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Health Relevance of Aerosols from Biomass Combustion in Comparison to Diesel Soot Indicated by Cytotoxicity Tests

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ABSTRACT: Biomass combustion exhibits relatively high particle emissions smaller 10 microns (PM 10). Under optimized conditions, these particles consist mainly of salts from ash constituents. Under poor combustion conditions, organic particulate matter is emitted additionally. The aim of the present project is to compare indicators for health effects of different types of combustion particles, i.e., mainly inorganic particles from optimized wood combustion, particles from wood burnt at incomplete combustion, and Diesel soot. The present paper presents different effects on cytotoxicity of particles during in-vitro tests of V79 lung cells of the Chinese hamster. For this purpose, particle emissions from an automatic wood furnace and from a Diesel engine were sampled on plane filters. The particles were extracted and added to the cell medium to test the respective toxicity. First tests reveal cytotoxic reactions for both types of particles, i.e., Diesel soot and mainly inorganic wood particles emitted during optimized combustion conditions. For a comparison, the cell survival rate was determined for both particle species as a function of particle mass concentration in the cell medium. The result reveals a significantly higher cytotoxicity for Diesel soot than for wood particles from optimized combustion, based on the same particle mass concentration.

INTRODUCTION

There is strong epidemiological evidence, that airborne particles are related to severe health effects [1–5]. In the focus of ongoing discussions are submicron particles, which are insufficiently filtered by nose and bronchia, and can therefore penetrate into the lung, where they are partially absorbed and can potentially cause damage of the lung tissue [6–8]. Recent studies have shown that Diesel soot particles, once present in the lung, can also enter into red blood cells [7, 8].

A comparison of particles from different combustion processes reveals that the distributions of ultra fine particles from wood combustion and Diesel engines are fairly similar and that their concentration is higher than for other sources like oil burners, coal fired power plants with particle filters, or gas turbines [9, 10]. In Switzerland, submicron particles in ambient air, which result mainly from combustion, are therefore dominated by the emissions from Diesel engines and wood combustion.

To describe the source and properties of combustion particles, the following types of particles can be distinguished [11]:

1. Particles from incomplete combustion such as soot and organic particles, which are present in Diesel exhaust, as well as in flue gas of incomplete wood combustion.
2. Inorganic particles resulting from ash constituents, in the case of native wood mainly found as salts like KCl, K_2SO_4, $CaCO_3$ and CaO [12].
3. In addition, volatile organic compounds such as polycyclic aromatic hydrocarbons (PAH) can be adsorbed on the surface of both particle types.

For automatic wood combustion, safely less than 5 wt.-% but preferably less than 1 wt.-% of organic matter in the combustion particles are aimed at and can be achieved under optimised conditions. Inorganic particles are mainly formed by the solid-vapour-particle path which yields in a high concentration of submicron fine-mode particles around 80 nm electrical diameter (measured by Scanning Mobility Particle Sizer SMPS and described as number size distribution) [12] as shown in Fig. 1.

In addition, the solid-to-particle path is also relevant (especially for bark), thus leading to coarse-mode particles between 5 µm and 20 µm aerodynamic diameter (measured by cascade impactor and described as mass size distribution as shown in Fig. 2) [12, 13]. If complete combustion is achieved, fine mode particles smaller than 100 nm are dominant for wood with low bark content. Further, an incomplete combustion or an entrainment of large particles at high local gas velocities can also lead to a strongly increased particle mass with coarse-mode particles larger than 1 µm [14].

It is therefore evident that the chemical composition of submicron particles from automatic wood furnaces differs significantly from Diesel soot. Their health effect, once penetrated into the lung, has not yet been well investigated. Due to the different morphology, a different impact on health is also possible.

As a first approach to distinguish the health effects of these different particle types, their cytotoxity has been investigated in the present study. A suitable method to study effects of cell damage without animal experiments is in-vitro testing on cell cultures. In the ongoing project, lung fibroblast cells of the Chinese hamster (V79 cells) have been selected [15]. With this cell type, cancerogenic effects can be studied as well in a later phase of the project.

