A LITERATURE REVIEW OF THE CURRENT STATE OF THE SCIENCE REGARDING SPECIES DIFFERENCES IN THE CONTROL OF, AND RESPONSE TO, THYROID HORMONE PERTURBATIONS. PART 1: A HUMAN HEALTH PERSPECTIVE

Report No:
RSA/ECPA001_THYROID

Report prepared for Sponsor:

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APRIL 2018

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1. Executive Summary

This review was commissioned by the European Crop Protection Association to address a series of questions raised following the issue of the draft guidance document (GD) from the joint ECHA/EFSA/JRC recommendations. The questions asked specifically related to the literature use by the draft guidance and whether or not it represented a state of the current science regarding chemical thyroid disruption. While the intention was that this document would present the laboratory animal data related to chemical induced disruption, while a second document would cover the human evidence, it was apparent in beginning the review that a document that ignored the human data was not going to be possible without seriously compromising the scientific content. This was because of the enormous amount of basic information, derived from human thyroid diseases and the clinical treatment of those diseases that was available and its incorporation was considered integral to understanding the laboratory animal data in order to place animal findings into the context of human relevance and risk.

Although there are numerous publications detailing the basic control of thyroid hormones it was decided that the first part of the review would require an introduction to the normal control and functioning of thyroid hormones in order for the reader to understand the subsequent sections dealing with perturbations in thyroid homeostasis. The review is structured to address the ECPA questions in order and covers species differences in the functional morphology and biology of the thyroid gland always with an eye to comparing the most common laboratory species for toxicity testing, the rat, with what we know about human thyroid responses. In terms of the effects of thyroid hormone perturbation there are two major endpoints of concern, namely neurodevelopmental effects on the foetus and neonate, and thyroid cancer. These have generally been separated where possible since the critical features of both, in terms of thyroid effects and the consequences of dysfunction in one part of the thyroid control process, are different between those acting in carcinogenesis and those determining foetal and postnatal development.

Section 4 of this review is an appraisal of the draft GDs and, on the whole, the GD provides an excellent roadmap for prospective registrants of new products and describes in detail what will be expected in terms of levels of proof, what it terms “lines of evidence”. Throughout, the draft GD illustrates its points with relevant literature references to clarify where necessary. However, it is highly selective in its use of the literature and in no way does it aspire to be a “state of the science” review of the area of endocrine disruption and neither is this within the stated scope of the document. Appendix A of the document is a summary of the information regarding thyroid physiology and provides a high-level opinion on how chemicals could secondarily affect the thyroid predominantly through a primary effect on the liver. While the draft GD does mention alternative modes of action, such as the sodium-iodide symporter, as potential thyroid targets, the degree of coverage would not permit any reader from understanding these processes, let alone the other thyroid targets that are present. It is clearly not a state of the science document, as regards the thyroid gland but it does take the opportunity of repeating a fallacy of there being effects on thyroid hormones in the absence of histopathology on the gland (page 96). It would be helpful to the critical reader if a relevant mammalian literature reference could be included to substantiate this statement since it has the potential to undermine one of the most sensitive and regularly conducted endpoints for most of the in vivo assays While this is disappointing it simply displays the perils of straying off the stated scope of the document into what appears, at face value, to be an informed survey of the literature.

Section 6 of the current review document details the literature evidence for species differences between human and rodents addressing each function in thyroid hormone synthesis, metabolism and excretion separately. It then covers the respective responses to chemicals known to affect thyroid homeostasis in the rodent using classic examples of rodent thyroid disruptors to illustrate the points made. Where possible, such as is the case for the sulphonamide drugs, the responses in rodents and humans can be directly compared under similar exposure conditions. Throughout the review statements are illustrated with clear chemical examples, such as those compounds operating through induction of hepatic metabolism and excretion that show qualitative differences between human and rat thyroid effects while for others, such as those chemical inhibiting thyroperoxidase which show significant quantitative differences in responses with humans being considerably more resistant to the thyroid disruption than are rodents.

Section 6.10 discusses the underlying principles of carcinogenesis as they apply to thyroid cancer in both human and the rodent, and addresses similarities and differences (summarized in Table 2), in particular the pivotal role of thyroid stimulating hormone (TSH) in rodent thyroid cancer and its secondary role in human cancer. There are a number of additional significant differences between the rodent and human in their basic thyroid biology and in their responses to the same or similar chemicals and the differences are exemplified with particular reference to iodine deficiency which induces goitre in humans under the sustained driving influence of TSH, even though thyroid cancer under these circumstances doesn't normally occur. In contrast non-genotoxic rodent thyroid cancer appears exquisitely dependent upon sustained TSH stimulus alone without the need for additional factors. This section concludes with a discussion of the overall relevance of chemically-induced rodent thyroid cancer to humans. Section 7 discusses the presence of thresholds for thyroid hormone changes for both neurodevelopmental and carcinogenic outcomes again by reference to literature examples of situations where threshold shave been clearly demonstrated for both outcomes.

Section 8 is a discussion of the effects on extra-thyroidal changes such as concurrent disease, concurrent systemic toxicity in other organs such as the liver and kidney, starvation, and heat and cold in affecting the concentrations of circulating thyroid hormones and thyroid morphology. All of these environmental changes can profoundly affect the turnover of thyroid hormones and at the very least organ toxicity, and food and water consumption, are situations that can occur regularly in routine toxicity studies as a consequence of the maximum tolerated dose (MTD) approaches that are applied in the conduct of such studies. A consideration of these factors will be critical if thyroid hormone measurements are routinely incorporated into toxicity studies.

Throughout the review, where possible, the discussion has been undertaken with reference to the WHO/IPCS MoA/human relevance framework and the draft GD also embraces the principles outlined in the numerous publications that have arisen out the initiative. This is a welcome move to introduce more objective approaches to the regulatory process and to lay solid factual foundations to regulatory decisions.

The final part of the current review is questioning whether or not the current testing strategies for detecting thyroid disrupting chemicals are adequate for the purpose, and alternative animal models, including in vitro screens, are discussed. In light of the two-tier approaches advocated in the draft GD additional assays that specifically target potential molecular initiating events, are to be welcomed as part of the second tier for establishing MoA and determining human relevance of the particular events. The review makes use of an extensive list of references, both current and some considerably older, in making the points throughout.

2. Introduction

This document was commissioned by the European Crop Protection Association (ECPA) to appraise the Association of the current (issued 7th December 2017) ECHA/EFSA/JRC draft guidance document (GD) in terms of its fitness for purpose and its use of the current science in arriving at its conclusions. In order to carry out this remit, the current document has summarised the current state of understanding regarding the control of thyroid hormone homeostasis, in both human and animal species, and incorporates a critical review of the publicly available literature regarding the chemical perturbation of thyroid hormones particularly in those animal species that are used in the non-clinical safety evaluation studies required for the determination of human safety.

The publication of the outcome of the Thyroid European Commission-ANSES Workshop (European Union 2017) and the ECHA EFSA JRC Endocrine Disruptor Draft guidance (European Food Safety Authority/European Chemicals Agency 2016) posed a number of critical questions regarding the entirety of the information used to arrive at their conclusions, with particular emphasis on the sensitivity and specificity of the assays currently used to detect thyroid effects and the relevance of the species used in the bioassays for subsequent human risk extrapolation. This paper attempts to address those questions, by providing a comprehensive review of the available literature and to place

this into the appropriate context with regard to the value, or otherwise, of current testing strategies. It also proposes an approach by which the effects seen can be objectively used in providing appropriate assessment of the risks associated with exposure of the human population to chemical entities that could potentially perturb thyroid hormone function.

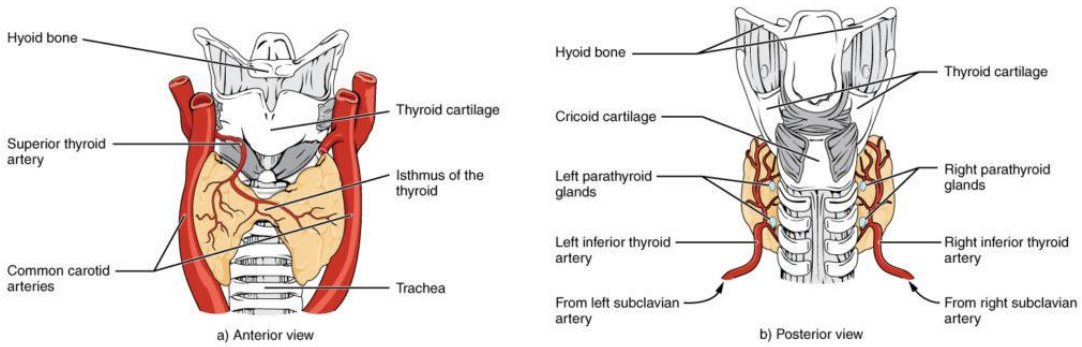
ECPA requested a focus on certain specific questions that are highlighted in italics before the respective relevant parts of the review.

3. Thyroid hormone function – the established understanding

This section summarises what is known about thyroid hormone production and secretion and is generally an amalgamation of the current knowledge derived from human and animal species. Where species differences exist, these are mentioned within the text.

The thyroid gland in mammals and birds (Fig. 1) is normally a bilobed organ with the two lobes being connected by an isthmus which lies on, and located ventro-laterally to, the trachea (McNabb and Darrass 2015). Histologically the gland consists of a number of follicles lined by low cuboidal epithelial cells and a follicular lumen containing colloid, composed of thyroglobulin, which appears pink in haematoxylin and eosin stained sections.

Fig. 1: Gross anatomy of the thyroid gland in mammals and birds - from https://courses.lumenlearning.com/ap2/chapter/the-thyroid-gland/

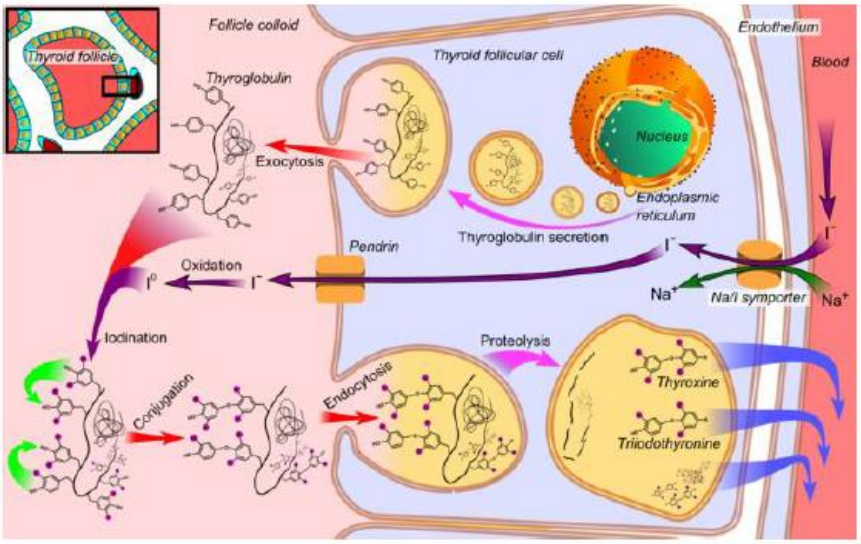


3.1. Thyroid hormone synthesis

Thyroglobulin is a glycoprotein made up of 134 tyrosine residues that is stored in the follicular lumen and which is the starting molecule for the subsequent synthesis of triiodothyronine (T3) and thyroxine (T4). Binding of thyroid stimulating hormone (TSH) to its receptor on the follicular cells triggers the up-regulation of the sodium-iodide symporter (NIS) on the basolateral membrane of follicular cells, resulting in an increase in the intracellular concentrations of iodine through a process known as iodine trapping (Rousset et al 2015). Once inside the cell the iodide is transported to the apical membrane of the follicular cell where it is acted upon by the enzyme thyroid peroxidase (TPO), an integral membrane protein present in the apical plasma membrane that catalyzes the sequential reactions needed for the formation of the respective thyroid hormones. TPO first oxidizes iodide to iodine, then iodinates tyrosine residues on thyroglobulin to produce mono- and diiodotyrosine, and finally links two tyrosine molecules together to produce T3 and T4. The peptide linkage between the thyroid hormones and thyroglobulin is then enzymatically cleaved, thyroid hormones are internalized at the apical surface of the thyroid epithelial cells by endocytosis, and hydrolytic enzymes within lysosomes

fuse with the endosomes to release the hormones which are subsequently actively exported into the circulation, through the basolateral membrane of the follicular cells via the monocarboxylate transporter 8 (Di Cosmo et al 2010). Once in the circulation the majority of the hormones reversibly complex with liver-derived binding proteins for transport to other tissues although a small proportion remains free in the plasma.

Fig. 2: Formation of hormones in the thyroid follicular cells – *from* https://courses.lumenlearning.com/boundless-ap/chapter/the-thyroid-gland/



T3 is the biologically active hormone and T4, the major thyroid hormone that is secreted from the thyroid gland, is considered a precursor or prohormone. Although all of the T4 is synthesised within the thyroid, in human beings between 40 - 80% of T3 (Bianco and Kim 2006; Bianco et al 2002; Gereben et al 2008; Gereben et al 2015) is made through deiodination of T4 in peripheral tissues, especially the liver, kidney, pituitary gland and muscle. An additional, inactive molecule, reverse T3 (rT3), is also made in peripheral tissue by the action of deiodinases on T4. T3 is produced by deiodination on the outer ring of the T4 molecule, while the inactive form, rT3 is produced through deiodination of the inner ring of the T4 molecule (Visser et al 2016). Production of rT3 is thought to be a means of controlling excess circulating T4 since it has no known biological activity. Approximately 20% of all of the bound T4 is converted to rT3 on a daily basis in the human liver and other extra-thyroidal tissues.

The deiodination reaction is catalysed by one of three enzymes called type 1, type 2 and type 3 iodothyronine deiodinases (Visser, 1988; Leonard and Visser 1986; Chanoine et al 1993). Type 1 deiodinase is the main enzyme expressed in the liver, kidney and thyroid, and the liver is considered to be the most important extra-thyroidal site for the production of T3 and for removal or rT3. Both T3 and rT3 are metabolized within the liver by 5- and 5'-deiodination respectively to produce the inactive 3,3'-di-iodothyronine (T2). In situations, such as illness, increased metabolic demand in general, or administration of drugs such as amiodarone or β-adrenergic medication, the proportions of T3 and rT3 made from T4 can change to accommodate the altered demands (Hackney et al 1995a; Narayana et al 2011; Barbesino 2014).

3.2. Tissue uptake, signal transduction, and metabolism of thyroid hormones

Tissue thyroid status depends not only on thyroid hormone uptake and secretion but also on metabolism, delivery of T3 to the nuclear receptors, and receptor expression and distribution within the target cell populations.

It had generally been assumed that thyroid hormone, due to its hydrophobicity, enters cells via passive diffusion but *in vitro* studies identified several membrane transporters belonging to the monocarboxylate, and organic anion, transporter families with the ability to actively transport thyroid hormones into cells (Janson et al 2005). A human genetic disorder, known as Allan-Herndon-Dudley Syndrome, was instructive in indicating the critical role of the monocarboxylate transporter 8 (MCT8) protein in actively sequestering hormones into target cells. (Dumitrescu et al 2004; Friesema et al 2004; Schwartz and Stevenson 2007), and severe neurologic deficits. The syndrome is due to a mutation in the MCT8 gene andit was shown that active thyroid hormone transport is required in certain tissues, especially the brain for normal functioning (Kersseboom and Visser 2011; Visser et al 2011).

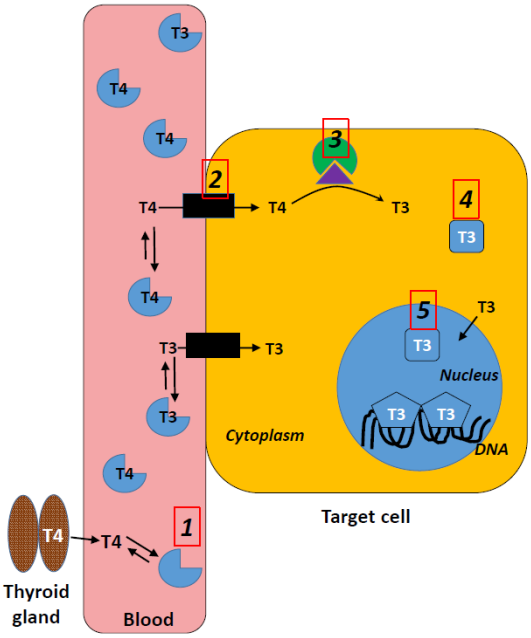
Table 1: Types of thyroid hormone transporters and their iodothyronine derivatives (Ahmed 2012)

Transporter	Iodothyronine derivatives	Specificity
MCT8	T3, T4, rT3, T2	+++
MCT10	T3. T4	++
OATP1A1	T3, T4, rT3, T2, T4S, T3S, rT3S, T2S	+
OATP1A2	T4, T3, rT3	
OATP1A3	T4, T3	
OATP1A4		
OATP1A5		
OATP1B1	T4, T3, T3S, T4S, rT3S	
OATP1B2	T3, T4	
OATP1B3	rT3, T4S, T3S, rT3S	
OATP1C1	T4, rT3, T3, T4S	++
OATP2B1	T4	+
OATP3A1 (V1/V2)		++
OATP4A1	T3, T4, rT3	+
OATP4C1	T3, T4	
OATP6B1		
OATP6C1		
LAT1	T3, T4, rT3, T2	
LAT2		
NTCP	T4, T3, T4S, T3S	++

Notes: The specificity is defined as high (+++) if the transporter only transports iodothyronine hormones, moderate (++) if it transports fewer than 5 other ligands, and low (+) if more than 5 ligands are known.

The MCT8 transporter has also been localised on the basolateral membrane of follicular epithelial cells suggesting that this protein might also act in the export of T4/T3 following its synthesis in the follicular epithelial cells (Friesma et al 2006). There are many known intracelllular thyroid hormone transporters, in addition to MCT8, and these are listed in Table 1 above (Ahmed 2012).

Fig. 3: Thyroid hormone secretion and transfer into cells (from Alshehri et al 2015)



1. = Plasma binding proteins (TBG, TTR, albumin); 2 = thyroid hormone transporters (MCT8, OCT14); 3. = iodothyronine deiodinases; 4. = cytosolic thyroid hormone binding proteins; 5. = thyroid hormone nuclear receptors.

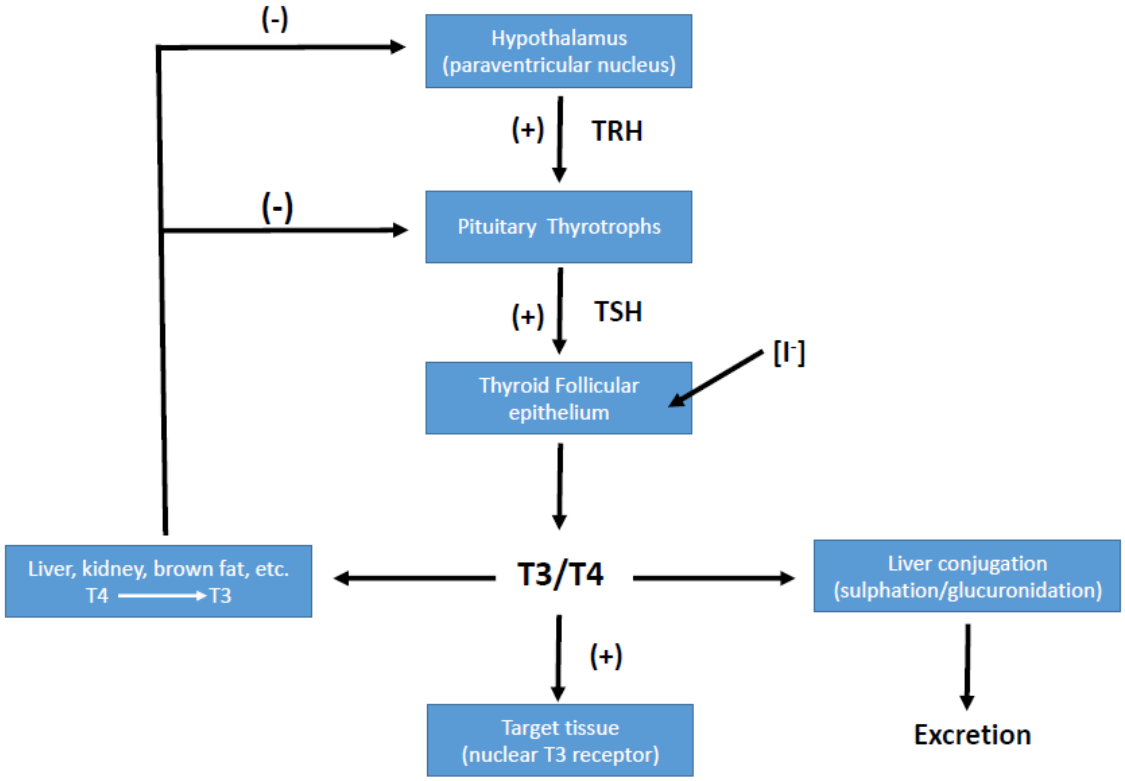
Once across the cell membrane and inside their target cells, cytosolic thyroid hormone binding proteins transfer the hormones to the nucleus where they dissociate from the carrier protein and bind to the nuclear T3 hormone receptor for subsequent cellular responses (Fig 3). Several different cytosolic proteins can act as thyroid transfer proteins including glutathione s-transferases (Ishigaki et al 1989; Kato et al 1989). It has been found that the thyroid nuclear receptor exists in several different forms, depending primarily on tissue type, some of which are able to bind T3 while others act to supress the activity of T3 (Mullur et al 2014). The different isoforms of the thyroid nuclear receptor are thought to exist to provide a pathway of tissue specific thyroid hormone action. The thyroid hormone receptor is part of the nuclear superfamily group that includes the retinoic acid receptor, retinoid X receptor, vitamin D, and the peroxisome proliferator activated receptor (Evans 1988). These receptors, on binding their respective ligand, are able to bind as monomers to thyroid response elements (TREs) on DNA, but the majority bind in the form of a heterodimer with the retinoid X receptor (RXR). Heterodimer formation is thought to enhance DNA binding affinity as well as providing target gene specificity (Kliewer et al 1992). The thyroid hormone/RXR heterodimer complex, on binding to its thyroid response element on DNA, is then able to stimulate or inhibit subsequent gene transcription within the target cell. There is considerable opportunity for crosstalk between the thyroid hormone receptor/RXR complex and other nuclear hormone receptors including the alpha and gamma forms of the peroxisome proliferator activated receptors (PPARα and PPARγ), and the liver X receptor (LXR) (Liu and Brent 2010). Since these nuclear receptors all form heterodimers with RXR, there is potential for competition for the limited amounts of RXR available (Hsu et al 1995; Liu et al 2007; Fattori et al 2015) that would result in depression of receptor activation by thyroid hormone and a possible inability or reduced ability to respond to elevated levels of thyroid hormone.

3.3. The hypothalamus/pituitary/thyroid axis (Fig. 4)

Thyroid releasing hormone, (TRH), secreted by the tanycytes within the hypothalamus, acts upon the pituitary gland, binding to G protein-coupled TRH receptors, resulting in an increase in intracellular cAMP, and subsequent thyrotropin (TSH) release from the thyrotrophs (Hershman 1974). Hormone signals that have modulatory effects on pituitary TSH secretion include dopamine, somatostatin, and leptin, and these molecules help to regulate thyroid hormone release at the level of the CNS (Scanlon et al 1979; Tanjasiri et al 1976; Seoane et al 2000; Ghamari-Langroudi et al 2010).

Thyroidectomy in rats results in a marked increase in serum TSH concentrations in response to the failure to produce T4 and T3. In experiments with thyroidectomised rats, where exogenous T4 was administered in the presence of selenium deficiency, TSH levels continued to remain high despite the maintenance of serum T4 concentrations identical to those observed in the serum of intact rats. In this experiment although serum T4 levels were normal, serum T3 levels remained low due to the diet-induced depression in deiodinase enzyme activities (selenium containing enzymes). This data confirmed the hypothesis that circulating T3, rather than T4, plays the critical role in regulating TSH secretion (Chanoine et al 1992; Abend et al 1991; Emerson et al 1989; Scanlon and Toft, 2000).

Fig. 4: Feedback control of thyroid hormone synthesis and release (adapted from Hill et al 1989).



Local tissue conversion of T4 to T3, by iodothyronine deiodinase type 2, provides negative feedback at the level of the thyrotrophs in the pituitary, and via the TRH secreting tanycytes in the hypothalamus (Fonseca et al 2013; Gereben et al 2008; Larsen and Zavacki 2012. High circulating thyroid hormone levels result in a reduction in TRH and TSH secretion, whereas low thyroid hormone

levels stimulate TRH release from the hypothalamus and TSH release from the pituitary which act to increase thyroid production of the hormones.

On release from the pituitary gland, TSH binds to G protein-coupled TSH receptors on the thyroid follicular cells, stimulating the production and secretion of T4 and lower levels of T3, and when required to be increased, e.g. by certain chemicals and drugs, TSH stimulation will lead to hypertrophy of the follicular cells to meet the extra demand (Parmentier et al 1989; Tani et al 2004). If increased thyroid hormone production by the existing follicular cell population alone is insufficient to correct the decreased circulating hormone levels, continued TSH stimulation results in a stimulation of follicular cell proliferation, hyperplasia of the follicles, and ultimately can result in the development of follicular cell neoplasms (Smith et al 1991).

Although originally it was thought that T3 and T4 simply diffused into target cells it is now clear that there are several ion-coupled, membrane transporters that are able to actively transport the thyroid hormones into target cells (Visser 2011). The MCT8 protein is involved in transporting T3 into cells throughout the body and mutations in the gene are associated with serious neurodevelopmental abnormalities in humans. Mouse models of MCT8 gene knockout show thyroid function study changes similar to those in patients with so-called Allan-Herndon-Dudley Syndrome, a condition brought about by a mutation of the transporter, but these mice show only minor changes in brain function in comparison with the human syndrome (Dumitrescu et al 2006; Trajkovic et al 2007; Visser et al 2011). The discrepancy between the response of inactivation of the MCT8 transporter in mice, and the serious consequences that result from the same mutation of the transporter in humans, is most likely due to the former having redundant thyroid hormone transporters that are able to compensate for the loss of the MCT8 gene. MCT8 is highly expressed in the hypothalamus and certain mutations have shown impairment of central hormone regulation and an abnormal thyroid hormone feedback (Alkemade et al 2011). Without a functioning MCT8 transporter in the brain, specific brain areas are unable to absorb T3 and become hypothyroid although the liver Is still able to respond through the use of alternative thyroid uptake systems. Mutated MCT8 also means that the excess TRH/TSH is produced due to impaired negative feedback in the hypothalamus results in tissue-specific hyperthyroidism, hyper-metabolism and profound weight loss (Heuer et al 2009). Treatment with the thyroid hormone analogue, diiodothyropropionic acid (DITPA), in both animal models and humans with inactivated MCT8 gene, results in a reduction in both serum TSH and serum T3, with a consequent improvement in weight gain and decreased metabolic rates (Di Cosmo et al 2009; Verge et al 2012).

3.4. Physiological function of thyroid hormones

Thyroid hormones regulate the metabolic processes essential for normal growth and differentiation in the developing organism, as well as determining the metabolic rate in the adult (Brent 2012b; Cheng et al 2010; Oetting and Yen 2007) particularly with regard to the regulation of energy metabolism within cells (Malik and Hodgson 2002; Iwen et al 2013; Liu and Brent 2010). In healthy humans, thyroid hormone status correlates with body weight and energy expenditure (Fox et al 2008; Iwen et al 2013; Knudsen et al 2005) and hyperthyroidism promotes a hypermetabolic state characterized by increased resting energy expenditure, weight loss, reduced cholesterol levels, increased lipolysis, and gluconeogenesis (Brent 2008; Motomura and Brent 1998). In contrast, hypothyroidism induces reductions in metabolic rate that are characterized by chronic fatigue, weight gain, increased cholesterol levels, reduced lipolysis, and reduced gluconeogenesis (Brent 2012a; Oppenheimer et al 1991). The effect of thyroid hormones on metabolism is achieved through interactions with receptors in the brain, white fat, brown fat, skeletal muscle, liver, and pancreas (Mullur et al 2014).

3.5. Thyroid hormones in neurodevelopment

During the initial stages of gestation/pregnancy, the foetus relies on maternal thyroid hormones for normal brain development and growth. Deprivation of the maternal thyroid hormones, in hypothyroidism, can have devastating effects on the foetus and in humans, dysfunction of thyroid hormones in brain development is most often mediated through mutations in thyroid hormone transporters or in the deiodinases that degrade excess T3 (Ahmed 2015). Human hypothyroidism is most commonly monitored through assessment of circulating TSH levels and where needed, maternal thyroid hormone supplementation (levothyroxine) given during pregnancy corrects for any deficit (Maraka et al 2017). The recommended fixed upper threshold for TSH concentration in humans in January 2017 was 2.5 mIU/L during the first trimester and 3.0 mIU/L during the second and third trimesters (Maraka et al 2017). According to these diagnostic criteria, subclinical hypothyroidism, defined as an elevated TSH concentration with concurrent normal thyroid hormone concentrations, was estimated to affect up to 15% of pregnancies in the US and 14% in Europe. This represented a fivefold increase in apparent prevalence of hypothyroidism compared with the 2-3% prevalence of subclinical hypothyroidism before these criteria were established, raising the possibility of overdiagnosis of subclinical hypothyroidism and subsequent discussions by the American Thyroid Association (Alexander et al 2017) have revised these estimations by increasing the TSH cut-off limit to 4.0 mIU/L.

Because of increased thyroid hormone production, increased renal iodine excretion, and foetal iodine requirements, dietary iodine requirements are higher in pregnancy than they are for non-pregnant adults (Glinoer 2007).

Patients with the human genetic disorder, Allan-Herndon-Dudley Syndrome, show low levels of serum T4 and rT3, elevated T3, and normal or slightly elevated serum thyrotropin (TSH) (Dumitrescu et al 2004; Friesema et al 2004; Schwartz and Stevenson 2007), and severe neurologic developmental deficits that develop before birth. The mutated MCT8 gene translates a dysfunctional protein that is unable to adequately transport sufficient T3 into the developing brain and prevents the normal formation and growth of nerve cells and its discovery was instrumental in showing the critical requirement of thyroid hormones in the developing brain and that active thyroid hormone transport was required in the brain to ensure normal development (Kersseboom and Visser 2011; Visser et al 2011).

Studies in MCT8 knockout mice show a dramatically reduced uptake of T3 into the brain in comparison with wild type mice with an intact MCT8 system (Dumitrescu et al 2006; Trajkovic et al 2007). Because mutations in the MCT8 transporter are associated with multiple neurologic abnormalities in humans, with developmental delays, and progression to quadriplegia (Bernal 2011), the transporter is thought to have a critical role in normal brain development, and thyroid hormone transporters in general show both a specific temporal, but also spatial, pattern of expression in the developing brain (Sharlin et al 2011; Van der Deure et al 2010, Visser et al 2011).

Experimental data on thyroid hormone transport suggests significant species differences exist between humans and experimental animals, especially with regard to the uptake of thyroid hormones by the brain. In contrast to the situation in man knockout mice, lacking the MCT8 transporter, fail to show motor deficits suggesting that alternate pathways for transporting thyroid hormones into the brain exist in this species (Di Cosmo et al 2010; Roberts et al 2008). It is now known that the organic ion transporter polypeptide-14 (OATP14) is the primary thyroid hormone transporter expressed in the endothelial cells at the blood-brain barrier, whereas MCT8 mediates thyroid hormone uptake into neurons (Friesma et al 2005). OATP14 mRNA and protein is strongly expressed in both rat and mouse cerebral microvessels, but not in human, while both MCT8 and OATP14 is present in mouse and rat tanycytes, the cells in the hypothalamus that are responsible for the secretion of TRH (Roberts et al 2008). While MCT8 is primarily concerned with transporting T3 into the brain, OATP14 has been found to primarily transport T4 (Pizzagalli et al 2002; Sugiyama et al 2003; Tohyama et al 2004), and the high microvessel expression of OATP14 in the rodent, as compared with the human brain, may explain the relatively mild neurophysiological consequences of deletion of the MCT8 transporter in Mct8-null mice. In Mct8 KO mice, OATP14 is thought to be able to compensate for the loss of MCT8

in transporting thyroid hormone into the brain without loss of function, in contrast to humans lacking functional MCT8, where the absence of an alternate transporter results in serious neurodevelopmental consequences.

MCT8-mediated T3 transport itself, rather than being generated following uptake of T4 into the brain is also consistent with the finding that brain development and function is normal in type 2 deiodinase knockout mice (Schneider et al 2001). Type 2 deiodinase is responsible for converting T4 to T3 and the fact that type 2 deiodinase knockout mice are phenotypically normal indicates that direct brain uptake of T3 from the circulation can compensate for the inability of the neurones themselves to generate T3 by deiodination of T4.

Conversion of the pre-hormone T4 to the active thyroid hormone, T3, occurs mainly in peripheral tissue via the enzymic activity of a group of iodothyronine deiodinases. Deiodinases generally are present at low levels in the brain, and while the type 1 deiodinase is found at high concentrations in the liver, within the brain the type 3 deiodinase generally mediates the degradation of thyroid hormones to inactive metabolites rather than deiodinases catalysing the conversion of T4 to T3. Especially high type 3 deiodinase activity has been demonstrated in the placenta and the pregnant uterus, as well as in different foetal tissues. This is considered to be a protective mechanism to prevent exposure of foetal tissues to high T3 levels at inappropriate times in development thus allowing the normal growth of these tissues. Paradoxically, T3 is only required at the differentiation stage of tissue development, and its presence at earlier stages, in conditions of maternal hyperthyroidism, has been linked with developmental abnormalities (Batra 2013). The critical role of type 2 and 3 deiodinases, and their differential expression at different stages in the developing foetus, is exemplified in cochlear development, since mice carrying either a type 2 or a type 3 deiodinase knockout have severe hearing loss (Ng et al 2004). At immature stages of foetal development, the type 3 enzyme limits stimulation by T3 whereas postnatally, a double switch occurs with a decline in the activity of the type 3 enzyme and a concomitant increase in the activity of type 2 deiodinase, resulting in a local T3 surge which is independent of serum T3 levels and which triggers the onset of auditory function (Ng et al 2004; Visser et al 2016).

4. ECPA Question: Consider the references in the draft guidance document – is this the state of the science? If not, what is?

The GD is intended to provide guidance for applicants and risk assessors on the implementation of the draft scientific criteria for determining endocrine disrupting chemicals and as such it appears to excellently provide ”point by point” guidance using relevant literature references but it clearly is not, and does not claim to be, a state of the science document. It does introduce the entire area of endocrine disruption and does a good job of introducing those assays having OECD guidelines, together with other assays not currently covered by guidelines but it clearly does not incorporate the most up to date scientific research in the area as a whole and in the thyroid gland in particular. It does address its stated scope and provides an invaluable source of information for assessors and dossier submitters that provides clear guidance on what evidence will currently constitute a chemical being classified as an endocrine disruptor of relevance to the human exposure situation.

On page 3 of the document the principles used to set out the guidance are detailed for assessing whether a substance meets the hazard based ED criteria. The strategy is based on the requirements outlined in the ED-criteria

'[…] that a substance shall be considered as having endocrine disrupting properties […] if:

(1) It shows an adverse effect in an intact organism or its progeny, which is a change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences;
(2) It has an endocrine mode of action. i.e. it alters the function(s) of the endocrine system;
(3) The adverse effect is a consequence of the endocrine mode of action.'

