(J. Endocrinol. Invest. 26: 950-955, 2003) ©2003, Editrice Kurtis.

In vitro assay of thyroid disruptors affecting TSH-stimulated adenylate cyclase activity

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ABSTRACT. Several natural or synthetic chemicals have been indicated as potential thyroid disruptors. The development of *in vitro* assays has been recommended to comprehensively assess the potential thyroid disrupting activity of a substance or a complex mixture. In this study, 12 substances suspected for acting as thyroid disruptors were tested for their ability to inhibit TSH-stimulated cAMP production *in vitro*. Those substances producing an inhibition were further studied to establish the level at which they interfere with this step of thyroid cell function. Using Chinese hamster ovary cells (CHO) transfected with the recombinant human TSH receptor, a dose-dependent inhibition of TSH-stimulated adenylate cyclase activity was produced by 1,1-bis-(4-chlorphenyl)-2,2,2-trichloroethan (DDT), Aroclor 1254 and *Melissa Officinalis*. All three substances also inhibited the cAMP production stimulated by TSH receptor antibody. *Melissa Officinalis* produced a significant inhibition of TSH binding to its receptor and of antibody binding to TSH, while no significant changes were produced by Aroclor 1254 or DDT in these assays. These data suggest that principles contained in *Melissa Officinalis* may block the binding of TSH to its receptor by acting both on the hormone and the receptor itself, while DDT and Aroclor 1254 affect cAMP production mainly at post-receptor step. In conclusion, we have developed a set of *in vitro* assays that allow investigation into the effect of thyroid disruptors on the TSH-mediated activation of the cAMP cascade. These assays may be useful to identify the mechanism of action of thyroid disruptors, coming beside and supporting animal studies or epidemiological surveys.

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INTRODUCTION

Environmental factors that interfere with thyroid function or thyroid hormone action, altering thyroid homeostasis, are defined thyroid disruptors (TDs). Although numerous studies in the literature indicate several natural or synthetic chemicals as potential TDs, the real clinical impact of these substances in humans is still a matter of controversy, the main concern deriving from potential deleterious effects of TDs on brain development during fetal and neonatal life (1-3). TDs can act at central level by interfering with TSH production or release, at thyroid level by affecting thyroid hormone synthesis or release, at peripheral level by interfering with thyroid hormone transport, metabolism or action on T_3 receptor. While experimental evidence obtained in animals indicates that several substances may act as TDs, data in humans are far from conclusive. Next to *in vivo* assays the development of *in vitro* assays has been recommended to comprehensively assess the potential thyroid-disrupting activity of a substance or a complex mixture of substances (4-6). To this purpose we developed a panel of *in vitro* assays to verify the effect and the possible mechanism of action of putative TDs on the first steps of thyroid gland activation, i.e. TSH-induced adenylate cyclase activation and cAMP production.

In order for the thyroid cell to synthesize cAMP, TSH has to bind to its specific receptor on the cell surface, a G protein-coupled receptor that has seven putative helical transmembrane domains with an extra-cellular amino terminus and an in-

Key-words: Thyroid disruptors, adenylate cyclase, thyrotropin, TSH receptor, TSH receptor-stimulating antibody, *Melissa Officinalis*, polychlorinated biphenyls, DDT.

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Accepted May 15, 2003.

tracellular carboxyl terminus (7). Upon binding of TSH, the activated receptor interacts with the Gs protein that, in turn, interacts with and activates several effectors, including adenylate cyclase, which catalyzes the production of cAMP. In this study, a panel of substances suspected for acting as TDs was tested for their ability to inhibit TSH-stimulated cAMP production *in vitro*. Those substances producing an inhibition were further studied to establish the level at which they interfere with this step of thyroid cell function.