The aim of the present project phase is the assessment of possibly different lung cell damage effects by the following types of combustion particles:

Wood type 1: mainly inorganic particles from a quasi-complete combustion of wood

Wood type 2: as 1 but from combustion based on a low-particle concept [16]

Wood type 3: particles from wood combustion with high concentration of organic matter

Diesel: Diesel soot from a modern type Diesel engine without particle filter.

The paper focuses on first test results of the comparison between Diesel particles and wood particles of type 1.

SAMPLE PREPARATION

An identical sampling procedure was used for an automatic wood furnace and a passenger car Diesel engine (Fig. 3, 4). The wood furnace was operated with wood chips and is described in detail elsewhere [12]. The Diesel car was operated with constant speed of 55 km/h on a test bench. For the purpose of repeated series of cell toxicity tests, the sampling of relatively large amounts of particles is needed. To obtain these amounts, a large fraction of the total flue gas volume was passed through plane filters of 300 mm x 400 mm in size. The particle mass sampled on each filter was typically 0.6 g to 1 g. For each test run, a total mass of approximately 20 g particles was sampled on the plane filters.

The particle loaded filters from Diesel and wood exhibit a significantly different colour (Fig. 5). The Diesel filter is black as a result of the soot particles, while the filter from the wood furnace exhibits a white colour even if loaded with a higher particle mass than the Diesel filter. The sampled wood particles from the automatic furnace contained therefore negligible black carbon content and consisted mainly of inorganic salts.

An extractive treatment of the particle samples was established, which achieves a relevant extraction of both particle types, i.e., inorganic particles as well as Diesel soot. A particle preparation should neither include a thermal treatment nor the utilisation of foreign liquids not available in the human body, as both can potentially effect undesired changes of the particle properties. These requirements were met by the following procedure (Fig. 6): The complete quartz filter including sampled particles was grinded in a mixer. The resulting homogenised mixture of filter material and combustion particles was then mixed with liquid cell culture medium. After an ultrasonic treatment and continuous mixing for 24 hours, the dominant fraction of particles was dispersed in the cell medium. The separation of the remaining filter material was performed with a syringe.

The cell medium treated by the above procedure contains mainly the sampled particles, with a minor contamination of residual filter fibre material. This particle loaded dispersion was diluted with untreated cell medium in different fractions and used in the following cell tests.

BIOLOGICAL CELL TESTS

The toxicity tests were performed according to XTT method [17] with V79 lung fibroblast cells of the Chinese hamster [15]. A reference cell culture was kept in cell medium without any treatment. To detect the effect of particle toxicity, the liquid cell medium of other standard cell cultures was replaced by cell medium containing particle extract in different dilutions. The maximum exposure was obtained by replacement of the cell medium by undiluted extract solution, i.e. the cell medium that was used to extract the particles from the filter samples. Under the assumption that all particles on the filter are dispersed in the extraction solution, the particle exposure can be quantified based on the total filter and particle mass added to the cell medium. To investigate possible effects of remaining filter fibres on cell toxicity, an extract solution of an empty filter was generated in a similar way as for the other filters, i.e. including all mixing and subsequent best possible filter fibre separation.

TEST RESULTS

The cell tests show toxicity for all types of particles, as soon as a certain concentration limit is exceeded. The direct test results for all particles types are given in Figs. 7–9. Here, the cell survival compared to an untreated control group is plotted as a function of the relative amount of particle extraction solution that was mixed to the cell medium. 100% extract concentration means that the cell medium was completely replaced by undiluted extract solution.

The most pronounced effect was observed for Diesel particles (Fig. 7), with a cell survival of zero in the most extreme case, i.e. all treated cells died at the maximum extraction solution concentration. A toxic effect was also visible for cell medium containing wood particles. However, the effect was less pronounced and slightly different for the two filter samples tested so far.

Unfortunately, the blind tests with an extract solution that was generated with empty filter material, showed a limited toxicity as well (Fig. 9). Obviously, the cells are also harmed by residual filter material in the cell medium, which could not be completely separated in the sample pre-treatment. This background effect accounts for a cell death rate of 20%–40%, i.e. only 60%–80% of the cells survived the blind test with residual empty filter material. As a consequence, the test results with combustion particles are not sensitive to low toxicity, since a fraction of the investigated cells are harmed by the residual filter material. A cell survival rate of less than 60%, on the other hand, indicates a significant toxic effect of the added particles.

