INCREASED PREVALENCE AND COINCIDENCE OF ANTINUCLEAR AND ANTITHYROID ANTIBODIES IN THE POPULATION EXPOSED TO HIGH LEVELS OF POLYCHLORINATED POLLUTANTS COCKTAIL

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Objectives. Because of well known association between the exposure to persistent organochlorinated pollutants (POPs) and impaired immune system, it was attempted to check possible coincidence of nuclear and thyroperoxidase antibodies with the levels of major POPs.

Methods. Antinuclear antibodies. (ANA) were estimated by indirect immunofluorescence test using Hep2- cells and thyroperoxidase antibodies (TPOab) by electrochemiluminiscent immunoassay in the cohort of 253 adults (82 males and 171 females) aged 21-75 years, among them 144 (46 males and 98 females) from the area polluted (POLL) by polychlorinated biphenyls (PCB) and 109 (36 males and 73 females) from the area of background pollutrion (BCGR). In the same cohort fifteen congeners of PCB and also total DDE (2,2'-*bis*(4-chlorophenyl)-1,1-dichloroethylene) and hexachlorobenzene (HCB) were estimated by high resolution gas chromatography/mass spectrometry.

Results. Prevalence of ANA only was significantly higher in POLL than in BCGR in males ($p<0.001$) and females ($p<0.001$) and the same was true for the prevalence of TPOab in males ($p<0.05$) and females ($p<0.01$) from POLL. In addition, also the prevalence of coincident ANA+TPOab in males ($p<0.001$) and females ($p<0.05$) was significantly higher in POLL. In a total of 253 pooled males and females from both areas and stratified in terms of PCB level quintiles. The prevalence of ANA in the 4th and 5th quintile of each among three pollutants (PCB, DDE and HCB) was significantly higher ($p<0.01$ or <0.001) and showed the parallel increase with the level of all pollutants.

Conclusions. Significantly increased prevalence of ANA either only or in coincidence with TPOab was found related to increasing level of PCB, DDE and HCB.

Key words: Antinuclear antibodies – Thyroperoxidase antibodies – Polychlorinated biphenyls – DDE - HCB

Antibodies to any nuclear components are called antinuclear antibodies (ANA). These are targeted against intracellular antigens of the cell nucleus such as double stranded and single stranded DNA, histones, nucleosomes, extractable nuclear antigens etc. The potential to generate measurable B cell and T cell autoimmunity to DNA and nucleosomes is being considered an inherited property of the normal immune system and most of these autoantibodies are produced non-specifically as a result of polyclonal B cell activation. From this follows that various species of ANA can be produced by subjects without any actually present clinically relevant disease,

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such as blood donors (Azizah et al. 1996; Spiewak and Stojek 2003) or general population without systemic autoimmune disease (Tan et al. 1997; Cooper et al. 2006). Several studies from various countries also appeared showing the association between juvenile idiopathic arthritis and autoimmune thyroid disorders (Stagi et al 2005; Harel et al. 2006; Unsal et al. 2008)

On the other hand, certain varieties of ANA are important in establishing the diagnosis of systemic lupus erythematosus (Haugbro et al. 2007). However, increasing evidence shows that significant number of subjects has one or more autoantibody following the exposure to organophosphate and organochlorine pesticides and that multiple mechanisms are involved in the initiation and progression of autoimmune disease as reviewed by Powell et al. (1999) and Holsapple (2002).

Within our previous large scale surveys of the population exposed for several decades to heavy industrial and agricultural pollution by persistent organochlorinated pollutants (POPs) very high serum concentrations of polychlorinated biphenyls (PCBs), pesticides (such as dichlophenyl-dichloroethylene – DDE, hexachlorobezene – HCB) as well as these of dioxin (TCDD) were found (Kocan et al. 1994, 2001; Chovancova et al. 2005; Petrik et al. 2006; Jursa et al. 2006; Hovander et al. 2006). In the subjects living in such polluted area we found also significantly increased prevalence of thyroid autoantibodies such as these against thyroperoxidase, thyroglobulin and thyrotropin receptor (Langer et al. 1998, 2003a, 2007a; Radikova et al. 2008) and of antibodies against insulin producing cells such as these to glutamic acid decarboxylase (Langer et al. 2003b). These findings were considered as signs of adverse effects of POPs on immune system possibly resulting in increased prevalence of thyroid autoimmune disorders (Langer et al. 2007b) and diabetes (Rádiková et al. 2004, 2008).

