Effects of Occupationally Relevant Zinc- and Copper-Containing Metal Fumes on Human THP-1 Macrophages and A549 Lung Epithelial Cells *in vitro*

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Occupationally Relevant Zinc- and Copper-Containing Metal Fumes Inhibit Human THP-1 Macrophage TNF and IL-6 Responses to Bacterial Stimuli

Jan Steffens, Sabrina Michael, Katharina Kuth, Henner Hollert, Miriam Du Marchie Sarvaas, Andrijana Nesic, Thomas Kraus, and Ralf Baumann*

Metal workers have an increased risk of severe lobar pneumonia due to exposure to metal fume particles, which lead to recent pneumococcal vaccination recommendations. To investigate the effects of metal fume-derived zinc oxide (ZnO) and copper oxide (CuO) particles on airway immune responses, human THP-1-derived macrophages are exposed in vitro to the bacterial pathogen-associated molecular patterns (PAMPs) lipopolysaccharide (LPS), lipoteichoic Acid (LTA), or peptidoglycan (PGN), together with particle suspensions. Particles are generated through metal inert gas (MIG) soldering. Spectrometric and microscopic analysis confirms CuO and ZnO as main components. Macrophage *IL-6* and *TNF* mRNAs are quantified by qPCR and secreted protein levels by electrochemiluminescent multi-spot assay. A dose-dependent increase in macrophage TNF and IL-6 mRNA (4 h) and protein (24 h) levels following exposure to PAMPs is significantly inhibited by 2 µg mL^{-1} CuO/ZnO particles ($n = 5$). Additionally, CuO/ZnO particles significantly inhibit TNF protein expression in unstimulated macrophages, while IL-6 protein levels are unaffected ($n = 5$). The presented in vitro immunotoxicity approach may extend existing new approach methodology (NAM) elements for chemical risk assessment and possibly exposure limit evaluation refinements. These findings implicate that CuO/ZnO particles suppress macrophage proinflammatory responses to PAMPs, potentially compromising lung immunity, underlining current vaccine recommendations and efforts for preventive occupational health guidelines.

1. Introduction

Long-term exposure (for several years) of metal workers or welders to metal fumes is associated with several health risks,[1] including cardiovascular diseases,[2,3] as well as airway diseases such as pulmonary carcinogenicity,[4] severe chronic obstructive pulmonary disease (COPD),[5,6] respiratory inflammation,[7,8] increased airway responsiveness,[9] bronchitis,[10,11] rhinitis and welding-related asthma,[12] and fibrosis.[13] In addition, metal fume exposure is reported to exacerbate the severity, frequency, and duration of respiratory tract infections in welding laborers, which leads to a higher risk of lobar pneumonia.[14–19] The severity of this issue is underscored by an increased mortality rate among metal workers, particularly welders, due to invasive pneumococcal disease (IPD).[20–22]

Pneumococcal disease is caused by several bacteria, such as the Gram-positive bacteria *Staphylococcal aureus* and particularly *Streptococcus pneumoniae*. Indeed, *Streptococcus pneumoniae*

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(with currently over 90 known distinct serotypes) is, to a large extent, responsible for community-acquired pneumonia,[23,24] mortality during flu outbreaks,[25,26] and admission of severe community-acquired pneumonia patients into intensive care units.[27,28] Pneumococcal vaccines that protect against clinically relevant serotypes of *Streptococcus pneumoniae* are recommended in the UK[29] and Germany[28,30] for adults aged 65 and above and people with certain health conditions (e.g., immunodeficiency, chronic diseases of the heart or respiratory organs).

As *Streptococcus pneumoniae* is also the main cause of severe lung pneumonia of long-term metal workers,[17,19,21,31] the Health and Safety Executive (HSE) in the United Kingdom[27] and the German Standing Committee on Vaccination (STIKO) of the Robert Koch Institute (RKI) both strongly recommend pneumococcal vaccines for metal workers since 2014 and 2016, respectively.[28,29,32] Taken together, the metal fumes seem to dampen the human lung immune responses to bacterial infection.