Such a significant toxic effect was found for all Diesel samples and for at least one wood particle sample. To further quantify this effect, the particle concentration in the cell medium has to be accounted for. Under the assumption that all particles on the filter were completely extracted to the cell medium solution, the concentration can be obtained based on the mass of material added to the cell medium during sample preparation.

The cell survival as a function of particle mass concentration in the filter medium is depicted in Fig. 10. It can be seen that a Diesel particle concentration in the cell medium above 3 mg/ml is sufficient to destroy more than 80%, in some cases 100%, of the investigated lung cells. The tested particle concentrations from wood combustion were higher than for Diesel, but did not reach the same levels of toxicity. A toxic effect, which was higher than the background level, is indicated above a concentration of 5 mg/ml. A significant toxicity was observed above 12 mg/ml for one filter sample.

Based on the comparison in Fig. 10, the wood particle concentration needs to be at least five times higher than for Diesel particles to cause similar toxicity effects (i.e., a destruction rate of 70% corresponding to 30% cell survival).

CONCLUSIONS AND FUTURE WORK

The present test results indicate a cell toxicity of both, Diesel soot and particles from wood combustion. However, the toxicity of the wood particles from optimised combustion conditions is less pronounced and at the border of the measurement significance in some tests. Unlike for wood particles, the toxicity of Diesel particles is always highly significant, with cell survival rates close to zero at the highest particle concentration in the cell medium. For the same particle mass concentration, Diesel particles are by far more toxic than wood particles from an automatic furnace.

This difference can be understood as a result of the different properties and chemical composition of these two particle types, which is also visible in the different colour of the filter samples and which has been described earlier. Diesel particles contain mainly unburned carbonaceous material with a low inorganic ash fraction. Particles from a state-of-the-art automatic wood furnace are dominantly salts, which arise from the inorganic biomass components. Although these salts form submicron particles, which can easily penetrate into the lung, their toxicity for lung cells is by far lower than for Diesel soot particles.

This statement is only verified for wood combustion with low or negligible emissions of unburned hydrocarbon material, as it is the case for the automatic furnace used for the present samples. It appears highly probable that incomplete combustion of biomass with high concentrations of organic particulate matter, which can occur in poorly designed or badly operated wood furnaces as well as in open fires, can result in a higher toxicity than the wood particles investigated in this study.

An investigation of wood particles with higher amount of organic matter is in progress. In addition, cell tests are planned for particles resulting from a wood combustion applying a low particle concept to further suppress the amount of inorganic particles [16]. The respective filter samples are available and will be used in an upcoming series of cell tests.

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- RCC Cytotest Cell Research GmbH, Darmstadt (Germany)