MATERIALS AND METHODS

Twelve substances, selected among those indicated as potential TDs, were obtained as follows: zineb, maneb, mancozeb, amitrol, ethylene thiourea, resorcinol (Riedel-De Haen, Sigma-Aldrich, Seelze, Germany); 3,5 dihydroxy benzoic acid, 3,4 dihydroxy benzoic acid, hexachlorobenzene (Fluka, Buchs SG, Switzerland); 1,1-bis-(4-chlorphenyl)-2,2,2-trichloroethan (DDT) (Aldrich, Sigma-Aldrich, Steinheim, Germany), Aroclor 1254 (Chem Service, West Chester, PA, USA), phthalic acid (Sigma, Sigma-Aldrich, Steinheim, Germany). Freeze-dried extract (FDE) of *Melissa Officinalis* was preparared by extracting powdered leaves with H_2O for 2 h at room temperature as previously described (8). After centrifugation and filtration, the clear solution was lyophilized. FDE was carefully weighed and dissolved in the appropriate assay buffer just before use.

Cell cultures

Chinese hamster ovary cells (CHO) clone K1 (CHO-K1) and CHO cells transfected with the recombinant human TSH receptor (CHO-R cells, clone JP-09) (9) were used in this study. Cells were grown on Petri dishes in RPMI 1640 medium supplemented with 10% fetal bovine serum as previously described (10). Cells were harvested using a trypsin-EDTA mixture and seeded (70,000 cells/well) in 24-well plates. Cells were fed fresh culture medium 24 h after seeding and used for the assays described below. Cell viability after incubation with IgG praparations was checked by Tripan Blue exclusion.

TSH-induced cAMP production assay in JP-09 cells

Before the assay the culture medium was aspirated and cells were washed with RPMI 1640 medium. TDs were diluted in hypotonic buffer containing 0.4% BSA (Sigma) and 0.5 mmol/l isobutylmethylxanthine (IBMX) (Sigma). A purified preparation of bovine TSH (bTSH, Sigma) was diluted in hypotonic buffer-BSA-IBMX to various concentrations as reported in the Results section. Cell cultures were incubated with TD alone, TSH alone, or TD plus TSH in a total volume 0,25 ml of hypotonic buffer-BSA-IBMX. Hypotonic buffer-BSA-IBMX alone was added to some cultures in each experiment to measure basal cAMP production. For each TD the lowest concentration of solvent necessary to dissolve the substance was used and kept constant at each TD dilution, including cultures without TD (control cells). After 1 h of incubation at 37 C in 5% CO_2-95% air atmosphere, cAMP was measured in the extra-cellular medium by a radioimmunoassay (RIA) developed in our laboratory (11). All experiments were performed in quadruplicate and the results (in pmoles per well) were expressed as the average of these. To evaluate the effect of TDs, an index of inhibition of TSH-dependent cAMP production was calculated according to the following formula:

$$1-\left[\frac{\text{cAMP (TD + TSH)} - \text{cAMP (TD)}}{\text{cAMP (TSH)} - \text{cAMP (control buffer)}} \times 100\right]$$

Table 1 - *Pre-incubation assay. Thyroid disruptors (TDs) tested, their concentrations and solvent used for each TD.*

Substance	Use	Concentration	Solvent
DDT	Insecticide	0.1-1-10-100 μM	0.1% DMSO
3,4 DHBA	Organic acid	1-10-100-1000 μM	RPMI 1640
3,5 DHBA	Organic acid	1-10-100-1000 μM	RPMI 1640
Phtalic acid	Plasticizer	1-10-100-1000 μM	95% ethanol
Maneb	Fungicide	0.1-1-10-100 μM	0.1% DMSO
Mancozeb	Fungicide	0.1-1-10-100 μM	0.1% DMSO
Zineb	Fungicide	0.1-1-10-100 μM	0.1% DMSO
ETU	Fungicide	1-10-100-1000 μM	RPMI 1640
Resorcinol	Rubber industry	1-10-100-1000 μM	95% ethanol
Arochlor 1254	Heat transfer fluid	0.1-1-10-100 μM	0.1% DMSO
Amitrol	Herbicide	1-10-100-1000 μM	0.1% DMSO
Melissa Officinalis	Plant extract	20-100-500-2000 μg/ml	H_2O