The link between the adverse effect and the endocrine mode of action (MoA) addressed in point (3) shall be established based on biological plausibility. The biological plausibility shall be determined in the light of current scientific knowledge and all available relevant scientific data by using a WoE approach".

The last sentence above in the GD is a critical one since its whole precept is dependent upon the scientific database being not only comprehensive, but also being critically balanced between the academic understanding of thyroid hormone disruption, obtained from non-guideline, *in vivo* and *in vitro,* laboratory studies, but also on practical experience gained through the evaluation of a broad range of chemical classes conducted following the strict guidelines laid down by the OECD TGs.

Appendix A of the draft guidance specifically addresses the problem of potential thyroid hormone disruption and is a pragmatic description of the current problems of species extrapolation between the rat and human in particular, but it also describes the evidence that would be taken to arrive at a conclusion of a relevant MoA to human risk, and how a registrant might approach disproving this human link, making excellent use of the WHO/IPCS MoA/human relevance framework approach advocated by Meek et al (2014b) and others.

Appendix A undertakes to aid the interpretation of any potential thyroid disrupting effect by setting out clearly stated decisions points on page 95 of the latest GD based upon the following "Using the current understanding of thyroid physiology and toxicology13 it is proposed that the following be applied when interpreting data from experimental animals:

1. It is presumed that substances that alter the circulating levels of T3 and/or T4 with concurrent histopathological findings in the thyroid would pose a hazard for human thyroid hormone insufficiency in adults as well as pre- and post-natal neurological development of offspring.
2. It is presumed that substances that alter the circulating levels of T3 and/or T4 without histopathological findings would still present a potential concern for neurodevelopment.
3. In the absence of substance-specific data which provide proof of the contrary, humans and rodents are presumed to be equally sensitive to thyroid-disruption (including cases where liver enzyme induction is responsible for increased TH clearance)."

Point 2 is an important statement if proven to be true. As such it warrants a literature reference to justify its inclusion as one of the three most important points in the decision-making process. It seems to suggest that only neurodevelopmental endpoints would be relevant in such circumstances and also that the control of thyroid hormones is somehow different in the thyroid carcinogenesis process than it is in the neurodevelopmental ones. There are many reasons why a histopathological investigation of thyroid glands, in the presence of decreased thyroid hormones, may not detect follicular hypertrophy/hyperplasia but I suspect the effect not being there is not one of them! This point is covered in detail in section 7.1 of this review

An objective discussion of the balance of pure and applied research is not within the stated scope of the draft GD and is clearly one of the objectives of this current review document.

5. ECPA Question: Is the way in which the references are used in the GD appropriate?

The literature references presented in the main text of the draft GD do appear to be appropriate for the intent and stated scope of the document. The literature listings are not extensive but the majority are relevant, and appear in the appropriate parts of the document to help clarify some of the more complex thinking behind the proposals. They are a pragmatic listing that does not attempt to introduce the reader to the most current scientific data being generated particularly in the human, but also in the experimental animal, field of thyroid research.

The GD is lacking particularly in its inclusion of the current state of thyroid scientific literature and, while heavily biased towards oestrogen and androgen disruption, it is still not comprehensive in its coverage of even these areas. But a state of the science review is not the intended scope of the GD!

The literature references used do appear to support the sections throughout the GD and offer clarification in those areas requiring it. They do not provide a “get-out” clause for industrial submitters to refute decisions of human relevance, but the GD does suggest appropriate actions, via their Tier 2 list of approaches, that can usefully be adopted to support MoA/human relevance cases that could possibly challenge suggestions of thyroid hormone disruption in other Tier tests.

6. ECPA Question: Comparison between human and key laboratory animal species (rodent, dog, monkey)

The physiological functioning and regulation of the pituitary-hypothalamic-thyroid system in all known mammalian species, birds and humans is qualitatively extremely similar (Choksi et al 2003; Bianco et al. 2002) and correct thyroid functioning and maturation during foetal organogenesis are essential for the development of critical organ systems, including the nervous system and reproductive tract (Jannini et al 1995; Metz et al 1996; Krassas 2000). However, the dynamics of the thyroid hormone control and turnover do differ substantially between the different mammalian species even though there are significant structural homologies between the various hormones (Imamura et al 1991; Tsykin and Schreiber 1993; Power et al 2000). Table 2 summarises the main thyroid features where differences are seen between human and rodent thyroid hormone control.

6.1. Species comparison of the functional morphology of the thyroid gland

Compared to the thyroid follicles in primates, which are large with abundant colloid and with follicular cells that are relatively flattened (low cuboidal), rodent follicles are considerably smaller, contain less thyroglobulin colloid and are most often lined by cuboidal, basophilic-staining epithelium indicative of a higher content of mRNA production, and by inference, higher rate of hormone synthesis, than the equivalent cells in those species having low eosinophilic epithelium such as primates including human. Within any single gland in both rats and mice, a small number of follicles will be large, lined by an attenuated epithelial layer and contain large amounts of thyroglobulin, in similarity with the majority of follicles in the primate and human gland, while other rodent follicles, the majority, will normally contain much smaller amounts of thyroglobulin colloid and have taller lining epithelium considered to represent more actively synthesising, and hormone secreting, cells. During times of increased hormone demand the number of active follicles, in the thyroid gland of rodents, increases while the number of resting “cold” follicles decreases. Because of this plasticity in the follicular response in

rodents, the morphological differences in the appearances of the follicles in the normal thyroid gland between control primates and rodents is supportive of the faster rates of thyroid hormone turnover in the rodent (US EPA 1998).

6.2. Species comparison of thyroid hormone binding proteins in the plasma

Once secreted into the plasma from the thyroid follicular cells, the vast majority (>90%) of the T3 and T4 are transported through the blood bound to thyroid hormone binding proteins. These liver-derived binding proteins, and the proportion of T3 and T4 that they are able to bind, varies significantly among animal species. T3 and T4, in different species, have been found to be able to reversibly bind to three different liver-derived binding proteins: thyroxine-binding globulin (TBG), transthyretin (TTR), also called thyroid-binding prealbumin, and albumin (Schussler 2000; Bartalena and Robbins 1993). Lipoproteins also bind a small fraction of the available thyroid hormones. TBG is a monomer and a member of the serine protease inhibitor (serpin) superfamily of proteins (Flink et al., 1986; Robbins, 2000) while TTR is a tetramer composed of four identical subunits each composed of 127 amino acids (Power et al., 2000). Albumin is a monomer that has substantial sequence homology with α-fetoprotein and vitamin D-binding proteins (Robbins, 2000). There is little overall amino acid sequence homology between the three major binding proteins.

In normal human plasma, there are three T4 binding proteins (Fig. 3) with respective hormone distribution being approximately 80% bound to TBG, 15% to TTR, and 5% to albumin and lipoproteins, while for T3 the respective proportions are 90% bound to TBG and the remainder to albumin and lipoproteins. The binding distribution of T4 and T3 appears to correlate exactly with the binding affinity of these hormones to their respective proteins in humans, and the affinity of both T4 and T3 for TBG is much higher than are their affinities for either albumin or TTR (Kaneko, 1989; Robbins, 2000). Although only about 0.3% or less of T3 and T4 circulates unbound, it is this free hormone fraction that is metabolically active at the tissue and cellular level although the presence of deiodinases within target tissues means that local intracellular production of T3 from T4 can occur and most probably is responsible for the majority of T3 required by these tissues.

Table 2: A comparison of thyroid function and control between humans, rats and mice (adapted from Choksi et al 2003; Jahnke et al 2004; Colnot & Dekant 2017; Lewandowski et al 2003).

Parameter	Human	Rat	Mouse
Half-life of T4	5-9 days	0.5-1 day	0.5-0.75 days
Half-life of T3	1 day	0.25 days	0.45 days
High affinity TBG	Present	Absent	Absent
Primary serum binding protein	TBG	Albumin	Albumin
Serum TSH levels (ng/ml)	0.05-0.5	0.6-6.0	unknown
Sex difference in serum TSH level?	Males = females	males>females	males>females
Sex ratio for thyroid cancer	Females>males	Males>females	Males>females
Effect of chronic TSH stimulation?	Goitre	Cancer	Cancer
Amount of T4 supplementation required in absence of functioning thyroid?	2.2 mg/kg bw/day	20 mg/kg bw/day	Unknown
Development of foetal HPT	TSH/T3 by week 20 of gestation	TH & TSH by day 17 gestation	Unknown
Effect of mutant/KO MCT8	Multiple severe neurological deficits	unknown	Normal
Morphology of the follicular epithelium	Low epithelium	Tall cuboidal epithelium	Tall cuboidal epithelium
Morphology of the follicles	Large, lots of colloid	Small, little colloid	Small, little colloid
T3 glucuronidation	Minor route	Major route	unknown
Type 2 deiodinase expression in thyroid	High	Very low/Absent	Very low/Absent
% of T4 eliminated in bile	10-15%	~50%	Unknown
Timing of thyroid nuclear receptor binding in foetus	Week 10-16 (of 39 weeks total)	Day 10-15 (of 21 days total)	Unknown

In humans, inherited or acquired variations in the concentration and/or affinity of these thyroid hormone binding proteins may produce substantial changes in serum total thyroid hormone levels but these changes do not result in hypothyroidism or hyperthyroidism because the concentration of the free thyroid hormone does not change. A deficiency in thyroid hormone binding proteins in humans is suspected when abnormally low serum total thyroid hormone concentrations are present in clinically normal (euthyroid) subjects in the presence of normal serum TSH. More specifically, low TBG is suggested to be the cause of the low total T4 levels in these circumstances as it is this protein that carries the majority of the serum hormones. Under these circumstances the assay of free T4/T3 is more diagnostic of a thyroid gland effect than measures of total hormone where decreases could occur during liver pathologies where decreased production of binding proteins could be the cause of the decreased total T4/T3 levels measured.

Compared to humans, albumin is the major thyroid hormone binding protein in adult rodents and while they do carry a TBG gene, the TBG protein is expressed at very low levels in adult animals (Vranckx et al 1990; Rouaze-Romet et al 1992; Tani et al 1994). Developmental studies in the rat have shown that TBG protein expression increases briefly postnatally in rodents, but then declines to very low levels during weaning to remain at these low levels for the remainder of the rat's life (Savu et al 1987, 1991; Vranckx et al 1990). Although the binding affinity of T3 and T4 for TBG in the adult rat is still higher than it is for TTR and albumin in the rat, the fact that serum TBG levels in the rat are so low means that both thyroid hormones, to all intents and purposes, bind only to TTR and albumin in the serum of adult rodents.

6.3. The role of transthyretin (TTR)

The protein that is currently known as transthyretin (TTR) was first described in 1942 in human serum (Kabat et al 1942) and cerebrospinal fluid (CSF) and as a result of its mobility during electrophoresis at pH 8.6, where it was the only serum protein that migrated ahead of albumin, it was named at the time as 'prealbumin' (Seibert and Nelson 1942).

TTR is a thyroid hormone binding protein that is synthesised in the liver, is secreted into the bloodstream and distributes thyroid hormones around the body (Alshehri et al 2015). A second source of TTR is the choroid plexus and at this site it is thought to be involved in the movement of thyroxine from the blood into the cerebrospinal fluid and the subsequent distribution of thyroid hormones in the brain. Adequate uptake of thyroid hormone into the developing brain is essential for normal maturation and differentiation of the nervous system in the foetus in both animals and humans. In human plasma thyroxine binding globulin has the highest affinity for binding T3 and T4 followed by transthyretin and finally albumin, and they carry respectively 75%, 15% and 10% of the thyroid hormones in human blood (Alshehri et al 2015). It has been argued that TTR is the main protein responsible for transporting T4 in rodent blood (Palha et al 1994) although this has been challenged in the light of data from TTR knockout mice where the mice are perfectly viable without phenotypic changes (see discussion below).

The vast majority of *in vivo* studies assessing the potential for chemically-induced thyroid hormone disruption are carried out in the rat and it is this species where most attention has been focussed in determining the relevance of any observed rodent thyroid effect for the human population. While humans have all three major binding proteins, healthy adult rodents have only albumin and TTR (Vranckx et al 1990a; 1990b; Savu et al 1991; Lewandowski et al., 2004). If TTR is the major binding protein in the rat then it is clear that factors other than hormone binding affinity and dissociation rates must explain the clear differences demonstrated between the hormone kinetics in the rat with T4 half-life being around 24h, versus 5-6 days in humans. It is equally true that the enhanced clearance rate in the rat is compensated for by a correspondingly higher production rate of thyroid hormones, with an equally greater basal TSH levels in the plasma, and a more active thyroid gland as a consequence (Lewandowski et al., 2004).

It is a perfectly viable hypothesis, supported by data, that the differing thyroid hormone kinetics between rats and humans make the former considerably more sensitive to the effects of many, if not the majority, of these chemical disruptors of thyroid homeostasis (McClain 1995; Colnot and Dekant 2017). A comparison of the data on the affinities and dissociation rates of thyroid hormones for the TTRs from rodents, humans and other animals have admittedly shown little variation (Chang et al 1999) but TTR is only one of the thyroid binding proteins in rodents. Albumin is the main carrier of thyroid hormones in the rat (Jahnke et al 2004) and differences in thyroid hormone binding is only one of the determining factors in the kinetics of thyroid hormone turnover between humans and rodents.

There are well described examples of both qualitative and quantitative differences in the responses of humans and rats to some of the drug induced thyroid hormone disrupters, such as with the sulphonamide antibiotics, where controlled exposures in both humans and rats have shown qualitative and significant quantitative differences in the thyroid responses (Capen 1999; McClain 1995; Colnot and Dekant 2017). Perhaps the best understood examples of drugs affecting thyroid hormone homeostasis in rodents and not in man are the rodent hepatic enzyme inducing drugs (Curran and

DeGroot 1991) where a considerable amount of controlled human exposure has been monitored for thyroid effects with drugs such as sodium phenobarbitone has failed to find such a relationship even though other anti-epileptics, such as carbamazepine and rifampin have been shown to be goitrogenic.

In an attempt to better understand the importance of TTR in rodents, TTR knockout mice have been produced and shown to be perfectly viable (Episkopou et al 1993; Richardson 2007) without neurodevelopmental abnormalities. If mice are like rats in that they make TBG in the foetus, then this thyroid hormone binding protein could, along with albumin, substitute for TTR at critical developmental stages in the knockout foetus, which are taken over exclusively by albumin postnatally. The mouse TTR knockout data therefore suggest that TTR, rather than being a key thyroid hormone transporter, as is claimed in the draft GD and in the publication by Alshehri et al (2015), has a secondary, or backup, role at most in the transport of thyroid hormones in rodents, and supports a conclusion that, in the adult rat/mouse, albumin with its relatively low affinity but high dissociation rates for carrying T4and T3, is the main thyroid binding protein in rodents (Palha et al 1994; 2000; 2002; Sousa et al 2005; Alshehri et al 2015). Considering the critical importance of thyroid hormones to brain development it is inconceivable that the absence of the major thyroid hormone transporter, as is claimed for TTR, in knockout mice would permit survival without significant neurological disorders. These results suggest that albumin is the most important thyroid hormone transporters in the mouse, and most probably also in the rat, under these circumstances.

6.4. Species differences in thyroid hormone metabolism

Thyroid hormones are metabolised in peripheral tissues by three enzyme systems, the iodothyronine deiodinases, by sulphate conjugation by sulphotransferases (SULT), and by conjugation with glucuronic acid catalysed by glucuronosyltransferases (UGT). The deiodinases and UGT enzymes are localised intracellularly within the endoplasmic reticulum while the SULTs are cytosolic enzymes (Dentice et al 2013; Radominska-Pandya et al 2005; Teubner et al 2007). Both pathways are responsible for the excretion of thyroid hormones in the rat, and the liver and kidney are major sites for this process (Vansell and Klaassen 2002; Visser et al. 1993). In the rat liver, there are a large number of UGT enzymes involved in conjugating various substrates, but with regards to the thyroid hormones, UGT1A1, UGT1A6 and UGT1A7 are reported to conjugate T4 while a number of different UGT2 enzymes conjugate T3 (Vansell and Klaassen 2002; Emi et al 2007).

T3 is not glucuronidated significantly in human liver or kidney although it is induced in certain diseases where circulating thyroid hormones increase, such as in hyperthyroidism, and where glucuronidation becomes more important (Findlay et al 2000; Visser 1996). The lack of hepatic T3 glucuronidation in humans suggests that either human liver doesn't express an enzyme homologous to rat UGT2B2, the enzyme primarily, although not solely, responsible for glucuronidating T3 in the rat, or that it does not accept T3 as a substrate. In humans therefore T3 appears to be metabolized predominantly by deiodination and sulfation. In contrast to the situation in humans, normal rat liver has been shown to have substantial T3 glucuronidation (Findlay et al 2000).

Laboratory animal studies show that there are both species-and gender-dependent variations in enzyme activity (Kelly, 2000) that can explain some of the gender specific thyroid hormone effects seen with enzyme inducing agents. The UGT enzymes have especial importance in the biology of thyroid hormones since they can be upregulated in the liver by a number of xenobiotics as part of the pleiotropic response that occurs following activation of several nuclear hormone receptors, including the constitutive androstane receptor (CAR) and PXR, and the impact of altered thyroid hormone homeostasis is considerable in terms of both thyroid cancer and neonatal and postnatal development in animal species susceptible to the induction of these enzymes (McClain et al 1988; 1989). Since the capacity for the hepatic induction of these enzymes in humans is low (Richardson et al 2014) while rodents are almost exquisitely responsive to chemically-induced activation of these nuclear hormone receptors, disruption of thyroid hormone homeostasis via induction of hepatic UDP-GT enzymes, as a MoA, is not thought to be relevant to human situation (McClean 1995).

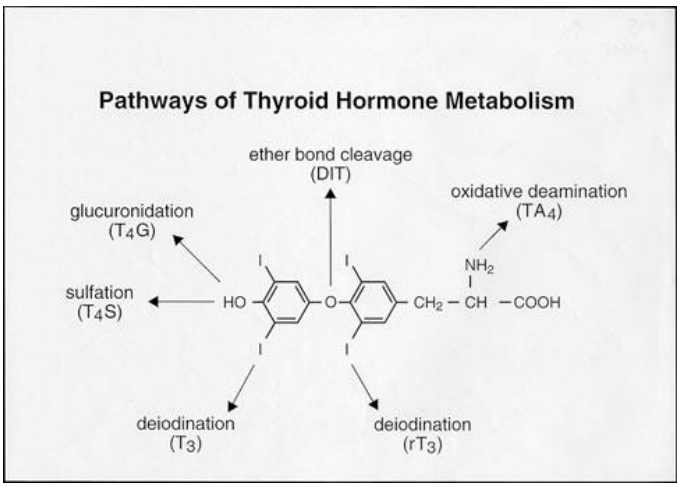
As with the UGTs, there are a number of SULT enzymes present in the liver that are involved with conjugation of thyroid hormones (Kester et al 1999). Whereas glucuronidation appears to facilitate the faecal excretion of thyroid hormones, sulfation initiates its degradation, allowing reutilization of the iodide for *de novo* thyroid hormone synthesis. In healthy humans, the sulphation pathway does not appear to contribute significantly to thyroid hormone metabolism, although its role increases in importance when Type I deiodinase activity is depressed (Visser 1994). Animal studies indicate that activation of the sulphation pathway inhibits T3 formation and increases the degradation of T4 and the inactive rT3 to additional inactive metabolites (Kelly, 2000). While minor in the adult human, T3-sulphate activity may be more important in the human foetus where, in the absence of foetal iodothyronine deiodinase to generate T3 from T4, sulphated T3 (T3S) can serve as a vital source of foetal T3 (Brucker-Davis, 1998).

Although type 2 deiodinase is normally expressed at high levels in human thyroid, and both mRNA and activity of type 2 deiodinase are induced by TSH, and agonistic TSH receptor antibodies circulating in patients with Graves' disease (Imai et al 2001; Murakami et al 2001), this enzyme is not expressed in normal rat or mouse thyroid (Wagner et al 2003). The consequences of a lack of expression of type 2 deiodinase in the rat are that intracellular conversion of T4 to T3 will not occur within the follicular cells, possibly as a result of the fact that rat follicular cells are already normally in a higher state of enzyme production than those present in humans and lack the need to increase their production rate of T3. Type 2 deiodinase can however be induced in conditions of hypothyroidism in both human and the rats in an attempt to increase the conversion of T4 to T3 (Wagner et al 2003).

6.5. Hepatic metabolism of thyroid hormones (Fig. 5).

Studies using ^{131}I labelled T4 have shown that in humans, the liver extracts between 5–10% of plasma T4 passing through the organ at any one time, a value that is considerably higher than could be accounted for by the amount of free T4 in the plasma delivered to the organ. This discrepancy shows that a substantial amount of protein bound T4, in addition to free T4, is absorbed directly into the cells (Mendel et al 1988). MCT8, a major thyroid hormone transporter has been localised to the membranes of hepatocytes and the thyroid hormone uptake occurs in a saturatable, energy and sodium-dependent, manner that enables the protein bound T4 and T3 to cross the hepatocyte membrane and to concentrate free intracellular hormones to a much higher level than those present in the plasma (Nishimura and Naito 2008). Although MCT8 is an important thyroid hormone transporter, several other proteins, such as the organic anion transporter protein 1C1 (OATP1C1), have been shown to transport thyroid hormones into cells throughout the body although they do tend to show a tissue specific pattern of expression (Abe et al 1998; Visser et al 2011).

Fig. 5: Thyroid hormone metabolism (from Visser et al 2016)



Thyroid function in a healthy individual is critically dependent upon a normally functioning liver to thyroid axis (Malik and Hodgson 2002) and while the major route for metabolism of T3 is through deiodination, significant removal of T3, rT3 and T4 occurs in the liver through conjugation reactions with sulphate and glucuronic acid (Fig. 2) catalysed respectively by iodothyronine sulfotransferase and iodothyronine glucuronyltransferase enzymes with the conjugates of the latter in particular, being excreted into the intestine through the bile (Visser 1996; Wu et al 2005).

There are some differences in the metabolism of T4 by the liver between rats and humans with approximately 50% of the T4 being eliminated via bile in rats, but only 10-15% in humans (Hill et al. 1989). However this difference does not appear to reflect a qualitative difference in metabolism, because the major metabolite in bile (glucuronide conjugate) remains the same in both species (Hard 1998). It does however suggest a kinetic difference in metabolism between the two species with the rat turning over T4 at an appreciably faster rate than in humans. This is consistent with the proposed biological differences in the transport of free and bound thyroid hormones between the two species. Iodothyronine glucuronides are rapidly excreted in large quantities in the bile with approximately 20% of human daily T4 production appearing in the faeces. This is not an end stage process as the conjugates are readily hydrolysed within the intestine by bacterial ß-glucuronidases, and some of the liberated hormone can be reabsorbed through enterohepatic cycling (Visser et al 1988).

Sulphated iodothyronine (T3S) levels are normally present at very low concentrations in plasma, bile and urine, because these conjugates are rapidly degraded by type 1 deiodinase. This has been taken to indicate that sulphate conjugation is a primary step leading to the irreversible inactivation of thyroid hormone (Visser 1994; Peeters et al 2005). Plasma concentrations, and biliary excretion, of T3S is significantly increased following inhibition of type 2 deiodinase activity with PTU or the iodine-containing radiocontrast medium, iopanoic acid, both during foetal development, and following fasting (Wu et al 2005; Visser 1994). Under these conditions, T3S may be acting as a reservoir of inactive hormone from which active T3 may be regenerated.

6.6. The importance of thyroid hormone sulphation

Serum concentrations of T3S are normally very low in healthy human subjects but are very high in the blood of the foetus and umbilical cord, and high in patients treated with the type 1 deiodinase inhibitor, triac, an acetic acid derivative of T4 (Eelkman et al 1989; Wu et al 2005). Similar high T3S/T3 ratios are also seen in some human cases of hypothyroidism, with the high sulphated T3 levels being due to a low peripheral type 1 deiodinase activity (Visser 1994; Peeters et al 2005).

An increase in T3S levels is also seen in rats when hepatic and renal type 1 deiodinase activities are decreased following exposure to enzyme inhibitors of the type 1 deiodinase enzymes, or in the presence of selenium deficiency, where marked increases in both serum and bile concentrations of iodothyronine sulphates occur (Visser 1994). These changes result from decreased clearance of the sulphated iodothyronines due to the absence of the type 1 deiodinases, but under conditions of hypothyroidism the inactivation of thyroid hormone by sulphation has been found to be reversible due to the presence of sulphatases in different tissues and in intestinal bacteria (Kester et al 2002). It is supposed therefore that the presence of relatively high concentrations of T3S in the foetus has an important function as a reservoir from which active T3 may be released in a tissue-specific, and time-dependent, manner (Santini et al 1992; Wu et al 1992; Darras et al 1999).

6.7. Species comparison of thyroid hormone and TSH half-life

The serum half-life of T4 and T3 in normal human adults is 5–9 days and 1 day, respectively (Choksi et al 2003) while in rats, the comparative values are 0.5–1 and 0.25 days, for T4 and T3 respectively. The plasma half-life of T4 and T3 in the dog has been estimated to be 8 –16 hours and 5-6 hours respectively (Kaptein et al 1993; 1994). The basis for the difference in half-lives is not completely understood, but it is proposed that the lack of the high-affinity T4 binding protein, TBG, in the adult rat plays a critical role since this leads to a higher serum level of free T4 in the rat and a greater tendency

for the bound hormones to dissociate as a consequence of the lower affinity binding that albumin and TTR has for the thyroid hormones. These increased concentrations of free hormones are thought to make the hormones far more susceptible to metabolism and excretion (U.S. EPA, 1998; Capen 1999), and to explain the very short half-lives that the thyroid hormones have in the rat and mouse in comparison to humans. The binding affinity of TBG for T4 in humans is approximately 1000 times greater than it is of TTR for T4, and it is well known that the % of free T4 is appreciable lower in species with high levels of TBG than it is in species without TBG (Capen 1999). Experiments have shown that thyroidectomised rats required 10 times more T4, equating to 20 μg/kg body weight (Frumess and Larsen 1975; Dohler et al 1979; Schlenker et al 2008), for full substitution as compared to an adult human requiring 2.2 μg/kg body weight (McAninch and Bianco 2016).

Comparative studies on the effects of perchlorate in the rat and human thyroid gland have also shown significant biochemical and physiologic differences in the relative responses. In an experiment by Yu et al. (2002) with perchlorate, inhibition of radiolabelled iodide uptake was 15%, 55%, and 65% at 1.0, 3.0, and 10 mg/kg perchlorate respectively at 1 day following perchlorate administration to the rat. However, by day 5, inhibition of iodide uptake had decreased to 0, 10%, and 30% at each respective dose level and after 14 days, inhibition of iodide uptake was only observed only at the top dose levels of 10 mg/kg. The data showed that the initial inhibition of iodide uptake by perchlorate in rats was similar to that observed in humans but that rats were able to compensate for the inhibition within 5 days of administration, most likely by increasing the expression of the sodium-iodide symporter on the follicular cells of the thyroid. A similar response was not observed in a 14-day human study with perchlorate administration (Greer et al. 2002). It was thought that compensation occurred in rats because of their smaller reserve capacity of thyroid hormones than humans and their more rapid turnover of circulating hormones.

The mitogenic hormone responsible for the normal production of thyroid hormones, and for the chemically induced thyroid hypertrophy and hyperplasia is TSH. It is this hormone that, on chronic stimulation in rodents, can lead to follicular neoplasia of the thyroid due to chemicals that interfere with thyroid hormone homeostasis (Hard 1998). The plasma levels of TSH in the male rat are approximately 6 ng/ml (Helmreich and Tylee 2011) which are about 3-fold higher than they are in the female rat (Kieffer et al 1976). This compares with values of 0.05 – 0.5 ng/ml in adult humans (Lewandowski et al 2003). In addition, production of both T4 and T3, in the rat, is appreciably higher than it is in man, due to their shorter half-life, and since production is driven by TSH levels, the higher plasma levels of TSH in rodents are thought to be responsible for the differences in follicle morphology seen between rodent and primates, including humans, with rodents having a follicular appearance consistent with a higher rate of thyroid hormone production and turnover.

6.8. Comparison of thyroid modifying chemicals in human and animal species

There is no doubt that adequate thyroid hormones are essential for the development of the foetus in both humans and laboratory animal species, as well as other vertebrate species (Williams 2008). It is also evident that thyroid hormone deficiency will result in neurodevelopmental deficits in humans and animals, the severity of which appears to be related directly to the severity of the hypothyroidism and the degree of thyroid hormone depression (Glinoer 2000; Haddow et al 1999; Klein et al 2001).

6.8.1. Thyroid modifying chemicals in humans

There are a number of environmental chemicals that have been shown to interfere with thyroid hormone homeostasis in humans that include polychlorinated biphenols (PCBs), bisphenol A, perchlorate, tetrachlorodibenzo-p-dioxin (TCDD), polychlorinated dibenzofuran (PCDF), pentachlorophenol (a breakdown product of hexachlorobenzene), triclosan, polybrominated and tetrabrominated diphenyl ethers (PBDEs) and other, naturally-occurring, chemicals such as soy isoflavones and thiocyanates in cruciferous vegetables (Miller et al 2009). Because of its ubiquitous distribution in the environment, there has been intensive study of the potential for perchlorate to cause human thyroid effects and, more importantly, neurodevelopmental deficits in the human population

and while a clear association with decreased T4 and increased TSH has described in women, no such association was found in men (Blount et al 2006).

There are a variety of drugs that have also been shown to inhibit peripheral production of T3 in humans including propylthiouracil (PTU), dexamethasone, propranolol, lithium, iodinated compounds such as the radiographic agents, iopanoic acid and ipodate, and the anti-arrhythmic drug amiodarone. PTU is a specific non-competitive inhibitor of type 1 deiodinase, while iopanoic acid and ipodate are competitive inhibitors not only of type 1 deiodinase but also of the type 2 enzyme. In addition, the radiographic agents have been shown to inhibit hepatic uptake of thyroid hormone (Dentice et al 2013). Amiodarone, and its metabolite desethylamidarone, may also interfere with peripheral thyroid hormone levels by a combination of inhibition of deiodinase activities and of tissue thyroid hormone uptake (Narayana et al 2011; Rosene et al 2010). The therapeutic use of lithium in the treatment of manic-depressive psychosis in humans has been known to be associated with the development of goitre for many years, and decreases in the rate of degradation of T4, and a decrease in serum T3 is seen in patients receiving high doses of lithium carbonate through a variety of mechanisms including enhancement of iodide induced inhibition of the NIS, inhibition of the deiodinases, and an exaggerated response of pituitary TSH release to TRH (Shopsin et al 1973; Andersen 1973; Carlson et al 1973). Little is known about the mechanisms by which propranolol and dexamethasone inhibit peripheral T3 production. In recognition of the thyroid inhibiting properties of these drugs, combinations of PTU, ipodate, dexamethasone and/or propranolol have been used to acutely decrease plasma T3 levels in patients with severe hyperthyroidism, known as a “thyrotoxic storm” (Carroll and Matfin 2010).

While a link has been established between the urinary concentrations of PCBs and human neurodevelopmental deficiencies, a study by Longnecker et al (2003) failed to establish a link between serum thyroid hormone deficits and PCB exposure even though this study was, wrongly, cited by Crofton (2008) as an example of a chemical-induced thyroid hormone effects operating in humans. Nevertheless, PCBs have been shown to alter thyroid hormone homeostasis in rats and to be associated with neurodevelopmental deficits (Gauger et al 2004).

6.8.2. Thyroid modifying chemicals in rodents

In comparison to the situation in humans, there are a far greater proportion of tested chemicals that have been shown to alter thyroid homeostasis in animals, mostly rodents, that include a number of phthalate esters, pregnenolone-16α-carbonitrile, benzodiazepines, calcium channel blockers, steroids, chlorinated hydrocarbons, such as chlordane and DDT, and polyhalogenated hydrocarbons such as PCBs and PBBs (Curran and DeGroot 1991; Capen 1999). Despite considerable variation in chemical structure and classes, for those chemicals that cause thyroid hormone effects in rodents, there are a limited number of ways (Fig. 5) in which they can affect thyroid hormone homeostasis either by direct effects on the thyroid gland itself or by effects on extra-thyroidal tissues, such as the liver, brain and kidney, responsible for the deiodination of T4 to T3 (Capen 1999).

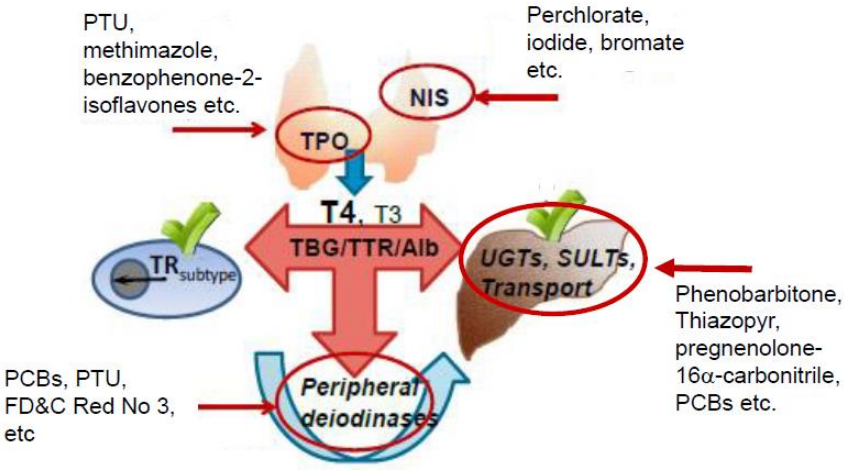
6.8.2.1. Competitive inhibitors of the sodium iodide symporter (NIS)

Chemicals such as pertechnetate, thiocyanate and perchlorate ions can directly affect the thyroid gland by acting as competitive inhibiters of the follicular cell NIS (Fig 5). This leads to a decrease in the availability of inorganic iodide in the thyroid gland and a consequential decrease in the synthesis and release of thyroid hormones (Merrell et al 2003; Capen 1999). When circulating levels of T4 and T3 decrease, compensatory release of TSH from the pituitary gland leads to hypertrophy, hyperplasia, thyroid gland growth and increases the chances of developing cancer (Fisher et al 2012).

6.8.2.2. Thyroperoxidase inhibitors

Certain chemical classes including thionamides such as 6-propylthiouracil and ethylene thiourea, some of the sulphonamide drug classes, and miscellaneous compounds such as amitrole, reversibly or irreversibly inhibit the thyroperoxidase catalysed incorporation of active iodide into thyroglobulin (Sarne 2016). Inhibition of thyroperoxidase reduces the production and release of T4 and T3 into the circulation, provoking a compensatory release of TSH from the pituitary gland and causing hypertrophy and hyperplasia in the thyroid follicular epithelium (Hard 1998).

Fig. 6: Known sites of action of chemicals in disrupting thyroid hormone homeostasis in rodents (from Paul 2014)



Notes: Abbreviations are TPO = thyroperoxidase; NIS = sodium/iodine symporter; TBG = thyroxine binding globulin; TTR = transthyretin; Alb = albumin; UGTs = UDP glucuronyltransferase; SULT = sulphotransferases; TR = thyroid hormone nuclear receptor; PCBs = polychlorinated biphenyls; PTU = propylthiourea;

6.8.2.3. Direct toxicity to follicular epithelium

Direct chemical toxicity to the thyroid follicular cells has been seen with PCBs (Collins et al 1977), which reduce the output of thyroid hormones, while lithium (Kibirge et al 2013; Bocchetta and Loviselli 2006) and excess iodide (Leung and Braverman 2014) inhibit the secretion of thyroid hormones from the follicular cells, induce retention of the hormones within the follicular lumen, seen as increased colloid, and produce a decrease in circulatory thyroid hormones (Green, 1978; Capen and Martin 1989). Both of these effects have been seen in humans. Amiodarone is an iodine-rich drug used to manage ventricular and supraventricular tachyarrhythmias, and is also associated with thyroid dysfunction, most probably through its effect as an iodide source (Minelli et al 1992).