FIGURES

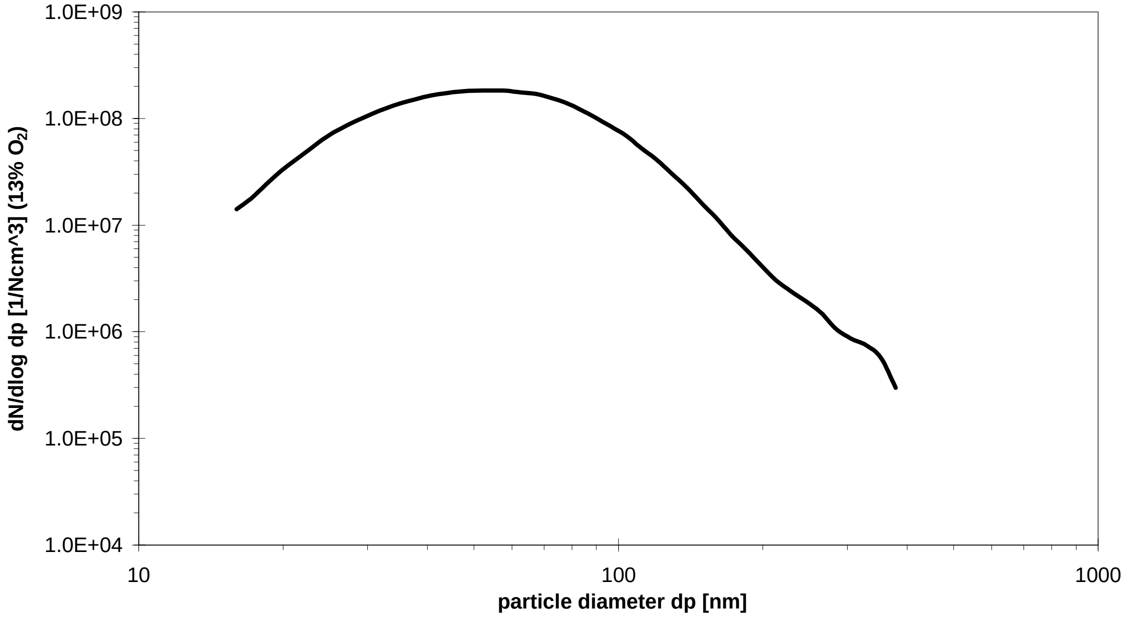

Fig. 1: Ultra fine particle spectrum measured with an SMPS in the flue gas of an automatic wood furnace [12].

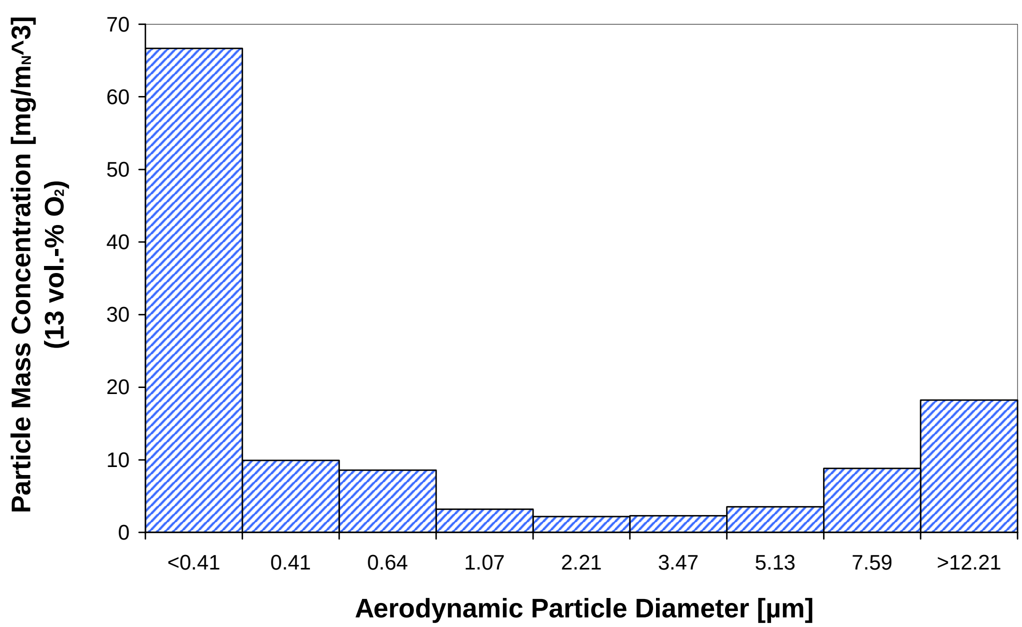

Fig. 2: Particle mass size distribution measured after an automatic wood furnace [12].