At the same time, however, our attention has been focused on few reports on increased prevalence of ANA in the population exposed to such organochlorinated pollutants. Thus, by indirect immunofluorescence using HEp-2 cells Jennings et al. (1988) found increased prevalence of ANA in 8 positive among 18 workers exposed to dioxin as a result of industrial accident vs. 0 positive among 15 subjects of portering staff and management ($p<0.01$). Similarly, positive HEp-2 cells ANA were found in 25/69 (36.2 %) Japanese exposed to the rice oil contaminated by dioxins, furans and coplanar-PCBs (Nagayama et al. 2001) and the same authors mentioned their previous paper written in Japanese (Tsuji et al. 1999) in which they reported the prevalence of ANA in 36/79 (45.6 %) Japanese subjects. At about the same time increased prevalence of ANA as estimated by similar method on HEp-2 was found in Canadian rural population involved in farming operations and exposed to phenoxyacetic acid, carbamate and organochlorine such as aldrin, chlordane, dieldrin, endrin, heptachlor and lindane (Rosenberg et al. 1999). This has been recently supported by Semchuk et al. (2007) who indicated female gender, BMI in the obese category and recent occupational use of trifluralin or fungicides as well as exposure to oilseed, poultry or dairy production as positive predictors of ANA prevalence as estimated by indirect immunofluorescence on HEp-2 cells. Moreover, among 137 African-American farmers from North Carolina with high plasma DDE levels Cooper et al. (2004) found 16 cases of positive ANA specific to Sm, double stranded DNA, SSA/Ro, SSB/La, histone, ribonucleoprotein, Scl-70, Jo-1 and centromeric antigens. Also in Eastern-Polish rural inhabitants Spiewak and Stolek (2003) observed increased prevalence of ANA to several recombinant nuclear antigens (double stranded DNA, RNP, Sm, SS-A, SS-B, Sci-70, CENP and Jo-1) which was higher than that in other random population. However, using an autoantibody panel including immunofluorescence assays Michalek et al. (1999) did not found any difference in the prevalence of ANA between 914 dioxin exposed Vietnam veterans and 1,186 referents.

As based on our previous findings described above, we assumed a general increase of several autoimmune disorders prevalence in the polluted area. From such reason, several findings by others presented above prompted us to search for the prevalence of ANA in the subjects from a certain area of East Slovakia heavily polluted by PCBs as well as by several other organochlorinated chemicals.

Subjects and Methods

Subjects. Original cohort examined consisted of 2046 adults (834 males and 1212 females) recruited from three East Slovakian districts. Among them was the district of Michalovce, the major part of which was previously found heavily polluted by polychlorinated biphenyls (PCBs) and other organochlorinated pollutants (POLL), while the other two upstream and upwind located districts of Svidnik and Stropkov were defined as areas of background pollution (BCGR). As described in detail elsewhere (Langer et al. 2007), the subjects were recruited by 28 a priori selected local practitioners (about 60 to 100 randomly selected subjects per each) and the examination by survey staff consisted of previ-

ously obtained questionnaire data, physical examination, thyroid ultrasound examination, obtaining 20 ml of blood and spot urine samples from all participants after signing an informed consent document. The procedure was approved by Institutional Review Board and by anonymous reviewers of European Commission.

For the estimation of ANA a cohort of 253 subjects has been selected among those 2046 examined subjects. The aim was to assemble a cohort consisting of about twice as much of females than males, slightly more of those from POLL than from BCGR and, at the same time, encompassing the majority of PCBs level and age range in these particular areas.

Thus, the selected final cohort consisted of 82 males aged 21-75 years (median 51), among them 46 from POLL and 36 from BCGR and of 171 females aged 21-77 years (median 48), among them 98 from POLL and 73 from BCGR. The range of PCBs levels for subjects from BCGR was 149 – 5628 ng/g serum lipid and for these from POLL was 507 – 32,273 ng/g.

Antinuclear antibodies. Antinuclear antibodies (ANA) were detected by indirect immunofluorescence test using Hep2 cells (Euroimmun, Germany), the sera being diluted 1:40.

