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Drug Metabolism and Disposition
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DRUG METABOLISM & DISPOSITION

Special Section on Bile Acids, Drug Metabolism, and Toxicity—Minireview

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

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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 thyroxine (T4) glucuronidation by UDP-glycosyltransferase (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 thyroid-stimulating hormone. With the identification of other key inducible drug processing genes and proteins involved in hepatic disposition of thyroid hormones, including uptake (e.g., organic anion transporter polypeptides) and efflux (e.g., multidrug resistance proteins) transporters, data exist that support transporters as additional target 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 (triiodothyronine 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 have contributed key information, yet the contributions of efflux transport (canalicular and basolateral) to the mechanism of individual, effective inducers also require 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 thyroid-stimulating hormone (TSH) as part of maintenance by the hypothalamus-pituitary-thyroid axis that regulates available T3 in the tissues. Circulating thyroid hormone consists mainly of thyroxine (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, whereas 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 pregnenolone-16α-carbonitrile (PCN) (Thompson and Klaassen, 1995) altered the biliary excretion of xenobiotics and

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

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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. UDP-glycosyltransferase (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; Crivellente 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; Giacomini et al., 2010; Marin, 2012; Halilbasic et al., 2013; Slitt, 2018; Felmlee 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 increased 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, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and polychlorinated biphenyl (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; Japundžić 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 benzypyrene (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 that 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

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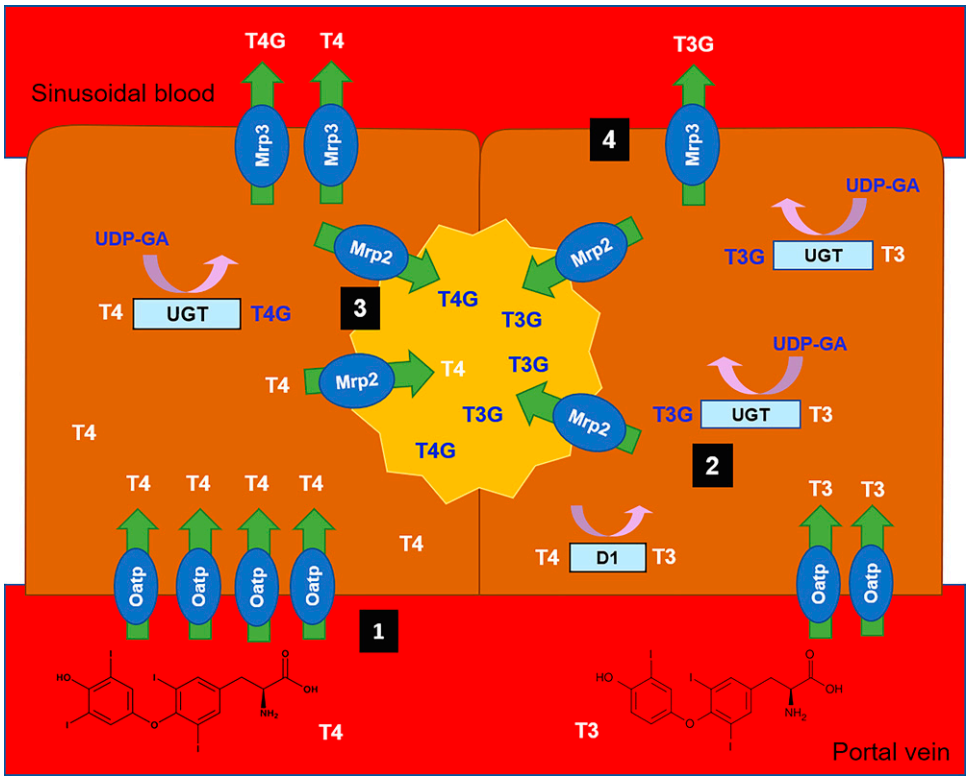

Fig. 1. A depiction of the sites of inducer activity in the alteration of thyroid hormone disposition. The numbers represent: (1) increased uptake of thyroid hormone into liver; (2) increased glucuronidation of thyroid hormone by UGT enzymes; (3) increases in the biliary excretion of conjugated and unconjugated 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.

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-16α-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 (i.e., Aroclor 1254 had largest effect on both), but not between the lesser decreases in serum triiodothyronine (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 (i.e., less steep for phenobarbital [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 (i.e., 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

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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.

Inducer	Receptor	Model	Effects	References
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)

KO, knockout; T3G, T3 glucuronide; T4G, T4 glucuronide.

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 site of more 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

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T4 glucuronidation may drive increases in circulating TSH and thyroid gland proliferation after 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 Sprague-Dawley (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 Fischer 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, in which 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, whereas 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 laboratory 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

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the return to circulation (Van Groen et al., 2021). In rats, hepatic uptake transporters include organic anion transporter polypeptides (Oatps), organic cation transporters, and sodium taurocholate cotransporting polypeptide (Ntcp), among others; and efflux transporters including the bile salt export pump, multidrug resistance protein (Mrps) (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 [^{125}I]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 [^{125}I]T4 and, within 1 minute, increases in uptake of [^{125}I]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 Kaneclor 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.

4-(3-pentylamino)-2,7-dimethyl-8-(2-methyl-4-methoxyphenyl)-pyrazolo-[1,5-a]-pyrimidine (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 (80× control) and T4 glucuronide along with a small increase in the unbound fraction of T4 in blood (~2×). 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 [^{125}I]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, in which T4 glucuronide was the major biliary metabolite, in control TR$^-$ rats the predominant T4 metabolite detected in bile was T3 sulfate, indicating that Mrp2 is responsible for elimination of T4 glucuronide in bile. In TR$^-$ rats, phenobarbital or DMP 904 did not increase the plasma clearance of [^{125}I]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 [^{125}I]T4 from the blood was increased at the end of 4 weeks; no effects on thyroid gland ^{125}I uptake or organification were apparent. To address further the role of glucuronidation versus biliary excretion of thyroid hormone following clobazam administration, two mutant rat strains, Gunn (UGT1A-deficient) and Eisai hyperbilirubinemic rats (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

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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-chain 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., 2010).

Current Hypothesis, Gaps, and Future Considerations

From these collective data, it is apparent there are two 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 Fig. 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 [^{125}I]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 in which 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 knockout 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 [^{125}I]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 versus 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

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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 and 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., 2020), and transporter expression can vary widely among individuals and populations (Amacher, 2016). Furthermore, 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 with the rat homologs, and there were differences in the UGT homologs that were induced in rat versus human three-dimensional microtissues (Plummer et al., 2020).

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) mean integrated in vitro assays that measure induction of thyroid hormone transporters and conjugating enzymes are likely required.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Vansell.

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