6.8.2.4. Inhibition/induction of iodothyronine 5'-deiodinases

There are a number of chemicals that alter thyroid hormone homeostasis either by inhibiting the 5' deiodinase catalysed conversion of T4 to T3 in peripheral tissues (Capen 1999) or, in rarer cases, by enhancing the conversion of T4 to T3 through induction of the deiodinases (Morse et al 1993).

The dye erythrosine, also known as FD & C Red No 3, was shown to increase thyroid follicular adenomas in rats in lifetime bioassays (Borzelleca et al 1987) and the compound induces thyroid gland hypertrophy and hyperplasia in shorter duration studies in the rat. The thyroid morphological

changes are accompanied by decreases in serum T3 and increases in rT3, T4 and TSH, due to inhibition of 5'-deiodinase (Capen 1997; 1999; Capen and Martin 1989).

PTU has also been shown to inhibit 5'deiodination (Cavalieri and Pitt-Rivers 1981) in addition to inhibiting thyroperoxidase (TPO), and its potency in inducing thyroid hypertrophy and hyperplasia is most probably a product of a combination of effects rather than down to any single MoA.

A common component of sun-cream, octyl-methoxycinnamate, when given over a five day period, has also induced a dose-dependent decrease in serum T3, T4, TSH, and in hepatic type 1 5'-deiodinase (Klammer et al 2007). In this study TSH receptor expression in the thyroid was also increased while hypothalamic TRH expression, and the activities of TPO and NIS in the thyroid were unaffected. Oral administration of the PCB, 3,3',4,4',5,5'-hexachlorobiphenyl (HCB), or a combination of HCB with 3,3',4,4'-tetrachlorobiphenyl (TCB), to pregnant rats from day 1 to day 18 post-gestation induced significant decreases in plasma thyroid hormones in the dams, and significant increases in brain type 2 5' deiodinase activity in foetuses (Day 20 of gestation) and neonates (Days 7 and 21 postpartum), and an associated reduction in circulating total, and free, T4 levels. To complicate matters further, treatment also induced hepatic T4 glucuronidation in both 20 day gestation foetuses, and in neonates (Morse et al 1993).

In the Morse et al (1993) study as described above, the induced decreases in plasma thyroid hormones were accompanied by a highly significant induction of type 2 thyroxine 5'-deiodinase activity in brain homogenates from 20 day post-gestation foetuses, and day 7 and 21 postpartum neonates (Morse et al 1993). The increase in deiodinase activity was interpreted as indicating that local hypothyroidism had occurred in the brains of the foetal and neonatal rats exposed to HCB. Since these effects occurred during a period in which thyroid hormones play an important role in brain maturation, the data may be relevant in explaining the mechanism of developmental neurotoxicity induced by PCBs. As this group of chemicals also induce hepatic glucuronidation, that increase clearance of thyroid hormones, this group of compounds, in common with many of the well worked chemical examples of thyroid hormone disrupters, exhibits more than simply one single molecular initiating event that could operate in concert (an additive effect) to magnify the resulting thyroid hormone perturbation seen with this chemical class (Crofton 2008).

6.8.2.5. Displacement of hormones binding to thyroid transport proteins/receptors

There is some evidence from *in vitro* competitive binding studies that certain chemicals are able to displace T4 from one of the serum thyroid binding proteins, TTR (Meerts et al 2000). Although opinion differs as to the relative importance of this binding protein in the rat the findings, if substantiated *in vivo,* would have the potential to stress the thyroid system, making it more likely that dysfunction could occur (Kohrle et al., 1989; Lueprasitsakul et al., 1990; Lans et al., 1993; Cheek et al., 1999; Chauhan et al., 2000; Ishihara et al., 2003). Because of the redundancy in thyroid hormone binding proteins in humans and rodents, it is questionable whether or not such displacement of T4 from TTR alone would lead to functional consequences. *In vivo* evidence with the flavonoid, EMD 49209, which appears to displace T4 from TTR *in vitro*, shows that it reduces tissue levels of T3 *in vivo,* not through any MoA involving displacement of T4 from TTR, but as a result of a shortage of T4 as a substrate for deiodinases (Schroder-van der Elst et al 1998). Until *in vivo* evidence is produced to support this hypothetical MoA it has to be concluded that, of the time of writing, this *in vitro* phenomenon has not been reciprocated in any known *in vivo* chemical-induced thyroid disrupter.

6.8.2.6. Induction of hepatic turnover/biliary excretion of thyroid hormones

At least in rodents, certain chemicals have been shown to induce the activity of the hepatic UGT and SULT enzymes responsible for the conjugation and removal, through biliary excretion, of thyroid hormones (Oppenheimer et al 1968; McClain et al 1989; Visser et al 1993; Hood and Klaassen 2000; Liu and Klaassen 1996). This results in decreased circulating free and bound thyroid hormones, a

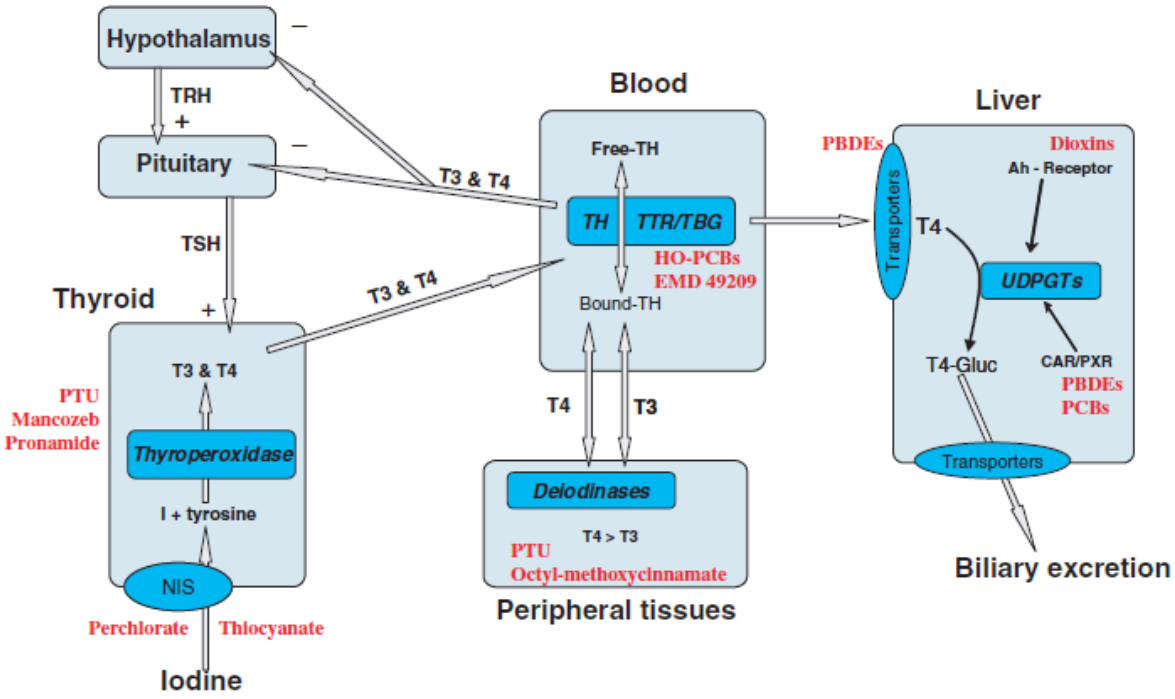
higher turnover and decreased half-life of circulating hormones, and a compensatory TRH and TSH increased release from the hypothalamus/pituitary acts on the follicular cells of the thyroid to induce hypertrophy and hyperplasia, and if sustained, thyroid follicular neoplasia (McClaIn 1989; Hill et al 1989; Thomas and Williams 1991; Williams 1995). In addition to chemical induction of hepatic SULTs, a number of drugs and environmental chemicals, such as salicylic acid, clomiphene, naturally occurring chemicals such as flavonoids and phytoestrogens, and environmental chemicals such as the disinfectant, pentachlorophenol, and the antibacterial product, triclosan, have been shown to inhibit hepatic SULTs and to have the potential to increase circulating levels of thyroid hormones (Schuur et al 1998; Wang et al 2004; Wang & James 2006). There is evidence that thyroid hormone perturbations, specifically those mediated via hepatic enzyme induction and increased biliary excretion, are a rodent only phenomenon that is not able to work in humans for a number of reasons relating to the significantly greater sensitivity of hepatic enzyme induction in rodents versus humans, and the much shorter half-life, and hence greater turnover, of thyroid hormones in rodents in comparison to humans (McClean 1995; McClean et al 1989; Colnet and Dekant 2017).

The other modes of action of chemicals in affecting thyroid hormone homeostasis are not inherently species specific but pharmacokinetic and metabolism differences may render some species more sensitive to the thyroid effects than others (Zimmermann and Galetti 2015).

6.8.2.7. Miscellaneous modes of action in disrupting thyroid hormone homeostasis

There is considerable debate as to whether or not chemical disrupters are able to bind directly to thyroid receptors *in vivo* but while it is clear that certain chemicals, such as tetrabromo-bisphenol A, bisphenol A and hydroxyl PCBs, can alter thyroid hormone-responsive genes in various tissues including liver and brown fat (Moriyama et al 2002; Gauger et al 2004; Bansal et al 2005; Kitamura et al 2005), the evidence exists that the changes observed are not induced by binding to the nuclear thyroid receptors (Cheek et al 1999; Ishihara et al 2003; Gauger et al 2004). In an *in vitro* thyroid receptor binding assay, Ishihara et al (2003) showed that while several chemicals, including diethylstilboestrol, pentachlorophenol and ioxynil, were able to displace thyroid hormone from TTR, none were able to displace thyroid hormone from its receptor. Marsh et al (1998) found that two synthesised polybrominated diphenyl ethers were able to bind, *in vitro,* to both the alpha and beta forms of the thyroid hormone receptor but this has not been repeated by other groups investigating competitive displacement of thyroid hormones from the receptors. However, most *in vitro* screens for thyroid hormone receptor binding have relied upon the ligand-binding domain of the receptor, and while clearly this is the site for activation of the receptor, binding to other parts of the receptor could potentially result in allosteric hindrance that would alter the subsequent functioning of the receptor. Indeed this has been shown at least *in vitro* to be the case for PCBs (Miyazaki et al 2008).

Fig. 7: Known MoAs (in blue boxes) for disruption of thyroid hormone production and release (from Crofton 2008)



6.9. Comparison of thyroid hormone involvement in neurodevelopment in humans and rodents

6.9.1. Thyroid hormone and neurodevelopment in humans

It has been over 120 years since a committee of the UK Royal College of Physicians in London linked the pivotal role of the thyroid gland to normal human brain development (Ord 1898). Many countries have now instigated a neonatal hypothyroidism screening test for the early diagnosis of hypothyroidism, in recognition of the latter as the most common preventable cause of mental retardation in the young (Bhatara et al 2002). Thyroid hormones are known to be essential for the normal maturation and functioning of the central nervous system although they also modulate a broad range of effects on virtually all tissues (Karapanou and Papadimitriou 2011). It was originally thought that thyroid hormones were only important for human neurodevelopment post-natally. This was based on the observation that circulating foetal levels of these hormones were very low, together with the fact that the placenta was thought to present a very efficient barrier to the transfer of thyroid hormones from the mother. This prejudice was supported by the findings that post-natal thyroid support therapy could correct many of the thyroid dependent conditions that were present at birth following maternal hypothyroidism due to low iodine (Glendenning 2008). However, it is now clear that thyroid hormones are essential for both foetal and post-natal neurodevelopment, and for the regulation of neuropsychological function in children and adults (Williams 2008).

Thyroid hormones have no influence on the very early developmental events in the human foetus, such as neural induction and the establishment of cellular polarity, but they are essential to regulate later processes, including neurogenesis, myelination, dendritic proliferation and synapse formation

(Bernal et al 2003; Bernal 2007; Zoeller and Rovet 2004). There are large numbers of genes transcribed following activation of the thyroid hormone nuclear receptor and the timing of the onset of thyroid hormone action is crucial for the correct development of the brain (Zoeller and Rovet 2004; Hindmarsh 2002). A condition known as endemic neurological cretinism in humans is due to maternal iodine deficiency, and resulting maternal hypothyroxinaemia, with low maternal T4 levels causing neurological hypothyroidism in the foetus, which results in profound mental retardation, cerebral spastic diplegia, deaf-mutism and squint in the absence of general signs of hypothyroidism (Porterfield and Hendrich 1993). Although iodine dependent neurodegenerative conditions, in neonates, can be prevented by iodine supplementation, iodine deficiency remains the commonest endocrine disorder in the human population and has been estimated to be the most frequent cause of preventable mental retardation (de Escobar et al 2004).

6.9.2. Comparison of the stages of thyroid hormone dependency in the rat and human foetus

There are three stages of thyroid hormone dependent neurological development in the foetus, with the first occurring between 16-20 weeks post-conception in humans, equivalent to day E17.5-18 in the rat, and before the foetus is able to synthesise its own thyroid hormones (Fig. 8). During this first stage thyroid hormones can only be delivered to the foetus via the placenta from maternally synthesised hormone (De Escobar et al 2000; 2004; Obregon et al 2007) and its presence stimulates neuronal proliferation and migration in the cerebral cortex, hippocampus, and medial ganglionic eminence (Narayanan and Narayanan 1985; Lucio et al 1997; Cuevas et al 2005; Auso et al 2004). The second stage occupies the remainder of pregnancy after the initiation of foetal thyroid hormone synthesis when the developing brain receives a dual supply of thyroid hormones from both a foetal and maternal origin. During this stage processes dependent upon thyroid hormones include neurogenesis, neurone migration, axonal outgrowth, dendritic branching and synaptogenesis, glial cell differentiation and migration, and the onset of myelination (de Escobar et al 2000; Porterfield and Hendrich 1993).

The third stage occurs between the neonatal and post-natal period when thyroid hormone supplies to the brain are entirely derived from the child (Fig. 8). During this stage, thyroid hormone is needed for the continuing maturation of the central nervous system that involves migration of the granule cells in the dentate gyrus and cerebellum, migration of the pyramidal cells in the cerebral cortex and the Purkinje cells of the cerebellum, and the maturation of the glial system.

Fig 8: Contribution of human maternal and foetal thyroid hormones to development (from Williams 2008).

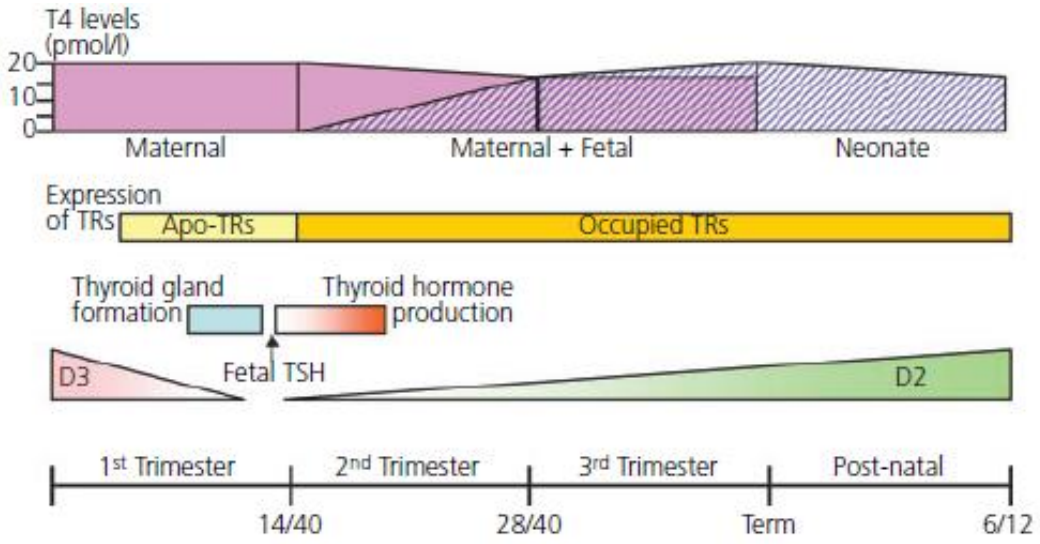


Fig. 8 above illustrates the respective contribution of foetal and maternal thyroid hormones at the respective times during gestation/pregnancy. The foetus is entirely dependent upon maternal thyroid hormone during the first trimester and entirely dependent upon its own hormone after birth. Foetal thyroid hormone production begins at the end of the first trimester peaking at term. The thyroid gland begins development midway through the first trimester and begins production of foetal hormone following a surge in foetal TSH at the end of the first trimester. D3 is an inactivating type 3 iodothyronine deiodinase which decreases during the first trimester allowing the initiation of production of the foetal hormone as concentrations of type 2 deiodinase (D2) increase after this period. TR represents thyroid hormone nuclear receptor which is able to bind thyroid hormone at the end of the first trimester. Type 3 iodothyronine deiodinase is thought to act to prevent thyroid hormone access to specific tissues at certain critical times and to prevent saturation of the thyroid hormone nuclear receptor (Bianco and Kim 2006; Bianco et al 2002). Type 3 deiodinase works by removing iodine from the inner ring of T4 or T3 and hence inactivates the hormone. It is expressed at its highest levels in the foetus whereas it is present only at low levels in adult tissue (Williams 2008).

The findings of neurodevelopmental problems following thyroid hormone deficiency in humans were subsequently confirmed and shown in animal studies (Koibuchi and Chin 2000; Thompson and Potter 2000; Morte et al 2002; Singh et al 2003). This data provided the critical evidence that thyroid hormones are essential in early (foetal) brain development, and that the associated neurological deficits depended upon the timing and severity of thyroid hormone insufficiency. Hence for chemicals that showed dose-response relationships in terms of their depression in circulating thyroid hormones, a threshold for the initiation of defects, based upon the threshold for thyroid hormone dependent effects, existed (Narayanan and Narayanan 1985). In the case of hypothyroidism occurring in adult rats, the neurological defects could be reversed by T4 supplementation (Ruiz-Marcos et al 1988) although this was not the case for perinatal and prenatal thyroid deficiency, where neurological deficits were generally permanent.

The order of development of the thyroid gland, during organogenesis, is similar for rodents, sheep, and humans, but the timing of various perinatal developmental events differs significantly between the species, with rats being born relatively immature, and hence late developmental events, that occur *in utero* in humans, only occur postnatally in rats. Because of their advanced state of development at birth, thyroid development in sheep, by comparison, occurs almost exclusively in utero (Hombach-Klonisch et al 2013).

The timing of the onset of thyroid receptor (TR) binding also differs significantly between species independent of their differing gestational times and can be first observed in the rat during mid-to late-gestation (average gestation is 21 days), in sheep during the latter two-thirds of gestation (average gestation is 20.5 weeks), and in humans between gestational weeks 10 and 16 (average gestation is 39 weeks) (Fisher and Brown, 2000).

6.10. Comparison of thyroid cancer in human and animal species

Thyroid cancer is the most common endocrine malignancy in the human population and incidence rates in most countries have been steadily increasing over the past few decades, particularly in women (Zimmermann and Galetti 2015). Known risk factors for human thyroid cancer are radiation exposure during childhood, exposure from nuclear accidents (Mazonakis et al 2007; Bounacer et al 1997), natural radiation, or medical imaging (Bard et al 1997), but suspected risk factors include obesity and the metabolic syndrome (Renehan, et al 2008; Rinaldi et al 2012), environmental pollutants (Hallgren and Darnerud 2002; Zhang et al 2008), a family history of thyroid cancer, or thyroid disorders (Franceschi et al 1999) and, for certain susceptible individuals, iodine intake (Zimmermann and Galetti 2015). While iodine deficiency together with a small number of drugs are known to induce thyroid growth (goitrogen) in humans, the only confirmed human thyroid carcinogen is ionizing radiation, and the available evidence would suggest that chemicals that induce thyroid

growth alone, in the absence of additional mutational events, will not lead to human thyroid cancer (McTiernan et al 1984; Holm et al 1988; Daminet and Ferguson 2003).

Chemically-induced rodent thyroid follicular cell cancer arises from one of two different processes that ultimately converge under the influence of continued TSH stimulation. The first process is found with chemicals that induce DNA damage/mutations specific to the thyroid, while the second is seen with chemicals that induce perturbations of hypothalamus/pituitary/thyroid axis with subsequent increased stimulation of thyroid cell growth by TSH. An example of a chemical that induces thyroid follicular cancer in rodents exclusively through a mutagenic MoA is N-Bis-(2-hydroxypropyl) nitrosamine as it is entirely devoid of effects on thyroid hormone production or activity (Hiasa et al 1991).

6.10.1 The association between iodide deficiency, goitre and thyroid cancer in the human population

The epidemiology of goitre and thyroid cancer is confusing, and confused, and the appropriate interpretation requires an in depth understanding of the molecular biology, underlying causes of thyroid cancer, and the clinical development and treatment of both goitre and cancer. One of the most scientifically enlightening causes of human thyroid disease is that resulting from iodine deficiency since it has been known for centuries that in such conditions, there will be a high incidence of thyroid hypertrophy in the local population. Humans living in areas of chronic iodide deficiency develop thyroid hypertrophy/hyperplasia manifest in the appearance of goitre (Zimmerman 2009; Kotwal et al 2007; Li and Eastman 2012), due to chronic deficiency in the production of thyroid hormones, and the continued stimulation of the thyroid gland by TSH released from the pituitary gland. Supplementation of dietary iodine is normally able to reverse the clinical symptoms of both goitre and the other physiological and neurological effects of hypothyroidism (Kotwal et al 2007; van der Haar 2007; Liesenkötter et al 1995).

6.10.1.1. The role of TSH in human thyroid cancer

The data for the role of TSH in human thyroid cancer contrasts dramatically with the situation in rats. There is a large volume of epidemiological data regarding thyroid cancer rates in areas of endemic goitre, where iodine deficiency would be expected to lead to chronic TSH stimulation of the thyroid gland. In the United States an extensive study compared regional rates of thyroid cancer mortality and endemic goitre over a 20-yr period both before, and after, the generalized introduction of iodinated salt (Pendergrast et al 1961). While the incidence of goitre significantly decreased in the areas previously affected by endemic goitre, there was no similar change in thyroid cancer mortality rates and hence no association between the two diseases. Similar results were obtained in Austria (Riccabona 1986) where large areas of the Tyrol have historically shown an abnormally high incidence of goitre due to iodine deficiency. While there are regions of the world with endemic goitre and a high incidence of thyroid cancer, there are also similar regions with a high incidence of goitre in the absence of similar high thyroid cancer rates, as well as areas without endemic goitre where the incidence of thyroid malignancies is higher than normal (Clements 1954, Riccabona 1987).

In contrast to the previous examples however, patients with goitres that arise, not from iodide deficiency but from congenital thyroid gland metabolic defects (i.e. mutations of various kinds), have been noted to have a markedly increased incidence of thyroid malignancies (Kitahara and Sosa 2016; Cooper et al 1981; McGirr et al 1959; Elman 1958). There is also an increased incidence of thyroid carcinoma in patients with thyrotoxicosis (Siegel and Lee, 1998; Verburg and Reiners 2010; Pendergrast et al 1961; Clements 1954). There are, however, no reports of an increased incidence of thyroid cancer in patients with thyroid hormone resistance where chronically high levels of circulating TSH exist, due to a non-functional negative feedback control in the hypothalamus/pituitary gland, and an inability to react to normal levels of circulating T3 (Yen 2003).

In those situations where there is an increased risk of thyroid malignancy in patients with glands showing metabolic defects, mutations, or in subjects with thyrotoxicosis, the thyroid cancers do not arise simply due to the hyperplastic stimulus of TSH alone but are complex combinations of genetic and hormonal factors. The finding that simple goitre can be fully reversed by supplementing the diet

with iodine shows that while the goitre is undoubtedly produced under a chronic TSH stimulus, the thyroid gland nevertheless retains its ability to return to normal despite many years of TSH stimulation. This is very different to the situation in rodents where the evidence points to the ability of sustained TSH stimulation alone, as can happen with chemically induced thyroid insufficiency, being able to induce neoplasia (Capen 1999). Indeed the implantation of TSH secreting pituitary tumours alone into recipient rats has been shown to be able to induce thyroid follicular neoplasms in the absence of any further treatment (Hill et al 1989).

6.10.1.2. The role of TSH in rodent cancer

Rats have higher basal levels of circulating TSH than are present in humans (Table 2; Lewandowski et al 2003) and in contrast to the situations in the human population, where plasma levels of TSH are roughly equal between men and women, male rats have higher normal levels of TSH than are present in female rats (Hill et al 1998). This finding correlates well with the observation that the height of the follicular epithelium is greater in males than in female rats, reflecting the greater TSH-induced activity in males versus females (Capen 1996). Male rats also show a higher spontaneous incidence of thyroid follicular cancer than do female rats (IARC 1999; Haseman et al 1985). In a survey of the outcome of mouse and rat carcinogenicity studies (Hurley et al 1998), twenty-four of the approximately 240 pesticides tested were found to induce thyroid follicular cell cancer, and of these twenty-two induced thyroid tumours only in rats, with the remaining two inducing thyroid cancer in both rats and mice. Additionally, chemically-induced thyroid neoplasms occurred far more frequently in male rats than in female rats, both with respect to the proportion of chemicals that induced thyroid tumours, and to final tumour incidence. In contrast to the situation in the rat, thyroid cancer incidences are greater in human females than in males, while TSH levels between men and women are the same suggesting some fundamental differences both in basal thyroid control and physiology but also in their responses to situations that cause thyroid cancer (Hill et al 1998; Parker et al 1997).

6.10.2 Comparative cancer studies in laboratory animals and humans for goitrogenic chemicals

The goitrogenic effects of sulphonamide drugs have been known for many years since the first demonstration of rat thyroid hypertrophy by the prototype sulphonamide, sulphaguanidine (MacKenzie and MacKenzie 1943; Mackenzie et al 1941; Astwood et al 1943). The sulphonamide drugs, as a class, induce thyroid changes in the rat and dog through inhibition of thyroperoxidase-catalysed binding of iodine to thyroglobulin and the resulting decrease in circulating thyroid hormones (Nishikawa 1983).The next generation of sulphonamides also turned out to be potent goitrogens in the rat, when a combination of sulfamethoxazole and trimethoprim was shown to cause dramatic decreases in T3 and T4, with compensatory increases in both circulating TSH and in the weight of the thyroid gland. While similar effects were also seen in the dog with this drug (Nishikawa 1983), monkeys and humans did not show the thyroid inhibitory effects, and when rhesus monkeys were given doses of sulfamethoxazole up to 300 mg/kg/day for 52 weeks there were no changes in either thyroid gland weight or histology (Swarm et al 1973). In contrast long-term administration of sulphonamides, at dose levels that result in prolonged stimulation of the thyroid gland by TSH, induces thyroid neoplasia in rats (Doerge and Decker 1994).

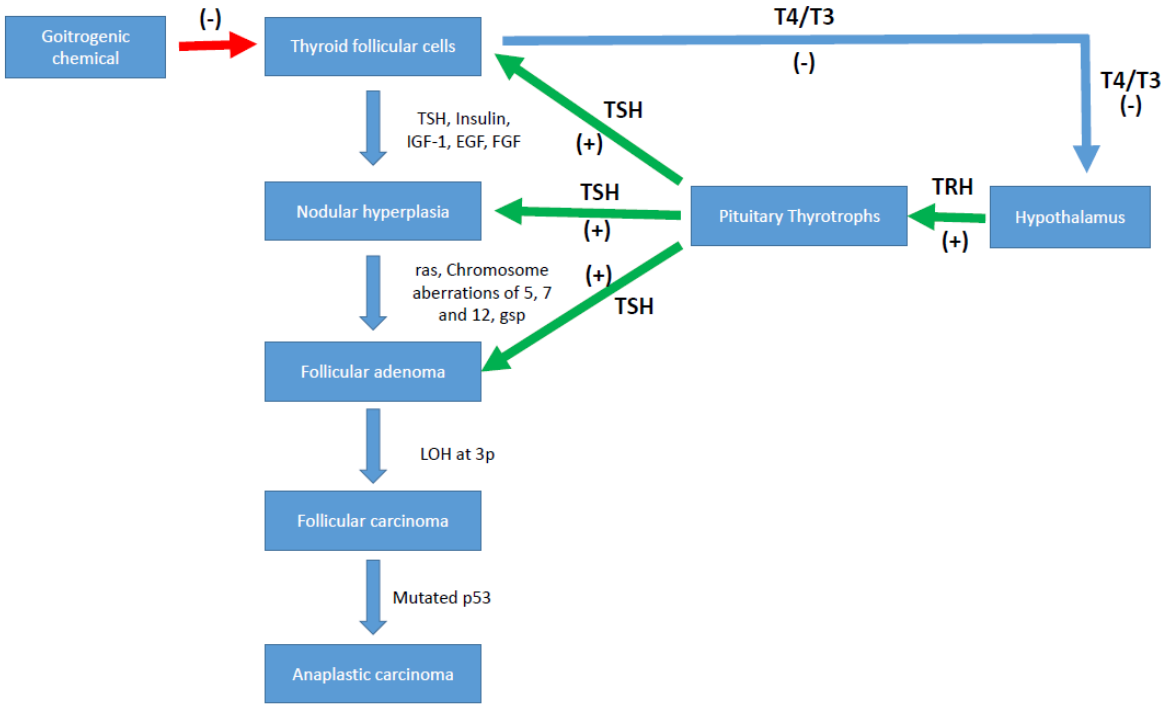
A similar species difference in sensitivity to the inhibitory effects of these drugs was found following *ex vivo* experiments in a thyroid peroxidase assay using PTU (another thyroperoxidase inhibitor) and the sulphonamide, sulphamonomethoxine. In this study, the concentration of PTU required to inhibit thyroperoxidase sourced from monkey thyroid was approximately 50x higher than that required for the same degree of inhibition in rat thyroperoxidase, while for sulphamonomethoxine the concentration required to inhibit 50% of the thyroperoxidase activity was 500 fold greater in the monkey enzyme than in rat thyroperoxidase (Takayama et al 1986). This data was strongly supportive of an intrinsic sensitivity difference in the enzyme between that derived from rat thyroid and that from monkey thyroid. These studies further demonstrated that, at least with thyroperoxidase inhibitors, significant differences in potency existed between sensitive species, such as rat, mouse and dog, and more resistant species that included non-human primates, human, guinea pig and chicken (Capen 1999). In

support of this data, only mild effects of sulfonamides have ever been observed on human thyroid function (Cohen et al 1980).

6.10.3. Thyroid hormone perturbation and thyroid cancer in rodents

In mammals in general, including humans, when demands for more thyroid hormone are small, existing thyroid follicular cells can meet any extra demand by increasing their synthesis and output, often becoming larger in size (hypertrophic). At least in rodents, inhibition of thyroid hormone synthesis and/or secretion, such as that occurring following the administration of certain chemicals, is responded to by increasing the number (hyperplasia) and size (hypertrophy) of thyroid follicular cells to enhance thyroid hormone output. This response is mediated through feedback release of TSH from the pituitary gland, and in the presence of continued thyroid hormone deficiency, and chronic TSH stimulation, there is an increase in the thyroid weight as a result of a combination of thyroid follicular hypertrophy and hyperplasia. Since the TSH-producing thyrotrophs in the pituitary gland are also producing, and secreting, at a greater rate than normal, they will also undergo hypertrophy and hyperplasia and where looked for, these are invariably seen in the adenohypophysis of the pituitary gland under conditions of moderate to severe thyroid hypertrophy (Norford et al 1993; Russfield 1967; Moriarty and Tobin 1976; Akosa et al 1982).

Fig. 9: The postulated progression of changes in rat thyroid follicular epithelium in the development of cancer from chronic exposure to a goitrogenic chemical



Notes: The red arrow indicates a thyroid disrupting chemical, the green arrows indicate a positive effect on the target cells while the blue arrow indicates the negative feedback response of the hypothalamus to low circulating T3/T4 levels.

In rodents, there is adequate evidence, with a comparatively large number of chemicals of various classes and diverse pharmacological, that continued thyroid stimulation by TSH alone eventually leads to neoplasia of the thyroid follicular cells (Hill et al 1998; Hurley et al 1998; Capen 1999). The proposed MoA for the production of rat thyroid cancers by goitrogenic chemicals is illustrated in Fig. 9. Although TSH is the main hormonal driver for follicular cell hypertrophy and hyperplasia, several other factors, including insulin, epidermal growth factor (EGF), fibroblast growth factor (FGR) and insulin-like growth factor (IGF), have been shown *in vitro* to also be mitogenic for the follicular thyroid epithelial cells (Maviel et al 1988; Logan et al 1992; Żerek-Meleń et al 1987).

The data therefore support a conclusion that intrinsic sensitivity differences occur between species, in terms of their sensitivity to thyroid hormone inhibition, and also strongly suggests that qualitative differences may also determine a cancer outcome with prolonged TSH stimulation being the main driver for rodent thyroid non-genotoxic cancer, whereas TSH is only able to promote cancer in humans in the presence of underlying thyroid problems such as inherited mutation associated metabolic disorders or thyrotoxicosis (Curran and DeGroot 1991).

6.10.4. Is chemically-induced rodent thyroid cancer relevant to man?

For regulatory purposes, and in the absence of MoA data to the contrary, chemical induced rodent thyroid tumours is presumed to be relevant to humans, and when information on differences in inter-species MoA is lacking, the default is to assume comparable carcinogenic sensitivity in rodents and humans (Hill et al 1998; Hard 1998). Where there are follicular neoplasms in the absence of evidence of follicular hyperplasia/ hypertrophy, or evidence of disruption of the thyroid-pituitary axis, such neoplasms are presumed to be relevant to humans and in terms of their dose-response relationships, the Environmental Protection Agency applies linearity when estimating the risk to thyroid cancer induced by chemical substances that either do not disrupt thyroid functioning, are mutagenic, or that lack MoA information. In terms of a neoplastic MoA for rodent thyroid tumours both IARC (1999; 2000) and the US Environmental Protection Agency (US EPA 1998) currently have established specific guidance for evaluating the human relevance of these tumours.

In order to show a thyroid-pituitary MoA for any particular chemical, the US EPA (1998) asks for evidence in the following five areas:

1. Increases in cellular growth (e.g., increased thyroid weight, hypertrophy or hyperplasia, proliferation detected by DNA labelling or mitotic indices)
2. Changes in thyroid and pituitary hormones (T3/T3, TSH)
3. Location of site of action (e.g., thyroid, liver, or peripheral, and enzyme target within the target organ);
4. Dose correlations among thyroid effects (cell proliferation/hypertrophy) and cancer;
5. Reversibility of effects when chemical dosing stops.

For those chemicals that are non-mutagenic and non-genotoxic, that reduce thyroid hormones acutely/chronically, and that increase TSH levels, in the absence of a proven MoA having no relevance to humans, the outcome is still considered to represent a human risk.

The increasing adoption of the WHO/IPCS principles of establishing MoA and human relevance for these findings have, more recently introduced a more stringent list of requirements that consider all possible alternative MoAs and that apply modified Bradford Hill criteria for estimating the strength of association of between the proposed MoA and the adverse outcome (Dellarco et al 2006; Boobis et al 2006; Meek 2008; Meek et al 2014). This has been a welcome step in applying objective scientific data to support arguments of non-adversity of effects, and non-relevance to the human population of animal toxicity results and has applied an objective approach to regulatory decision making that takes

into account non-guideline investigative study data in addition to GLP conducted guideline study data to arrive at their final conclusions.

