Toxicological validation during the development of new catalytic systems using air / liquid interface cell exposure system

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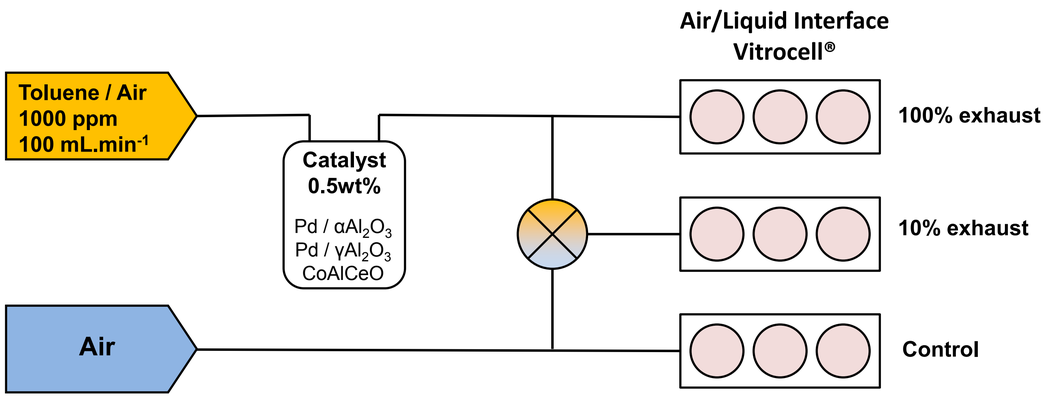
INTRODUCTION

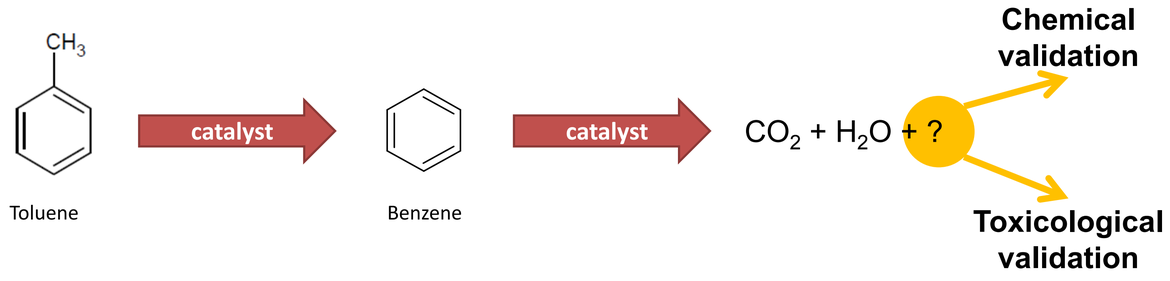
Volatile Organic Compounds (VOCs), including Benzene, Toluene, Ethylbenzene and Xylenes (BTEX) are industrial solvents frequently used and emitted into the atmosphere. Toluene is one of the most used despite its major and direct impact on human health. It is therefore fundamental to minimize emissions directly at source.

Catalytic oxidation represents an efficient remediation technique in order to reduce VOCs emission directly at the source, but it can release byproducts. Catalyst performance is usually evaluated using dedicated analytical chemistry methods measuring VOC conversion or CO_2 emission. Precious metals such as gold are the most effective catalysts, but they are very expensively. It is necessary to develop less expensive alternatives by keeping a good efficiency. In this study, several catalysts with aluminum doped with low contents of precious metals were developed for the oxidation of the toluene (i.e. Pd / αAl_2O_3, Pd/ γAl_2O_3 and CoAlCeO).

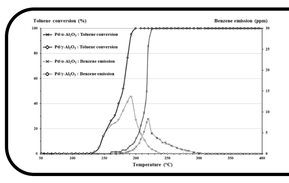
The aim of this study was to determine the most efficient catalyst for toluene oxidation. Thereby A549 lung cells were exposed using an air/liquid interface (ALI) system for 1h to a 1000ppm stream of toluene or to gas mixtures issued from its oxidation. Following the exposure, toxicological tests were conducted to validate the best performing catalyst for toluene remediation.

EXPERIMENTAL DESIGN



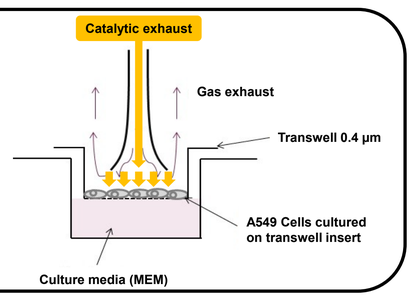


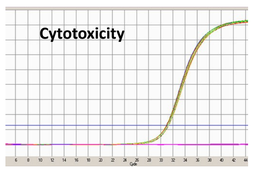
METHODS



Determination of catalyst temperature to obtain 100% of toluene conversion. Light-off curve were obtained by heating catalyst between 50 and 400°C with a temperature ramp of 1.5°C.min^{-1}, while measuring toluene conversion by µGC/MS.

