

A Weight of Evidence Review on the Mode of Action, Adversity, and the Human Relevance of Xylene’s Observed Thyroid Effects in Rats

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Introduction

Xylene substances (**Table 1**) have wide industrial and consumer uses and are currently undergoing dossier and substance evaluation under Registration, Evaluation, Authorization and Restriction of Chemicals (REACH). New repeated dose toxicity study data on xylenes identify the thyroid as a potential target organ, and therefore a weight of evidence review (WoE) is provided to investigate whether xylene-mediated changes on the hypothalamus-pituitary-thyroid (HPT) axis are secondary to liver enzyme induction and are of a magnitude that is relevant for neurological human health concerns.

Table 1. Chemical ID of Xylene Isoforms, Xylene, and Xylene Reaction Mass (RM) Substances

Xylene Isomers								
Substance	ortho (o)-xylene		meta (m)-xylene		para (p)-xylene			
CAS #	95-47-6		108-38-3		106-42-3			
EC #	202-422-2		203-576-3		203-396-5			
	Typical conc. ^a	Conc. Range ^a	Typical conc.	Conc. range	Typical conc.	Conc. range		
	98%	>98%	98%	>98%	95%	>90-100 %		
Structure								
Xylene and Xylene Reaction Mass (RM) Substances								
Substance	Xylene		RM EB ^b and Xylene		RM EB ^b and m-Xylene		RM EB ^b , m-Xylene and p-Xylene	
CAS #	1330-20-7		-		-		-	
EC #	215-535-7		905-588-0		905-570-2		905-562-9	
Constituent	Typical conc.	Conc. range	Typical conc.	Conc. range	Typical conc.	Conc. range	Typical conc.	Conc. range
o-Xylene	20%	20 - 30%	>10%	5 - 60%	5%	< 10%	2.5%	< 10%
m-Xylene	50%	40 - 60%	>10%	10 - 60%	>10%	10 - 79.9%	>10%	10 - 65%
p-Xylene	20%	15 - 30%	>10%	5 - 60%	5%	< 10%	>10%	7 - 40%
EB ^b	-	>3 - <10%	>10%	10 - 75%	>10%	10 - 60%	>10%	10 - 60%

^a All typical concentrations and concentration ranges are (w/w); ^b EB – ethylbenzene.

Methods

The review used published data from the literature, from multiple newly generated guideline OECD 422, OECD 408 and OECD 414 studies with xylene isomers and xylene reaction mass substances, and data from the United States Environmental Protection Agency (US EPA) Toxicity Forecasting (ToxCast US EPA ToxCast database¹) in combination with metabolomic analysis (MetaMap®Tox)² to compile the WoE for xylene mediated effects on the HPT axis.

The WOE review included the following:

- Review of effects on the Liver
- Mode of Action on the thyroid
- Review of effects on the thyroid, and xylene metabolomics

