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Cytotoxicity assessment of exhaust gases and analysis of volatile organic compounds emitted from a diesel engine

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ABSTRACT

Hydrocarbons contained in exhaust gases emitted by vehicles determine their actual toxicity. This study investigated the exhaust emissions from a passenger car equipped with a compression-ignition engine that meets the Euro 6 standard. Exhaust gas samples were collected on a chassis dynamometer during engine operation at idle, at maximum torque, and at maximum useful power. The cytotoxic activity of the exhaust gases was determined using the BAT-CELL method in a viability test of L929 cell line after 24 hours of exposure to the exhaust gas mixture. The results showed a decrease in cell proliferation. It was also observed that the tested cells exhibited morphological changes, such as disruption of the cell membrane integrity. Volatile organic compounds (VOCs) were identified using gas chromatography. It was found that the permissible concentration of benzene was exceeded by up to several hundred times. Among the tested samples, the exhaust gases emitted during the maximum torque phase were the most cytotoxic. It was concluded that the current legal standards and the methodology used to control exhaust gas composition are insufficient from the perspective of human health protection.

Keywords: internal combustion engine, emission, cells viability, benzene.

INTRODUCTION

Air pollution continues to be one of the main environmental and health threats in the modern world [1]. One of the significant sources of toxic emissions into the atmosphere is exhaust gases emitted by internal combustion engine vehicles – both compression-ignition (CI) and spark-ignition (SI) [2]. These emissions include not only conventional gaseous pollutants (such as NO_x, CO, CO_2, or particulate matter PM) but also volatile organic compounds (VOCs), including toxic and carcinogenic substances such as benzene, formaldehyde, acetaldehyde, and toluene [3]. The qualitative and quantitative composition of VOCs depends on many factors, including the type of engine, combustion conditions, and engine load [4]. In recent years, in addition to conventional emission assessment methods, increasing attention has been paid to the direct impact of exhaust gases on living cells under in vitro conditions [5]. These methods enable the evaluation of the actual cytotoxic potential of gas mixtures emitted by vehicles, regardless of whether emission standards are formally met. One of the modern in vitro methods for assessing the toxicity of exhaust gas mixtures is the BAT-CELL method (Bio-Ambient-Tests method to assess the cytotoxic impact of exhaust gas mixtures) [6], which allows for direct exposure of cell cultures to exhaust gas samples under controlled conditions.

The cytotoxicity of exhaust gases results from the presence of multiple chemical compound groups, including VOCs and polycyclic aromatic hydrocarbons (PAHs), whose concentration and composition vary depending on engine operating conditions. Notably, even vehicles that meet strict emission standards (e.g., Euro 6) can emit gas

mixtures with significant toxic effects, especially under increased engine load [7].

The aim of this study was to assess the cytotoxicity of exhaust gases emitted by a BMW 520dX passenger car equipped with a Euro 6-compliant diesel engine. The tests were carried out under laboratory conditions at three engine load levels (idle, maximum torque, maximum power), using the L929 cell line viability test and analysis of VOC presence using gas chromatography. The study attempts to answer the question of whether current emission standards actually protect human health against exposure to toxic exhaust gas components, and whether a correlation can be identified between the presence of VOCs and the observed level of cytotoxicity of exhaust gas mixtures.

MATERIALS AND METHODS

Research object

The source of the exhaust gases under investigation was a BMW 520dX passenger car manufactured in 2018. The vehicle is equipped with a four-cylinder, turbocharged compression-ignition engine with a displacement of 1995 cm³. According to the manufacturer's specifications, the engine has a power output of 140 kW at 4000 rpm and a maximum torque of 400 Nm in the 1750–2500 rpm range. Mechanical energy is transmitted to all four wheels via the xDrive all-wheel-drive system. At the time of testing, the vehicle had a mileage of 197,000 km and held a valid technical inspection certificate issued by a certified vehicle inspection station. The tested vehicle complied with the Euro 6 emissions standard.

