Increased thyroid volume and prevalence of thyroid disorders in an area heavily polluted by polychlorinated biphenyls

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Abstract

Objective: To evaluate whether long-term exposure to heavy environmental pollution with polychlorinated biphenyls (PCBs) could result in impairment of thyroid status as evaluated by an epidemiological field survey.

Methods: Thyroid volume (ThV) was measured by ultrasound in 238 employees of a factory (EMP) which previously produced PCBs and 454 adolescents from the surrounding area polluted by PCBs. Controls (C) were 572 adults and 965 adolescents from much less polluted areas. In the 238 EMP and various numbers (shown in parentheses) of adult C the levels of thyroid-stimulating hormone (TSH), thyroxine, thyroglobulin and thyroid antibodies (anti-peroxidase (TPO Ab), anti-thyroglobulin (Tg Ab), TSH receptor (TSHR Ab)) were estimated in serum, while only TSH and TPO Ab were measured in 269 and 171 adolescents from polluted and control areas respectively. In several subjects in whom thyroid disease was suspected, total tri-iodothyronine or free thyroxine and tri-iodothyronine were measured. In a total of 362 adults and adolescents the urinary iodine was estimated.

Results: Using the Mann-Whitney test, ThV in EMP (mean ± s.e. = 18.85 ± 0.69 ml, median = 17.3 ml, upper quartile = 22.9 ml, n = 238) was significantly higher (P < 0.001) than that in C (13.47 ± 0.48 ml, 11.5 ml, 15.3 ml, n = 486 respectively). Similarly, ThV in adolescents from the polluted area (9.37 ± 0.17 ml, 8.9 ml, 11.0 ml, n = 454 respectively) was significantly higher (P < 0.001) than that in C (8.07 ± 0.10 ml, 7.6 ml, 9.6 ml, n = 965 respectively).

In adults, a significantly increased prevalence of TPO Ab (P < 0.05) was found (using the chi-square test) in EMP women of all ages (54/190) vs C women (70/282), in EMP women aged 31–50 years (40/117 vs 70/282 respectively) and those aged 41–50 years (28/77 vs 54/215 respectively). Compared with C, there was also a higher prevalence of Tg Ab in EMP women aged 31–60 years (36/169 vs 50/342 respectively) and of TSHR Ab (P < 0.001) in the group of EMP men and women (25/238) vs sex- and age-matched C (6/238).

No difference between EMP and C was found in the level of thyroxine (mean ± s.d. = 116.1 ± 31.2 nmol/l, n = 238 vs 112.2 ± 37.0 nmol/l, n = 460 respectively). TSH in the range 0.1–4.5 mU/l (1.56 ± 0.86 mU/l, n = 219 vs 1.51 ± 0.84 mU/l, n = 460 respectively), prevalence of TSH >4.5 (14/238 vs 28/498 respectively) and <0.1 mU/l (5/238 vs 10/498 respectively). The prevalence of individuals without any defined clinical or laboratory signs of thyroid disorders among EMP who had worked in the factory for 21–35 years (43/129, 33.6%) was significantly lower than that in twice as many matched C (118/256, 46.1%, P < 0.025) or in EMP who had worked for only 11–20 years (36/73, 49.3%, P < 0.05).

In adolescents, no difference was found in the prevalence of TPO Ab or TSH >4.5 mU/l between the polluted (17/269, 6.3% and 2/243, 0.8% respectively) and C areas (15/171, 8.5% and 4/140, 2.8% respectively). The median values of urinary iodine were in the optimal range (μg per dl/number of cases) and about the same in polluted (12.6/90 and 11.4/55) and C areas (14.1/80, 13.2/82 and 13.4/55).

Conclusions: Since iodine intake in Slovakia is considered sufficient as a result of 45 years of well-monitored iodine prophylaxis, the increased ThV and prevalence of thyroid disorders in the polluted areas presumably results from long-term exposure to toxic substances rather than from a difference in life-long iodine intake. The increased prevalence of some thyroid antibodies may be related to the known immunomodulatory effects of PCBs.
Introduction

Several toxic effects of polychlorinated biphenyls (PCBs), dioxins and furans in laboratory and wild animals (carcinogenicity, teratogenicity, immunotoxicity etc.) are well known (1). Since these substances also interact with several functions of the endocrine system, they have been called endocrine disruptors (2). Although a variety of harmful effects have been found in human subjects after accidental or occupational exposure, recent reviews concluded that little is known about the effects of chronic low-level environmental exposure, and that further epidemiological studies should be undertaken (2, 3).