The different metals used in metal construction operations, such as iron (Fe), chromium (Cr), nickel (Ni), zinc (Zn), copper (Cu), and manganese (Mn), cause different health implications. In the present study, the focus lays on the immunotoxicological effects of Zn and Cu for several reasons: ZnO nanostructure research is ongoing as ZnO nanostructures are advantageous for many applications in sensing, photocatalysis, functional textiles, and cosmetic industries.[33] Furthermore, the role of Zn and Cu in modern joining technology, particularly in the automotive industry, is increasing,[34] and therefore, metal workers are more frequently exposed. Short-term exposure (for several hours or days) to Zn and ZnO fumes, and in some reports also to Cu and CuO fumes, cause metal fume fever (MFF), a medical condition characterized by fever, headache, and respiratory symptoms.[35–39] In previous studies, we found in healthy individuals significant systemic increases of acute phase proteins after a short-term (6 h) exposure to occupationally relevant welding fumes containing ZnO and CuO at a combined dose of 2.5 mg m^{-3},[40,41] but not after inhalation of welding fumes containing either iron, nickel, chromium[42] or aluminum.[40] Likewise, we found comparable systemic effects for the inhalation of metal fumes containing "only Zn" (1.2 mg m^{-3}) or "only Cu" (0.34 mg m^{-3}).[43,44] The systemic elevation of acute phase responses was confirmed by another research group, which exposed healthy individuals for four consecutive days to either 0.5, 1, or 2 mg m^{-3} pure nano-sized ZnO particle concentrations, with significant effects already at 1.0 mg m^{-3}. As this concentration is well below the occupational exposure limit for ZnO in many countries, this group has recommended to reassess the exposure limit value for ZnO at workplaces.[45,46] Other groups showed for workers in field studies at workplaces significant systemic increases of CRP and/or IL-6 after short-term exposures to welding fume (not explicitly Zn or Cu) and/or other occupational air pollutants.[47,48] In a further study, we showed that short-term exposure to ZnO- and CuO-containing metal fumes caused early nasal increases of acute phase proteins,[49] thereby confirming that the inflammation starts in the airways. Moreover, in vitro, comparative toxicity studies using 24 manufactured nanoparticles showed that Cu- and Zn-based nanomaterials were the most toxic nanoparticles for human macrophages.[50] Specifically, ZnO nanoparticles have been linked to lysosomal destabilization, thereby disturbing macrophage functionality.[13] Taken together, ZnO and CuO affect macrophages, potentially compromising their responses to bacterial lung infections.

To enable a mechanistic investigation of the potentially inhibitory effects of metal fume exposure on lung immune responses to bacterial infection, in the current study, we used a simplified in vitro model: Human macrophages, as important lung immune cell type,[51–54] were exposed to three different pathogen-associated molecular patterns (PAMPs) (lipopolysaccharide (LPS), lipoteichoic acid (LTA) or peptidoglycan (PGN)) to simulate an infection scenario with gram-positive or -negative bacteria, in the presence or absence of metal fume-derived ZnO and CuO particle suspensions.

2. Experimental Section

2.1. Metal Fume Particle Generation

To generate the CuO/ZnO particles, metal inert gas (MIG) soldering was manually performed by a skilled welder within the confines of a funnel-shaped fume hood. Utilizing a hot-dip zinc-coated steel sheet (EN 10346: DX51D+Z275) as the base material, the filler metal comprised 96% Cu, 1% Mn, and 3% silicon (Si) (CuSi3Mn;) (Bercoweld S3, Bedra, Germany). Argon served as the shielding gas during this procedure. The resulting fumes were captured within the fume hood and collected as metal particle dust, following established methodologies.[40]

2.2. Metal Fume Particle Characterization by Electron Microscopy

A suspension of CuO/ZnO particles was prepared using ethanol and ultra-pure water as solvents. 100 and 2 µg mL^{-1} of CuO/ZnO of the metal fume particles were resuspended in ethanol and ultrapure water, respectively. Subsequently, the CuO/ZnO particle suspensions underwent sonication in an ultrasonic bath for 30 minutes, followed by storage in a freezer at -20 °C. Prior to application, the suspensions were thoroughly mixed by vortexing. The prepared suspension was then applied onto a sample grid and evaporated under a fume hood to obtain a thin layer of particles. Scanning electron microscope (SEM) images of the particles were acquired using a field emission scanning electron microscopy (SEM; Zeiss Gemini 982 SEM, Zeiss, Oberkochen, Germany). Elemental analysis of the welding fume particles was conducted using energy-dispersive X-ray spectroscopy (EDX) with an Oxford ISIS EDS detector (Wiesbaden, Germany).

2.3. Metal Fume Particle Characterization by Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Initially, 50 mg of the metal fume particles were added to an aqueous solution within a decomposition vessel. This solution consisted of 1 mL of rhodium-ICP standard solution, 7 mL of hydrogen peroxide, and 5 mL of aqua regia (75% (v/v) HCl, 25% (v/v) HNO_3). Subsequently, microwave digestion was performed for 1 h. Following digestion, the chilled particle suspension was diluted to a ratio of 1:10 using aqua bidest and then

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filtered. To the resulting solution, 20 µL of nitric acid and 100 µL of rhodium-ICP were added to 9.88 mL of the filtered solution. Analysis of these solutions was conducted using the ICP Mass Spectrometer ELAN DRC II (PerkinElmer Inc., Waltham, Massachusetts, USA). Quantification was achieved by comparing the results to measured standards, including 10% (v/v) ICP-multi-element standard solution (ME) VI and 10% (v/v) Sn-ICP Standard 1%. Based on the determined values, the aqueous-available fraction of the metals was calculated.

