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APPLICATION OF BAT-CELL BIO-AMBIENT TESTS IN EXHAUST GAS EMISSIONS EXAMINATIONS FOR EURO 4 AND EURO 6 COMBUSTION ENGINES

The introduction of the subsequent European emission standards raises controversy among vehicle manufacturers and researchers. In order to meet the legal requirements, it is necessary to continually improve not only the cars but also the costly measurement facilities. In addition, current methods do not give a clear answer to the harmful effects of exhaust gases on living organisms. By using the appropriate *In vitro* method to directly assess the toxicity of gaseous mixtures, one can obtain simple, reliable results that can be used to validate the introduction of ever more stringent Euro standards.

1. INTRODUCTION

In the atmospheric air, apart from permanent constituents such as nitrogen, oxygen or argon, there are also variable components. Among them is atmospheric air pollution, i.e. all those substances in solid, liquid and gaseous state, whose share in the air exceeds the average content in the pure atmospheric air. It can adversely affect living organisms. According to scientists, a substance harmful to living organisms, which has a detrimental effect on tissues, organs, or biological processes, is defined as a toxic substance [1].Real toxicity refers to the real and direct effect on a living organism. Among the toxic substances present in atmospheric air few of them can be distinguish: SO_2 sulfur dioxide, NO_X nitrogen oxides, CO carbon monoxide, NH_3 ammonia, suspended particulate matter (total - TSP, fine particles - PM 10, PM 2.5), heavy metals, not metal Volatile Organic Compounds (NMVOC) and persistent organic pollutants. All these substances, as required by the European Union, should be subject to continuous monitoring.

One of the main sources of air pollution, which is a threat to the natural environment, health, and even human life, is road transport. From the beginning of the emergence of vehicles with combustion engines on the roads, the trend in the worldwide automotive

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industry is to strive to minimize the harmful effect of exhaust gases on the environment. In Europe, it manifests itself in the adoption of directives which introduce increasingly stringent European emission standards for commercial vehicles. Standards that set acceptable levels of toxic combustion products have undergone an evolution for over 20 years. The regulations specify the maximum concentrations of the individual exhaust gases that may be emitted into the atmosphere by vehicles. The difference between the first and the last standards is the reduction of harmful emissions that reach over 90% (see Table 1 and 2). In order to meet the restrictions imposed, vehicle manufacturers are forced to look for new design solutions, use modern technologies to enhance fuel efficiency, and purify gases emitted by internal combustion engines. Introducing new, stricter emission reduction standards from motor vehicles also forces the continual investment in research and measuring equipment used in R & D laboratories. Furthermore, it is necessary to supplement technical knowledge on new exhaust gas analysis procedures and methods [2]. In order to meet legal requirements, research methods must be continuously assessed and developed in the face of constant changes in both measuring equipment and computer programs.

Table 1. Emission values for new gasoline vehicles [3]

	Valid since	CO [g/km]	HC [g/km]	NOx [g/km]	HC+NOx	PM [g/km]
Euro 1	12/92	2.72	-	-	0.97	-
Euro 2	01/97	2.20	-	-	0.5	-
Euro 3	01/00	2.30	0.20	0.15	-	-
Euro 4	01/05	1.00	0.10	0.08	-	-
Euro 5	09/09	1.00	0.10	0.06	-	0.005*
Euro 6	08/14	1.00	0.10	0.06	-	0.005*

*with direct injection

Table 2. Emission values for new diesel vehicles [3]

	Valid since	CO [g/km]	HC [g/km]	NOx [g/km]	HC+NOx [g/km]	PM [g/km]
Euro 1	01/92	3.16	-	-	1.13	0.14
Euro 2	01/96	1.00	0.15	0.55	0.70	0.08
Euro 3	01/00	0.64	0.06	0.50	0.56	0.05
Euro 4	01/05	0.50	0.05	0.25	0.30	-
Euro 5	09/09	0.50	0.05	0.18	0.23	0.005
Euro 6	08/14	0.50	0.09	0.08	0.17	0.005

