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Numerical optimization of the BAT-CELL Bio-Ambient-Tests method for engine exhausts toxicity evaluation

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The BAT-CELL Bio-Ambient-Tests method is based on the assessment of the influence of the actual toxicity of various types of gas mixtures on living cells, taking into account the additive synergism. Work has been carried out on the application of the BAT-CELL method for testing engine exhaust gases. The application of computational fluid mechanics using Ansys Fluent made it possible to analyse the flow of engine exhaust gases through the aspiration system used, including analysis of shear stress values and their uniformity distribution on the bottom wall of the sampler containing cell culture on the bottom wall of the sampler. The appropriate flow rate of exhaust gases through the aspiration system and the shape of aspiration tubing for the sampler were selected in order to enable uniform contact of gas particles with the cell surface and not to damage them mechanically. The simulation results were verified in real-life tests and confirmed the theoretical assumptions.

Key words: *internal combustion engine, Euro norms, new method, in-vitro tests*

1. Introduction

In accordance with the principle of sustainable development, propagated in Europe since the beginning of the 19th century, the economic development must lead to an improvement in the quality of the natural environment by, among other things, limiting the harmful effects of production and consumption on the state of the environment and protecting natural resources. At a time when motorisation is one of the most important trends in the development of society, attention should be paid to the aspect of exposure of a human body to the negative effects of such progress of civilisation. The progress of motorisation in the world has forced the introduction of certain legal restrictions on the control of exhaust emissions. Euro emission standards have been in force in Europe since 1992. The control is carried out by means of various measurement methods, thanks to which the concentration of emitted compounds subject to standards is determined, including hydrocarbons (HC), nitrogen oxides (NO_x), carbon monoxide (CO) and particulate matter (PM/PN). However, all the methods used do not give direct and clear results on their actual toxicity. Actual toxicity should be understood as a harmful effect of a substance on living organisms – tissues, organs or biological processes. It seems appropriate to use a method that will make it possible to determine the actual impact of toxic substances on a living organism in a relatively quick, unambiguous and objective manner. Moreover, the currently binding exhaust emission standards do not take into account many harmful compounds, directly unlimited (Fig. 1), which determine toxicity of exhaust gases. In the group of hydrocarbons we can distinguish compounds from polycyclic aromatic hydrocarbons (PAH) and volatile organic compounds (VOC), such as benzo(a)pyrene or benzene, which exhibit, inter alia, carcinogenic and mutagenic effects even at very low concentrations, which has been repeatedly scientifically proven [3, 18, 21–23].

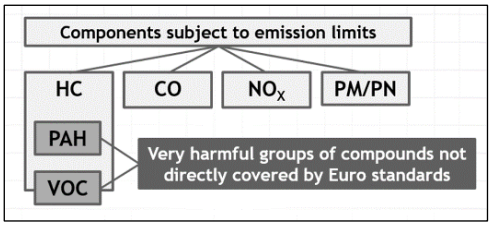

Fig. 1. Location of VOCs and PAHs among the limited components of exhaust gases [19]

This paper proposes a method for measuring the real toxicity of engine exhaust gases, which may be a supplement or alternative to the currently used methods. The method takes into account synergistic interactions of compounds without the need to identify them qualitatively and quantitatively. Moreover, in a relatively simple way, both in terms of conducting the experiment and interpretation of results, it allows for obtaining an answer to the question whether a given gas mixture may cause toxic effects in our body.

2. Review of exhaust emission test methods

Research on exhaust emission measurement methods in the context of changing Euro emission standards is an extremely important and topical issue. Commonly used exhaust emission measurement methods can be divided into those using exhaust gas analysers [33] (including portable emissions measurement system PEMS), analytical methods (e.g. chromatography, spectroscopy) and less popular calculation methods [28]. These methods can be used for testing exhaust emissions in stationary conditions on a chassis dynamometer or in road traffic conditions.

