Journal of Polish CIMEEAC
Faculty of Ocean Engineering & Ship Technology
GDAŃSK UNIVERSITY OF TECHNOLOGY

THE CYTOTOXICITY VERIFICATION OF THE VOCs REMOVAL BY MEANS OF PHOTOCATALYSIS

Anna Czarny, Ewa Zaczyńska

Ludwik Hirszfeld
Institute of Immunology and Experimental Therapy
Polish Academy of Sciencesul. Rudolfa Weigla 12
53-114 Wrocław tel. +48-71-337 1172
czarny@iitd.pan.wroc.pl, ezacz@iitd.pan.wroc.pl

Aleksander Górniak, Anna Janicka

Wroclaw University of Technology
Department Vehicle Engineering
Braci Gierymskich 164 Street , 51-640 Wrocław
tel +48/(71) 347 79 26, fax +48/(71) 347 79 18
e-mail: aleksander.gorniak@pwr.wroc.pl, anna.janicka@pwr.wroc.pl,

Maciej Zawiślak

Wroclaw University of Technology
Faculty of Mechanical Engineering
Department of Machine Design and Research
Lukasiewicza 7/9
50-371 Wroclaw
e-mail: maciej.zawislak@pwr.wroc.pl

Abstract

This paper constitutes the approach to correlate the results of gas purification properties performed with aid of photocatalysis with the cytotoxicity test results. The gas purification by means of photocatalysis is a subject well known a profusely described in literature. Easiness and continence of application causes the photocatalysis to be used in variety of industries, however this paper considers only automotive industry application. The effectiveness of photocatalysis have been proven experimentally by vast number of researchers. However, the literature contains only the information about the level of volatile organic compounds identification and reduction. The toxicity measure in terms of living cells was not yet fully tested and described in literature. Therefore, the standard chromatography test which enables quantitative identification of volatile organic compounds was confronted with a cytotoxicity tests revealing the virtual toxicity of gases before and after photocatalysis.

Keywords: *Cytotoxicity, photocatalysis, volatile organic compounds*

1 Introduction

One of the most hazardous for human chemical substances are a compounds in which the main, primordial sources are processes of anthropogenic nature. In the case of a passenger compartment of a vehicle the predominant source of hazardous volatile organic compounds (VOC) occurs as a consequence of a co – emission of outer sources (mobile and stationary emitors) as well as inner sources (emission of materials resulting from the vehicle exploitation – for example cigarette smoke). The lower boiling temperature of the substance (greater volatility), the greater level of hazard of subjecting a human organism to it. Low boiling aromatic hydrocarbon such as benzene, ethylbenzene, toluene and xylene (BTX) as well as their isomers appears to be toxic, mutagenic, and carcinogenic for the cells of respiratory system [1]. Therefore, the attempt to purify air aspirated into a car cabin is fully justified. In general, the gas purification can be obtained by means of variety of methods, three of which are most commonly used. One of them is absorption of volatile organic compounds on an active carbon, but this is rather a VOCs displacement than disposal. The second procedure is bifurcation which is sluggish and its results are rather doubtful. The third is a high temperature oxidation which occurs at temperatures of 200 to 1000℃ – effective but also expensive. The alternative is utilisation of photocatalysis which is more effective, environmental friendly and free from economic issues [2]. The most common catalytic agent is titanium dioxide TiO_2 due to its capability to degrade a wide spectrum of chemical individuals when illuminated with UV light or when in the vicinity of ultraviolet [3], [4], [5], [6].

One of procedure for effectiveness determination of a photocatalysis in terms of VOCs reduction is for example a chromatography test. Samples of a polluted air can be gathered before and after implementation of catalytic agent and UV light. Confrontation of those results indicates the level of gas purification abilities of a catalytic agent in given conditions (temperature, UV light length, surface of catalytic agent etc.). However, this effectiveness have never been confronted with actual impact on human health. To the best of this papers authors' knowledge there were no investigation confronting the VOCs reduction with the impact on human respiratory system. Even if a portion of compounds was reduced because of photocatalysis, it does not mean that they vanish. It is obvious that it was converted in different compounds which can be hazardous to humans as well. Therefore, in this sense, it is not enough adequate to state that reduction of VOCs is always related to human health protection. For this reason an approach to correlate the effectiveness of the photocatalysis with impact on human health arose. The verification was done with aid of cytotoxicity tests by the novel method of BAT-CELL Bio-Ambient Tests.