At present, most of the researchers are investigating vehicles in real traffic conditions. Measurements made during approval are based on NEDC tests, which only to some extent reflect conditions during normal vehicle operation, and thus may be unreliable due to their stationary nature and often questionable course of the individual test steps [2,4,5]. However, they are useful for vehicle users, for example, to compare different cars of the same class, in order to choose a good brand for themselves. Most often emission measurements of toxic exhaust gases are carried out using a system of mobile exhaust gas analyzers that are mounted on the vehicle - PEMS [4,6]. Such analyzers allow for a comprehensive real-time

onboard emission measurement in road conditions of vehicles powered by different fuels. It should be noted, however, that the concentration of the individual components of the exhaust gas resulting from the measurements does not give a direct, readable result of their toxicity, and therefore of a negative effect on the living organism. It seems reasonable to use a method that will allow a relatively quick, unequivocal and objective way to determine the actual impact of toxic substances on a living organism. Among the methods used to assess the toxicity of the atmosphere, *In vitro* methods, i.e. biological tests on living, isolated cells, appear to be appropriate. By effecting a given gas mixture on living cells, it is possible to obtain a reliable and unambiguous answer to whether the test mixture is harmful to the organism or not.

2. MATERIAL AND METHOD

In order to investigate the actual exhaust gas toxicity, measurements were made for two Euro 4 and Euro 6 passenger cars of two different brands which belong to the middle class. The vehicles were propelled with gasoline engines, had a valid periodical inspection and were therefore deemed fit and authorized for traffic. The research was carried out using the innovative method of direct toxicity assessment of BAT-CELL *Bio-Ambient Tests*, based on *In vitro* tests [7,8].

The study included usability of a human lung carcinoma cell line A549 and a mice fibroblast cell line of subcutaneous adipose tissue L929. After multiplying the cells to the desired number, they were placed in special specimens in the culture fluid and stored in an incubator until testing began. The exhaust gases were taken to the test bags at a distance of about 20 cm from the exhaust pipe of the passenger car at idling speed. Immediately prior to the start of the test, the culture fluid was removed from the live cell samplers. After setting the appropriate flow, temperature and pressure parameters in the measuring apparatus (Fig. 1), a proper test was started. Thanks to numerical methods used in fluid mechanics, the flow parameters matched to the shape of the probe so as to enable equal contact of gas particles with the cell surface of the cell culture. This allows do not damage the cells by mechanical means. The chamber with cells is in a controlled environment of temperature and pressure chosen for the specificity of the applied cell culture so that maintain vital functions of cells. Exposure time was 3.5, 7, 10, 15 and 20 minutes respectively. A test was performed for each cell line. After the test, the cells were re-flooded with culture fluid and then subjected to the procedure of gas-cell impact assessment at the Institute of Immunology and Experimental Research of the Polish Academy of Sciences. For this purpose, the Bürker chamber was used to determine the number of particles per unit volume of liquid.

The advantages of the BAT-CELL *Bio-Ambient Tests* method, and at the same time what distinguishes it from other such methods are: ease of experimentation, ability to work outside the research laboratory, mobility of measuring apparatus, elimination of culture fluid, i.e. barrier separating cells from direct effect on the non-gaseous mixture tested and reduction of cell exposure time to toxins. Repeatability of the method was estimated at 5%, which is relatively small compared to other biological methods.

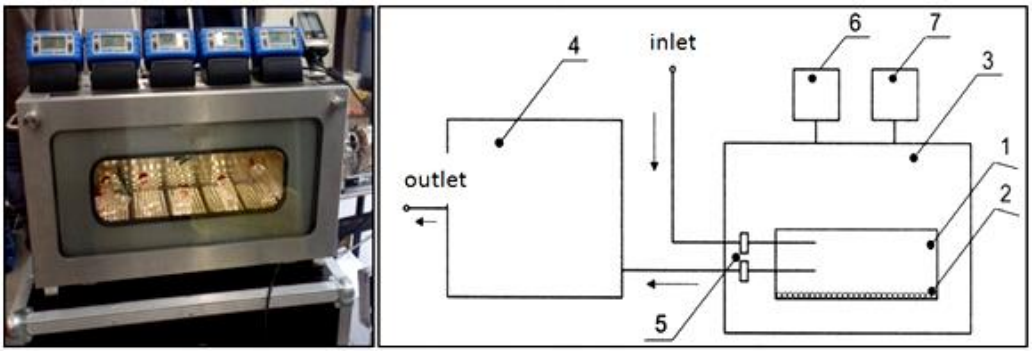

Fig. 1. A measurement system for gas toxicity evaluation by BAT-CELL (1 cell sampler, 2 cell without culture fluid, 3 BAT-CELL cell, 4 aspirator, 5 antibacterial filters, 6 and 7 temperature and pressure sensors)