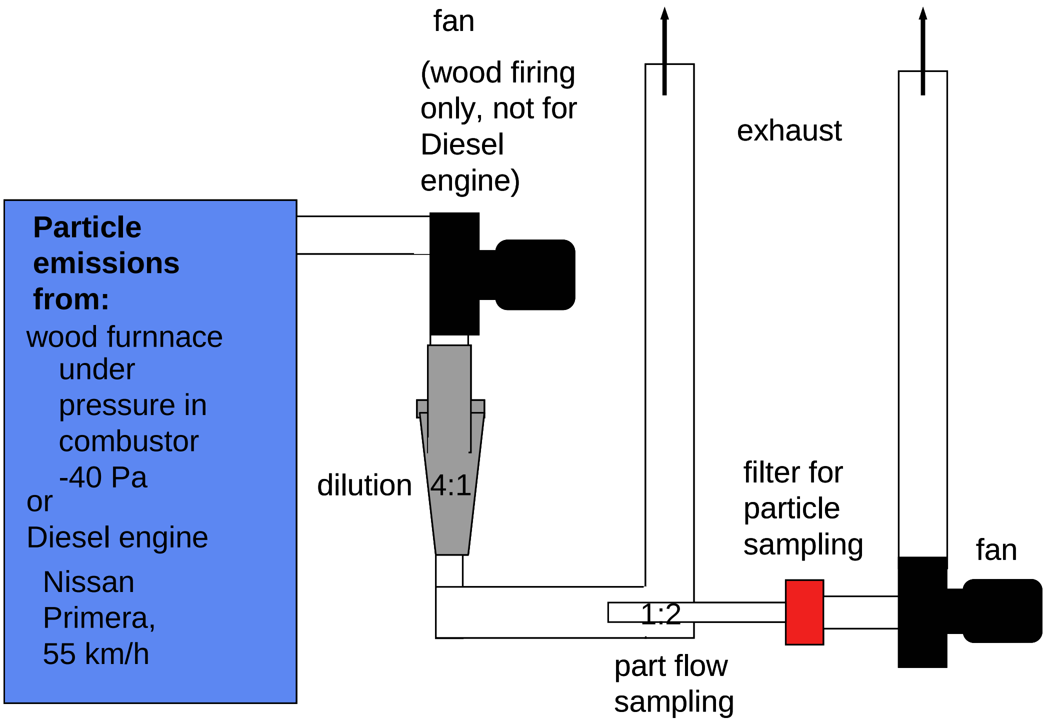

Fig. 3: Schematic view of particle sampling.

Fig. 4: Particle sampling from the exhaust gas of a Diesel car without internal particle filter according to Euro 3 emission standard (Nissan Primera 2003) on the test-bench at EMPA Dübendorf.

Fig. 5: Filters (300 mm x 400 mm) after particle sampling. Left from Diesel engine (filter loaded with 0.6 g particles), right from almost-complete wood combustion (filter loaded with 1 g particles).

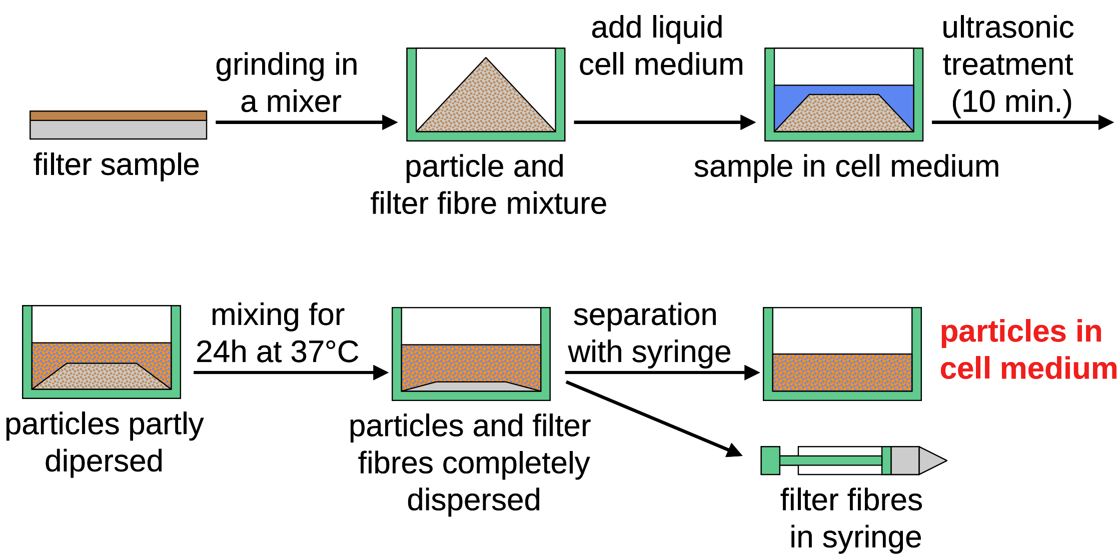

Fig. 6: Sample preparation for cell tests.

