Title: Mechanisms by Which Inducers of Drug Metabolizing Enzymes Alter Thyroid
Hormones in Rats

Author: Nichole R Vansell, PhD, DABT

Pfizer Inc, Groton, CT (NRV)

No author has an actual or perceived conflict of interest with the contents of this article

Running Title: Mechanisms of Induction of Altered Thyroid Hormones

Corresponding Author: Nichole R. Vansell, Pfizer Inc, Eastern Point Road, Groton, CT

06340; nichole.vansell@pfizer.com; 860-271-9954

Text pages: 15

Number of tables: 1

Number of figures: 1

Number of references: 142

Words in Abstract: 248

Nonstandard abbreviations: TSH, thyroid stimulating hormone; T4, thyroxine; T3, triiodothyronine PCN, pregnenolone-16 α -carbonitrile; UGT, UDP-glycosyltransferase; CAR, constitutive androgen receptor; PXR, pregnane X receptor; AhR, aryl hydrocarbon receptor; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; PCB, polychlorinated biphenyl; AhR, aryl hydrocarbon receptor; PB, phenobarbital; DDT, dichlorodiphenyltrichloroethane; 3-MC, 3-methylcholanthrene; SD, Sprague Dawley; Oatp, organic anion transporter polypeptide; Ntcp, sodium taurocholate cotransporting polypeptide; Mrp, multidrug resistance protein; LAT, L-type transporters; DMP 904, 4-(3-pentylamino)-2,7-dimethyl-8-(2-methyl-4-methoxyphenyl)-pyrazolo-[1,5-a]-pyrimidine

Abstract

Increased disposition of thyroid hormones is a way that xenobiotics may alter thyroid homeostasis and in rats, produce thyroid follicular adenoma/carcinoma. This capacity is historically attributed to induction of T4 glucuronidation by UGT enzymes, and cytochrome P450 induction is often a surrogate. However, gaps exist in correlating the effectiveness of certain chemical inducers at increasing T4 glucuronidation with decreases in systemic T4 and resulting increases in TSH. With the identification of other key inducible drug processing genes and proteins involved in hepatic disposition of thyroid hormones, including uptake (eg, Oatps) and efflux (eg, Mrps) transporters, data exist that support transporters as additional targets sites of induction. These data are reviewed herein and indicate an increase in hepatic uptake of thyroid hormones, as well as increased biliary excretion of iodothyronine conjugates, represent critical activities that differentiate inducer effectiveness in disrupting thyroid hormones in rats. Increased membrane transport of thyroid hormones, likely in conjunction with induced glucuronidation of thyroid hormone (T3 more relevant than T4), provide a better indication of thyroid disrupting potential than consideration of UGT induction alone. Because coordinate regulation of these targets is inconsistent among inducers belonging to various classes and among species, and there are disparities between in vitro assays and in vivo responses, further work is required to identify specific and relevant inducible thyroid hormone uptake transporters. Data from Mrp2-null animals has contributed key information, yet the contributions of efflux transport (canalicular and basolateral) to the mechanism of individual, effective inducers also requires further study.

Significance Statement:

Key advances in understanding the target sites for altered disposition of thyroid hormones have occurred in the last 2 decades to better inform potential sites of action of inducing chemicals. Ultimately, the knowledge of inducible thyroid hormone transport into and out of liver, beyond induction of glucuronidation, should be considered and applied to screening and risk assessment paradigms when assessing an inducer's potential to alter thyroid homeostasis in nonclinical species and humans.

Introduction

Thyroid hormones are essential for growth and development and regulate a multitude of metabolic processes. Thyroid hormones are formed from thyroglobulin in the thyroid gland, where production and secretion are stimulated by TSH as part of maintenance by the hypothalamus-pituitary-thyroid axis that regulates available T3 in the tissues. Circulating thyroid hormone consists mainly of T4, which undergoes outer ring deiodination to form the active hormone T3, or which can be deactivated primarily by inner ring deiodination, sulfation, or glucuronidation, and, along with subsequent metabolites, excreted (Wu et al., 2005; van der Spek et al., 2017). Some hydrolysis of glucuronides occurs in the intestine with the resultant hormone reabsorbed into systemic circulation while the remaining conjugated hormones are eliminated in the feces. Liver plays an essential role in determining thyroid hormone bioavailability (Piantanida et al., 2020). In addition to deiodinase activity, the liver extracts 5–10% of plasma T4 during a single passage, thus influencing T4 blood levels; liver also

synthesizes the major thyroid hormone binding proteins, which provide a rapidly exchangeable pool of circulating thyroid hormone.

The phenomenon that exposure to chemicals can increase the biliary excretion and disposition of drugs and endogenous substances became evident over 50 years ago (Conney, 1967; Goldstein and Taurog, 1968; Klaassen and Plaa, 1968; Klaassen, 1970b; Klaassen, 1970a). It was recognized that some chemicals such as phenobarbital (Oppenheimer et al., 1968) and PCN (Thompson and Klaassen, 1995) altered the biliary excretion of xenobiotics and endogenous substances (like thyroid hormone). The presumption that thyroid hormones cross membranes by passive diffusion such that glucuronidation is the rate limiting step in thyroid hormone disposition (Bastomsky, 1973; Bastomsky and Papapetrou, 1973), an identified correlation between chemically induced T4 glucuronidation activity and the lowering of systemic T4 (McClain et al., 1989; De Sandro et al., 1991; Barter and Klaassen, 1992a; Barter and Klaassen, 1992b; Liu et al., 1995), and the association of an increase in circulating TSH produced by some of these chemicals with thyroid gland stimulation, follicular cell proliferation, or growth (Japundzic et al., 1976; Hood et al., 1999a; Hood et al., 1999b) built the basis for a mechanism of action for rat thyroid gland tumorigenesis that has been referenced for years. UGT enzyme induction, hepatocellular hypertrophy, and associated changes in thyroid gland morphology demonstrated in rodents has been the cited mechanism of xenobiotics ranging from therapeutics (Masubuchi et al., 1997; Burns-Naas et al., 2005) to herbicides (Dellarco et al., 2006; Bomann et al., 2021), pesticides (Christenson et al., 1996; European Food Safety et al., 2019), and other chemicals, and this mechanism is often understood to be less relevant to adult human risk assessment based on inherent differences in

sensitivity and responsiveness (Curran and Degroot, 1991; McClain, 1995; Capen, 1997; Cheek et al., 1999; Capen, 2001; Meek et al., 2003; Crofton and Zoeller, 2005).

A tremendous expansion of knowledge following the sequencing and cloning of gene products of enzymes (Mackenzie et al., 2005; Zanger and Schwab, 2013; Coughtrie, 2016; Meech et al., 2019; Xie and Xie, 2020) and membrane transporters (Hagenbuch and Meier, 2004; The International Transporter Consortium, 2010; Marin, 2012; Halilbasic et al., 2013; Slitt, 2018; Felmler et al., 2020; Van Groen et al., 2021) involved in the metabolism and disposition of chemicals occurred at the end of the last century and allowed for the development of more thorough tools, such as genetically modified animals, and strategies to study the tissue expression, function, and selectivity of exogenous and endogenous substrates for these enzymes and membrane transporters (Dunn and Klaassen, 1998; Rausch-Derra et al., 2001; Cherrington et al., 2002; Guo et al., 2002; Johnson and Klaassen, 2002; Li et al., 2002; Shelby et al., 2003; Alnouti and Klaassen, 2006; Klaassen and Lu, 2008; Gong et al., 2011; Hammer et al., 2021). This included the demonstration that uptake of thyroid hormones into tissues is not through passive diffusion but by a carrier mediated process (Krenning et al., 1978; Friesema et al., 1999; Hennemann et al., 2001; Visser et al., 2011; Groeneweg et al., 2020). Likewise, the molecular basis for the chemical regulation of expression of these enzyme and transporter gene families was elucidated and attributed to the action of xenobiotic-sensing receptors that act as ligand activated transcription factors to regulate the expression of cytochrome P450s and other drug metabolizing enzymes and transporters (Rausch-Derra et al., 2001; Sueyoshi and Negishi, 2001; Klaassen and Slitt, 2005; Tirona and Kim, 2005; Buckley and Klaassen, 2009; Köhle and Bock, 2009; Klaassen and Aleksunes, 2010; Aleksunes and Klaassen, 2012; Chai et al., 2013; Amacher, 2016; Hakkola et al., 2018; Küblbeck et al., 2020). The classification of differential

responses according to activation of these receptors and the coordinate regulation of responsive genes has furthered the information available to understand how the induced disposition of thyroid hormones disrupts thyroid homeostasis. Applying this understanding to existing data collected from animal models and other integrated physiology systems provides a basis for which to reassess the specific mechanisms of action of chemical inducers.

Comprehensive reviews of the various modes of action of chemicals on thyroid function and the contribution of this to human risk assessment, considering adverse outcome-based approaches and testing strategies, have been published recently (Murk et al., 2013; Noyes et al., 2019; Foster et al., 2021). This mini review focuses on data generated from experimental rat models that characterized the altered disposition of thyroid hormones in the presence of inducing chemicals to bring forward a current understanding of the likely pathway(s) by which these chemicals act, as depicted in **Figure 1**. Given the sensitivity of rats to perturbations in thyroid homeostasis and tumorigenesis by inducers and their status as the most common species for nonclinical safety testing, rats are an appropriate species to delineate relevant and potentially coordinating pathways. Whereas the best explanation for the mode of actions for some inducers may in part be the increased conjugation of thyroid hormone, for others the functional site of action is an increase in hepatic uptake or increase excretion of conjugates into bile. Implications for this in future hypothesis building and testing will be considered.

Historical Hypothesis Building: An Extrathyroidal Mechanism

Some thyroid toxicants exert their effects by direct action on the thyroid gland to impair thyroid hormone synthesis or secretion, such as propylthiouracil or methimazole, or desensitize regulation by the hypothalamic-pituitary axis (Capen, 1994). Certain other xenobiotics that

affect drug metabolizing enzymes, and are now identified as activators for xenobiotic-sensing transcription factors such as the constitutive androgen receptor (CAR), the pregnane X receptor (PXR), and the aryl hydrocarbon receptor (AhR) (Kretschmer and Baldwin, 2005; Tirona and Kim, 2005; Küblbeck et al., 2020), were historically demonstrated to increase the peripheral elimination of T4 (Cavalieri and Pitt-Rivers, 1981; Curran and Degroot, 1991) and enhance the biliary clearance of iodothyronine glucuronides in rats.