DMSO: dimethyl sulfoxide; DDT: 1,1-bis-(4-chlorphenyl)-2,2,2-trichloroethan; 3,4 DHBA: 3,4 dihydroxy benzoic acid, hexachlorobenzene; 3,5 DHBA: 3,5 dihydroxy benzoic acid, hexachlorobenzene; ETU: ethylene thiourea.

Pre-incubation assay

This assay was performed to verify whether TD could affect cAMP production by non-specific interference with cellular functions. JP-09 cells were incubated overnight with 4 concentrations of various TDs at 37 C, as shown in Table 1. Subsequent incubation cells were washed with RPMI 1640 and then used in the TSH-induced cAMP production assay as described above. Inhibition observed by this assay was considered non-specific and only concentrations of TD that did not produce any inhibition of cAMP production after pre-incubation were used for further experiments.

Co-incubation assay

In this assay JP-09 cells were incubated with hypotonic medium alone or with hypotonic medium containing TDs at the highest concentration that did not produce inhibition in the pre-incubation assay, without and with bTSH, as described in the cAMP production assay. TDs that exhibited significant inhibition of cAMP production during the co-incubation assay were further tested at lower concentrations and at various concentrations of bTSH to obtain a dose-response curve.

TSAb-induced cAMP production assay

TSH receptor stimulating antibody (TSAb) were obtained from the serum of a patient with Graves' disease and purified using a commercial kit (Mab Trap G II, Pharmacia Biotech. Inc., Piscataway, NJ). The assay was performed using JP-09 cells and 0.25 mg/ml TSAb as a stimulator of TSH receptor in place of TSH. The remaining steps were performed following the same protocol described for the co-incubation assay.

Forskolin-induced cAMP production assay

This assay was performed using both JP-09 and CHO-K1 cells and 10 μM forskolin (Sigma) as a direct stimulator of adenylate cyclase activity in place of TSH. The remaining steps were performed following the same protocol described for the co-incubation assay.

Immunometric assay of TSH

To test whether TDs would interfere with TSH antigenic properties, 10 mU/l human TSH were assayed in the absence and in the presence of each TD at the highest concentration that produced inhibition of cAMP production in the co-incubation assay. A third generation TSH assay was employed using a commercial kit (Immulite 2000, Diagnostic Product Corporation, Los Angeles, CA).

Assay of TSH receptor blocking activity

To test whether TDs would interfere with TSH binding to the specific receptor, radioactive ^{125}I-bTSH was incubated with human TSH receptor in the absence and in the presence of each TD at the highest concentration that produced inhibition of cAMP production in the co-incubation assay. To this purpose the materials of commercial kit (TRAK assay, B.R.A.H.M.S., Berlin, Germany) were used.

RESULTS

TSH-induced cAMP production assay in JP-09 cells

In the pre-incubation assay only three substances (DDT, Aroclor 1254 and *Melissa Officinalis*), at the highest concentration used, exhibited significant inhibition of TSH-induced cAMP production (data not shown). These concentrations were not used for further experiments since inhibition could derive from non-specific interference with cell function.

During the co-incubation assay, DDT, Aroclor 1254 and *Melissa Officinalis* inhibited TSH-induced cAMP production. None of the other TDs produced significant inhibition at the highest concentration tested (range of inhibition 0-6%). Figure 1 shows the dose response curves of TSH-induced cAMP production at various concentrations of DDT, Aroclor 1254 and *Melissa Officinalis*. A dose-dependent increase in cAMP production was produced by 3 concentrations of TSH. At each TSH concentration TDs produced an inhibition that was proportional to the TD concentration. These three substances were further investigated to understand the mechanism by which they inhibit cAMP production.