2 Test station

The test was performed on a specially designed test station (see Fig. 1) which simulates closed flow within a vehicle cabin. The test station is additionally equipped in aspiration socked for chromatography samples collection. The socked are located on both sides of the test chamber (in frond and behind it). Inasmuch the valves enables to direct flow in open and closed cycle, for the sake of this research only a closed flow was considered.

The volume of the closed loop pipeline is 150 litters. During this studies it was assumed that the air – pollution mixture shall be composed of 90% of air and 10% of pollution. Therefore, 15 litters of pollutions was introduced to the test station. A reference level was established before each test by performing the experiment without installation of the tested element. The flow rate is set to be 1 m/s of polluted air which is proportional to mass flow rate of 2 – 3 g/s of the mixture. The catalytic agent was installed inside of the test chamber and illuminated with set of UV light with light length of 365nm (Fig. 1 b).

a) b)

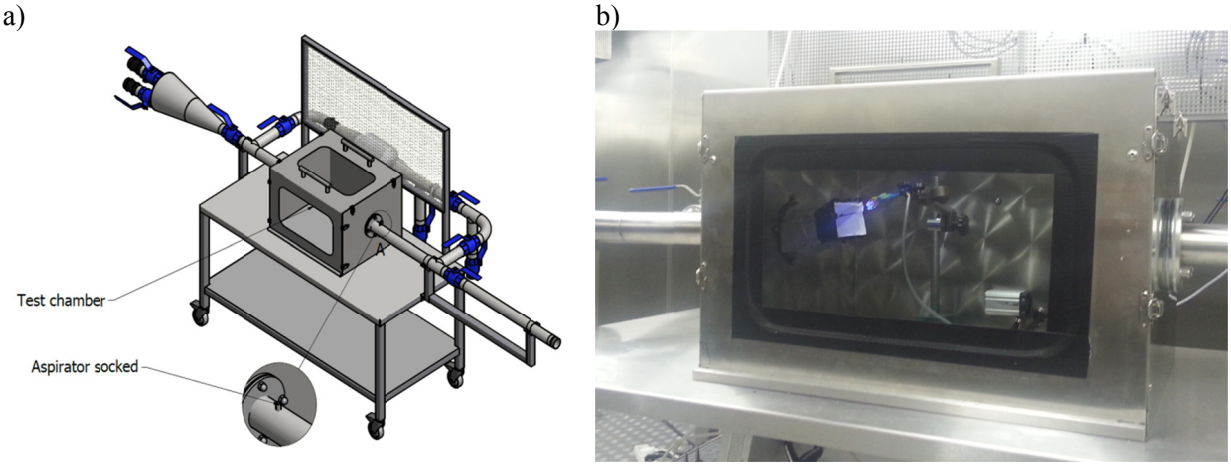


Fig. 1. The test station

Samples were collected by YEARS aspirator, model ASP-3 II, flow rate adjusted to 30 dm^3/h, the amount of gas collected is 10 dm^3. The gas was absorbed on active carbon Anasorb® SKC CSC. Activated carbon is poured into a glass tube of 5 cm^3 and later it is emerged in 2cm^3 of carbon disulfide. Volatile organic compounds in the samples was determined by gas chromatography according to the test procedure of the Emission Research Laboratory No. 1/2010 using the gas chromatograph Varian 450 - GC with flame ionization detector (FID), and a column Varian VF-WAXms 30mx0,25mm ID DF: 0.25 um. The work was performed at the set temperature of the column 373 K (110° C), the dispenser 523 K (250° C) and detectors 423 K (150° C). YEARS aspirator, model ASP-3 II, flow rate adjusted to 30 dm3/h, the amount of gas collected is 10 dm3, collected samples. The gas was absorbed on active carbon Anasorb® SKC CSC. Activated carbon is poured into a glass tube of 5 cm^3 and later it is emerged in 2cm^3 of carbon disulfide. The glass tube is sealed with a stopper. Extraction takes place in a period of 20 min. After every few minutes the contents of the bottle was shaken in order to ensure adequate mixing of the material. Then 5µl of solution is gathered from above of the carbon layer. Gathered sample is injected into the chromatograph. Compounds designated as "residuals" have been converted to the concentration corresponding to n-pentatonic acid. In other words residuals are the compounds existing within the gas mixtures but was not identified by the chromatograph. The total relative error of the method was estimated at 20% (according to PN - EN ISO 16017-1: 2006).