2.4. Optimal Concentration of Cu/Zn Metal Fumes for In Vitro Study of Cellular Responses

The concentration of 2 µg mL^{-1} did not result in significant macrophage cell death. To assess the adequacy of this in vitro concentration for accurately simulating actual occupational conditions, the methodology was applied proposed by McCarrick et al. to compare in vitro and in vivo exposure concentrations.[55] The concentration of 2 µg mL^{-1} of CuO/ZnO particles, equivalating to 1.1 µg cm^{-2} (calculation based on: 24-well plate; 1 mL medium volume, 1.82 cm^2 growth surface area), falls within their investigated range considering MPPD modeling (deposition, clearance, retention) and is relatively low. McCarrick defines short-term exposure as 6 h to 1 week and long-term exposure as 1 year to 45 years. This concentration exceeds the alveolar retention after 1 week of exposure to the occupational exposure limit (OEL) of 5 mg m^{-3} (0.083 µg cm^{-2}) but is lower than the retention after 1 year (2.85 µg cm^{-2}). It also corresponds to the tracheobronchial retention of 0.89 µg cm^{-2} after 6 h at the OEL, indicating relevance for short-term scenarios. Furthermore, 1.1 µg cm^{-2} exceeds retention levels observed in both tracheobronchial (0.023 µg cm^{-2}) and alveolar regions (0.16 µg cm^{-2}) after 1 week of exposure to low-end concentrations (0.05 mg m^{-3}). This suggests its use for investigating both short- and long-term exposure effects, as it represents a potential concentration in different lung regions based on exposure duration and concentration. While 1.1 µg cm^{-2} equals the tracheobronchial retention level of 1.15 µg cm^{-2} long-term, it remains below the alveolar retention of 2.85 µg cm^{-2} after one year. It is essential to note that in vitro concentrations often represent a bolus dose, while in vivo deposition typically occurs over an extended exposure period, such as several hours. Additionally, in vitro systems frequently require higher concentrations to produce observable effects due to their relative simplicity compared to in vivo conditions.[55]

2.5. Cell Culture Conditions and Exposure Experiments

Monocytic THP-1 cells were cultured in RPMI 1640 medium (Gibco, Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA), supplemented with 10% low-endotoxin fetal bovine serum (FBS) Sera Plus (PAN Biotech, Aidenbach, Germany), 2 mm L-glutamine, 100 U mL^{-1} penicillin, and 100 µg mL^{-1} streptomycin (Gibco, Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA), within a 5% CO_2 humidified atmosphere at 37 °C. To induce monocytic differentiation, cells were exposed to 100 nm phorbol 12-myristate 13-acetate (PMA; Merck KGaA, Darmstadt, Germany) at a concentration of 5.5×10^5 cells mL^{-1} for 48 h.

Table 1. Exposure substances and conditions overview. The following table summarizes the concentrations of the exposure substances used and their respective exposure conditions. The symbols "+" and "++" indicate lower and higher concentrations, respectively. The abbreviations used are as follows: LPS, LTA, PGN, and CuO/ZnO (Cu/Zn-containing metal fume particles).

	Substance [ng mL^{-1}]						
	LPS		LTA		PGN		CuO/ZnO
Concentration	2	20	100	1000	1000	3162	2000
Exposure level	+	++	+	++	+	++	+

Following differentiation to macrophages, the PMA-containing medium was replaced with assay medium comprising 1% FBS and 1% penicillin/streptomycin. After an additional 24 h incubation, the adherent macrophages were subjected to various cell culture media suspensions (as delineated in **Table 1**) under a 5% CO_2-humidified atmosphere at 37 °C.

To assess macrophage responses to bacterial stimuli, PAMPs were employed instead of live bacteria. LPS from *Escherichia coli* O111:B4 (Merck KGaA, Darmstadt, Germany) was used to represent Gram-negative bacteria, while LTA (InvivoGen, Toulouse, France) represented Gram-positive bacteria, and PGN (InvivoGen, Toulouse, France) served as representative of Gram-positive and Gram-negative bacteria, respectively. Both LTA and PGN were obtained from *Staphylococcus aureus* because of their commercial availability and low endotoxin levels. *Staphylococcus aureus*, similar to *Streptococcus pneumoniae*, is a well-known cause of community-acquired pneumonia and can be preceded by influenza infection, thus promoting the emergence of low-virulent strains.[56] Moreover, the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) underlines the clinical importance of this bacterial strain.[57] The chemical structure and properties of LTA from *Staphylococcus aureus* and *Streptococcus pneumoniae* are identical, and their signaling effects were reported to be similar.[58,59]

For RNA purification, cells were harvested using QIAzol (Qiagen GmbH, Hilden, Germany) lysis buffer at 0 and 4 h. The resulting homogenized samples were then transferred to clean tubes pre-cooled at 4 °C and stored at -80 °C until further processing.