In various experiments starting in the late 1960s, drugs and chemicals like phenobarbital, PCN, polycyclic aromatic hydrocarbons, TCDD, and PCB, were shown to stimulate the hepatocellular binding of T4 (Bernstein et al., 1968), increase the plasma disappearance and biliary excretion of T4 (Bernstein et al., 1968; Japundžić, 1969; Bastomsky, 1974; Japundzic et al., 1976; Bastomsky, 1977), and in some cases produce goitrogenic effects on the thyroid gland. When thyroidectomized rats were maintained on a constant replacement dose of T4 and administered phenobarbital, they exhibited a marked fall in serum protein bound iodine, leading to the conclusion that the effects of phenobarbital on thyroid function are the result of an increased peripheral disposition of thyroid hormone (Oppenheimer et al., 1968). Using both in vivo and ex vivo techniques, some differential effects were observed among the inducers phenobarbital and DDT versus benzopyrene (Goldstein and Taurog, 1968) and other aromatic hydrocarbons (Bastomsky, 1974) in the relative amount of T4 glucuronide present in bile and the increase in T4 glucuronidation activity, leading some to postulate phenobarbital was likely enhancing T4 excretion by increasing bile flow rather than by UGT induction. Comparing biliary excretion of T4 versus T4 glucuronide in Gunn rats genetically deficient in UGT1A enzymes that glucuronidate T4 (Visser et al., 1993a), it was concluded that the rate limiting

step in the biliary excretion of T4 is the formation of the glucuronide conjugate (Bastomsky, 1973; Bastomsky and Papapetrou, 1973).

On this background, the causal link between changes in thyroid gland morphology, including the promotion of thyroid follicular tumors, and increased hepatic UGT activity toward T4 in rats by certain chemicals was hypothesized (Hiasa et al., 1982; Comer et al., 1985; McClain, 1989). Exogenous supplementation with T4 blocked the thyroid tumor promoting action of phenobarbital in rats, and the promoting effect was proportional to plasma TSH concentrations (McClain et al., 1988). This, in addition to demonstration that hepatic uptake, bile flow, and cumulative excretion of an administered dose of [125 I]T4 were increased in phenobarbital-dosed rats, as was liver microsomal T4 UGT activity (McClain et al., 1989), supported the conclusion that the effect of phenobarbital on thyroid function in rats is primarily a result of its induction of T4 glucuronidation.

The Klaassen laboratory built upon prior and parallel work delineating the regulation of biliary excretion based on differential inducibility and substrate specificity to carry out further systematic experiments better defining the plausibility of T4 UGT induction as a mode of action for thyroid disruption across multiple classes of chemical inducers and individual UGT enzymes. The extrathyroidal mechanism of action of prototypical inducers phenobarbital, 3-methylcholanthrene (3-MC), pregnenolone-16 α -carbonitrile (PCN), and the polychlorinated biphenyl (PCB) mixture Aroclor 1254 in lowering serum T4 was demonstrated using thyroidectomized rats supplemented with thyroid hormone replacement (Barter and Klaassen, 1992b), and induction of microsomal T4 glucuronidation was demonstrated across known substrate classes and of inducers (Barter and Klaassen, 1992a; Barter and Klaassen, 1994; Liu et al., 1995). A good inverse correlation was present between decreased serum T4 and T4 UGT

activity when considering all inducers (ie, Aroclor 1254 had largest effect on both), but not between the lesser decreases in serum T3 and increased T4 UGT activity, indicating that reductions in T3, when present, were not a direct consequence of decreased T4. The general maintenance of serum T3 concentrations in these studies relative to T4 concentrations (some inconsistent decreases in serum total T3 but not free T3) did not appear to be result of differential or tissue specific effects on deiodination (Hood and Klaassen, 2000b).

Dose response studies affirmed the inverse correlation between effects on serum T4 and T4 UGT activity but a difference in regression plot slope (ie, less steep for PB and PCN than PCB or 3-MC) suggested different mechanisms were contributing to the decrease in T4, and the potential influence of compensatory increases in serum TSH. Whereas serum TSH could be correlated to proliferation of thyroid follicular cells and growth, a poor correlation existed between the ability of an inducer to ablate serum T4 concentrations and produce an increase in serum TSH in rats (ie, PCB lowered serum T4 and increased T4 UGT activity but did not appreciably increase TSH) (Barter and Klaassen, 1994; Liu et al., 1995; Hood et al., 1999b). This disconnect applied to biliary excretion of T4 as well. Administration of PCN, 3-MC, or PCB reduced total serum T4 and increased the overall amount and rate of excretion of T4 into bile (Vansell and Klaassen, 2001). PCN, however, had the least effect on the excretion of T4 glucuronide compared with the other inducers, but the largest effect on serum TSH. Other reports of differential induction responses in T4 UGT activity in rats that were correlated with serum T4 lowering but not compensatory increases in TSH occurred, and differences in activity against T4 were also evident (Saito et al., 1991; Van Birgelen et al., 1995). Although profiles of individual inducers were variable among different experimental conditions (De Sandro et al., 1991), markers of hepatic enzyme induction like higher liver weights or increases

in activity against known cytochrome P450 substrates generally provided evidence of expected pharmacodynamics. It was therefore evident that alternate sites of action than glucuronidation for enzyme inducer lowering of circulating T4 and increasing TSH needed consideration.

Key Advances Driving Alternate Hypotheses

A series of studies proceeded using experimental conditions and animal models with reduced or differential glucuronidation capacity, and later, hepatic transporter capacity, to determine the contribution of increased T4 glucuronidation in the disruption of thyroid homeostasis observed with these inducers. Select comparative responses generated by some inducers described in this and subsequent sections are provided in **Table 1**.

Correlations between rat T3 UGT activity and increased TSH

Because T3 and T4 are substrates for different UGT enzymes (van Raaij et al., 1993; Visser et al., 1993a), whether induced T3 glucuronidation could be the more site of action than induced T4 glucuronidation was investigated. The PXR activator PCN was effective at increasing T4 glucuronidation (Barter and Klaassen, 1994; Liu et al., 1995; Hood et al., 1999b; Hood et al., 2003), biliary excretion of T4 glucuronide (Japundzic et al., 1976; Vansell and Klaassen, 2001), and inducing UGT1A1 mRNA (Vansell and Klaassen, 2002b; Shelby et al., 2003). PCN also uniquely increased microsomal T3 glucuronidation (Hood and Klaassen, 2000a) and biliary excretion of T3 glucuronide in rats (Vansell and Klaassen, 2002a), and consistently and significantly increased serum TSH in each study where measured. These effects on T3 and TSH were in contrast to the PCB mixture Aroclor 1254 and 3-MC, despite the effectiveness of those inducers in lowering of serum T4. Furthermore, PCN was identified as a promoter of

thyroid follicular adenomas in rats, whereas Aroclor 1254 was not (Vansell et al., 2004). These data indicate that induction of T3 glucuronidation instead of T4 glucuronidation may drive increases in circulating TSH and thyroid gland proliferation following exposure to hepatic enzyme inducers.

UGT2B enzymes are considered responsible for T3 glucuronidation in rats (Beetstra et al., 1991; Visser et al., 1993b), and UGT2B2 (androsterone UGT) is thought to be the primary responsible enzyme. Therefore, Fischer 344 rats deficient in UGT2B2 activity (Corser et al., 1987; Haque et al., 1991; Visser et al., 1991) were compared with SD rats to determine whether this enzyme mediated the thyroid-disruption response of some inducers (Richardson and Klaassen, 2010b). Although basal microsomal T3 glucuronidation in Fischer 344 rats was approximately half the activity in SD rats (androsterone glucuronidation was absent), T3 UGT activity was still present in Fischer 344 rats and inducible by PCN to an equivalent extent as in SD rats, along with increased TSH and thyroid follicular cell proliferation, suggesting another UGT is capable of T3 glucuronidation. Importantly, there was no appreciable effect in T3 glucuronidation with 3-MC or PCB and no increase in TSH in these Fishcher 344 rats, although lower serum T4 and/or T3 and increased T4 UGT activity occurred, consistent with prior results. Which UGT enzyme is the target of PCN-induced increases in T3 glucuronidation remains unconfirmed. Among UGT2B family mRNAs, PCN induced UGT2B1 in rat liver, although not to the extent of CAR activators (Shelby and Klaassen, 2006). Another PXR ligand, spironolactone, increased excretion of thyroid hormone, increased T4 UGT activity, and caused thyroid tumors in rats (Lumb et al., 1978; Semler et al., 1989), and was reported to increase UGT2B1 protein (Catania et al., 2003), but T3 glucuronidation activity was not specifically demonstrated. The herbicide, trifluralin, increased both T4 and T3 conjugates in

bile, as well as induced UGT1A6, 1A1, and 2B1 mRNA in rats (Saghir et al., 2008), and the benzodiazepine clobazam also upregulated UGT2B1/2 in association with changes in thyroid hormones (Miyawaki et al., 2012)

Experiments in Gunn rat with absent T4 UGT induction

Gunn rats deficient in UGT1A glucuronidation capacity provided mixed historical data indicating they were not responsive to 3-MC nor PCN induction of UGT activity against known substrates, α -naphthol and digitoxigenin monodigitoxioside (Watkins and Klaassen, 1982), respectively, but did have some constitutive T4 UGT activity (Barter and Klaassen, 1992a), consistent with Bastomsky and Papapetrou (1973). More direct investigations were completed in this model to evaluate the contribution of inducible T4 UGT activity under conditions where it would be reduced or absent. Despite the absence of constitutive or inducible T4 UGT activity in Gunn rats in this study, PCN, 3-MC, and PCB produced decreases in serum T4 and increases in TSH similar to Wistar rats where T4 UGT activity was substantially increased (Richardson and Klaassen, 2010a). Likewise, Kato and colleagues (2005; 2007) found that decreased total T4 after administration of PB or Kanechlor 500, a commercial PCB mixture, to Gunn rats was also not dependent on glucuronidation of T4, as a significant decrease in serum T4 occurred in both Wistar and Gunn rats but induction of T4 UGT was observed only in Wistars. This indicated that lowering of serum T4 did not correlate with induction of T4 UGT activity and did not correlate with T3 UGT activity in Gunn or Fisher 344 rats for 3-MC and PCB. Therefore, it was concluded that events other than T4 glucuronidation, potentially T3 glucuronidation, must be involved in decreasing the circulating T4 in this model.

Experiments with PCB congeners

PCB mixtures, such as Aroclor 1254, are produced by random chlorination of the biphenyl molecule, which produces many different congeners with varying degrees of chlorination that dictate their receptor binding properties and thus toxicity profiles (Safe et al., 1985; Hansen, 1998). Because coplanar (no ortho position chlorination) congeners have affinity for the AhR, noncoplanar (di-ortho position chlorination) share properties with phenobarbital, and mono-ortho coplanar PCB congeners induce both CYP1A and CYP2B, these chemicals provide good tools for assessing different degrees of responses to inducible genes in rodents.