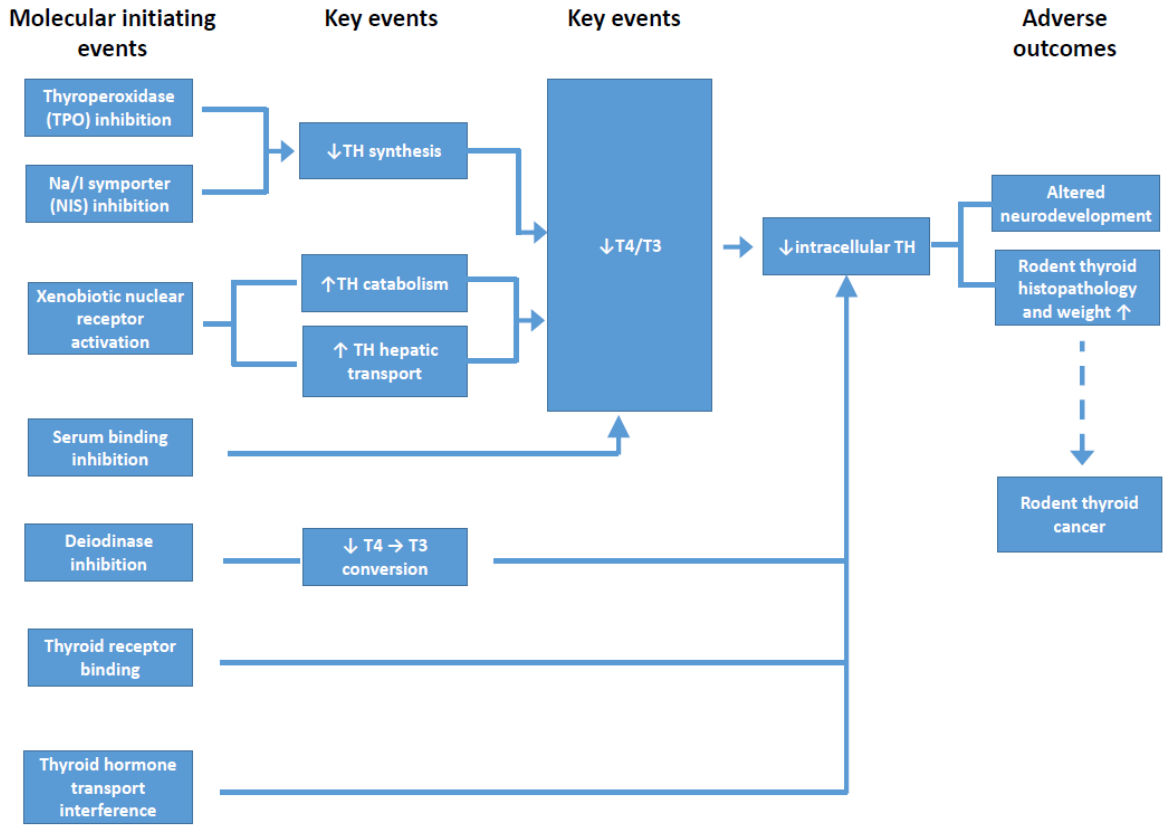
6.11. Cross-species extrapolation for thyroid effects

Inter-species extrapolation of adverse thyroid effects, normally detected in laboratory animal studies, to human exposures, is an exacting science requiring firstly a thorough demonstration of the MoA of the toxicity in the target laboratory animal species, most often the rat. Once accepted as a plausible explanation a reasoned argument would then be made, based upon sound principles, as to the human relevance of the laboratory animal MoA. Acceptable approaches to this problem have been widely publicised in the WHO/IPCS guidelines for establishing a weight of evidence conclusion to the exercise (Meek et al 2003; Boobis et al 2006) and this approach has been included in the draft GD (ECHA/EFSA/JRC 2017). There are clearly situations in which the effects of a chemical in the rat are similar to what would be predicted in humans, and perchlorate is a clear example of this principle (Wolff 1998).

For some chemicals that affect thyroid function however the situation may be very different and there may be little data to support cross-species extrapolation (Crofton 2004). Both *in vivo* and *in vitro* studies show that sodium phenobarbitone, pregnenolone-16α-carbonitrile (PCN), acetochlor and PCBs, activate nuclear receptors such as the constitutive androstane receptor (CAR) and the pregnane X receptor (PXR) either individually, or as dimers and that administration of these chemicals to rodents leads to an up-regulation of hepatic catabolic enzymes, stimulation of the biliary elimination of thyroid hormones, a decrease in circulating thyroid hormones (Schuetz et al. 1998; Liu et al 1996; Hood and Klaassen 2000), and thyroid hypertrophy and hyperplasia. Although human liver does contain both CAR and PXR (Moore et al 2000; Omiecinski et al 2011) there are significant species differences in response to those chemicals that activate these nuclear receptors both in terms of sensitivity of response (Elcombe et al 2014) and in terms of specificity with rodent PXR being activated by PCN, but not by rifampicin, whereas human PXR is activated by rifampicin but not by PCN (Kliewer et al. 2002). Additionally, while PCBs in general are agonistic to PXR in rats, *in vitro* data in human hepatocytes show that high concentrations of PCB-153 are antagonists to the human PXR (Tabb et al. 2004).

Not to make too much of a point about it, significant species differences exist between human and rodents in terms of the thyroid binding proteins in the circulation where the relative importance of TTR, TBG and albumin for transporting thyroid hormones accounts for significant differences in thyroid hormone half-life and turnover, with rodents showing appreciably higher rates of production and loss of thyroid hormones than do humans, a situation that leads to increased overall sensitivity, and in some cases species specific differences, in chemical perturbation of the HPT axis (Capen 1997; Hill et al 1998).

Fig. 10: An adverse outcome pathway analysis of chemically-induced thyroid hormone disruption (from Paul 2014).



7. ECPA Question: Is there evidence for a threshold/correlation for adversity linked to serum thyroid hormone levels (T3, T4, TSH and sulphated thyroid hormone), between species

Thyroid hormone disruption has been the subject of adverse outcome pathway (AOP) analysis (Paul 2014). Fig 10 above is taken from a presentation that clearly outlines the current understanding of the molecular initiating events (MIEs) that lead to altered hormone homeostasis (Crofton 2008; Murk et al 2013). Although the AOP above might suggest that any single chemical would act through a single molecular initiating event, many rodent thyroid-acting chemicals disrupt multiple MIEs and for several the ultimate thyroid disruption is thought to be considerably greater as a result of these multiple hits on its function. PCBs exemplify this principle where they have been shown to displace thyroid hormone from its binding protein, to inhibit deiodinase activity, and to increase thyroid hormone catabolism by inducing hepatic glucuronidation (Barter and Klaassen 1992; 1994; Brouwer 1989; Hood and Klaassen 2000).

While each of the MIEs in Fig 10 will operate essentially independent of the administered dose of a thyroid disrupting chemical, activation of subsequent key events would be expected to follow classical dose-response relationships with higher dose levels inducing greater changes in each key event, and a threshold dose level below which each respective key event will not be triggered (Dellarco et al 2006). Hence each key event would be expected to follow conventional dose-response relationships dependent upon the degree of change in the previous key event with clearly defined exposure thresholds for triggering subsequent events. In the absence of a sufficient change in the previous key

event the downstream key event would not occur. An alternative way of looking at this is in terms of chemically induced depressions in thyroid hormones where TSH release from the pituitary gland only occurs when circulating levels of T3 achieve a threshold level that stimulates the receptors in the hypothalamus to release TRH.

Comparative studies on the effects of perchlorate in the rat and human thyroid gland (Yu et al. 2002) have shown dose related inhibition of uptake of radiolabelled iodide of 15%, 55%, and 65% at 1.0, 3.0, and 10 mg/kg perchlorate respectively on day 1 following initiation of perchlorate administration to the rat but that the homeostasis was reset in animals killed after 28 days where inhibition of iodide uptake was only observed at the top dose levels of 10 mg/kg. This adaptation of the thyroid system to prolonged administration is somewhat different to the way in which other organs deal with toxicity but shows clear adaptation probably by increasing the expression of the sodium-iodide symporter on the follicular cells of the thyroid. A similar response was not observed in a 14-day study with perchlorate administration in human volunteers (Greer et al. 2002) and the species difference exhibited was thought to have occurred in rats because of their smaller reserve capacity of thyroid hormones than humans.

7.1. Thyroid hormone changes in the absence of histological changes

The negative feedback control of circulating free T3 on the hypothalamus and pituitary plays an essential role in ensuring that there is sufficient circulating thyroid hormones to satisfy the daily needs of the body. The circulating T3 concentrations are a product of the deiodination of T4 through the action of hepatic 5' deiodinases together with small amounts of T3 being released directly from the thyroid gland. Hence circulatory T3 levels are maintained by a large pool of protein bound T4 which can be deiodinised in peripheral tissues, mostly the liver, when the concentration of free circulating T3 falls below acceptable limits. In addition to TSH controlling the production and release of T4/T3 from the thyroid gland, it is also responsible for the control of the activity of peripheral tissue deiodinases to modulate the conversion of T3 from T4 (Hoermann et al 2015) and hence TSH acts to integrate the peripheral and central elements of thyroid hormone homeostasis into one overarching control system. This is the normal physiological control of thyroid hormones and it operates within defined limits of circulating hormones, but not within a narrow band of concentrations since it is clear that release of the master controller, TSH, follows a circadian rhythm of peaks and troughs in the circulation throughout the day (Fisher 1996). Indeed, the differences between peak and nadir levels of TSH in humans have been shown to differ by as much as ±50% around a mean value (Hoermann et al 2015; Fisher 1996). Despite these daily fluctuations in serum TSH levels, those of T3 and T4 remain essentially within acceptable limits underlining the critical importance of an adequate concentration of thyroid hormones in maintaining the normal physiology in the body (Bianco and Kim 2006).

It is stated in the draft GD (page 96; Appendix A) that "A decrease in T4 (total or free) in the absence of other histological changes and/or hormonal evidence of hypothyroidism is a relatively frequent observation in experimental toxicological studies, particularly in rodents." (ECHA/EFSA/JRC 2017). It is unclear exactly where this statement has come from and within what context it is meant since it is not referenced in the text. However, sporadic changes in single parameters, in the multitude of clinical chemistry endpoints measured in routine toxicity studies, is indeed a common observation and, in the absence of expected accompanying changes in linked parameters, is normally discounted (Hamada et al 1998; Lewis et al 2002). As with all parameters in such studies, the difficulty lies in linking the effects, in this case a decrease in T4, to any functional consequence since thyroid control, like most physiological systems in the body, operates within tolerances rather than being dependent upon excursions outside of narrow ranges. Considering the critical importance of thyroid hormones, there is an inbuilt redundancy in terms of high circulating T4 levels, which allows the maintenance of T3 homeostasis within the limits required for adequate physiological control. Hence a degree of decrease in T4 will still permit adequate levels of T3 to be maintained without triggering compensatory TSH release and thyroid histological changes. Therefore, in the absence of supporting evidence of histopathological effects in the thyroid gland or pituitary, or expected associated changes in TSH and T3, isolated changes in T4 need to be placed into the context of the study as a whole, and interpreted

with an eye to the duration of the study and dose-response relationships for the observed change, as it would for any other potentially adverse endpoint.

While decreases in T4 can be diagnostic of an endocrine effect, there are a number of non-physiological, and non-endocrinological, reasons why a decrease in a single parameter should not be considered biologically/toxicologically relevant (Lewis et al 2002). Despite the fact that decreases in T4 in human cases can be adverse with regard to pre- and post-natal neurological development and thyroid pathology, they are always accompanied by concomitant hormonal and/or tissue changes that may or may not be described due to the constraints placed upon clinical studies (Colnot and Dekant 2017). There are no such restrictions on laboratory animal toxicity studies and complete tissue histopathology and a broad range of hormonal and clinical chemistry assessments will accompany any appropriately OECD guide-lined test, such that expected accompanying pathophysiological endpoints will be present, alongside the change in the measured parameter, to enable that change to be placed into context.

In a situation where a chemical is suspected of affecting thyroid homeostasis then specialised studies to establish any functional consequences of the changes, including MoA and human relevance focussed experiments, might help clarify whether or not the observed change was accompanied by functional consequences. Studies such as the EOGRTS or DNT would help to clarify the biological significance of particular hormonal changes, but since hormonal disruption generally follows classical dose response relationships (Klaassen and Hood 2001; Ren et al 1988; Liu et al 1995), small changes in circulating hormones, that occur at relatively low doses of chemicals, would not be expected to result in functional consequences when the considerable redundancy in function that exists for the thyroid control system is taken into account.

A discussion of the topic of isolated thyroid hormone changes on studies has been covered in an excellent recent publication by Colnot and Dekant (2017) where they outline the considerations that would normally be taken when arriving at a decision regarding the treatment-relatedness or otherwise of a change in thyroid hormones. Hence a difference in thyroid hormone levels between controls and treated animals is unlikely to represent a treatment-related effect if it is transient and occurs in the absence of dose-response or if it occurs in one or a few animals. A difference in thyroid hormone levels is less likely related to treatment even if statistically significant if it remains within the range of hormone levels present in the serum of control animals from other studies conducted in the same laboratory (historical controls).

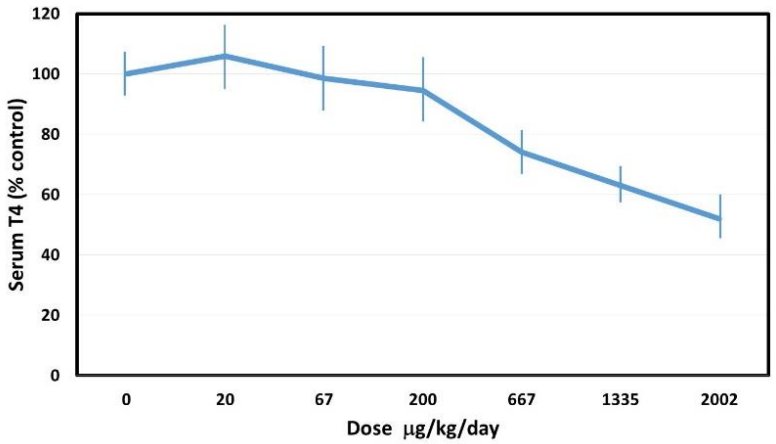
Since it is well known that circulating thyroid hormone levels are subject to a number of confounders such as generalised stress including body weight and food consumption effects, altered thyroid hormone levels are less likely to be specific and more likely to be secondary to systemic stress effects if there is no alteration in the general function of the thyroid as determined by thyroid weights and histology (DeVito et al., 1999; Choksi et al., 2003) since these endpoints are less sensitive to these secondary effects (St Germain and Galton, 1985; Cavalieri, 1991). Altered caloric intake is known to affect thyroid hormones, and reduced food and water intake is commonly observed on toxicity studies in rodents, some of the thyroid hormone changes observed, when unaccompanied by supportive changes in histology and thyroid weight may be secondary to inanition induced by the use of maximum tolerated doses or, considering the impact of disease on thyroid hormone homeostasis, be secondary to toxicity in other organs such as the kidney or liver.

7.2. Evidence for dose response/threshold relationships for thyroid hormone effects

7.2.1. Evidence in animal studies

A series of dose response studies on a range of chemical mixtures, known to disrupt thyroid hormone homeostasis, were carried out on female Long-Evans rats dosed via gavage with a number of poly-halogenated aromatic hydrocarbons for 4 consecutive days and serum total T4 was measured in samples collected 24 hr after the last dose (Crofton et al 2005). These studies clearly showed a threshold dose for the mixtures (Fig. 11), below which no effects on serum T4 were observed (Crofton et al 2005).

Fig 11: Dose dependent decrease in serum T4 concentrations following exposure to a mixture of polycyclic halogenated hydrocarbons (PHAH) – redrawn from Crofton et al 2005)



A dose response study was also carried out under the auspices of the National Toxicology Program where 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153) was dosed by oral gavage to female Harlan Sprague-Dawley rats at dose levels of 10, 100, 300, 1,000, or 3,000 µg/kg 5 days per week for periods of up to 105 weeks (NTP 2006). Assessments carried out on this study included free and total thyroid hormones and potential cancer outcomes, but not thyroid organ weight.

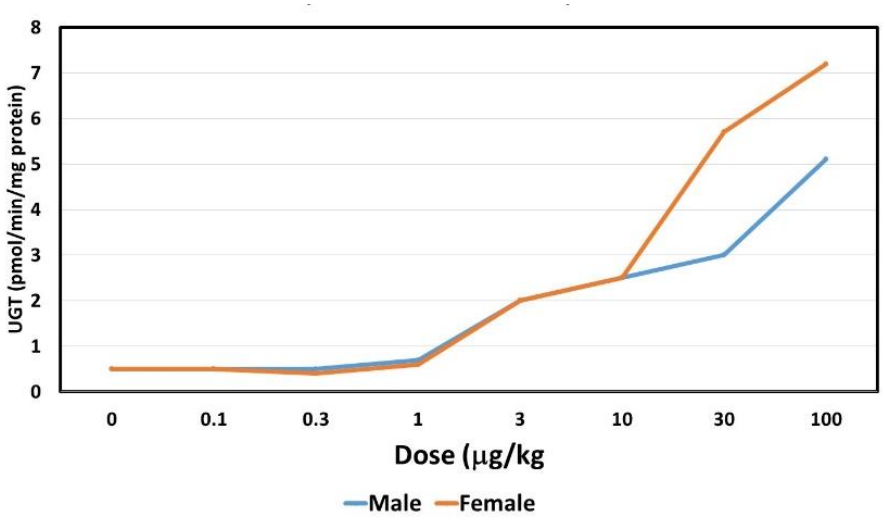
Serum total T4, free T4, and total T3 concentrations in the 3,000 µg/kg group only were significantly lower than those in the vehicle controls at the 14-week interim evaluation. At the 53-week interim evaluation, serum total T4 and free T4 concentrations in the 3,000 µg/kg group only were significantly lower than in the vehicle controls but there were no changes in total or free T3 levels. At 2 years, the incidences of minimal to mild follicular cell hypertrophy were significantly increased in the 300 µg/kg and 3,000 µg/kg (core and stop-exposure) groups although follicular hypertrophy was recorded at all dose levels including the controls (incidences of 5/51, 9/52, 9/53, 12/53, 10/53, 17/51, 12/49 at the 0, 10, 100, 300, 1000 and 3000 μg/kg).

Once again this study clearly illustrates a threshold for thyroid hormone disruption at all time points measured (NOEL at 1000 μg/kg), but not for histopathology of follicular hypertrophy at the 2 year time period. The proposed AOP for thyroid changes predicts that hormone changes would be a more sensitive endpoint than histopathology and that the latter should occur following activation of the respective MIE. The fact that neither of these was seen in this study is most probably explained as an artefact of the time points where the various endpoints were measured and emphasises the fact that bolting on thyroid hormone measure to a standard study design is most probably not going to give supportive information for establishing MoA. This data strongly supports the conclusion that specially

designed, preferably short term, studies would most likely provide the information that would give the most informative data on which to address prospective MoAs.

A similar study was conducted with another polyhalogenated hydrocarbon, PCB 126, which was dosed by oral gavage to rats daily for four days at a range of dose levels (Fig. 12) from 0.1 to 100 μg/kg/day (Craft et al 2002). The study measured effects on hepatic glucuronyltransferase and on serum T4 levels, and while showing clear thresholds for dose levels where reductions in serum T4 occurred, it also demonstrated a concomitant induction of hepatic UGT which showed a LOEL at 3 μg/kg which corresponded to a similar LOEL for reductions in serum T4 (Fig. 11).

Fig. 12: Hepatic UGT levels in rats following dosing with PCB 126 for 4 days (Craft et al 2002)



Studies in rapidly growing, prepubertal, cynomolgus monkeys, made hypothyroid by the administration of methimazole in drinking water, showed a clear dose response in the concentration of exogenous T4 required to correct the inhibition of lower leg growth rate, and to stabilise serum levels of T3, T4 and TSH. (Ren et al 1988). Hypothyroid monkeys showed a 65% decrease in lower leg growth rate, decreased T3, T4 and IGF-1, and increased TSH compared to non-hypothyroid monkeys of similar age. Following administration of exogenous T4 in this experiment, lower leg growth rate increased significantly, in a dose dependent manner, such that T4, given at dose levels of 4 and 8 μg/kg/day had 56% and 73% increases in lower leg growth rate respectively compared to controls, this change being accompanied by a return of the circulating thyroid hormone levels to normal.

7.2.2. Evidence in human studies

The evidence for thresholds in the effects of T4/T3 is also seen with TSH in human studies. In a study where PTU was administered to hypothyroid patients receiving 0.2 mg/day of T4 (Saberi et al 1974), PTU induced a fall in mean serum T3 concentrations from a mean value of 84 ng/ 100 ml to 70 ng/100 ml (a 17% decrease) after 1 day, and to 63 ng/100 ml (a 25% decrease) after 2 days of PTU. Under these conditions, and following these drops in serum T3, serum TSH concentrations did not increase. Hypothyroid patients receiving T4 at a lower dose of 0.1 mg/day showed a similar decrease in serum T3 following PTU administration which was accompanied by increases in TSH from pre-study levels of 29.6 μU/ml to a peak of 40 μU/ml on day 5-6 of administration of PTU. These results suggest that at least in hypothyroid patients, there is a tolerance before a TSH response occurs following decreases in circulating T3, and a threshold for decreasing circulatory thyroid hormones, below which no compensatory release of TSH will occur from the pituitary.

7.3. Evidence for dose responses/thresholds for neurodevelopmental disorders of thyroid homeostasis

It is clear from both human and animal studies that foetal development is critically dependent upon the correct concentration of thyroid hormones at the right phase in the foetal development. For many years human hypothyroidism during pregnancy has been known to produce severe neurological abnormalities in the offspring of affected individuals that cannot be corrected by thyroid hormone supplementation post-partum. However, the severity of the neurological deficits of neonatal hypothyroidism in humans are dependent upon the severity of the maternal hypothyroidism, and when detected are largely preventable by immediate thyroid hormone replacement (Williams 2008; Alexander et al 2017). However, untreated human neonates exhibit growth retardation and general features of hypothyroidism with mental retardation, tremor, spasticity, and speech and language deficits.

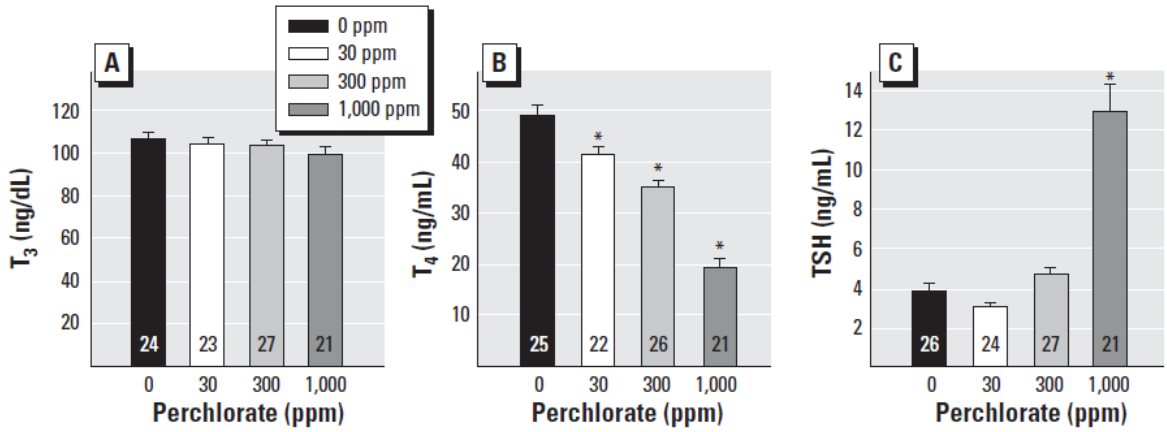
In human beings, pregnancy has a significant impact on the thyroid gland and its function. During pregnancy, the thyroid gland increases in size by 10% in countries with healthy iodine levels in the diets, but by 20% to 40% in areas of iodine deficiency. Production of T4 and T3 has been reported to increase by nearly 50% on non-pregnant women, and dietary demand for iodide increases proportionally to meet these needs (Alexander et al 2017). Subclinical thyroid disease can become clinical during pregnancy and it is for these reasons that TSH levels are monitored in women during pregnancy. Because thyroid hormone supply to the foetus during the first trimester is dependent entirely on the mother thyroid hormone deficiency at this time can have severe effects on the developing nervous system (Bath et al 2013). Results of the Avon Longitudinal Study of Parents and Children (ALSPAC) survey showed that a higher proportion of children born to women with an iodine status of less than 150 μg/g/day had sub-optimum cognitive outcomes than did those born to women in the 150 μg/g/day or more group (Bath et al 2013). Since iodine is essential for the production of the thyroid hormones and iodine deficiency is known to result in hypothyroidism, these data demonstrate a clear threshold for dietary iodine intake below which an increased risk to the developing foetus results.

Subclinical hypothyroidism in pregnant humans has been internationally defined as an upper limits of serum TSH levels of 4 mIU/L (Alexander et al 2017). Levels of TSH higher than this incur recommended therapeutic intervention, typically using the soluble thyroxine substitute, levothyroxine or LT4 (Maraka et al 2017). A typical dose of LT4 is 50 μg/day. This was shown to correct thyroid hormone deficiencies, to minimise the risk of neurodeficiencies in children, and to decrease pregnancy loss associated with low thyroid hormone levels. Adequate iodine intake is essential for the production of thyroid hormones and the World Health Organisation recommends a daily intake of 250 μg for pregnant and lactating women (Alexander et al 2017). There are many parts of the world where iodine deficiency is endemic and associated with increased pregnancy loss and cretinism in offspring and goitre in adults (de Benoist et al 2004). There have been two non-randomised trials in which neurodevelopmental outcomes were improved in children from mildly to moderately iodine-deficient areas whose mothers received iodine supplementation early in pregnancy (Berbel et al 2009; O'Donnell et al 2002)

Data in rats on chemicals that disrupt the thyroid hormone homeostasis has shown that while high doses of potent thyrotoxic chemicals will produce profound neurodevelopment abnormalities, lower doses, where decreases in circulating thyroid hormones are less, are also associated with less severe developmental defects (Auso et al. 2004; Crofton 2004; Crofton et al. 2000; Goldey et al. 1995a, 1995b; Goodman and Gilbert 2007; Morreale de Escobar 2003). These results support the concept of conventional dose response relationships of cause and effect, and although MIEs would be expected to be activated on exposure, irrespective of dose, downstream key events would be expected to follow normal dose-response relationships which would only be triggered once the previous key event reached a particular threshold.

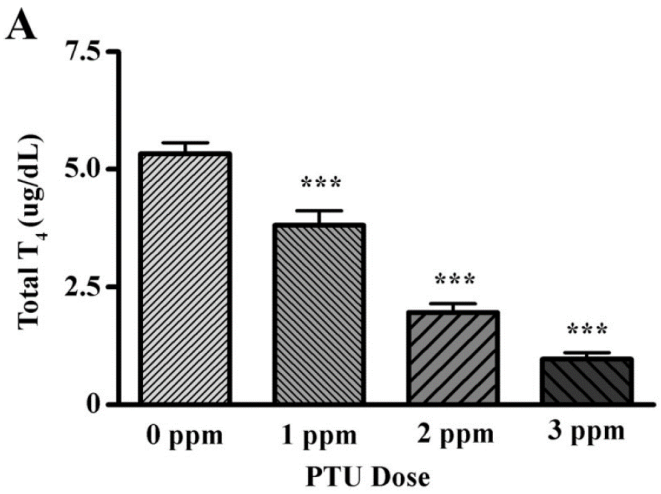
Experimental data supports the contention of thresholds for developmental abnormalities and in a study in pregnant rats, a 50% decrease in maternal T4 was shown to affect cochlear maturation in rats resulting in them being born deaf (Crofton 2004). A study in which pregnant rats were made hypothyroid by perchlorate administered in the drinking water, at dose levels up to 1000 ppm beginning on GD6 and sacrificed on PND30 (Gilbert and Sui 2008), found that T4 in the dams was reduced by 16%, 28%, and 60% in the 30, 300, and 1,000-ppm dose groups respectively accompanied by compensatory increases in TSH at the high-dose only supporting the threshold concept of preceding key events where the reductions in T4 (preceding key event), at the two lower dose levels were insufficient to trigger the subsequent key event, TSH release. Interestingly, in this study there were no changes in T3 values at any of the dose levels illustrating the degree of redundancy in the system that allows sufficient levels of T3 to be maintained despite decreases in T4. This study illustrates the expected dose-response relationships in terms of decreasing T4 and increasing TSH although the relationship was less obvious for T3 levels (Fig. 13).

Fig. 13: Changes in circulating hormone levels in rats dosed with perchlorate (Gilbert and Sui 2008)



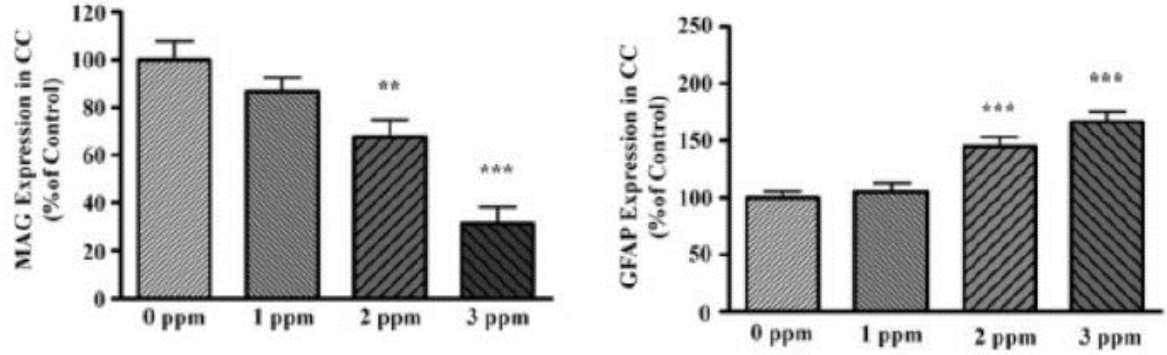
Another study where two week old rats were given PTU in the drinking water at dose levels of 0, 1, 2 and 3 ppm (Sharlin et al 2008) decreased T4 levels at all administered doses in a dose-dependent manner producing a 28%, a 63% and a 82% decrease in total T4 at the 1, 2, and 3 ppm dose levels respectively in PND 15 pups (Fig. 14).

Fig. 14: Dose dependent decrease in total T4 in the serum (from Sharlin et al 2008)



The decrease in T4 showed a direct correlation with a dose-dependent decrease in the density of oligodendrocytes, and an increase in the density of glial fibrillary acidic protein-positive astrocytes in both the corpus callosum and anterior commissure (Fig. 15). Although linear regression analyses demonstrated a strong positive correlation with each of these parameters, the 1 ppm dose level of PTU was a NOEL for both oligonucleotide and astrocytic responses even though T4 decreases were seen at all exposure levels. This again supports the concept of a threshold degree of decrease in total T4 levels below which there are no neurodevelopmental deficits presumably because of the maintenance of T3 values sufficient to satisfy the requirements of the developing foetuses.

Fig. 15: Dose dependent decrease in oligonucleocytes (identified by presence of myelin associated glycoprotein expression - MAG) and increase in GFAP astrocytes in the corpus callosum (CC) in rats exposed to PTU (from Sharlin et al 2008)



An interesting chemical example which appears to demonstrate thresholds for decreases in circulating maternal thyroid hormones not affecting the offspring is seen with the fungicide, mancozeb in two developmental toxicity studies and one EOGRTS study (Axelstad et al 2011). This compound was extensively discussed in the thyroid disruption workshop that took place in April 2017, and an account of the discussion was published in the final report of the workshop (Kortenkamp et al 2017). Mancozeb is thought to be thyrotoxic via a degradation product, ethylene thiourea, which is a known rodent goitrogen and carcinogen acting through inhibition of thyroperoxidase (Marinovich et al 1997; National Toxicology Program 1992). The maximum dose level of mancozeb permissible in the developmental neurotoxicity study was limited by maternal toxicity consisting of significant body weight loss and hind limb paralysis but the top dose levels used were still considered adequate because it depressed maternal thyroid hormone levels by ~50% and dose levels lower than those used in the study had been shown to induce thyroid weight increases, decreased thyroid hormones and thyroid gland hypertrophy and hyperplasia, in a previous ninety day rat study. None of the three studies showed effects on the developmental and behavioural parameters assessed in the pups and foetuses on the study and mancozeb is not a developmental neurotoxicant despite clear evidence of maternal thyroid effects. This data supports the concept of a threshold for depression of circulating thyroid hormones in the dam below which there are no toxicological consequences for the pups.

In summary it is apparent that both the decreases in thyroid hormones and any subsequent neurodevelopmental abnormalities arising from the altered thyroid hormone homeostasis show expected dose response relationships with clear NOELs and thresholds for responses below which no biological effect is seen.

7.4. Is there evidence for a threshold for chemicals inducing thyroid cancer through thyroid hormone disruption?

The presence or absence of a threshold for the induction of rodent thyroid cancer by chemical exposure is dependent upon the MoA of the particular chemical in inducing thyroid hormone dysfunction (McClain 1995). Clearly it would be difficult to argue any thresholds for chemicals inducing thyroid cancer through mechanisms involving mutational events but for most other non-genotoxic modes of action for producing thyroid cancer, it is the chronic effect of high TSH levels that stimulate the thyroid gland to undergo growth and sustained cell hyperplasia, the latter outcome classically increasing the chances of the hyper-proliferative follicular epithelial cells acquiring spontaneous mutations that lead eventually, through promotional and progressional changes, to cancer (Cohen and Ellwein 1991; Foster 1997).

Since the hypothalamus/pituitary sensing system for thyroid hormones is considered to be responsive only to free T3, and that this represents <10% of the total circulating T3, there is also considerable redundancy in terms of measuring any reduction in total serum thyroid hormones before a TSH response is invoked. This latter comment argues for the importance of measuring both total and free thyroid hormones when assessing potential thyroid modulating effects of a particular chemical (Kioukia et al 2000; Dayan 2001; van der Watt 2008; Li et al 2014). Furthermore, the whole postulate regarding non-genotoxic MoAs for cancer demands a sustained condition of stimulation of the target cell population (Paynter et al 1988), and the degree of thyroid dysfunction produced by a chemical over a prolonged period of time would present a significant clinical problem to affected individuals before such exposure would increase the risk for neoplasia in humans.

In an EPA series of case examples of thyroid carcinogens (EPA 2014), a thionamide chemical that inhibited both thyroperoxidase and deiodinase activity was found to cause an increase in thyroid follicular cancer in both rats (Table 3) and mice (Table 4), and a corresponding dose related increase in serum TSH. In the article the chemicals were anonymised so that their exact structure could not be obtained. The compound was stated to be non-mutagenic by standard assays and to inhibit the synthesis of T4 within the thyroid, and the subsequent conversion of T4 to the active T3 form in the peripheral tissues. The compound also induced an increase in TSH secreting pituitary adenomas in the rat oncogenicity study. The compound has also been dosed to humans and monkeys and, with regard to altered TSH levels, exposed humans showed no TSH imbalance, while monkeys showed minor TSH increases but were considerably less sensitive on a mg/kg basis than were rats (EPA 2014). Table 3 shows the outcome of a two-year carcinogenicity study with the compound and demonstrated a clear threshold for development of thyroid follicular cancer at 120 mg/kg/day for both male and female rats. A two-year study in B6C3F1 mice was also carcinogenic for the compound but the NOEL was considerably higher than was seen in the rat study (Table 4) suggesting a differential sensitivity between the two, rodent, species.