Definitely, the most common method of measurement in the conditions of real vehicle traffic is the method using a special mobile scientific and research apparatus of the PEMS type, appropriately installed in a vehicle [5, 8, 9, 11, 15, 20, 24, 26, 30]. It makes it possible to measure the con-

centration of individual pollutants and the energy consumption of traffic, while measuring the mass flow rate of exhaust gases from a power unit. The apparatus may be used for testing vehicles of various types and homologation categories. Moreover, it provides instantaneous values of the measured parameters, which allows for quick conclusions from the conducted tests.

A common method of measuring pollutant emissions in stationary conditions on a chassis dynamometer are exhaust gas analysers [27, 31]. These are measuring instruments intended for measuring the content of exhaust gas components such as carbon monoxide, carbon dioxide, nitrogen oxides, hydrocarbons, oxygen. The most popular of them are non-dispersive infrared (NDIR) analysers, using spectrometric methods, which consist in measuring with a photometer the total absorption of radiation in a quite narrow band of wavelengths, characteristic of the compound in question. Other analysers used are flame ionisation detection (FID) for the determination of hydrocarbons and methane and chemiluminescent (CLD) for the determination of nitrogen oxides. Other methods used to measure exhaust emissions are analytical methods, mainly chromatographic (including flame ionisation or chemiluminescence detection) and spectroscopic [1, 6, 10, 13, 16, 25, 32]. Among them we can distinguish the less known ones, e.g. the electronic method for the detection of nitric oxide emissions, which is based on absorption spectroscopy using a diode laser [7, 29], or the method using a specialised spectroscopic remote sensing device developed by the University of Denver [4].

Testing of gaseous mixtures by means of in vitro tests is a new method, not commonly used. So far, the only Polish author of papers published in Switzerland, based on tests on living cells exposed to gaseous mixtures, including exhaust gases, is Czerwiński [2]. In this type of research, the key issue is not the selection of an appropriate test, but the selection of appropriate parameters of cell exposure to harmful substances and the knowledge of the dose of the tested gases causing the toxic effect.

3. BAT-CELL method

Innovative, patented BAT-CELL Bio-Ambient-Tests method (Patent. Poland, No 220670. Method for the measurement of the effects of gaseous mixtures on living cells: Int. Cl. C12M 1/34, C12M 1/36, C12M 1/38, G01N 33/00. Application no. 400646 of 04.09.2012. published 30.11. 2015) consists in evaluating the effects of the actual toxicity of different types of gas mixtures on living cells, taking into account additive synergism. It allows direct contact between the test gas and the cell surface, thanks to the elimination of a physicochemical barrier in the form of culture fluid, which distinguishes it among other direct methods of this type.

The cell culture, devoid of the culture fluid, is placed in a sterile closed chamber (sampler). In particular, the fibroblast-like cell line obtained from mouse subcutaneous adipose tissue L929 is dedicated to this type of study. Subsequently, the gas is introduced into the sampler through an inlet tube through an antibacterial filter and by means of an aspiration system (Fig. 2).

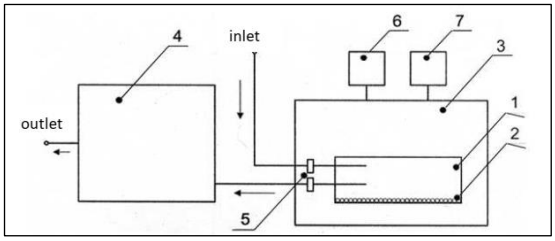

Fig. 2. Diagram of a system for assessing the toxicity of gas mixtures using the BAT-CELL Bio-Ambient-Tests method (1 – sterile sampler, 2 – cell line devoid of culture fluid, 3 – conditioning chamber equipped with pressure (6) and temperature (7) sensors, 4 – aspirating system, 5 – antibacterial filters) [12]

After exposure of the cell culture to the gas, it is flooded with culture fluid and the toxic effect of the gas on the culture is examined by standard toxicological tests according to standard procedures. The exposure time is chosen individually depending on the type of gas mixture. Flow parameters shall be chosen to the shape of the sampler in such a way that uniform contact of the gas molecules with the cell surface is ensured and the cells are not mechanically damaged. Numerical methods of fluid mechanics are used for this purpose.