Simultaneously a cytotoxicity tests were performed in order to confront the gas VOCs removability with the gas impact on human health. Here the authors, patented research methodology was used – *BAT-CELL Bio-Ambient Tests*. Methodology of BAT-CELL Bio-Ambient Tests is the procedure of measure the actual impact of toxic gas mixtures to living cells, therefore, this method belongs to a group of direct methods. The cells are left without the culture fluid and placed within sterile closed chamber which is subsequently inflated with

tested toxic gas. After the exposition of the cell line to the toxic gas the cells are immersed in culture fluid and the toxicity is evaluated by counting the dead cells.

The BAT – CELL measurement device is shown in Fig. 2. The device contains five sterile closed chambers enclosed within one rectangular conditioning hosing which in turn is equipped in an antibacterial filter. The device is additionally equipped in an aspiratory system (aspiratory panel) connected with each of sterilely closed chambers in order to provide inflow and outflow of polluted gas. The live cells are located inside of each of sterile chambers. In order to evaluate an impact of a gas mixture to the human respiratory system and skin (mostly exposed to the gases) tests were performed on two groups of cell lines detailed below:

- L929 – fibroblast cell line derived from the subcutaneous adipose tissue of C3H mice (ATCC CCL 1)
- A549 - epithelial cell line of human lung carcinoma cells (ATCC CCL 185)

The plant cell culture of L929 and A459 having a density of 10^6 $^{cells}/_{ml}$, incubated for 24 hours at 37° C in a 5% CO_2. After this time, the cell supernatant was removed and the cell monolayer transported to the laboratory of the Department of Vehicle Engineering, Technical University of Wroclaw, where with the help of a dedicated bench sampling of BAT-CELL ® the toxicity was evaluated. Cells devoid of culture fluid within the duration of the test (30 min) constitutes the reference for tested gases.

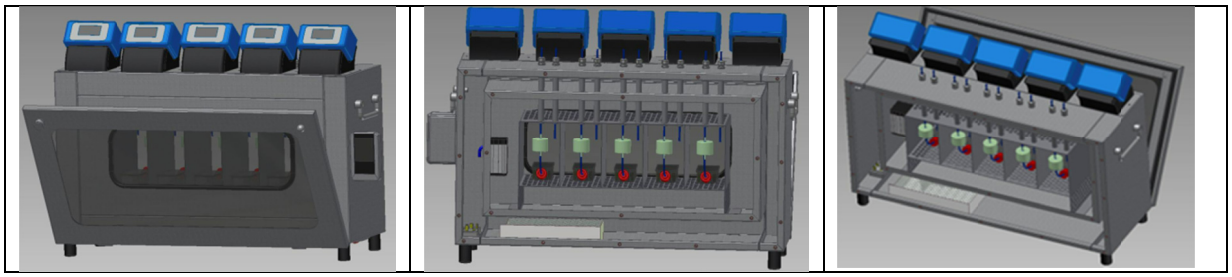


Fig. 2. BAT – CELL device

3 Results and discussion

As a result of qualitative and quantitative study of the gas samples taken in parallel (on the test bench) before and after the application of a catalytic agent, 9 groups of volatile organic compounds was identified. Samples were collected after 30 min (time of exposure of the cells). All of them, in addition to pentane, constitute a group of toxic aromatic hydrocarbons with known toxicity properties. As it can be seen in Fig. 3 the photocatalysis caused reduction of every compound that was detected during the chromatography test.

