being approximately between 2 and 12 ng/ml.

As shown in the previous Fig. 2C, the level of TE in 444 middle aged males remained relatively stable, this age group has been taken for the estimation of possible effects of EDs on the level of TE. Fig. 3A shows that in spite of extraordinary large range of blood PCBs level (such as 100 to 100.000 ng/g lipids), there was no statistically detectable correlation with TE. Similarly, no correlation has been found between TE and DDE (Fig. 3B). In contrast, however, highly significant decrease ($p<0.001$) of TE has been observed with increasing level of HCB (Fig. 3C).

EDs in mothers and newborns

When comparing PCBs level in mothers and in cord blood of their newborns, highly significant correlation has been found (Fig. 4A). Although highly significant correlation has been found also between mothers and newborns after 16 months of suckling, the level in those newborns has been twice as high as that in mothers (Fig. 4B). For instance, the level of 2000 ng/ml in the mother corresponded to that of 4000 ng/ml in the suckling.

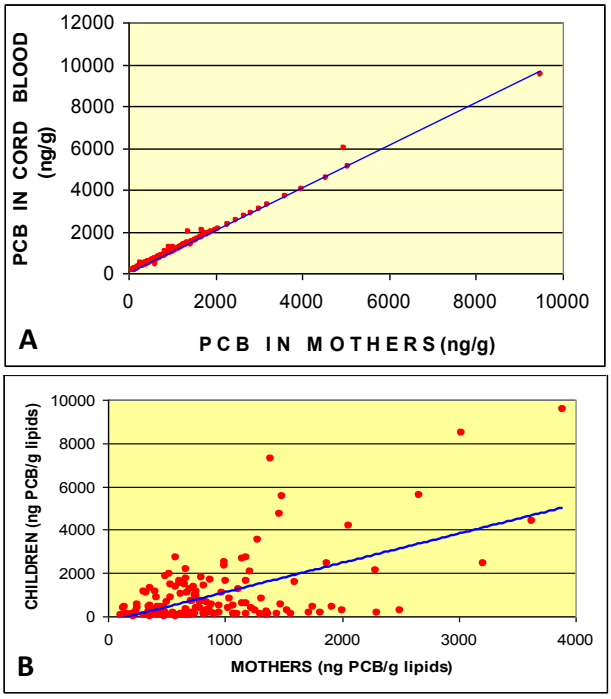

Fig. 4. Correlation between PCB serum levels (ng/ng lipids) in mothers and PCB levels (ng/ng lipids) in blood cord of their newborns (A) or PCB serum levels (ng/ng lipids) in the same children aged 16 months (r=0.586) (B).

EDs and human genome

Recently, interrelations between EDs pollution and human genome have been published. Vezina et al. (2004) have shown that metabolism of xenobiotics is under genetic control indicating that some individuals may be sensitive or resistant to chemical agents. Using genome-wide association studies (GWAS), Lee et al. (2006) have shown that at least 40 genetic loci are associated with the type 2 diabetes.

The ability of environmental factors to promote a phenotype or disease state not only in the individuals exposed but also in subsequent progeny for multiple generations is termed as transgeneration inheritance. The majority of environmental factors such as nutrition or toxicants such as EDs do not promote genetic mutations or alterations in DNA sequence. In contrast, these factors have a capacity to alter the epigenome. Epimutations in the germ line that become permanently programmed can allow transmission of epigenetic transgenerational phenotypes. The above review provides an overview of the epigenetics and biology of how environmental factors may promote transgenerational phenotypes and disease.

In the highly polluted Eastern Slovakia (Dutta et al. 2008, 2012; Mitra et al. 2012) the well defined cohort, consisting of about 46 months old prepubertal girls (belonging to the third generation born after the beginning of heavy PCB and pesticide pollutions), has been classified in two groups: 1. high PCBs group with a PCBs level of 3.02±1.3 ng/mg serum lipid (mainly from industrial pollution); 2. low PCB group with a PCB level of 0.06±0.03 ng/mg serum lipid significantly lower than the sum of other POPs (DDT, DDE, HCB and HCH) and 3. OTP group (other POPs group) with the PCB levels of 0.06±0.03 ng/mg serum lipid (mainly from agricultural pollution).

In conclusion, a highly significant set of 162 differentially expressed genes between high and low PCBs groups ($p<0.00001$) have been identified. The results showed that cell-to-cell signaling and interaction, cellular movement, cell signaling, molecular transport, as well as vitamin and mineral metabolisms were the major molecular and cellular functions associated with the differentially altered gene set in high PCBs-exposed children.