Preparing the vehicle for testing

Preparatory steps included a visual inspection of the selected vehicle, carried out using a scissor lift. The inspection covered the power unit and exhaust system, looking for any irregularities such as leaks, uneven engine operation, missing components, fluid leaks, or engine control unit (ECU) error codes. No issues were found. The chassis dynamometer used for the tests was a MAHA MSR 1050, a dual-axle setup with eddy current brakes. This configuration allowed for precise testing of a vehicle with all-wheel drive. The eddy current braking technology enabled the application of load to the internal combustion engine, which is crucial for turbocharged engines and ensures high measurement accuracy. The rollers had a diameter of 762 mm..

Immediately before driving onto the chassis dynamometer, the engine and exhaust after-treatment system were brought to operating temperature. This was important due to the direct correlation between the efficiency of the catalytic converter and its temperature [8]. Operating the engine below its optimal temperature leads to incomplete combustion, in which only part of the supplied fuel is burned, thereby increasing the hydrocarbon content in the exhaust gases [9]. For the tested vehicle, proper operating temperatures were defined as 100 °C for the coolant and 90 °C for the engine oil. These temperatures were reached by driving a designated route for approximately 20 minutes.

Communication between the dynamometer control computer and the engine control unit was established via the OBD-II diagnostic port, allowing real-time readings of engine speed and oil temperature. The composition of the exhaust gases was monitored using a MAHA MET 6.3 exhaust gas analyzer, capable of measuring carbon monoxide (CO), carbon dioxide (CO_2), oxygen (O_2), and hydrocarbons (HC). The analyzer was connected to the vehicle's exhaust pipe via a hose with a metal extension. The analyzer was also integrated with the dynamometer control system. However, the data obtained from this device were not included in the final analysis due to insufficient measurement accuracy.

Before exhaust gas sampling, the vehicle's external performance characteristics were determined (Figure 1). This process was repeated three times to identify the engine speeds corresponding to peak torque and maximum useful power – values that are characteristic for every combustion engine. Alongside idle speed, these data points define a broad operating range for the engine, enabling potential correlations between engine operating parameters and exhaust gas toxicity.

Testing revealed a maximum torque of 401.8 Nm, indicated by the orange curve, achieved at 2545 rpm – close to the manufacturer's specifications. Maximum power, represented by the red curve, was measured at 140.7 kW (191.3 BHP – break horsepower) at 4040 rpm. A correction factor of 1% was applied according to DIN 70020, accounting for deviations from standardized environmental conditions (ambient temperature and air pressure). Idle speed was measured at 800 rpm.

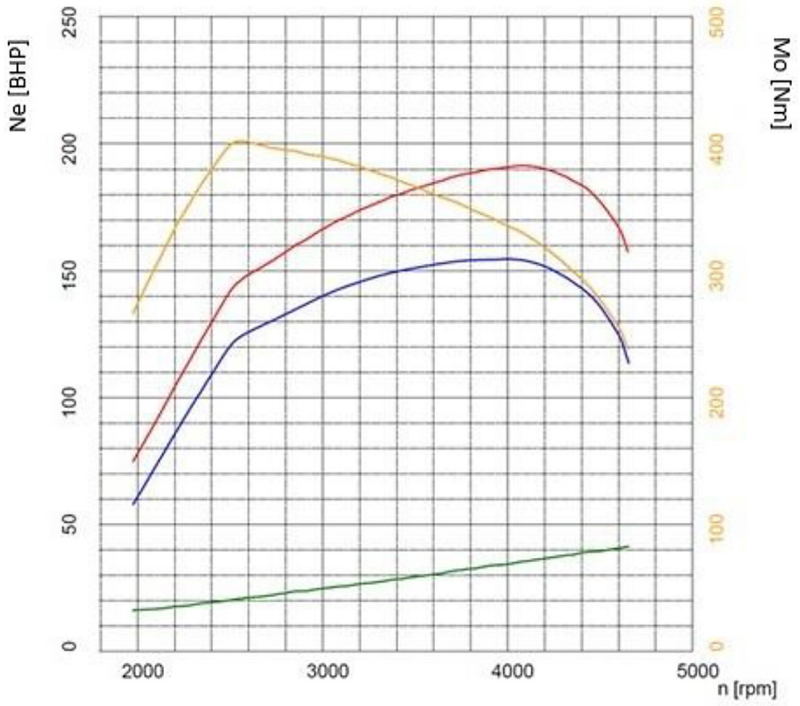

Figure 1. External engine characteristics; Ne-wheels [BHP], Ne-losses [BHP], Ne-norm [BHP], Max Mo [Nm]