2.6. Cell Viability and Toxicological Assessment

The toxicity of the exposure substances on THP-1 macrophages was evaluated using the 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) cell proliferation assay following the manufacturer's instructions (MTT# 30–101K, ATCC Rockville, MD, USA). THP-1 cells were seeded in triplicate at a density of 10^3 cells mL^{-1} and incubated for 24 h at 37 °C in a 5% CO_2 incubator. Subsequently, the testing reagents were added: 2, 4, 6, 8, and 16 µg mL^{-1} CuO/ZnO particles, LPS at 2 ng mL^{-1} (+ 2 µg mL^{-1} CuO/ZnO particles), LTA at 100 ng mL^{-1} (+ 2 µg mL^{-1} CuO/ZnO particles), PGN at 1000 ng mL^{-1} (+ 2 µg mL^{-1} CuO/ZnO particles). RPMI 1640 medium served as a control to establish the baseline for absorbance readings. After incubation of 24 h, 10 µL of the MTT reagent was added

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Table 2. Sequences of the mRNA qPCR primer pairs. The mRNA primer pairs chosen for the RT-qPCR analysis are shown below. The applied concentration of each primer was 0.5 µm for qPCR measurements. The abbreviations used in the table are as follows: F ((forward) – [5′-primer sequence-3′]), R ((reverse) – [5′-primer sequence-3′]).

Gene	Accession	Sequence (5′→ 3′)	References
TNF	NM_000594.4	F – CTGCTGCACTTTGGAGTGAT	[60]
		R – AGATGATCTGACTGCCTGGG	
IL-6	NM_000600.3	F – TCGAGCCCACCGGGAACGAA	[61]
		R – GTGGCTGTCTGTGTGGGGCG	
GAPDH	NM_002046.3	F – GGAGAAGGCTGGGGCTCAT	[62]
		R – TGATGGCATGGACTGTGGTC	

and incubated for an additional 4 h, followed by the addition of 100 µL of the detergent reagent. Following an overnight incubation period in the dark, absorbance readings were obtained at 570/670 nm using an automatic ELISA plate reader SpectraMax iD3 (Molecular Devices, San Jose, USA). The optical density (OD) was measured for cultures exposed to both exposure and non-exposure media, as detailed in Table 1.

2.7. Cell Sample Preparation and Gene Expression Analysis

The total RNA of the cells was extracted using a phenol-based lysis buffer, QIAzol, as part of the miRNeasy Mini Kit (Qiagen GmbH, Hilden, Germany), following the manufacturer's protocol. Subsequently, 1 µg of total RNA was reverse-transcribed using the Applied Biosystems High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA). Quantitative real-time Polymerase Chain Reaction (qPCR) was conducted using 5 µL of cDNA in 20 µL reaction volumes (in duplicates) with the SensiFAST SYBR Lo-ROX Kit (Bioline GmbH, Luckenwalde, Germany) on the StepOnePlus Real-Time PCR System (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA). Primer pairs for *IL-6*, *TNF*, and Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) genes are detailed in **Table 2**. The qPCR protocol consisted of an initial denaturation step at 95 °C for 3 min, followed by 40 cycles of denaturation at 95 °C for 5 s, annealing at 62 °C for 10 s, and extension at 72 °C for 20 s. *GAPDH* served as the reference gene for data normalization.

2.8. Multispot Electrochemiluminescence Immunoassay (ECLIA) for TNF and IL-6

For quantifying TNF and IL-6 protein levels, supernatants were collected 24 h post-treatment and stored immediately at -80 °C. The supernatants were thawed only once right before analysis to ensure accuracy. The detection of TNF and IL-6 was performed using the Meso Scale Discovery V-PLEX following the provided manufacturer's instructions. Measurements were conducted on the MESO QuickPlex SQ 120 instrument, using DISCOVERY WORKBENCH 4.0 software for analysis. The reported protein levels of IL-6 and TNF are the average from three separate experiments, presented with their standard error of the mean.

2.9. Statistical Analysis

Experimental data were subjected to statistical analysis using GraphPad Prism software version 10 (GraphPad Software, Inc., San Diego, USA). A two-tailed *P*-value of less than 0.05 was considered statistically significant. Data obtained from RT-qPCR was expressed as relative quantification (RQ; $2^{-\Delta\Delta Ct}$) of the mean, with RQ_{min} and RQ_{max} representing the range of possible RQ values based on the standard error of the mean ($2^{(-\Delta\Delta Ct) \pm \text{standard error of the mean}}$) for each sample. The standard error of the mean of -6CT was determined by five independent experiments ($n = 5$). Statistical analysis of -log2(fold change) was conducted by using the Shapiro-Wilk test, followed by ratio-paired t-test analysis.