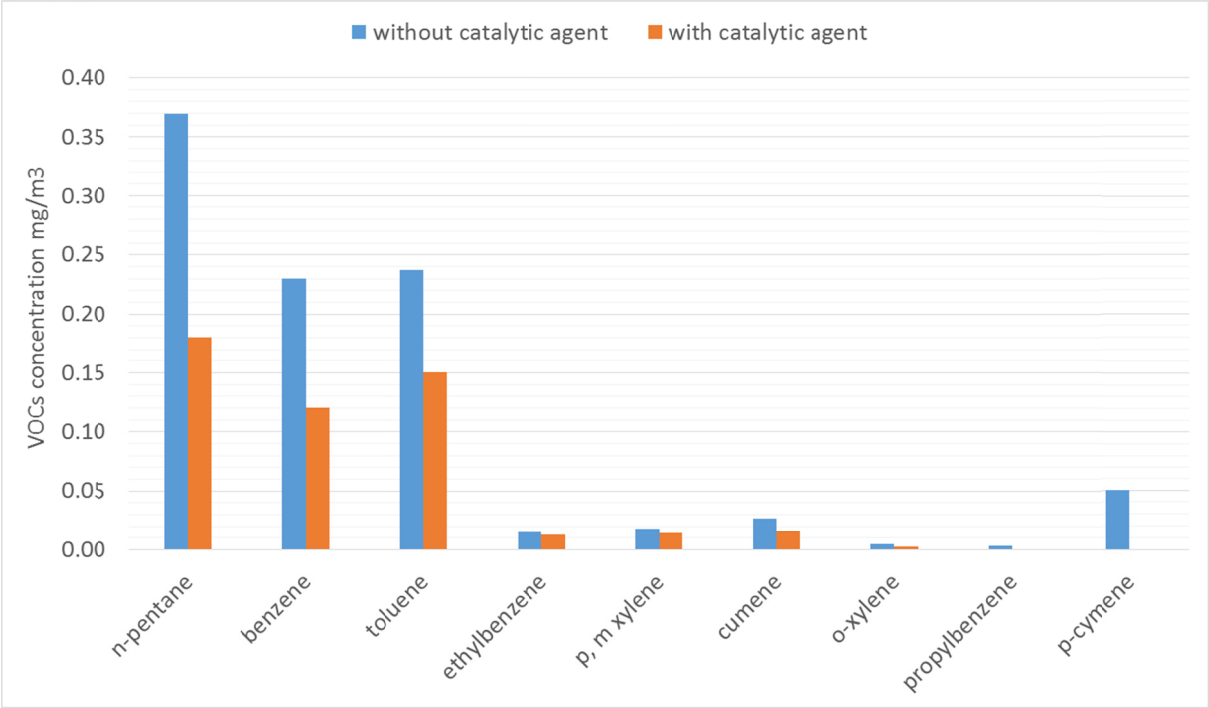


Fig. 3. Reduction of VOCs before and after introduction of the titanium dioxide

It appeared moreover, that the compounds represent different vulnerability to the photocatalysis. As it can be seen in Fig. 4 the highest percentage change of a compound was detected in the case of p-cymene which was almost entirely reduced. The reduction of other compounds vary ranging from 20 to 55%. The exception here is propylobenzyne which was generated probably as a product of photocatalytic reactions. In general, after summation of all VOCs participating in the photocatalysis their reduction reached 46%, while considering only compounds in the BTX group the reduction was 39%.