Method of exhaust gas sampling

To collect gas samples, a silicone hose with a metal extension was inserted into the vehicle's exhaust pipe. The other end was connected to inert gas sampling bags, each with a capacity of 10 dm^3. These bags were placed in a special aspiration system with a vacuum chamber (Figure 2).

Engine speeds corresponding to maximum torque (2500 rpm) and maximum power (4000 rpm) were enforced using the chassis dynamometer's control system. This ensured high stability of operating points with a tolerance of less than 1%. For each selected operating point, exhaust gases were collected into two separate bags: one for the analysis of volatile organic compounds (VOCs) and the other for in vitro toxicity testing. Throughout the sampling process, exhaust gas composition was continuously monitored using the MAHA MET 6.3 analyzer.

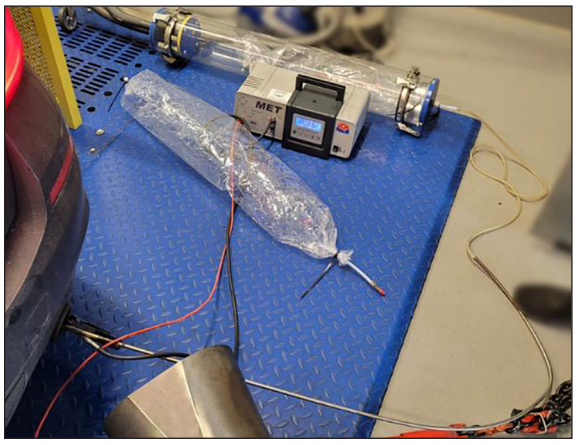

Figure 2. Exhaust gas sampling; one sample bag collected, the other placed in the vacuum chamber

Cell culture

The study employed the adherent fibroblast cell line L929 (ECACC 85011425), originally derived from murine subcutaneous adipose tissue. This cell line serves as a reference model for toxicity evaluation of biomaterials, in accordance with ISO-10993:5 guidelines [10].

Cell culture procedure

The cultivation process followed standardized operating protocols [11]. Cells were maintained in Minimum Essential Medium (MEM) supplemented with Earle's salts (Capricorn Scientific, Ebsdorfergrund, Germany), enriched with 10% fetal bovine serum (FBS, Sigma-Aldrich, St. Louis, MO, USA), 1% L-glutamine, penicillin, streptomycin, and the pH buffering agent HEPES (Sigma-Aldrich). Incubation was performed in a humidified atmosphere at 37 °C and 5% CO_2 using a CELL 50 Comfort S CO_2

incubator. Subculturing was conducted at 70% confluence with 0.25% trypsin-EDTA (Sigma-Aldrich). Microscopic observations were carried out using an AE31E trino inverted microscope with a Moticam Pro S5 Lite camera, offering up to 400× magnification.

Exposure to exhaust gases

Twenty-four hours prior to exhaust gas exposure, cells were seeded in adherent flasks with a surface area of 25 cm² at a density of 28,000 cells/cm². Cell density was determined using the EveTM automatic cell counter (NanoEn Tek Inc.).

The BAT-CELL Bio-Ambient-Tests method (Polish patent No. 220670) was employed to assess the cytotoxic impact of exhaust gas mixtures [6]. In this method, the culture medium is removed, and the cells are placed in a sterile sampling chamber. Exhaust gases are introduced via an aspiration system through an inlet equipped with an antibacterial filter, which blocks particulate contaminants. After the exposure period, fresh culture medium is reintroduced, and toxicological assays such as viability tests are conducted to assess cellular responses. The exposure duration varies depending on the gas mixture; in this study, cells were exposed to exhaust gases for 7.5 minutes [12]. Gas flow parameters were optimized to ensure even distribution across the cell surface while preventing mechanical damage, with a constant flow rate of 150 cm³/min [13].