The conditioning chamber (Fig. 3) is equipped with pressure and temperature sensors to ensure that the vital functions of the cell culture are maintained. The elimination of the culture fluid is possible by maintaining physical parameters appropriate to the requirements of the cell line, a line-safe residence time for the cells outside the incubator atmosphere and no nutrient supply. The culture fluid additionally has an antibiotic function for the cells, therefore the sampler with the cell line is additionally protected at the inlet with an antibacterial filter [12].

Fig. 3. BAT-CELL chamber

4. CFD numerical analysis

Thanks to the application of computational fluid mechanics with the Ansys Fluent software, it was possible to select an appropriate flow rate and shape of the aspiration tubing for the cell culture sampler.

The simulations were carried out for two values of the flue gas flow rate and the shape of the aspiration pipes for the sampler containing the cell culture.

Simulations were carried out for two flow rates – 150 and 250 cm^3/min. Four solutions of the shape of aspiration tubing were proposed, differing in the angle of inclination of the tubing in relation to the bottom wall of the sampler, and thus in their length, as well as in the angle of truncation of the outlet duct (Fig. 4). The length of the aspiration tubing (the section inside the sampler) varied from 18 to 35 mm, depending on the model.

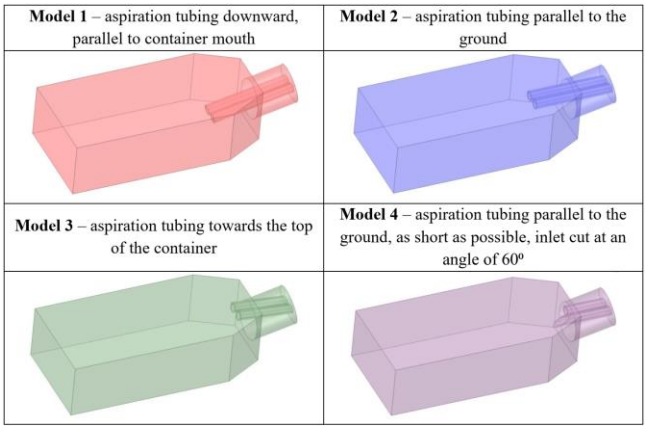

Fig. 4. Different solutions of the shape of aspiration tubing

For the purpose of the simulation, the sampler construction was simplified. The 3D spatial model was discretised. A tetrahedral mesh with an element size of 0.001 m was applied. In order to compact the mesh locally, a wall layer was applied to the lower wall of the sampler.

For a flow rate of 250 cm^3/min, a flow velocity of 0.5895 m/s was calculated, while for a flow rate of 150 cm^3/min the value was 0.3537 m/s. For both cases, the Reynolds number was calculated (an example of calculation for the higher flow rate is given – formula 1). The obtained values $Re_1 = 73$ and $Re_2 = 121$ testified to laminar flow. In laminar motion, the fluid elements move along straight or gently curved paths, depending on the shape of the rigid walls that give shape to all the current lines. In laminar flow, therefore, there is an exchange of mass, and with it an exchange of momentum on a microscopic scale, which is the cause of the occurrence of tangential stresses. In laminar flow, characterised by the dominance of viscous forces over inertial forces, any random disturbances arising are damped, so that the flow is static (stable). Such motion can occur as long as the Reynolds number does not exceed the critical value $Re_{kr} \leq 2300$ [14]. The Reynolds number values were calculated for the medium, which was air. The obtained parameters were set under flow simulation conditions.

$$Re_2 = \frac{\rho \cdot V \cdot d_h}{\mu} = \frac{1.225\,\frac{kg}{m^3} \cdot 0.5895\,\frac{m}{s} \cdot 0.003\,m}{0.000017894\,\frac{kg}{m \cdot s}} = 121 \qquad (1)$$

where: ρ – the density of the medium, V – flow velocity, d_h – hydraulic diameter, μ – dynamic viscosity.