TSAb-induced cAMP production assay

All three substances inhibited cAMP production stimulated by TSAb (Table 2). Inhibition produced by extracts of *Melissa Officinalis* after TSAb stimulation was about one half of that observed after TSH stimulation (45.4 vs 87.1%, respectively). Aroclor 1254 and DDT inhibited TSAb and TSH to a similar extent (Aroclor: 88.8 vs 84.7%, respectively, DDT: 48.8 vs 60.5%).

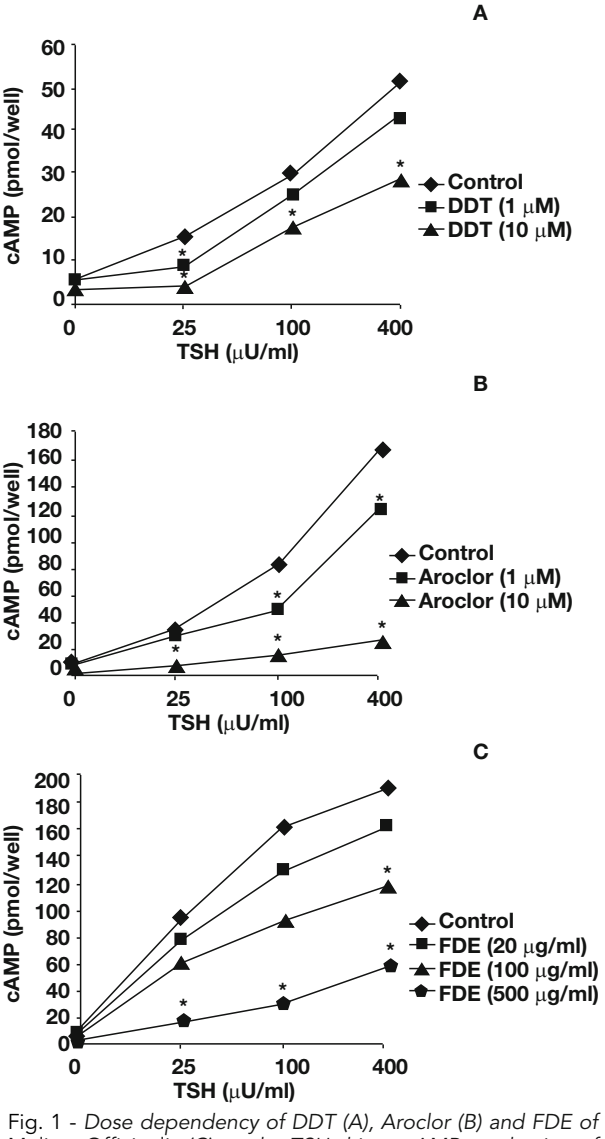

Fig. 1 - *Dose dependency of DDT (A), Aroclor (B) and FDE of* Melissa Officinalis *(C) on the TSH-driven cAMP production of JP-09 cells. Three different concentrations of TSH and thyroid disruptors were tested. Control indicates that, at each TSH concentration tested, each thyroid disruptor was replaced by its own solvent. For each thyroid disruptor, a dose-dependent inhibition of cAMP production is observed. *Student's t-test vs control:* $p<0.05$. *See text for abbreviations.*

Table 2 - *Results of various assays expressed as percent inhibition (mean±SD) produced by thyroid disruptors (TD) vs controls. Control indicates that each endocrine disruptor was replaced by its own solvent.*

Test	*Melissa Officinalis* (500 µg/ml)	DDT (10 µM)	Aroclor (100 µM)
JP-09 + TSH	87.1±4.8%*	60.5±4.8%*	84.7±3.1%*
JP-09 + TSAb	45.4±14.8%*	48.8±9.4%*	88.8±1.2%*
JP-09 + Forsk	12.5±11.3%	40.7±10.3%*	58.6± 16.7%*
CHO-K1 + Forsk	6.1±42.8%	56.3±0.4%*	59.3±4.5%*
TSH assay	23.9±2.5%*	3.2±0.7%	6.4±3.3%
TSH-R assay	64±2.9%*	0%	0%

