Relevance of the assessment of thyroidal activity in the (water) environment:

A deskresearch

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Rhine Water Works
The Netherlands

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Contents

1 Introduction

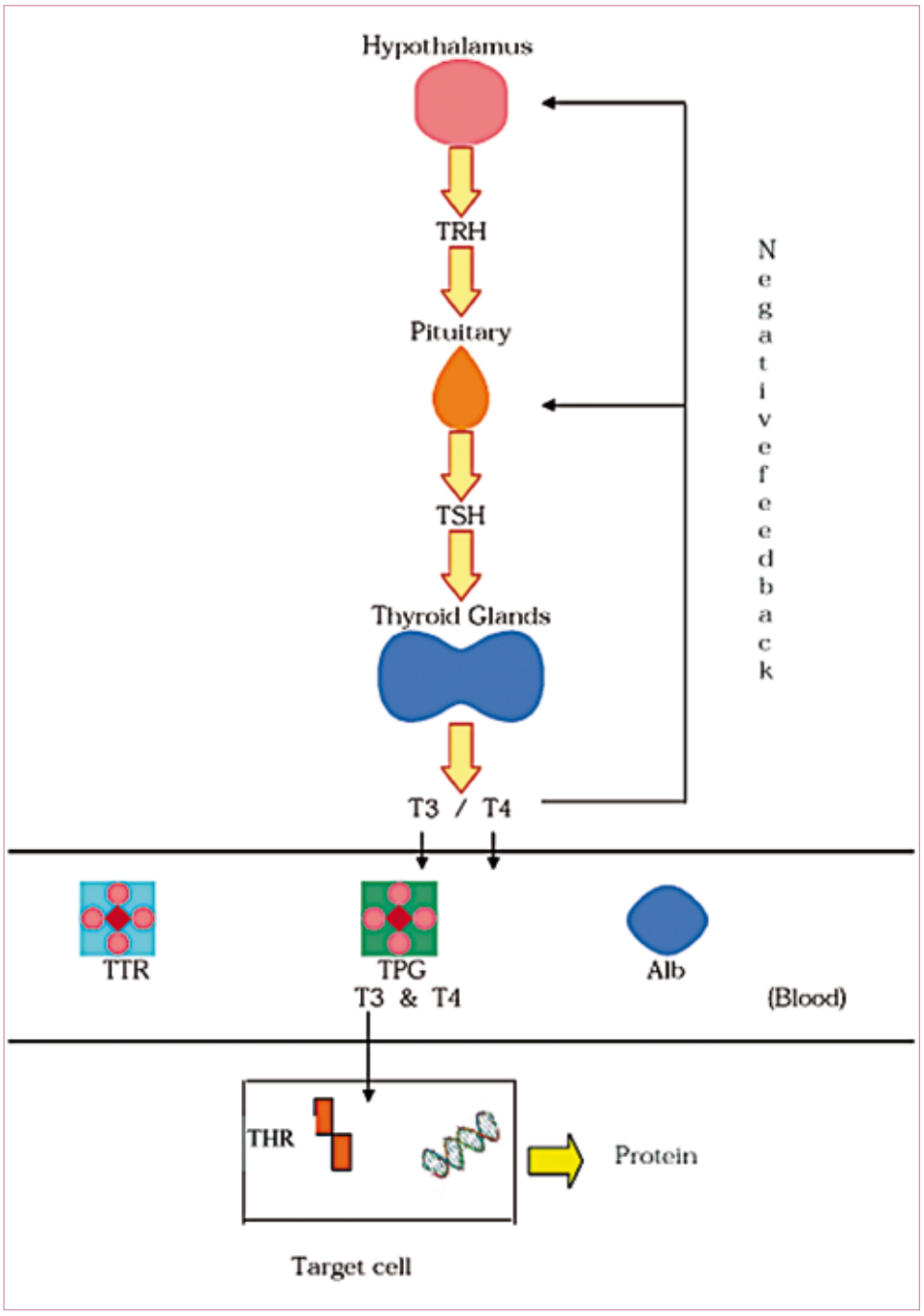
In view of the alarming reports in the media and scientific literature regarding effects of endocrine disrupting compounds (EDC) on wild life and humans, much effort is at the present time put into the development of analytical methods and bioassays in order to be able to detect and quantify known, suspected and unknown compounds in the environment, and in food or feed. Until recently, most attention was focussed at EDC showing estrogenic activity. However, in particular in humans there are three main hormonal systems with an overall influence on functioning and well-being. These comprise the estrogen/androgen system (Hypothalamus-pituitary-sex organ system, HPS), the glucocorticoid system (Hypothalamus-pituitary-adrenal system, HPA), and the Hypothalamus-pituitary-thyroid system, HPT). Each of these systems involves several organs and hormones, tightly regulated with respect to secretion and negative feedback. Details can be found in the literature and in text books (a, b). This report aims to give an overview of the HPT system in man and the putative disturbing effects of environmental pollutants, as well as potential bioassays and analytical methods for the assessment of EDC showing thyroidal effects.