One of the best understood effects of such organochlorines on the thyroid is a decrease in the plasma thyroxine (T4) level due to the displacement of T4 from protein binding (4) and increased hepatic metabolism of T4 resulting from the induction of UDP-glucuronosyltransferase (5).

Thyroid ultrastructural alterations in rats after PCB feeding have been found, suggesting an interference in the interaction between the accumulated numerous colloid droplets and irregularly shaped lysosomal bodies in the expanded cytoplasmic area. Since these were distinct from those observed after short- and long-term thyroid-stimulating hormone (TSH) stimulation, they were considered to result from direct damage to the thyroid (6). Direct effects of PCBs on the thyroid are supported by the decreased effect of TSH on T4 and triiodothyronine (T3) release from the thyroid in rats pretreated with PCBs when compared with controls (7).

Because of a structural similarity to T4, PCBs may also interfere with the transport of T4 into cells and T4 to T3 conversion. They may also mimic thyroid hormone action and even modulate the mechanism of T3 binding to its nuclear receptor and resulting gene expression (8).

In neonates, perinatal exposure to maternal milk containing polychlorinated dioxins and furans resulted in increased TSH levels after 11 weeks (9) and, in addition, in mother–infant pairs such exposure resulted in a decreased T4 level in blood (10). Irreversible neurologic damage resembling that seen in those exposed to thyroid hormone deficiency in utero and/or in infancy was also observed (2, 11). In occupationally exposed workers, an increased prevalence of thyroid disorders and thyroid cancer (12, 13) has been reported.

In the CHEMKO factory (East Slovakia), 20000 tonnes of PCBs were produced in 1955–1985, the chemical nature of PCBs manufactured closely resembling that of the well known Arochlor 1242. Solid waste made up of about 900 tonnes of PCBs polluting the surrounding area still exists in the vicinity of the factory, while liquid waste was dumped directly into the Laborec (pronounced Laborets) river, without any protective treatment. In addition to airborne pollution, this resulted in contamination of underground sources of drinking water, superficial water, including one large artificial resort lake, and a large artificial fish pond, with resultant contamination of the food chain, which is still persisting at a high level.

Most affected was the area surrounding the factory and the town of Michalovce (pronounced Mykhalovtse), including the resort lake (Fig. 1). During the previous political regime, the population was kept uninformed about the high risk, and any scientific or public information on the subject has been strictly banned.

Although production was terminated in 1985, very high levels of total PCBs in human samples from Michalovce were still found by congener specific analysis.
in 1990–1994 (14, 15), e.g. in adipose tissue (average 12 300 ng/g lipids), serum (range 1160–9600 ng/g lipids) and human milk (average 1360 ng/g lipids). The most abundant were the congeners PCB-28, -118, -138, -153 and -180. Similarly, in CHEMKO employees the average toxic equivalent (TEQ) of 137.7 pg/g lipids for cumulated serum levels of the most toxic coplanar PCBs (PCB-126 and PCB-169), polychlorinated dibenzodioxins and dibenzofurans was found (15). In 1996, a group of employees with persisting high contamination was found with serum TEQ about 600 pg/g lipids (16). The above levels in serum were up to 10 times higher (14) and the those in human milk about 3–100 times higher than the values reported from several European and overseas countries (15), which shows that this territory is among the most heavily polluted and the population among the least protected currently known.

In this investigation we have used an epidemiological field survey to evaluate whether the long-term heavy exposure of CHEMKO employees and adolescents from Michalovce might have resulted in some impairment of thyroid status.