The Navier-Stokes equations (equation 2) [14] were used to describe the flow of a Newtonian viscous fluid in the studied system. These equations describe the principle of conservation of momentum for a moving fluid. According to them, changes in the fluid element depend only on mass forces, external pressure and internal viscous forces in the fluid. In addition to normal stresses, tangential stresses occurring on the walls of the viscous fluid element can also be considered.

$$\frac{\partial v}{\partial t} + v \cdot \nabla v = F - \frac{1}{\rho}\nabla p + v\nabla^2 v \qquad (2)$$

where: $(v \cdot \nabla)v$ – convection: the transfer of local momentum with the movement of a fluid, F – creation of momentum due to mass forces (gravity), $\frac{1}{\rho}\nabla p$ – change of momentum due to pressure forces, $v\nabla^2 v$ – friction forces: dissipation of momentum due to friction processes.

The results show maps and graphs of shear stress distribution on the bottom wall of the sampler depending on the set flow rate value and shape of aspiration tubing (Fig. 5–8). Due to the wide range of stress values in this case, the range of scale values on individual maps and graphs was not standardised, as the exact stress distribution and maximum values of tangential stresses for individual models would not be visible. Therefore, the results are presented unaltered. The diagram of the dependence of shear stress values on the position of a given point in the plane of the bottom wall of the sampler shows the values at a given node of the element mesh.

Maximum values of tangential stresses for particular models of aspiration tubing are presented in the Fig. 9.

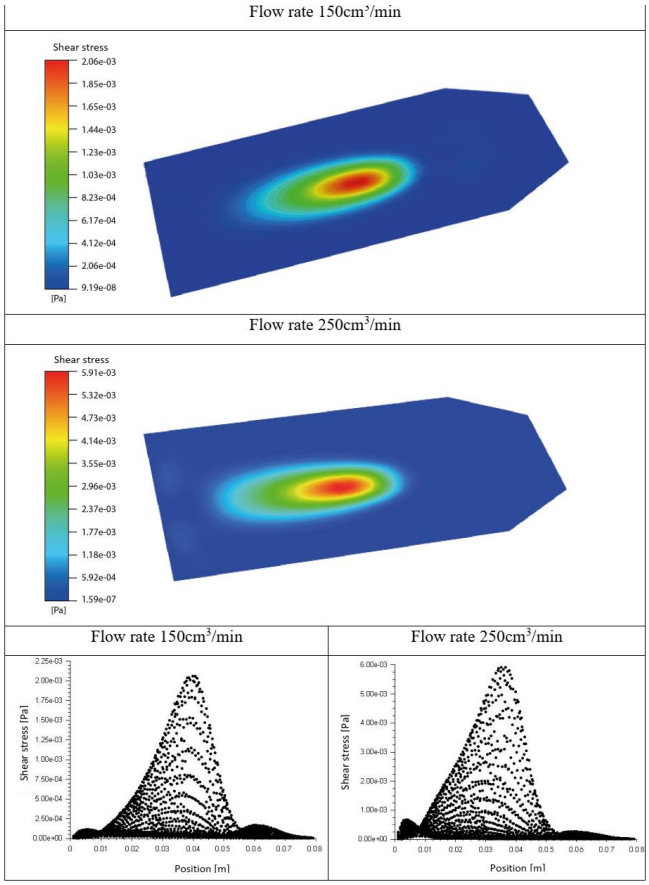

Fig. 5. Maps and diagrams of shear stress distribution on the bottom wall of the test specimen for model 1

The lowest stress values were observed for model 3, in which the designed tubing were directed upwards. On the basis of the analysis performed, it was concluded that the direction of aspiration tubing has an influence on the values of shear stresses. Reducing the flow rate to 150 cm^3/min will reduce the shear stress values several times. Directing the inlet aspiration tubing towards the top of the sampler or cutting its tip at an angle leads to a more uniform distribution of shear stresses on the bottom wall of the container.