When the effects of individual congeners representing the coplanar (PCB 126), noncoplanar (PCB 95 and 99), and mixed (PCB 118) categories were compared with the PCB mixtures Aroclor 1254 and Aroclor 1242 for their effectiveness in reducing serum T4, PB-type and mixed-type PCBs were most effective (Martin and Klaassen, 2010). However, when congeners were assessed for induction of T4 glucuronidation and excretion, there was no good correlation between the ability of various individual PCB congeners to increase T4 glucuronidation and their ability to lower serum T4; that is, the coplanar type congener PCB 126 and TCDD itself most effectively increased T4 UGT activity, but were less effective at reducing serum T4 than PCB 99, which had no effect on T4 UGT activity (Martin et al., 2012). This was consistent with differences reported using PCB 126 and another PB-like congener PCB 153 in rats (Craft et al., 2002) Furthermore, whereas PCB mixtures and congeners all significantly reduced serum T4, not all of them significantly increased the in vivo excretion of T4 glucuronide into bile or increased the UGT activity toward T4 in vitro. Somewhat surprisingly, only the PCB congeners with TCDD-like activity, namely PCB 118 and PCB 126, significantly increased the biliary excretion of T4 glucuronide in vivo, while the PB-type

congeners, PCB 95 and PCB 99, did not (Martin et al., 2012). This suggests that the PB-like activity of the mixtures and congeners is responsible for affecting pathways that are not related to T4 UGT induction. However, Aroclor 1254, PCB 99, and PCB 118 administration to rats produced the most rapid disappearance of [125 I]T4 from the plasma (77% within 15 minutes), which suggests that the PB-like activity of these compounds is important for increased T4 clearance. It was hypothesized that these compounds may increase tissue uptake of T4 by increasing thyroid hormone transport across cell membranes.

Based on a lack of T4 UGT induction despite decreased serum T4, Saito et al. (1991) had proposed the alignment with earlier suggestions that reduced serum T4 (protein bound and free) by DDT and PB was probably due to increased production of bile (Goldstein and Taurog, 1968; Bastomsky, 1974). They also proposed that the more substantial increases in T4 UGT activity produced by 3-MC and PCB were better associated with T4 lowering, and that in the case of PCB, a marked difference between serum total and free T4 concentrations was likely due to inhibition of T4 binding to plasma binding protein by PCB, as reported by Bastomsky (1974). The previously summarized Gunn rat studies with phenobarbital and Kanechlor 500 (Kato et al., 2005; Kato et al., 2007) also demonstrated increased hepatic uptake and accumulation of administered [125 I]T4; decreases in serum T4 were attributed mainly to hepatic accumulation secondary to inhibition of T4 binding to transthyretin. Later studies in that lab evaluating phenobarbital (Kato et al., 2010) and transthyretin-null mice administered Kanechlor 500 (Kato et al., 2019) concluded that decreases of the serum T4 in these conditions occurred independent of transthyretin binding, and that resulting T4 accumulation in liver occurs through the development of liver hypertrophy and the promotion of T4 transport from serum to liver.

Combined, these data triggered further initial investigations of the uptake of T4 following exposure to inducers, and the relationship to the expression of hepatic transporters in rats.

Evidence for hepatic transporter upregulation

In humans, the active transport of thyroid hormones across cell membranes has been demonstrated in vitro and was recently comprehensively reviewed (Groeneweg et al., 2020). Hepatic uptake transporters are localized to the sinusoidal basolateral membrane; efflux transporters to the canalicular apical membrane for biliary excretion, or basolateral membrane for the return to circulation (Van Groen et al., 2021). In rats, hepatic uptake transporters include Oatps, organic cation transporters, and Ntcp, among others; and efflux transporters including the bile salt export pump, Mrps, and multidrug resistance proteins (Klaassen and Aleksunes, 2010). Primarily based on studies in transfected cells, transport proteins thought to mediate thyroid hormone uptake in rats include rat Oatp1a1, Oatp1a4, and Oatp1b2, as well as Ntcp, two L-type transporters (LAT1 and LAT2), and a monocarboxylate transporter MCT8 (Visser et al., 2011). Inducibility of uptake transporters has been demonstrated, and they are under the regulation of the xenobiotic-activated receptors, as with other drug metabolizing enzymes (Kretschmer and Baldwin, 2005; Klaassen and Aleksunes, 2010; Wang et al., 2012; Chai et al., 2013; Amacher, 2016). Examples of inducers of thyroid hormone glucuronidation that are also known to induce hepatic transport expression in rats or mice include PXR ligands PCN and spironolactone and CAR activators phenobarbital and PCB 99 (Hagenbuch et al., 2001; Rausch-Derra et al., 2001; Guo et al., 2002; Cheng et al., 2005), whereas some substrates for the AhR decrease uptake transporter expression (Guo et al., 2002). PXR ligands increase rat hepatic protein levels of the canalicular efflux transporter Mrp2 (Johnson and Klaassen, 2002)

and CAR activators increase sinusoidal efflux protein Mrp3 (Cherrington et al., 2002); reports of induction by CAR activators in rat are mixed (Schrenk et al., 2001; Johnson and Klaassen, 2002). In the case of Mrp2 induction by PCN and Mrp3 induction by PB, these appear to be independent of PXR and CAR, respectively; increases in Mrp2 protein following PCN exposure in rats were attributed to de novo protein synthesis and not to changes in mRNA stability or protein degradation (Cherrington et al., 2003; Jones et al., 2005).

Based on differential UGT induction and effectiveness of inducers to lower serum T4, it was hypothesized that certain inducers, particularly those with CAR activity, may increase tissue uptake of T4 and/or efflux of thyroid hormone conjugates by increasing thyroid hormone transport across cell membranes. To better characterize which tissue uptake was responsible for lowering serum T4 following PCB administration in rats, and any association with increases in membrane transporters, PCB mixtures Aroclor 1254 or Aroclor 1242 were administered to rats for 7 days, followed by injection with [¹²⁵I]T4 and a sampling time course for blood and tissue collection (Martin, 2003). Consistent with observations in other studies (Vansell and Klaassen, 2001; Martin et al., 2012), Aroclor 1254 and Aroclor 1242 produced a rapid reduction in serum [¹²⁵I]T4, and within 1 minute, increases in uptake of [¹²⁵I]T4 into liver by 105% and 54%, respectively, compared with control. Evaluation of protein concentrations for uptake transporters Oatp1a4 and Oatp1a5 indicated either no effect, or suppression (Oatp1a4 decreased 87% by Aroclor 1254). In contrast, Aroclor 1254 and Aroclor 1242 produced significant increases in Mrp2 protein of 108% and 92%, respectively, relative to control. These data suggest that the enhanced plasma disappearance of T4 by PCB mixtures is due to an enhanced uptake of T4 into the liver, although potentially not by induction of Oatp1a4 or

Oatp1a5. Upregulation of Mrp2 as well as increased liver size also could contribute to increased biliary excretion of T4 glucuronide produced by PCBs.

Similarly, with the PCB mixture Kanechlor 500, the rapid decrease in administered serum [125 I]T4 was accompanied by increased liver T4 accumulation (Kato et al., 2017). These investigators demonstrated liver selective T4 accumulation that was associated with an increase in the expression of influx transporters LAT1 and Oatp1a4, and the basolateral efflux transport Mrp3, with no effect on Oatp1a1, Oatp1a5, Oatp1b2, Ntcp, LAT2, or Mrp2. They further suggested that Kanechlor 500-mediated decreases in serum T4 in rodents occurs through increased liver volume and enhancement of hepatic T4 accumulation, induction of T4 glucuronidation, and/or increase in Mrp3-mediated T4 conjugate excretion into sinusoidal blood.

DMP 904, a potent and selective corticotropin releasing factor type 1 receptor antagonist that produced thyroid follicular cell hypertrophy and hyperplasia in 3-month rat toxicity studies, also decreased serum T4 and increased serum TSH within 12 hours; serum T4 was undetectable in 3 days and TSH continued to increase through 5 days of administration; serum T3 also decreased over time (Wong et al., 2005). Total body clearance of [125 I]T4 was increased as a result of increased biliary clearance of unconjugated T4 (80x control) and T4 glucuronide along with a small increase in the unbound fraction of T4 in blood (~2x).

Induction of UGT1A1 and UGT1A2 mRNA, uptake transporter Oatp1a4 mRNA and Oatp1a4 protein, and the canalicular transporter Mrp2 mRNA were demonstrated. These data suggest that DMP 904 is an agonist of CAR and PXR, and that the decreased serum T4 and T3 resulted from increased hepatobiliary clearance. This was consistent with results of a whole body autoradiography study that demonstrated an increase in the concentration of 125 I in the liver of

DMP 904 dosed rats, suggesting that the marked reductions in serum T4 may result largely from an increased uptake of T4 into the liver.

In follow up studies utilizing the Groningen Yellow transport deficient (TR⁻) rat that lacks functional Mrp2 protein (Paulusma et al., 1996), the contribution of Mrp2 to biliary excretion of administered [¹²⁵I]T4 to control Wistar and TR⁻ rats, or rats that were dosed with DMP 904 or phenobarbital, was evaluated (Lecureux et al., 2009). Compared with control Wistar rats where T4 glucuronide was the major biliary metabolite, in control TR⁻ rats the predominant T4 metabolite detected in bile was T3 sulfate, indicating Mrp2 is responsible for elimination of T4 glucuronide in bile. In TR⁻ rats, phenobarbital or DMP 904 did not increase the plasma clearance of [¹²⁵I]T4 as in Wistars, and there was a marked decrease in biliary clearance of T4 and its metabolites in TR⁻ rats. This mitigated but did not prevent phenobarbital- and DMP 904-induced decreases in serum T4 concentrations, although there was no concomitant increase in serum TSH in TR⁻ rats. These results indicate the importance of biliary excretion in inducer-mediated effects on T4 homeostasis in rats but a direct correlation with the decrease in serum T4 in TR⁻ rats was lacking. Higher constitutive expression of Mrp3 and upregulation of Mrp3 by DMP 904 and phenobarbital were reported in these rats and likely contributed to some hepatic efflux of T4 and metabolites.

Additional noteworthy experiments were conducted with clobazam. Clobazam administered to SD rats for 4 weeks resulted in increased T4 UGT activity, modest decreases in circulating thyroid hormone (T3 more so than T4), increased TSH, thyroid gland follicular hypertrophy, and increased thyroid and liver weights (Miyawaki et al., 2003). Clearance of [¹²⁵I]T4 from the blood was increased at the end of 4 weeks; no effects on thyroid gland ¹²⁵I uptake or organification were apparent. To address further the role of glucuronidation versus biliary

excretion of thyroid hormone following clobazam administration, 2 mutant rat strains, Gunn (UGT1A-deficient) and EHBR (an Mrp2-deficient SD rat) were used (Miyawaki et al., 2012). Clobazam produced a slight decrease in plasma T4, and/or T3, and thyroid cellular hypertrophy in SD and Gunn rats, but not in EHBR rats. Clobazam altered neither plasma TSH nor thyroid morphology in EHBR rats. Hepatic UGTs 1A1, 1A6, 2B1/2, and Mrp2 mRNA and protein were upregulated in SD rats by clobazam; in Gunn rats only UGT2B1/2 and Mrp2 mRNA and protein were upregulated; in EHBR rats, all hepatic UGTs were upregulated, but not Mrp2. Like DMP 904, clobazam acts as an inducer of CYP3A1/2 and CYP2B1, which means that activation of nuclear receptors, PXR and CAR simultaneously occurs (Miyawaki et al., 2012). These results again indicate that the presence of Mrp2 is associated with the development of the thyroid cellular hypertrophy following exposure to some inducers. UGT2B1/2 appear to work with Mrp2 function to enable elimination of thyroid hormones that disrupts homeostasis.