Exposure of A549 cells to gas released from the catalytic procedure. The Vitrocell® system was coupled with the VOCs oxidation experimental setup in a flow rate of 5 mL.min^{-1} in order to achieve a 1h cell exposure with the three catalysts Pd/α-Al_2O_3, Pd/γ-Al_2O_3 and CeCo/Al_2O_3.





Xenobiotic Metabolising Enzymes

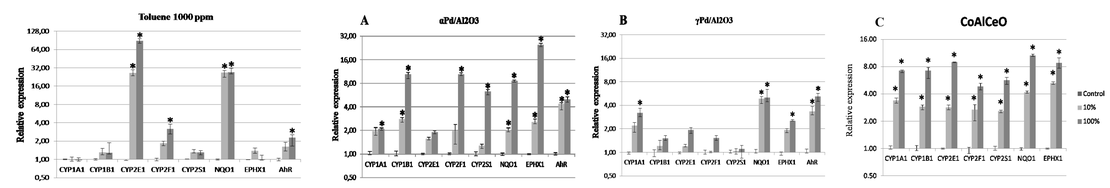
Toxicological validation using cytotoxicity and expression levels by RT-qPCR of cytochromes family genes and their promotors followed by characterization of by-products by µGC/MS.

RESULTS & DISCUSSION

Cytotoxicity

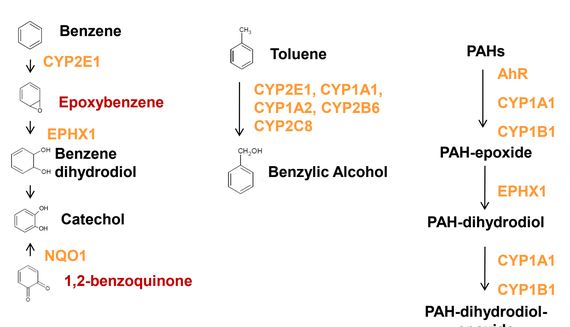
Cytotoxicity study performed with LDH test did not show any significant changes in the membrane integrity of cells exposed to catalytic emissions. This verification of the absence of significant cell death allows us to consider the underlying pathophysiological mechanisms of toxicity.

Expression of Xenobiotic Metabolizing Enzymes (XMEs)



Relative expression of XME genes after exposure to toluene or exhaust from catalytic oxidation of toluene

A549 cells exposed for 1h to 100 or 1000ppm of toluene activated the classical pathway of toluene metabolization. *CYP1A1*, *CYP1B1*, *CYP2S1* and *EPHX1* were not induced while an increase of *NQO1* and *CYP2E1* gene expression level were observed at both concentrations. After catalytic degradation of toluene, these genes were less expressed in cells exposed to catalytic exhausts than in cells exposed to toluene. On the other hand, in the three catalytic process, cells exposed to catalytic exhaust showed an induction of many genes not involved in toluene metabolism such as *CYP1A1*, *CYP1B1*, *CYP2S1* and *EPHX1*.



Metabolic activation of Toluene, Benzene and PAHs

	Toluene	Pd/α-Al_2O_3	Pd/γ-Al_2O_3	CoAlCeO
CYP1A1	=	+	+	++
CYP1B1	=	++	=	++
CYP2E1	+++	=	=	++
CYP2F1	+	++	=	++
CYP2S1	=	++	=	++
CYP3A4	=	=	=	=
CYP2B6	=	=	=	=
AhR	+	++	++	++
PXR	=	=	=	=
CAR	=	=	=	=
NQO1	+++	++	++	++
EPHX1	=	+++	+	+

Our study is a first step in the validation of new catalyst. The purpose of the toxicological validation is to measure the efficiency of toluene degradation by comparing gene induction in pure toluene exposure and after catalytic oxidation. In addition it gives information on the composition of the by-products. Results showed an increase in gene induction of *CYP1A1*, *CYP1B1* and *CYP2E1*. CYP1 subfamily members are the major cytochromes involved in PAH bioactivation [1] specially *CYP1A1* and *CYP1B1* [2, 3]. Even though *CYP2S1* belongs to the CYP2 family, it is involved in PAH metabolism as in the case of naphthalene exposure [4]. In addition, our results reported an increase in AHR gene expression when compared to control. This receptor is a cytochrome inducer (*CYP1A1*, *CYP1B1* and CYP2E1) regulated by polycyclic aromatic hydrocarbons [5]. The increase in the expression of cytochromes and their regulatory receptor confirms the presence of PAHs in the catalytic exhausts after oxidation of toluene.

CONCLUSION

This study validated in a first step the suitability of using Vitrocell® system as an innovative, direct and dynamic model of ALI exposure in the development of new catalysts, showing the presence of chemically undetected byproducts. CYP1A1 and CYP1B1 activation indicates the presence of PAHs, which were not detected by chemical analysis by µGC.

Our study highlighted the importance to reduce toluene, VOC known for its direct effects on human health and allowed the conduction of a toxicological validation in order to determine the more suitable catalyst for toluene oxidation. In a second step, the comparison of the catalysts showed that less organic compounds metabolizing genes were induced with Pd/γ-Al_2O_3 making it more efficient for toluene remediation.

ACKNOWLEDGEMENTS

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