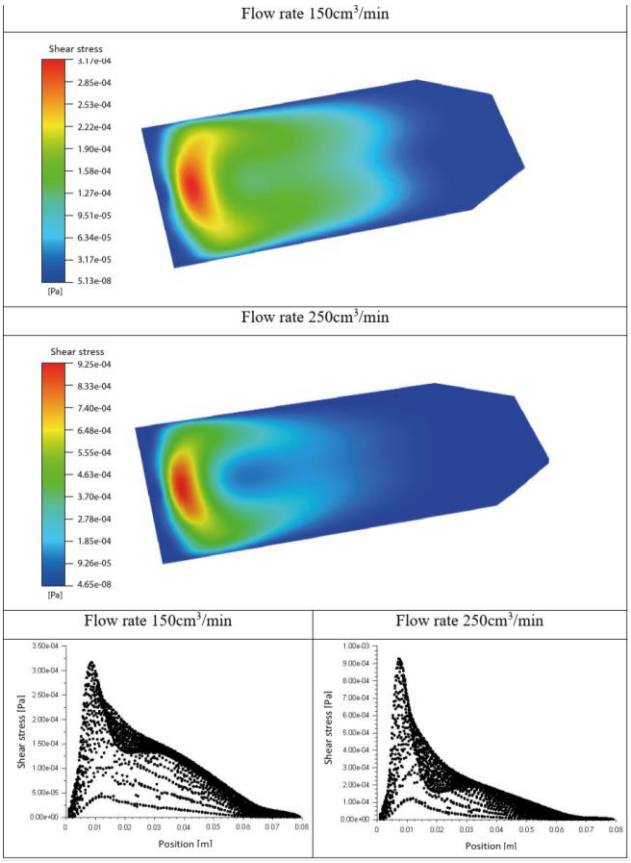

Fig. 6. Maps and diagrams of shear stress distribution on the bottom wall of the specimen for model 2

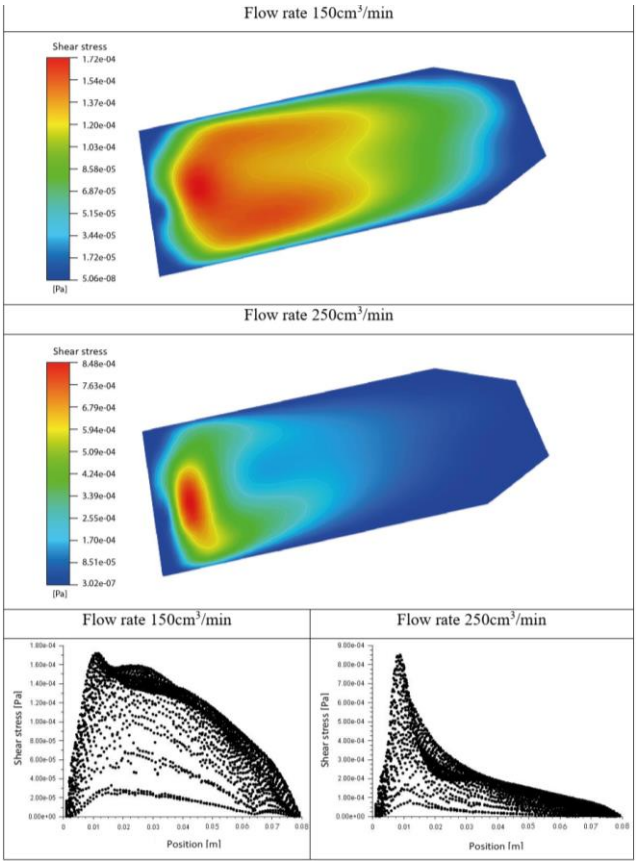

Fig. 8. Maps and diagrams of shear stress distribution on the lower wall of the sampler for model 4