Protein concentration in the supernatant was determined through three independent experiments ($n = 3$). The statistical analysis was conducted using the Shapiro-Wilk test, followed by ratio-paired t-test analysis. Results were presented as mean ± standard error of the mean.

Cell viability results were obtained from three independent experiments ($n = 3$). Statistical testing was carried out using the Shapiro-Wilk test, followed by the ratio-paired t-test analysis. Results were presented as mean ± standard error of the mean.

For concentrations of CuO/ZnO particles demonstrating a minimum of two viabilities lower than 50% of the control condition, the IC_{50} value (inhibitory concentration 50; concentration causing 50% cell mortality) was determined. The X-values underwent a logarithmic transformation, and non-linear regression analysis was conducted using a sigmoidal dose-response model with variable slope, setting bottom and top constraints at 0 and 100, respectively. The values were expressed as mean ± 95% confidence intervals (CI).

3. Results and Discussion

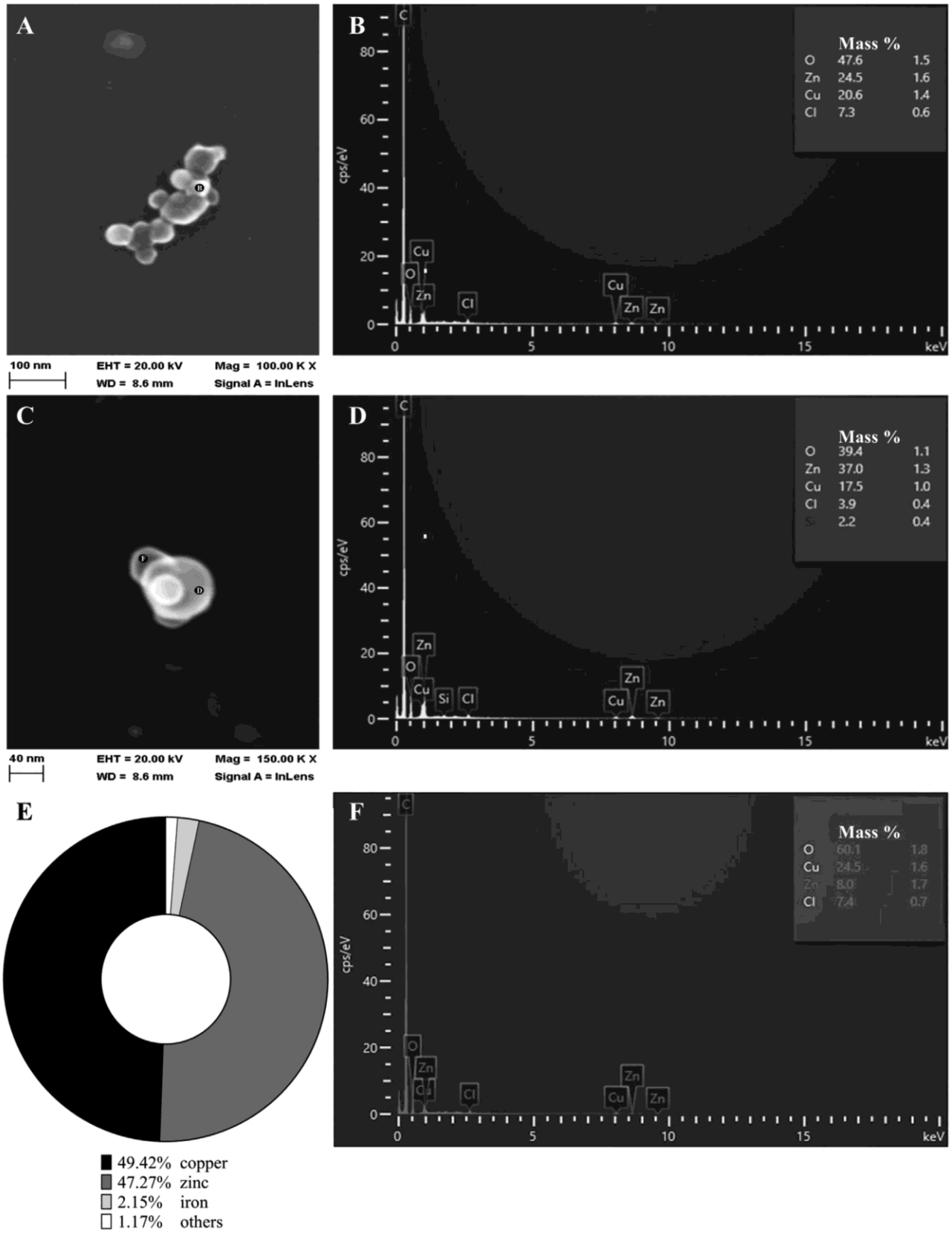
3.1. Physicochemical Analysis of the Metal Fume Particles

