

## REVIEW

## From naturally occurring goitrogens to the effects of anthropogenic endocrine disruptors on the thyroid in Slovakia

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### Abstract

**The objective is to describe the effect of mandatory and well monitored prophylaxis of endemic goitre in Slovakia with the use of iodised salt for past 50 years and previous experimental attempts to assess a possible participation of naturally occurring goitrogens in the etiology of goitre. Previous observations by others showed a striking decrease of goitre prevalence in schoolchildren as early as few years after starting the prophylaxis in Slovakia and this success has been confirmed by a European study conducted by others in mid nineties. In the meantime, however, massive industrialisation of Slovakia and environmental negligence of the administration resulted in heavy airborne and mainly waterborne pollution of the food chain in certain areas around large chemical factories by industrial waste including polychlorinated biphenyls (PCB). Since Slovakia has been recently evaluated as an iodine replete country as based on the findings of the low thyroid volume by ultrasound and high urinary iodine, it became possible to evaluate the participation of factors different from iodine deficiency in the thyroid growth. Recent field surveys including an extensive international study PCBRIK repeatedly showed highly increased blood PCB level in subjects living in the polluted area and, at the same time, increased thyroid volume by ultrasound, increased frequency of positive thyroid antibodies and impaired glucose tolerance. (Ref. 59.)**

**Key words:** goitrogens, anthropogenic endocrine disruptors, thyroid gland, Slovakia.

Each population living in the landlocked territory of Slovakia since ancient times has been presumably a target of iodine deficiency which resulted in endemic goitre. In early fifties, the high prevalence of goitre has been supported by excellent survey conducted by Podoba (senior) and his colleagues (Podoba, 1962). They examined about 5 percent of actual total population in Slovakia (e.g. 160 thousands) in 612 villages regularly distributed over the whole territory and found up to 70 % of goitres in females in mountainous areas and 30–40 % in lowlands. In males, the prevalence was about 30 percent of that found in females.

Due to high consumption of cabbage in some mountainous areas, in early fifties we started to search for possible participation of cabbage goitrogens as discovered in 1928 by Chesney and co-workers in rabbits and later understood by Astwood et al (1949) who, among others, identified L-5-vinyl-2-thioxazolidone (VTO) as antithyroid substance in plant seeds of Brassica family. Later, in several species of Brassica family (including cabbage, kohlrabi, cauliflower etc.) we found thiocyanate which also shows certain antithyroid activity (Michajlovskij and Langer, 1956). It was suggested to use a simple chemical estimation of this substance in

blood as a useful marker of that time still unidentified group of more potent antithyroid substances called “Brassica-factor” (Langer, 1964). This suggestion was based on our findings which showed that the level of thiocyanate in serum is progressively increasing during the period of repeated daily consumption of rela-

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tively small amounts of cabbage and that in several thousands of examined subjects the level of thiocyanate in serum was the highest in September and October which coincides with the increased seasonal consumption of cabbage.

Nevertheless, since iodine deficiency is the main etiological factor resulting in increased thyroid volume, we definitely concluded that we cannot demonstrate any possible additional effects of Brassica plants on thyroid volume in certain areas and certain seasons of increased cabbage consumption. This was essentially because that time the assessment of thyroid size by a simple semiquantitative palpation was the only method available for thyroid volume estimation. Nevertheless, our only valid observation on the effect of raw cabbage on the thyroid activity in human beings was a decreased radioiodine uptake by thyroid of human volunteers after daily consumption of 500 g cabbage for 14 days as compared to the uptake before such period of cabbage consumption (Langer et al, 1971).