Thyroid antibodies. Thyroperoxidase antibodies (TPOAb) were estimated by highly sensitive electrochemiluminiscent immunoassay using automatic system Elecsys (Roche, Germany). The cut/off level used for positive values was 37 IU/ml.

Organochlorinated pollutants. Fifteen PCBs congeners (IUPAC numbers 28, 52, 101, 105, 114, 118, 123, 138^{+163}, 153, 156^{+171}, 157, 167, 170, 180 and 189) and also *p,p'*-DDE (2,2'-*bis*(4-chlorophenyl)-1,1-dichloroethylene), *p,p'*-DDT (2,2'-*bis*(4-chlorophenyl)-1,1,1-trichloro-ethane), hexachlorobenzene (HCB) as well as α-, β- and γ-hexachlorocyclohexane (HCH) were determined in serum as described in more detail elsewhere (Kocan et al. 1994b, 2001; Pavuk et al., 2004) by high resolution gas chromatography using an HP 6890 (Agilent, Palo Alto, CA) equipped with a Ni-63 micro-electron capture detector and a 60 m DB-5 capillary column (J&W Scientific, Folsom, MA**).** Sum of all individual PCB congeners was calculated as a sum of PCBs including half LODs for non-detected CBs.

Statistical evaluation. The differences in the prevalence of ANA or TPOab between all males and females from BCGR and POLL as well as between groups of pooled genders stratified either in terms of PCB, DDE and HCB quintiles or in terms of age were evaluated with the aid of Yates' chi-square test.

Results

As shown in Table 1, total prevalence of ANA only was significantly higher in the polluted area than that in the area of background pollution both in females (62/98 = 63.2 % vs. 16/73 = 21.9 % ; $p<0.001$) and in males (21/46 = 45.6 % vs. 2/36 = 5.5 % ; $p<0.001$) and the same was true for the total prevalence of TPOab only in females (49/98 = 50.0 % vs. 24/73 = 32.8 % ; $p<0.01$) and in males (13/46 = 28.2 % vs. 3/36 = 8.3 % ; $p<0.01$). In addition, also the prevalence of coincident ANA+TPOab in females (26/98 = 26.5 % vs. 6/73 ; $p<0.01$) and in males (7/46 = 15.2 % vs. 0/36 = 0 % ;

Table 1

Prevalence of positive ANA and TPOab among the total number (pooled genders) of examined subjects and prevalence of positive TPOab among the total number of ANA positive subjects

Sex	Area	Subjects (total number)	Positive ANA		Positive TPOab		
			Total	%	Total	Coincident with ANA	
						Total	%
Females	POLL	98	62[3)]	63.2	49[5)]	26[8)]	53.1
	BCGR	73	16	21.9	24	6	25.0
Males	POLL	46	21[3)]	45.6	13[4)]	7[9)]	53.8
	BCGR	36	2	5.5	3	0	0.0

Statistical significance between POLL and BCGR
in the prevalence of ANA only: [1)] = $p<0.05$; [3)] = $p< 0.001$;
in the prevalence of TPOab only: [4)] $p<0.05$; [5)] $p<0.01$;
in the prevalence of conicident ANA+TPOab: [8)] $p<0.05$; [9)] $p<0.001$;

Table 2

Number of ANA positive cases in individual quintiles of individual persistent organochlorinated pollutants and their coincidence with TPOab

PCB level quintiles		PCB level range (ng/g lipid)	Number of ANA and TPOab positive cases in quintiles of stratified level of individual pollutants		
Quin-tile	Number of subjects[A)]		PCB[B)]	DDE[B)]	HCB[b)]
1	50 (44)	149 - 679	13 (5 = 38.4 %)	11 (5 = 45.4 %)	11 (9 = 81.8 %)
2	50 (38)	683 – 1012	17 (9 = 52.9 %)	17 (8 = 47.0 %)	18 (7 = 38.8 %)
3	51 (23)	1027 – 1508	17 (6 = 35.3 %)	19[1)] (7 = 36.8 %)	19[1)] (9 = 47.4 %)
4	51 (2)	1517 – 2734	26[2)] (15 = 57.6 %)	24[2)] (12 = 50.0 %)	23[2)] (11 = 47.8 %)
5	51 (1)	2740 – 32,273	28[2)] (11 = 39.3 %)	30[3)] (13 = 43.3 %)	24[3)] (10 = 41.7 %)