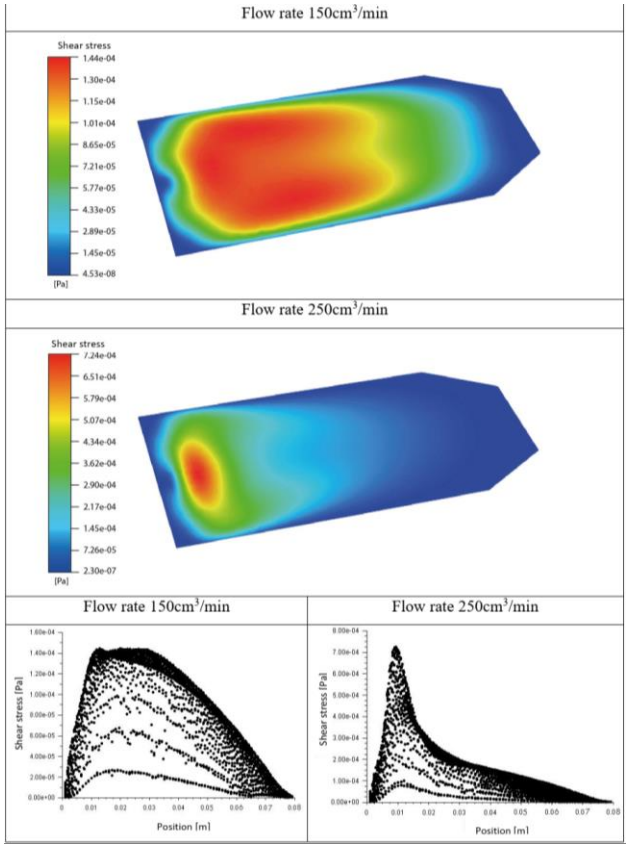

Fig. 7. Maps and diagrams of shear stress distribution on the lower wall of the sampler for model 3

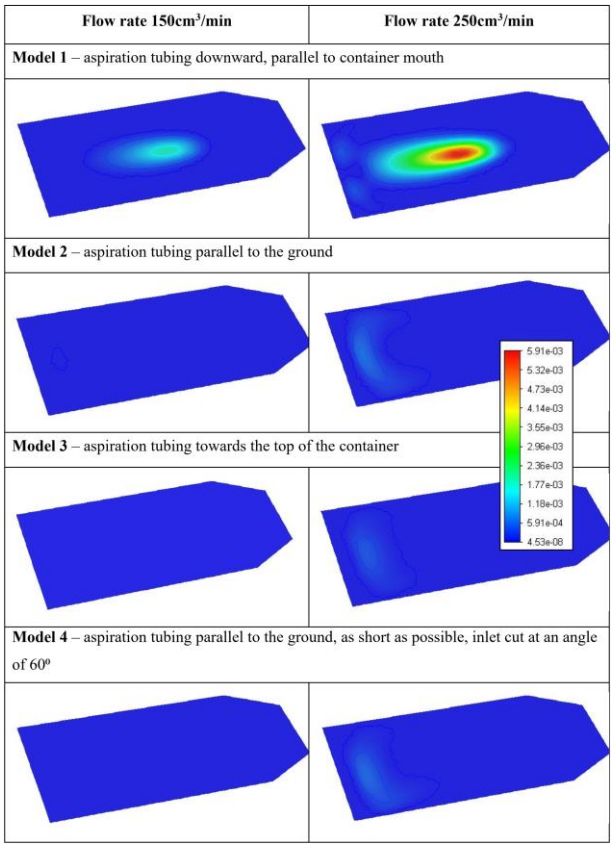

Fig. 9. Comparison of shear stress distributions on the lower wall of the sampler for individual models

In order to compare the models, a table containing distribution maps of shear stress values with a unified measurement scale is also presented (Table 1). Its range is determined by the highest and lowest values of shear stresses selected on the basis of the previous analysis.