This method has been patented by the scientists of the Faculty of Mechanical Engineering of the Wrocław University of Science and Technology in cooperation with the Polish Academy of Sciences (Polish Patent No. 220670. *Method of measuring the effect of gas mixtures on living cells:* Int. Cl. C12M 1/34, C12M 1/36, C12M 1/38, G01N 33/00, 400646 from 04.09.2012) [9].

3. RESULTS

The results of the toxicity assessment of the investigated gases may be presented as a plot of the cell number per milliliter with respect to the exposure time or as a percentage of degraded cells. Figure 2 shows the corresponding graph for two cell lines simultaneously, taking into account two different Euro standards. The number of cells per milliliter is to be understood as the ability of the cells to multiply.

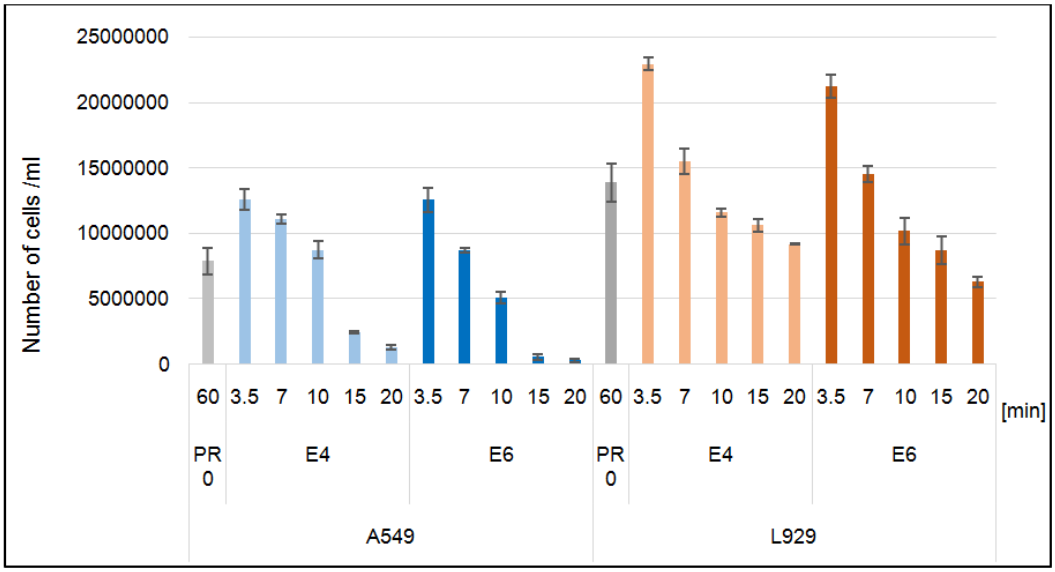

Fig. 2. Test results – number of cells in 1 ml with respect to exposure time [min]

Based on the results obtained (Fig. 2), both human and mouse cell lines for Euro 6 compliant vehicles are less favorable than those of Euro 4. The cell multiplication capacity for both lines is higher for the previously introduced and less stringent Euro 4 standards. Furthermore, studies have shown that there are measurement points where the percentage of degenerated cells for a Euro 6 compliant car is several times higher than that of Euro 4 (Table 3).

Table 3. Study results - percentage of degenerated cells

Cell line	Exposure time [min]	Euro standards	Degenerate cells [%]
L929	15	Euro 4	24
		Euro 6	44
	20	Euro 4	24
		Euro 6	84

Figure 3 shows the imaging results of the tests - microscopic magnification of the examined cells after the tests. Over a 20-minute exposure time, there is a clear loss and deformation of the cells for both cell lines compared to the shorter exposure time of 3.5 minutes. Cells are therefore degraded (destroyed) with respect to their exposure time. A549 cells are more sensitive to exhaust gas than L929 cells.