Table 3: Incidence of thyroid follicular cell tumours in F344 rats in a 2-year study of compound 1 (EPA 2014).

Dose	**0**		**120**		**240**		**480**	
Sex	M	F	M	F	M	F	M	F
Total No. rats	49	50	49	50	47	48	45	46
Follicular adenoma	1	0	1	0	10*	14*	34*	28*
Follicular carcinoma	1	0	0	0	4*	3*	8*	7*
Adenoma+carcinoma	2	0	1	0	14*	17*	42*	35*

* $p<0.05$

Table 4: Incidence of thyroid follicular neoplasms in B6C3F1 mice in a 2-year study of compound 1 (EPA 2014).

Dose	0		120		240		480	
Sex	M	F	M	F	M	F	M	F
Total No. rats	48	50	46	47	44	47	47	46
Follicular adenoma	1	2	1	1	2	0	16*	11*
Follicular carcinoma	0	0	0	0	0	0	0	1
Adenoma+carcinoma	1	2	1	1	2	1	16*	11*

* $p<0.05$

In summary, since the mitogenic stimulus for any chemical that targets the rodent thyroid gland is sustained TSH stimulation, there would be no cancer risk from non-genotoxic chemicals at exposure levels that fail to decrease circulating thyroid hormone levels sufficiently to provoke the TSH release from the pituitary gland (McClain 1995). For those rodent thyroid gland carcinogens, a threshold dose for thyroid hormone depression will exist based upon a lack of TSH induced cell proliferation in the thyroid follicular cells, below which there will be no hazard for the development of thyroid cancer in the rodent.

8. ECPA Question: Are there non-endocrine causes of the same adverse effects e.g. stress, starvation, environmental factors such as temperature?

The sensitive and tightly regulated thyroid hormone feedback control system, thyroid gland autoregulation, and the large intra- and extra-thyroidal storage pools of thyroid hormone under normal conditions are able to provide a constant supply of free thyroid hormone to peripheral tissues in the face of perturbations imposed by the external environment, chemicals and drugs, and a variety of diseases processes. However, under certain situations the homeostatic control is disturbed and compensatory regulation of synthesis and secretion can occur. This section details the secondary response of the thyroid to particular stresses unrelated to a direct effect of the particular stress on the thyroid gland itself.

It is especially important, in the context of laboratory animal studies, to understand those responses that affect thyroid hormones under stress situations since many animal safety evaluation studies are conducted at a range of dose levels that might include, at the top dose level, exposures that can compromise the normal physiology of the rat. The use of a maximum tolerated dose level can significantly influence the normal hormonal homeostasis (Carr and Kolbye 1991; Downes and Foster 2015; Apostolou 1990; Carr and Kolbye 1991; Haseman and Seilkop 1992; McConnell 1989), not as a primary effect of the chemical, but as a secondary effect of being exposed to concentrations of chemicals that inhibit food consumption, or cause disease in organs in addition to, or other than, direct toxicity to the thyroid gland. Hence the interpretation of any thyroid hormone changes under circumstances of multi-organ pathology, or significant effects on food and water consumption, will need to be very carefully evaluated to eliminate the secondary consequences of these confounding influences on circulating thyroid hormone levels.

TSH secretion by the pituitary gland thyrotrophs, and their sensitivity to TRH stimulation, has been shown in humans to be affected by renal failure, starvation, sleep deprivation, depression, and hormones, including cortisol, growth hormone, and sex steroids (Gary et al 1996; Jackson 1982).

8.1. Thyroid hormone changes in disease states

In many chronic illnesses in humans, defects occur in thyroid hormone metabolism, resulting in the "sick euthyroid syndrome" characterized by a normal total T4, normal/high free T4, low total T3, low free T3 and an elevated rT3 (Malik and Hodgson 2002). These changes reflect a reduction in type 1 deiodinase activity, an increase in type 3 deiodinase activity (Bianco et al 2002) and changes in the plasma concentration of thyroid-binding proteins and free fatty acids which displace thyroid hormones from binding proteins. There are also non-thyroidal influences on the hypothalamic-pituitary-thyroid axis such as cortisol inhibiting TSH secretion (Camacho and Dwarkanathan 1999).

Increased levels of rT3, indicating greater conversion of T4, have been observed in humans in starvation, anorexia nervosa, severe trauma and haemorrhagic shock, hepatic dysfunction, postoperative states, severe infection, and in burn patients (Chopra 1976; Boelaert and Franklyn 2005; Jabbar et al 2017). Since rT3 is an inactive form of thyroid hormone, an increased conversion of T4 suggests that certain disease states induce a lowering of the metabolic rates and recuing caloric requirements as part of the natural physiological processes involved in coping with the disease.

8.2. Specific thyroid hormone changes in liver disease

The main transporter protein for thyroid hormones in humans, TBG, is an acute phase protein made in the liver, and in human cases of acute hepatitis, patients show elevated serum levels of total T4, due to increased production of TBG, but with normal levels of free T4. As the severity of hepatitis increases with impending liver failure, low total serum T4 levels are thought to reflect the reduced hepatocellular synthesis of TBG (Kano et al 1987). While serum T3 levels in patients with acute hepatitis can be very variable, the free T3:T4 ratio appears to correlate negatively with the severity of the liver disease reflecting diminished type 1 deiodinase activity, resulting in a reduced conversion of T4 to T3. Some case histories have shown an association of acute hepatic failure with the development of goitre that resolved with improvement in liver function (Hegedus 1986).

Data on thyroid hormone changes in laboratory animals on routine regulatory toxicity studies, in the presence of liver damage, is not available and it is not possible to comment on whether or not thyroid hormone changes would be induced in situations of liver damage.

8.3. Effects of temperature on thyroid hormones

8.3.1. Effects in humans

Changes in the outside temperature will alter TSH secretion in humans and as a consequence the serum concentration of thyroid hormones and their metabolism. These changes are thought to be mediated centrally via the pituitary/hypothalamus and peripherally by effects on the rates of thyroid hormone degradation, through increased loss in the faeces, and by alterations in thyroid hormone receptor expression in the tissues. Such changes would be expected to decrease the metabolic rates in peripheral tissues.

In cell systems *in vitro,* changes in temperature have been shown to affect the binding affinity of T4 to its serum binding proteins and this could also function *in vivo* under conditions of extreme cold or extreme heat (Bernstein and Oppenheimer 1966). In a study of males living in Northern Finland, serum free T3 levels were shown to be lower in February than in August, and TSH levels were higher in December than at other times of the year. Serum free T3 levels correlated significantly with the mean outdoor temperature of the preceding month but serum TSH levels failed to show any correlation with the mean temperature of the month or with free T3. Low serum free T3 in winter suggests that thyroid hormone degradation was accelerated in the cold (Leppäluoto et al 1998). The interpretation of some of the human studies may have been confounded by additional variables such as altered daylight, activity levels, living conditions, and sleep deprivation accompanying prolonged residence in Arctic and Antarctic regions (Hackney et al 1995 a, b).

8.3.2. Effects in laboratory animals

The overall effects of environmental temperature have been easier to demonstrate in animals than in humans but profound species differences in thermal regulation may mean that the findings in animal models may not apply to humans (Silva and Larsson 1985). Cold exposure in animals leads to thyroid gland hyperplasia, enhanced hormonal secretion, degradation, and excretion, accompanied by an increased demand for dietary iodine (Goglia et al 1983). The prompt activation of pituitary TSH secretion after cold exposure of the rats (Panda and Turner 1975; Emerson and Utiger 1975) is thought to be due to a direct effect on the hypothalamus (Anderson 1964).

Exposure to cold has also resulted in increased secretion of TRH (Szabo and Frohman 1977), and a reduced response of pituitary TSH release to administered TRH (Hefco et al 1975). Cold exposure in laboratory studies in the rat is associated with increased rates of T4 and T3 deiodination, increased conversion of T4 to T3, enhanced hepatic binding, and increased biliary and faecal clearance of all of the iodothyronines (Balsam and Sexton 1975; Bernal and Escobar del Rey 1975; Tsukahara et al 1997; Galton and Nisula 1969; Balsam and Leppo 1974). Finally, thyroid hormone effects may be enhanced by alterations in co-activators which are able to enhance the activity of thyroid hormone receptors on gene activation (Puigserver et al 1998).

When rats were exposed to high environmental temperatures of 34^{o}C for three weeks, there was a rapid and simultaneous decrease in hypothalamic TRH, plasma TSH, plasma T4 and thyroid activity by the 36th hour of heat exposure suggesting an effect mediated via the hypothalamus. On prolonged exposure, by day 9, there was a rebound in thyroid activity due to a peak in circulating TSH in response to the marked decrease in plasma T4 seen at this time. From day 9 to the end of the study on day 21, all thyroid parameters returned to control levels indicating adaptation to the increased temperature regime (Rousset and Cure 1975). This result in the laboratory was mirrored by a similar effect in human subjects where a decrease in the elevated serum TSH level associated with primary hypothyroidism was induced by increases in body temperature (O'Malley et al 1980).

8.4. The relationship of fasting and obesity to thyroid hormones

In periods of limited food availability, there is central downregulation of the HPT axis, and serum T4 and T3 levels fall during fasting both in humans (Chan et al 2003) and in rodents (Ahlma et al 1996; Legradi et al 1997) to downregulate the metabolic rate in affected animals/individuals. In rats, fasting has been shown to decrease both pituitary type 2 deiodinase activity and liver type 1 deiodinase activity, and the reduction correlates with reduced peripheral T3 isolated from liver homogenates (Boelen et al 2006; 2008). Despite this reduction in pituitary and liver T3, hypothalamic type 2 deiodinase activity increases with fasting, resulting in a stimulation of release of the appetite-related proteins, neuropeptide Y (NPY) and agouti-related peptide (AgRP) from the arcuate nucleus. Thus, despite fasting-associated reductions in peripheral thyroid hormone levels, there is still a localized increase in T3 within the hypothalamus with a marked increase in orexigenic (hunger) signals, which in turn act upon the paraventricular nucleus to decrease TRH and, in consequence, TSH production. Humans who are anorexic, or who undergo severe caloric restriction, exhibit similar reductions in thyroid hormones, which are thought to be protective of vital energy stores (Langouche et al 2014; Reinehr 2010; Warren 2011). The administration of leptin (Da Veiga et al 2004), or α-MSH (Fekete et al 2000), has been shown to abolish the fasting-induced reductions in TRH and restore normal circulating TSH levels and humans and mice with mutations in the leptin receptor or in the leptin molecule itself exhibit central hypothyroidism are prone to severe obesity (Ohtake et al 1977; Clement et al 1998), which is ameliorated, at least in leptin-deficient humans, by the administration of exogenous leptin (Farooqi et al 2002).

In humans, there is a clear relationship between body weight, obesity and hypothyroidism (Amin et al 2011) and thyroid hormones are thought to directly affect appetite via their actions on the brain. Administration of TRH and TSH directly into the brain of rodents causes a reduction in food intake (Lin et al 1983; Vijayan and McCann 1977; Suzuki et al 1982) and similar effects on food intake are seen following peripheral administration of TRH (Choi et al 2002). In contrast, central and peripheral administration of T3 increases food intake and consequentially induces body weight gain (Kong et al 2004; Ishii et al 2003; 2008).

In summary therefore, any situation that significantly alters the normal intake of food by a laboratory animal will be expected to affect thyroid hormone homeostasis, not as a direct effect on the thyroid but secondarily to effects on food consumption.

9. ECPA Question: What are the non-thyroid hormone activities of the thyroid hormone transport proteins? Are there quantitative and/or qualitative species differences?

9.1. Thyroid hormone binding proteins as acute phase proteins

Since the liver is responsible for metabolism of thyroid hormones essentially any toxicity, including chemical-induced hypertrophy and enzyme induction, will affect thyroid hormone turnover, alter circulating thyroid hormone levels, and potentially feed back to the hypothalamus/pituitary to alter thyroid hormone production. Acute phase proteins are proteins that are increased or decreased in the presence of inflammatory conditions in general (Cray et al 2009). The vast majority of acute phase proteins are made in the liver and any condition that affects their hepatic production and release, irrespective of species, will alter the concentrations of these proteins (Ross et al 1983). TBG is an acute phase protein and hence in conditions such as hepatic necrosis, where total serum protein is decreased because of compromised liver function, the serum concentration of the thyroid binding proteins will be decreased and the circulating total T4 and T3 has also been shown to decrease (Schussler et al 1978; Neto and Zantut-Wittmann 2016). While the exact identity of the thyroid binding proteins differs between different species, they are all manufactured and released from the liver and will consequently be altered, and induce alterations in circulating thyroid hormones, in the presence of compromised hepatic function (Huang and Liaw 1995).

In human cases of acute liver disease, patients show elevated serum levels of total T4 but with normal concentrations of free T4, due to increased production of TBG as an acute phase response (Jayachandran et al 2016; Neto and Zantut-Wittmann 2016; Gardner et al 1982). In severe cases of hepatitis low total T4 levels are thought to reflect the reduced hepatocellular synthesis of TBG (Kano et al 1987). While serum T3 levels in patients with acute hepatitis can be very variable, the free T3:T4 ratio appears to correlate negatively with the severity of the liver disease reflecting diminished type 1 deiodinase activity, resulting in a reduced conversion of T4 to T3. Some case histories have shown an association of acute hepatic failure with the development of goitre that resolved with improvement in liver function (Hegedus 1986).

Conditions of elevated oestrogen, including pregnancy, induce an increase in serum thyroxine binding globulin (TBG), and similarly, when exogenous oestrogen was administered to rats, elevations in TBG and total T4 were observed (Ain et al 1987). Whether this is an adaptation to impending pregnancy and the need for thyroid hormones by the developing foetus is open to speculation.

10. ECPA Question: What are the remaining uncertainties? How may they be addressed?

In order to arrive at a decision as to whether or not a given chemical is indicating thyroid disrupting properties, the draft GD suggest a two-tier approach based upon the use of the OECD Conceptual Framework levels 1 – 5 (page 9 of draft GD 2017) to categorise the various data. In terms of human

health assessment, levels 2 and 3 list the specific *in vitro* and *in vivo* assays respectively that would be used to discount a generalised endocrine effect using specifically designed assays, while level 4 lists the guideline studies that would normally be routinely conducted in any submission passage for pesticides and biocides. While the majority of the assays mentioned in the various levels have OECD test guidelines already issued, there are a minority of the *in vitro* assays that are not guideline at the time of writing.

In order to adequately address the question of the remaining uncertainties, it is necessary to break it down into two separate questions that relate to whether or not we are asking basic questions of understanding, that could be used in a TIER 2 MoA type of analysis, and where the basic biology of thyroid hormone control will be questioned, or whether we are questioning the adequacy of the current testing strategies for evaluating potential thyroid disrupting chemicals which would constitute part of the TIER 1 assessment. Currently much of the debate has muddled the two parts together and has resulted in polarised views as to the adequacy of each.

10.1. The current assays and their assessment of thyroid effects

As can be seen from Table 5 the current mandated toxicity assays carry a limited assessment of thyroid effects of chemicals dependent largely upon the assessment of thyroid gland weight and thyroid gland histopathology.

Table 5: The current assays and their assessment of thyroid effects (modified from European Union 2017).

OECD TG	Study Title	Thyroid Endpoints Measured
407	Repeated dose 28-day oral toxicity study	Histopathology of Thyroid gland liver and CNS & PNS Weight of thyroid gland liver & brain
408	Repeated dose 90-day oral toxicity study in rodents	Histopathology of Thyroid gland CNS, PNS & liver. Weight of thyroid gland, liver & brain
451-3	Chronic toxicity & carcinogenicity	Histopathology of Thyroid gland, CNS, PNS and liver
414	Prenatal developmental toxicity study	No thyroid-related endpoints are included
415	One-generation reproductive toxicity study	No thyroid related endpoints are included
416	Two-generation reproductive toxicity study	Thyroid gland histopathology in P0 and F1 parents
421	Reproductive screening test	T4 from PND13 offspring and P0 adult males; T4 may also be measured in pups on PND4 and dams on PND13; optional thyroid weight and histopathology
422	Combined 28-day/reproductive screening assay	As TG421
426	Developmental neurotoxicity study	No thyroid but assessment of developing

		brain
443	Extended one generation reproductive toxicity study	T4/TSH in F1 offspring from cohort 1A at term (PND22); Thyroid gland weight and histopathology; when the DNT cohort is included, assessment of developing brain

Clearly the mandatory inclusion of additional thyroid endpoints to many of these guidelines would enhance theirvalue without placing extra burden on animal experimentation and would go a long way to filling in perceived shortcomings in the current test battery.

While the pivotal endpoint of thyroid gland carcinogenesis is fairly well covered by the lifetime chronic toxicity/carcinogenicity test, there is concern that potential neurodevelopmental deficits, induced by low levels of circulating thyroid hormones may not be so adequately assessed in the current available test guidelines (EU 2017). While no guideline studies fully assess this issue, TG 426, the DNT study, and TG 443, the EOGRTS (with DNT cohort) should, with modifications, be able to adequately address this. Hence the inclusion of thyroid hormone measurements into TG426 would significantly improve the guidance document insofar as thyroid assessments are concerned. TG 443 has the possibility to include a DNT cohort and if so the endpoints currently included in TG 426 would strengthen any concern that the neurodevelopmental effects of hypothyroidism were being adequately assessed. Furthermore, several behavioural studies performed according to these guidelines have not been able to show any adverse effects in behaviour or on brain histopathology, even in severely hypothyroxinemic animals. This has led scientists in the field of neurotoxicology and thyroid disruption to suggest that new endpoints which are more sensitive to perinatal thyroid disruption should be added to these guidelines, in order to better assess adverse effects on neurodevelopment (OECD 2006, Harry et al 2014).

There has also been criticism that the behavioural assays currently included into the TG are not sensitive enough to detect neurodevelopmental effects when they may be occurring (Harry et al 2014; EU 2017). This conclusion is based upon the findings that some chemicals, that clearly affect the hypothalamic/pituitary/thyroid axis, have not shown neurobehavioural effects in specifically designed studies. One example that has been used to exemplify this conclusion is the fungicide, mancozeb. This compound has shown thyroid effects in terms of hypertrophy and hyperplasia and thyroid gland weight increases in a number of toxicity studies but did not affect nervous system development in specifically conducted developmental toxicity studies in the rat (, Axelstad et al 2011). The mode of action of mancozeb in affecting the thyroid gland is thought to be due to its conversion to ethylene thiourea, a known thyroid toxic chemical (Graham et al 1975) and once again, a EOGRT study with a DNT cohort carried out with ETU failed to show adverse effects on brain development or on the neurobehavioural parameters that were assessed, even though thyroid hypertrophy was present in the adult rats at both the mid and high dose levels (Marty et al 2013b, in DRAR).

There are several alternative interpretations for these data. If we accept that severe hypothyroidism can lead to serious neurodevelopmental deficits, and there is sound evidence for a limited number of chemicals to support this conclusion, then the following arguments can be used to explain the lack of behavioural effects in these studies:

1. The thyroid disrupting effects of mancozeb/ETU on the dams were not severe enough to lead to altered brain development in the offspring.
2. The offspring were not hypothyroid themselves in the postnatal period (due to limited milk transfer) and since much brain development occurs postnatally in rats, the prenatal hypothyroxinemia was not severe enough to disrupt brain development.

3. The neurobehavioural assessment methods were not sensitive enough to detect subtle changes in brain development.
4. The decreases in circulating T4 levels lead to a compensatory upregulation of peripheral deiodinase activity, leading to an increased conversion of T4 to the active T3 sufficient to provide enough T3 for normal brain development in the pups.

With the exception of explanation 3, the other alternatives imply a threshold level of thyroid hormone inhibition in the dams, below which no consequences to pup neurodevelopment occur. The data also support the conclusion that chemically-induced thyroid effects in the dams occur at significantly lower chemical concentrations than do thyroid hormone induced neurodevelopmental deficits in the pups (Gilbert and Sui 2008). An alternative way of stating this is that the sensitivity of the adult thyroid gland in demonstrating the effects of chemically-induced thyroid hormone lowering is considerably greater than the induction of neurodevelopmental effects in the pups such that the degree of thyroid hormone decreases in the dams needs to be considerably lower to induce neurodevelopmental effects in the offspring. Human data from mothers living in iodine deficient parts of the world would support this conclusion where the % of hypothyroid mothers present in the population greatly exceed the incidence of neurological deficits in the children of the affected mothers (Alexander et al 2017).

Explanation point 3, namely that the neurobehavioural assays currently in use are simply not sensitive enough to detect subtle changes in brain development in the pups, is a criticism that can be levelled at almost every measured endpoint in any of the current guidance documents. The introduction of behavioural endpoints into guidance documents is always accompanied by significant efforts at validation of the introduced methods and indeed this was the case with those recommended in the developmental neurotoxicity test, TG426, and the EOGRTS, TG443, cohorts 2A and 2B. The neurobehavioural assays are conducted in addition to extensive, and specifically targeted, neuro-histopathological and hormonal assessment and provides the most comprehensive test system currently available. Clearly the guidelines are not immutable, and when sufficient information becomes available to warrant additional assays, then these do become incorporated, albeit following prolonged and extensive validation exercises to ensure the quality of the additional assays.

10.2. The need for additional screens for chemicals that might disrupt the hypothalamus/pituitary/ thyroid (HPT) axis?

There has been much criticism that current testing strategies, for detecting thyroid disrupting chemicals, lack both specificity and sensitivity, and that the rodent assays currently employed may not necessarily predict outcomes in the human population (European Food Safety Authority/European Chemicals Agency 2016; European Union 2017).

A consequence of this debate has resulted in proposals for the greater incorporation of *in vitro* cell and protein binding assays, of basic molecular interactions of chemicals with components of the HPT axis, into the screening toolbox for detecting thyroid interfering chemicals (Murk et al 2013). There is no doubt that *in vitro* assays of various designs have advanced our basic understanding of biological processes immeasurably and they are an essential part in developing MoA and human relevance arguments, and in formulating adverse outcome pathways. However, the assays need to fill current gaps in the toolbox rather than simply adding unnecessarily to the already large list of testing assays needed to ensure the safe use of chemicals in society. The formulation of adverse outcome pathways is one way in which gaps in current understanding of thyroid hormone perturbation can be identified and novel assays naturally fit as part of the current TIER system where the primary tiers depend upon properly validated animal studies which can be supported in a TIER 2 testing strategy that uses the most appropriate assays for addressing problems raised following the TIER 1 assessment, as described in the draft GD. While there exist a plethora of various *in vitro* assays, their use as screens for chemical toxicity, in the absence of any hypothesis driven selection of the appropriate assay, could raise more questions than answers, generate unnecessary extra work to understand exactly what the *in vitro* screen is showing, and involve additional unnecessary experimentation to clarify what any

single *in vitro* screen might mean in the context of the biology of the whole animal (Colnot and Dekant 2017; Hulme and Trevithick 2010).

10.2. Are there refinements that can be made to current hormone assessments?

10.2.1. When to measure hormones?

Evidence suggests that for chemicals that depress thyroid hormones, the degree of depression gets progressively less as the duration of suppression increases (Yu et al 2002). This is thought to be due to a resetting of the homeostatic level at which the thyroid hormone feedback system works. This is particularly true of elevations in TSH that tend to occur shortly following exposure to thyroid affecting chemicals but which can return to normal values within weeks of exposure. The same effect is seen with the depression of serum T4 and T3 which tend to return to normal values on continued exposure. Hence the question arises regarding the most appropriate time for measuring these hormones and what consequences the reset system might have on the long-term health of the animal. It is clear that although hormone levels return to near normal levels on prolonged dosing, the system maintains this at a higher metabolic level than does the HPT axis in untreated animals and as such would be expected to induce a sustained level of stress that long term could increase the chances of developing diseases including thyroid cancer (Vansell et al 2004). Hence a careful evaluation of the right time, or right times, for evaluating thyroid hormone levels in animal studies is needed to avoid situations where morphological thyroid changes might be seen in the apparent absence of effects on thyroid hormones (Colnot and Dekant 2017). It is not within the scope of this review to recommend sampling times but in order to generate interpretable data, a thorough knowledge of the pharmacokinetics of the chemical under test, together with the known circadian control for some of the hormones is essential prerequisite for such studies.

10.2.2. Should thyroid hormone measures be incorporated into routine toxicity studies?

There have been suggestions that thyroid hormone measures could be incorporated into guideline toxicity studies as a routine rather than when indicated by a confirmed knowledge of a chemical class thyroid effect or some indication that the thyroid gland was a potential target. While there are clearly advantages to gaining the maximum amount of information from animal studies but there does need to be a measure of caution when advocating such additions. Thyroid hormone assays are not currently straightforward, and they require a degree of expertise not routinely present in many laboratories conducting guideline studies. Routine incorporation might well generate data that becomes uninterpretable without supporting evidence from histopathology and could throw up false positive data arising from inexperience of the conducting laboratory. Conducting laboratories need to have the necessary historical background data to understand the limits within normality that exist in laboratory animals. The timing of blood sampling for hormone measures needs to be carefully planned in the knowledge of the circadian control that some of the hormones operate under.

Lastly and perhaps most importantly, the occurrence of confounding factors, such as organ toxicity and reductions in food consumption that are frequently seen in toxicity studies, are known to affect thyroid hormone levels independent of any primary effect on the thyroid gland itself and ruling these factors out can be challenging without embarking on an extensive investigative study. There is also evidence of adaptation to thyroid inhibition where hormone depression is seen early in studies but which recover on prolonged exposures. For an unknown compound, the question would always arise as to when, in the conduct of the study, it might be appropriate to sample for potential thyroid hormone changes. Since these are likely to be different dependent upon the inhibitory MoA triggered, and the individual chemical involved, the routine incorporation of thyroid hormone measures to guideline toxicity studies presents almost insurmountable difficulties if it is to generate sensible and interpretable data that adequately assesses potential thyroid effects.

Histopathology of the thyroid gland is routinely carried out as part of acute, chronic and lifetime toxicity bioassays, and arguably is the most sensitive biomarker of thyroid hormonal disruption, at least when incorporated into a routine toxicity study (NTP 2002). Any sign of a thyroid effect by histopathology could then be best approached by undertaking specifically designed investigative *in vivo* and *in vitro*

toxicity studies to investigate potential thyroid effects by targeting the hormonal endpoints relevant to the thyroid.

10.3. Is the rat the appropriate species? Should we explore different animal models?

Various alternative animal models to the rat have been proposed for testing of both the potential carcinogenic and the developmental effects of chemicals disrupting the HPT axis. The inherent advantages of the rat as a model for human risk assessment refer back to our familiarity with the vagaries of the species and our extensive understanding of its similarities, and differences in terms of biology, to humans. Clearly alternative animal species, and *in vitro* and *ex vivo* systems, exist for assessing potential thyroid hormone disrupting properties but they all have drawbacks and limitations which are discussed in the relevant sections in this and other documents (Sadamatsu et al 2006; Brent 2012b).

10.3.1 The sheep as a model for human thyroid effects

There has been some discussion as to the sheep being a more appropriate animal model to represent human health hazard with regard to disruption of thyroid hormone homeostasis (European Union 1917; page 23) especially where neurodevelopmental toxicity is concerned. The study quoted to reinforce this statement was Leghait et al (2010) where fipronil, a compound known to produce significant thyroid hypertrophy in rats, was studied in sheep. The underlying reason for regarding the sheep as a more representative model for man, is based upon the greater similarity of thyroid binding protein in the plasma of sheep where TBG is the major serum transporter for thyroid hormones, whereas in the rat this protein is, to all intents and purposes, absent in the adult.

Leghait et al (2010) found that fipronil failed to produce thyroid toxicity in the sheep but the conclusions to the study were somewhat compromised because the formation of the sulphone active metabolite of fipronil was appreciable less in the sheep than it is in the rat. This failure highlights the problems of embarking on studies in species where the database is seriously limited, as in the case of the sheep.

The metabolism of thyroid hormones has been studied in the pregnant sheep, both in the dam and in the developing foetus (Fisher et al 1972) and the paper speculates that, on the basis of thyroid metabolism, the foetal sheep has greater similarity to human foetuses than does the rat system. While this may well be the case, the lack of detailed information on the other, not inconsiderable, variables determining chemical-induced thyroid toxicity, together with the impracticality of using such a large species for toxicity studies, compound supply alone would most likely deter such studies, would mean that at best it might be considered as a second species in place of the canine, but only after a significant period of data gathering on the current paucity of basic biochemical and biological parameters pertinent to the conduct of toxicity studies.

In spite of this drawback, a recent study has been reported that looked at the effect of exposing pregnant ewes to sewage sludge-fertilized pastures that succeeded in showing that pre-conceptual exposure of the dam increased the relative thyroid organ weights in male foetuses and decreased expression of the sodium iodide symporter in the thyroid glands (Hombach-Klonisch, et al 2013).

10.3.2. The contribution of knockout mice to understanding human relevance of thyroid hormone disruption

Several transgenic mouse models of thyroid cancer exist that contain various genes found to be overexpressed in human thyroid cancer, that are placed under the expression of the thyroglobulin (Tg)

promoter, and that subsequently allow thyroid-specific over expression of the specific transgene and the subsequent development of thyroid cancer (Kim and Zhu 2009).

Of particular relevance for potential species differences in response has been the MCT8 knockout mouse which has shown little neurodegenerative effects () despite the human condition affecting the MCT8 transporter, where serious and profound neurological pathologies are produced in the mutation of the MCT8 gene in Allan-Herndon-Dudley Syndrome (Dumitrescu et al 2004; Friesema et al 2004; Schwartz and Stevenson 2007). In knockout mice, lacking the MCT8 transporter, they fail to show motor deficits suggesting that alternate pathways for transporting thyroid hormones into the brain exist in this species (Di Cosmo et al 2010; Roberts et al 2008). It is now known that the organic ion transporter polypeptide-14 (OATP14) is the primary thyroid hormone transporter responsible (Friesma et al 2005) and OATP14 mRNA and protein is strongly expressed in both rat and mouse cerebral microvessels, but not in human. The high expression of OATP14 in the rodent brain, as compared with the human, is one explanation for the relatively mild neurophysiological consequences of deletion of the MCT8 transporter in Mct8-null mice. The absence of an alternate transporter in the developing human brain results in serious neurodevelopmental consequences seen in individuals carrying the mutated MCT8 gene.

RET/PTC gene rearrangements have been found to be consistently involved in human thyroid cancer, and transgenic mice carrying over-expression of this gene combination have indeed been found to develop thyroid carcinogenesis (Santoro et al 1996; Jhiang et al 1996). Several other genes, such as the NTRK1, BRAF and RAS genes, are also known to be involved in the development of human cancer, and while mice transgenically designed to overexpress these have been found to reciprocate the biology, morphology and behaviour of human thyroid cancer and to help in the understand the basic biology of the process (Knostman et al 2007; Knauf et al 2005; Vitagliano et al 2006), they have been singly unsuccessful in informing on the relative susceptibility of chemically induced thyroid cancer in either laboratory animals or in man simply because that was not what they were designed to do. Their whole purpose was to develop a model that could be used to study the development of human thyroid cancer with a view to better understand the pathophysiology and molecular biological changes leading to cancer.

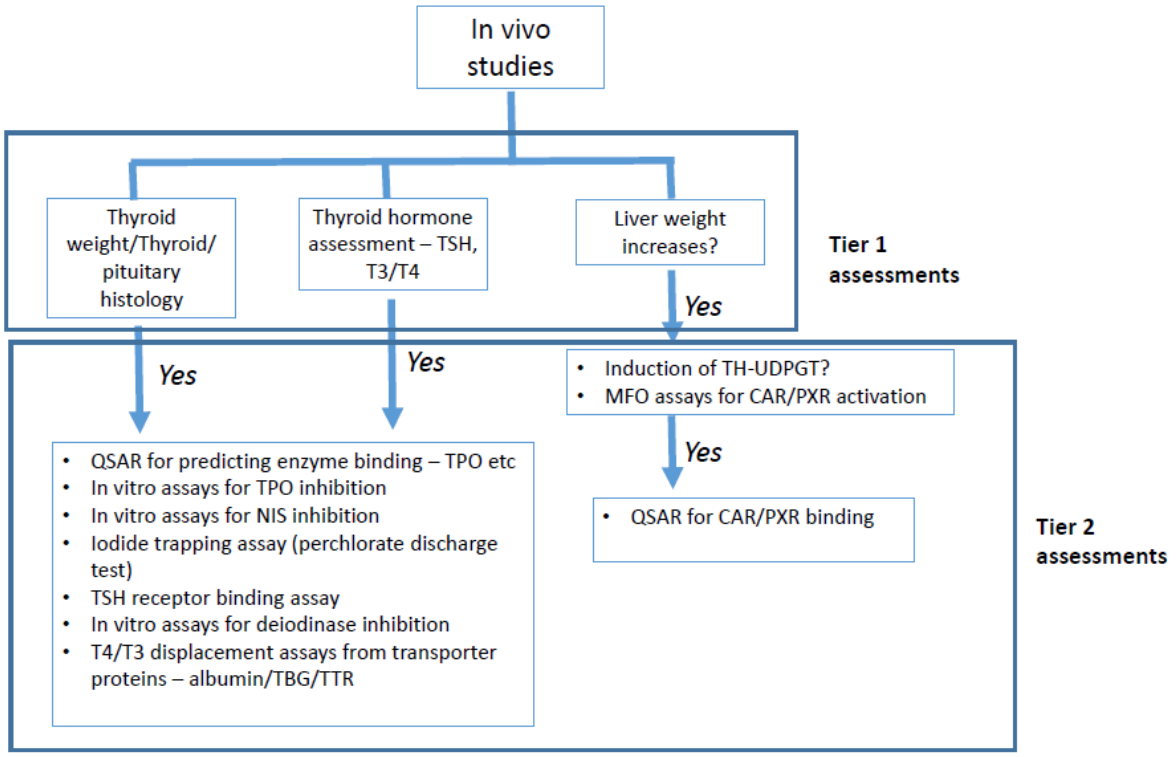
Type 2 iodothyronine deiodinase is essential for maintaining normal local concentrations of free T3 under different physiological and pathophysiological situations and has a critical role in the correct operation of the negative feedback control of TSH/TRH release from the pituitary and hypothalamus (Schneider et al 2001). In an attempt to better characterise the respective roles of the three iodothyronine deiodinase enzymes, knockout mice have been generated for each of the enzymes (Schneider et al 2001; 2006; Marsili et al 2011). Mice bearing the knocked-out gene for iodothyronine Type 2 deiodinase show incomplete development of the inner ear (Ng et al 2004), and bone and muscle malformations resulting from a deficiency in the local conversion of T3 in cochlear cells, myoblasts and osteoblasts respectively (Dentice et al 2010; Bassett et al 2010). The type 2 deiodinase KO mouse also shows impaired embryonic development of brown adipose tissue, and as a consequence the mice suffer a permanent thermogenic defect (Hall et al 2010; de Jesus et al 2001) which leaves them susceptible to hypothermia during cold challenge, and a greater susceptibility to diet-induced obesity at ambient temperatures (Castillo et al 2011).

11. A proposal for a testing strategy for assessing thyroid disrupting effects of a chemical

In the event of a chemical inducing thyroid hypertrophy/hyperplasia, or measured decreases in circulatory thyroid hormones, in rodent toxicity studies, a mode of action analysis and human relevance framework in the context of the recommended IPCS guidance should be conducted (Boobis et al. 2006) together with an adverse outcome pathway constructed to identify missing data from the proposed pathway as recommended by Vinken et al (2017).