[A)] – pooled genders; the numbers in brackets show the number of subjects from background area alloted to the appropriate PCB level quintile)

[B)] – numbers in brackets show the number of positive TPOab among those with positive ANA in the appropriate quintiles of PCB, DDE and HCB

Statistical significance versus the first quintile: [1)] $p<0.05$; [2)] $p<0,01$; [3)] $p<0.001$

Table 3

Prevalence of ANA in young and old subjects from the background and polluted area as related to the mean level of PCB

Subjects		Background area				Polluted area			
		Total number	ANA		Mean PCB[1)]	Total number	ANA		Mean PCB[1)]
			Number	%			Number	%	
Females	Young[A)]	14	2	14.3	495	26	19	73.1[3)]	1367
	Old [B)]	59	14	23.7	761	72	48	59.7[3)]	3313
Males	Young[A)]	7	1	14.3	611	9	6	66.6[1)]	2332
	Old [B)]	29	1	3.4	1293	37	15	40.5[3)]	5384

[A)] = young subjects including those aged 40 years; [<40] = old subjects including those aged 41 years

Table 4

The range of serum level of PCB, DDE and HCB as stratified in terms of quintiles of each individual substance levels

Quintiles ofPCB, DDE, HCB	PCB (ng/g lipid)	DDE (ng/g lipid)	HCB (ng/g lipid)
	min-max level	min-max level	min-max level
1	149 – 679	185 – 909	50 – 332
2	683 – 1012	917 – 1545	339 – 624
3	1027 – 1508	1547 – 2636	631 – 998
4	1517 – 2734	2647 – 3878	1012 - 1513
5	2740 – 32,272	3881 – 14,434	1568 – 12,915

$p<0.001$) was significantly higher in the polluted area. Thus, it may be concluded that the prevalence of all types antibodies investigated was higher in the polluted area than that in the area of background pollution.

Table 2 shows the increase of ANA prevalence in a total of 253 pooled males and females which were examined in both areas and stratified in terms of PCB level to five quintiles. However, the same Table 2 also

shows that the majority of subjects allotted to lower PCB quintiles were from the background area (e.g. 44/50 in the first, 38/50 in the second quintile). In contrast, however, this ratio was inversed in the upper quintiles in which negligibly few persons were from background area (e.g. 2/51 in the fourth and 1/51 in the fifth quintile), while in the middle third quintile the ratio between areas was around 1:1 (exactly 23/51:27/51). From this follows that, at the same time, Table 2 also shows the interrelation between the areas and PCB level in the groups of subjects consisting of both genders by somewhat different way than that presented in Table 1.

In addition, Table 2 shows that the number of subjects with positive ANA was increasing in parallel with the increasing levels of PCB, DDE and HCB. At this occasion, however, it should be noted that the levels of both DDE and HCB are also increasing in parallel with these of PCB. This is supported by the authentic data on the level of individual pollutants (PCB, DDE and HCB) as shown in Table 4, while in Table 2 the level of PCB is being used as an indirect marker of DDE and HCB levels.

Table 3 shows the increased prevalence of ANA in young and old males and females from POLL as compared to the appropriate groups from the BCGR ($p<0.05$ to $p<0.001$) It may be also seen that in all groups of subjects shown in this Table the mean PCB level POLL is about 3-5 times higher than that in BCGR.

Discussion

One of the main aims of this study was to evaluate the possibility whether the increased prevalence of ANA in the cohort from polluted area is associated with the exposure to high environmental organochlorines and thus from xenobiotic-induced autoimmunity. It is well known that the identification or diagnosis of autoimmune disease is usually bound to the presence of specific autoantibodies on one hand and appropriate clinical symptoms on the other. However, in certain initial stages of autoimmune disease the clinical symptoms could be still more or less latent.

Although antinuclear antibodies are the serological hallmark of systemic lupus erythematosus and some other specific lesions (systemic sclerosis, rheumatoid arthritis etc.), we attempted to evaluate whether increased prevalence of ANA could result also from well known immunotoxic effect of organochlorines. Actually, we repeatedly found increased prevalence of thyroid antibodies in the polluted area (Langer et al. 1998, 2007a; Rádiková et al. 2008) and thus we raised the question whether, similarly to that of thyroid antibodies, also the increased prevalence of ANA may result from the impairment of immune system due to the immunotoxic effect of organochlorines, though the simultaneous presence of some specific clinical or subclinical lesions (e.g. systemic lupus erythematosus, systemic sclerosis, rheumatoid arthritis etc.) resulting from ANA cannot be excluded.