Other experiments with inducers that lower circulating thyroid hormone and produce thyroid tumors or other morphologic changes in rat thyroid have generated different responses in the expression of transport proteins, despite evidence of acting on the same hepatic transcription factors. A mixture of short chained chlorinated paraffins administered to SD rats decreased hepatic T4 concentrations, but increased hepatic T3 concentrations; whereas there was an increase in CYP2B1 and UGT1A1 mRNA and protein, indicating CAR activation, increased hepatic transporter mRNA and protein expression was limited to OAT2, with no effect on Ntcp, Oatp1a4, Mrp2, Mrp3, Sult1a1, Sult1b1, or transthyretin mRNAs, or Type I deiodinase protein (Saghir et al., 2008). There was also no apparent increase in Oatp1a1, Oatp1a4, Mrp2, or Mdr1 in rats administered the CAR/PXR activator triclosan, although T4 and more so T3

were both reduced in circulation and UGT1A1, CYP2B, and CYP3A mRNA were induced (Paul et al., 2009).

Current Hypothesis, Gaps, and Future Considerations

From these collective data it is apparent there are 2 predominantly effective pathways for the disruption in thyroid homeostasis produced in rats by inducers of drug metabolizing enzymes and transporters, and these likely work in coordination to alter the disposition of thyroid hormones and disrupt homeostasis (depicted in **Figure 1**):

1. An increase in the uptake of thyroid hormone (T4 and/or T3) into liver results in rapid clearance of T4 from circulation and subsequent enhanced conjugation and biliary excretion. This mode of action seems most closely associated with CAR activation or combined CAR/PXR regulation. A specific T4 uptake transporter induced in rats is possibly Oatp1a4, but more likely to be an undetermined transporter given that known significant inducers of Oatp1a4, like PCN, do not cause such a dramatic increase in plasma disappearance of [¹²⁵I]T4 as PCB mixtures or congeners with phenobarbital-like activity (Vansell and Klaassen, 2001; Guo et al., 2002; Vansell and Klaassen, 2002b; Martin et al., 2012). This action is likely also supplemented with an increase in the excretion of T4/T3 glucuronide or unconjugated T4 into the bile via induction of Mrp2, or potentially sinusoidal efflux of iodothyronines via Mrp3, as demonstrated in studies with DMP 904 and Kanechlor 500 (Lecureux et al., 2009; Kato et al., 2017) However, it is clear in some cases, as with PCBs (Martin, 2003; Martin et al., 2012), the resulting significant and sustained lowering of serum T4 in rats does not correlate to the extent of increase in T4 glucuronide activity or biliary clearance and is presently better attributed to

increased hepatic uptake. This lowering of T4 also does not necessarily produce a substantial TSH response detectable in the circulation and associated with hypertrophy/hyperplasia of the thyroid. As represented by an inducer like phenobarbital where data are sometimes conflicting, the differences in whether reductions in T4 elicit increases in TSH and subsequent thyroid gland morphology may be related to potency, pharmacokinetics, the dose response relationship, variable physiology among rat strains, or other combined attributes differing among inducers and experimental designs.

2. An induction in the glucuronidation of thyroid hormone and biliary excretion of glucuronide conjugates occurs that is substantial enough to lower systemic thyroid hormone concentrations. Whereas inducers binding to AhR are effective at increasing T4 glucuronidation and produce some lowering of circulating T4 in rats, only when there is an increase in T3 glucuronide formation and biliary excretion, does there appear to be an interruption of negative feedback resulting in significant increases in serum TSH and stimulation of the thyroid gland. Induction of T3 glucuronidation seems most closely tied to PXR activation and inducers such as PCN (Vansell and Klaassen, 2002a), however given that CAR activators induce hepatic UGT2B1 mRNA, which could contribute to T3 glucuronidation (Shelby and Klaassen, 2006), they may also increase this activity. Although in humans the majority of available T3 is formed through peripheral deiodination of secreted T4, in rats, it appears the thyroid secretes T4 and T3 in a more equal proportion (Chanoine et al., 1993), meaning rat sensitivity could result from increased removal of T3 from circulation secondary to induction of the responsible UGT, or potentially through increased hepatic uptake of T3 itself. As such, increased TSH and

thyroid gland activation may also be the consequence of increasing both T4 glucuronidation and T3 glucuronidation simultaneously, in conjunction with some amount of increased hepatic uptake and increased canalicular export by Mrp2. The significance of the loss of function of Mrp2 was demonstrated with clobazam in studies with EHBR rats where UGT2B1/2 induction still occurred but increases in TSH did not (Miyawaki et al., 2012). Because there are no selective inducers of only T3 but not T4 glucuronidation identified in rats, and inducers of T3 glucuronidation and biliary excretion, such as PCN, have not been studied yet in rat models absent of efflux transporter function, proper experiments to draw conclusions are still needed.

Several key experiments would address relevant unknowns related to this mode of action of inducers. First, further loss of function experiments would enable a more direct assessment of the significance of Oatp1a4 and Mrp2 by using PCB mixtures/congeners or PXR activators like PCN. Although some inducers affecting thyroid hormone produced no effect on Mrp2 mRNA expression, other translational regulatory mechanisms exist for Mrp2 in rats. With an inducer like PCN that increases T3 glucuronidation, an assessment in the absence of Mrp2-facilitated excretion in EHBR or TR- rats would provide a more definitive determination of the critical target protein, particularly should absence of Mrp2 excretion eliminate PCN-mediated alterations in thyroid hormones. Likewise, if Mrp2 is important to the increased biliary excretion of T4 glucuronide observed in response to some PCBs, it should not occur in these rats. Similarly, in the absence of a rat knock-out of Oatp1a4, competitive substrate studies for liver uptake of T4 (or T3) using digoxin could also be useful to determine the contribution of influx transporters in the induction of thyroid hormone disposition.

Second, further characterization of effects on T3 glucuronidation and T3 transport would be useful, using selective PB-like inducers such as PCB 99. Although it is a prototypical inducer, the potency of phenobarbital relative to other CAR class activators is less and disparate; PCB 99 has been shown to increase the elimination of [¹²⁵I]T4 from circulation, induce mRNA and/or protein for Oatp1a4, UGT2B1, Mrp3, and very effectively lower serum T4. Thus far, PCN, PCB (Aroclor 1254), and 3-MC are the only inducers that have been evaluated for specific effects on T3 glucuronidation and biliary excretion directly in vivo. Assessment of the differential responses in rats against T4 vs T3 as substrates for inducers from other classes that alter thyroid hormones would establish confidence in the specific target of action. If UGT2B1 induction is responsible for increased glucuronidation of T3, then this should be demonstrated in vivo by PCB 99 administration. Likewise, the effectiveness of PCB 99-mediated reduction in serum T4 in absence of T4 glucuronidation in Gunn rats would offer supporting evidence of the contribution of other disposition activities.

Beyond this, comprehensive identification of which transporters in rats are responsible for the influx and efflux of T4, T3, and their metabolites into liver, bile, and sinusoidal blood continues to be important to address knowledge gaps. For example, some inducers (DMP 904, temelastine) increase the biliary excretion of unconjugated T4 instead of conjugated hormone, or in addition to T4 glucuronide (PCN); what is the basis for this differential profile despite potentially overlapping transcription factor activation? Excretion of unconjugated T4 into bile was lower but present in control TR⁻ rats (Lecureux et al., 2009), thereby suggesting that Mrp2 is unlikely to be the only transporter involved in the biliary excretion of unconjugated T4. What is the contribution of sinusoidal efflux, and/or the extent to which uptake and excretion are coordinately inhibited or induced? For example, inducers that are very effective

at increasing UGT activity such as TCDD and PCB 126 are not as effective at lower serum T4 or provoking TSH increases, but this may be secondary to the simultaneous suppression of Oatp1a4 uptake by TCDD-like inducers (Rausch-Derra et al., 2001; Guo et al., 2002). This knowledge would also be helpful in interpreting data generated in mutant models, given that compensatory mechanisms often exist. For example, Oatp1a1 and Oatp1a4 are lower, whereas the expression of Mrp3 is higher in UGT1A-deficient Gunn rats (Higuchi et al., 2004).

Ultimately, a better understanding of what prevents the significant lowering of circulating T4 by certain enzyme inducers from eliciting an increase in TSH, whereas inducers less effective at T4 lowering interfere with negative feedback, is also needed. Relative maintenance of circulating T3 concentrations is generally present with all inducers of T4 disposition but whether T3 maintenance is the result of TSH activation of the thyroid or what prevents it is unclear. In the case of the PCB mixture Aroclor 1254, administration to rats in an initiation-promotion model resulted in a lowering of serum T4 to nearly undetectable levels for 5 months, with a transient increase in TSH, but no evidence of any proliferative lesions consistent with TSH stimulation of the thyroid (Vansell et al., 2004). The specific reason this fails to occur with the PCB and TCDD like class of inducers remains unknown. Hypotheses that local deiodination in the pituitary is maintained or that some thyromimetic inducers blunt responsiveness at the level of the thyroid receptor have been postulated but not demonstrated in rats. It may be that comparative studies among rat strains with different tonic activity of the thyroid-pituitary axis could provide some insight into responses in these models (Lecureux et al., 2009).

Extrapolation of this mechanism to other nonclinical species and humans is challenging both in translation of the relevant pathways of thyroid hormone transport and metabolism, and in the

regulation of these drug transporters and enzymes by nuclear receptors. There is both overlap within a species and variability between species for thyroid hormone drug metabolizing enzymes and transporters, and orthologs do not always exist or recognize the same substrates (Shiratani et al., 2008; Van Groen et al., 2021). In the case of humans, transport and glucuronidation of thyroid hormone in vitro has been demonstrated by many OATPs and UGT enzymes (Tong et al., 2007; Meech et al., 2019; Plummer et al., 2021), and transporter expression can vary widely among individuals and populations (Amacher, 2016). Further, whereas regulation of enzymes and transporters by nuclear receptors occurs across species, the expression response in liver may vary among species. In particular, this is true for regulation by CAR and PXR, where there are distinct differences in the amino acid sequences of the ligand-binding domains among rodents, rabbits, and humans (Wang et al., 2012; Amacher, 2016; Hakkola et al., 2020; Hammer et al., 2021) that result in differences in ligand preferences among species. Recently, using CAR response element sequence analysis of UGT promoters, it was found that human UGT promoters contained a higher proportion of consensus CAR response elements compared to the rat homologs, and there were differences in the UGT homologs that were induced in rat versus human 3D microtissues (Plummer et al., 2021).