2 Physiology

The thyroid system comprises a pathway from hypothalamus, pituitary and thyroid gland, secreting the main thyroid hormones 3,5,3'-triiodo-thyronine (T_3) and 3,5,3',5'-tetraiodothyronine, also termed thyroxine (T_4), which in turn are transported in blood to their biological targets [a,b]. It starts with thyrotrophin releasing hormone (TRH), a tripeptide synthesized and released by the hypothalamus in a daily rhythm. When reaching the pituitary, the synthesis and secretion of thyroid stimulating hormone (TSH), a glycoprotein, is stimulated. In the thyroid gland iodide uptake from food takes place, iodide is oxidized to iodine, which in turn is coupled to tyrosine residues at either 3 or 4 positions of thyroglobulin (TG) within the cells of the thyroid gland. Upon binding of TSH, TG degrading enzymes become active, releasing iodinated tyrosine residues T_3 and T_4 into the extracellular fluid, the blood. The ratio of T_3 to T_4 in the gland cells is about 1:10. Once in the blood, the thyroid hormones are bound to their corresponding binding proteins: thyroid binding globulin (TBG), transthyretin (TTR) and albumin (Alb), and then transported to target organs/cells.

The above mentioned transport proteins serve several purposes: 1) because thyroid hormones are highly insoluble in aqueous solution, their binding to these proteins leads to increased concentrations; 2) protein-bound hormones are protected against degradation by existing enzymes and this leads to a decreased clearance rate; 3) protein-bound hormones are readily transported to target cells. In blood the greater part of the thyroid hormones exist in bound form, where T_4 is for about 0.03 % in the free form and T_3 for about 0.3 % [a,b]. The ratio between bound and free thyroid hormones, as for hormones of other classes, is in a delicate equilibrium under the control of negative feedback regulation. In this aspect, T_4 in the pituitary is converted into T_3 and any rise in T_3 above normal levels leads to down-regulation of TSH secretion and hence to less synthesis of thyroid hormones in the gland. The thyroid pathway including negative feedback regulation is depicted in Figure 1.

Figure 1: Negative feedback regulation in the hypothalamus-pituitary-thyroid (HPT) system.



Shown is the regulation in the HPT system, including negative feedback control by T3/T4 in blood.

For a long time only the free fraction of thyroid hormones was considered biologically active, where is was thought that due to the non-polar nature T3 and T4 were mainly entering cells by diffusion. However, it seems that specific membrane transporters and receptors mediate uptake processes for protein bound thyroid hormones and also play a significant role in their function at target cells [c-f].