Student's t-test vs controls in the absence of TD. *$p<0.05$. CHO-K1: Chinese Hamster ovary cells (CHO) clone K1; JP-09: CHO cells transfected with the recombinant human TSH receptor (clone JP-09); Forsk: forskoline; TSAb: TSH-receptor stimulating antibody; TSH assay: immunometric assay of TSH; TSH-R: assay of TSH receptor blocking activity. DDT: 1,1-bis-(4-chlorphenyl)-2,2,2-trichloroethan.

Forskolin-induced cAMP production assay

This assay was performed using both JP-09 cells (that display the TSH-receptor) and CHO-K1 cells (no TSH receptor) to make sure that the effect on cAMP production was totally unrelated to the presence of the TSH receptor. No significant inhibition of forskolin-mediated cAMP production was produced by *Melissa Officinalis*. Inhibition of cAMP production was observed in the presence of Aroclor 1254 and DDT, with no difference between the two cell clones (Table 2).

Immunometric assay of TSH

These experiments were devised to test the conformational changes of the TSH molecule that might interfere with the hormone antigenicity and possibly with its biological activity. In the presence of *Melissa Officinalis* a significant inhibition (23.9%, $p<0.05$) of antibody binding to TSH was observed, while Aroclor 1254 and DDT did not interfere with the TSH assay (Table 2).

Assay of TSH receptor blocking activity

A clear inhibition of TSH binding was observed in the presence of *Melissa Officinalis* (64%) while no significant changes were produced by Aroclor 1254 or DDT (Table 2).

DISCUSSION

During the years a large number of endocrine disruptors have been pointed out for their potential ability to induce thyroid hormone imbalance. Many natural substances and drugs have long been known to cause goiter or thyroid dysfunction (2, 3). More recently, environmental pollutants, such as pesticides and industrial compounds, have attracted the attention of the debate on public health. Indeed, many observations of wildlife suggest a thyroid-disrupting activity of these substances and their possible relevance to human health due to their widespread distribution in soil and water (1, 12-20). TDs can act at several levels: a) at central level by interfering with TSH production or release; b) at thyroid level by affecting thyroid hormone synthesis or release; c) at peripheral level by interfering with thyroid hormone transport, metabolism or action on T_3 receptor. Thyroid hormone secretion is sustained by TSH that binds to a specific receptor and stimulates Gs protein that activates adenylate cyclase, and in some species, including human, Gq protein that stimulates the inositolphosphate pathway. These enzymes activate most thyroid functions, including iodide uptake and organification (7), thyroid hormone synthesis, thyroglobulin proteolysis (21), which results in thyroid hormone secretion, and thyroidal deiodinases (22), which help conserve iodide stores. TSH also stimulates many other pathways of thyroid cell metabolism: oxygen consumption, glucose and fatty acid utilization, the synthesis of nucleic acids and structural proteins. Any impairment of thyroid hormone synthesis leads to an increase in serum TSH concentrations that is the main causative factor of thyroid enlargement, i.e. goiter formation. The latter mechanism may, to a certain extent, compensate for thyroid deficiency.