Although sensitivity to inducer-mediated thyroid disruption leading to increased TSH and tumorigenesis is established to be greater in rats versus mice, and even less relevant in humans due in part to the lack of thyroxine-binding globulin, shorter half-life of thyroid hormone, and higher TSH in rats, data exist indicating that selective inducers of PXR (rifampin) and CAR activators (carbamazepine, phenytoin) can reduce human blood levels of thyroid hormone (Noyes et al., 2019; Foster et al., 2021; Van Groen et al., 2021). This indicates there is responsiveness in humans to hepatic enzyme inducers of thyroid hormone disposition. As the

responsible key events in the pathway for thyroid disruption by inducers are better elucidated in rodents, there is value in exploring further the possibility that differential susceptibility to these responses in other species and humans may be in part related to differences in a chemical's activation of relevant enzymes and transporters among those species. Data from mice suggest that differential responses to certain classes of hepatic UGT inducers could be responsible for species differences in response to hypothyroxemia (Craft et al., 2002; Hood et al., 2003; Shiratani et al., 2008; Buckley and Klaassen, 2009). Continued use of model systems, such as humanized mice, will allow analyses of the integrated nuclear receptor responses to chemicals for different species to enable a thorough evaluation of species selectivity.

To conclude, the complex interactions and regulation of hepatic uptake, conjugation, and the contribution of biliary versus sinusoidal efflux pose a challenge for establishing distinct molecular initiating events that broadly hold true across classes of inducers. Use of knockout rat models has reinforced that in the case of PCN and PB, functioning CAR or PXR are required to elicit thyroid disrupting effects (Haines et al., 2018; Haines et al., 2019). However, in vitro screening paradigms that rely solely on these upstream targets for nuclear receptor activation may fail to reveal accurate potential for modulation. Complexities such as nuclear-receptor independent regulation of Mrp2 and 3, and the absence of clear structure–activity relationship with respect to CAR activation among PCB congeners (Küblbeck et al., 2020) means integrated in vitro assays that measure induction of thyroid hormone transporters and conjugating enzymes are likely required.

Authorship Contribution:

Wrote or contributed to the writing of the manuscript: Vansell, NR

Table 1. Responses for select inducers in comparative rat models. Several inducers have been evaluated in standard rat models as well as loss of function models to enable comparative assessments on thyroid hormone effects and targets. Differences from control or baseline conditions are represented by (↑), (↓), or (↔), or both if data conflict. T4G = T4 glucuronide; T3G = T3 glucuronide; KO = knockout

Inducer	Receptor	Model	Effects	Reference(s)
PCN	PXR	SD Rat	↓T4, ↓T3, ↑TSH, ↑T4G biliary excretion, ↑T3G biliary excretion, ↑T4 UGT activity, ↑T3 UGT activity, ↑UGT1A1, ↑UGT2B1, ↑Oatp1a4, ↑Mrp2, ↑ thyroid proliferation, ↑ thyroid tumors	(Barter and Klaassen, 1994; Liu et al., 1995; Hood et al., 1999b; Hood and Klaassen, 2000a; Vansell and Klaassen, 2001; Guo et al., 2002; Johnson and Klaassen, 2002; Vansell and Klaassen, 2002a; Vansell et al., 2004; Shelby and Klaassen, 2006)
		Gunn Rat	↓T4 ↓T3 ↑TSH ↔T4 UGT activity, ↑T3 UGT activity, ↑thyroid proliferation	(Richardson and Klaassen, 2010a)
		PXR KO Rat	↔T4, ↔T3, ↔TSH, ↔thyroid proliferation	(Haines et al., 2018)
PB	CAR	SD or Wistar Rat	↓T4, ↓T3, ↑/↔TSH, ↑T4 clearance, ↑/↔T4G biliary excretion, ↑T4 in liver, ↑T4 UGT activity, ↑T3 UGT activity, ↑/↔UGT1A1, ↑/↔UGT1A6, ↑UGT2B1, ↑Oatp1a4, ↑/↔Mrp2, ↑thyroid proliferation, ↑ thyroid tumors	(Hiasa et al., 1982; McClain et al., 1989; Barter and Klaassen, 1994; Liu et al., 1995; Hood et al., 1999b; Hood and Klaassen, 2000a; Hagenbuch et al., 2001; Guo et al., 2002; Johnson and Klaassen, 2002; Kato et al., 2005; Lecureux et al., 2009; Kato et al., 2010)
		Gunn Rat	↓T4, ↓T3, ↔TSH, ↔T4 UGT activity, ↑UGT2B1	(Kato et al., 2005)
		TR- Rat	↓T4, ↓T3, ↔TSH, ↔T4 clearance, ↔T4G biliary excretion, ↑T4 UGT activity, ↑Mrp3	(Lecureux et al., 2009)
		PXR/CAR KO	↔T4, ↔T3, ↔TSH, ↔ thyroid proliferation	(Haines et al., 2019)

Kanechlor 500	CAR/PXR	Wistar Rat	↓T4, ↔TSH, ↑T4 UGT, ↑T4 clearance, ↑T4G biliary excretion, ↑T4 in liver; ↑UGT1A1, ↑UGT1A6, ↑UGT2B1, ↑Oatp1a4, ↑LAT1, ↑Mrp3, ↔ Oatp1a1, Oatp1a5, Oatp1b2, Ntcp, LAT2, or Mrp2	(Kato et al., 2004; Kato et al., 2007; Kato et al., 2017)
		Gunn Rat	↓T4, ↔TSH, ↔T4 UGT, ↑T4 clearance, ↑T4 liver, ↑UGT2B1	(Kato et al., 2004; Kato et al., 2007)
DMP 904	CAR/PXR	SD Rat	↓↓T4, ↓T3, ↑TSH, ↑↑T4 clearance, ↑T4 biliary excretion, ↑T4G biliary excretion, ↑T4 UGT activity, ↑UGT1A1/2, ↑ Oatp1a4 (mRNA and protein), ↑Mrp2	(Wong et al., 2005; Lecureux et al., 2009)
		TR- Rat	↓T4, ↓T3, ↔TSH, ↔T4 clearance, ↔T4 biliary clearance, ↑T4 UGT activity, ↑Mrp3	(Lecureux et al., 2009)
Clobazam	CAR/PXR	SD Rat	↓T4, ↓T3, ↑TSH, ↑T4 clearance, ↑T4 UGT activity, ↑UGT1A1, ↑UGT1A6, ↑UGT2B1/2, ↑Mrp2 , thyroid hypertrophy	(Miyawaki et al., 2003; Miyawaki et al., 2012)
		Gunn Rat	↓T4, ↓T3, ↑TSH, ↑UGT2B1/2, ↑Mrp2 , thyroid hypertrophy	(Miyawaki et al., 2012)
		EHBR Rat	↔T4, ↔T3, ↔TSH, ↑UGT1A1, ↑UGT1A6, ↑UGT2B1/2, ↔ hypertrophy	(Miyawaki et al., 2012)

References

- Aleksunes LM and Klaassen CD (2012) Coordinated regulation of hepatic phase I and II drug-metabolizing genes and transporters using AhR-, CAR-, PXR-, PPAR α -, and Nrf2-null mice. *Drug Metabolism and Disposition* **40**:1366-1379.
- Alnouti Y and Klaassen CD (2006) Tissue distribution and ontogeny of sulfotransferase enzymes in mice. *Toxicological Sciences* **93**:242-255.
- Amacher DE (2016) The regulation of human hepatic drug transporter expression by activation of xenobiotic-sensing nuclear receptors. *Expert Opinion on Drug Metabolism & Toxicology* **12**:1463-1477.
- Barter RA and Klaassen CD (1992a) Rat liver microsomal UDP-glucuronosyltransferase activity toward thyroxine: Characterization, induction, and form specificity. *Toxicology and Applied Pharmacology* **115**:261-267.
- Barter RA and Klaassen CD (1992b) UDP-glucuronosyltransferase inducers reduce thyroid hormone levels in rats by an extrathyroidal mechanism. *Toxicology and Applied Pharmacology* **113**:36-42.
- Barter RA and Klaassen CD (1994) Reduction of thyroid hormone levels and alteration of thyroid function by four representative UDP-glucuronosyltransferase inducers in rats. *Toxicology and Applied Pharmacology* **128**:9-17.
- Bastomsky CH (1973) The biliary excretion of thyroxine and its glucuronic acid conjugate in normal and gunn rats. *Endocrinology* **92**:35-40.

- Bastomsky CH (1974) Effects of a polychlorinated biphenyl mixture (aroclor 1254) and DDT on biliary thyroxine excretion in rats. *Endocrinology* **95**:1150-1155.
- Bastomsky CH (1977) Enhanced thyroxine metabolism and high uptake goiters in rats after a single dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Endocrinology* **101**:292-296.
- Bastomsky CH and Papapetrou PD (1973) The effect of methylcholanthrene on biliary thyroxine excretion in normal and Gunn rats. *Journal of Endocrinology* **56**:267-273.
- Beetstra JB, van Engelen JG, Karels P, van der Hoek HJ, de Jong M, Docter R, Krenning EP, Hennemann G, Brouwer A, and Visser TJ (1991) Thyroxine and 3,3',5-triiodothyronine are glucuronidated in rat liver by different uridine diphosphate-glucuronyltransferases. *Endocrinology* **128**:741-746.
- Bernstein G, Artz SA, Hasen J, and Oppenheimer JH (1968) Hepatic accumulation of 125I-thyroxine in the rat: augmentation by phenobarbital and chlordane. *Endocrinology* **82**:406-409.
- Bomann W, Tinwell H, Jenkinson P, and Kluxen FM (2021) Metribuzin-induced non-adverse liver changes result in rodent-specific non-adverse thyroid effects via uridine 5'-diphospho-glucuronosyltransferase (UDPGT, UGT) modulation. *Regulatory Toxicology and Pharmacology* **122**:104884.
- Buckley DB and Klaassen CD (2009) Induction of mouse UDP-glucuronosyltransferase mRNA expression in liver and intestine by activators of aryl-hydrocarbon receptor, constitutive androstane receptor, pregnane X receptor, peroxisome proliferator-

activated receptor α , and nuclear factor eryth. *Drug Metabolism and Disposition* **37**:847-856.

Burns-Naas LA, Zorbas M, Jessen B, Evering W, Stevens G, Ivett JL, Ryan TE, Cook JC, Capen CC, Chen M, Furman G, Theiss JC, Webber S, Wu E, Shetty B, Gasser R, and McClain RM (2005) Increase in thyroid follicular cell tumors in nelfinavir-treated rats observed in a 2-year carcinogenicity study is consistent with a rat-specific mechanism of thyroid neoplasia. *Hum Exp Toxicol* **24**:643-654.