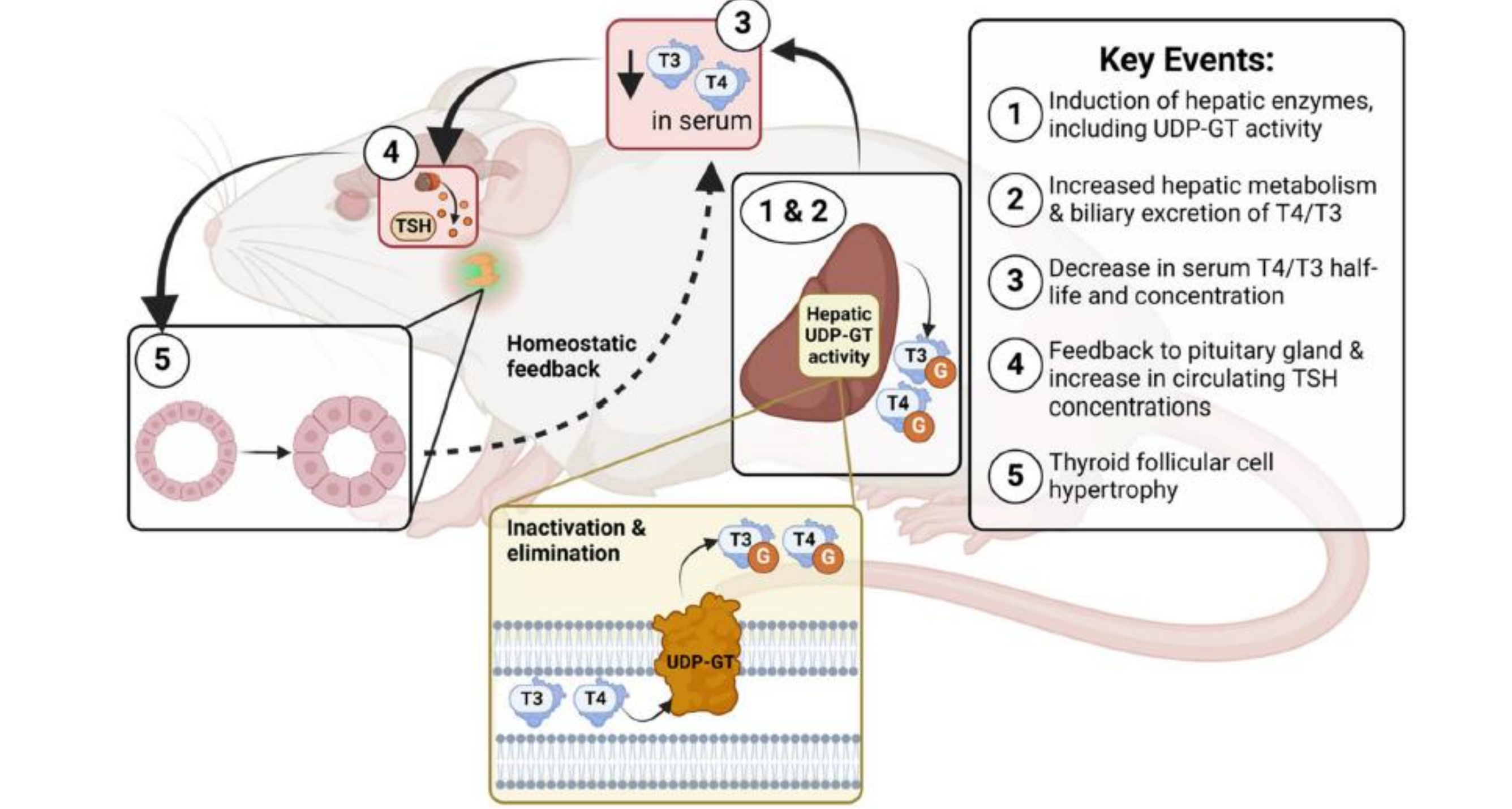
Review of Liver Effects

There is clear *in vivo* evidence in the rat of liver enzyme induction can increase metabolism of thyroid hormones. Oral gavage repeated dose toxicity studies with xylene exposure show increased liver weights in the rat (**Table 2**)³. Exposure to xylene causes centrilobular hepatocyte hypertrophy in the rat, and electron microscopy analysis has confirmed increased smooth endoplasmic reticulum proliferation in hepatocytes⁴. This is a reversible process consistent with an adaptive response in the liver in response to xenobiotic exposure⁵. The magnitude of hypertrophy observed in hepatocytes was mild, completely consistent with a normal physiological adaptive response, and occurs in the absence of liver injury.

Mode of Action on Thyroid

Figure 1. Depiction of the key events occurring in an indirect thyroid MOA in rodents

UDP-GT: uridine 5'-diphospho-glucuronosyltransferase



Once the serum T4 levels are reestablished to their “normal” physiological range, homeostasis is reestablished so the compensatory signals through the HPT axis are shut off. A disruption of this homeostatic feedback, through sustained and escalating UDP-GT induction leading to constant TSH secretion (**Figure 1**), and continued thyroid hormone biosynthesis, is a critical step that can lead to escalating thyroid pathology (e.g. follicular cell hyperplasia) in rats. However, this is not observed in humans and not considered a human-relevant mechanism.