Table 1. Maximum shear stresses for various aspiration pipe shapes

Model	Maximum shear stress on the bottom wall of the container [Pa]	
	for the flow rate 150 cm^3/min	for the flow rate 250 cm^3/min
1	$2.06 \cdot 10^{-3}$	$5.91 \cdot 10^{-3}$
2	$3.17 \cdot 10^{-4}$	$9.25 \cdot 10^{-4}$
3	$1.44 \cdot 10^{-4}$	$7.24 \cdot 10^{-4}$
4	$1.72 \cdot 10^{-4}$	$8.48 \cdot 10^{-4}$

Due to the lowest maximum values of shear stress and the most uniform distribution of values on the surface of the bottom wall of the sampler, model 3 and a gas flow rate of 150 cm^3/min were selected for the real tests.

5. Real model

The sampler used is a standard container used for adherent cell culture, made of polystyrene (Fig. 10). The inner surface of the bottom wall of the sampler enables proper adhesion of cells to the substrate, thus their movement inside the sampler is not possible. It should be noted that the samplers used in the BAT-CELL method are subject to constructional modifications by the manufacturer over time. Therefore, it is important to simulate the flow of tested gases through the sampler before commencing the main tests.

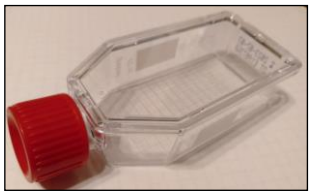

Fig. 10. Sterile closed cell culture container

For cell exposure, the method requires changing the sampler stopper to a different one, which contains aspiration tubing – inlet and outlet. The tubes are made of rigid Teflon, which is resistant to elevated temperature (max. 260°C), in which liquid sterilisation of exchangeable stoppers equipped with tubing. The diameter of the tubing has been selected according to the size of the upper surface of the stopper, so that two holes can be drilled in it. The outer diameter of the tubing was 4 mm and the inner diameter was 3 mm. The tubings were fixed in the holes of the stopper using a suitable VOC-free adhesive. When designing the shape of the tubing, care had to be taken to ensure that they did not mechanically damage the lower wall with the adherent cell culture when the stopper was screwed on. Because of the downward slope of the sampler inlet, it was necessary to design appropriately long aspiration tubing. An important issue was the selection of the angle of the aspiration tubing in relation to the bottom wall of the sampler and their shape.

The actual model of the sampler with the visible aspiration tubing facing upwards inside the sampler is shown in Fig. 11. Antibacterial filters are screwed to the external ends of the aspiration tubing via a specially selected connector.

The selection of an appropriate flow rate is aimed only at the exchange of air, the velocity of which will not cause mechanical damage to the cells, but will lead to a continuous supply of fresh fumes. Therefore, it is not important to select an exact value of the flow rate, but one that does not exceed the critical value that ruptures the cells.

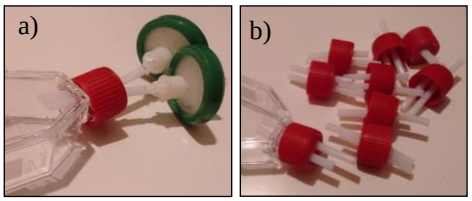

Fig. 11. a) The actual model of the sampler with aspiration tubing terminated with filters, b) prototype series of identical interchangeable caps

The value of the flow rate of exhaust gases flowing through the sampler inlet/outlet system equal to 250 cm^3/min in the first test experiment was selected on the basis of previous experiments [12, 17]. The second tested flow rate value of 150 cm^3/min was selected based on the first trial test, during which cell rupture from the adherent substrate was observed (Fig. 12a, b). The lack of uniformity of cell distribution after exposure indicated that the set flow rate was too high. Previous analyses [12, 17] did not show the problem of cell rupture for a flow rate value of 250 cm^3/min. The reason was most likely due to discrepancies in the methods of adhesion of a given cell line to the sampler substrate.