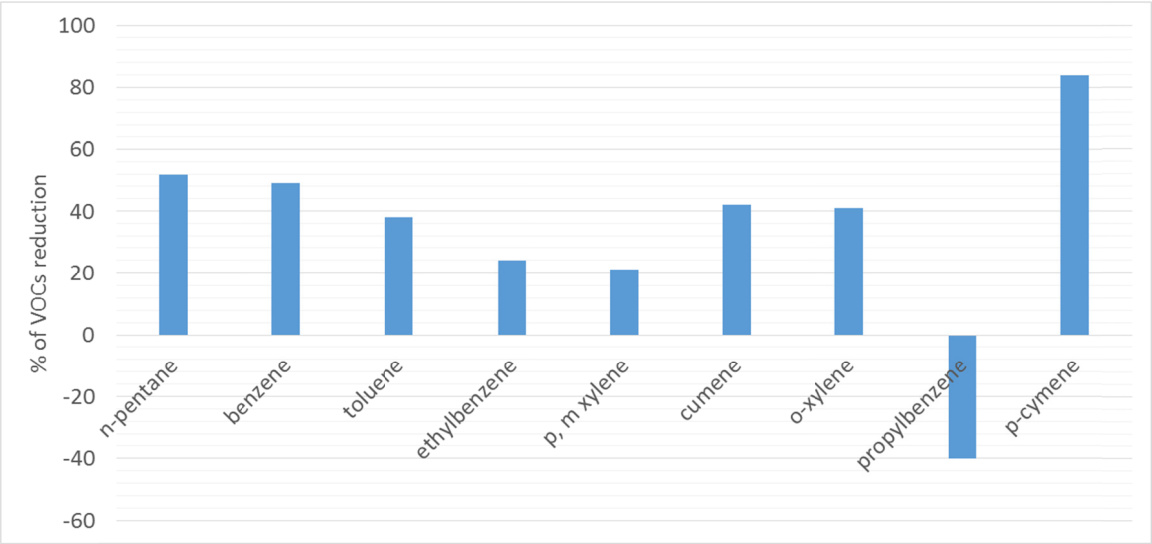


Fig. 4. Percentage reduction of VOCs by means of photocatalysis

In the case of tests carried out on the cell line of epithelial human lung cells (A459) shown in Fig. 5 a, introducing on a cell line mixtures of air with the exhaust gas led after 30 minutes of exposure to reduce the amount of cells by 38% to the reference sample. After introduction to the catalytic agent the amount of intact cells increased on average by 13% (with three

consecutive repetitions of the experiment. In the case of tests carried out on the subcutaneous tissue of mice (L292), shown in Fig. 5 b introducing on a cell line mixtures of air with the exhaust gas led after 30 minutes of exposure to reduce the amount of cells by 41% compared to the reference. After an introduction to the catalytic agent the amount of intact cells increased on average by 8%.