The particle size distribution of the occupationally relevant metal fume was previously reported to be in the range between 40 and 250 nm, with the modal size at 124 nm and 33% of the particles being smaller than 100 nm, representing ultrafine metal particles.[40] The metal fume particle suspension was analyzed using ICP-MS, EDX, and SEM (**Figure 1**). ICP-MS analysis showed that the metal fume particles consisted of 49.42% Cu, 47.27% Zn, 2.15% Fe, and 1.17% residual components (Figure 1E). EDX analysis confirmed CuO and ZnO as the primary components of the single particles and particle clusters (Figure 1B,D,F), and we therefore refer to these welding fume particles as CuO/ZnO particles. In addition, SEM examination of the welding fume particles in suspension identified spherical particle shapes (Figure 1A,C). This shape is characteristic of metals undergoing first evaporation at high temperatures, followed by condensation, during metal joining processes.[63,64]

The generation of metal fume particles during metal-joining processes results in significant compositional variability due to factors such as filler material, base material, flux components, shielding gas, and process parameters.[11] This variability explains the observed differences between the analyses of the entire sample (ICP-MS) (Figure 1E) and the individual particle

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sites (EDX) (Figure 1B,D,F). ICP-MS analysis of the metal fume particles represents the average particle composition of the entire sample, which revealed similar amounts of Cu and Zn (Figure 1E). In contrast, EDX analyses (Figure 1B,D,F) taken from concrete measurement sites (the locations are depicted in Figure 1A,C) showed varying Cu-to-Zn ratios and thus a heterogeneity of the individual metal fume particles. As stated, these disparities stem from the inherently stochastic nature of particle formation during metal-joining processes. The presented findings underscore the importance of a dual analysis approach using ICP-MS and EDX for fully characterizing metal fume particles.[65,66]

3.2. Cytotoxicity Testing of CuO/ZnO Particles and/or PAMPs Exposure on THP-1 Macrophages

To determine the toxicity of these CuO/ZnO particles on human THP-1 macrophages, different concentrations of the particles were tested by the MTT assay using three independent experiments ($n = 3$). The MTT assay showed a dose-dependent decrease in cell viability after a 24 h exposure to increasing concentrations of CuO/ZnO particles. Specifically, concentrations of 4, 8, and 16 μg mL^{-1} resulted in significant decreases in the cell viability, down to 70.01 ± 1.6%, 38.64 ± 0.3%, and 21.58 ± 0.8%, respectively, compared to the nonexposed control at 24 h (**Figure 2**A). The concentration of 2 μg mL^{-1} did not result in significant cell death (97.52 ± 3% viability). The IC_{50} value, as the concentration at which 50% of cells exhibited toxicological effects, was determined to be 6.728 μg mL^{-1} (Figure 2C).

A cellular pathogen-host infection model was simulated by inducing pro-inflammatory responses in the THP-1-derived macrophages using the PAMPs LPS, LTA, and PGN. When THP-1 macrophages were treated with PAMPs (LPS, LTA, or PGN) alone, the cell viabilities remained relatively high, ranging from 91.95 ± 0.9% to 94.43 ± 1.0% (Figure 2B). However, co-exposure to 2 μg mL^{-1} CuO/ZnO particles alongside PAMPs for 24 h resulted in decreased cell viabilities: 87.36 ± 1.0% for LPS-activated cells, 86.15 ± 0.8.8% for LTA-activated cells, and 86.7 ± 1.8% for PGN-activated cells.

3.3. Inflammatory Effects of PAMPs on THP-1 Macrophages in the Absence or Presence of CuO/ZnO Particles

THP-1 macrophages exposed in vitro to the bacterial components LPS (2 or 20 ng mL^{-1}), LTA (100 or 1000 ng mL^{-1}), or PGN (1000 or 3162 ng mL^{-1}) showed a dose-dependent increase of *TNF* and *IL-6* mRNA expression at 4 h in comparison to the corresponding unexposed control at 4 h (**Figures 3–5**, panels A and C; $n = 5$). This effect was more pronounced for LPS and LTA than for PGN but for all 3 PAMPs distinct. Compared to cells exposed to PAMPs alone, co-exposure to 2 μg mL^{-1} CuO/ZnO particles alongside PAMPs resulted in significant inhibition of the expression of *TNF* mRNA and *IL-6* mRNA levels (Figures 3–5, A and C; $n = 5$). In order to confirm a biological significance, we next investigated whether corresponding effects could also be detected for secreted TNF and IL-6 protein levels (in the in vitro macrophage culture supernatants) after 24 h of exposure. Indeed, a dose-dependent increase of TNF and IL-6 protein levels following exposure to bacterial compounds (LPS, LTA, or PGN) was inhibited by 2 μg mL^{-1} CuO/ZnO particles (Figures 3–5 and B and D; $n = 5$). This inhibition of the innate immune response was more pronounced for TNF protein than for IL-6, but in both cases distinct.