The exposure chamber was fitted with sensors for temperature and pressure monitoring to maintain cell viability during treatment. The culture medium was safely removed owing to physical conditions specifically adapted to preserve cell integrity outside the incubator environment for a limited duration. An antibacterial filter also protected the sampler's gas inlet. Each experimental condition involving simulated exhaust emissions was performed in triplicate, accompanied by two control groups: one with cells kept under laboratory conditions and another exposed to filtered ambient air.

The BAT-CELL Bio-Ambient-Tests method demonstrated a repeatability rate of 5%, which is relatively low when compared to other biological testing methods [14]. The method's error was previously determined based on average deviations recorded across multiple experiments.

Trypan blue test

Twenty-four hours following exposure to the exhaust gas mixture [12], cytotoxicity was assessed using the trypan blue dye exclusion method, in conjunction with the Eve™ automatic cell counter (NanoEn Tek Inc.). Both total and viable cell counts were obtained, and cell viability was expressed as the percentage of viable cells relative to the air-exposed control group.

Gas chromatography

The qualitative and quantitative characterization of volatile organic compounds (VOCs) was carried out using gas chromatography. Air samples were collected using a dual-channel ASP II aspirator (LAT), with VOCs adsorbed onto SKC LOT 120 sorbent at a gas flow rate of 30 dm³/h. Samples containing activated carbon were stored below 20 °C until laboratory analysis. Carbon disulfide was employed as the desorbing solvent. VOC analysis was performed using a VARIAN 450GC gas chromatograph equipped with a flame-ionization detector and a capillary column (Varian VF-WAXms, 30 m × 0.25 mm ID, DF: 0.25 µm). Analytical parameters included a column temperature of 373 K (110 °C), injector temperature of 423 K (150 °C), and detector temperature of 423 K (150 °C). All instruments were calibrated before VOC analysis. Calibration and desorption coefficients were determined to evaluate method recovery. The overall relative error associated with VOC quantification was estimated at approximately 20% [15].

RESULTS

Viability tests

The cell viability 24h after exposure to the tested exhaust gases is shown in Figure 3. The percentages reported for samples exposed to exhaust gases are an average of three tests. Error bars (standard deviation) are marked on the graph. In the control samples (Control LAB and Control AIR), cell viability was recorded at 92–96%, which is an expected and proper value. For the sample corresponding to maximum power (Max Ne), cell viability remained at the level of the control samples – 92%. A slightly lower value of 85% was observed for the idling sample. The lowest viability, 42%, was