In terms of possible thyroid effects mediated via increased clearance through the liver, the testing strategy outlined in Fig. 16 below addresses all known thyroid modes of action, and suggests a way of ruling out alternative MOAs through experimentation in appropriate assays.

Fig. 16: Suggested testing strategy to confirm a thyroid mode of action



If either of the three tier 1 effects are seen *in vivo* studies in rodents, additional assessments (new studies may have to be carried out) would then be commissioned as tier 2 assessments to specifically investigate the proposed mode of action and/or eliminate alternative modes of action. The elimination of alternative pathways is a necessary part of the IPCS process and while validated *in vitro* assays do not currently exist for all of the endpoints listed in the left-hand box, experimental *in vitro* cell assays are available for all of the endpoints listed. The proposed mode of action would need to be subject to Bradford Hill causality/association test to verify its strength, and then the human relevance framework would be applied by answering the three pivotal questions as described in Meek et al (2013):

1. Is the weight of evidence sufficient to establish a MOA in animals?
2. Can human relevance of the MOA be reasonably excluded on the basis of fundamental qualitative differences in key events between animals and humans?
3. Can human relevance of the MOA be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between animals and humans?

The establishment of a plausible MOA would identify some mechanisms which are known to have either qualitative species differences between rodents and humans, or quantitative species differences whereby humans have been shown to be more or less sensitive to the toxicity seen with the given chemical. An example of the latter is the activation of the CAR/PXR nuclear receptor in the rodent whereby induction of hepatic UDPGT leads to an increased rate of biliary excretion of T4 through glucuronidation, a resultant decrease in circulating T4, and a compensatory hypertrophy/hyperplasia of the thyroid follicular epithelium through TSH stimulation (Dellarco et al

2006). While human liver does contain the CAR/PXR receptor, there are significant quantitative differences that make activation considerably less sensitive in human than it is in the rodent with considerably higher dose levels need to produce the same effect.

12. Literature References

Abe, T., Kakyo, M., Sakagami, H., Tokui, T., Nishio, T., Tanemoto, M., Nomura, H., Hebert, S.C., Matsuno, S., Kondo, H. and Yawo, H. (1998). Molecular characterization and tissue distribution of a new organic anion transporter subtype (oatp3) that transports thyroid hormones and taurocholate and comparison with oatp2. *J. Biol. Chem.* 273: 22395–22401

Abend, S. L., Fang, S.L., Alex, S., Braverman, L.E. and Leonard, J.L. (1991). Rapid alteration in circulating free thyroxine modulates pituitary 5' deiodinase and basal thyrotropin secretion in the rat. *J. Clin. Invest.* 88: 898-903.

Ahlma, R.S., Prabakaran, D., Mantzoros, C., et al., (1996). Role of leptin in the neuroendocrine response to fasting. *Nature* 382, 6588, pp. 250–252.

Ahmed, R.G. (2012). Maternal–newborn thyroid dysfunction. In: Ahmed RG, editor. In developmental neuroendocrinology. Saarbrücken, Germany: LAP LAMBERT Academic Publishing GmbH & Co KG; 2012. p. 1–369.

Ahmed, R.G. (2015). Hypothyroidism and brain developmental players. *Thyroid Res.* 8:2 DOI 10.1186/s13044-015-0013-7.

Ain, K.B., Mori, Y. and Refetoff, S. (1987). Reduced clearance rate of thyroxine-binding globulin (TBG) with increased sialylation: a mechanism for estrogen-induced elevation of serum TBG concentration. *J. Clin. Endocrinol. Metab.* 65: 689-696.

Alshehri, B., D'Souza, D.G., Lee, J.Y., Petratos, S. and Richardson, S.J. (2015). The diversity of mechanisms influenced by transthyretin in neurobiology: development, disease and endocrine disruption. *J Neuroendocrinol.* 27: 303-323.

Alkemade, A., Friesema, E.C., Kalsbeek, A., Swaab, D.F., Visser, T.J. and Fliers, E. (2011). Expression of thyroid hormone transporters in the human hypothalamus. *J. Clin. Endocrinol. Metab*. 96: E967–E971

Akosa, B.T., Sleight, S.D., Nachreiner, R.F. and Aust, S.D. (1982). Effects of purified polybrominated biphenyl congeners on the thyroid and pituitary glands in rats. *J. Am. Coll. Toxicol.* 1: 23-36.

Alexander, E.K., Elizabeth N. Pearce, E.N., Brent, G.A., Brown, R.S., Chen, H., Dosiou, C., Grobman, W.A., Laurberg, P., Lazarus, J.H., Mandel, S.J., Peeters, R.P., and Sullivan, S. (2017). 2017 Guidelines of the American Thyroid Association for the Diagnosis and Management of Thyroid Disease During Pregnancy and the Postpartum. Thyroid 27; 315-389

Amin, A., Dhillo, W.S. and Murphy, K.G. (2013). The Central Effects of Thyroid Hormones on Appetite. *J. Thyroid Res.* Vol. 2011 Article ID 306510, 7 pages, 2011. doi:10.4061/2011/306510

Andersen, B.F. (1973). Iodide perchlorate discharge test in lithium-treated patients. *Acta. Endocrinol.* 73: 35-46.

Andersson, B. (1964). Hypothalamic temperature and thyroid action. C. F. S. G. 18 (eds), Brain-Thyroid Relationships, pp. 35-50.

Antonangeli, L., Maccherini, D., Cavaliere, R., Di Giulio, C., Reinhardt, B., Pinchera, A. and Aghini-Lombardi, F. (2002). Comparison of two different doses of iodide in the prevention of gestational goiter in marginal iodine deficiency: a longitudinal study. *Eur. J. Endocrinol*. 147:29–34.

Apostolou, A. (1990). Relevance of maximum tolerated dose to human carcinogenic risk. *Regul. Toxicol. Pharmacol.* 11: 68–80.

Astwood, E.B., Sullivan, J., Bissell, A. and Tyslowity, R. (1943). Action of certain sulfonamides and of thiourea upon the function of the thyroid gland of the rat. *Endocrinol.* 32; 210-225.

Auso, E., Lavado-Autric, R., Cuevas, E., Escobar Del Rey, F., Morreale De Escobar, G. and Berbel, P. (2004). A moderate and transient deficiency of maternal thyroid function at the beginning of foetal neocorticogenesis alters neuronal migration. *Endocrinol.* 145: 4037–4047.

Axelstad M, Boberg J, Nellemann C, Kiersgaard M, Jacobsen PR, Christiansen S, Hougaard KS, Hass U. (2011). Exposure to the widely used fungicide mancozeb causes thyroid hormone disruption in rat dams but no behavioural effects in the offspring. *Toxicol. Sci.* 120: 439-446.

Balsam, A. and Sexton, F.C. (1975). Increased metabolism of iodothyronines in the rat after short-term cold adaptation. *Endocrinology* 97: 385-392.

Balsam, A. and Leppo, L.E. (1974). Augmentation of the peripheral metabolism of L-triiodothyronine and L-thyroxine after acclimation to cold. Multifocal stimulation of the binding of iodothyronines by tissues. *J. Clin. Invest*. 53: 980-

Barbesino G. (2010). Drugs affecting thyroid function. *Thyroid* 20: 763-770.

Bard D, Verger P, Hubert P. (1997). Chernobyl, 10 years after: health consequences. *Epidemiol. Rev.* 19:187–204.

Bartalena, L. and Robbins, J. (1993). Thyroid hormone transport proteins. *Clin. Lab. Med.* 13: 583-598.

Barter, R.A. and Klaassen, C.D. (1992). UDP glucuronosyltransferase inducers reduce thyroid hormone levels in rats by an extrathyroidal mechanism. *Tox.Appl. Pharmacol.* 113; 36-42

Barter, R.A. and Klaassen, C.D. (1994). Reduction of thyroid hormone levels and alteration of thyroid function by four representative UDP glucuronosyltransferase inducers in rats. *Tox.Appl. Pharmacol.* 128; 9-17

Bassett, J.H., Boyde, A., Howell, P.G., Bassett, R.H., Galliford, T.M., Archanco, M., et al. (2010). Optimal bone strength and mineralization requires the type 2 iodothyronine deiodinase in osteoblasts. *Proc Natl Acad Sci U S A.* 107: 7604-7609

Batra, C.M. (2013). Fetal and neonatal thyrotoxicosis. *Indian J. Endocrinol. Metab.* 17(Suppl1): S50–S54.

Beckett, G.J., Alison Russell, A., Nicol, F., Sahu, P., Wolf, C. R. and Arthur, J.R. (1992). Effect of selenium deficiency on hepatic type I 5-iodothyronine deiodinase activity and hepatic thyroid hormone levels in the rat. *Biochem. J.* 282: 483-486.

Berbel, P., Mestre, J.L., Santamaria, A., Palazon, I., Franco, A., Graells, M., Gonzalez-Torga, A., de Escobar, G.M. (2009). Delayed neurobehavioral development in children born to pregnant women with mild hypothyroxinemia during the first month of gestation: the importance of early iodine supplementation. *Thyroid* 19; 511–519.

Bernal, J., Guadano-Ferraz, A., Morte, B. (2003). Perspectives in the study of thyroid hormone action on brain development and function. *Thyroid* 13: 1005–1012.

Bernal, J. (2007). Thyroid hormone receptors in brain development and function. *Nat. Clin. Pract. Endocrinol. Metab*. 3: 249–259.

Bernal, J. (2011). Thyroid hormone transport in developing brain. *Curr. Opin. Endocrinol. Diabetes Obes.* 18: 295–299

Bernal, J. and Escobar del Rey, F. (1975). Effect of the exposure to cold on the extrathyroidal conversion of L-thyroxine to triiodo-L-thyronine, and on intramitochondrial -glycerophosphate dehydrogenase activity in thyroidectomized rats on L-thyroxine. *Acta Endocrinol* 78: 481-

Bernstein, G. and Oppenheimer, J.H. (1966). Factors influencing the concentration of free and total thyroxine in patients with non-thyroidal disease. *J. Clin. Endocrinol. Metab*. 26: 195- 1966.

Bianco, A.C. and Kim, B.W. (2006). Deiodinases: implications of the local control of thyroid hormone action. *J. Clin. Invest.* 116: 2571-2579.

Bianco, A.C., Salvatore, D., Gereben, B., Berry, M.J. and Larsen, P.R. (2002). Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. *Endocr Rev*. 23; 38-89.

Blumberg, B., Sabbagh, W. Jr., Juguilon, H., Bolado, J.Jr., van Meter, C.M., Ong, E.S., et al. (1998). SXR, a novel steroid and xenobiotic-sensing nuclear receptor. *Genes Dev.* 12: 3195–3205.

Bocchetta, A. and Loviselli, A. (2006). Lithium treatment and thyroid abnormalities. *Clin. Pract. Epidemiol. Mental Hlth.* 2: 23-

Boelen, A., Kwakkel, J., Vos, X.G., Wiersinga, W.M. and Fliers, E. (2006). Differential effects of leptin and refeeding on the fasting-induced decrease of pituitary type 2 deiodinase and thyroid hormone receptor beta2 mRNA expression in mice. *J. Endocrinol.* 190: 537–544

Boelen, A., Wiersinga, W.M. and Fliers, E. (2008). Fasting-induced changes in the hypothalamus-pituitary-thyroid axis. *Thyroid* 18: 123–129.

Boelaert, K. and Franklyn, J.A. (2005). Thyroid hormone in health and disease. *J. Endocrinol.* 187: 1-15.

Boobis, A.R., Cohen, S.M., Dellarco, V., McGregor, D., Meek, M.E., Vickers, C., Willcocks, D. and Farland, W. (2006). IPCS framework for analyzing the relevance of a cancer mode of action for humans. *Crit. Rev. Toxicol.* 36: 781–792.

Borzelleca, J.F., Capen, C. C. and Hallagan, J.B. (1987). Lifetime toxicity/carcinogenicity study of FD & C Red No. 3 (erythrosine) in rats. *Food Chem. Toxicol.* 25: 723-733.

Bounacer, A., Wicker, R., Caillou, B., Cailleux, A.F., Sarasin, A., Schlumberger, M., et al. (1997). High prevalence of activating ret proto-oncogene rearrangements, in thyroid tumors from patients who had received external radiation. *Oncogene.* 15: 1263–1273.

Brent, G.A. (2008). Clinical practice. Graves' disease. *N. Engl. J. Med*. 358: 2594–2605

Brent, G.A. (2012a). Hypothyroidism and thyroiditis. In: Williams Textbook of Endocrinology, edited by Melmed, S.P., Larsen, P.R., Kronenberg, H.M., editors. Philadelphia, PA: Elsevier

Brent, G.A. (2012b). Mechanisms of thyroid hormone action. *J. Clin. Invest*. 122: 3035–3043.

Brouwer, A. (1989). Inhibition of thyroid hormone transport in plasma of rats by polychlorinated biphenyls. *Arch. Toxicol.* 13 (Suppl): 440-445.

Brucker-Davis, F. (1998). Effects of environmental synthetic chemicals on thyroid function. *Thyroid* 8: 827–856.

Camacho, P.M. and Dwarkanathan, A.A. (1999). Sick euthyroid syndrome. What to do when thyroid function tests are abnormal in critically ill patients. *Postgrad. Med.* 105: 215–219.

Capen, C. (1996). Toxic responses of the endocrine system. In: Klaassen, CD editor. Casarett and Doull's toxicology: the basic science of poisons, 5th ed. New York: McGraw-Hill. pp 623–624

Capen, C.C. (1999). Thyroid and parathyroid toxicology. IN: Endocrine and Hormonal Toxicology. EDS. P.W. Harvey, K.C. Rush and A.Cockburn. Wiley Press. pp.33-66.

Capen, C.C. and Martin, S.L. (1989). The effects of xenobiotics on the structure and function of thyroid follicular and C-cells. *Toxicol. Pathol.* 17: 266-293.

Carlson, H.E., Temple, R. and Robbins, J. (1973). Effect of lithium on thyroxine disappearance in man. *J. Clin. Endocrinol. Metab.* 36: 1251-.

Carroll, R. and Matfin, G. (2010). Endocrine and metabolic emergencies: thyroid storm. *Ther. Adv. Endocrinol. Metab.* 1: 139–145.

Carr C.J. and Kolbye, A.C. Jr. (1991).A critique of the use of the maximum tolerated dose in bioassays to assess cancer risks from chemicals. *Reg. Toxicol. Pharmacol.* 14: 78-87

Castillo, M., Hall, J.A., Correa-Medina, M., Ueta, C., Kang, H.W., Cohen, D.E., et al. (2011). Disruption of thyroid hormone activation in type 2 deiodinase knockout mice causes obesity with glucose intolerance and liver steatosis only at thermoneutrality. *Diabetes.* 60: 1082-1089.

Cavalieri, R. R. and Pitt-Rivers, R. (1981). The effects of drugs on the distribution and metabolism of thyroid hormones. *Pharmacol. Rev*. 33: 55–80.

Chan, J.L., Heist, K., DePaoli, A.M., Veldhuis, J.D. and Mantzoros, C.S. (2003). The role of falling leptin levels in the neuroendocrine and metabolic adaptation to short-term starvation in healthy men," *J. Clin. Invest.* 111: 1409–1421

Chang, L., Munro, S.L., Richardson, S.J. and Schreiber, G. (1999). Evolution of thyroid hormone binding by transthyretins in birds and mammals. *Eur. J. Biochem.* 259: 534–542.

Chanoine, J. P., Safran, M., Farwell, A.P., Tranter, P., Ekenbarger, D., Dubord, S., Alex, S., Arthur, J.R., Beckett, G.J., Braverman, L.E. and Leonard, J.R. (1992). Selenium deficiency and type II 5'-deiodinase regulation in the euthyroid and hypothyroid rat: evidence of a direct effect of thyroxine. *Endocrinology.* 131: 479-484.

Chanoine, J-P., Braverman, L.E., Farwell, A.P., Safran, M., Alex, S., Dubord, S. and Leonard, J.L. (1993). The Thyroid Gland Is a Major Source of Circulating T3 in the Rat. *J.Clin. Invest.* 91: 2709-2713.

Chauhan, K. R., Kodavanti, P. R. & McKinney, J. D. (2000). Assessing the role of ortho-substitution on polychlorinated biphenyl binding to transthyretin, a thyroxine transport protein. Toxicol.Appl. Pharmacol. 162: 10–21.

Cheek, A. O., Kow, K., Chen, J. & McLachlan, J. A. (1999). Potential mechanisms of thyroid disruption in humans: interaction of organochlorine compounds with thyroid receptor, transthyretin, and thyroid-binding globulin. *Environment. Hlth. Perspect.* 107: 273–278.

Cheng, S.Y., Leonard, J.L. and Davis P.J. (2010). Molecular aspects of thyroid hormone actions. *Endocr Rev*. 31: 139–170.

Choi, Y.H., Hartzell, D., Azain, M.J. and Baile, C.A. (2002). TRH decreases food intake and increases water intake and body temperature in rats. *Physiol. Behav.* 77: 1–4

Choksi, N.Y., Jahnke, G.D., St. Hilaire, C. and Shelby, M. (2003). Role of Thyroid Hormones in Human and Laboratory Animal Reproductive Health. *Birth Defects Research* (Part B) 68:479–491.
Chopra, I.J. (1976). An assessment of daily production and significance of thyroidal secretion of 3, 3', 5'-triiodothyronine (reverse T3) in man. *J. Clin. Invest.* 58: 32–40.

Clements, F.W. (1954). The relationship of thyrotoxicosis and carcinoma of the thyroid to endemic goitre. *Med. J. Aust.* 41: 894-897.

Clement, K. Vaisse, C., Lahlou, N., et al., (1998). A mutation in ´ the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature,* 392: 6674, 398–401.

Cohen, H.N., Beastall, G.H., Ratcliffe, W.A., et al. (1980). Effects on human thyroid function of sulfonamide and trimethoprim combination drugs. *Br. Med. J*. 81:646–647

Cohen, S.M. and Ellwein, L.B. (1991). Genetic errors, cell proliferation, and carcinogenesis. *Cancer Res.* 51: 6493-6505.

Collins, W.T. Jr., Capen, C.C., Kasza, L., Carter, C. and Dailey, R.E. (1977). Effect of Polychlorinated Biphenyl (PCB) on the Thyroid Gland of Rats. Ultrastructural and Biochemical Investigations. *Am. J. Pathol.* 89: 119-136.

Colnot, T. and Dekant, W. (2017). Approaches for grouping of pesticides into cumulative assessment groups for risk assessment of pesticide residues in food. *Regul. Toxicol. Pharmacol.* 83: 89-99.

Cooper, D.S., Axelrod, L., DeGroot, L.J., Vickery, Jr. A.l. and Maloof, F. (1981). Congenital goitre and development of metastatic follicular carcinoma with evidence for a leak of non-hormonal iodide: clinical, pathological, kinetic, and biochemical studies and a review of the literature. *J. Clin. Endocrinol. Metab*. 52: 924-932.

Craft, E.S., DeVito, M.J. and Crofton, K.M. (2002). Comparative responsiveness of hypothyroxinemia and hepatic enzyme induction in Long-Evans rats versus C57BL/6J mice exposed to TCDD like and phenobarbital-like polychlorinated biphenyl congeners. *Toxicol. Sci.* 68: 372–380.

Cray, C., Zaias, J. and Altman, N.H. (2009). Acute Phase Response in Animals: A Review. *Comp Med.* 59: 517–526.

Crofton, K.M. (2004). Developmental disruption of thyroid hormone: correlations with hearing dysfunction in rats. *Risk Anal.* 24: 1665–1671.

Crofton, K.M. (2008). Thyroid disrupting chemicals: mechanisms and mixtures. *Int. J. Androl.* 31: 209-223.

Crofton, K.M., Craft, E.S., Hedge, J.M., Gennings, C., Simmons, J.E., Carchman, R.A., Carter, W.H. Jr. and DeVito, M.J. (2005). Thyroid-hormone-disrupting chemicals: evidence for dose-dependent additivity or synergism. *Environ. Health Perspect.* 113: 1549-1554

Crofton, K.M., Ding, D., Padich, R., Taylor, M. and Henderson, D. (2000). Hearing loss following exposure during development to polychlorinated biphenyls: a cochlear site of action. *Hear. Res*. 144: 196–204.

Cuevas, E., Auso, E., Telefont, M., Morreale de Escobar, G., Sotelo, C. and Berbel, P. (2005). Transient maternal hypothyroxinemia at onset of corticogenesis alters tangential migration of medial ganglionic eminence-derived neurons. *Eur. J. Neurosci*. 22: 541–551.

Curran, P. G. and DeGroot, L.J. (1991). The effect of hepatic enzyme-inducing drugs on thyroid hormones and the thyroid gland. *Endocr. Rev.* 12: 135-150.

Daminet, S. and Ferguson, D.C. (2003). Influence of Drugs on Thyroid Function in Dogs. *J. Vet. Intern. Med.* 17: 463–472.

Darras, V.M., Hume, R. and Visser, T.J. (1999). Regulation of thyroid hormone metabolism during fetal development. *Mol. Cell Endocrinol.* 151: 37-47.

Da Veiga, M.A., Oliveira Kde, J., Curty, F.H. and de Moura, C.C. (2004). Thyroid hormones modulate the endocrine and autocrine/paracrine actions of leptin on thyrotropin secretion. *J. Endocrinol.* 183: 243–247.

Dayan, C.M. (2001). Interpretation of thyroid function tests. *Lancet* 357 (9256): 619–624
de Escobar, G. M., Obregon, M.J. and del Rey, F. E. (2000). Is neuropsychological development related to maternal hypothyroidism or to maternal hypothyroxinemia? *J. Clin. Endocrinol. Metab*. 85: 3975–3987

de Benoist, B., Andersson, M., Egli, I., Takkouche, B. and Allen, H. (2004). Iodine status worldwide. WHO Global Database on Iodine Deficiency. http://apps.who.int/iris/bitstream/handle/10665/43010/9241592001.pdf;jsessionid=2F2F0EF063EBDD806CC3672B11DE10D3?sequence=1 Accessed 08/04/2018

de Escobar, G.M., Obregon, M.J. and del Rey, F.E. (2004). Maternal thyroid hormones early in pregnancy and fetal brain development. *Best Pract. Res. Clin. Endocrinol. Metab*. 18: 225–248

de Jesus, L.A., Carvalho, S.D., Ribeiro, M.O., Schneider, M., Kim, S.W., Harney, J.W., et al. (2001). The type 2 iodothyronine deiodinase is essential for adaptive thermogenesis in brown adipose tissue. *J. Clin. Invest.* 108: 1379-1385.

Dellarco, V.L., McGregor, D., Berry, C., Cohen, S.M. and Boobis, A.R. (2006). Thiazopyr and thyroid disruption: Case study within the context of the 2006 IPCS human relevance framework for analysis of a cancer mode of action. *Crit. Rev. Toxicol.* 36: 793-801.

Dentice , M., Marsili, A., Ambrosio, R., Guardiola, O., Sibilio, A., Paik, J.H., et al. (2010). The FoxO3/type 2 deiodinase pathway is required for normal mouse myogenesis and muscle regeneration. *J Clin Invest.* 120: 4021-4030.

Dentice, M., Marsili, A., Zavacki, A-M., Larsen, P.R. and Salvatore, D. (2013). The deiodinases and the control of intracellular thyroid hormone signaling during cellular differentiation. *Biochim Biophys Acta.* 1830: 3937–3945.

Di Cosmo, C., Liao, X.H., Dumitrescu, A.M., Weiss, R.E. and Refetoff, S. (2009). A thyroid hormone analog with reduced dependence on the monocarboxylate transporter 8 for tissue transport. *Endocrinology* 150: 4450–4458

Di Cosmo, C., Liao, X.H., Dumitrescu, A.M., Philp, N.J., Weiss, R.E. and Refetoff, S. (2010). Mice deficient in MCT8 reveal a mechanism regulating thyroid hormone secretion. *J. Clin. Invest.* 120: 3377-3388.

Doerge, D.R. and Decker, C.J. (1994). Inhibition of peroxidase-catalyzed reactions by arylamines: Mechanism for the anti-thyroid action of sulfamethazine. *Chem. Res. Toxicol*. 7: 164–169.

Dohler, K.D., Wong, C.C. and von zur Muhlen, A. (1979). The rat as a model for the study of drug effects on thyroid function: consideration of methodological problems. *Pharmacol. Ther. B*, 5: 305-318.

Downes, N. and Foster, J.R. (2015). Regulatory Forum Opinion Piece: Carcinogen Risk Assessment: The Move from Screens to Science. *Toxicol. Pathol.* 43: 1064-1073.

Dumitrescu, A.M., Liao, X.H., Best, T.B., Brockmann, K. and Refetoff, S. (2004). A novel syndrome combining thyroid and neurological abnormalities is associated with mutations in a monocarboxylate transporter gene. *Am. J. Hum. Genet.* 74: 168–175.

Dumitrescu, A.M., Liao, X.H., Weiss, R.E., Millen, K. and Refetoff, S. (2006). Tissue-specific thyroid hormone deprivation and excess in monocarboxylate transporter (MCT) 8-deficient mice. *Endocrinology* 147: 4036–4043

Eelkman R.S.J., Kaptein, E. and Visser, T.J. (1989). Serum triiodothyronine sulfate in man measured by radioimmunoassay. *J. Clin. Endocrinol. Metab.* 69: 552-556.

Elcombe, C. R., Peffer, R.C., Wolf, D.C., Bailey, J., Bars, R., Bell, D., Cattley, R.C., Ferguson, S.S., Geter, D., Goetz, A., Goodman, J.I., Hester, S., Jacobs, A., Omiecinski, C.J., Schoeny, R., Xie, W., and Lake, B.G. (2014). Mode of action and human relevance analysis for nuclear receptor-mediated liver toxicity: A case study with phenobarbital as a model constitutive androstane receptor (CAR) activator. *Crit. Rev. Toxicol.* 44: 64–82.

European Food Safety Authority/European Chemicals Agency (2017). Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 16 528/2012 and (EC) No 1107/2009. Draft for public consultation. Drafted by EFSA and ECHA staff, with support from JRC 7 December 2017.

Elman, D.S. (1958). Familial association of nerve deafness with nodular goitre and thyroid carcinoma. *N. Engl. J. Med.* 259: 219-223.

Emerson, C.H. and Utiger, R.D. (1975). Plasma thyrotropin-releasing hormone concentrations in the rat. *J. Clin. Invest.* 56: 1564-1570

Emerson, C. H., Lew, R., Braverman, L.E. and DeVito, W.J. (1989). Serum thyrotropin concentrations are more highly correlated with serum triiodothyronine concentrations than with serum thyroxine concentrations in thyroid hormone-infused thyroidectomized rats. *Endocrinology*. 124: 2415-2418.

Emi, Y., Ikushiro, S. and Kato, Y. (2007). Thyroxine-Metabolizing Rat Uridine Diphosphate Glucuronosyltransferase 1A7 Is Regulated by Thyroid Hormone Receptor. *Endocrinol.* 148: 6124-6133.

EPA (2014). Appendix A. Case studies of compounds. https://www.epa.gov/sites/production/files/2014-11/documents/thyroid_appx.pdf accessed 30/10/2017

Episkopou, V., Maeda, S., Nishiguchi, S., Shimada, K., Gaitanaris, G.A., Gottesman, M.E. and Robertson, E.J. (1993). Disruption of the transthyretin gene results in mice with depressed levels of plasma retinol and thyroid hormone. *Proc Natl Acad Sci USA* 90: 2375–2379.

Epstein, Y., Udassin, R. and Sack, J. (1979). Serum 3,5,3′-triiodothyronine and 3,3′,5′-triiodothyronine concentrations during acute heat load. *J. Clin. Endocrinol. Metab.* 49: 677-678.

European Union (2017). Supporting the organisation of a workshop on thyroid disruption – Final Report. Framework Contract ENV.A.3/FRA/2014/0029 on implementation of the Community strategy on Endocrine Disrupters https://publications.europa.eu/en/publication-detail/-/publication/472d2c88-a8b1-11e7-837e-01aa75ed71a1/language-en accessed 26/10/2017

Evans, R.M. (1988). The steroid and thyroid hormone receptor superfamily. *Science* 240: 889–895.

Farooqi, I.S., Matarese, G., Lord, G.M. et al., (2002). Beneficial effects of leptin on obesity, T cell hypo-responsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. *J. Clin. Invest.* 110: 1093–1103

Fattori, J., Campos, J.L.O., Doratioto, T.R., Assis, L.M., Vitorino, M.T., Polikarpov, I., Xavier-Neto, J. and Figueira, A.C.M. (2015). RXR Agonist Modulates TR: Corepressor Dissociation Upon 9-cis Retinoic Acid Treatment. *Mol. Endocrinol.* 29: 258–273.

Fekete, C., Mihaly, E., Luo, L.G., Kelly, J., Clausen, J.T., Mao, Q., Rand, W.M., Moss, L.G., Kuhar, M., Emerson, C.H., Jackson, I.M. and Lechan, R.M. (2000). Association of cocaine- and amphetamine-regulated transcript immunoreactive elements with thyrotropin-releasing hormone-synthesizing neurons in the hypothalamic paraventricular nucleus and its role in the regulation of the hypothalamic-pituitary-thyroid axis during fasting. *J. Neurosci*. 20: 9224–9234.

Findlay, K.A.B., Kaptein, E., Vissser, T.J. and Burchell, B. (2000). Characterization of the uridine diphosphate-glucuronosyltransferase-catalyzing thyroid hormone glucuronidation in man. *J. Clin. Endocrinol. Metab.* 85: 2879–2883.

Fisher, D.A. (1996). Physiological variations in thyroid hormones: physiological and pathophysiological considerations. *Clin. Chem.* 42: 135-139.

Fisher, D.A. and Brown, R.S. (2000). Thyroid physiology in the perinatal period and during childhood. In: Braverman, L.E., Utiger, R.D., editors. Werner and Ingbar's The Thyroid, 8th ed. Philadelphia: Lippincott Williams & Wilkins. pp 959–972.

Fisher, D.A., Dussault, J.H., Erenberg, A. and Lam, R.W. (1972).Thyroxine and Triiodothyronine Metabolism in Maternal and Fetal Sheep. *Pediat. Res.* 6: 894-899.

Fitch, N.J.S., Akbari, M.T. and Ramsden, D.B. (1991). An inherited non-amyloidogenic transthyretin variant, [Ser6]-TTR, with increased thyroxine-binding affinity, characterized by DNA sequencing. *J Endocrinol*. 129: 309-313.

Flink, I.L., Bailey, T.J., Gustafson, T.A., Markham, B.E. and Morkin, E. (1986). Complete amino acid sequence of human thyroxine-binding globulin deduced from cloned DNA: close homology to the serine antiproteases. *Proc Natl Acad Sci USA* 83: 7708–7712.

Fonseca, T.L., Correa-Medina, M., Campos, M.P., Wittmann, G., Werneck-de-Castro, J.P., Arrojo e Drigo, R., Mora-Garzon, M., Ueta, C.B., Caicedo, A., Fekete, C., Gereben, B., Lechan, R.M. and Bianco, A.C. (2013). Coordination of hypothalamic and pituitary T3 production regulates TSH expression. *J. Clin. Invest*. 123: 1492–1500.

Foster, J.R. (1997). The role of cell proliferation in chemically induced carcinogenesis. *J. Comp. Pathol.* 116: 113-144.

Fox, C.S., Pencina, M.J., D'Agostino, R.B., Murabito, J.M., Seely, E.W., Pearce, E.N. and Vasan, R.S. (2008). Relations of thyroid function to body weight: cross-sectional and longitudinal observations in a community-based sample. *Arch. Intern. Med* .168: 587–592.

Franceschi, S., Preston-Martin, S., Dal Maso, L., Negri, E., La Vecchia, C., Mack, W.J., et al. (1999). A pooled analysis of case–control studies of thyroid cancer. IV. Benign thyroid diseases. *Cancer Causes Control*. 10: 583–595.

Friesema, E.C., Grueters, A., Biebermann, H., Krude, H., von Moers, A., Reeser, M., Barrett, T.G., Mancilla, E.E., Svensson, J., Kester, M.H., Kuiper, G.G., Balkassmi, S., Uitterlinden, A.G., Koehrle, J.,

Rodien, P., Halestrap, A.P. and Visser, T.J. (2004). Association between mutations in a thyroid hormone transporter and severe X-linked psychomotor retardation. *Lancet*. 364(9443): 1435–1437.

Friesema, E.C., Jansen, J. and Visser, T.J. (2005). Thyroid hormone transporters. *Biochem. Soc. Trans.* 33: 228-232.

Friesema, E.C., Kuiper, G.G., Jansen, J., Visser, T.J. and Kester, M.H. (2006). Thyroid hormone transport by the human monocarboxylate transporter 8 and its rate-limiting role in intracellular metabolism. *Mol. Endocrinol.* 20: 2761–2772.

Frumess, R.D. and Larsen, P.R. (1975). Correlation of serum triiodothyronine (T3) and thyroxine (T4) with biologic effects of thyroid hormone replacement in propylthiouracil-treated rats. *Metabolism.*24: 547-554.

Galanti, M.R., Sparen, P., Karlsson, A., Grimelius, L. and Ekbom, A. (1995). Is residence in areas of endemic goitre a risk factor for thyroid cancer? *Internat. J. Cancer* 61: 615-621.

Galton, V.A. and Nisula, B.C. (1969). Thyroxine metabolism and thyroid function in the cold-adapted rat. *Endocrinology* 85: 79-92.

Gardner, D.F., Carithers, R.L.Jr., and Utiger, R.D.(1982). Thyroid function tests in patients with acute and resolved hepatitis B virus infection," *Ann. Int. Med.* 96: 450–452

Gary, K.A., Winokur, A., Douglas, S.D., Kapoor, S., Zaugg, L. and Dinges, D.F. (1996). Total sleep deprivation and the thyroid axis: effects of sleep and waking activity. *Aviat. Space Environ. Med*. 67: 513–519.

Gauger, K.J., Kato, Y., Haraguchi, K., Lehmler, H-J., Robertson, L.W., Bansal, R. and Zoeller, R.T. (2004). Polychlorinated biphenyls (PCBs) exert thyroid hormone-like effects in the fetal rat brain but do not bind to thyroid hormone receptors. *Environ. Hlth. Perspect.* 112: 516-523.

Gereben, B., Zavacki, A.M., Ribich, S., Kim, B.W., Huang, S.A., Simonides, W.S., et al. (2008). Cellular and molecular basis of deiodinase-regulated thyroid hormone signaling. *Endocrin.Rev.* 29: 898-938.

Gereben, B., McAninch, E.A., Ribeiro, M.O. and Bianco, A.C. (2015). Scope and limitations of iodothyronine deiodinases in hypothyroidism. *Nat Rev Endocrinol.* 11: 642-652.

Ghamari-Langroudi, M., Vella, K.R., Srisai, D., Sugrue, M.L., Hollenberg, A.N. and Cone, R.D. (2010). Regulation of thyrotropin-releasing hormone-expressing neurons in paraventricular nucleus of the hypothalamus by signals of adiposity. *Mol. Endocrinol*. 24: 2366–2381

Gilbert, M.E. and Sui, L. (2008). Developmental exposure to perchlorate alters synaptic transmission in hippocampus of the adult rat. *Environ. Health Perspect*. 116: 752–760.

Glatt H. (2000). Sulfotransferases in the bioactivation of xenobiotics. *Chem. Biol. Interact*. 129: 141-170.