Furthermore, exposure to CuO/ZnO particles, without the presence of any PAMPs, significantly inhibited macrophage *IL-6* mRNA and *TNF* mRNA expression at 4 h, and macrophage TNF protein secretion at 24 h, while IL-6 protein secretion at 24 h was not affected (Figures 3–5, A, B, C, and D; $n = 5$).

3.4. Metal Fume Exposure and Its Impact on Antibacterial Macrophage Function and Lung Immunity

While human *short*-term exposures to ZnO fumes have been found to increase levels of IL-6, TNF, and IL-8 in bronchoalveolar lavage (BAL) fluid,[35,36,38,39,67,68] analysis of blood and induced sputum from long-term welders showed no difference in inflammatory markers from unexposed controls.[69] Thus, long-term inhalation and corresponding higher doses of metal fumes may dampen the normal pro-inflammatory response to inhaled particulate.[69] This observation is in line with the fact that the incidence, severity, and death rates of bacterial pneumonia are higher among long-term metal workers than in the general population.[14–21] For these reasons, pneumococcal vaccines are recommended for long-term metal workers in England and Germany.[17,19,21 27–29,31,32] Thus far, the concrete molecular mechanisms for the dampening of lung immune responses by metal fumes have not been fully elucidated.

Lanone et al. showed that Cu- and Zn-based nanoparticles were, among 24 metal nanoparticles, the most toxic for human macrophages.[50] In the current study, we found a significant inhibition of innate macrophage responses to gram-positive and gram-negative bacterial PAMPs in the presence of occupationally relevant CuO/ZnO particles. The inhibition could be consistently shown for macrophage mRNA expression and protein secretion of two cytokines, TNF and IL-6. Both cytokines are highly important for the immune defense against bacterial lung infections. TNF, crucial for leukocyte recruitment and activation for pathogen clearance,[70] has been shown to play an essential role in the protection against gram-positive *Streptococcus pneumoniae* infections in murine models. In mice intranasally infected with *Streptococcus pneumoniae*, TNF knockout (KO) (or pre-treatment with a neutralizing anti-TNF monoclonal antibody) led to higher bacterial load in the lungs, and earlier death from pneumococcal pneumonia in comparison to control wild-type mice (or to mice

Figure 1. Physicochemical analysis of the metal fume particles. A,C) SEM micrographs of metal fume particles in aqueous solution. Metal fume particles were suspended in an aqueous solution for SEM analysis. The particles are shown at A) 100 000× or C) 150 000× magnification. The images reveal spherical particle shapes. A,C) Contain three black circles with B,D, or F) white letters to denote measurement sites of the respective EDX data. B,D,F) EDX spectra of metal fume particles. EDX mapping analysis illustrates the spatial distribution of elements within a particle cluster, confirming the presence of oxygen, Zn, and Cu as significant components. E) ICP-MS analysis of metal fume particles. The metal fume predominantly consists of 49.42% Cu and 47.27% Zn, with minor contributions from iron (2.15%) and residual components (1.17%), indicating a complex chemical composition.

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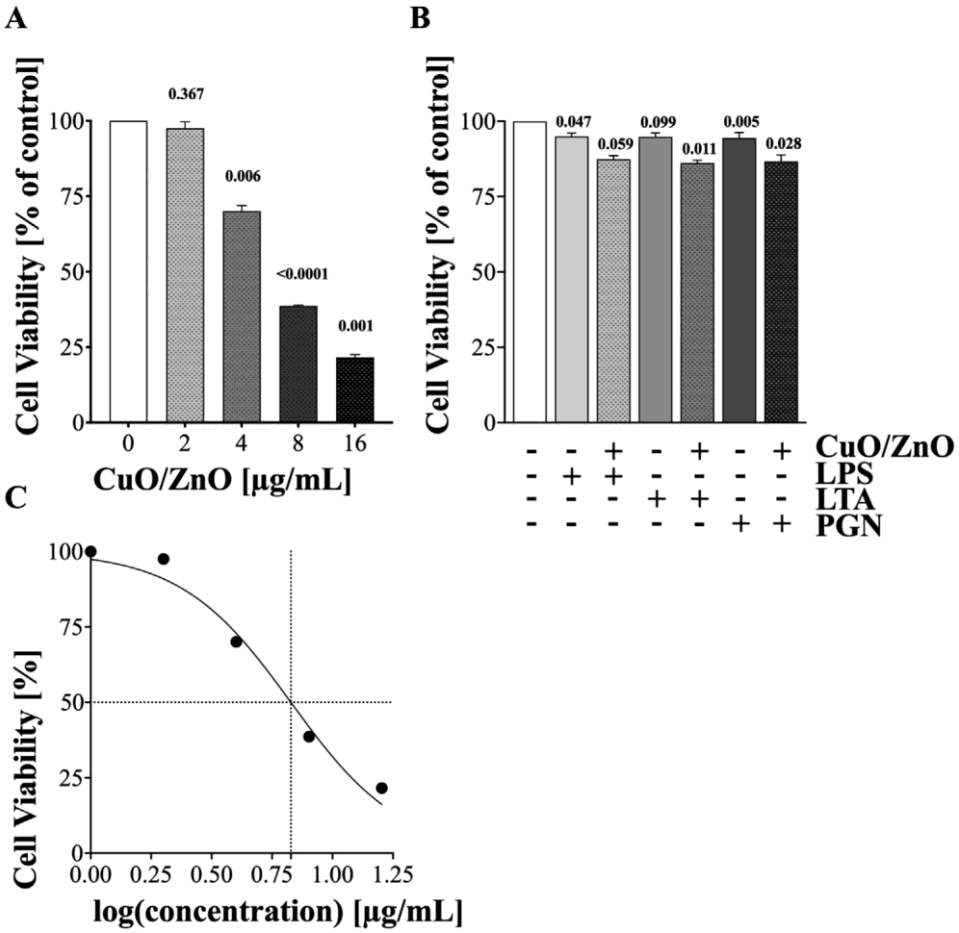