Patients and methods

Subjects

Two groups of adults were examined (the numbers in parentheses show: (i) the number of examined subjects, (ii) the number of thyroid volume (ThV) estimations, (iii) the number of blood samples obtained): first, employees of the CHEMKO factory (238/238/238; 48 males and 190 females; age range 22–65 years, median 44), 71.8% being employed for more than 15 years and 53.4% for more than 20 years; secondly, a control group (school teachers, parents of examined pupils, staff from local hospitals) from three much less polluted areas (572/486/523; 70 males and 502 females; age range 22–68 years, median 43). In addition, for 184 out of 238 employees (29 males and 155 females; age range 22–61 years, median 43) we were able to identify two controls matched by age and sex and to compare these two groups.

The data on smoking and alcohol consumption obtained by questionnaires did not reveal any remarkable differences between CHEMKO employees (16.3% smokers and 2.5% admitting daily drinking) and controls (17.4% and 3.2% respectively). The remaining subjects drank alcohol only on an occasional, social basis.

ThV was also estimated in two groups of 17-year-old adolescents: first, subjects from the most polluted city of Michalovce (454/454/269; 190 boys (41.8%) and 264 girls) who were born in the period of maximal pollution (1979–1980); secondly, control subjects of the same age (965/965/171; 434 boys (44.9%) and 531 girls) from five much less polluted areas. Less than 3% of adolescents admitted occasional smoking for 1–3 years, while the majority admitted occasionally drinking alcohol; drug abuse in rural areas is still negligible.

The project has been approved by the Ethical Committee, Faculty of Medicine in Košice. All adults gave their oral informed consent. The parents of adolescents gave their written informed consent to obtain blood samples, which was also confirmed by the oral consent of the adolescents themselves.

Methods

Thyroid volume

Thyroid examination was performed using a real-time instrument (Sonoline SI-400, Siemens, Munich, Germany) using a 7.5 mHz linear transducer. The ThV for each lobe (ml) was calculated according to the ellipsoid formula: width (cm) × length (cm) × thickness (cm) × correction factor 0.479 (17). All examinations in the adults and those in 72.4% of adolescents were performed by MT, who has >15 years experience in clinical work and field surveys using thyroid sonography (18, 19). The remaining 27.6% of examinations in adolescents were performed by GF who has similar experience. The difference between two observers or between the repeated examinations of ThV by the same observer was less than 5%.

Urinary iodine

Urinary iodine in 171 adults and 192 adolescents was kindly estimated by Professor F Vertongen (University of Brussels, Brussels, Belgium) by the colorimetric ceric ion arsenious acid wet ash method using a Technicon autoanalyzer (20, 21).

Blood sampling and estimation of hormones and thyroid antibodies

All subjects were instructed in advance not to eat anything in the morning. The blood was withdrawn with an S/Monovette vacutainer (Sarstedt, Nümbrecht, Germany), and after centrifugation several aliquots of serum were transported frozen on dry ice to the laboratory. They were kept frozen at −20°C. In all blood samples the concentrations of TSH, T₄ and autoantibodies against thyroid peroxidase (TPO Ab), thyroglobulin (Tg Ab) and TSH receptor (TSHR Ab) were measured. Moreover, in a limited number of subjects in whom thyroid disease was suspected, T₃ (n = 376) or free T₄ and T₃ (n = 159 each) were measured in all CHEMKO employees and in the subjects from one control area (n = 198). The level of thyroglobulin was also measured. For all estimations the commercial kits made by Brahms GmBH (Berlin, Germany) were used, e.g. DYNOTest TSH (upper normal reference range 4.5 mU/l), DYNOTest anti-TPO on for TPO Ab (upper normal reference range 70U/ml), THYRAK-Assay for Tg Ab (upper normal reference range 150 U/ml) and TRAK-Assay for TSHR Ab (upper normal reference range 10U/l).

Definition of normal thyroid

For the purpose of epidemiological evaluation of the prevalence of thyroid
disorder vs normal thyroid, the latter was arbitrarily defined as: (i) negative personal history, (ii) negative routine clinical examination, (iii) negative ultrasound examination, (iv) normal plasma TSH and thyroid hormone concentrations, (v) negative thyroid antibodies, (vi) ThV <22.0 ml.