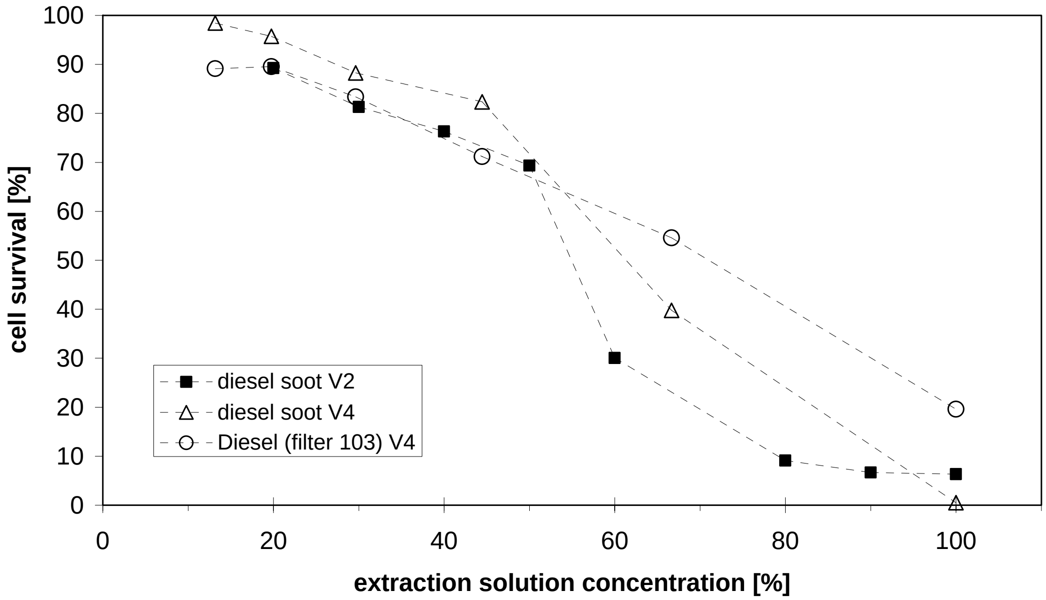

Fig. 7: Toxicity test results for Diesel particles.

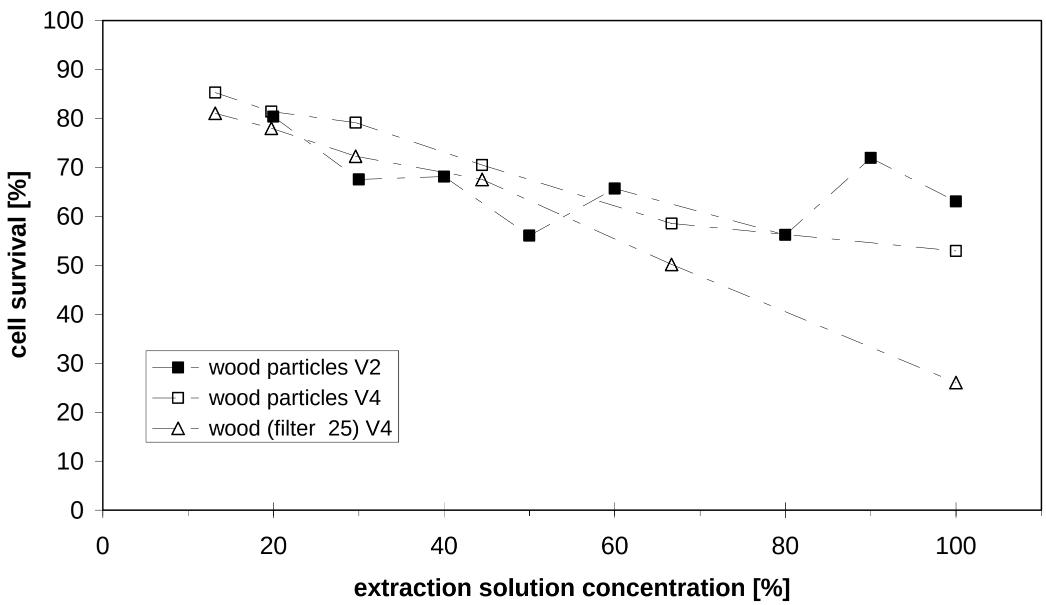

Fig. 8: Toxicity test results for particles from an automatic wood furnace.

