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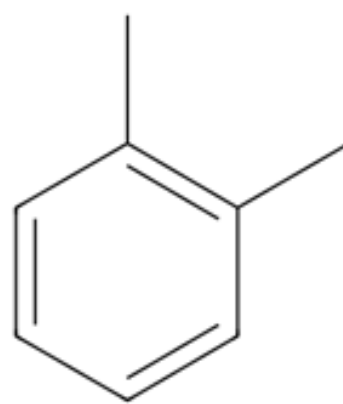
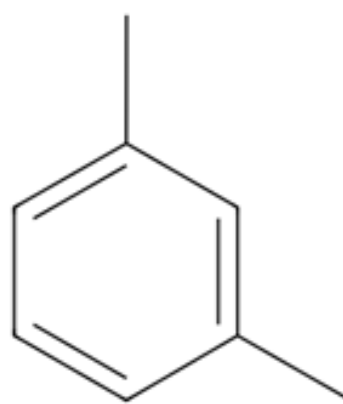
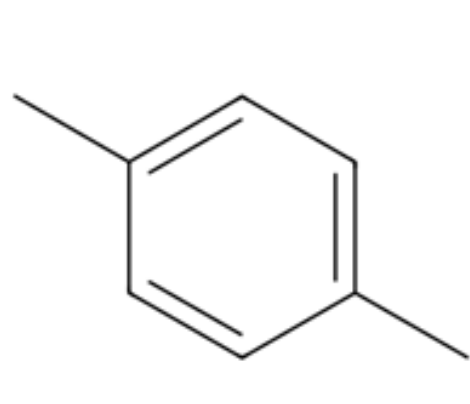
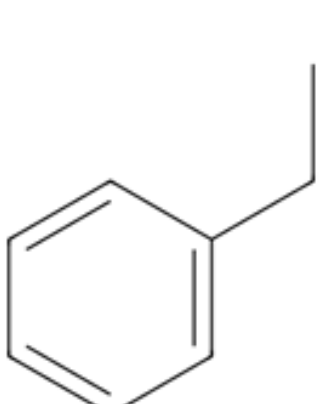
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Introduction

The REACH legislation defines the information requirements for hazard assessment of industrial chemicals. Read-Across is a tool that can be used between chemicals with similar physical-chemical and biological properties to potentially fulfill the information requirements.

The aim of this study¹ was to investigate the metabolism between the three xylene isomers (ortho- (o-), meta- (m), and para- (p) xylene), and ethylbenzene, as single substrates with structural similarities (see [Table 1](#)); and to further investigate whether a mixture representing the most typical composition of the REACH registered reaction mass of ethylbenzene (EC No. 905-588-0) and xylene would influence single substrate enzymatic kinetics. Reaction mass of ethylbenzene and xylene (EC No. 905-588-0) has a typical composition of 15% o-xylene, 37% m-xylene, 21% p-xylene and 27% ethylbenzene. Substrate and protein dependency were measured with a time course to determine Michaelis-Menten kinetics (Km and Vmax) of each single substrate. Data were then used to design an experiment with the mixture (reaction mass of ethylbenzene and xylene) where metabolism of each isomer in the mixture could be compared to the metabolism of each isomer alone to determine if exposure to the mixture affected the system’s ability to metabolize the substrate.