Statistical evaluation After testing the normality of ThV distribution, the differences between individual groups were evaluated with the non-parametric Mann–Whitney test. The results are shown as means ± s.e. or as means ± s.d., as indicated below. The differences in the prevalence of individual thyroid antibodies, TSH level and thyroid disorders were evaluated using chi-squared in respect of the above cut-off points. The level of significance was $P < 0.05$.

Results

Urinary iodine The median values from all areas were in the optimal range 10.0–20.0 μg/dl (20) and were similar (median in μg per dl/number of estimations) in adults from CHEMKO (12.6/90) and from the pooled control areas (14.1/80), as well in adolescents from Michalovce (11.4/55) and two control areas (13.2/82 and 13.4/55).

Thyroid volume in adults Figure 2 shows the distribution of ThV, which was significantly higher ($P < 0.001$) in 238 employees of CHEMKO (mean ± s.e. = 18.85 ± 0.69 ml; median = 17.3 ml; upper quartile = 22.8 ml) than in 486 controls (mean ± s.e. = 13.47 ± 0.48 ml; median = 11.3 ml; upper quartile = 15.5 ml). The most remarkable difference was found in the number of values above the cut-off level of 22.1 ml (65/238 or 27.3% in CHEMKO workers vs 32/486 or 6.6% in controls; $P < 0.02$), which was arbitrarily selected and closely resembled the upper quartile (e.g. 22.8 ml) as found in the CHEMKO group. For 184 out of 238 employees we were able to identify two controls matched by age and sex, the same difference in ThV ($P < 0.001$, not shown) being found between CHEMKO employees (mean ± s.e. = 18.48 ± 0.66 ml; median = 17.0 ml; upper quartile = 22.9 ml) and controls (mean ± s.e. = 13.49 ± 0.38 ml; median = 11.8 ml; upper quartile = 14.6 ml).

Thyroid volume in adolescents Since the percentage of boys was approximately the same in both the control and polluted groups (see Methods) and since no significant differences were found between ThV in boys and girls (see below), the two groups were considered as approximately sex-matched. Figure 3 shows that ThV in the pooled control areas (mean ± s.e.) was 8.33 ± 0.09 ml (median = 7.7 ml, upper quartile = 9.8 ml, $n = 965$), while that in the polluted city of Michalovce was significantly higher ($P < 0.001$), being 9.41 ± 0.15 ml (median 9.0 ml, upper quartile 11.0 ml, $n = 454$). Similarly, the ThV values as expressed per square meter of body surface were 4.82 ± 0.07 ml/m$^2$ for boys, 5.16 ± 0.08 ml/m$^2$ for girls and 5.01 ± 0.05 ml/m$^2$ for pooled sexes from the control areas and 5.39 ± 0.13 ml/m$^2$ for boys, 5.59 ± 0.11 ml/m$^2$ for girls and 5.51 ± 0.08 ml/m$^2$ for pooled sexes from the polluted area, all corresponding values in the polluted area being significantly higher than those in the controls ($P < 0.001$). Even in adolescents, the greatest difference was found in the range of high values, the number of these >11.1 ml being 112/454 (24.6%) in the polluted area vs 151/965 (15.6%) in controls ($P < 0.001$).

Prevalence of autoantibodies and hypoechogenicity As shown in Table 1, the percentage of individual antibody prevalence in all age- and sex-matched adult
groups was higher in CHEMKO employees, significant differences ($P < 0.05$) for TPO Ab being found in women of all ages, and in women 31–50 and 41–50 years old, while the difference for Tg Ab was significant ($P < 0.05$) only in women 31–60 years old. The prevalence of TSHR Ab in CHEMKO employees (25/238) was significantly higher ($P < 0.001$) than in sex- and age-matched control subjects (6/238).

In limited numbers of adolescents, the prevalence of TPO Ab was very low and no significant differences between the polluted (17/269 = 6.3%) and control areas (15/171 = 8.7%) were found. The prevalence of hypocholesterinemia in adolescents found in the polluted area (35/454 = 7.7%) was the same as in controls (71/965 = 7.4%).