From such reasons we changed our original aim and started to investigate the chemical nature of "Brassica factor" as well as the metabolism and mechanism of action of individual goitrogenic substances. Using ether extraction, ultraviolet spectrophotometry and chromatography, we found L-5-vinyl-2-thioxazolidone which was previously known only as a component of seeds, also in edible parts of "Brassica factor" (Langer and Gschwendtová, 1969). Later a goitrogenic effect of allylthiocyanate, a most widespread mustard oil in plant kingdom was demonstrated in rats (Langer and Štolc, 1965) and later also that of other isothiocyanates (e.g. methyl- and butylisothiocyanate) *in vitro* (Langer and Greer, 1968).

Based on these findings, it was subsequently demonstrated in rats that the chemical nature of "Brassica factor" may be explained as a synergistic effect of thiocyanate, isothiocyanates and thioxazolidones (Langer, 1964, 1966). Later a goitrogenic activity of synthetic thiocarbonyl derivatives and 2-thiohydantoins was found. Such thiocarbonyl derivatives may be obtained by the reaction of isothiocyanates with amino acids under mild temperature and 2-thiohydantoins originate from a spontaneous conversion of those thiocarbonyl derivatives *in vitro*. However, such metabolic steps may also spontaneously occur *in vivo* after ingestion of exogenous isothiocyanates and thus it has been suggested as one of possible mechanisms of antithyroid effects of these substances (Langer et al, 1964).

Later we found new metabolite of L-5-vinyl-2-thioxazolidone in urine, defined its structure as L-5-vinyl-4-hydroxy-2-thioxazolidone (Michajlovskij and Langer, 1971) and demonstrated its goitrogenic activity in rats (Langer and Michajlovskij, 1969). Further studies showed that thiocyanate is displacing thyroxine from the binding to plasma proteins thus increasing the level of free thyroxine (Michajlovskij and Langer, 1974) and also changing the distribution of thyroxine in the organism (Langer et al, 1976). The single peroral dose of thiocyanate resulted in a short and striking inhibition of radioiodine uptake by thyroid in rats followed by a period of increased radioiodine uptake resulting presumably from increased thyrotropic function of the pituitary (Langer, 1968).

### **Slovakia among European countries with the most successful iodine prophylaxis**

In the meantime (e.g. within 1955–1985), however, the epidemiological status of endemic goitre in Slovakia dramatically changed namely due to well organised and monitored production and strictly monitored mandatory consumption of iodised salt which has been proposed to government authorities in early fifties by Podoba (Senior). As early as 5 years after the beginning of countrywide iodised salt distribution the prevalence of goiters in adolescents considerably decreased (Németh et al, 1955). Finally, the European Thyromobil Study in 1995–1996 which collected data on thyroid volume in about 7500 adolescents from 12 European countries showed that Slovakia belongs to few countries with the lowest thyroid volume in schoolchildren and highest iodine concentration in urine, Podoba (junior) being one of the main participants conducting this study (Delange et al, 1997).

However, such study was only possible thanks to thyroid volumetry by ultrasound which, since the beginning of eighties, replaced the obsolete semiquantitative examinations of thyroid size by palpation and thus became a new generation methods suitable for the studies on epidemiology of thyroid disorders. The evaluation of thyroid volumes in 6324 inhabitants of Slovakia aged from 7 to 70 years (Tajtaková et al, 1998, 2000) also showed relatively small values, the 75th percentile being 5.2 ml (10 yr), 10.2 ml (17 yr), 16.3 ml (31–40 yr) and 19.9 ml (61–70 yr).

### **Large scale industrialisation of Slovakia and heavy environmental pollution**

Simultaneously with the improvement of iodine intake by Slovak population the deterioration and heavy chemical pollution of the environment was silently going on which was namely due to massive industrialisation in fifties and sixties and unlimited use of pesticides when attempting to reach the highest possible yields of grain. During the previous regime any scientific or even public information about possible environmental pollution resulting from these activities has been very strictly banned, no preventive measures were undertaken and thus the uninformed population used contaminated drinking water and foods for decades. At the same time, however, the Western countries paid increasing attention namely to the chemical pollution of environment.