Glendenning, P. (2008). Management of Thyroid Dysfunction during Pregnancy and Postpartum: An Endocrine Society Clinical Practice Guideline. *Clin. Biochem. Rev.* 29: 83–85.

Glinoer, D. (2000). Thyroid disease during pregnancy. In: Werner & Ingbar's The Thyroid: A Fundamental and Clinical Text (Braverman LE, Utiger RD, eds). Philadelphia:Lippincott Williams & Wilkins, pp. 1013–1027.

Glinoer, D. (2007) The importance of iodine nutrition during pregnancy. *Public Health Nutr* 10; 1542–1546.

Glinoer, D., De Nayer, P., Delange, F., Lemone, M., Toppet, V., Spehl, M., Grun, J.P., Kinthaert, J. and Lejeune, B. (1995). A randomized trial for the treatment of mild iodine deficiency during pregnancy: maternal, neonatal effects. *J. Clin. Endocrinol. Metab.* 80: 258–269.

Goglia, F., Liverini, G., De Leo, T. and Barletta, A. (1983). Thyroid state and mitochondrial population during cold exposure. *Pflugers Arch.* 396: 49-53.

Goldey, E.S., Kehn, L.S., Lau, C., Rehnberg, G.L. and Crofton, K.M. (1995a). Developmental exposure to polychlorinated biphenyls (Aroclor 1254) reduces circulating thyroid hormone concentrations and causes hearing deficits in rats. *Toxicol. Appl. Pharmacol.* 135: 77–88.

Goldey, E.S., Kehn, L.S., Rehnberg, G.L. and Crofton, K.M. (1995b). Effects of developmental hypothyroidism on auditory and motor function in the rat. *Toxicol. Appl. Pharmacol.* 135: 67–76.

Goodman, J.H. and Gilbert, M.E. (2007). Modest thyroid hormone insufficiency during development induces a cellular malformation in the corpus callosum: a model of cortical dysplasia. *Endocrinology* 148: 2593–2597.

Graham, S.L., Davis, K.J., Hansen, W.H. and Graham, C.H. (1975). Effects of prolonged ethylene thiourea ingestion on the thyroid of the rat. *Food Cosm. Toxicol.* 13; 493-499,

Green, W.L. (1978). Mechanisms of action of antithyroid compounds. In: The Thyroid. Werner, S.C. and Ingbar, S.H., eds. New York: Harper and Row, pp. 77-87

Greer, M.A., Goodman, G., Pleus, R.C. and Greer, S.E. (2002). Health effects assessment for environmental perchlorate contamination: The dose response for inhibition of thyroidal radioiodine uptake in humans. *Environ. Health Perspect.* 110: 927-937

Hackney, A.C., Feith, S., Pozos, R. and Seale, J. (1995a). Effects of high altitude and cold exposure on resting thyroid hormone concentrations. *Aviat. Space Environ. Med*. 66: 325-329

Hackney, A.C., Hogdon, J.A., Hesslink, R. Jr. and Trygg, K. (1995b). Thyroid hormone responses to military winter exercise in the Arctic region. *Arctic Med. Res*. 54: 82-90.

Haddow, J.E., Palomaki, G.E., Allan, W.C., Williams, J.R., Knight, G.J., Gagnon, J., O'Heir, C.E., Mitchell, M.L., Hermos, R.J., Waisbren, S.E., Faix, J.D. and Klein, R.Z. (1999). Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. *N. Engl. J. Med.* 341: 549–555.

Hall, J.A., Ribich, S., Christoffolete, M.A., Simovic, G., Correa-Medina, M., Patti, M.E., et al. (2010). Absence of thyroid hormone activation during development underlies a permanent defect in adaptive thermogenesis. *Endocrinology.* 151: 4573-4582.

Hallgren, S. and Darnerud, P.O. (2002). Polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) and chlorinated paraffins (CPs) in rats - testing interactions and mechanisms for thyroid hormone effects. *Toxicology*. 177: 227–243.

Hamada, C., Yoshino, K., Abe, I., Matsumoto, K., Nomura, M. and Yoshimura, I. (1998). Detection of an Outlier and Evaluation of its Influence in Chronic Toxicity Studies. *Ther. Innov. Reg. Sci.* 32: 201-212.

Haseman, J. and Seilkop, S. K. (1992). An examination of the association between maximum-tolerated dose and carcinogenicity in 326 long-term studies in rats and mice. *Fundam. Appl. Toxicol.* 19: 207–213.

Haseman, J.K., Huff, J.E., Rao, G.N., Arnold, J.E., Boorman, G.A, and McConnell, E.E. (1985). Neoplasms observed in untreated and corn oil gavage control groups of F344/N rats and (C57BL/6N x C3H/HeN)F1 (B6C3F1) mice. *JNCI* 75: 975-984

Hefco, E., Krulich, L., Illner, P. and Larsen, P.R. (1975). Effect of acute exposure to cold on the activity of the hypothalamic-pituitary-thyroid system. *Endocrinol.* 97: 1185-1192.

Hegedus, L. (1986). Thyroid gland volume and thyroid function during and after acute hepatitis infection. *Metabolism* 35: 495–498.

Helmreich, D.L. and Tyree, D. (2011). Thyroid hormone regulation by stress and behavioural differences in adult male rats. *Horm. Behav*. 60: 284-291.

Hershman, J.M. (1974). Clinical application of thyrotropin-releasing hormone. *N. Engl. J. Med.* 290: 886–890

Herwig, A., Campbell, G., Mayer, C-D., Boelen, A., Anderson, R.A., Ross, A.W., Mercer, J.G. and Barrett, P. (2014). A thyroid Hormone Challenge in Hypothyroid Rats Identifies T3 Regulated Genes in the Hypothalamus and in Models with Altered Energy Balance and Glucose Homeostasis. *Thyroid* 24: 1575-1593.

Heuer, H. and Visser, T.J. (2009). Minireview: pathophysiological importance of thyroid hormone transporters. *Endocrinol.* 150: 1078–1083

Hiasa, Y., Kitahori, Y., Kitamura, M., Nishioka, H., Vane, K., Fukumoto, M., Ohshima, M., Nakaoka, S., Nishii, S. (1991). Relationships between serum thyroid stimulating hormone levels and the development of thyroid tumors in rats treated with N-bis-(2-hydroxypropyl)nitrosamine. *Carcinogenesis* 12: 873-877.

Hill, R.N., Erdreich, L.S., Paynter, O.E., Roberts, P.A., Rosenthal, S.L. and Wilkinson, C.F. (1989). Thyroid follicular cell carcinogenesis. *Fundam. Appl. Toxicol.* 12: 629-697.

Hill, R.N., Crisp, T.M., Hurley, P.M., Rosenthal, S.L. and Singh, D.V., (1998). Risk Assessment of Thyroid Follicular Cell Tumors. *Environ. Health Perspect.* 106: 447-457.

Hindmarsh, P. (2002). Optimisation of thyroxine dose in congenital hypothyroidism. *Arch. Dis. Child*. 86: 73–75.

Hoermann, R., Midgley, J.E.M., Larisch, R. and Dietrich, J.W. (2015). Homeostatic Control of the Thyroid–Pituitary Axis: Perspectives for Diagnosis and Treatment. *Front. Endocrinol*. 6: 177. doi: 10.3389/fendo.2015.00177.

Holm, L.E., Wiklund, KE., Lundell, G.E. et al. (1988). Thyroid cancer after diagnostic doses of iodine-131: a retrospective cohort study. *J. Natl. Cancer Inst.* 80: 1132-1138.

Hombach-Klonisch, S., Danescu, A., Begum, F., Amezaga, M.R., Rhind, S.M., Sharpe, R.M., Evans, N.P., Bellingham, M., Cotinot, C., Mandon-Pepin, B., Fowler, P.A. and Klonisch, T. (2013). Peri-conceptional changes in maternal exposure to sewage sludge chemicals disturbs foetal thyroid gland development in sheep. *Molecular and Cellular Endocrinology* 367:98–108.

Hood, A. and Klaassen, C.D. (2000a). Effects of microsomal enzyme inducers on outer ring deiodinase activity towards thyroid hormones in various rat tissues. *Toxicol. Appl. Pharmacol.* 163; 240-248

Hood, A. and Klaassen, C. D. (2000b). Differential effects of microsomal enzyme inducers on in vitro thyroxine (T(4)) and triiodothyronine (T(3)) glucuronidation. *Toxicol. Sci*. 55: 78–84.

Hsu, J.H., Zavacki, A.M., Harney, J.W. and Brent, G.A. (1995). Retinoid-X receptor (RXR) differentially augments thyroid hormone response in cell lines as a function of the response element and endogenous RXR content. *Endocrinol.* 136: 421–430.

Huang, M.J. and Liaw, Y.F. (1995). Clinical associations between thyroid and liver diseases. *J. Gastroenterol. Hepatol.* 10: 344-350.

Hulme, E.C. and Trevethick. M.A. (2010). Ligand binding assays at equilibrium: validation and interpretation. *Br. J. Pharmacol.* 161: 1219–1237.

Hurley, P. M., Hill, R.N. and Whiting, R.J. (1998). Mode of Carcinogenic Action of Pesticides Inducing Thyroid Follicular Cell Tumors in Rodents. *Environ. Hlth. Perspect.*106: 437-445.

IARC (1999). Species Differences in Thyroid, Kidney and Urinary Bladder Carcinogenesis. IARC Scientific Publications No. 147. Edited by C.C. Capen, E. Dybing, J.M. Rice and J.D. Wilbourn. https://monographs.iarc.fr/ENG/Publications/pub147/IARCpub147.pdf Accessed 30/10/2017

Imai, Y., Toyoda, N., Maeda, A., Kadobayashi, T., Fangzheng, G., Nishikawa, M., et al. (2001). Type 2 iodothyronine deiodinase expression is upregulated by the protein kinase A dependent pathway and is downregulated by the protein kinase C-dependent pathway in cultured human thyroid cells. *Thyroid.* 11: 899-907.

Ishigaki, S., Abramovitz, M. and Listowsky, I. (1989). Glutathione-S-transferases are major cytosolic thyroid hormone binding proteins. *Arch. Biochem. Biophys.* 273: 265-272.

Ishihara, A., Sawatsubashi, S. & Yamauchi, K. (2003) Endocrine disrupting chemicals: interference of thyroid hormone binding to transthyretins and to thyroid hormone receptors. *Mol. Cell. Endocrinol*. 199: 105–117.

Ishii, S., Kamegai, J., Tamura, H., Shimizu, T., Sugihara, H. and Oikawa, S. (2003). Hypothalamic neuropeptide Y/Y1 receptor pathway activated by a reduction in circulating leptin, but not by an increase in circulating ghrelin, contributes to hyperphagia associated with triiodothyronine-induced thyrotoxicosis. *Neuroendocrinol*. 78: 321–330.

Ishii, S., Kamegai, J., Tamura, H., Shimizu, T., Sugihara, H. and Oikawa, S. (2008). Triiodothyronine (T3) stimulates food intake via enhanced hypothalamic AMP-activated kinase activity. *Regulatory Peptides* 151: 1–3: 164–169.

Iwen, K.A., Schroder, E. and Brabant, G. (2013). Thyroid hormone and the metabolic syndrome. *Eur. Thyroid J*. 2: 83–92.

Jabbar, A., Pingitore, A., Pearce, S.H.S., Zaman, A., Iervasi, G. and Razvi, S. (2017). Thyroid hormones and cardiovascular disease. *Nat. Rev. Cardiology* 14: 39–55.

Jackson, I.M.D. (1982). Thyrotropin-releasing hormone. *N. Engl. J. Med.* 306: 145–155

Jacobson, D.R., Alves, I.L., Saraiva, M.J., Thibodeau, S.N. and Buxbaum, J.N. (1995). Transthyretin Ser 6 gene frequency in individuals without amyloidosis. Hum. Gen. 95: 308-312.

Jahnke, G.D., Choksi, N.Y., Moore, J.A. and Shelby, M.D. (2004). Thyroid Toxicants: Assessing Reproductive Health Effects. *Environ. Health Perspect*. 112: 363–368.

Jannini, E.A., Ulisse, S. and D'Armiento, M. (1995). Thyroid hormone and male gonadal function. *Endocr. Rev.* 16: 443–459.

Jansen, J., Friesema, E.C., Milici, C. and Visser, T.J. (2005). Thyroid hormone transporters in health and disease. *Thyroid* 15: 757–768.

Jayachandran, C., Suchetha, A., Mundinamane, D.B., Apoorva S. M., Bhat, D. and Lalwani, M. (2016). Acute phase proteins. *J. Chem. Pharm. Res.* 8: 365-370.

Jhiang, S.M., Sagartz, J.E., Tong, Q., Parker-Thornburg, J., Capen, C.C., Cho, J.Y., Xing, S. and Ledent, C. (1996) Targeted expression of the ret/PTC1 oncogene induces papillary thyroid carcinomas. *Endocrinol.* 137: 375–378.

Kabat, E.A., Moore, D.H. and Landow H. (1942). An electrophoretic study of the protein components in cerebrospinal fluid and their relationship to the serum proteins. *J Clin Invest* 21: 571–577.

Kaptein, E.M., Hays, M.T. and Ferguson, D.C. (1994). Thyroid hormone metabolism: A comparative evaluation. *Vet. Clin. North Am. Small Anim. Pract.* 24: 431–463.

Kaptein, E.M., Moore, G.E., Ferguson, D.C., et al. (1993). Thyroxine and triiodothyronine distribution and metabolism in thyroxine-replaced athyreotic dogs and normal humans. *Am. J. Phys. (Endocrinol. Metab.)* 264: E90–E100.

Kaneko, J.J. (1989). Thyroid function. In: Kaneko J.J., editor. Clinical biochemistry of domestic animals. New York: Academic Press, Inc. p 634–635.

Kano, T., Kojima, T., Takahashi, T. and Muto, Y. (1987). Serum thyroid hormone levels in patients with fulminant hepatitis: usefulness of rT3 and the rT3/T3 ratio as prognostic indices. *Gastroenterol. Jpn.* 22: 344–353.

Kato, H., Fukuda, T., Parkison, C., McPhie, P. and Cheng, S-Y. (1989). Cytosolic thyroid hormone-binding protein is a monomer of pyruvate kinase. *Proc. Natl. Acad. Sci. USA* 86: 7861-7865.

Karapanou, O. and Papadimitriou, A. (2011). Thyroid hormone transporters in the human. *Hormones* 10: 270-279.

Kelly, G. (2000). Peripheral metabolism of thyroid hormones: a review. *Altern. Med. Rev*. 5: 306–333.

Kersseboom, S. and Visser, T.J. (2011). MCT8: from gene to disease and therapeutic approach. *Ann. Endocrinol.* 72: 77–81.

Kester, M.H., Kaptein, E., Van Dijk, C.H., Roest, T.J., Tibboel, D., Coughtrie, M.W., et al. (2002). Characterization of iodothyronine sulfatase activities in human and rat liver and placenta. *Endocrinol.*143: 814-819.

Kester, M.H.A., Van Dijk, C.H., Tibboel, D., Hood, A.M., Rose, N.J.M., Meinl, W., Pabel, U., Glatt, H., Falany, C.H., Coughtrie, M.W.H. and Visser, T.J. (1999). Sulfation of thyroid hormone by estrogen sulfotransferase. *J. Clin. Endocrinol. Metab.* 84; 2577-2580.

Kibirige, D., Luzinda, K. and Ssekitoleko, R. (2013). Spectrum of lithium induced thyroid abnormalities: a current perspective. *Thyroid Res.* 6: 3 https://thyroidresearchjournal.biomedcentral.com/articles/10.1186/1756-6614-6-3 98: 295-304.

Kieffer, J.D., Mover, H., Federico, P. and Maloof, F. (1976). Pituitary–thyroid axis in neonatal and adult rats: comparison of the sexes *Endocrinol*.98: 295-304.

Kim, C.S. and Zhu, X. (2009). Lessons from Mouse Models of Thyroid Cancer. *Thyroid* 19: 1317-1331.

Kioukia, N., Bekris, S., Antoniou, K., Papadopoulou-Daifoti, Z. and Christofidis, I. (2000). Effects of chronic mild stress (CMS) on thyroid hormone function in two rat strains. *Psychoneuroendocrinol.* 25: 247-257.

Kitahara, C.M. and Sosa, J.A. (2016). The changing incidence of thyroid cancer. *Nat.Rev. Endocrinol.* 12, 646–653.

Klaassen, C.D. and Hood, A.M. (2001). Effects of microsomal enzyme inducers on thyroid follicular cell proliferation and thyroid hormone metabolism. *Toxicol. Pathol.* 29: 34-40.

Klammer, H., Schlecht, C., Wuttke, W., Schmutzler, C., Gotthardt, I., Köhrle, J. and Jarry, H. (2007). Effects of a 5-day treatment with the UV-filter octyl-methoxycinnamate (OMC) on the function of the hypothalamo-pituitary-thyroid function in rats. *Toxicol.* 238: 192-199.

Klein, R.Z., Sargent, J.D., Larsen, P.R., Waisbren, S.E., Haddow, J.E. and Mitchell, M.L. (2001). Relation of severity of maternal hypothyroidism to cognitive development of offspring. *J. Med. Screen.* 8: 18–20.

Kliewer, S.A., Umesono, K., Mangelsdorf, D.J. and Evans, R.M. (1992). Retinoid X receptor interacts with nuclear receptors in retinoic acid, thyroid hormone, and vitamin D3 signalling. *Nature* 355: 446–449.

Kliewer, S.A., Goodwin, B. and Willson, T.M. (2002).The nuclear pregnane X receptor: a key regulator of xenobiotic metabolism. *Endocrin. Rev.* 23: 687–702.

Koibuchi, N. and Chin, W.W. (2000). Thyroid hormone action and brain development. *Trends Endocrinol Metab*. 11: 123–128.

Kong, W,M,. Martin, N.M., Smith, K.L. et al., (2004). Triiodothyronine stimulates food intake via the hypothalamic ventromedial nucleus independent of changes in energy expenditure. *Endocrinol*. 145: 5252–5258.

Kohrle, J., Fang, S. L., Yang, Y., Irmscher, K., Hesch, R. D., Pino, S., Alex, S. & Braverman, L. E. (1989) Rapid effects of the flavonoid EMD 21388 on serum thyroid hormone binding and thyrotropin regulation in the rat. *Endocrinology* 125: 532–537.

Kortenkamp, A., Martin, O., Baynes, A., Silva, E., Axelstad, M. and Hass, U. (2017). Supporting the organisation of a workshop on thyroid disruption – Final Report. Framework Contract ENV.A.3/FRA/2014/0029 on implementation of the Community strategy on Endocrine Disrupters. Written by Brunel University London, Institute of Environment, Health and Societies National Food Institute, Technical University of Denmark. September– 2017. https://publications.europa.eu/en/publication-detail/-/publication/472d2c88-a8b1-11e7-837e-01aa75ed71a1/language-en Accessed 10/11/2017.

Kotwal, A., Priya, R. and Qadeer, I. (2007). Goitre and other iodine deficiency disorders: A systematic review of epidemiological studies to deconstruct the complex web. *Arch. Med. Res.* 38: 1-14.

Knauf, J.A., Ma, X., Smith, E.P., Zhang, L., Mitsutake, N., Liao X.H., Refetoff, S., Nikiforov, Y.E. and Fagin, J.A. (2005). Targeted expression of BRAFV600E in thyroid cells of transgenic mice results in papillary thyroid cancers that undergo dedifferentiation. *Cancer Res*. 65: 4238–4245

Knostman, K.A., Jhiang, S.M. and Capen, C.C. (2007). Genetic alterations in thyroid cancer: the role of mouse models. *Vet. Pathol.* 44: 1–14

Knudsen, N., Laurberg, P., Rasmussen, L.B., Bulow, I., Perrild, H., Ovesen, L. and Jorgensen, T. (2005). Small differences in thyroid function may be important for body mass index and the occurrence of obesity in the population. *J. Clin. Endocrinol. Metab*. 90: 4019–4024.

Krassas, G.E. (2000). Thyroid disease and female reproduction. *Fertil. Steril*. 74: 1063–1070

Langouche, L., Vander, P. S., Marques, M., Boelen, A., Wouters, P.J., Casaer, M.P. and Van den Berghe, G. (2013). Impact of early nutrient restriction during critical illness on the nonthyroidal illness syndrome and its relation with outcome: a randomized, controlled clinical study. *J. Clin. Endocrinol. Metab*. 98: 1006–1013.

Lans, M. C., Klasson-Wehler, E., Willemsen, M., Meussen, E., Safe, S. & Brouwer, A. (1993) Structure-dependent, competitive interaction of hydroxy-polychlorobiphenyls, -dibenzo-pdioxins and -dibenzofurans with human transthyretin. *Chemico-Biol. Interact*. 88: 7–21.

Larsen, P.R. and Zavacki, A.M. (2012). The role of the iodothyronine deiodinases in the physiology and pathophysiology of thyroid hormone action. *Eur. Thyroid J*. 1: 232–242

Leghait, J., Gayrard, V., Toutain, P.L., Picard-Hagen, N. and Viguié, C. (2010). Is the mechanisms of fipronil-induced thyroid disruption specific of the rat: Re-evaluation of fipronil thyroid toxicity in sheep? *Toxicol Lett.* 194: 51-57.

Legradi, G., Emerson, C.H., Ahlma, R.S., Flier, J.S. and Lechan, R.M. (1997). Leptin prevents fasting-induced suppression of prothyrotropin-releasing hormone messenger ribonucleic acid in neurons of the hypothalamic paraventricular nucleus," *Endocrinol.* 138: 2569–2576.

Leonard, J. L. and Visser, T. J. (1986). in Thyroid Hormone Metabolism. (Heinemann, G., ed.), pp. 189-229, Marcel Dekker, New York and London

Leppäluoto, J., Sikkilä, K. and Hassi, J. (1998). Seasonal variation of serum TSH and thyroid hormones in males living in subarctic environmental conditions. *Int. J. Circumpolar Health*. 57 Suppl 1: 383-385.

Leung, A.M. and Braverman, L.E. (2014). Consequences of excess iodine. *Nat. Rev. Endocrinol.* 10: 136-142.

Lewandowski, T.A., Seeley, M.R. and Beck, B.D. (2004) Interspecies differences in susceptibility to perturbation of thyroid homeostasis: a case study with perchlorate. *Regul. Toxicol. Pharmacol.* 39: 348-362.

Lewis, R.W., Billington, R., Debryune, E., Gamer, A., Lang, B. and Carpanini, F. (2002). Recognition of adverse and non-adverse Effects in Toxicity Studies. *Toxicol. Pathol.* 30: 66–74

Li, M. and Eastman, C.J. (2012). The changing epidemiology of iodine deficiency. *Nature Reviews Endocrinology* 8, 434–440.

Li, H., Yuan, X., Liu, L., Zhou, J., Li, C., Yang, P., Bu, L., Zhang, M. and Qu, S. (2014). Clinical Evaluation of Various Thyroid Hormones on Thyroid Function. *Int. J. Endocrinol.* 2014: Article ID 618572, 5 pages, doi:10.1155/2014/618572

Liesenkötter, K.P., Göpel, W., Bogner, U., Stach, B. and Grüters, A. (1995). Earliest prevention of endemic goitre by iodine supplementation during pregnancy. *Eur. J. Endocrinol*. 134: 443-448

Lin, M.T., Chu, P.C. and Leu, S.Y. (1983). Effects of TSH, TRH, LH and LHRH on thermoregulation and food and water intake in the rat. *Neuroendocrinol*. 37: 206–211.

Liu, Y.Y. and Brent, G.A. (2010). Thyroid hormone crosstalk with nuclear receptor signalling in metabolic regulation. *Trends Endocrinol. Metab.* 21: 166–173.

Liu, Y.Y., Heymann, R.S., Moatamed, F., Schultz, J.J., Sobel, D. and Brent, G.A. (2007). A mutant thyroid hormone receptor alpha antagonizes peroxisome proliferator-activated receptor alpha signaling in vivo and impairs fatty acid oxidation. *Endocrinology* 148: 1206–1217

Liu, L. and Klaassen, C. D. (1996). Regulation of hepatic sulfotransferases by steroidal chemicals in rats. *Drug Metab. Dispos*. 24: 854–858.

Liu, J., Liu, Y., Barter, R.A. and Klaassen CD. (1995). Alteration of thyroid homeostasis by UDP-glucuronosyltransferase inducers in rats: a dose-response study. *J. Pharmacol. Exp. Ther.* 273: 977-985.

Ljunggren, J.G., Klalner, G. and Tryselius, M. (1977). The effect of body temperature on thyroid hormone levels in patients with nonthyroidal illness. *Acta Med. Scand.* 202: 459-462.

Logan, A., Black, E.G., Gonzalez, A.M., Buscaglia, M. and Sheppard, M.C. (1992). Basic fibroblast growth factor: an autocrine mitogen of rat thyroid follicular cells? *Endocrinology* 130: 2363–2372.

Longnecker, M. P., Wolff, M. S., Gladen, B. C., Brock, J. W., Grandjean, P., Jacobson, J. L., Korrick, S.A., Rogan, W.J., Weisglas-Kuperus, N., Hertz-Picciotto, I., Ayotte, P., Stewart, P., Winneke, G., Charles, M.J., Jacobson, S.W., Dewailly, E., Boersma, E.R., Altshul, L.M., Heinzow, B., Pagano, J.J. and Jensen, A.A. (2003). Comparison of polychlorinated biphenyl levels across studies of human neurodevelopment. *Environ. Hlth. Perspect.* 111: 65–70.

Lucio, R.A., Garcia, J.V., Ramon Cerezo, J., Pacheco, P., Innocenti, G.M. and Berbel, P. (1997). The development of auditory callosal connections in normal and hypothyroid rats. *Cereb. Cortex* 7: 303–316.

Lueprasitsakul, W., Alex, S., Fang, S. L., Pino, S., Irmscher, K., Kohrle, J. & Braverman, L. E. (1990) Flavonoid administration immediately displaces thyroxine (T4) from serum transthyretin, increases serum free T4, and decreases serum thyrotropin in the rat. *Endocrinol.* 126: 2890–2895.

McAninch, E.A. and Bianco, A.C. (2016). The History and Future of Treatment of Hypothyroidism. *Ann. Intern. Med.* 164: 50–56.

McClain, R.M. (1989). The significance of hepatic microsomal enzyme induction and altered thyroid function in rats: implications for thyroid gland neoplasia. *Toxicol. Pathol.* 17: 294-306.

McClain, R.M. (1995). Mechanistic considerations for the relevance of animal data on thyroid neoplasia to human risk assessment. *Mutat. Res*. 333: 131-142.

McClain, R. M., Levin, A. A., Posch, R. and Downing, J. C. (1989). The effect of phenobarbital on the metabolism and excretion of thyroxine in rats. *Toxicol. Appl. Pharmacol*. 99: 216–228.

McClain, R.M., Posch, R.C., Bosakowski, T. and Armstrong, J.M. (1988). Studies on the mode of action for thyroid gland tumor promotion in rats by phenobarbital. *Toxicol. Appl. Pharmacol.* 94: 254-265.

McConnell, E. E. (1989). The maximum tolerated dose: The debate. *J. Am. Col. Toxicol*. 8: 1115–1120.

McGirr, E.M., Clement, W.E., Currie, A.R. and Kennedy, J.S. (1959). Impaired dehalogenase activity as cause of goitre with malignant changes. *Scott. Med. J*. 4: 232-241.

McNabb, F.M.A. and Darras, V.M. (2014). Anatomy, Embryology, and Histology of Thyroid Glands. IN Sturkie's Avian Physiology (Sixth Edition), 2014 ed by: C. Scanes, pp. 535-547. Academic Press, New York, London.

McTiernan, A.M., Weiss, N.S. and Daling, J.R. (1984). Incidence of thyroid cancer in women in relation to previous exposure to radiation therapy and history of thyroid disease. *J. Natl. Cancer Inst*. 73: 575-581.

Maciel, R.M., Moses, A.C., Villone, G., Tramontano, D. and Ingbar, S.H. (1988). Demonstration of the production and physiological role of insulin-like growth factor II in rat thyroid follicular cells in culture. *J. Clin. Invest.* 82: 1546–1553.

Mackenzie, C. and Mackenzie, J.B. (1943): Effect of sulfonamides and thioureas on the thyroid gland and basal metabolism. *Endocrinology* 32: 185-209.

Mackenzie, J.B., Mackenzie, C. G. and McCollum, E. V. (1941): The effect of sulfanyl guanidine on the thyroid in the rat. *Science* 94: 518-519.

Malik, R. and Hodgson, H. (2002). The relationship between the thyroid gland and the liver. *Q. J. Med.* 95: 559–569.

Maraka, S., Mwangi, R., McCoy, R.G., Yao, X., Sangaralingham , L.R., Singh, N.M., O'Keeffe, D.T., Espinosa De Ycaza, A.E., Rodriguez-Gutierrez, R., Coddington, C.C. III., Stan, M.N., Brito, J.P. and Montori, V.M. (2017). Thyroid hormone treatment among pregnant women with subclinical hypothyroidism: US National Assessment. *BMJ* 356:i6865 doi: 10.1136/bmj.i6865

Marinovich, M., Guizzetti, M., Ghilardi, F., Viviani, B., Corsini, E. and Galli, C.L. (1997). Thyroid peroxidase as toxicity target for dithiocarbamates. *Arch. Toxicol.* 71: 508-512.

Marsh, G., Bergman, A., Bladh, L.G., Gillner, M. and Jakobsson, E. (1998). Synthesis of p-hydroxybromo- diphenyl ethers and binding to the thyroid receptor. *Organohalogen Compounds* 37: 305-308.

Marsili, A., Sanchez, E., Singru, P., Harney, J.W., Zavacki, A.M., Lechan, R.M. and Larsen, P.R. (2011). Thyroxine-induced expression of pyroglutamyl peptidase II and inhibition of TSH release precedes suppression of TRH mRNA and requires type 2 deiodinase. *J. Endocrinol.* 211: 73-78.

Mazonakis, M., Tzedakis, A., Damilakis, J. and Gourtsoyiannis, N. (2007). Thyroid dose from common head and neck CT examinations in children: is there an excess risk for thyroid cancer induction? *Eur Radiol.* 17: 1352–1357.

Meek, M.E. (2008). Recent developments in frameworks to consider human relevance of hypothesized modes of action for tumours in animals. *Environ. Mol. Mutagen.* 49: 110–116.

Meek, M. E., Bucher, J. R., Cohen, S. M., Dellarco, V., Hill, R. N., Lehman-McKeeman, L. D., Longfellow, D. G., Pastoor, T., Seed, J. and Patton, D. E. (2003). A framework for human relevance analysis of information on carcinogenic modes of action. *CRC Crit. Rev. Toxicol*. 33: 591–653.

Meek, M.E., Palermo, C.M., Bachman, A.N., North, C.M. and Lewis, R.J. (2014a). Mode of action human relevance (species concordance) framework: Evolution of the Bradford Hill considerations and comparative analysis of weight of evidence. *J. Appl. Toxicol.* 34: 595–606

Meek, M.E., Boobis, A., Cote, I., Dellarco, V., Fotakis, G., Munn, S., Seed, J. and Vickers, C. (2014b). New developments in the evolution and application of the WHO/IPCS framework on mode of action/ species concordance analysis. *J. Appl. Toxicol.* 34: 1-18.

Meerts, I.A., van Zanden, J.J., Luijks, E.A., van Leeuwen-Bol, I., Marsh, G., Jakobsson, E., Bergman, A., and Brouwer, A. (2000). Potent competitive interactions of some brominated flame retardants and related compounds wuth human transthyretin in vitro. *Toxicol. Sci.* 56: 96-104.

Mendel, C.M. and Weisiger, R.A. (1990). Thyroxine uptake by perfused rat liver. No evidence for facilitation by five different thyroxine-binding proteins. *J. Clin Invest*. 86: 1840–1847.

Mendel, C.M. (1989). The free hormone hypothesis – a physiologically based mathematical-model. *Endocr. Rev.* 10: 232–274.

Mendel, C.M., Cavalieri, R.R. and Weisiger, R.A. (1988). Uptake of thyroxine by the perfused rat liver: implications for the free hormone hypothesis. *Am. J. Physiol.* 255: E110–E119.

Merrill, E.A., Clewell, R.A., Gearhart, J.M., Robinson, P.J., Sterner, T.R., Yu, K.O., Mattie, D.R. and Fisher, J.W. (2003). PBPK Predictions of Perchlorate Distribution and Its Effect on Thyroid Uptake of Radioiodide in the Male Rat. *Toxicol. Sci.* 73: 256-269.

Metz, L.D., Seidler, F.J., McCook, E.C. and Slotkin, T.A. (1996). Cardiac alpha-adrenergic receptor expression is regulated by thyroid hormone during a critical developmental period. *J Mol. Cell Cardiol*. 28: 1033–1044.

Miller, M.D., Crofton, K.M., Rice, D.C. and Zoeller, R.T. (2009). Thyroid-disrupting chemicals: interpreting upstream biomarkers of adverse outcomes. *Environ. Health Perspect.* 117: 1033-1041.

Minelli, R., Gardini, E., Bianconi, L., Salvi, M. and Roti, E. (1992). Subclinical hypothyroidism, overt thyrotoxicosis and subclinical hypothyroidism: the subsequent phases of thyroid function in a patient chronically treated with amiodarone. *J. Endocrinol. Invest*. 15: 853–855.

Miyazaki, W., Iwasaki, T., Takeshita, A., Tohyama, C. and Koibuchi, N. (2008). Identification of the functional domain of thyroid hormone receptor responsible for polychlorinated biphenyl-mediated suppression of its action in vitro. *Environ. Health Perspect.* 116: 1231–1236.

Monk, J.A., Sims, N.A., Dziegielewska, K.M., Weiss, R.E., Ramsay, R.G. and Richardson, S.J. (2013). Delayed development of specific thyroid hormone-regulated events in transthyretin null mice. *Am. J. Physiol. Endocrinol. Metab.* 304: E23–E31.

Moore, L.B., Parks, D.J., Jones, S.A., Bledsoe, R.K., Consler, T.G., Stimmel, J.B., Goodwin, B., Liddle, C., Blanchard, S.G., Willson, T.M., Collins, J.L. and Kliewer, S.A. (2000). Orphan Nuclear Receptors Constitutive Androstane Receptor and Pregnane X Receptor Share Xenobiotic and Steroid Ligands*. J..Biol. Chem.* 275: 15122-15127.

Moreno, M., Lombardi, A., Beneduce, L., Silvestri, E., Pinna, G., Goglia, F. and Lanni, A. (2002). Are the Effects of T3 on Resting Metabolic Rate in Euthyroid Rats Entirely Caused by T3 Itself? *Endocrinol*.143: 504–510.

Moriarty, G.C. and Tobin, R.B. (1976). An immunocytochemical study of TSH storage in rat thyroidectomy cells with and without D or L thyroxine treatment. *J. Histochem. Cytochem.* 24: 1140-1149.

Morreale de Escobar, G. (2003). Maternal hypothyroxinemia versus hypothyroidism and potential neurodevelopmental. *Ann. Endocrinol. (Paris)* 64: 51–52.

Morse, D. C., Groen, D., Veerman, M., van Amerongen, C. J., Koeter, H. B., Smits van Prooije, A. E., Visser, T. J., Koeman, J. H. and Brouwer, A. (1993). Interference of polychlorinated biphenyls in hepatic and brain thyroid hormone metabolism in foetal and neonatal rats. *Toxicol. Appl. Pharmacol.* 122: 27–33.