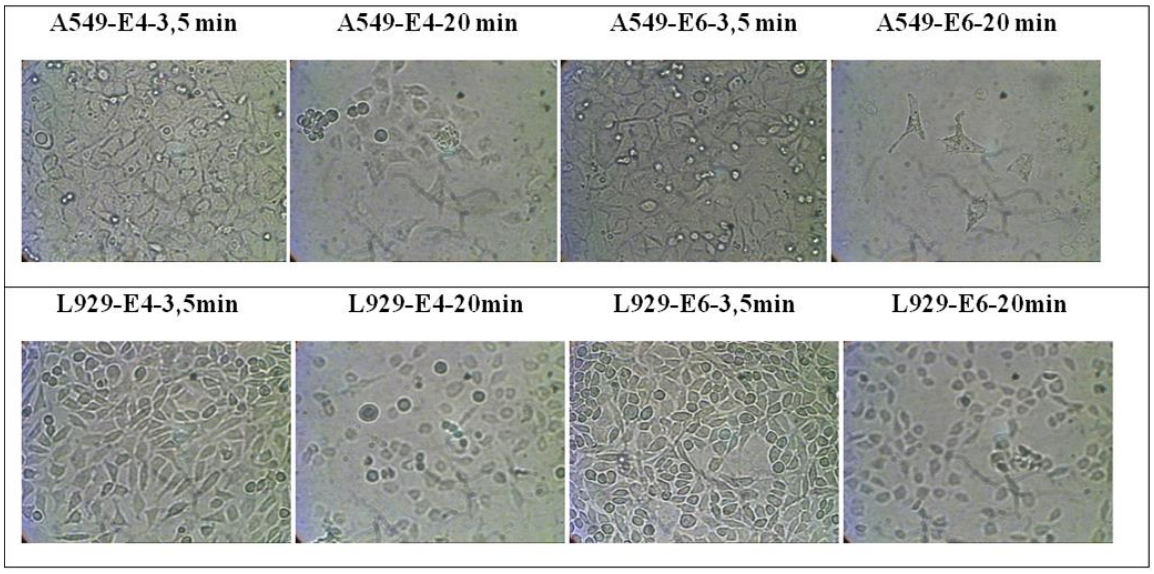

Fig. 3. A549 and L929 cell lines at 3.5 and 20 minute exposure time for Euro 4 and Euro 6 vehicles - microscope view

4. DISCUSSION

The exhaust gas toxicity studies proposed by this method showed the direct impact on the living organism of exposure to toxic combustion products. The results of the test showed that after a few minutes of exposure to a mixture of gases on living cells, they undergo irreversible degeneration. Bearing in mind that research has been carried out, among

the other, on human lung epithelial cells through which respiratory gas diffuses between the lung and capillary glands in the body, the results can be considered as meaningful and adequate.

The presented method allowed to obtain a reliable result of the toxic effect of the gas mixture on living tissues. Although the research was conducted under stationary conditions, the results showed interesting differences between the studied objects. This may indicate that it is not necessary to carry out complex dynamic tests to determine the level of pollutants toxicity.

The increasing number (Fig. 4) and the average age [2] of cars in each of the following years, despite the development of new technologies and attempts to neutralize the negative effects of combustion, still cause high emissions of toxins into the atmosphere. Moreover, the automotive industry is one of the main sources of this emission (Fig. 5). According to the automotive reports of 2016, in both diesel and petrol engines, emission standards for individual components are considerably exceeded. Figure 6 shows the limits imposed by Euro standards and the measured concentrations of nitrogen oxides emitted into the atmosphere.