TBG

Thyroid binding globulin is a high-affinity, low-capacity transport protein with a MW of about 63,000 Da that is synthesized in the liver at a rate of about 16 mg per day. Its concentration in blood in normal physiological conditions is about 200 – 350 nmol/L. It consists of 4 subunits and has one specific binding site. It can bind 200 – 300 nmol of thyroid hormones per liter. About 70 percent of circulating T4 and 77 percent of T3 is normally bound to TBG. Its affinity for T4 is about 2.5 x 107 L/mol.

TTR

Transthyretin, also termed thyxoxine binding prealbumin or TTR, is a stable tetramer consisting of 4 identical monomeric subunits and with a MW of about 54,000 Da. It contains two binding sites, one showing high affinity, the other showing low affinity for thyroid hormones. Its concentration in blood is about 5 µmol/L providing a capacity of about 10 µmol/L. Of the total amount of thyroid hormones about 10 percent of T4 and about 8 percent of T3 is bound to TTR in normal conditions. The affinity of T4 is about 6.2 x 1010 L/mol. TTR is synthesized in liver and choroid plexus, where in the latter case it is mainly secreted into the brain and cerebrospinal fluid.

Albumin

This blood protein is a low-affinity, high-capacity transport protein for thyroid hormones, as well as many other substances. Of the total circulating thyroid hormones it binds about 20 percent of T4 and about 15 percent of T3. Albumin is synthesized in the liver in high concentrations.

It should be noted that for all three transport proteins the affinity of T3 is about 10-fold lower than of T4.

Thyroid hormones

The main thyroid hormones T4 and T3 and for a smaller part rT3 are synthesized in the thyroid gland and peripherally as described above. The total daily production is about 0.8 µg/L, 0.012 µg/L and 0.003 µg/L in serum, respectively. Degradation products are eliminated in faeces. Although T4 is present in the highest concentration, it has been found that T3 is the more biological active form in target cells.

Thyroid hormone receptor

The receptor for thyroid hormones, THR, has been found in cytoplasm and nucleus. It has a molecular weight of 60 – 65,000 Da. Upon binding of T3 dimerisation occurs and the dimeric form binds to

thyroid hormone response elements (THRE) at the DNA, leading to transcription and the synthesis of proteins. The highest levels of THR are found in pituitary and muscle.

Physiological function of thyroid hormones

Thyroid hormones, in particular T_4, are considered the ultimate regulator of metabolism and their function was recognized as far back as the late 1800s [g]. Thyroid hormone affects carbohydrate and lipid metabolism, protein synthesis and breakdown, cardiovascular and renal function, brain development and maintenance, etc. Low levels of thyroid hormone in animals result in arrest of development [h]. In humans, especially in babies, hypothyroidism leads to cretinism, a condition characterized by mental and physical retardation. However, also in adults hypothyroidism has serious effects, such as tiredness, lethargy, obesitas, hair loss, attention deficit. In contrast, hyperthyroidism leads to muscle protein catabolism, restlessness, weight loss, mental slowness and emotional instability, increased heartbeat and, eventually, cardiovascular diseases. Many people suffer from thyroid disorders and thyroid hormones or derivatives present the 2nd most prescribed drugs [g]. Fortunately, especially hypothyroidism can be readily treated by administration of thyroid hormones. Before T_4 could be produced synthetically in 1926 (discovered by C.R. Harington) thyroid gland extracts were successfully given to hypothyroidal patients. Later on it was found that in some cases a combination therapy of T_4 plus T_3 worked better.

Apart from genetic defects, there are several physiological situations that may lead to changes in levels of thyroid hormones, TRH, TSH and/or transport proteins. Thyroid function in normal conditions is predominantly regulated by proper intake of iodine from food. For that reason iodine is added to salt to compensate for inappropriate alimentary uptake. Additionally, in pregnancy and hepatitis, for example, TBG is increased, whereas during starvation TSH and consequently thyroid hormones are decreased. Further, TTR levels in CSF are clearly related to Alzheimer, Parkinson and depression. The autoimmune disorders Graves disease and Hashimotos disease are characterized by a hyperthyroidal and hypothyroidal state, respectively [i].