Xylene Liver Data

Table 2. Summary of liver changes for technical xylene

Substance		Males				Females			
o-xylene									
Condie et al. (1988)	Dose (mg/kg/day)	0	250	1000	2000	0	250	1000	2000
	Absolute weights				+			+	+
	Relative weights							+	+
Hypertrophy									
Britton (2020)	Dose (mg/kg/day)	0	100	300	1000	0	100	300	1000
	Absolute weights				+			+	+
	Relative weights			+	+			+	+
Hypertrophy									
-									
-									
+									
++									
p-xylene									
Condie et al. (1988)	Dose (mg/kg/day)	0	250	1000	2000	0	250	1000	2000
	Absolute weights				+		+	+	+
	Relative weights							+	+
Hypertrophy									
Wolfe (1988a)	Dose (mg/kg/day)	0	100	200	800	0	100	200	800
	Absolute weights								
	Relative weights								
Hypertrophy									
-									
-									
+									
m-xylene									
Condie et al. (1988)	Dose (mg/kg/day)	0	250	1000	2000	0	250	1000	2000
	Absolute weights			+	+			+	+
	Relative weights			+	+			+	+
Hypertrophy									
Wolfe (1988a)	Dose (mg/kg/day)	0	100	200	800	0	100	200	800
	Absolute weights								
	Relative weights								
Hypertrophy									
-									
-									
-									
Mixed xylene									
Flick et al. (2021)	Dose (mg/kg/day)	0	300	1000		0	300	1000	
	Absolute weights		+	+			+	+	
	Relative weights		+	+			+	+	
Hypertrophy									
Condie et al. (1988)	Dose (mg/kg/day)	0	150	750	1500	0	150	750	1000
	Absolute weights		+	+	+		+	+	+
	Relative weights		+	+	+		+	+	+
Hypertrophy									
van Vessem (2023)	Dose (mg/kg/day)	0	100	300	1000	0	100	300	1000
	Absolute weights				+				+
	Relative weights				++				+
Hypertrophy									
-									
-									
-									
Reaction mass ethylbenzene and xylene									
Peeters and van Erp (2023)	Dose (mg/kg/day)	0	100	300	1000	0	100	300	1000
	Absolute weights			+	+			+	+
	Relative weights			+	+			+	+
Hypertrophy									
van Vessem (2023)	Dose (mg/kg/day)	0	100	300	1000	0	100	300	1000
	Absolute weights				+				+
	Relative weights								
Hypertrophy									
-									
Not evaluated									
-									
Reaction mass ethylbenzene, m-xylene and p-xylene									
Doomen-van den Hoven and van de Heuvel (2024)	Dose (mg/kg/day)	0	100	300	1000	0	100	300	1000
	Absolute weights				+				+
	Relative weights				+				+
Hypertrophy									
-									
-									
-									
+									
Reaction mass ethylbenzene, m-xylene and p-xylene									
Doomen-van den Hoven and Hoogendoorn (2024)	Dose (mg/kg/day)	0	100	300	1000	0	100	300	1000
	Absolute weights				+				+
	Relative weights			+	+			+	+
Hypertrophy									
-									
-									
-									
+									

Liver weights:
+ denotes p < 0.05;

Hypertrophy:
- denotes no incidence
+ denotes minimal/grade 1
++ denotes slight/mild/grade 2
+++ denotes moderate

For a list of full references used in **Table 2**, please scan QR code at bottom right of poster.

Review of Thyroid Effects

Studies in the rat have not revealed consistent effects after exposure to xylene isomers, mixed xylene, or xylene reaction mass substances. Only minimal to slight increases in thyroid follicular cell hypertrophy have been observed in some repeated-dose studies . There were no irreversible or severe correlated pathological effects in the thyroid. None of the observed effects were observed in the offspring in reproductive toxicity studies.

The lack of reliable and consistent trends in *in vivo* studies provide evidence that Xylene’s effects on the thyroid are weak and non-adverse, and that the measured outcomes are in fact the result of a normal functioning HPT in magnitudes well below the published literature thresholds for developmental neurotoxicological outcomes established for thyroidal changes in animals and humans.