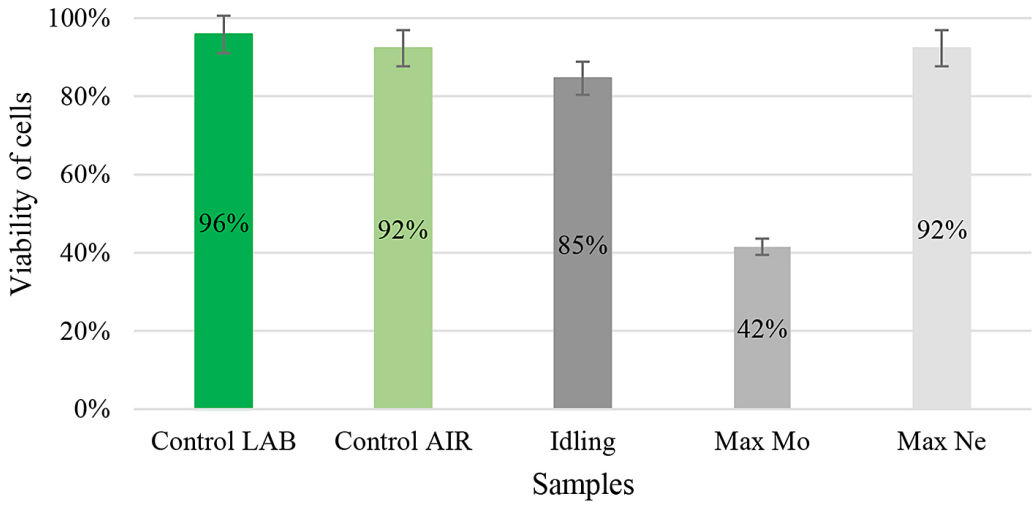

Figure 3. Viability of cells after exposure to diesel exhaust gases

recorded for the maximum torque sample (Max Mo), which is more than twice lower than the viability observed in the control samples.

Imaging of cells

Images of the cells taken 24 hours after exposure (Figure 4) were captured using the same optical microscope that was employed to monitor the samples during their growth. It was found that even at 400x magnification, it was possible to infer changes in the external morphology and confluence of the cells following exposure to harmful gases. In the control sample, healthy fibroblasts with a typical spindle-like shape growing in a monolayer were observed. The remaining images show cells 24 hours after exposure to exhaust gas mixtures. In

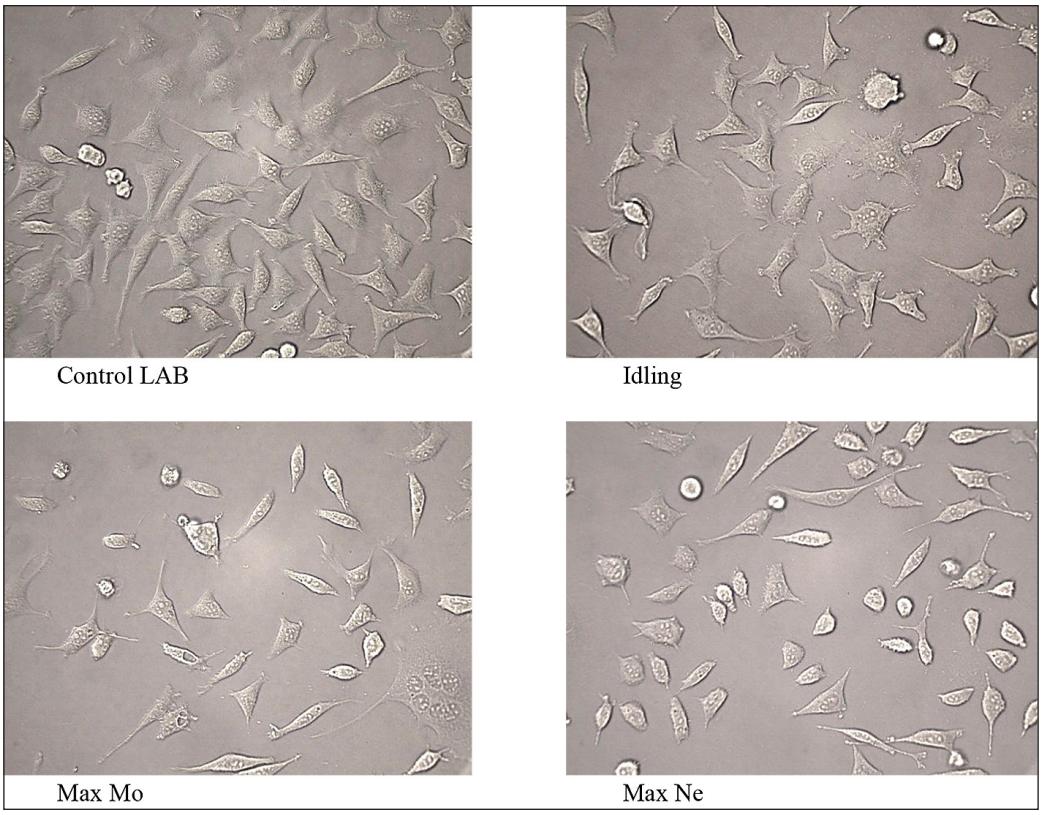

Figure 4. Viability of cells after exposure to diesel exhaust gases

Table 1. Concentration of VOCs in the exhaust gases compared with the standards

Samples	Compound concentration in the exhaust [mg/m^3]		
	Benzene	2-butanol	Sum of VOCs
Idling	31	-	31
Max Mo	287	68	355
Max Ne	183	36	219
NDS	1.6	300	