**Levels of $T_4$, TSH and thyroglobulin**

The level of total $T_4$ (means ± S.D.) in all CHEMKO employees (116.1 ± 31.2 nmol/l, $n = 238$) was the same as that in 498 controls (112.2 ± 37.0 nmol/l). No significant difference was found in the prevalence of TSH >4.5 mU/l between CHEMKO employees (14/238 = 5.9%, 6/14 TPO Ab negative) and adult controls (28/498 = 5.6%, 9/28 TPO Ab negative) or between adolescents from polluted (2/243 = 0.8%) and control areas (4/140 = 2.8%). Also, the prevalence of TSH <0.1 mU/l was about the same in CHEMKO workers (5/238 = 2.1%, 2/5 TSHR Ab negative) and in controls (10/498 = 2.0%, 6/10 TSHR Ab negative). In the remaining adults from CHEMKO (mean ± S.D. = 1.56 ± 0.86 mU/l, $n = 219$) and controls (1.51 ± 0.84 mU/l, $n = 460$) no significant difference in TSH level was found. In addition, the prevalence of thyroglobulin levels >70 ng/ml in the CHEMKO group (12/238, 5.0%) was the same as in one control area (9/198, 4.7%).

**Prevalence of total thyroid disorders**

The prevalence of normal thyroids (as defined in Methods) in the group of employees who had worked in CHEMKO for 21–35 years (age 49 ± 8 years, mean ± S.D.) was 43/128 (33.6%). This was significantly lower than that either in the group who worked there for 11–20 years

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Group</th>
<th>CHEMKO</th>
<th>Control</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPO Ab</td>
<td>All men + women</td>
<td>62/238 (26.0%)</td>
<td>107/517 (20.7%)</td>
<td>&gt;0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>All women</td>
<td>54/190 (28.4%)</td>
<td>99/482 (20.5%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Women 31–50 years</td>
<td>40/117 (34.2%)</td>
<td>70/282 (24.8%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Women 41–50 years</td>
<td>28/77 (36.4%)</td>
<td>54/215 (25.1%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Tg Ab</td>
<td>Women 31–60 years</td>
<td>36/169 (21.3%)</td>
<td>50/342 (14.8%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>TSHR Ab&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Matched subjects</td>
<td>25/238 (10.5%)</td>
<td>6/238 (2.5%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<sup>a</sup> The difference was slightly below the limit of significance.

<sup>b</sup> The prevalence in 238 CHEMKO employees vs 238 age- and sex-matched control subjects.
Thus the prevalence of thyroid disorders was 66.4% in those employed at CHEMKO for 21–35 years, compared with 53.9% in matched controls and 50.7% in those at CHEMKO for 11–20 years (50.7%). The prevalence of individual thyroid disorders in each group is shown in Table 2.

Discussion

It seems clear that the ThV in CHEMKO employees who were occupationally exposed to long-term heavy contamination with PCBs and other organochlorines (polychlorinated dibenzodioxins and dibenzofurans), as well as that in the adolescents from the heavily polluted area of Michalovce, was significantly increased as compared with the control groups. The same is apparently true for the increased prevalence of total thyroid disorders in a group of CHEMKO employees. Since the level of urinary iodine was sufficient and about the same in all areas examined, the difference in ThV in individual areas does not appear to be caused by a regional difference in iodine intake. The assumption of a sufficient iodine intake in Slovakia since the early 1950s is based on the mandatory consumption of iodized salt produced by a sole state factory, and on the embargo on importing any other salt, the actual concentration of iodine in salt being monitored by about 1000 analyses of salt samples from the distribution network per year performed by public health laboratories. In addition, Slovakia was recently evaluated by the European ThyroMobil Study as a country with sufficient iodine intake and low ThV in schoolchildren (21). Finally, the ThV values in our control adult and adolescent groups were very low, also supporting a life-long sufficient iodine intake. If, as our data indicate, PCB-induced thyroid enlargement occurs with a sufficient iodine intake, it would occur to an even greater extent under conditions of iodine deficiency.

Since global pollution by PCBs is well known, a completely pollution-free area does not exist in Slovakia and thus even the control population examined may be under some influence from PCBs and other organochlorines (e.g. pesticides etc.). However, this probably did not play any considerable role in evaluating our data, since the analytical data we have obtained so far have shown that the levels of PCBs in adipose tissue, serum and human milk from the polluted city of Michalovce and employees of the CHEMKO factory were about three to four times higher than those from the control areas (14–16).