In vitro data from the United States Environmental Protection Agency (US EPA) Toxicity Forecasting (ToxCast) provides no evidence of xylene interaction with thyroidal functionality.

Xylene Metabolomics

Metabolomics analysis (MetaMap®Tox)⁶ of xylene showed a dose-dependent, slight to strong effect on the metabolome in both sexes of the rat. The comparison with patterns in MetaMap®Tox database (**Table 3**) showed matches or weak matches for liver effects, such as liver toxicity and paracetamol-like toxicity in animals of both sexes. In female animals, a further weak match was observed for a pattern indicating indirect effects on the thyroid (increased thyroid hormone excretion due to the liver enzyme induction), supporting the organ weight and histopathology data in repeat dose toxicity studies.

Importantly, no patterns were observed that were consistent with direct thyroid-acting substance toxicity.

Table 3. MetaMap®Tox metabolomics summary results for technical xylene

Metabolite parameters	300 mg/kg/day		1000 mg/kg/day	
	Females	Males	Females	Males
Number down regulated	7	9	10	12
Number up regulated	21	12	85	58
Number total	28	21	95	70
% down regulated	2.7	3.4	3.8	4.5
% up regulated	8.0	4.5	32.2	21.9
% total	10.6	7.9	36.0	26.4
Profile Strength	1.2	1.02	2.36	1.82
Observed MetaMap®Tox Pattern Ranking Assessment				
Only metabolite matches of 75- <90% were observed with each of the present modalities				
Males			Females	
300 mg/kg/day	1000 mg/kg/day		300 mg/kg/day	1000 mg/kg/day
No matches	Liver, enzyme induction Liver toxicity		No matches	Thyroid indirect, liver enzyme induction Liver, enzyme induction d14 Liver toxicity Liver, enzyme induction Kidney, organic anion transporter inhibition

Discussion & Conclusion

The data and various lines of scientific evidence presented herein individually and collectively demonstrate that xylene’s mediated changes in the HPT axis, *via* a secondary extra-thyroidal MOA (i.e. liver enzyme induction), do not raise a human health concern with regards to developmental neurotoxicity. As such, the available toxicological data do not support classification of xylene as a known or suspected endocrine disruptor, specifically through the thyroid modality, per Regulations Commission Delegated Regulation (EU) 2023/ 707 of 19 December 2022 amending Regulation (EC) No 1272/2008 and do not support the need for a neurotoxicological cohort evaluation in any subsequent EOGRTS.

1. Fowles JR, Banton MI, Boogaard PJ, Ketelslegers HB, Rohde AM. 2016. Assessment of petroleum streams for thyroid toxicity. Toxicol Lett. 254:52–62. doi: 10.1016/j.toxlet.2016.05.001;
2. US Environmental Protection Agency (EPA). 2024. Comptox chemicals dashboard v2.4.1. Data retrieved 13 May 2024;
3. Condie LW, Hill JR, Borzelleca JF. 1988. Oral toxicology studies with xylene isomers and mixed xylenes. Drug Chem Toxicol. 11(4):329–354. doi: 10.3109/01480548809018107;
4. Rydzynski K, Korsak Z, Jedlinska U, Sokal JA. 1992. The toxic effects of combined exposure of toluene and m-xylene in animals. IV. Liver ultrastructure after subchronic inhalatory exposure. Int J Occup Med Environ Health. 5(1):35–42;
5. Feldman D, Swarm RL, Becker J. 1980. Elimination of excess smooth endoplasmic reticulum after phenobarbital administration. J Histochem Cytochem. 28(9):997–1006. doi: 10.1177/28.9.7410819;
6. Kamp H, Kocabas NA, Faulhammer F, Synhaeve N, Rushton E, Flick B, Gird V, Sperber S, Higgins LG, Penman MG, et al. 2024. Utility of in vivo metabolomics to support read-across for UVCB substances under REACH. Arch Toxicol. 98(3):755–768. doi: 10.1007/s00204-023-03638-6