In early fifties three giant chemical plants started to produce large scale artificial fibers (Chemlon Humenné), chemically processed wood products (Bukóza Vranov) and various organic chemicals including polychlorinated biphenyls, explosives etc. (Chemko Strážske). Liquid waste has been disposed to the local rivers without any preventive measures and also the airborne pollution does not seem to be negligible. Such environmental negligence resulted in heavy contamination of soil, superficial and underground waters and namely of food chain. Such circumstances remained strictly latent for decades and thus the present status should be considered as a sequel or outcome of

previous regime and should not be considered a responsibility of the present management of those chemical plants.

Some idea about the magnitude of pollution may be obtained from the data published in the daily paper "Národní Obroda" in August 1995. Thus, in 1990 (first year after the fall of previous regime in former Czechoslovakia) 5920 tons of organic chemicals and 1744 tons of insoluble waste has been disposed to the river Ondava from the factory Bukóza. Moreover, in 1998 it was found that the sediments of the open channel from the factory Chemko to the river Laborec contain several hundred tons of polychlorinated biphenyls. Although this channel is no more in use, it is being occasionally filled by rain water or melting snow and thus some toxic material is still being flushed down to the river and to the artificial lake of Širava (Kočan et al, 2001).

### Endocrine disruptors

A half century ago the Swiss chemist Paul Muller won the Nobel prize for the discovery of DDT which appeared to be an excellent insecticide namely for the fighting malaria. Later several similar chlorinated compounds were synthesised which showed insecticide, pesticide or fungicide activity. Nevertheless, the mankind possibly forgot that all such -icides could be effective also in the animals and human beings. Strong objections against environmental pollution started to appear after the alligators in the lake Apopka (Florida) (Guillette et al, 1991) and cormorans at the Californian costs vanished (Gilbertson et al, 1991) and after thyroid hyperplasia has been found in a great percentage of salmon in lake Michigan (Sonstegard and Leatherland, 1974).

During last several decades the global environment has been polluted by organochlorinated pesticides, insecticides, industrial chemicals (mainly polychlorinated biphenyls or PCBs) and byproducts of large scale chemical syntheses (such as dioxins and furans). The structure of most of them closely resembles that of thyroid hormones or synthetic nonsteroid estrogens such as diethylstilbestrol. These substances were recently called "endocrine disruptors" which were defined as "exogenous agents that interfere with the production, release, transport, metabolism, binding, action or elimination of natural hormones" (Kavlock et al, 1996). In addition to endocrine disruption, the main toxic effects include carcinogenicity, immunotoxicity, dermal- and hepatotoxicity, teratogenicity, neurobehavioral effects and numerous metabolic effects.

The effects of endocrine disruptors are either receptor mediated or non-receptor mediated. Most of them are mediated by Ah-receptor (Ah=aryl hydrocarbon) or by the members of steroid/thyroid/retinoid receptor superfamily, both of them functioning as ligand-activated transcription factors (Safe, 1995; Hansen, 1998). After the binding of disruptor ligand to the receptor, the translocation of such complex to nucleus results which is followed by the binding to specific DNA sequences resulting in the expression or inhibition of specific genes.

One of the non-receptor mediated effects, for instance, is the displacement of thyroid hormone from plasma protein binding.

Originally, the views prevailed on the main toxic effects of so called "coplanar" PCB congeners (with coplanar orientation of both phenyls thus resembling the structure of most toxic 2,3,7,8-tetrachlorodibenzo-para-dioxin), but recently such opinion has been revised by Hansen (1998) who turned the attention to so called "ortho-chlorinated" congeners the effect of which is apparently non-receptor mediated.

The effects of endocrine disruptors are based on three mechanisms: 1) reversible binding to specific molecules (receptors, transport proteins, enzymes etc.), 2) irreversible covalent reaction with target molecules (DNA, proteins), 3) accumulation in adipose tissues and possibly also in lipid containing cellular structures (e.g. cell membranes etc.).