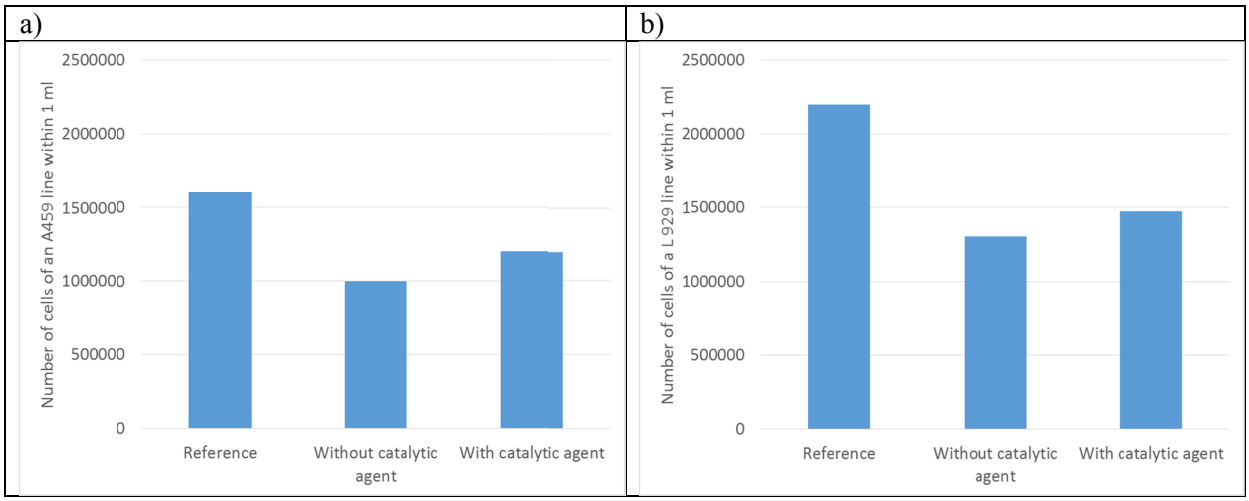
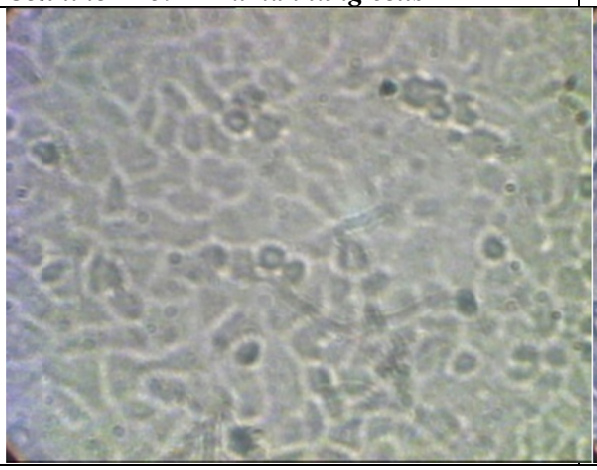
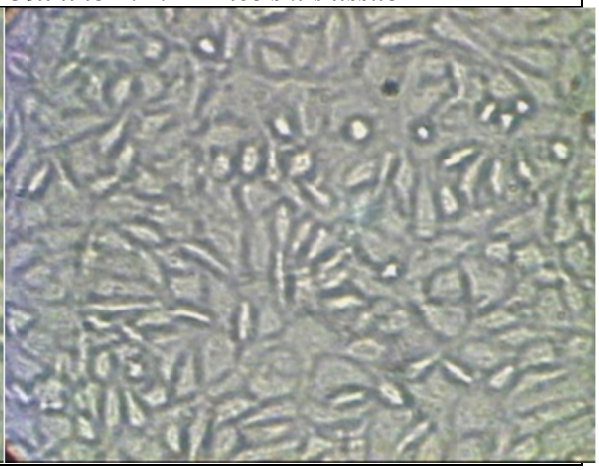
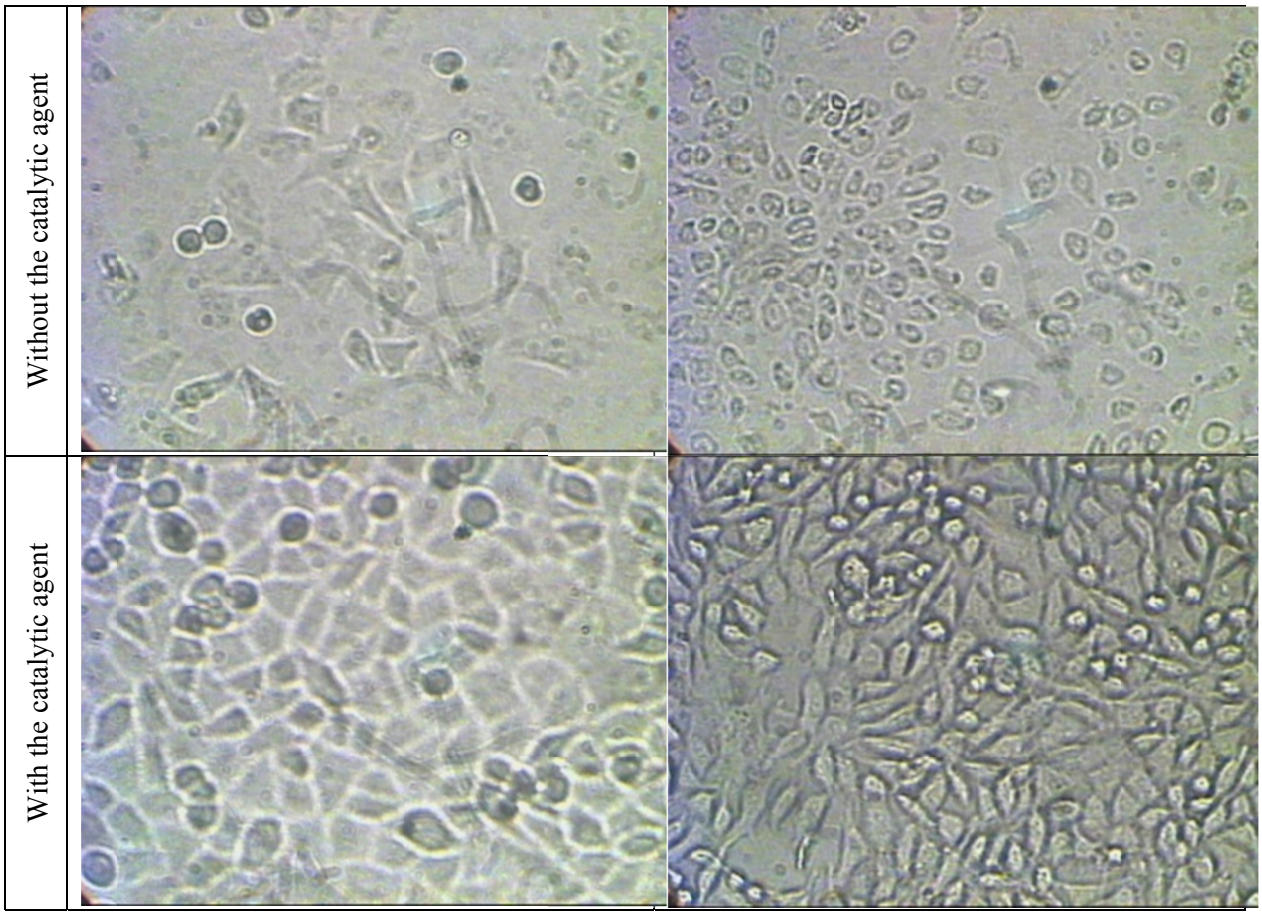