This study was undertaken to standardize an assay that allows the identification of exogenous substances capable of interfering with TSH-induced cAMP production. To this purpose we have modified a previous assay that was set up in our laboratory to measure TSH receptor blocking antibodies in sera from patients with autoimmune thyroid diseases (10). CHO-K1 cells transfected with the human cDNA of TSH receptor (JP-09 cells) were employed to reproduce *in vitro* the first steps of adenylate cyclase activation in man. To better understand the mechanism of action of TDs acting at this level, we developed a set of assays further exploring some of the possible sites of attack of the substances. First, we have selected a panel of 12 substances among those that, based on current literature, might inhibit thyroid function or growth. Any toxic substance may affect cell function by acting on innumerable sites that would inhibit cAMP production in a non-specific manner. To rule out this possibility we have preliminarily measured the TSH-induced adenylate cyclase activity retained by cells after prolonged pre-incubation (overnight) with each substance. The substance concentrations that showed a non-specific inhibitory effect by this means were not employed in following experiments.

Three out of 12 potential TDs (25%) turned out to be inhibitors of TSH-induced adenylate cyclase activity: freeze-dried extracts of *Melissa Officinalis*, DDT and Aroclor 1254.

Melissa Officinalis has been studied in the past for its ability to inhibit the response of thyroid cells to TSH *in vivo* and *in vitro* (8). The inhibitory action of *Melissa Officinalis* was attributed to its ability to interact with the protein moiety of the TSH molecule, to form products of increased molecular size and altered configuration that are unable to bind to the TSH receptor. Our study confirms that extracts of *Melissa Officinalis* are potent inhibitors of TSH-induced adenylate cyclase activity in CHO cells transfected with the human TSH receptor. This inhibition was not observed when cells were incubated with forskolin, a stimulator of adenylate cyclase that bypasses the TSH receptor, thus indicating that the extracts of *Melissa Officinalis* interfere with the hormone binding to the receptor. This was confirmed by direct measurement of TSH binding to its receptor that was strongly inhibited in the presence of the extracts. We also observed that the extracts of *Melissa Officinalis* modify the antigenic properties of TSH, although to a much lesser extent as compared to the inhibition of binding and biological activity. Furthermore, cAMP production induced by TSAb was also inhibited by *Melissa Officinalis*. Overall, these data suggest that principles contained in *Melissa Officinalis* may block the binding of TSH to its receptor by acting both on the hormone and the receptor itself.

Aroclor 1254 is a mixture of polychlorinated biphenyls (PCBs), industrial chemicals that were largely used as fire retardants in past years, and that are still present at high levels in the soil and waters of many countries, since they are very resistant to biodegradation. In addition they tend to accumulate in organisms, particularly in those placed at the top of the food chain. Current evidence suggests that these compounds could have a direct effect on the thyroid, as indicated by the histologic changes observed in the glands of animals exposed to PCBs (23), resulting in primary thyroid hypofunction. The mechanism by which PCBs would produce such an effect is not known. Our results suggest that PCBs may block thyroid function, and possibly growth, by inhibiting cAMP production. Indeed, we observed an inhibition of adenylate cyclase activity stimulated by TSH or TSAb and, though to a lesser extent, by forskolin, both in the presence and in the absence of the TSH receptor. However, Aroclor 1254 did not interfere with the TSH binding to its receptor or with the antigenic properties of the TSH molecule. Thus, it is conceivable that Aroclor 1254 affects cAMP production at the level of TSH-receptor and at post-receptor step. These observations are consistent with earlier *in vivo* studies (24) showing that in rats PCB exposure impairs the ability of thyroid to respond to TSH.

DDT is an organochlorine insecticide having severely adverse effects on the sexual development and reproductive capacity of many animal species (25). Though DDT is banned in developed countries, it is still used in third world countries with endemic malaria. Like PCBs it is fat-soluble and accumulates in organisms. There is abundant literature concerning the possible effects of DDT on thyroid hormone balance (1), but the results are discordant, probably depending on the level and the duration of exposure, the animal species studied and the presence of co-contaminants. In this study we observed an inhibition of cAMP production by DDT, which was independent of the presence of TSH receptor since it was observed both in JP-09 and CHO-K1 cells, stimulated either by TSH, TSAb or forskolin. Like Aroclor 1254, DDT did not interfere with the TSH-receptor binding assay or with the TSH immunometric assay.

