

A Systematic Review and Weight of Evidence Evaluation on the Immunosuppressive Potential of Xylene

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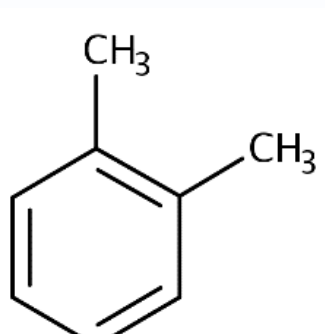
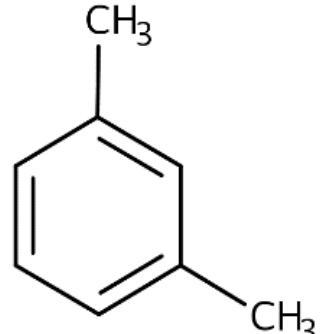
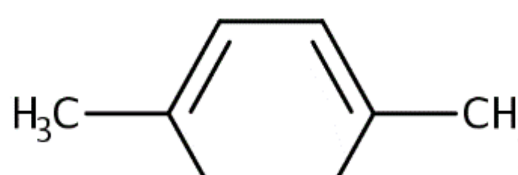
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Introduction

Xylene, an aromatic hydrocarbon organic solvent (Table 1), is currently undergoing dossier evaluation under Registration, Evaluation, Authorization and Restriction of Chemicals (REACH). Several spurious, isolated, and/or inconsistent immune system findings have been reported across the dataset. LOA XWG and Cefic APA* have evaluated the dataset in its totality to examine the potential for xylene-mediated immunosuppression.

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.

As a legacy industrial chemical with a significant amount of existing animal and human data, the xylene substances present a unique opportunity for a weight of evidence (WoE) review to investigate whether additional animal toxicology testing is truly necessary to meet the specified regulatory data requirements or if it would be contradictory to the ECHA commitment to the 3R principles.

*Lower Olefins and Aromatics Xylene Working Group and CEFIC Aromatics Producers Association

Methods

A systematic literature review and WoE approach evaluation was conducted that included consideration of all available immunotoxicity information on xylene derived from relevant in vitro, in vivo, and epidemiology approaches including previously unpublished new studies of xylene substances. Literature searches were conducted on Pro Quest and PubMed, with a total of 2703 unique hits identified. All studies were reviewed for relevancy which concluded that ultimately 8 studies were considered for inclusion for further discussion. Note that no study was excluded from the review based on a data quality assessment and any strengths or limitations of these published studies were reviewed.

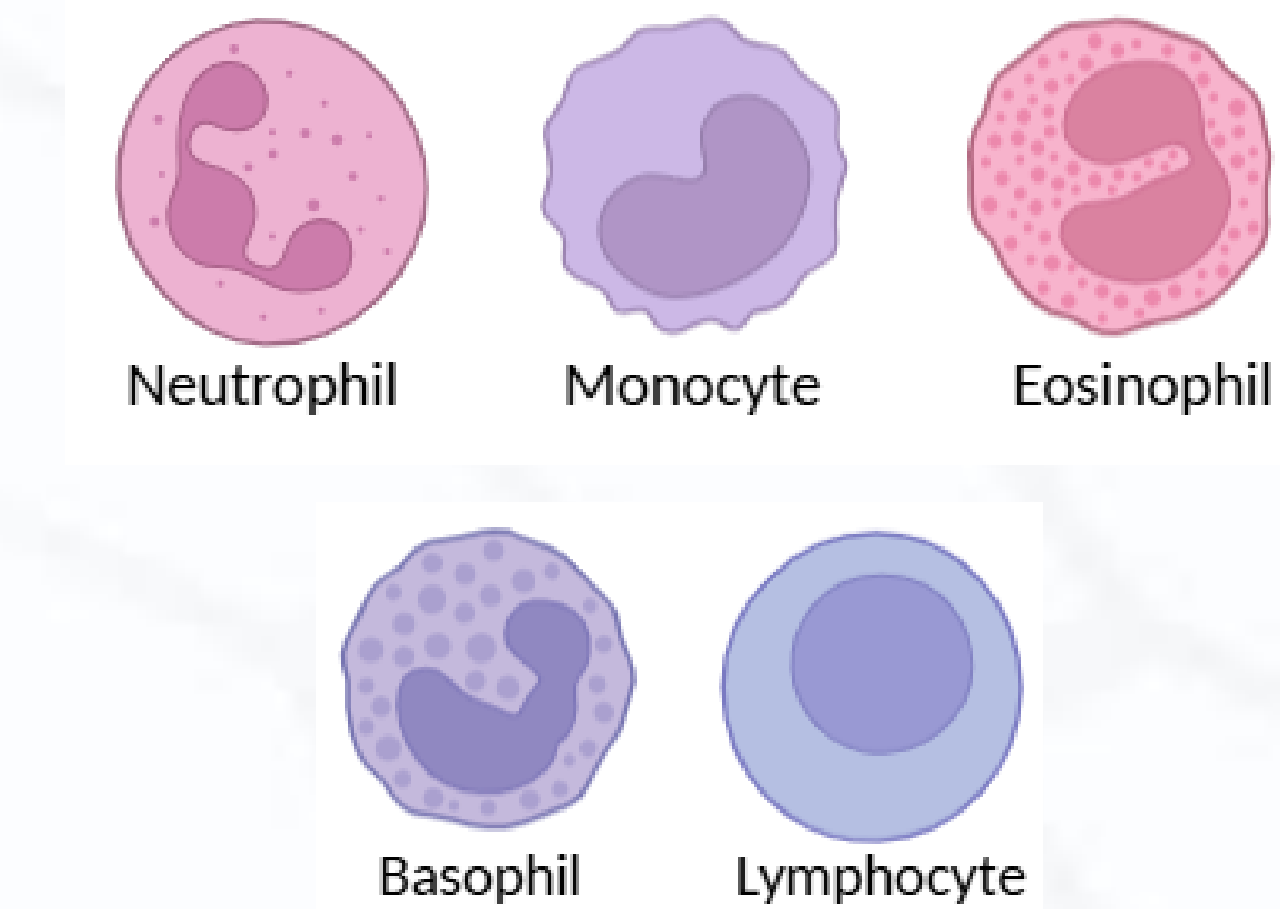
The specific mechanisms/modes of action of xylene and xylene metabolites that would indicate immunosuppression were reviewed including the following endpoints:

- Haematology
- Clinical chemistry markers such as globulin
- Immune system tissues including thymus, lymph nodes, spleen and thymus

Haematology Effects

Table 2. Summary of Key Haematological Effects (WBC counts and lymphocytes)

Total White Blood Cell Count	
Reference/Guideline/Test Substance	Effect/Reasoning
Gerding (2024) ¹¹ OECD TG 422 (Oral) <i>m</i> -xylene	↓ 33% at 300mg/kg/day in females No effect at top dose.
Condie et al (1988) ¹² OECD TG 408 (Oral) Technical xylene	↓ 15% at 150mg/kg/day in males No effect at top two doses. ↑ 35% and 48% at top two doses in females No other immunotoxicity changes observed
Yin et al (2024) ¹³ Non guideline (Oral) Xylene (unclear identity)	↓ WBC counts compared to controls. Insufficient details reported. Conflation of xylene with benzene effects.
Korsak et al (1994) ¹⁴ Non guideline (Inhalation) <i>m</i> -xylene	↑ 35% in top dose group. Pre-exposure haematology data NR. Findings inconsistent with Korsak et al (1992).
Lymphocyte Count	
Reference/Guideline/Test Substance	Effect/Reasoning
Korsak et al (1992) ¹⁵ Non guideline (Inhalation) <i>m</i> -xylene	↓ 25% in male exposure group Guidance from WHO (2015) changes in lymphocytes without changes in total WBC counts considered non-adverse