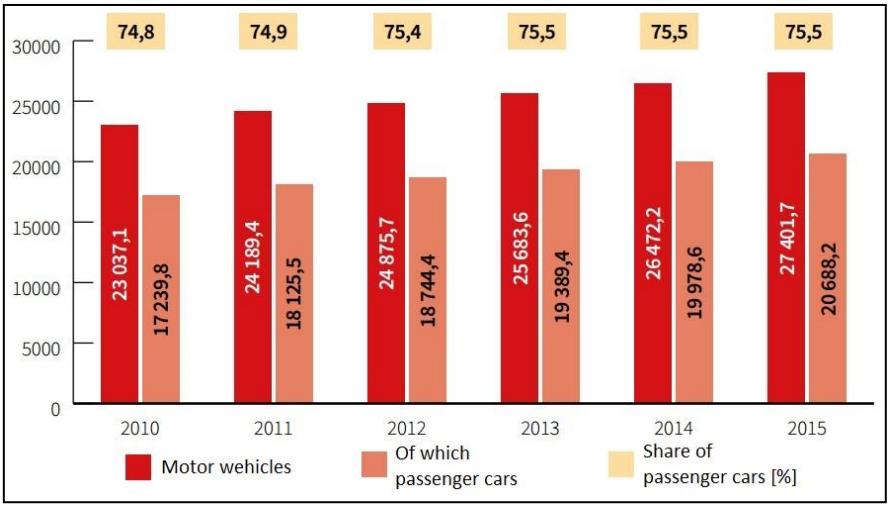

Fig. 4 Motor vehicles in Poland (000 units) [10]

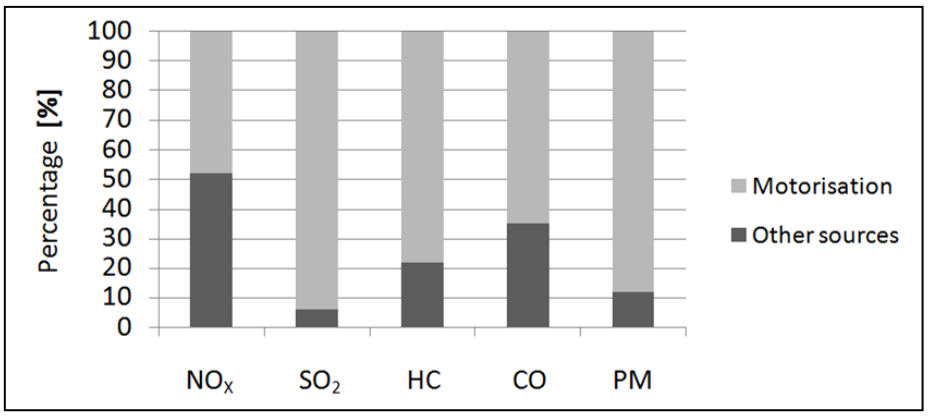

Fig. 5. Road transport share in the emission of major toxins into the atmosphere [11]

As can be seen from the data, despite the continual tightening of European emission standards, there is still a large problem of exceeding the imposed limits and high emission of toxic compounds. Car manufacturers do not keep up with the constant changes in imposed standards. The effects of emission of toxic compounds continue to be felt, as evidenced by the carried out research. The question then is whether, to be sure, the introduction of increasingly stringent Euro standards is justified and serves to improve the quality of atmospheric air and, consequently, our health? Are continuous changes in legislation on how to set maximum allowable concentrations of chemicals indicates how, and to what extent they affect a living organism? Does reducing emissions lead to real reductions in gas toxicity and therefore to human health risks?