Many drugs similarly may influence the thyroidal status of patients. For example, estrogens as in oral contraceptives and high-dose corticosteroids increase TBG and TTR levels, respectively. In contrast, TBG is raised upon drug therapy with anabolic steroids, phenytoin, phenylbutazone, high-dose corticosteroids; and salicylates lead to decreased TBG and TTR (f). Uptake of thyroid hormones, required for proper physiological functions, is inhibited by several drugs. An overview of effects of drugs is given in Table 1.

Table 1: Effects of drugs on the thyroid system.

Agent	[TBG]	[TTR]	[TH]	Displacement from binding sites	Inhibition TH-uptake Inhibition	Inhibition Transport	Competition with T_4 for binding to TTR
Estrogens	+	-					
Heroin	+						
Methadone	+						
Perphenazine	+						
5-Fluorouracil	+		+				
Clofibrate	+						
Androgens	-	+					
Anabolic steroids	-	+					
Glucocorticoids	-	+					

Agent	[TBG]	[TTR]	[TH]	Displacement from binding sites	Inhibition TH-uptake Inhibition	Inhibition Transport	Competition with T_4 for binding to TTR
Furosemide				+			
Cholecystographic Agents			-	+	+	+	
Amiodarone			-				
Aminoglutethimide			-				
Tolbutamide			-				
Sulfonamides			-				
Mitotane			+				
Phenytoin			-				
Carbamazepine			-				
Phenobarbital			-				
Fenclofenac				+			
Rifampicine			-				
Salicylates				+			+
Nicotinic acid				+			
Sulfobromophthalein				+		+	
Bilirubin				+		+	
Indocyanine green				+		+	
Diphenylhydantoin					+		
Flufenamic acid					+		+
Meclofenamic acid					+		+
Mefenamic acid				+	+		+
2,3-Dimethyldiphenylamine					+		
Diclofenac					+		+
Phloretin					+		
Nifedipine					+		
Verapamil					+		
Diltiazem					+		
CMPF					+		
Indoxylsulfate					+		
NEFAs					+		
Diflunisal							+
Milnirone							+
Ethacrynic acid							+
Indomethacin							+
Sulindac							+
Fenoprofen							+
Ibuprofen							+
Oxyphenylbutazone							+
Iopanoic acid							+
Flavonoids							+

Legend: The above data comprise a compilation from literature.
[TBG] = concentration TBG; [TTR] = concentration TTR; [TH] = concentration thyroid hormones; + = increase in concentration / inhibition of optake TH into cells/ transport of TH / competition with T4 for binding to TTR.

Assays for thyroidal status

In clinical chemistry the thyroidal status of patients is generally rather important. Laboratory diagnosis of thyroid disease may be divided into 1) tests measuring the level of thyroid function; 2) tests indicating the cause of thyroid dysfunction. As outlined previously, the thyroid systems function at several levels from hypothalamus through pituitary to thyroid gland and thyroid hormones, including transport by proteins to target cells and binding to THR and DNA. For each of these levels analytical as well as functional tests have been developed. Tests which are most widely used in routine clinical practice involve: a) total circulating concentrations of thyroid hormones T_3 and T_4; b) serum protein bound iodine; c) thyroid hormone binding tests; d) concentrations of circulating free (unbound) thyroid hormones or the calculated free thyroxine index; e) dynamic tests of thyroid function; f) tests of peripheral tissue function; g) tests of hypothalamic/pituitary function from TRH and TSH concentrations; h) uptake by cells. For details, see the textbook of Hall en Griffin [a]. It should be noted that conventionally components of the TPH systems are quantified using immunoassays, whereas free thyroid hormones are determined by equilibrium dialysis [a, b].