The clinical relevance of these findings cannot be inferred at present. In adults the thyroid gland is relatively resistant to mild TDs, since it is able to compensate through TSH-induced gland hyperplasia and stimulation of hormonal synthesis. This mechanism of protection may be impaired if TSH function is inhibited by an exogenous compound, leading to overt hypothyroidism. However, this compensatory mechanism may prove insufficient to maintain normal thyroid function in conditions of iodine deficiency that still affects many populations across the world. The main concern derives from TDs acting during development since thyroid hormones have a pivotal role for the development of the central nervous system in the fetus and neonate (26-30). Prenatal exposure to TDs has a potential for the most damaging effects, particularly on the central nervous system, and even transient defects in thyroid hormone availability could result in permanent brain damage.

In conclusion, we have developed a set of *in vitro* assays that allow the investigation into the effect of putative TDs on the TSH-mediated activation of the cAMP cascade. These assays may be useful to identify the mechanism of action of TDs, coming beside and supporting animal studies or epidemiological surveys.

ACKNOWLEDGMENTS

This work was supported by the following grants: Ministero dell'Università e della Ricerca Scientifica, Centro di Eccellenza

AmbiSEN "Effects of environmental chemical agents on the endocrine and nervous system"; Ministero dell'Università e della Ricerca Scientifica, progetti di interesse nazionale "Le malattie della tiroide: dalle basi molecolari alla clinica" e "Meccanismi molecolari nella patogenesi del nodulo tiroideo".