Tan et al. (1997) reported that positive ANA as estimated by indirect fluorescence using HEp-2 cells and sera dilution of 1:40 were found in 9 among 16 subjects (56.2 %) with self-reported thyroid disease. Similarly, in this study based on the same sera dilution we found a total of 42 (66.6 %) ANA positive subjects among a total of 62 TPOab positive. It appeared that our cohort showed slightly higher prevalence of ANA than that by Tan et al. (1997) possibly because it consisted of subjects with increased TPOab thus including clinical as well as subclinical cases of thyroid disorders, while that reported by Tan et al. (1997) included only overt and clinically positive self-reported cases.

Our findings confirmed the coincidence of both TPOab and ANA from which it may be concluded that both result from possible impairment of immune system. This observation may be confirmed by several reports by others. There are several findings about the coincidence of ANA with thyroid antibodies (mainly with TPOab). Recently, among 145 children (115 with juvenile idiopathic arthritis [JIA], 17 with lupus erythematosus, 5 with juvenile dermatomyelosis, 4 with sclerodermia and 4 others) there were 6 children (3 with JIA 3 with lupus) who had HEp-2 positive ANA (dilution 1:160 to 1:2560) and also positive TPOab as well as thyroblobulin antibodies (TGab)l. Thus, in 100 % children with thyroid antibodies ANA were found, while among the rest of 139 patients the prevalence of ANA was 34,5 % (Madrid et al. 2009). Pedro et al. (2006) found 91 % prevalence of anti-dsDNA among 51 adults with autoimmune thyroid disease.

From increased prevalence of antithyroid, anti glutamic acid decarboxylase and antinuclear antibodies in subjects from polluted area with high serum levels of various organochlorinated pollutants, it may be suggested that this could result from immunomodulatory disrupting effects of such toxicants.

There are several findings about the coincidence of ANA with thyroid antibodies (mainly with TPOab). Recently Madrid et al. (2009) evaluated the coincidence of TPOab and ANA (estimated by HEp-2 cells at sera

dilution of 1:160 to 1:2560) in 145 children (115 with juvenile idiopathic arthritis [JIA]), 17 with lupus erythematosus, 5 with juvenile dermatomyelosis, 4 with sclerodermia and 4 others) there were 6 children (3 with JIA 3 with lupus) and, among them, there were 6 children (3 with JIA 3 with lupus) also positive TPOab as well as thyroblobulin antibodies (TGab). Thus, in 100 % children with thyroid antibodies also ANA were found, while among the rest od 139 patients the prevalence of ANA was 34,5 % . However, among 81 children with JIA only 4 cases were found with positive TPOab (Unsal et al. 2008). Among 70 adults with active rheumatoid arthritis 26 cases (37 %) were found with positive TPOab (Altzeni et al. 2008) and among 51 adults with autoimmune thyroid disease. Pedro et al. (2006) found 91 % prevalence of anti-ssDNA antibodies among 51 adults with autoimmune thyroid disease.

In conclusion, we found significant coincidence of ANA (as estimated by HEp-2 cells at sera dilution of 1:40) and thyroperoxidase antibodies which was further significantly associated with the level persistent organochlorinated pollutants such as polychlorinated biphenyls (PCB), dichlorophenyl-dichloroethylene (DDE) and hexachlorobenzene (HCB).