Morte, B., Manzano, J., Scanlan, T., Vennstrom, B. and Bernal. J. (2002). Deletion of the thyroid hormone receptor alpha 1 prevents the structural alterations of the cerebellum induced by hypothyroidism. *Proc Natl Acad Sci U S A*. 99: 3985-3989.

Motomura, K. and Brent, G.A.(1998). Mechanisms of thyroid hormone action. Implications for the clinical manifestation of thyrotoxicosis. *Endocrinol. Metab. Clin. N. Am*. 27: 1–23.

Mullur, R., Liu, Y-Y. and Brent, G.A. (2014). Thyroid Hormone Regulation of Metabolism. *Physiol. Rev.* 94: 355–382.

Murakami, M., Araki, O., Hosoi, Y., Kamiya, Y., Morimura, T., Ogiwara, T., et al. (2001). Expression and regulation of type II iodothyronine deiodinase in human thyroid gland. *Endocrinology* 142: 2961-2967.

Murrell, J.R., Schoner, R.G., Liepnieks, J.J., Rosen, H.N., Moses, A.C. and Benson, M.D. (1992). Production and functional analysis of normal and variant recombinant human transthyretin proteins. *J. Biol. Chem*. 267: 16595-16600.

Narayana, S.K., Woods, D.R. and Boos, C.J. (2011). Management of Amiodarone-Related Thyroid Problems. *Ther. Adv. Endocrinol. Metab.* 2: 115–126.

Narayanan, C.H. and Narayanan, Y. (1985). Cell formation in the motor nucleus and mesencephalic nucleus of the trigeminal nerve of rats made hypothyroid by propylthiouracil. *Exp. Brain Res*. 59: 257–266.

National Toxicology Program (1992). National Toxicology Program Technical Report Series No. 388. NTP technical report on the perinatal toxicology and carcinogenesis studies of ethylene thiourea (CAS No. 96-45-7) in F344/n rats and B6C3F1 mice (feed studies). https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr388.pdf

Neto, A.M. and Zantut-Wittmann, D.E. (2016). Abnormalities of Thyroid Hormone Metabolism during Systemic Illness: The Low T3 Syndrome in Different Clinical Settings," *Int. J. Endocrinol.* 2016, Article ID 2157583, 9 pages, 2016. doi:10.1155/2016/2157583.

Nishikawa, S. (1983). Effects of sulfonamide on the pituitary-thyroid gland. 1. Morphological changes of thyroid gland and variation in plasma thyroxine and triiodothyronine. *J. Toxicol. Pathol.*8: 47-59.

Nishimura, M. and Naito, S. (2008). Tissue-specific mRNA expression profiles of human solute carrier transporter superfamilies. *Drug Metab. Pharmacokinet*. 23: 22–44.

Ng, L., Goodyear, R.J., Woods, C.A., Schneider, M.J., Diamond, E., Richardson, G.P., Kelley, M.W., St. Germain, D.L., Galton, V.A. and Forrest, D. (2004). Hearing loss and retarded cochlear development in mice lacking type 2 iodothyronine deiodinase. *Proc Nat. Acad. Sci.USA.* 101: 3474–3479

Nohr SB, Laurberg P 2000 Opposite variations in maternal and neonatal thyroid function induced by iodine supplementation during pregnancy. *J. Clin. Endocrinol. Metab.* 85: 623–627.

Norford, D.C., Meuten, D.J., Cullen, J.M. and Collins J.J. (1993). Pituitary and Thyroid Gland Lesions Induced by 2-Mercaptobenzimidazole (2-MBI) Inhalation in Male Fischer-344 Rats. *Toxicol. Pathol.* 21: 456-464.

NTP (2006). NTP Technical report on the toxicology and carcinogenesis studies of 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153) (CAS No. 35065-27-1) in female Harlan Sprague-Dawley rats (gavage studies). NTP: TR 529. NIH Publication No. 06-4465 National Institutes of Health Public Health Service U.S. Department of Health and Human Services.

Obregon, M.J., Calvo, R.M., Del Rey, F.E. and de Escobar, G.M. (2007). Ontogenesis of thyroid function and interactions with maternal function. *Endocr Dev.* 10: 86–98.

OECD (2012). OECD Guideline for the Testing of Chemicals. Extended One-Generation Reproductive Toxicity Study. TG443. https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd-tg443-508.pdf Accessed 30/10/2017.

Oetting, A. and Yen, P.M. (2007). New insights into thyroid hormone action. *Best Pract. Res. Clin. Endocrinol. Metab*. 21: 193–208.

O'Donnell, K.J., Rakeman, M.A., Zhi-Hong, D., Xue-Yi, C., Mei, Z.Y., DeLong, N., Brenner, G., Tai, M., Dong, W. and DeLong, G.R. (2002). Effects of iodine supplementation during pregnancy on child growth and development at school age. *Dev. Med. Child Neurol.* 44: 76–81.

O'Malley, B.P., Davies, T.J. and Rosenthal, F.D. (1980). TSH responses to temperature in primary hypothyroidism. *Clin. Endocrinol*. 13: 87-94.

Omiecinski, C.J., Vanden Heuvel, J.P., Perdew, G.H. and Peters, J.M. (2011). Xenobiotic Metabolism, Disposition, and Regulation by Receptors: From Biochemical Phenomenon to Predictors of Major Toxicities. *Toxicol. Sci.* 120: S49-S75.

Oppenheimer, J. H., Bernstein, G. and Surks, M. I. (1968). Increased thyroxine turnover and thyroidal function after stimulation of hepatocellular binding of thyroxine by phenobarbital. *J. Clin. Invest.* 47: 1399–1406.

Oppenheimer, J.H., Schwartz, H.L., Lane, J.T. and Thompson, M.P. (1991). Functional relationship of thyroid hormone-induced lipogenesis, lipolysis, and thermogenesis in the rat. *J. Clin. Invest*. 87: 125–132.

Ord, W.M. (1898). The Bradshaw Lecture on Myxoedema and Allied Disorders: Delivered before the Royal College of Physicians. *Br. Med. J*. 2: 1473-1479.

Ohtake, M., Bray, G.A. and Azukizawa, M. (1977). Studies on hypothermia and thyroid function in the obese (ob/ob) mouse," *Am. J. Physiol*. 2: 110– 115.

Paynter, O.E., Burin, G.J., Jaeger, R.B. and Gregorio, C.A. (1988). Goitrogens and thyroid follicular cell neoplasia: evidence for a threshold process. *Regul. Toxicol. Pharmacol*. 8: 102-119.

Palha, J.A., Fernandes, R., de Escobar, G.M., Episkopou, V., Gottesman, M. and Saraiva, M.J. (2000). Transthyretin regulates thyroid hormone levels in the choroid plexus, but not in the brain parenchyma: study in a transthyretin null mouse model. *Endocrinology* 141: 3267–3272.

Palha, J., Nissanov, J., Fernandes, R., Sousa, J., Bertrand, L., Dratman, M., Morreale de Escobar, G., Gottesman, M. and Saraiva, M. (2002). Thyroid hormone distribution in the mouse brain: the role of transthyretin. *Neuroscience* 113: 837–847.

Palha, J.A., Episkopoull, V., Maeda, S., Shimada, K., Gottesman, M.E. and Saraiva, M.J.M. (1994). Thyroid Hormone Metabolism in a Transthyretin-null Mouse Strain. *J.Biol. Chem.* 269: 33135-33139.

Panda, J.N. and Turner, C.W. (1975). Effect of thyroidectomy and low environmental temperature (4.4^{o}C) upon plasma and pituitary thyrotrophin in the rat. *Acta Endocrinol*. 54: 485-493.

Parker, S.L., Tong, T., Bolden, S. and Wingo, P.A. (1997). Cancer statistics. *CA-Cancer J. Clin.* 47: 5-27.

Parmentier, M., Libert, F., Maenhaut, C., Lefort, A., Gérard, C., Perret, J., Van Sande, J., Dumont, J.E. and Vassart, G. (1989). "Molecular cloning of the thyrotropin receptor". *Science.* 246 (4937): 1620–1622.

Paul, K. (2014). Using adverse outcome pathway analysis to identify gaps in high-throughput screening for thyroid disruption. Bayer CropScience. https://ntp.niehs.nih.gov/iccvam/meetings/aop-wksp-2014/presentations/paul-508.pdf . Accessed 29/10/2017

Pedersen, K.M., Laurberg, P., Iversen, E., Knudsen, P.R., Gregersen, H.E., Rasmussen, O.S., Larsen, K.R., Eriksen, G.M. and Johannesen, P.L. (1993) Amelioration of some pregnancy associated variations in thyroid function by iodine supplementation. *J. Clin. Endocrinol. Metab*. 77: 1078–1083.

Peeters, R.P., Kester, M.H., Wouters, P.J., Kaptein, E., van Toor, H., Visser, T.J., et al. (2005). Increased thyroxine sulfate levels in critically ill patients as a result of a decreased hepatic type I deiodinase activity. *J. Clin. Endocrinol. Metab.* 90: 6460-6465.

Pendergrast, W.J., Milmore, B.K. and Marcus, S.C. (1961). Thyroid cancer and thyrotoxicosis in the United States: their relation to endemic goitre *J. Chronic Dis*. 13: 22-38.

Pizzagalli, F., Hagenbuch, B., Stieger, B., Klenk, U., Folkers, G. and Meier, P.J. (2002). Identification of a novel human organic anion transporting polypeptide as a high affinity thyroxine transporter. *Mol. Endocrinol.* 16: 2283–2296.

Porterfield, S.P. and Hendrich, C.E. (1993). The role of thyroid hormones in prenatal and neonatal neurological development – current perspectives. *Endocr Rev.* 14: 94–106.

Power, D.M., Elias, N.P., Richardson, S.J., Mendes, J., Soares, C.M. and Santos, C.R.A. (2000). Evolution of the thyroid hormone-binding protein, transthyretin. *Gen. Comp. Endocrinol.* 119: 241–255.

Puigserver, P., Wu, Z., Park, C.W., Graves, R., Wright, M. and Spiegelman, B.M. (1998). A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell* 92: 892-839.

Radominska-Pandya, A., Ouzzine, M., Fournel-Gigleux, S. and Magdalou, J. (2005). Structure of UDP-glucuronosyltransferases in membranes. *Methods Enzymol.* 400: 116-147.

Reinehr, T. (2010). Obesity and thyroid function. *Mol. Cell Endocrinol.* 316: 165–171.

Ren, S.G., Malozowski, S., Simoni, C., Garcia, H., Caruso-Nicoletti, M., Cutler, G.B. Jr. and Cassorla, F. (1988). Dose-response relationship between thyroid hormone and growth velocity in cynomolgus monkeys. *J. Clin. Endocrinol. Metab.* 66: 1010-1013.

Renehan, A.G., Tyson, M., Egger, M., Heller, R.F. and Zwahlen, M. (2008). Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. *Lancet.* 371: 569–578.

Ribeiro, R.C., Kushner, P.J. and Baxter, J.D. (1995). The nuclear hormone receptor gene superfamily. *Ann. Rev. Med.* 46: 443–453.

Riccabona, G. (1986). Thyroid cancer and other surgical problems in the areas of endemic goitre. In: Dunn, J.T., Pretell, E.A., Daza, C.H., Viteri, F.E. (eds) Towards the Eradication of Endemic Goitre, Cretenism, and Iodine Deficiency. Pan American Health Organization, Washington DC, p 68

Riccabona, G. (1987). Correlation of thyroid cancer with endemic goitre. In: Thyroid Cancer, Its Epidemiology, Clinical Features and Treatment. Springer-Verlag, Berlin, p 6-14.

Richardson, S.J. (2007). Cell and molecular biology of transthyretin and thyroid hormones. *Int. Rev. Cytol*. 258: 137–193.

Richardson, V.M., Ferguson, S.S., Sey, Y.M. and DeVito, M.J. (2014). In vitro metabolism of thyroxine by rat and human hepatocytes. *Xenobiotica* 44: 391-403.

Rinaldi, S., Lise, M., Clavel-Chapelon, F., Boutron-Ruault, M.C., Guillas, G., Overvad, K., et al. (2012). Body size and risk of differentiated thyroid carcinomas: findings from the EPIC study. *Int. J. Canc.* 131: E1004–E1014.

Robbins, J. (2000). Thyroid hormone transport proteins and the physiology of hormone binding. In: Braverman, L.E., Utiger, R.D., editors. Werner and Ingbar's, the thyroid, 8th ed. Philadelphia: Lippincott Williams & Wilkins. Pp. 105–120.

Robbins, J. (2002). Transthyretin from discovery to now. *Clin. Chem. Lab. Med.* 40: 1183–1190.

Roberts, L.M., Woodford, K., Zhou, M., Black, D.S., Haggerty, J.E., Tate, E.H., Grindstaff, K.K., Mengesha, W., Raman, C., Zerangue, N. (2008). Expression of the thyroid hormone transporters monocarboxylate transporter-8 (SLC16A2) and organic ion transporter-14 (SLCO1C1) at the blood-brain barrier. *Endocrinology.* 149: 6251-6261.

Rosene, M.L., Wittmann, G., Arrojo e Drigo, R., Singru, P.S., Lechan, R.M. and Bianco, A.C. (2010). Inhibition of the Type 2 Iodothyronine Deiodinase Underlies the Elevated Plasma TSH Associated with Amiodarone Treatment. *Endocrinol*. 151: 5961–5970.

Ross, D.S., Daniels, G.H. and Dienstag, J.L. and Ridgway, E.C. (1983). Elevated thyroxine levels due to increased thyroxine-binding globulin in acute hepatitis. *Am. J. Med.* 74: 564-569.

Rouaze-Romet, M., Savu, L., Vranckx, R., Bleiberg-Daniel, F., Le Moullac, B., Gouache, P. and Nunez, E.A. (1992). Re-expression of thyroxine-binding globulin in post-weaning rats during protein or energy malnutrition. *Acta Endocrinol (Copenh)* 127: 441–448.

Rousset, B. and Cure, M. (1975). Variations of rat thyroid activity during exposure to high environmental temperature (34°C). Relation between hypothalamic pituitary and thyroid hormone levels. *Pflugers Arch*. 354: 101-115.

Rousset B, Dupuy C, Miot F, et al. Chapter 2 Thyroid Hormone Synthesis And Secretion. [Updated 2015 Sep 2]. In: De Groot LJ, Chrousos G, Dungan K, et al., editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK285550/

Ruiz-Marcos, A., Abella, P.C., Garcia, A.G., Escobar del Rey, F. and de Escobar, G.M. (1988). Rapid effects of adult-onset hypothyroidism on dendritic spines of pyramidal cells of the rat cerebral cortex. *Exp. Brain Res.* 73: 583-588.

Russfield, A.B. (1967). Pathology of the endocrine glands, ovary and testis of rats and mice. In: Pathology of Laboratory Rats and Mice, E. Cotchin and F.J. Roe (eds). Blackwell Scientific Publications, Oxford, pp. 391-403.

Saberi, M., Sterling, F.H. and Utiger, R.D. (1974). Reduction in Extrathyroidal Triiodothyronine Production by Propylthiouracil in Man. *J.Clin. Invest.* 55: 218-223.

Sadamatsu, M., Kanai, H., Xu, X., Liu, Y. and Kato, N. (2006). Review of animal models for autism: implication of thyroid hormone. *Congenital Abnormalities* 46: 1–9.

Santini, F., Chopra, I.J., Wu, S.Y., Solomon, D.H. and Chua, T.G.N. (1992). Metabolism of 3,5,3'-triiodothyronine sulfate by tissues of the fetal rat: a consideration of the role of desulfation of 3,5,3'-triiodothyronine sulfate as a source of T3. *Pediatr Res.* 31: 541-544.

Santoro, M., Chiappetta, G., Cerrato, A., Salvatore, D., Zhang, L., Manzo, G., Picone, A., Portella, G., Santelli, G., Vecchio, G. and Fusco, A. (1996) Development of thyroid papillary carcinomas secondary to tissue-specific expression of the RET/PTC1 oncogene in transgenic mice. *Oncogene* 12: 1821–1826.

Sarne, D. (2016). Effects of the Environment, Chemicals and Drugs on Thyroid Function. [Updated 2016 Sep 27]. In: De Groot LJ, Chrousos G, Dungan K, et al., editors. Endotext [Internet]. South

Dartmouth (MA): MDText.com, Inc.; 2000-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK285560/

Savu, L., Vranckx, R., Maya, M. and Nunez, E.A. (1987). A thyroxine binding globulin (TBG)-like protein in the sera of developing and adult rats. *Biochem Biophys Res Commun* 148: 1165–1173.

Savu, L., Vranckx, R., Rouaze-Romet, M., Maya, M., Nunez, E,A,, Reton, J. and Flink, I.L. (1991). A senescence up-regulated protein: the rat thyroxine-binding globulin (TBG). *Biochim Biophys Acta* 1097: 19–22.

Scanlon, M.F. and Toft, A.D. (2000). Regulation of thyrotropin secretion In: Braverman, L.E., Utiger, R.D., editors. *Werner and Ingbar's, the thyroid*, 8th ed. Philadelphia: Lippincott Williams & Wilkins. pp 234–253.

Schuetz, E.G., Brimer, C. and Schuetz, J.D. (1998). Environmental xenobiotics and the antihormones cyproterone acetate and spironolactone use the nuclear hormone pregnenolone X receptor to activate the CYP3A23 hormone response element. *Mol. Pharmacol.* 54: 1113–1117.

Schlenker, E.H., Hora, M., Liu, Y., Redetzke, R.A., Morkin, E. and Gerdes, A.M. (2008). Effects of thyroidectomy, T4, and DITPA replacement on brain blood vessel density in adult rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 294: R1504-1509.

Schneider, M.J., Fiering, S.N., Pallud, S.E., Parlow, A.F., St Germain, D.L. and Galton, V.A. (2001). Targeted disruption of the type 2 selenodeiodinase gene (DIO2) results in a phenotype of pituitary resistance to T4. *Mol Endocrinol.* 15: 2137-2148.

Schneider, M.J., Fiering, S.N., Thai, B., Wu, S.Y., St Germain, E., Parlow, A.F., St Germain, D.L. and Galton, V.A. (2006). Targeted disruption of the type 1 selenodeiodinase gene (Dio1) results in marked changes in thyroid hormone economy in mice. *Endocrinol.* 147: 580-589.

Schroder-van der Elst, J. P., van der Heide, D., Rokos, H., Morreale de Escobar, G. & Kohrle, J. (1998). Synthetic flavonoids cross the placenta in the rat and are found in foetal brain. *Am. J. Physiol.* 274: E253–E256.

Schussler, G.C. (2000). The thyroxine-binding proteins. *Thyroid.* 10: 141-149.

Schussler, G.C., Schaffner, F. and Korn, F. (1978). Increased serum thyroid hormone binding and decreased free hormone in chronic active liver disease. *N. Engl. J. Med.* 299: 510-515.

Schuur, A. G., Legger, F. F., van Meeteren, M. E., Moonen, M. J., van Leeuwen-Bol, I., Bergman, A., Visser, T. J. and Brouwer, A. (1998). In vitro inhibition of thyroid hormone sulfation by hydroxylated metabolites of halogenated aromatic hydrocarbons. *Chem. Res. Toxicol*. 11: 1075–1081.

Schwartz, C.E. and Stevenson, R.E. (2007). The MCT8 thyroid hormone transporter and Allan-Herndon-Dudley syndrome. *Best Pract. Res. Clin. Endocrinol. Metab.* 21: 307–321.

Seibert, F.B. and Nelson, J.W. (1942). Electrophoretic study of the blood protein response in tuberculosis. *J. Biol. Chem.* 143: 29–38.

Seoane, L.M., Carro, E., Tovar, S., Casanueva, F.F. and Dieguez, C. (2000). Regulation of in vivo TSH secretion by leptin. *Regul. Pept.* 92: 25–29.

Sharlin, D.S., Tighe, D., Gilbert, M.E. and Zoeller, R.T. (2008). The balance between oligodendrocyte and astrocyte production in major white matter tracts is linearly related to serum total thyroxine. *Endocrinology* 149: 2527–2536.

Sharlin, D.S., Visser, T.J. and Forrest, D. (2011). Developmental and cell-specific expression of thyroid hormone transporters in the mouse cochlea. *Endocrinology* 152: 5053–5064.

Shopsin, B., Shenkman, L., Blum, M. and Hollander CS. (1973). Iodine and lithium-induced hypothyroidism. Documentation of synergism. *Am. J. Med.* 55: 695-699.

Siegel, R.D. and Lee, S.L. (1998). Toxic nodular goitre. Toxic adenoma and toxic multinodular goitre. *Endocrinol. Metab. Clin. North Am.* 27: 151-168.

Silva, J.E. and Larsen, P.R. (1985). Potential of brown adipose tissue type II thyroxine 5'-deiodinase as a local and systemic source of triiodothyronine in rats. *J. Clin. Invest*. 76: 2296-2305.

Singh, R., Upadhyay, G., Kumar, S., Kapoor, A., Kumar, A., Tiwari, M. and Godbole, M.M. (2003). Hypothyroidism alters the expression of Bcl-2 family genes to induce enhanced apoptosis in the developing cerebellum. *J. Endocrinol.* 176: 39-46.

Smith, P.F., Grossman, S.J., Gerson, R.J., Gordon, L.R., Deluca, J.G., Majka, J.A., Wang, R.W., Germershausen, J.I. and Macdonald, J.S. (1991). Studies on the Mechanism of Simvastatin-Induced Thyroid Hypertrophy and Follicular Cell Adenoma in the Rat. *Toxicol. Pathol.* 19: 197-205.

Sonich-Mullin, C., Fielder, R., Wiltse, J. Baetcke, K., Dempsey, J., Fenner-Crisp, P., Grant, D., Hartley, M., Knaap, A., Kroese, D., Mangelsdorf, I., Meek, E., Rice, J.M. and Younes, M. (2001). IPCS Conceptual Framework for Evaluating a Mode of Action for Chemical Carcinogenesis. *Reg. Toxicol. Pharmacol.* 34: 146–152.

Sousa, J., de Escobar, G.M., Oliveira, P., Saraiva, M. and Palha, J. (2005). Transthyretin is not necessary for thyroid hormone metabolism in conditions of increased hormone demand. *J. Endocrinol*. 187: 257–266.

Sugiyama, D., Kusuhara, H., Taniguchi, H., Ishikawa, S., Nozaki, Y., Aburatani, H. and Sugiyama, Y. (2003). Functional characterization of rat brain-specific organic anion transporter (Oatp14) at the blood-brain barrier: high affinity transporter for thyroxine. *J. Biol. Chem*. 278: 43489 – 43495.

Suzuki, T., Kohno, H., Sakurada, T., Tadano, T. and Kisara, K. (1982). Intracranial injection of thyrotropin releasing hormone (TRH) suppresses starvation-induced feeding and drinking in rats. *Pharmacol. Biochem. Behav.* 17: 249–253.

Swarm, R.I., Roberts, G.K.S. Levy, A.C. and Hines, L.R. (1973). Observations on the thyroid glands in rats following administration of sulphomethoxazole and trimethoprim. *Toxicol. Appl. Pharmacol.* 24: 351-363.

Szabo, M. and Frohman, L.A. (1977). Suppression of cold-stimulated thyrotropin secretion by antiserum to thyrotropin-releasing hormone. *Endocrinology* 101: 1023-1033.

Tabb, M.M., Kholodovych, V., Grun, F., Zhou, C., Welsh, W.J. and Blumberg, B. (2004). Highly chlorinated PCBs inhibit the human xenobiotic response mediated by the steroid and xenobiotic receptor (SXR). *Environ Health Perspect.* 112: 163–169.

Takayama, S., Aihara, K., Onodera, T. and Akimoto, T. (1986). Anti-thyroid effects of propylthiouracil ans sulphamonomethoxine in rats and monkeys. *Toxicol. Appl. Pharmacol.* 82: 191-199.

Tani, Y., Mori, Y., Miura, Y., Okamoto, H., Inagaki, A., Saito, H. and Oiso, Y. (1994). Molecular cloning of the rat thyroxine-binding globulin gene and analysis of its promoter activity. *Endocrinology* 135: 2731–2736.

Tani, Y., Maronpot, R.R., Foley, J.F., Haseman, J.K., Walker, N.J. and Nyska, A. (2004). Follicular epithelial cell hypertrophy induced by chronic oral administration of 2,3,7,8-tetrachlorodibenzo-p-dioxin in female Harlan Sprague-Dawley rats. *Toxicol Pathol.* 32: 41-49.

Tanjasiri, P., Kozbur, X. and Florsheim, W.H.(1976). Somatostatin in the physiologic feedback control of thyrotropin secretion. *Life Sci*. 19: 657–660.

Thomas, G.A. and Williams, E.D. (1991). Evidence for and possible mechanisms of non-genotoxic carcinogenesis in the rodent thyroid. *Mutat. Res*. 248: 357-370.

Thompson, C.C. and Potter, G.B. (2000). Thyroid hormone action in neural development. *Cereb. Cortex* 10: 939-945.

Tohyama, K., Kusuhara, H., and Sugiyama, Y. (2004). Involvement of multi-specific organic anion transporter, Oatp14 (Slc21a14), in the transport of thyroxine across the blood-brain barrier. *Endocrinology* 145: 4384 – 4391.

Trajkovic, M., Visser, T.J., Mittag, J., Horn, S., Lukas, J., Darras, V.M., Raivich, G., Bauer, K. and Heuer, H. (2007). Abnormal thyroid hormone metabolism in mice lacking the monocarboxylate transporter 8. *J. Clin. Invest*. 117: 627– 635.

Trajkovic, M., Visser, T.J., Mittag, J., Horn, S., Lukas, J., Darras, V.M., Raivich, G., Bauer, K. and Heuer, H. (2007). Abnormal thyroid hormone metabolism in mice lacking the monocarboxylate transporter-8. *J. Clin. Invest.* 117: 627–635.

Tsukahara, F., Uchida, Y., Ohba, K., Nomoto, T. and Muraki, T. (1997). Defective stimulation of thyroxin 5'-deiodinase activity by cold exposure and norepinephrine in brown adipose tissue of monosodium glutamate-obese mice. *Horm. Metab. Res*. 29: 496-500.

Uemichi, T., Murrell, J.R., Zeldemrust, S., and Benson, M.D. (1992). A new mutant transthyretin (Arg10) associated with familial amyloid polyneuropathy. J. Med. Genet. 29: 888-891.

U.S. EPA. (1998). Assessment of thyroid follicular cell tumors. Washington, DC: EPA Document No. DC EPA/630/R-97/002.

Van Der Haar, F. (2007). Goitre and other iodine deficiency disorders: a systematic review of epidemiological studies to deconstruct the complex web. *Arch Med Res.* 38: 586-587.

Van der Deure, W.M., Peeters, R.P. and Visser, T.J. (2010). Molecular aspects of thyroid hormone transporters, including MCT8, MCT10, and OATPs, and the effects of genetic variation in these transporters. *J. Mol. Endocrinol.* 44: 1–11.

van der Watt, G., Haarburger, D. and Berman P (2008). Euthyroid patient with elevated serum free thyroxine. *Clin. Chem.* 54: 1239–1241.

Vansell, N.R. and Klaassen, C.D. (2002). Increase in rat liver UDP glucuronosyltransferase mRNA by microsomal enzyme inducers that enhance thyroid hormone glucuronidation. *Drug Metab. Dispos.* 30: 240–246.

Vansell, N.R., Muppidi, J.R., Habeebu, S.M. and Klaassen, C.D. (2004). Promotion of Thyroid Tumors in Rats by Pregnenolone-16aCarbonitrile (PCN) and Polychlorinated Biphenyl (PCB). *Toxicol. Sci.* 81: 50–59.

Verburg, F.A. and Reiners, C. (2010). The association between multinodular goitre and thyroid cancer. *Minerva Endocrinol.* 35: 187-192.

Verge, C.F., Konrad, D., Cohen, M., Di Cosmo, C., Dumitrescu, A.M., Marcinkowski, T., Hameed, S., Hamilton, J., Weiss, R.E. and Refetoff, S. (2012). Diiodothyropropionic acid (DITPA) in the treatment of MCT8 deficiency. *J. Clin. Endocrinol. Metab.* 97: 4515–4523.

Vijayan, E. and McCann, S.M. (1977). Suppression of feeding and drinking activity in rats following intraventricular injection of thyrotropin releasing hormone (TRH). *Endocrinol*. 100: 1727–1729.

Mathieu Vinken, M., Knapen, D., Vergauwen, L., Hengstler, J.G., Angrish, M. and Whelan, M. (2017). Adverse outcome pathways: a concise introduction for toxicologists. *Arch Toxicol.* 91; 3697–3707.

Visser, T. J. (1988) in Hormones and Their Actions, Part I (Cooke, B. A., King, R. J. B. & Van der Molen, H. J., eds.), pp. 81-103, Elsevier Science Publishers, Amsterdam

Visser, T.J. (1994). Role of sulfation in thyroid hormone metabolism. *Chem. Biol. Interact.* 92: 293-303.

Visser, T.J. (1996). Pathways of thyroid hormone metabolism. *Acta Med. Austriaca*. 23: 10-16.

Visser, T.J., Kaptein, E., van Raaij, J.A., Joe, C.T., Ebner, T. and Burchell, B. (1993). Multiple UDP-glucuronyltransferases for the glucuronidation of thyroid hormone with preference for 3,3',5'-triiodothyronine (reverse T3). *FEBS Lett* 315: 65–68.

Visser, T. J., Kaptein, E., van Toor, H., van Raaij, J. A., van den Berg, K. J., Joe, C. T., van Engelen, J. G. and Brouwer, A. (1993). Glucuronidation of thyroid hormone in rat liver: effects of in vivo treatment with microsomal enzyme inducers and in vitro assay conditions. *Endocrinol.* 133: 2177–2186.

Visser, W.E., Visser,T.J. and Peeters, R.P. (2016). Metabolism of thyroid hormone. http://www.thyroidmanager.org/wp-content/uploads/chapters/metabolism-of-thyroid-hormone.pdf Accessed 21st Oct 2017.

Visser, W.E., Friesema, E.C. and Visser, T.J. (2011). Minireview: thyroid hormone transporters: the knowns and the unknowns. *Mol. Endocrinol*. 25: 1–14.

Visser, T.J., Rutgers, M., de Herder, W.W., Rooda, S.J. and Hazenberg, M.P. (1988). Hepatic metabolism, biliary clearance and enterohepatic circulation of thyroid hormone. *Acta Med Austriaca.* 15 Suppl 1: 37-39.

Vitagliano, D., Portella, G., Troncone, G., Francione, A., Rossi, C., Bruno, A., Giorgini, A., Coluzzi, S., Nappi, T.C., Rothstein, J.L., Pasquinelli, R., Chiappetta, G., Terracciano, D., Macchia, V., Melillo, R.M., Fusco, A., Santoro, M. (2006) Thyroid targeting of the N-ras(Gln61Lys) oncogene in transgenic mice results in follicular tumors that progress to poorly differentiated carcinomas. *Oncogene* 25: 5467–5474.

Vranckx, R., Rouaze, M., Savu, L., Nunez, E.A., Beaumont, C., Flink, I.L. (1990a). The hepatic biosynthesis of rat thyroxine binding globulin (TBG): demonstration, ontogenesis, and up-regulation in experimental hypothyroidism. *Biochem. Biophys. Res. Commun.* 167: 317–322.

Vranckx, R., Savu, L., Maya, M. and Nunez, E.A. (1990b). Characterization of a major development-regulated serum thyroxine-binding globulin in the euthyroid mouse. *Biochem. J.* 271: 373–379.

Vranckx, R., Savu, L., Maya, M., Rouaze-Romet, M. and Nunez, E.A. (1990). Immunological quantification of rat and mouse thyroxine-binding globulins. Ontongenesis and sex-dependence of the circulating levels of the thyroxine-binding globulins. *Acta. Endocrinol.* 123: 649–656.

Wagner, M.S., Morimoto, R., Dora, J.M., Benneman, A., Pavan, R. and Maia, A.L. (2003). Hypothyroidism induces type 2 iodothyronine deiodinase expression in mouse heart and testis. *J. Mol. Endocrinol*. 31: 541-550.

Wang, L. Q., Falany, C. N. and James, M. O. (2004) Triclosan as a substrate and inhibitor of 3'-phosphoadenosine 5'-phosphosulphate-sulphotransferase and UDP-glucuronosyl transferase in human liver fractions. *Drug Metab. Dispos.* 32: 1162–1169.

Wang, L. Q. and James, M. O. (2006) Inhibition of sulfotransferases by xenobiotics. *Curr. Drug Metab.* 7: 83–104.

Warren, M.P. (2011). Endocrine manifestations of eating disorders. *J. Clin. Endocrinol. Metab*. 96: 333–343.

Williams, E.D. (1995) Mechanisms and pathogenesis of thyroid cancer in animals and man. *Mutat. Res*. 333: 123-129.

Williams, G.R. (2008). Neurodevelopmental and neurophysiological actions of thyroid hormone. *J. Neuroendocrinol.* 20: 784-794.

Wolff, J. (1998). Perchlorate and the thyroid gland. *Pharmacol Rev.* 50: 89–105.

Wong, C-C., Döhler, K-D., Atkinson, M.J., Geerlings, H., Hesch, R-D. and von zur Mühlen, A. (1983). Influence of age, strain and season on diurnal periodicity of thyroid stimulating hormone, thyroxine, triiodothyronine and parathyroid hormone in the serum of male laboratory rats. *Acta Endocrinol*. 102 377-385.

Wu, S.Y., Green, W.L., Huang, W.S., Hays, M.T. and Chopra, I.J. (2005). Alternate pathways of thyroid hormone metabolism. *Thyroid.* 15: 943-958.

Wu, S., Polk, D., Wong, S., Reviczky, A., Vu, R., Fisher, D.A. (1992). Thyroxine sulfate is a major thyroid hormone metabolite and a potential intermediate in the monodeiodination pathways in fetal sheep. *Endocrinology* 131: 1751-1756.

Yen, P.M. (2003). Molecular basis of resistance to thyroid hormone. *Trends Endocrinol. Metab.* 14: 327-333.

Yu, K.O., Narayanan, L., Mattie, D.R., Godfrey, R.J., Todd, P.N., Sterner, T.R., Mahle, D.A., Lumpkin, M.H. and Fisher. J.W. (2002). The pharmacokinetics of perchlorate and its effect on the hypothalamus-pituitary-thyroid axis in the male rat. *Toxicol. Appl. Pharmacol.* 182: 148-159.

Żerek-Meleń, G., Lewiński, A., Pawlikowski, M. SewerynekJ, E. and Kunert-Radek, J. (1987). Influence of somatostatin and epidermal growth factor (EGF) on the proliferation of follicular cells in the organ-cultured rat thyroid. *Res. Exp.Med.* 187: 415–421.

Zhang, Y., Guo, G.L., Han, X., Zhu, C., Kilfoy, B.A., Zhu, Y., Kilfoy, B.A., Zhu, Y., Boyle, P. and Zheng, T. (2008). Do polybrominated diphenyl ethers (PBDEs) increase the risk of thyroid cancer? *Biosci. Hypotheses.* 1: 195–199.

Zimmermann, M. B. (2009). Iodine deficiency. *Endocrine Rev*. 30, 376–408.

Zimmermann, M.B. and Galetti, V. (2015). Iodine intake as a risk factor for thyroid cancer: a comprehensive review of animal and human studies. *Thyroid Res.* 8: 8. DOI 10.1186/s13044-015-0020-8

Zoeller, R.T. and Rovet, J. (2004). Timing of thyroid hormone action in the developing brain: clinical observations and experimental findings. *J. Neuroendocrinol.* 16: 809–818.