In addition to clinical chemistry, also the development of drugs in the pharmaceutical area involves the study of effects of putative new medicines on endocrine systems, for example, thyroid hormone analogues for the treatment of hypothyroidism by replacement therapy; or, in contrast, undesirable side effects on the thyroid systems of drugs for different purposes. For such studies, either the end effect on thyroid hormone levels or the binding of certain compounds to transport proteins (TBG, TTR) and/or the receptor (THR) and consequently the displacement of endogenous thyroid hormones is examined. For an overview of drugs affecting the thyroid system see Table 1. In addition, components of the thyroids system are used in investigations into the relation between disorders and the thyroid system. It has been found, for example, that obesitas and insuline resistance (diabetes type 2) are both related to decreased levels of T_3 [j], similarly as cardiac deseases are accompanied by low T_3 syndrome (k). Thyroid hormones also have a hypocholesterolemic effect (l) and hypothyroidism is in some cases related to affective illness, which can be alleviated by thyroid hormone replacement therapy (m). In 2002, Colburn (n) noted that ADHD syndrome and autism may be caused by an imbalance in thyroid hormones initiated already in utero.

3 Environmental influences

Already in the 1960s an effect of synthetic chemicals on the endocrine system in wild life was noted. Initially, attention was focused on compounds showing estrogenic activity, but the additional observation of an enlarged thyroid gland made thyroid hormones also a subject of investigation. In the 1970s it was observed that exposure to mixtures of PCBs resulted in lowered levels of thyroid hormones and enlargement of the thyroid gland (o). It was hypothesized that structural similarities between endogenous hormones and chemicals could lead to disturbances of the HPT system. There are several mechanisms whereby chemicals may affect the HPT system: a) at the level of uptake of iodide; b) oxidation of iodide to iodine; c) iodination of tyrosines on thyroglobulin and deiodination of T_4 to T_3; d) displacement of thyroid hormones from their transport proteins; e) displacement of T_3 from its receptor; f) interference with metabolism and excretion [p]. In view of the importance of the HPT system in animals and humans many studies were performed into effects of various compounds, in particular PCBs, dioxins, metabolites thereof, pesticides, flame retardants, etc. Especially because such substances are widely present and often highly persistent. Some examples are given below.

In 2001, Zoeller (q) reported that PCBs are disruptors of thyroid hormone action resulting in decreased T_4, but without effect on T_3. It was also mentioned that some PCBs or metabolites as (ant)agonists affect human brain development, motor function, visual memory, shorter gestation, lower birthweights, IQ, behavior and hearing. Using computer graphics, Rickenbauer (r) predicted that selected PCBs and hydroxylated metabolites would show similar or even higher affinity for TTR than T_4 itself. His findings were supported by testing in competitive binding experiments. The effect of PCBs on the fetal brain due to an effect on thyroid signalling was also mentioned by Gauger (s). In wildlife, environmental agents with endocrine activity may lead to abnormal thyroid function, sex alternations, poor hatching success, lowered fertility, limited growth (t). A highly active group of compounds comprise the polybrominated flame retardants (PBDEs) showing both estrogenic and thyroidal effects, although the mechanisms are not fully elucidated (u). Organochlorines as thyroid disruptors are described by Wade (v). In their study male rats were administered low concentrations of 16 different organochlorine compounds and it was found that, although not always statistically significant, levels of T_3, T_4, TSH, T_3-uptake as well as gland cells and hepatic metabolism were all changed. Possible mechanisms are discussed. Further, it was also concluded that MRL or NOEL values are not sufficient to ensure protection. Colborn (n) describes effects of thyroid disruptors on various species. In amphibians PCBs result in several disorders with respect to metamorphose, vitellogenin production and brain development. In birds, especially fish-eating birds in the US Great Lakes, chemicals, e.g. PCB congeners have been determined and found to affect embryos and behaviour, increased T_4 levels and an extended breeding cycle. In fish, thyroid hormones direct swimming behaviour and migration. Pesticides such as carbaryl, endosulfan, and malathion appeared to have profound but varying effects on levels of T_3 and T_4 in fresh water catfish. In polar bears PCBs and DDEs have been detected and related to T_4. Thyroid hormones disturbed behaviour and brain development in red deer. In 2004, Renner (w) reported that PCBs act as thyroid hormones interfering with their biological action in children and foetus. Disorders resulting from exposure include mental deficits. It was mentioned that PCBs show no binding to the THR and mechanisms are reportedly unknown. A particularly interesting compound in this regard is perchlorate. High levels of this compound up to 92 ppb, have been found in breast milk from nursing mothers in the U.S., and