The matched control group of 5 girls was used to determine how the children may be affected by two different exposure scenarios. It appeared that in both PCB and OTP group twelve genes and two unidentified sets associated with connective tissue disorders, genetic disorders, skeletal muscular disorders, and neurological diseases were regulated differentially with respect to the control group. The analyses also pointed out possible organ-specific effects such as cardiotoxicity, hepatotoxicity, and nephrotoxicity in high PCB exposed subjects. A few notable genes, such as BCL2, PON1, and ITGB1, were significantly altered in this study. The related pathway analysis explained their plausible involvement in the respective disease processes, mentioned above.

The ability of environmental factors to promote a phenotype or disease state not only in the individual exposed but also in subsequent progeny for multiple generations is termed transgenerational inheritance. Although the majority of environmental factors such as EDs do not promote genetic mutations or alterations in DNA sequence, but they have the capacity to alter the epigenome. Such epimutations in the germ line that become permanently programmed can allow transmission of epigenetic transgenerational phenotypes as reviewed by Skinner et al. (2010).

EDs at the beginning of millennium?

Considering the great number of data available on EDs and their long term global use, there cannot be any doubts that all continents and oceans have been "pretty peppered" by such persistent substances as originally designed to kill harmful insects and pests or to serve various industrial purposes. Within the rush and multiple problems of everyday life, we nearly did not realize that we are starting to live in a world in which man-made chemicals have become a part of everyday life. Step by step it appeared clear that such chemicals are attempting to disrupt not only some individual metabolic reactions, but also our general health status. Innumerable large scale epidemiological consequences resulting from harmful effects of various EDs are now well documented.

Actually, in 2009 the Endocrine Society issued the Scientific Statement (Diamanti-Kandarakis et al. 2009) saying that: "Endocrine disrupting chemicals (EDCs) were originally thought to exert actions primarily through nuclear hormone receptors, including estrogen receptors (ERs), androgen receptors (ARs), progesterone receptors, thyroid receptors (TRs), and retinoid receptors, among others. Today, basic scientific research shows that the mechanisms are much broader than originally recognized. Thus, EDCs may act via nuclear receptors, non-nuclear steroid hormone receptors (e.g. membrane ERs), nonsteroid receptors (e.g. *neurotransmitter receptors*: such as serotonin, dopamine, norepineohrine receptors, and *orphan receptors*: such as AhR), enzymatic pathways involved in steroid biosynthesis and/or metabolism, and numerous other mechanisms that converge upon endocrine and reproductive systems. *Thus, from the physiological perspective, an endocrine-disrupting substance is a compound, either natural or synthetic, which through environmental or inappropriate developmental exposures, alters the hormonal and homeostatic systems that enable the organism to communicate with and respond to its environment.*"

At the same time, the above mentioned "Statement" recommended that "our chemical policies at local, state and national levels, as well as globally, need to be formulated, financed and implemented to ensure the best public health". Unfortunately, such recommendation, although being supported by numerous data obtained by scientific research, still seems to stay only at the level of recommendation and without any visible effective action of international community Just about 80 years after the first use of DDT to eradicated mosquitoes, it appears that the use of numerous chemical compounds acting by a similar, if not by the same way as that DDT, already resulted in several world wide epidemics of serious human diseases.

Nowadays, we do not have enough data to answer the question, what will happen within the next millennium. It seems that possibly some of the first attempts how to describe the present status of the problem and how to outline its dimensions appeared just two years ago in a comprehensive monograph entitled "State of the Science of Endocrine Disrupting Chemicals 2012" edited by a group of giants in environmental science such as Ake Bergman, Jerrold J. Heindel, Susan Jobling, Karen A. Kid and R. Thomas Zoeller, outstanding and well known experts in current status and perspectives of fighting that "nine-headed dragon" called "Endocrine Disruptors" which is attempting to jeopardize the mankind.

This book appears an assessment of "current state of art" in the field of EDs as prepared by World Health Organization (WHO) for the United Nations Environment Program (UNEP) and, at least it appears to be the first fundamental manual to serve a "sparkle of hope" for all those who feel they can do something for our gorgeous and beloved blue planet.

Is there still time to fight those EDs? Or should we just wait and follow what will go on and what will happen? Is there somebody who will start at least somehow without being considered crazy?

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