Capen CC (1994) Mechanisms of chemical injury of thyroid gland. *Prog Clin Biol Res* **387**:173-191.

Capen CC (1997) Mechanistic data and risk assessment of selected toxic end points of the thyroid gland. *Toxicol Pathol* **25**:39-48.

Capen CC (2001) Overview of structural and functional lesions in endocrine organs of animals. *Toxicologic Pathology* **29**:8-33.

Catania VA, Luquita MG, Sánchez Pozzi EJ, Ikushiro S, Emi Y, Iyanagi T, and Mottino AD (2003) Effect of spironolactone on the expression of rat hepatic UDP-glucuronosyltransferase. *Biochem Pharmacol* **66**:171-177.

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

Chai X, Zeng S, and Xie W (2013) Nuclear receptors PXR and CAR: implications for drug metabolism regulation, pharmacogenomics and beyond. *Expert Opinion on Drug Metabolism & Toxicology* **9**:253-266.

Chanoine JP, Braverman LE, Farwell AP, Safran M, Alex S, Dubord S, and Leonard JL (1993)

The thyroid gland is a major source of circulating T3 in the rat. *J Clin Invest* **91**:2709-2713.

Cheek AO, Kow K, Chen J, and McLachlan JA (1999) Potential mechanisms of thyroid

disruption in humans: interaction of organochlorine compounds with thyroid receptor, transthyretin, and thyroid-binding globulin. *Environmental health perspectives* **107**:273-278.

Cheng X, Maher J, Dieter MZ, and Klaassen CD (2005) Regulation of mouse organic anion-transporting polypeptides (Oatps) in liver by prototypical microsomal enzyme inducers that activate distinct transcription factor pathways. *Drug Metab Dispos* **33**:1276-1282.

Cherrington NJ, Hartley DP, Li N, Johnson DR, and Klaassen CD (2002) Organ distribution of multidrug resistance proteins 1, 2, and 3 (Mrp1, 2, and 3) mRNA and hepatic induction of Mrp3 by constitutive androstane receptor activators in rats. *Journal of Pharmacology and Experimental Therapeutics* **300**:97-104.

Cherrington NJ, Slitt AL, Maher JM, Zhang X-X, Zhang J, Huang W, Wan Y-JY, Moore DD, and Klaassen CD (2003) Induction of multidrug resistance protein 3 (mrpe) in vivo is independent of constitutive androstane receptor. *Drug Metabolism and Disposition* **31**:1315-1319.

Christenson WR, Becker BD, Wahle BS, Moore KD, Dass PD, Lake SG, Van Goethem DL, Stuart BP, Sangha GK, and Thyssen JH (1996) Evidence of chemical stimulation of hepatic metabolism by an experimental acetanilide (FOE 5043) indirectly mediating

reductions in circulating thyroid hormone levels in the male rat. *Fundamental and Applied Toxicology* **29**:251-259.

Comer CP, Chengelis CP, Levin S, and Kotsonis FN (1985) Changes in thyroidal function and liver UDP glucuronosyltransferase activity in rats following administration of a novel imidazole (SC-37211). *Toxicology and Applied Pharmacology* **80**:427-436.

Conney AH (1967) Pharmacological implications of microsomal enzyme induction. *Pharmacological Reviews* **19**:317-366.

Corser RB, Coughtrie MWH, Jackson MR, and Burchell B (1987) The molecular basis of the inherited deficiency of androsterone UDP-glucuronyltransferase in Wistar rats. *FEBS Letters* **213**:448-452.

Coughtrie MWH (2016) Function and organization of the human cytosolic sulfotransferase (SULT) family. *Chemico-Biological Interactions* **259**:2-7.

Craft ES, DeVito MJ, and Crofton KM (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. *Toxicological Sciences* **68**:372-380.

Crofton KM and Zoeller RT (2005) Mode of action: neurotoxicity induced by thyroid hormone disruption during development--hearing loss resulting from exposure to PHAHs. *Crit Rev Toxicol* **35**:757-769.

Curran PG and Degroot LJ (1991) The effect of hepatic enzyme-inducing drugs on thyroid hormones and the thyroid gland*. *Endocrine Reviews* **12**:135-150.

- De Sandro V, Chevrier M, Boddaert A, Melcion C, Cordier A, and Richert L (1991) Comparison of the effects of propylthiouracil, amiodarone, diphenylhydantoin, phenobarbital, and 3-methylcholanthrene on hepatic and renal T4 metabolism and thyroid gland function in rats. *Toxicol Appl Pharmacol* **111**:263-278.
- Dellarco VL, McGregor D, Berry SC, Cohen SM, and Boobis AR (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.
- Dunn RT and Klaassen C (1998) Tissue-specific expression of rat sulfotransferase messenger RNAs. *Drug metabolism and disposition: the biological fate of chemicals* **26** **6**:598-604.
- European Food Safety A, Crivellente F, Hart A, Hernandez-Jerez AF, Hougaard Bennekou S, Pedersen R, Terron A, Wolterink G, and Mohimont L (2019) Establishment of cumulative assessment groups of pesticides for their effects on the thyroid. *EFSA Journal* **17**:e05801.
- Felmlee MA, Jones RS, Rodriguez-Cruz V, Follman KE, and Morris ME (2020) Monocarboxylate transporters (SLC16): function, regulation, and role in health and disease. *Pharmacological Reviews* **72**:466-485.
- Foster JR, Tinwell H, and Melching-Kollmuss S (2021) A review of species differences in the control of, and response to, chemical-induced thyroid hormone perturbations leading to thyroid cancer. *Archives of Toxicology* **95**:807-836.

- Friesema ECH, Docter R, Moerings EPCM, Stieger B, Hagenbuch B, Meier PJ, Krenning EP, Hennemann G, and Visser TJ (1999) Identification of Thyroid Hormone Transporters. *Biochemical and Biophysical Research Communications* **254**:497-501.
- Goldstein JA and Taurog A (1968) Enhanced biliary excretion of thyroxine glucuronide in rats pretreated with benzpyrene. *Biochemical Pharmacology* **17**:1049-1065.
- Gong L, Aranibar N, Han Y-H, Zhang Y, Lecureux L, Bhaskaran V, Khandelwal P, Klaassen CD, and Lehman-McKeeman LD (2011) Characterization of organic anion-transporting polypeptide (Oatp) 1a1 and 1a4 null mice reveals altered transport function and urinary metabolomic profiles. *Toxicological Sciences* **122**:587-597.
- Groeneweg S, Van Geest FS, Peeters RP, Heuer H, and Visser WE (2020) Thyroid hormone transporters. *Endocrine Reviews* **41**:146-201.
- Guo GL, Choudhuri S, and Klaassen CD (2002) Induction profile of rat organic anion transporting polypeptide 2 (oatp2) by prototypical drug-metabolizing enzyme inducers that activate gene expression through ligand-activated transcription factor pathways. *Journal of Pharmacology and Experimental Therapeutics* **300**:206-212.
- Hagenbuch B and Meier PJ (2004) Organic anion transporting polypeptides of the OATP/SLC21 family: phylogenetic classification as OATP/SLCO superfamily, new nomenclature and molecular/functional properties. *Pflügers Archiv European Journal of Physiology* **447**:653-665.

- Hagenbuch N, Reichel C, Stieger B, Cattori V, Fattinger KE, Landmann L, Meier PJ, and Kullak-Ublick GA (2001) Effect of phenobarbital on the expression of bile salt and organic anion transporters of rat liver. *Journal of Hepatology* **34**:881-887.
- Haines C, Chatham LR, Vardy A, Elcombe CR, Foster JR, and Lake BG (2018) Comparison of the hepatic and thyroid gland effects of sodium phenobarbital in wild type and constitutive androstane receptor (CAR) knockout rats and pregnenolone-16 α -carbonitrile in wild type and pregnane X receptor (PXR) knockout rats. *Toxicology* **400-401**:20-27.
- Haines C, Chatham LR, Vardy A, Elcombe CR, Foster JR, and Lake BG (2019) Comparison of the hepatic and thyroid gland effects of sodium phenobarbital and pregnenolone-16 α -carbonitrile in wild-type and constitutive androstane receptor (CAR)/pregnane X receptor (PXR) knockout rats. *Xenobiotica* **49**:227-238.
- Hakkola J, Bernasconi C, Coecke S, Richert L, Andersson TB, and Pelkonen O (2018) Cytochrome P450 induction and xeno-sensing receptors pregnane X receptor, constitutive androstane receptor, aryl hydrocarbon receptor and peroxisome proliferator-activated receptor α at the crossroads of toxicokinetics and toxicodynamics. *Basic & Clinical Pharmacology & Toxicology* **123**:42-50.
- Hakkola J, Hukkanen J, Turpeinen M, and Pelkonen O (2020) Inhibition and induction of CYP enzymes in humans: an update. *Archives of Toxicology* **94**:3671-3722.
- Halilbasic E, Claudel T, and Trauner M (2013) Bile acid transporters and regulatory nuclear receptors in the liver and beyond. *Journal of Hepatology* **58**:155-168.

- Hammer H, Schmidt F, Marx-Stoelting P, Pötz O, and Braeuning A (2021) Cross-species analysis of hepatic cytochrome P450 and transport protein expression. *Archives of Toxicology* **95**:117-133.
- Hansen LG (1998) Stepping backward to improve assessment of PCB congener toxicities. *Environ Health Perspect* **106 Suppl 1**:171-189.
- Haque SJ, Petersen DD, Nebert DW, and Mackenzie PI (1991) Isolation, sequence, and developmental expression of rat UGT2B2: the gene encoding a constitutive UDP glucuronosyltransferase that metabolizes etiocholanolone and androsterone. *DNA Cell Biol* **10**:515-524.
- Hennemann G, Docter R, Friesema ECH, De Jong M, Krenning EP, and Visser TJ (2001) Plasma membrane transport of thyroid hormones and its role in thyroid hormone metabolism and bioavailability. *Endocrine Reviews* **22**:451-476.
- Hiasa Y, Kitahori Y, Ohshima M, Fujita T, Yuasa T, Konishi N, and Miyashiro A (1982) Promoting effects of phenobarbital and barbital on development of thyroid tumors in rats treated with N-bis(2-hydroxypropyl)nitrosamine. *Carcinogenesis* **3**:1187-1190.
- Higuchi K, Kobayashi Y, Kuroda M, Tanaka Y, Itani T, Araki J, Mifuji R, Kaito M, and Adachi Y (2004) Modulation of organic anion transporting polypeptide 1 and multidrug resistance protein 3 expression in the liver and kidney of Gunn rats. *Hepatol Res* **29**:60-66.
- Hood A, Allen ML, Liu Y, Liu J, and Klaassen CD (2003) Induction of T4 UDP-GT activity, serum thyroid stimulating hormone, and thyroid follicular cell proliferation in mice

treated with microsomal enzyme inducers. *Toxicology and Applied Pharmacology* **188**:6-13.