We cannot derive a definite explanation for the mechanism by which PCBs produce an increased ThV and increased prevalence of thyroid disorders from this field epidemiological survey alone. It should be emphasized that we were examining a steady state resulting from decades of exposure to the toxic effects of organochlorines for a population with a certain genetic background. This may be at least partly supported by the same levels of TSH and T4 in CHEMKO employees and controls.

Some effects on the thyroid might even result from a multiple non-specific toxicity. Polychlorinated organic compounds are not typical anti-thyroid substances which inhibit the biosynthesis of thyroid hormones.
resulting in a decrease in their blood level and a compensatory increase in TSH secretion. Among the primary effects of PCBs on thyroid hormone metabolism demonstrated so far are the displacement of thyroid hormones from plasma protein binding, an increase in their conjugation in the liver and interference with their intracellular metabolism (4, 5, 7, 8, 11).

An important characteristic of organochlorines is that they are highly lipophilic. Thus, in plasma they are bound to plasma lipids and they are stored for decades in adipose tissue. Such lipophilic compounds may accumulate in the cell membranes and damage their fluidity, as shown in renal tubular membranes (22), and stimulate free radical toxicity to destroy cellular structures, as shown in the testes (23). Similar changes in the thyroid, if any, may facilitate the communication between thyroid autoantigens and circulating immunocompetent cells. This possibility is supported by our preliminary findings (M Tajtákova & P Langer, unpublished data) that PCBs are detectable in human thyroids from a polluted area (25–45 ng/g wet weight, the most abundant congeners being PCB-108, -138, -153 and -180).

Since most of the examined employees of CHEMKO were exposed to PCB without any protection for several decades, the levels of organochlorines in their tissues were apparently very high, as demonstrated in several of them and also in people from Michalovce (14–16).

One of the most significant general effects of highly lipophilic organochlorines may be the immunomodulation, as related to the increased prevalence of various autoantibodies (e.g. anti-myelin, anti-smooth muscle etc.) in exposed groups (24), and the increased prevalence of autoimmune diabetes mellitus and high fasting serum glucose levels in Agent Orange sprayers among Vietnam veterans and in the cohort involved in the Seveso accident (25). The increased prevalence of thyroid antibodies in the present study might result from similar effects of PCBs and other organochlorines. This view is in agreement with the findings of an increased prevalence of anti-peroxidase antibodies in the areas of Kentucky contaminated by polycyclic hydrocarbons (26). The participation of immunomodulatory effects of organochlorines may be supported by our previous finding of a decreased level of β2-microglobulin in CHEMKO employees (27).

Although the most significant finding of the present study was the increase in thyroid volume in the CHEMKO employees, there was also an increased prevalence of thyroid disorders. These presumably resulted from long-term direct effects of organochlorines on thyroid structure, including the deterioration of cell membranes (6, 28), interference with peripheral thyroid hormone metabolism, immunomodulatory and other non-specific effects. These effects may have resulted in temporary or periodic variations in the effect of TSH. Resulting from such long-term actions, a new steady state was presumably derived which might not be manifested by the persisting detectable abnormalities in hormone levels or thyroid function. Another possibility is that long-term organochlorine effects on the thyroid selectively exacerbate pre-existing thyroid disease or even a pre-existing hereditary acquired predisposition to such disease.

Even estrogen-like effects on the thyroid cannot be ruled out, since several of more than 200 PCB congeners show estrogenic effects, estrogen receptors are present in human thyroid tissue (29), and striking estrogen-receptor-mediated potencies of various xenobiotic combinations including PCBs have recently been described (30). A role of estrogens in the stimulation of normal and malignant thyroid growth is suggested by the inhibitory effect of the non-steroidal anti-estrogen, tamoxifen, on the growth and migration of human cancer cells (31).

Our main aim was to utilize this opportunity to study the population in an exceptionally PCB-polluted area and to evaluate some possible effects of organochlorines on the thyroid. However, the majority of questions remain unanswered.

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