Table 1: Chemical structure of the xylene isomers and ethylbenzene

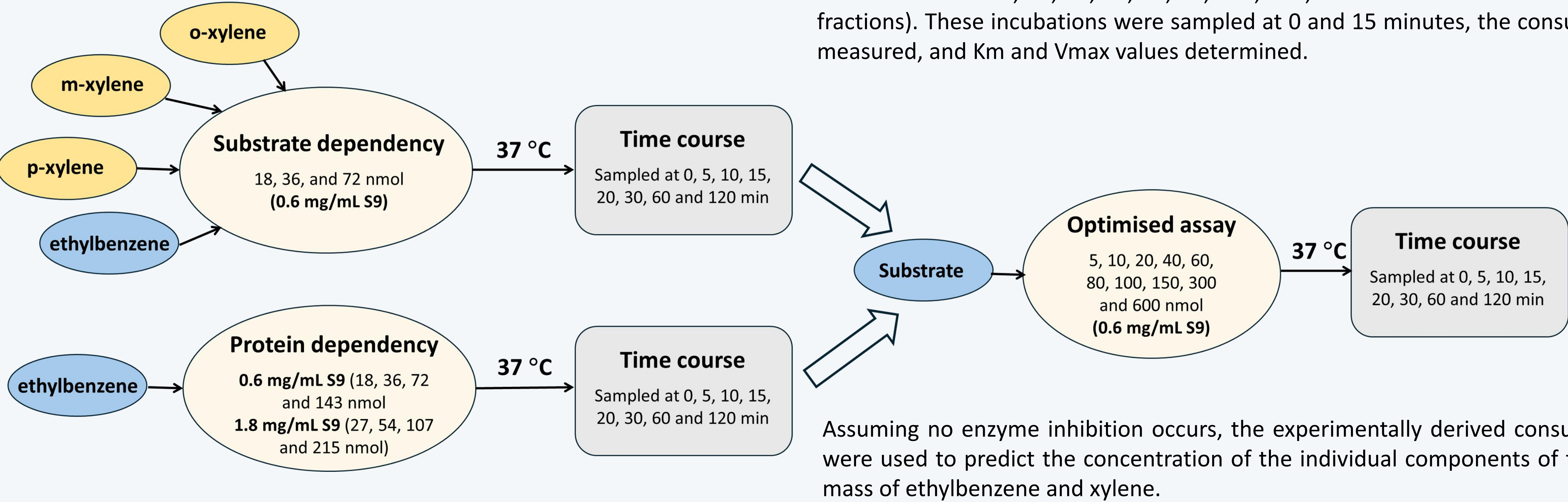
Substance	ortho (o)-xylene	meta (m)-xylene	para (p)-xylene	Ethylbenzene
CAS RN	95-47-6	108-38-3	106-42-3	100-41-4
EC No.	202-422-2	203-576-3	203-396-5	202-849-4
Structure				

Methods

A GC-MS method was validated to detect each of the single substrates, o-xylene, m-xylene, p-xylene, and ethylbenzene, and their respective major phase I metabolites: 2-methylbenzyl alcohol (2-MBA), 3-MBA, 4-MBA and 1-phenyl ethanol (1-PE).

To optimize the assay (see [Fig. 1](#)), single substrates (o-xylene, m-xylene, p-xylene and ethylbenzene) were incubated with rat S9 fractions at 37 °C (substrate dependency) and samples taken at various timepoints (time course). Ethylbenzene was additionally assayed with varying concentrations at two protein concentrations using the same sampling schedule (protein dependency).

Figure 1: Optimising the assay



Finally, a mixture representing the reaction mass of ethylbenzene and xylene was incubated according to the optimized experimental conditions, and the concentration of the separate substrates and the consumption rates measured.

Results

Incubations of the single substrates demonstrated similarity between the substances o-xylene, m-xylene, p-xylene and ethylbenzene in terms of Michaelis-Menten kinetics; i.e. the consumption rates and the determined Km and Vmax values were similar across all four single substrates (see [Table 2](#)).

Table 2: Michaelis-Menten kinetics of the xylene isomers and ethylbenzene

Parameter	o-xylene	m-xylene	p-xylene	ethylbenzene
Vmax [min ⁻¹]	5.35	4.34	3.51	5.02
Km [nmol]	140	110	100	125
Km/Vmax calculated	26.2	25.3	28.5	24.9

The individual single substrate rates (see [Table 2](#)) were used to predict the experimental substrate turnover values for substrate concentrations for the mixture representing the reaction mass of ethylbenzene and xylene (see [Table 3](#)).

The experimental consumption rate for the mixture representing reaction mass of ethylbenzene and xylene was then measured and was in agreement with predicted values (see [Table 3](#)), demonstrating that metabolism is not affected by incubating substrates alone or as a mixture representing a reaction mass substance; therefore, no enzyme inhibition took place.

Table 3: Comparison of predicted vs experimental substrate concentration at T15 for mixture representing the reaction mass ethylbenzene and xylene

Substrate	T _{0min} Experimental mean value (measured)	T _{15min} Calculated (predicted)	T _{15min} Experimental mean value (measured)	Experimental accuracy
	[nmol]	[nmol]	[nmol]	[%]
o-xylene	6.89	4.14	3.86	93.2
m-xylene	16.4	11.6	10.5	90.5
p-xylene	9.07	6.55	6.41	97.7
ethylbenzene	11.4	7.81	6.43	82.3

Discussion & Conclusion

Under the experimental conditions chosen, the results supported the hypothesis that metabolism rates of ethylbenzene and the three xylene isomers do not differ, regardless of whether they are exposed as single isomeric substrates or as a mixture representing the reaction mass of ethylbenzene and xylene (EC No. 905-588-0).

Therefore, due to the metabolic and structural similarities, it is feasible to use data from isomeric xylene substances to read-across to each other and to xylene reaction mass substances. This mechanistic work therefore supports the 3Rs (replacement, reduction, refinement) as a principle for read-across purposes in REACH, supporting registrant obligations to use animals as a last resort.