Hood A, Hashmi R, and Klaassen CD (1999a) Effects of microsomal enzyme inducers on thyroid-follicular cell proliferation, hyperplasia, and hypertrophy. *Toxicology and Applied Pharmacology* **160**:163-170.

Hood A and Klaassen CD (2000a) Differential effects of microsomal enzyme inducers on in vitro thyroxine (T(4)) and triiodothyronine (T(3)) glucuronidation. *Toxicol Sci* **55**:78-84.

Hood A and Klaassen CD (2000b) Effects of microsomal enzyme Inducers on outer-ring deiodinase activity toward thyroid hormones in various rat tissues. *Toxicology and Applied Pharmacology* **163**:240-248.

Hood A, Liu J, and Klaassen CD (1999b) Effects of phenobarbital, pregnenolone-16alpha-carbonitrile, and propylthiouracil on thyroid follicular cell proliferation. *Toxicol Sci* **50**:45-53.

Japundžić MM (1969) The goitrogenic effect of phenobarbital-Na on the rat thyroid. *Cells Tissues Organs* **74**:88-96.

Japundzic MM, Bastomsky CH, and Japundzic IP (1976) Enhanced biliary thyroxine excretion in rats treated with pregnenolone 16 α carbonitrile. *Acta Endocrinologica* **81**:110-119.

Johnson DR and Klaassen CD (2002) Regulation of rat multidrug resistance protein 2 by classes of prototypical microsomal enzyme inducers that activate distinct transcription pathways. *Toxicol Sci* **67**:182-189.

- Jones BR, Li W, Cao J, Hoffman TA, Gerk PM, and Vore M (2005) The role of protein synthesis and degradation in the post-transcriptional regulation of rat multidrug resistance-associated protein 2 (Mrp2, Abcc2). *Mol Pharmacol* **68**:701-710.
- Kato Y, Fujii A, Haraguchi K, Fujii Y, Atobe K, Endo T, Kimura O, Koga N, Ohta C, Yamada S, and Degawa M (2017) Possible mechanism for the polychlorinated biphenyl-induced liver-selective accumulation of thyroxine in rats. *The Journal of Toxicological Sciences* **42**:663-669.
- Kato Y, Ikushiro S-I, Takiguchi R, Haraguchi K, Koga N, Uchida S, Sakaki T, Yamada S, Kanno J, and Degawa M (2007) A Novel Mechanism for Polychlorinated Biphenyl-Induced Decrease in Serum Thyroxine Level in Rats. *Drug Metabolism and Disposition* **35**:1949-1955.
- Kato Y, Ikushiro S, Haraguchi K, Yamazaki T, Ito Y, Suzuki H, Kimura R, Yamada S, Inoue T, and Degawa M (2004) A possible mechanism for decrease in serum thyroxine level by polychlorinated biphenyls in Wistar and Gunn rats. *Toxicol Sci* **81**:309-315.
- Kato Y, Suzuki H, Haraguchi K, Ikushiro S, Ito Y, Uchida S, Yamada S, and Degawa M (2010) A possible mechanism for the decrease in serum thyroxine level by phenobarbital in rodents. *Toxicology and Applied Pharmacology* **249**:238-246.
- Kato Y, Suzuki H, Ikushiro S, Yamada S, and Degawa M (2005) Decrease in serum thyroxine level by phenobarbital in rats is not necessarily dependent on increase in hepatic UDP-glucuronosyltransferase. *Drug Metabolism and Disposition* **33**:1608-1612.

- Kato Y, Tamaki S, Haraguchi K, Ikushiro SI, Fujii Y, Ohta C, Atobe K, Kimura O, Endo T, Koga N, Yamada S, and Degawa M (2019) Kanechlor 500-mediated changes in serum and hepatic thyroxine levels primarily occur in a transthyretin-unrelated manner. *J Appl Toxicol* **39**:1701-1709.
- Klaassen CD (1970a) Effects of phenobarbital on the plasma disappearance and biliary excretion of drugs in rats. *Journal of Pharmacology and Experimental Therapeutics* **175**:289-300.
- Klaassen CD (1970b) Plasma disappearance and biliary excretion of sulfobromophthalein and phenol-3,6-dibromophthalein disulfonate after microsomal enzyme induction. *Biochemical Pharmacology* **19**:1241-1249.
- Klaassen CD and Aleksunes LM (2010) Xenobiotic, bile acid, and cholesterol transporters: Function and regulation. *Pharmacological Reviews* **62**:1-96.
- Klaassen CD and Lu H (2008) Xenobiotic transporters: ascribing function from gene knockout and mutation studies. *Toxicological Sciences* **101**:186-196.
- Klaassen CD and Plaa GL (1968) Studies on the mechanism of phenobarbital-enhanced sulfobromophthalein disappearance. *Journal of Pharmacology and Experimental Therapeutics* **161**:361-366.
- Klaassen CD and Slitt AL (2005) Regulation of hepatic transporters by xenobiotic receptors. *Current Drug Metabolism* **6**:309-328.

- Köhle C and Bock KW (2009) Coordinate regulation of human drug-metabolizing enzymes, and conjugate transporters by the Ah receptor, pregnane X receptor and constitutive androstane receptor. *Biochemical Pharmacology* **77**:689-699.
- Krenning EP, Docter R, Bernard HF, Visser TJ, and Hennemann G (1978) Active transport of triiodothyronine (T3) into isolated rat liver cells. *FEBS Lett* **91**:113-116.
- Kretschmer XC and Baldwin WS (2005) CAR and PXR: Xenosensors of endocrine disrupters? *Chemico-Biological Interactions* **155**:111-128.
- Küblbeck J, Niskanen J, and Honkakoski P (2020) Metabolism-disrupting chemicals and the constitutive androstane receptor CAR. *Cells* **9**:2306.
- Lecureux L, Dieter MZ, Nelson DM, Watson L, Wong H, Gemzik B, Klaassen CD, and Lehman-Mckeeman LD (2009) Hepatobiliary disposition of thyroid hormone in Mrp2-deficient TR⁻ rats: reduced biliary excretion of thyroxine glucuronide does not prevent xenobiotic-induced hypothyroidism. *Toxicological Sciences* **108**:482-491.
- Li N, Hartley DP, Cherrington NJ, and Klaassen CD (2002) Tissue expression, ontogeny, and inducibility of rat organic anion transporting polypeptide 4. *Journal of Pharmacology and Experimental Therapeutics* **301**:551-560.
- Liu J, Liu Y, Barter RA, and Klaassen CD (1995) Alteration of thyroid homeostasis by UDP-glucuronosyltransferase inducers in rats: a dose-response study. *Journal of Pharmacology and Experimental Therapeutics* **273**:977-985.
- Lumb G, Newberne P, Rust JH, and Wagner B (1978) Effects in animals of chronic administration of spironolactone--a review. *J Environ Pathol Toxicol* **1**:641-660.

- Mackenzie PI, Walter Bock K, Burchell B, Guillemette C, Ikushiro S-i, Iyanagi T, Miners JO, Owens IS, and Nebert DW (2005) Nomenclature update for the mammalian UDP glycosyltransferase (UGT) gene superfamily. *Pharmacogenetics and Genomics* **15**:677-685.
- Marin JJG (2012) Plasma membrane transporters in modern liver pharmacology. *Scientifica* **2012**:1-15.
- Martin L and Klaassen CD (2010) Differential effects of polychlorinated biphenyl congeners on serum thyroid hormone levels in rats. *Toxicological Sciences* **117**:36-44.
- Martin LA (2003) Differential effects of polychlorinated biphenyl (PCB) mixtures and congeners on the disposition of thyroxine (T4) in rats, Rutgers University.
- Martin LA, Wilson DT, Reuhl KR, Gallo MA, and Klaassen CD (2012) Polychlorinated biphenyl congeners that increase the glucuronidation and biliary excretion of thyroxine are distinct from the congeners that enhance the serum disappearance of thyroxine. *Drug Metabolism and Disposition* **40**:588-595.
- Masubuchi N, Hakusui H, and Okazaki O (1997) Effects of proton pump inhibitors on thyroid hormone metabolism in rats: a comparison of UDP-glucuronyltransferase induction. *Biochem Pharmacol* **54**:1225-1231.
- McClain R (1995) Mechanistic considerations for the relevance of animal data on thyroid neoplasia to human risk assessment. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* **333**:131-142.

- McClain RM (1989) The significance of hepatic microsomal enzyme induction and altered thyroid function in rats: implications for thyroid gland neoplasia. *Toxicologic Pathology* **17**:294-306.
- McClain RM, Levin AA, Posch R, and Downing JC (1989) The effect of phenobarbital on the metabolism and excretion of thyroxine in rats. *Toxicology and Applied Pharmacology* **99**:216-228.
- McClain RM, Posch RC, Bosakowski T, and Armstrong JM (1988) Studies on the mode of action for thyroid gland tumor promotion in rats by phenobarbital. *Toxicol Appl Pharmacol* **94**:254-265.
- Meech R, Hu DG, McKinnon RA, Mubarakah SN, Haines AZ, Nair PC, Rowland A, and Mackenzie PI (2019) The UDP-glycosyltransferase (UGT) superfamily: new members, new functions, and novel paradigms. *Physiol Rev* **99**:1153-1222.
- Meek ME, Bucher JR, Cohen SM, Dellarco V, Hill RN, Lehman-McKeeman LD, Longfellow DG, Pastoor T, Seed J, and Patton DE (2003) A framework for human relevance analysis of information on carcinogenic modes of action. *Crit Rev Toxicol* **33**:591-653.
- Miyawaki I, Moriyasu M, Funabashi H, Yasuba M, and Matsuoka N (2003) Mechanism of clobazam-induced thyroidal oncogenesis in male rats. *Toxicology Letters* **145**:291-301.
- Miyawaki I, Tamura A, Matsumoto I, Inada H, Kunitatsu T, Kimura J, and Funabashi H (2012) The effects of clobazam treatment in rats on the expression of genes and proteins encoding glucuronosyltransferase 1A/2B (UGT1A/2B) and multidrug

resistance-associated protein-2 (MRP2), and development of thyroid follicular cell hypertrophy. *Toxicol Appl Pharmacol* **265**:351-359.

Murk AJ, Rijntjes E, Blaauboer BJ, Clewell R, Crofton KM, Dingemans MML, David Furlow J, Kavlock R, Köhrle J, Opitz R, Traas T, Visser TJ, Xia M, and Gutleb AC (2013) Mechanism-based testing strategy using in vitro approaches for identification of thyroid hormone disrupting chemicals. *Toxicology in Vitro* **27**:1320-1346.