consequently babies are exposed to 20 times higher than the safe dose recently recommended by a National Academy of Sciences committee (x). Children from mothers with high perchlorate levels often exhibit hypothyroidism and increased TSH. Similarly, it has been found in cow milk leading to damage to the thyroid gland. Perchlorate has natural and anthropogenic sources. In fertilizers it amounts to a content of 1%, it originates from rocket fuel production and has been detected in drinking water supplies in the U.S. (y, z). Another suspected group of compounds with thyroidal activity comprises plasticizers and phthalates, that are commonly used in industry. For example, bisphenol A (a monomer of polycarbonate plastics, produced in an amount of about 800 million kg annually in the U.S.) has been shown to disrupt thyroid hormone action as an antagonist of T_3 at the nuclear level (aa). Various pesticides may also pose a problem with regard to thyroidal activity; e.g. animals chronically exposed to resmethrin and sumithrin exhibit increased thyroid gland weight, decreased T_3 and T_4 as well as increased TSH (bb).

In contrast to end effects, more specific actions of thyroid hormones have been assessed by determining the affinity of chemicals for transport proteins and receptors as well as the displacement of endogenous thyroid hormones. In addition, (bio)assays may be used to predict potential thyroidal activity of environmental samples. These items will be discussed below.

4

Assays for thyroidal activity of environmental pollutants

In view of the risk of thyroid and thyroid-related disorders as a result of exposure to environmental pollutants it appears highly important to provide (bio)assays to assess such activity before humans and animals come into contact by ingestion of contaminated food and/or water. In principle, any component of the HPT system may be used to develop bioassays. However, regarding the uptake of pollutants by an organism the first components that are encountered are the blood transport proteins, TBG, TTR and Albumin. As a tool for intracellular effects, the THR or membrane transporters may be used. One of the first assays directed to assess interactions of environmental pollutants with thyroid hormone transport protein TTR or TBG was developed by Lans (cc, dd). The proteins were purified from human and rodent blood and used together with 125I-T_4 in a competitive radiobinding assay. Several hydroxylated PCBs and some PCDDs and PCDFs showed inhibition of T_4 binding to and consequently affinity for TTR, but not TBG. A review of bioanalytical screening methods for dioxins and dioxin-like compounds is given in Behnisch (ee). Listed are AhR-based assays, enzyme induction bioassays using cell lines which express AhR-mediated CYP1A induction (EROD), CALUX, cell proliferation-based assays, DNA binding, AhR ligand binding and enzyme immunoassays. Advantages and disadvantages of each bioassay as well as some parameters such as detection limit, linear working range and EC_{50} for TCDD as a model compound are discussed. For the assessment of toxic and biochemical effects induced by PCBs/PCDDs/PCDFs, the group of Brouwer used the Ah-receptor (ff). Several physiological, developmental, behavioural effects as well as effects on thyroid hormone levels and thyroid hormone metabolism were related to exposure to the above compounds. In addition, the results of various studies discussed in the scope of a workshop are described. Especially alarming is the exposure of fetus or baby via the mother because of the high sensitivity of developing brain and organs for such pollutants. To study the effect of pollutants on the thyroid system at the nuclear level, Moriyama (aa) used nuclear THR from rat liver as well as a gene expression system comprising cloned cells from human embryonic kidney and human hepatoblastoma. Incubation with 125I-T_3 as radioligand and bisphenol A (BPA) as a model compound demonstrated weak affinity of BPA for THR, but clear suppression of transcriptional activities. Further experiments with BPA were conducted by Zoeller (gg). In short, pregnant rats were provided with BPA (various concentrations) in their diet. New born pups were evaluated for effects on serum T_4/THS levels (immunoassay) and TH-related gene expression in the brain using in situ hybridisation techniques. It was concluded that BPA exerts selective TH antagonism leading to increased T_4, hyperthyroidism, in offspring. In medicine several drugs used have similarity to environmental pollutants. For example, milrinone, a inotropic agent administered for cardiac diseases, is a non-iodinated bipyridine. In a study performed by Davis (hh) milnirone was examined for activity as an inhibitor of iodothyronine binding by human transport proteins in competitive radioligand binding assays combined with electrophoresis. It was found that this compound, having structural homology to thyroid hormones, has affinity for TTR and displaced both T_4 and T_3 from their binding site. In vitro testing of outdoor and indoor airborne particulate matter containing hydroxylated PCBs, using a competitive TTR assay, was performed by Heussen (ii) who concluded that in this assay T_4 could be displaced from TTR. Transport of thyroid hormones by TTR and the influence structural conformation of PCBs on binding was also subject of a study of Chauhan (jj). Using a competitive TTR assay with T_3 and T_4 as ligands, it was shown that both chlorinated and brominated compounds exhibited affinity for TTR, those having a di-meta-substitution on both rings having the highest affinity. A similar report was published by Meerts