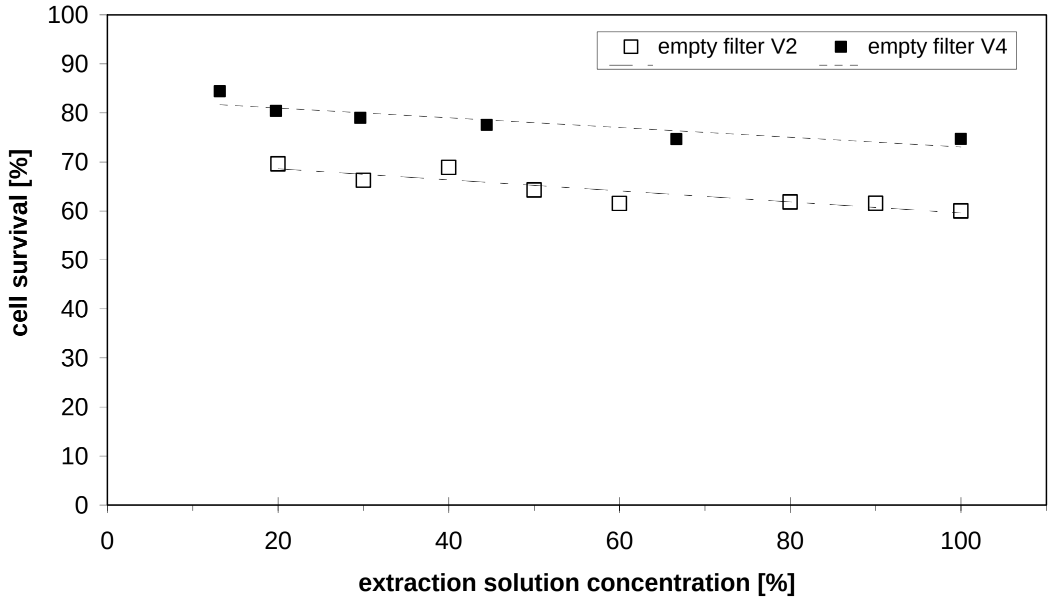

Fig. 9: Toxicity test results for control test with empty filter material.

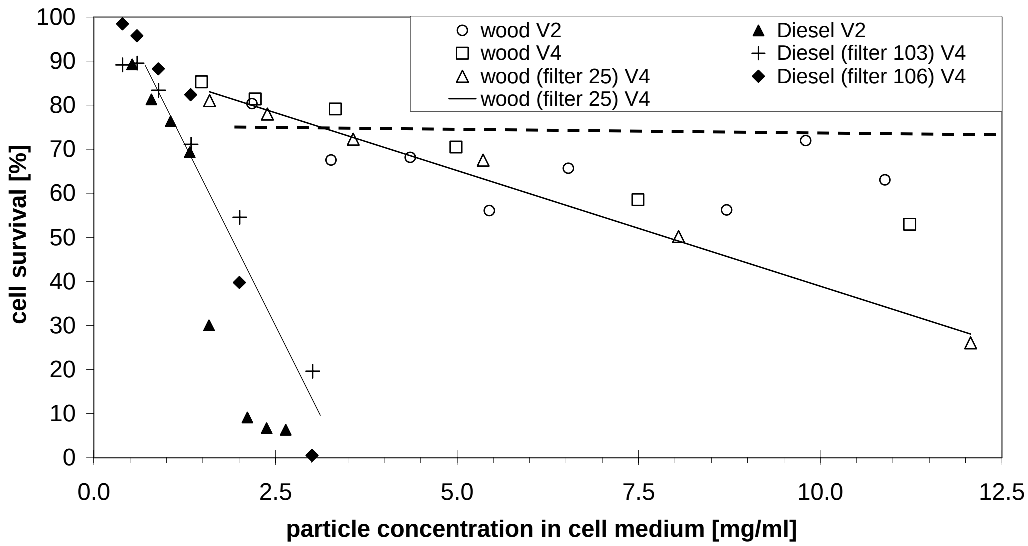

Fig. 10: Cell survival as a function of particle concentration, compared for Diesel and wood particles, respectively. The background toxicity level, which is caused by residual filter fibres, is indicated as dashed line.