Noyes PD, Friedman KP, Browne P, Haselman JT, Gilbert ME, Hornung MW, Barone S, Crofton KM, Laws SC, Stoker TE, Simmons SO, Tietge JE, and Degitz SJ (2019) Evaluating chemicals for thyroid disruption: opportunities and challenges with in vitro testing and adverse outcome pathway approaches. *Environmental Health Perspectives* **127**:095001.

Oppenheimer JH, Bernstein G, and Surks MI (1968) Increased thyroxine turnover and thyroidal function after stimulation of hepatocellular binding of thyroxine by phenobarbital. *Journal of Clinical Investigation* **47**:1399-1406.

Paul KB, Hedge JM, DeVito MJ, and Crofton KM (2009) Short-term exposure to triclosan decreases thyroxine in vivo via upregulation of hepatic catabolism in young Long-Evans rats. *Toxicological Sciences* **113**:367-379.

Paulusma CC, Bosma PJ, Zaman GJ, Bakker CT, Otter M, Scheffer GL, Scheper RJ, Borst P, and Oude Elferink RP (1996) Congenital jaundice in rats with a mutation in a multidrug resistance-associated protein gene. *Science* **271**:1126-1128.

- Piantanida E, Ippolito S, Gallo D, Masiello E, Premoli P, Cusini C, Rosetti S, Sabatino J, Segato S, Trimarchi F, Bartalena L, and Tanda ML (2020) The interplay between thyroid and liver: implications for clinical practice. *Journal of Endocrinological Investigation* **43**:885-899.
- Plummer S, Beaumont B, Elcombe M, Wallace S, Wright J, McInnes EF, Currie RA, and Cowie D (2021) Species differences in phenobarbital-mediated UGT gene induction in rat and human liver microtissues. *Toxicology Reports* **8**:155-161.
- Rausch-Derra LC, Hartley DP, Meier PJ, and Klaassen CD (2001) Differential effects of microsomal enzyme-inducing chemicals on the hepatic expression of rat organic anion transporters, OATP1 and OATP2. *Hepatology* **33**:1469-1478.
- Richardson TA and Klaassen CD (2010a) Disruption of thyroid hormone homeostasis in Ugt1a-deficient Gunn rats by microsomal enzyme inducers is not due to enhanced thyroxine glucuronidation. *Toxicology and Applied Pharmacology* **248**:38-44.
- Richardson TA and Klaassen CD (2010b) Role of UDP-glucuronosyltransferase (UGT) 2B2 in metabolism of triiodothyronine: effect of microsomal enzyme inducers in Sprague Dawley and UGT2B2-deficient Fischer 344 rats. *Toxicological Sciences* **116**:413-421.
- Safe S, Bandiera S, Sawyer T, Robertson L, Safe L, Parkinson A, Thomas PE, Ryan DE, Reik LM, Levin W, and et al. (1985) PCBs: structure-function relationships and mechanism of action. *Environ Health Perspect* **60**:47-56.
- Saghir SA, Charles GD, Bartels MJ, Kan LHL, Dryzga MD, Brzak KA, and Clark AJ (2008) Mechanism of trifluralin-induced thyroid tumors in rats. *Toxicology Letters* **180**:38-45.

- Saito K, Kaneko H, Sato K, Yoshitake A, and Yamada H (1991) Hepatic UDP-glucuronyltransferase(s) activity toward thyroid hormones in rats: Induction and effects on serum thyroid hormone levels following treatment with various enzyme inducers. *Toxicology and Applied Pharmacology* **111**:99-106.
- Schrenk D, Baus PR, Ermel N, Klein C, Vorderstemann B, and Kauffmann H-M (2001) Up-regulation of transporters of the MRP family by drugs and toxins. *Toxicology Letters* **120**:51-57.
- Semler DE, Chengelis CP, and Radzialowski FM (1989) The effects of chronic ingestion of spironolactone on serum thyrotropin and thyroid hormones in the male rat. *Toxicology and Applied Pharmacology* **98**:263-268.
- Shelby MK, Cherrington NJ, Vansell NR, and Klaassen CD (2003) Tissue mRNA expression of the rat UDP-glucuronosyltransferase gene family. *Drug Metab Dispos* **31**:326-333.
- Shelby MK and Klaassen CD (2006) Induction of rat UDP-glucuronosyltransferases in liver and duodenum by microsomal enzyme inducers that activate various transcriptional pathways. *Drug Metabolism and Disposition* **34**:1772-1778.
- Shiratani H, Katoh M, Nakajima M, and Yokoi T (2008) Species differences in UDP-glucuronosyltransferase activities in mice and rats. *Drug Metabolism and Disposition* **36**:1745-1752.
- Slitt AL (2018) Absorption, distribution, and excretion of toxicants, in: *Casarett & Doull's Toxicology: The Basic Science of Poisons, Ninth Edition* (Klaassen CD ed), pp 1696, McGraw-Hill Education.

Sueyoshi T and Negishi M (2001) Phenobarbital response elements of cytochrome P450 genes and nuclear receptors. *Annu Rev Pharmacol Toxicol* **41**:123-143.

The International Transporter Consortium (2010) Membrane transporters in drug development. *Nature Reviews Drug Discovery* **9**:215-236.

Thompson TN and Klaassen CD (1995) The effects of hepatic microsomal enzyme inducers on the pharmacokinetics of ouabain after portal and systemic administration to rats. *Journal of Pharmacy and Pharmacology* **47**:1041-1047.

Tirona RG and Kim RB (2005) Nuclear receptors and drug disposition gene regulation. *J Pharm Sci* **94**:1169-1186.

Tong Z, Li H, Goljer I, McConnell O, and Chandrasekaran A (2007) In vitro glucuronidation of thyroxine and triiodothyronine by liver microsomes and recombinant human UDP-glucuronosyltransferases. *Drug Metabolism and Disposition* **35**:2203-2210.

Van Birgelen AP, Smit EA, Kampen IM, Groeneveld CN, Fase KM, Van der Kolk J, Poiger H, Van den Berg M, Koeman JH, and Brouwer A (1995) Subchronic effects of 2,3,7,8-TCDD or PCBs on thyroid hormone metabolism: use in risk assessment. *Eur J Pharmacol* **293**:77-85.

van der Spek AH, Fliers E, and Boelen A (2017) The classic pathways of thyroid hormone metabolism. *Molecular and Cellular Endocrinology* **458**:29-38.

Van Groen BD, Nicolaï J, Kuik AC, Van Cruchten S, Van Peer E, Smits A, Schmidt S, De Wildt SN, Allegaert K, De Schaepdrijver L, Annaert P, and Badée J (2021) Ontogeny

of hepatic transporters and drug-metabolizing enzymes in humans and in nonclinical species. *Pharmacological Reviews* **73**:597-678.

van Raaij JA, Kaptein E, Visser TJ, and van den Berg KJ (1993) Increased glucuronidation of thyroid hormone in hexachlorobenzene-treated rats. *Biochem Pharmacol* **45**:627-631.

Vansell NR and Klaassen CD (2001) Increased biliary excretion of thyroxine by microsomal enzyme inducers. *Toxicology and Applied Pharmacology* **176**:187-194.

Vansell NR and Klaassen CD (2002a) Effect of microsomal enzyme inducers on the biliary excretion of triiodothyronine (T(3)) and its metabolites. *Toxicol Sci* **65**:184-191.

Vansell NR and Klaassen CD (2002b) Increase in rat liver UDP-glucuronosyltransferase mRNA by microsomal enzyme inducers that enhance thyroid hormone glucuronidation. *Drug Metab Dispos* **30**:240-246.

Vansell NR, Muppidi R JR, Habeebu SM, and Klaassen CD (2004) Promotion of thyroid tumors in rats by pregnenolone-16 α -carbonitrile (PCN) and polychlorinated biphenyl (PCB). *Toxicological Sciences* **81**:50-59.

Visser TJ, Kaptein E, and Harpur ES (1991) Differential expression and ciprofibrate induction of hepatic UDP-glucuronyltransferases for thyroxine and triiodothyronine in Fischer rats. *Biochem Pharmacol* **42**:444-446.

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

- Visser TJ, Kaptein E, van Toor H, van Raaij JA, van den Berg KJ, Joe CT, van Engelen JG, and Brouwer A (1993b) Glucuronidation of thyroid hormone in rat liver: effects of in vivo treatment with microsomal enzyme inducers and in vitro assay conditions. *Endocrinology* **133**:2177-2186.
- Visser WE, Friesema ECH, and Visser TJ (2011) Minireview: thyroid hormone transporters: the knowns and the unknowns. *Molecular Endocrinology* **25**:1-14.
- Wang Y-M, Ong SS, Chai SC, and Chen T (2012) Role of CAR and PXR in xenobiotic sensing and metabolism. *Expert Opinion on Drug Metabolism & Toxicology* **8**:803-817.
- Watkins JB and Klaassen CD (1982) Induction of UDP-glucuronosyltransferase activities in Gunn, heterozygous, and Wistar rat livers by pregnenolone-16 alpha-carbonitrile. *Drug Metab Dispos* **10**:590-594.
- Wong H, Lehman-McKeeman LD, Grubb MF, Grossman SJ, Bhaskaran VM, Solon EG, Shen HSL, Gerson RJ, Car BD, Zhao B, and Gemzik B (2005) Increased hepatobiliary clearance of unconjugated thyroxine determines DMP 904-induced alterations in thyroid hormone homeostasis in rats. *Toxicological Sciences* **84**:232-242.
- Wu SY, Green WL, Huang WS, Hays MT, and Chopra IJ (2005) Alternate pathways of thyroid hormone metabolism. *Thyroid* **15**:943-958.
- Xie Y and Xie W (2020) The role of sulfotransferases in liver diseases. *Drug Metabolism and Disposition* **48**:742-749.

Zanger UM and Schwab M (2013) Cytochrome P450 enzymes in drug metabolism: Regulation of gene expression, enzyme activities, and impact of genetic variation. *Pharmacology & Therapeutics* **138**:103-141.

Footnotes:

This work received no external funding.

Nichole R. Vansell, Pfizer Inc, Eastern Point Road, Groton, CT 06340; nichole.vansell@pfizer.com; 860-271-9954

Figure captions

Figure 1. A depiction of the sites of inducer activity in the alteration of thyroid hormone disposition. The numbers represent: 1) increased uptake of thyroid hormone into liver; 2) increased glucuronidation of thyroid hormone by UGT enzymes; 3) increases in the biliary excretion of conjugated and unconjugated thyroid hormones; 4) increased efflux of thyroid hormone and conjugates into circulation. Those inducers that produce the most substantial increase in T4 clearance from circulation appear to primarily target site 1, uptake, whereas inducers that are most effective at increasing serum TSH target site 2. However, all activities likely work in coordination to facilitate alterations in circulating thyroid hormone.

Figure 1