REFERENCES

1. Brucker-Davis F. Effects of environmental synthetic chemicals on thyroid function. Thyroid 1998, 8: 827-56.
2. Kohrle J. Flavonoids as a risk factor for goiter and hypothyroidism. In: Pèter F, Wiersinga W, Hostalek U eds. The thyroid and environment, Merk European Thyroid Symposium. Stuttgart: Schattauer. 2000, 41-63.
3. Gaitan E. Environmental natural goitrogens. In: Pèter F, Wiersinga W, Hostalek U eds. The thyroid and environment, Merk European Thyroid Symposium. Stuttgart: Schattauer. 2000, 69-78.
4. Zacharewski T. Identification and assessment of endocrine disruptors: limitations of in vivo and in vitro assays. Environ Health Perspect 1998, 2: 577-82.
5. De Vito M, Biegel L, Brouwer R. Screening methods for thyroid hormone disruptors. Environ Health Perspect 1999,107: 407-15.
6. Colborn T. Clues from wildlife to create an assay for thyroid system disruption. Environ Health Perspect 2002, 3: 363-7.
7. Tonacchera M, Van Sande J, Parma J, et al. TSH receptor and disease. Clin Endocrinol (Oxf) 1996, 44: 621-33.
8. Auf'mkolk M, Amir SM, Winterhoff H, Sourgens H, Hesch RD, Ingbar SH. Inhibition by certain plant extracts of the binding and adenylate cyclase stimulatory effect of bovine thyrotropin in human thyroid membranes. Endocrinology 1984, 115: 527-34.
9. Perret J, Ludgate M, Libert F, et al. Stable expression of the human TSH receptor in CHO cells and characterization of differentially expressing clones. Biochem Biophys Res Commun 1990, 171: 1044-50.
10. Chiovato L, Vitti P, Bendinelli G, et al. Detection of antibodies blocking thyrotropin effect using Chinese hamster ovary cells transfected with the cloned human TSH receptor. J Endocrinol Invest 1994, 17: 809-16.
11. Vitti P, Valente WA, Ambesi-Impiombato FS, Fenzi GF, Pinchera A, Kohn LD. Graves' IgG stimulation of continuously cultured rat thyroid cells: a sensitive and potentially useful clinical assay. J Endocrinol Invest 1982, 5: 179-82.
12. Jefferies D.J., French M.C. Avian thyroid: effect of p,p'-DDT on size and activity. Science 1969, 166: 1278-80.
13. Moccia RD, Leatherland JF, Sonstegard RA. Increasing requency of thyroid goiters in Coho salmon (Oncorhynchus kisutch) in the Great Lakes. Science 1977, 198: 425-6.
14. Koopman-Esseboom C, Morse DC, Weisglas-Kuperus N, et al. Effects of dioxins and polychlorinated biphenyls on thyroid hormone status of pregnant women and their infants. Pediatr Res 1994, 36: 468-73.
15. Leatherland JF. Changes in thyroid hormone economy following consumption of environmentally contaminated Great Lakes fish. Toxicol Ind Health 1998, 14: 41-57.
16. Osius N, Karmaus W, Kruse H, Witten J. Exposure to polychlorinated biphenyls and levels of thyroid hormones in children. Environ Health Perspect 1999, 107: 843-94
17. Longnecker MP, Gladen BC, Patterson DG Jr, Rogan WJ. Polychlorinated biphenyl (PCB) exposure in relation to thyroid hormone levels in neonates. Epidemiology 2000, 11: 249-54.
18. Matsuura N, Uchiyama T, Tada H, et al. Effects of dioxins and polychlorinated biphenyls (PCBs) on thyroid function in infants born in Japan – the second report from research on environmental health. Chemosphere 2001, 45: 1167-71.
19. Persky V, Turyk M, Anderson HA, et al. Great Lakes Consortium. The effects of PCB exposure and fish consumption on endogenous hormones. Environ Health Perspect 2001. 109: 1275-83.
20. Sandau CD, Ayotte P, Dewailly E, Duffe J, Norstrom RJ. Pentachlorophenol and hydroxylated polychlorinated biphenyl metabolites in umbilical cord plasma of neonates from coastal populations in Quebec. Environ Health Perspect 2002, 110: 411-7.
21. Marinò M, McCluskey RT. Role of thyroglobulin endocytic pathways in the control of thyroid hormone release. Am J Physiol Cell Physiol 2000, 279: 1295-306.
22. Santini F, Chiovato L, Lapi P, et al. The location and the regulation of the type I-iodothyronine 5'-monodeiodinase (type I-MD) in the rat thyroid: studies using a specific anti-type I-MD antibody. Mol Cell Endocrinol 1995, 110: 195-203.
23. Schumacher U, Zahler S, Horny HP, Heidemann G, Skirnisson K, Welsch U. Histological investigations on the thyroid glands of marine mammals (Phoca vitulina, Phocoena phocoena) and the possible implications of marine pollution. J Wildl Dis 1993, 29: 103-8.
24. Byrne JJ, Carbone JP, Hanson EA. Hypothyroidism and abnormalities in the kinetics of thyroid hormone metabolism in rats treated chronically with polychlorinated biphenyl and polybrominated biphenyl. Endocrinology 1987, 121: 520-7.
25. Turusov V, Rakitsky V, Tomatis L. Dichlorodiphenyltrichloroethane (DDT): ubiquity, persistence, and risks. Environ Health Perspect 2002, 110: 125-8.
26. Bigsby R, Chapin RE, Daston GP, et al. Evaluating the effects of endocrine disruptors on endocrine function during development. Environ Health Perspect 1999, 4: 613-8.
27. Porterfield SP. Thyroidal dysfunction and environmental chemicals – potential impact on brain development. Environ Health Perspect 2000, 3: 433-8.
28. Bernal J. Action of thyroid hormone in brain. J Endocrinol Invest 2002, 25: 268-88.
29. Howdeshell KL. A model of the development of the brain as a construct of the thyroid system. Environ Health Perspect 2002, 3: 337-48.
30. Zoeller TR, Dowling AL, Iannacone EA, Gauger KJ, Bansal R. Thyroid hormone, brain development, and the environment. Environ Health Perspect 2002, 3: 355-61.