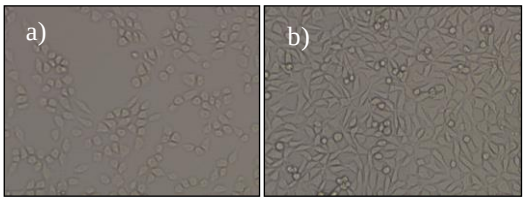

Fig. 12. a) cells detached from the substrate, b) cells multiplying properly

The test gases flowing through the aspiration system exert specific internal forces on the bottom of the adherent cell culture probe. These forces are divided into tangential and normal stresses. The effect of cell detachment from the substrate is related to the occurrence of shear stresses. Therefore, the distribution of shear stresses on the lower wall of the sampler was analysed. The aim was to obtain as uniform a distribution as possible, which would give information that the cell culture would not be disturbed at any point and that no mechanical damage would occur and the tests would have to be repeated.

The research, which confirmed the correctness of BAT-CELL method validation, concerned the survival rate of cells subjected to exposure to engine exhaust fumes emitted from petrol vehicles meeting Euro 3 and Euro 6 emission standards. One of the methods used to determine cell survival rate is the method using a haemocytometer – a glass plate with engraved lines forming a grid, with the help of which it is possible to count degenerated cells. Example results are presented in the diagram below (Fig. 13) [19].

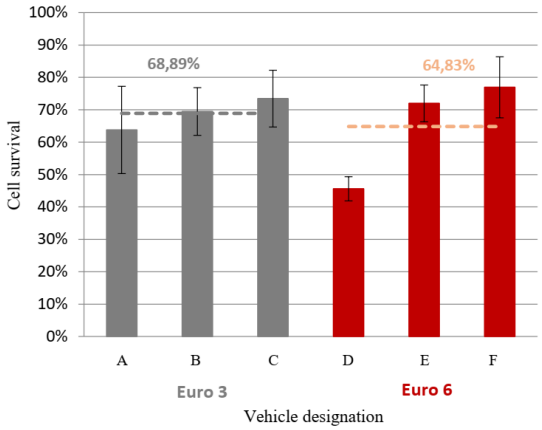

Fig. 13. Mean cell survival calculated with using a hemocytometer [19]

For the tested vehicles complying with the Euro 3 standard, the cell survival was found to be approximately 4% higher in comparison with the vehicles complying with the Euro 6 standard, which means higher toxicity of exhaust gases for the tested vehicles of newer generation [19].

6. Summary and conclusions

Validation of the BAT-CELL Bio-Ambient-Tests method performed by numerical analysis of the exhaust gas flow through the aspiration system was verified in real tests, and simulation results confirmed theoretical assumptions.

Appropriate selection of flow rate values, aimed at air exchange leading to continuous supply of fresh exhaust gases, as well as the shape of aspiration tubing allowed to eliminate the effect of cell detachment from the adherent sampler substrate, allowing for their proper multiplication and elimination of mechanical damage to the cells.

In addition to the sampler design, parameters such as exposure time of the cell culture to the engine exhaust gas and the exhaust gas collection method were validated.

While continuing the research, actions should be taken to improve the BAT-CELL Bio-Ambient-Tests method. Above all, the cell culture samplers for the method should be standardised, the sampling method for the gas mixtures tested should be standardised and a procedure should be developed for the in vitro method used to assess cell cytotoxicity. Undoubtedly, it is also necessary to verify the method on a sufficient number of vehicles to confirm the reliability of performing real toxicity tests of engine exhaust gases on living cells in order to use the method commercially as an alternative to those currently used. Nevertheless, it should be stressed that the test showed that it is worth considering the validity of introducing successively more restrictive exhaust emission standards, which take into account only selected compounds or groups of compounds, but not necessarily those which determine the toxicity of the exhaust gas mixture. Moreover, it is important to take into account the interactions of particular compounds with each other, which may, for example, intensify the toxic effect.

Acknowledgements

The work is a part of the research of the doctoral dissertation *The method for the assessment of the toxicity of engine exhaust gases in the aspect of the analysis of the development of emission standard*.

Nomenclature

CLD	chemiluminescence detector
CO	carbon monoxide
FID	flame ionization detector
HC	hydrocarbons
NDIR	nondispersive infrared sensor
NO_x	oxides of nitrogen
PAH	polycyclic aromatic hydrocarbons
PM	particulate matter
PN	number of particulate matter
VOC	volatile organic compounds

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