### Effects of PCB on the thyroid

One of less commonly known and recently emerged effects are these on the thyroid function. Among the main antithyroid effects found in animals belongs a decrease of blood thyroxine level either due to a displacement of thyroxine from plasma binding proteins or increased conjugation rate in the liver or both (Schuur et al, 1997). Although some authors found a reciprocal increase of thyrotropin (TSH), recent views suggest that some PCB congeners are suppressing TSH release in spite of profound decrease of thyroxine level (Tilson and Kodavanti, 1997). In addition, thyroid ultrastructural alterations were found which were distinct from those observed after TSH stimulation, so they are considered to result from a direct PCB effect on the thyroid tissue (Capen and Martin, 1989). Finally, PCB may also interfere with the transport of thyroxine into the cell, thyroxine to triiodothyronine conversion, thyroid hormone action and even modulate the triiodothyronine binding to its nuclear receptor.

As recently reviewed (Brucker-Davis, 1998; Brouwer et al, 1998; Karmaus, 2001), several organochlorinated substances inhibit the binding of thyroxine to plasma carrier proteins also in man. They also decreased the level of total and free thyroxine in plasma, but the data about the influence on TSH level are contradictory. PCBs appeared to be inducers of liver UDP-glucuronosyltransferase and thus of increased biliary excretion of thyroxine- and triiodothyronine-glucuronate. Because of the structural similarity, PCB may also interfere with the transport of thyroxine into the cell, with the conversion of thyroxine to triiodothyronine, may mimic the thyroid hormone action and, finally, even interfere with the binding of triiodothyronine to its nuclear receptor.

From several recent reviews it appears that less is known about possible effects of chronic, low background level general exposure of human population and further field studies in this respect should be undertaken. Among the first reports was the finding of 11 percent prevalence of hypothyroidism in workers from polybrominated biphenyls producing factory (Bahn et al, 1980). From collected data on 13482 workers exposed to chlorophenoxy herbicides or chlorophenols who were followed for 17 years it was found, among others, that 4 of them died from

thyroid cancer which was significantly more than no case of thyroid cancer in 4908 workers with unknown exposure or probably exposed (Saracci et al, 1991). Thirty five years after the accident in chemical factory in Hamburg during which the workers were contaminated by dioxin, significantly higher percentage of thyroid diseases (7 % vs 1.2 %) was found in the exposed group of 158 workers compared to 161 referents (Zober et al, 1994). Significantly increased incidence of thyroid cancer (3 new cases within 10 years among 5003 inhabitants) and significantly higher mortality for neoplasms of unknown origin was found in a Spanish village located closely to organochlorinated substances producing factory (Grimalt et al, 1994). Three cases of thyroid cancer were found among 81 survivors 28 years after Yusho accident with PCB poisoned rice oil in Japan (Tsuji et al, 1999). In a statistical study based on excellent Norwegian cancer register the data on a total of 1.2 million women with a total of 2409 thyroid cancers were evaluated. Among them the risk of thyroid cancer was significantly increased in a group of 40,839 women married to fishery workers with 174 thyroid cancers (Frich et al, 1997). According to our opinion, this might be related to increased concentration of organochlorinated substances in fish.

It should be emphasised that the employees of the factory Chemko Strážske and the population living in a vicinity have been exposed to heavy pollution for more than four decades without any effective protection. This may be strongly supported by very high PCB levels in their serum ( $7300 \pm 871$  ng/g lipids) as compared to the cases examined 13 years after Yucheng disease in Taiwan ( $2820 \pm 295$  ng/g) (Guo et al, 1997), to crab consumers in Norway (1481 ng/g) (Johansen et al, 1996) and to fishermen's wives in Sweden consuming contaminated fish from Baltic sea ( $574$ – $1610$  ng/g) (Hagmar et al, 2001 a), while the same authors did not find any similar difference in male fishermen (Hagmar et al, 2001 b). Several of these and other observations show the contaminated fish to be one of the most important PCB sources in human nutrition (Persky et al, 2001).