(kk). Here several brominated flame retardants were tested for possible interaction with 125I-T_4 in binding to TTR. Structure-activity relationships are discussed. In addition different PBDE congeners were subjected to treatment by microsomal extracts and it was found that the metabolites competed with binding of T_4 to TTR. Similarly, synthetic hydroxylated PBDEs showed thyroid-like potencies. With regard to hydrolylated PCBs and PBDEs bound to TTR it was noted that such complexes may facilitate transfer across the placenta and the blood-brain barrier, leading to relatively high levels in the fetus. This hypothesis was verified in rats administered 14C-labelled or unlabeled 4-OH-CB107 during pregnancy. In the fetus radioactivity was found in plasma bound to TTR, and T_4 as well as free T_4 were significantly decreased [ll]. TBG as the binding protein in a radioligand assay (125I-T_4) was used in a clinical study to determine the binding of thyroid hormones and derivatives [mm]. Similarly, Munro [nn] tested various drugs (n=26) on binding to TTR and TBG using 125I-T_4. It will be appreciated that medicinal drugs often show similarities to pesticides, however, analogous binding studies have until now not been reported. Most of the above mentioned studies used radioactively labelled ligand in competitive binding assays, which poses problems with regard to safety and disposal hazards. Those utilizing molecular-biology techniques and genetic engineering are laborious, time-consuming and expensive and require advanced facilities. An elegant solution to these disadvantages was found in the employment of the SPR-based biosensor assay using the Biacore® . Herein the specific interaction of any binding pair results in an enhancement of the SPR signal. This method has proven to be highly sensitive and versatile. Results of the development of a biosensor assay using a T_4-derivative and either TTR or TBG in a competitive format on the Biacore® have been published (oo). It was concluded that several pharmaceuticals, pesticides, PCBs, PHAHs, PBBEs, and metabolites thereof showed affinity for TTR, whereas TBG reacted with only very few of the compounds tested. The advantage of this biosensor assay is its high sensitivity, specificity and high throughput. Disadvantage are the high costs of the apparatus. Based on the Biacore® biosensor assay our present work is aimed at transferring the TTR and/or TBG-based bioassay onto microtiter plate using fluorescence for detection (FITC-labeled T_4). Preliminary results showed that several phenolic compounds, among which bisphenol A, alkylphenols, PCP, as well as some pesticides and pharmaceuticals, have affinity for TTR. The TBG-based assay and one using pregnancy serum are now under development.