References

Akins JR, Waldrep K, Bernert JT: The estimation of total serum lipids by completely 'summation' method. Clin Chim Acta **62**, 559–564, 1989

Ayotte P, Dewailly E, Ryan JJ, Bruneau S, Lebel G: PCBs and dioxin-like compounds in plasma of adult Inuit living in Nunavik (arctic Québec). Chemosphere **34**, 1459–1468, 1997

Azizah MR, Azila MN, Zulkifli MN, Norita TY: The prevalence of antinuclear, anti-ds DNA, anti-SM and anti-RNP antibodies in a group of healthy blood donors. Asian Pac J Allergy Immunol **14**:125–128, 1996

Brunn J, Block U, Ruf G, Bos I, Kunze WP, Scriba PC: Volumetrie der Schilddrüsenlappen mittels real time Sonographie. Dtsche med Wochenschrift **106**,1138–1140, 1981

Chovancová J, Kocan A, Jursa S: PCDDs, PDFs and dioxin-like PCBs in food of animal origin (Slovakia). Chemosphere **61**,1305–1311, 2005

Cooper GS, Martin SA, Longnecker MP, Sandler DP, Germolec DP: Associations between plasma DDE levels and immunologic measures in African-American farmers in North Carolina. Environ Health Perspect **112,** 1080–1084, 2004

Cooper GS, Parks CG, Schur PS, Fraser PA: Occupational and environmental associations with antinuclear antibodies in a general population sample. J Toxicol Environ Health **69,** 2063–2069. 2006

Fangstrom, B, Athanasiadou M, Grandjean P, Weihe P, Bergman A: Hydroxylated PCB metabolites and PCBs in serum from pregnant Faroese women. Environ. Health Perspect. **110**, 895–899, 2002

Guo YL, Yu M-L, Lin L-Y, Hsu CC, Rogan WJ: Chloracne, goiter, arthritis and anemia after polychlorinated biphenyl poisoning: 14-year follow-up of the Taiwan Yucheng cohort. Environ Health Perspect . **107**, 715–719, 1999

Haugbro K, Nossent JC, Winkler T, Figenschau Y, Rekvig OP: Anti-dsDNA antibodies and disease classification in antinuclear antibody positive patients: the role of analytical diversity. Ann Rheum Dis **63**, 386–394, 2007

Holsapple MP: Autoimmunity by pesticides: a critical review of the state of the science. Toxicol Lett **127**, 101–109, 2002

Hovander L, Linderholm L, Athanasiadou M, Athanassiadis I, Bignert A, Fangström B., Kocan A, Petrik J, Trnovec T, Bergman Å. Levels of PCBs and their metabolites in the serum of residents of a highly contaminated area in eastern Slovakia. Environ Sci Technol **40**, 3696–3703, 2006

Kavlock RJ, Daston GP, DeRosa C, Fenner-Crisp P, Gray LE, Kaattari S et al: Research needs for the risk assessment of health and environmental effects of endocrine disruptors. Environ Health Perspect **104**, suppl. 4, 15–40, 1996

Kocan A, Petrik J, Drobna B, Chovancova J: Levels of PCBs and some organochlorinated pesticides in the human population of selected areas of the Slovak republic. Chemosphere **29**, 2315–2325, 1994.

Kocan A, Petrik J, Jursa S, Chovancova J, Drobna B: Environmental contamination with polychlorinated biphenyls in the area of their former manufacture in Slovakia. Chemosphere **93**, 595–560, 2001.

Langer P, Tajtakova M, Fodor G, Kocan A, Bohov P, Michalek J, Kreze A: Increased thyroid volume and prevalence of thyroid disorders in an area heavily polluted by polychlorinated biphenyls. Eur J Endocrinol **139**, 402–409, 1998

Langer P, Tajtakova M, Bohov P, Klimes I: Possible role of genetic factors in thyroid growth rate and in the assessment of upper limit of normal thyroid volume. In: iodine-replete adolescents. Thyroid **9**, 552–562, 1999

Langer P, Kocan A, Tajtakova M, Petrik J, Chovancova J, Drobna B, Jursa S, Pavuk M, Koska J, Trnovec T, Sebokova E, Klimes I: 2003a. Possible effects of polychlorinated biphenyls and organochlorinated pesticides on the thyroid after long-term exposure to heavy environmental pollution. J Occup Environ Med **45,** 526–532, 2003

Langer P: Persistent organochlorinated pollutants (PCB, DDE, HCB, dioxins, furan) and the thyroid – Review 2008. Endocrine Regul **42**, 79–104, 2008

Langer P, Tajtakova M, Kocan A, Petrik J, Koska J, Ksinantova L, Radikova Z., Ukropec J, Imrich R, Huckova M, Chovancova J, Drobna B, Jursa S, Vlcek M, Bergman A, Athanasiadou M, Hovander l, Shishiba Y, Trnovec T, Sebokova E, Klimes I..Thyroid ultrasound volume, structure and function after long-term high exposure of large population to polychlorinated biphenyls, pesticides and dioxins. Chemosphere **69**, 118–127, 2007