the sample corresponding to engine idling, cells exhibited visibly reduced confluence, indicating an inhibition of cell proliferation. Morphological changes were also apparent, including more rounded and swollen cell shapes. In the Max Mo condition, in addition to decreased confluence, degeneration of the cell membranes in some cells was observed. No significant alterations in external cell morphology were detected in the Max Ne condition; the image displays cells at various stages of the cell cycle.

Chemical analysis of VOCs

Concentrations of individual compounds present in the exhaust gas mixture were calculated based on the data read from the chromatograms. Two different volatile organic compounds were determined during engine operation at the given parameters: benzene and 2-butanol (Table 1). The highest concentrations of compounds were recorded during maximum torque (Max Mo). The sum of concentrations of individual compounds was 355 mg/m^3. The NDS (maximum allowable concentration) benzene value of 1.6 mg/m^3 was exceeded times in all samples [16]. For 2-butanol the recorded NDS values were normal.

DISCUSSION

The objective of the conducted research was to assess the cytotoxicity of exhaust gases emitted by a BMW 520dX xDrive diesel-powered vehicle, as well as to perform a qualitative and quantitative analysis of the presence of VOCs. Exhaust gases were collected under three different engine operating conditions: idle, at maximum torque (Max Mo), and at maximum engine power (Max Ne). The results did not indicate any correlation suggesting that increased concentrations of benzene or a combination of benzene and 2-butanol in the exhaust gases lead to reduced cell viability. For the two loaded engine conditions, Max Mo and Max Ne, the presence of benzene and 2-butanol was detected in similar ratios, specifically 4:1 and 5:1, respectively. The morphological changes observed in cells exposed to the Max Mo sample – including membrane damage and altered cell shape – are puzzling, as no similar changes were noted for the Max Ne sample. Several explanations are possible. It is conceivable that morphological changes in cells only become apparent at higher VOC concentrations, or that the identified compounds interact with other chemical groups (e.g., polycyclic aromatic hydrocarbons, PAHs), enhancing their cytotoxic effects. Another possibility is the presence of more toxic compound groups (e.g., PAHs), which, without interacting with VOCs, are independently responsible for the observed cytotoxic effects. Clearly, identifying PAH compounds would be beneficial in this study, as they are known to be key contributors to the toxicity of exhaust gas mixtures, as repeatedly demonstrated in scientific literature[17]. Future research should definitely incorporate methods for detecting PAH compounds.

Nevertheless, it can be assumed that the emission of 2-butanol may depend on the engine load level and may be associated with the intensification of the combustion process. Benzene, classified as a Group 1 carcinogen, is a well-known component of motor vehicle emissions, and its presence in diesel exhaust is consistent with published data [18]. While the presence of 2-butanol is less frequently reported, it has been documented by other authors as a product of incomplete combustion under dynamic engine operation conditions [19].

CONCLUSIONS

The results of this study confirm that even new vehicles that comply with current emission standards (Euro 6) can emit compounds with harmful

biological effects. This is especially important in the context of evaluating the health risks of urban populations exposed to exhaust gases, particularly under conditions of heavy traffic [20].

Based on the conducted research, the following conclusions were drawn:

- exhaust gases emitted by a diesel engine operating at the speed corresponding to maximum torque reduced the viability of L929 cells by more than half;
- the presence of 2-butanol in the exhaust gases depended on the engine load – its emission was only detected under load conditions;
- the study for this engine did not demonstrate a correlation between VOC presence and reduced cell viability.

The conducted research highlights the need for further investigations into the biological effects of exhaust gases on living organisms, as well as efforts to identify correlations between specific exhaust components and their impact on cellular damage. Moreover, it is worth considering whether current legal regulations on emission limits genuinely address the problem of air pollution caused by internal combustion engine emissions.

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