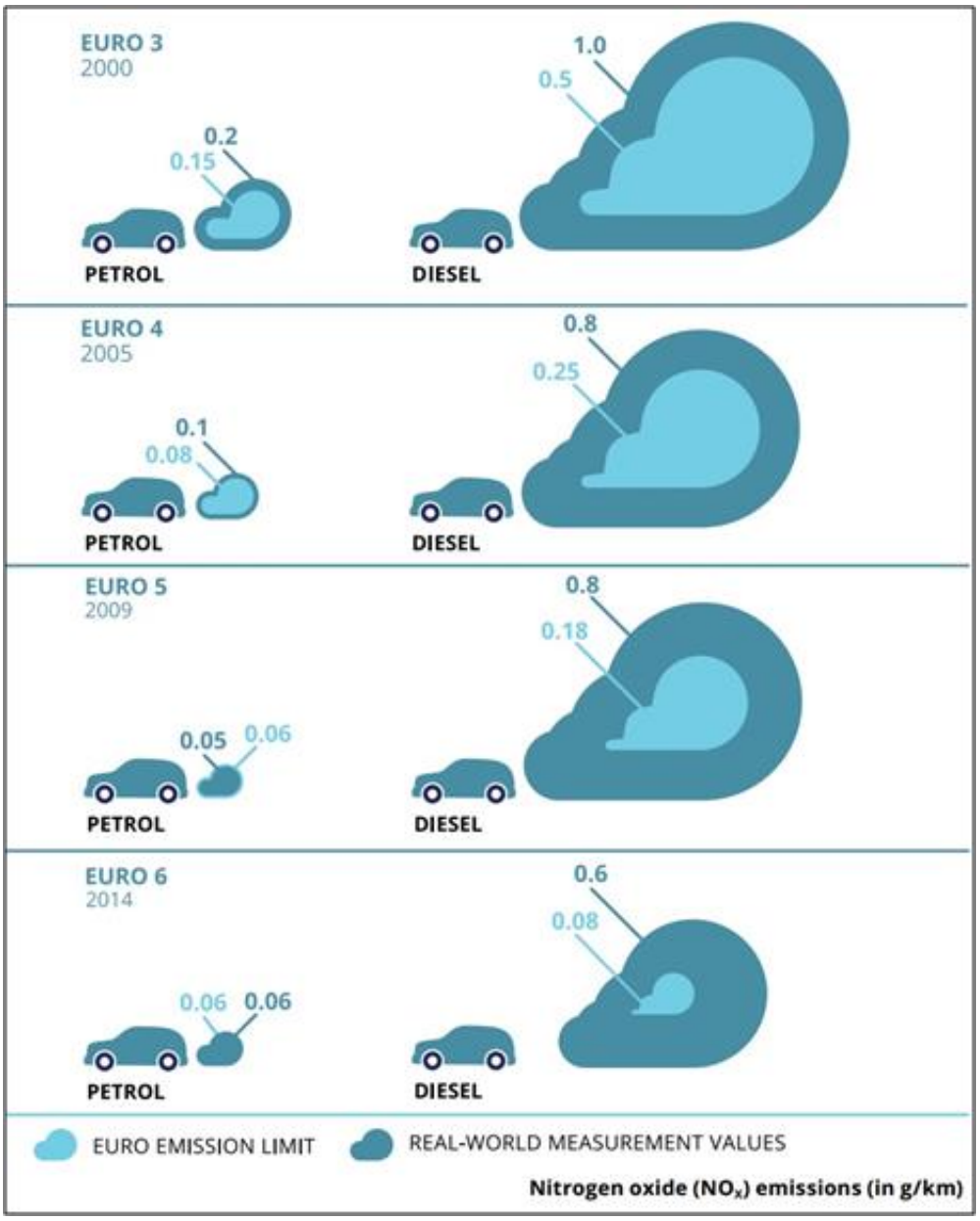

Fig. 6. Comparison of NO_X standards and emissions for different Euro classes [12]

5. CONCLUSIONS

The above discussion, though potentially controversial, is worth examining and establishing a pattern for road transport ecology, aiming at a real improvement in the quality of life. The *In vitro* BAT-CELL *Bio-Ambient Tests* have shown that there is a possibility of an alternative assessment of engine exhaust qualities compared to the hitherto used (Euro norm criterion).

It should be however emphasized that the research with the utilization of BAT-CELL *Bio-Ambient Tests* consisting on evaluating of exhaust gases toxicity produced by engines fulfilling subsequent Euro norms requires advanced and carefully designs experiments. Numbers of variables must be included – technical data of the vehicle, operating points of the engine, etc. Never the less the preliminary research presented in this paper should open the discussion of the scientific circles on:

- the issue of the appropriateness of introducing newer and more stringent engine emission standards,
- use appropriate measuring equipment to control vehicles before being allowed to market, providing a reliable and indisputable indication of toxicity, i.e. negative and undesirable effects of exhaust gases on the living organism.

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