Madrid CA, Fernandez AG, Munoz ML, Rodruguez MAM, Munoz PM, Nolina JG-C: Alteraciones tiroideas y anfermedades reumaticas infantiles. Anales de Pediatria **70**, 53–56, 2009

Nagayama J, Tsuji H, Iida T, Hirakawa H, Matsueda T, Ohki M: Effects of contamination level of dioxins and related chemicals on thyroid hormone and immune response system in patients with "Yusho". Chemosphere **43**, 1005–1010, 2001

Pedro AB, Romaldini JH, Americo C, Takei K: Association of circulating antibodies against doublestranded and singlestranded DNA with thyroid autoantibodies in Graves' disease and Hashimoto's thyroiditis patients. Exp Clin Endocrinol Diabetes **114**: 35–38, 2006

Powell JJ, Van de Water J, Gershvin ME: Evidence for the role of environmental agents in the initiation or progression of autoimmune condition. Environ Health Perspect **107**, suppl. 5, 667–673, 1999

Pratt EL, Tarantini MD, Wagner D, Pescovitz OR, Bowyer S, Shapiro AD: Prevalence of elevated antithyroid antibodies and antinuclear antibodies in children with immune thrombocytopenic purpura. Am J Haematol **89,** 175–149, 2005

Radikova Z, Koska J, Ksinantova L, Imrich R, Kocan A, Petrik J, Huckova M, Wsolova L., Langer P, Trnovec T, Sebokova E, Klimes I: Increased frequency of diabetes and other forms of dysglycemia in the population of specific areas of eastern Slovakia chronically exposed to contamination with polychlorinated biphenyls (PCB). Organohalogen Compounds **66**, 3547–3551, 2004

Radikova Z, Koska J, Huckova M, Ksinantova L, Imrich R, Vigas M, Trnovec T, Langer P, Sebokova E, Klimes I: Insulin sensitivity indices: A proposal of cut-off points for simple identification of insulin-resistant subjects. Exp Clin Endocrinol Diabetes **114**, 249–256, 2006

Radikova Z, Kocan A, Huckova M, Langer P, Trnovec T, Sebokova E, Klimes I: Environmental human contamination with persistent organochlorine pollutants and development of diabetes mellitus. A minireview. In: PCBs – Human and Environmental Disposition and Toxicology (Eds. L. Hansen, L. Robertson), pp. 107-113. University of Illinois Press, Urbana, ILL 2007

Radikova Z, Tajtakova M, Kocan A, Trnovec T, Sebokova E, Klimes I, Langer P: Possible effects of environmental nitrates and toxic organochlorines on human thyroid in highly polluted areas in Slovakia. Thyroid **18,** 353–362, 2008

Rosenberg AM, Semchuk KM, McDuffie HH, Ledingham DFL, Cordeiro DM, Cessna AJ, Irvine DG, Senthilselvan A, Dosman JA: Prevalence of antinuclear antibodies in a rural population. J Toxicol Environ Health A **25**, 225–236, 1999

Semchuk KM, Rosenberg AM, McDuffe HH, Cessna AJ, Pahwa P, Irvine DG: Antinuclear antibodies and bromoxynil exposure in a rural sample. J Toxicol Environ Health A **25**, 225–236, 2007

Spiewak R, Stojek N: Antinuclear antibodies among eastern-Polish rural inhabitants. Ann Agric Environ Med **10**, 207–209, 2003

Stagi S, Giani T, Simioni G, Falcini F: Thyroid function, autoimmune thyroiditis and coeliac disease in juvenile idiopathis arhritis. Rheumatology **44**, 517–520, 2005

Tan EM, Feltkamp TEW, Smolen JS, Butcher B, Dawkins R, Fritzleer MJ et al: Range of antinuclear antibodies in "healthy" individuals. Arthritis Rheum **40,** 1601–1611, 1997

Unsal E, Oren O, Salar K, Makay B, Abaci A, Ozhan B, Bober E: The frequency of autoimmune thyroid disorders innjuvenile arthritis. Turk J Pediatr **50,** 462–465, 2008