5 Relevance of pollutants

Among the numerous compounds present in surface water and effluents of wastewater treatment plants, only a limited number has been tested for thyroidal activity. Among these, PCBs, PBDEs, metabolites thereof, and phenolic compounds show the highest affinity in the tests used. However, these compounds are for the greater part associated with sediment and particulate matter due to their non-polar nature and, because of the treatment techniques routinely applied the risk of them being found in drinking water is negligible. This does not apply to e.g. nonylphenol and bisphenol A, both being rather soluble in water and active in thyroid assays [pp). Frequently, surveys are conducted in Dutch surface water and effluents to assess water quality status and the occurrence of new priority contaminants (qq, rr, ss). One drawback of the analytical methods used in these surveys is that mainly GC-MS is used. LC-MS would capture more polar compounds and it is suggested to be used in further studies. That would give more insight into the degradation of PCBs and PBDEs and the presence of metabolites in water. Further, although the above compounds are not expected to be present in drinking water, they might be produced during the production process if chlorination is used. Another origin of substances with thyroidal activity may be the plastic pipes (PE, PEX, HDPE, PVC) used for transport of drinking water. Some phenolic compounds are able to diffuse from the walls of the pipes into the drinking water (tt, uu). Additional pollutants comprise pharmaceuticals (vv, ww, xx). Little if any is know about the activity of pharmaceuticals present in surface (and drinking water) as thyroid-like substances. The same applies to pesticides and/or their metabolites. In our preliminary study for several of these substances an affinity for TTR was found, e.g. fibrates, ibuprofen, 2,4-D, 2,4,5-T and 2,4-DB. Further research is needed to assess thyroidal activity of pharmaceuticals/drugs and pesticides in order to get more insight into these groups of compounds. Studies should also comprise combinations of all kinds of compounds.

6 Conclusion

For water quality control, especially for the production of drinking water, it is required to assess not only the presence and quantity of numerous chemicals, but also to have a measure for toxicity. In view of the recent findings of endocrine activity in environmental and food samples and possible effects on ecology, animals and especially humans, it is of paramount importance to have reliable bioassays available for monitoring purposes. As discussed above, methods have been developed for testing of samples for estrogenic and thyroidal activity. In particular the presence of compounds showing thyroidal activity in environmental compartments and the exposure of humans to such substances may have several adverse effects. Effects on the foetus and young children due to transfer from the mother by the placenta and breast milk may comprise mental deficits and behavioural disorders, but also adults may suffer from thyroid-related conditions such as attention deficits, (chronic) fatigue, obesitas, diabetes, and the disease of Alzheimer or Parkinson at later age. Therefore environmental samples should be assessed for thyroidal activity. As mentioned above most of the methods developed for this purpose use either cell culture or radioactive ligand binding assays, which have various disadvantages. The biosensor assays on the Biacore® seem promising with regard to sensitivity and specificity for high throughput screening of samples. Nevertheless, the result is an integrated effect measurement for thyroidal activity and individual components still have to be analysed and reassayed. It is proposed to combine the Biacore® with either HPLC, GC and/or MS in an automated fashion. Any sample exhibiting affinity for TTR and/or TBG might then be further analysed and characterised. An alternative may be a TTR and/or TBG binding assay in microtiter plate format for ease of handling, high throughput and low costs. Once the causing agents have been characterized, measures can be taken to reduce their levels or even eliminate their presence. Overall, it is important to extend the work under development in order to achieve useful bioassays for assessing endocrine and in particular thyroidal activity in environmental or, optionally, food samples of individual compounds as well as combinations of several classes of compounds. In this regard, it should also be emphasized that for toxicity studies MRL and NOEL may not be sufficient as strict measures for protection assessment. In addition, although environmental pollutants having putative thyroidal activity may be present at low or very low levels, combinations of several compounds might have an unexpected, chronic, effect.

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