Fig. 5. The amount of dead cells;
a) line of epithelial human lung cells (A459), b) subcutaneous tissue of mice (L292)

The microscopic picture of the cell lines showed clear differences in the appearance of the cells exposed to the gas mixture relative to the control (Tab. 1). In the case of human lungs and mice skin the cells were shrunken, coming off the plate, reflecting the negative (toxic) effects of gases on the cell lines. Introduction of the catalytic agent apparently caused less damage (compared to the experiment carried out without its use) caused by the action of the gas mixture to the cells exposed. On the basis of preliminary studies it can therefore be concluded that the use of titanium dioxide in the ventilation system of the vehicle could be beneficial for the cytotoxic effect of a mixture of air with exhaust fumes on human lung and skin.

Tab. 1. Microscopic picture of the cell lines

	Cell line A459 – Human lung cells	***Cell line L929 – Mice skin tissue***
Reference cell line		



4 Conclusion

Considering the results of the experiment deliberated in this paper it can be concluded that

1. Evaluation of a gas mixtures toxicity appears to be complicated problem and poorly recognized in the worldwide literature.
2. Toxicity of gas mixtures (i.e. its influence on human health) does not depend directly only on the gas composition, but also on the mixtures interconnections and ingredients proportions.
3. The BAT – CELL Bio – Ambient Test is an innovative direct method, which enables toxicity evaluation of gas mixtures.
4. Basing upon gathered test results the usefulness of the toxicity evaluation for air directed inside to the car cabin has been demonstrated.
5. Inasmuch VOCs concentration identified in the gas mixture as a consequence of the active nozzle utilisation was decreased for over 46% (in the case of VOCs summation) and almost for 40% in the case of BTX (summation of benzene, toluene and xylene concentration), the improvement in mixture toxicity of the cell line A459 was 13% and 8% for the cell line of L929. This demonstrates the lack of proportionate link between the change in the chemical composition of the gas mixture and the impact on human health.
6. The research should be continued due to its ability to reveal a full impact of the established solution on the human health as well as wildly understood public health protection.

5 Acknowledgment

The results presented in the paper was provided and developed during the POIG.01.04.00-02-154/13 and POIG.04.05.02-00-030/12-00 projects realization.

References

[1] Janicka, A., *Ocena toksyczności mikroatmosfery środowiska wnętrza pojazdu samochodowego*. Wrocław: Oficyna Wydawnicza Politechniki Wrocławskiej, 2013

[2] Lin Lin, Yuchao, Chai, Bin Zhao, Wei Wei, Dannong He, Belin He, Qunwei Tang, *Photocatalytic oxidation for degradation of VOCs*, Open Journal of Inorganic Chemistry, 2013, 3, 14-25

[3]Bhatkhande, B.S., Pangarkar, V.G. and Beenackers, *Photocatalytic degradation for environmental applications A review*, Journal of Chemical Technology and Biotechnology, 2001, 77, 102-116

[4] Fu, X., Zeltner, W.A. and Anderson, M.A., *Applications in photocatalytic purification of air*, Science, 1996, 445-461

[5] Peral, J., Domenech, S. and Ollis, D.F., *Heterogeneous photocatalysis for purification, decontamination and deodorization of air*, Journal of Chemical Technology and Biotechnology, 1997, 70, 117-140

[6] Mills, A. and Le Hunte, S., An overview of semiconductor photocatalysis, Journal of Photochemistry and Photobiology A: Chemistry, 1997, 108, 1-35