Osius et al (1999) found increased 95 percentile value of TSH and free triiodothyronine level in 57 second grade school-children living 20 km from the toxic waste incinerator plant as compared to 583 children living in nearby areas, while no difference in free thyroxine level was observed. However, statistically significant was only a positive association of some PCB congeners with TSH and negative one with free triiodothyronine level. Nagayama et al (2001) did not find any changes of serum levels of total thyroxine, total triiodothyronine and TSH level in 16 Yusho survivors with the concentrations of polychlorinated dioxins, furans and coplanar PCB in serum about 7 times higher than in normal Japanese population. Sala et al (2001) reported a negative correlation between hexachlorobenzene and total thyroxine level in serum, while no interrelation was found with TSH level.

Nevertheless, due to their low body weight, the human newborns and sucklings are considered the most exposed fraction of human population (Pluim et al, 1993; Koopman-Esseboom et al, 1994).

### First results of the examination of exposed population in East Slovakia

During the first preliminary survey in 1993-94 (Langer et al, 1998) a total of 238 employees of Chemko factory has been examined as well as 486 control adults from various areas of East Slovakia (Bardejov, Košice, Moldava) and Orava. With the aid of ultrasonographic volumetry significantly higher ( $p < 0.001$ ) thyroid volume has been found in Chemko employees (mean  $\pm$  SE =  $18.85 \pm 0.69$  ml) as compared to controls ( $13.47 \pm 0.48$  ml;  $N = 486$ ). Similarly, in 17-yr old adolescents ( $N = 454$ ) from the polluted city of Michalovce the thyroid volume (mean  $\pm$  SE =  $9.37 \pm 0.17$  ml) was significantly higher ( $p < 0.001$ ) than that in 965 17-yr old control adolescents from the area of background pollution around the cities of Bardejov, Košice, Ružomberok and Trstená ( $8.07 \pm 0.10$  ml). The increase in adolescents was less remarkable presumably because a shorter duration of exposure.

In addition, in Chemko employees an increased prevalence of positive autoantibodies against thyroperoxidase (34.2 % vs 25.2 % in females aged 31–50 yr,  $p < 0.05$ ), against thyroglobulin (21.3 % vs 14.6 % in females aged 31–60,  $p < 0.05$ ) and against TSH-receptor (10.5 % vs 2.5 % in all 238 employees against twice as much of age and sex matched controls) (Langer et al, 1998). Retrospectively (Langer et al, 2002), we also found a highly increased ( $p < 0.001$ ) frequency of positive anti-GAD antibodies (GAD = glutamic acid decarboxylase) in 240 Chemko employees (40.4 %) compared to 704 controls (10.5 %). These findings strongly support the immunomodulatory effects of PCBs which are well known from animal experiments and observations in freely living animals, while the studies in human population did not yet yield conclusive data. This problem has been recently discussed by Baccarelli et al (2002) who found significantly decreased IgG level in the population exposed to dioxin after the accident in Seveso (Italy) more than 20 years ago, while the level of IgM, IgA, C3 and C4 did not show any difference as compared to control population.

Finally, we started to follow the idea based on the view that, if there exist some carcinogenic effects of PCBs, there should be some increased prevalence of positive levels of some tumour markers in the polluted area. Thus, we started to estimate the level of some broad spectrum markers such as  $\alpha$ -fetoprotein, thymidine kinase and  $\beta$ 2-microglobulin as well as of thyroglobulin as a possible marker of increased rate of thyroid growth. However, so far we did not find any considerable differences in the prevalence of  $\alpha$ -fetoprotein, thymidine kinase and thyroglobulin, but we observed significantly higher frequency of low serum levels of  $\beta$ 2-microglobulin in Chemko factory employees which might be related either to some impairment of renal tubules resembling that due to the effect of some heavy metals (e.g. cadmium or mercury) or to immunomodulation (Langer et al, 1997, 2001).