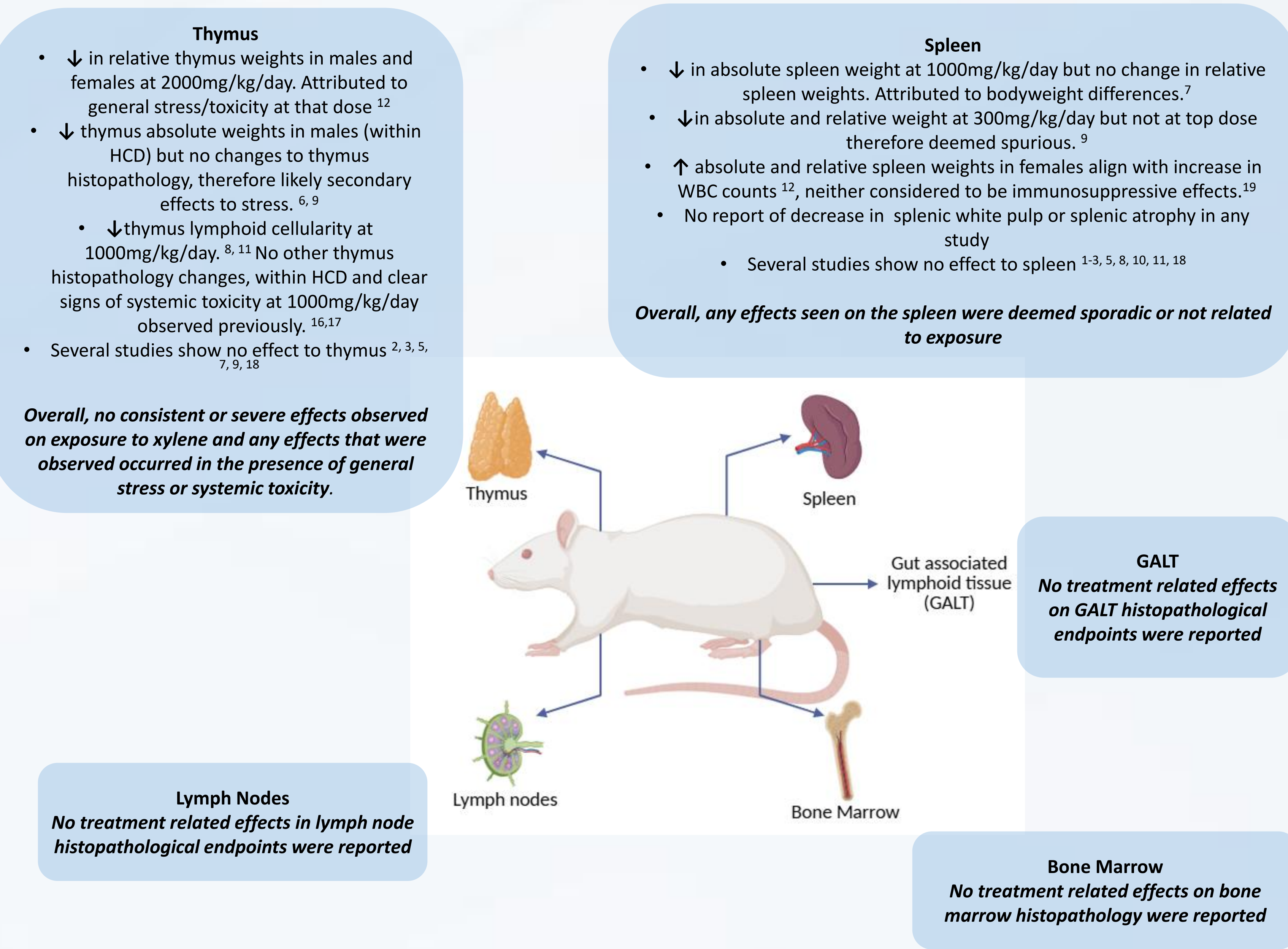
Globulin Effects

Table 3. Summary of Xylene Globulin Effects

Reference/Guideline/Test Substance	Globulin	Albumin: Globulin Ratio	Reasoning
Wolfe (1988a) ¹ OECD TG 408 (Oral) <i>m</i> -xylene	No statistically significant differences in either sex up to 800mg/kg/day	No statistically significant differences in either sex up to 800mg/kg/day	No effect
Britton (2020) ³ OECD TG 408 (Oral) <i>o</i> -xylene	↓ in female rat globulin levels at all doses.	↑ at all dose levels in female rats. Albumin levels also increased at all dose levels.	↓ immunoglobulin levels could be considered immunosuppressive, globulin levels within HCD and ↑ in albumin levels makes the ratio difficult to interpret. No effects observed in males.
Wolfe (1988b) ² OECD TG 408 (Oral) <i>p</i> -xylene	No statistically significant differences in either sex up to 800mg/kg/day	No statistically significant differences in either sex up to 800mg/kg/day	No effect

Immune System Tissue Effects

Repeated dose guideline studies included analysis of spleen and thymus organ weights and thymus, spleen, lymph node and bone marrow histopathological examinations. Any effects observed in the studies reviewed are summarised below.



Discussion & Conclusion

Multiple high-quality guideline in vivo studies have repeatedly interrogated xylene’s immunotoxicity potential including systemic hematological and clinical chemistry markers as well as histopathological evaluations of bone marrow, lymph nodes, spleen, and thymus. Several statistically spurious, isolated, and/or inconsistent immune system findings have been reported across the dataset but overall, the dataset in its totality demonstrated no signal of xylene-mediated immunosuppression.

In conclusion, the WoE supports that that xylene substances are not immunosuppressants and does not support the need for an immunotoxicity cohort evaluation in any subsequent EOGRTs per Regulations Commission Delegated Regulation (EU) 2015/ 282 of 20 February 2015 amending Regulation (EC) No 1907/2006.

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