At the same time the analyses in human serum and environmental samples showed extraordinary high levels of PCBs in human serum and environmental samples from the polluted area

(Kočan et al, 1994, 2001). Although the production of PCBs was terminated in 1985, very high levels of total PCBs in human serum from polluted area were still found during our survey conducted in 1998. Thus, in 101 Chemko factory employees the level (mean±SE) of total PCBs was 7300±871 ng per g of serum lipids which means per about 150 ml of serum) compared to that in 360 control subjects (2045±147 ng/g;  $p<0.001$ ). In the polluted area also very high PCB levels were found even in the air (100–1700 pg/m<sup>3</sup>), superficial or drinking waters (6–92 ng/l), river, lake and channel sediments (10–4100 µg/g dry matter), agricultural soil (2–28 ng/g dry matter), fish from polluted resort lake Šírava (average 375,000 ng/g lipids), wild animals (34–331 ng/g lipids), cow milk (average 52 ng/g), lard (average 49 ng/g lipids), pork and beef (range 5–50 ng/g lipids). In addition, the average of 12,300 ng/g lipids was previously found in human adipose tissue and average of 1360 ng/g lipids in human milk.

However, during the PCB production also considerable amounts of most toxic polychlorinated dibenzofurans and dioxins were possibly generated. Three sera obtained from the employees of chemical factory were analysed in the Centers for Disease Control and Prevention (Atlanta, Georgia) under the U.S.-Slovak co-operation project in which the average toxic equivalent (TEQ) of 137.7 pg/g lipids for cumulated serum levels of the most toxic coplanar PCB (No. 126 and 169), PCD and PCF were found (Kočan et al, 1996). However, several hundreds of sera are currently being analysed within the project PCB RISK (see below).

### Perspectives

Based on the above described results a large scale project has been submitted to the European Commission which has been finally approved and included into 5th Framework Program under the acronym PCB RISK (Trnovec et al, 2002). The fundamental step was the extensive examination of about 1000 adults from polluted area around Michalovce and Strážske and about 1000 control subjects from control areas around Stropkov, Svidník and Giraltovce as well as of about 200 children aged 9 years from each area. In addition to basal data on health and social status, education and professional history, also the thyroid was examined by ultrasound and several aliquots of plasma and urine were obtained from each subject. In addition to the original project, peroral glucose tolerance tests were performed in more than 1200 adults (Klimeš et al, 2002).

Currently the analyses of serum aliquots from adults and children are in progress in several laboratories from seven European countries, e.g. congener specific estimations of PCB, hexachlorobenzene, hexachlorocyclohexane, DDT and DDE as well as of polychlorinated dibenzodioxins and dibenzofurans. In addition, several hormones (TSH, free thyroxine, total triiodothyronine, insulin, and testosterone in males only), autoantibodies (anti-thyroperoxidase, anti-TSH receptor and anti glutamic acid decarboxylase or anti-GAD) and some tumour markers (thyroglobulin,  $\alpha$ -fetoprotein, carcinoembryonic antigen,  $\beta_2$ -microglobulin) are being estimated. Further analyses are con-

cerned with the serum level of hydroxylated and methylsulpho-nated PCB metabolites, of dioxin-like toxic equivalents and estrogenic and anti-estrogenic activity of plasma. Finally, the levels of some heavy metals and microelements, e.g. Cd, Pb, Hg, Mn, Zn, Se in plasma of adults and children are being estimated.

After preliminary evaluation of data obtained within this project the previous findings of increased thyroid volume and frequency of anti-thyroperoxidase antibodies in polluted area were confirmed. At the same time, considerably increased level of total PCB and individual congeners in polluted area were found as compared to in controls which also supported the previous findings. However, it appears that the extensive and large scale survey will bring several new and hard data which will contribute to the elucidation of PCB effects on human health status.

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