Figure 2. Evaluation of the cytotoxicity of CuO/ZnO particles and/or PAMPs on THP-1 macrophages. A) Dose-dependent cytotoxicity of CuO/ZnO particles. THP-1 macrophages were exposed to increasing concentrations of CuO/ZnO particles (2, 4, 8, and 16 µg mL^{-1}) for 24 h. The viability, determined by the MTT assay, decreased dose-dependently (97.52 ± 3%, 70.01 ± 1.6%, 38.64 ± 0.3%, and 21.58 ± 0.8%, respectively). B) Immunotoxic effects of CuO/ZnO particles on PAMP-exposed THP-1 macrophages. At exposure with PAMPs (LPS at 2 ng mL^{-1}, LTA at 100 ng mL^{-1}, or PGN at 1000 ng mL^{-1}) alone, high cell viabilities were detected at 24 h (ranging from 91.95 ± 0.9% to 94.43 ± 1.0%), and co-exposure with 2 µg mL^{-1} CuO/ZnO particles resulted in decreased viabilities (87.36 ± 1.0% for LPS-, 86.15 ± 0.8. 8% for LTA-, and 86.7 ± 1.8% for PGN-activated cells). C) The IC_{50} concentration for the macrophage cytotoxicity of CuO/ZnO particle exposure. This value was determined to be 6.728 µg mL^{-1} at 24 h after exposure using the MTT cell proliferation test.

without anti-TNF pre-treatment, respectively).[71,72] In addition, during pneumonia in mice caused by either gram-negative *Escherichia coli* or *Pseudomonas aeruginosa*, TNF deficiency significantly increased bacterial burdens in the lungs 100 000- to 10 000 000-fold and consequently increased mortality.[73] IL-6 activates the acute phase response and the specific immune system, e.g., Th17 cells, which are important for clearance of *Streptococcus pneumoniae* in the airways.[74] After *Streptococcus pneumoniae* infection, IL-$6^{-/-}$ mice showed an attenuated acute-phase protein response, higher bacterial loads in the lungs, and earlier death due to pneumococcal pneumonia compared to IL-$6^{+/+}$ mice.[75] Both murine cytokines, TNF and IL-6, were also reported to contribute to the clearance of *Staphylococcus aureus*.[76,77] Consistent with these mice model results, anti-TNF biologics and anti-IL-6 biologics, therapeutically applied in humans to treat inflammatory and/or autoimmune diseases, are both independently reported to contribute to an increased risk of serious infections, including pneumonia, sepsis, and/or reactivation of tuberculosis.[78–81] The current study shows that CuO/ZnO particles inhibit the two cytokines TNF and IL-6, both of which are essential for anti-bacterial lung defense. This provides a potential mechanistic explanation on a molecular level for the dampening of the innate lung immune system, which may occur in long-term metal workers. The suppression of antibacterial lung immunity by another metal, chromium, has already been reported. Exposure of rats to stainless steel welding fumes led to significant lung injury, delayed bacterial clearance, and altered immune responses. In a follow-up study, soluble chromium in stainless steel welding fumes was identified as the primary component responsible for the described suppression of lung defense responses.[82,83] Future studies could further investigate whether exposure to Cu, Zn, and/or other metals (single or in combination) is immunosuppressive and whether metal exposure affects, for example, the expression of the pattern recognition receptors TLR4 (for LPS) or TLR2 (for LTA) on the surface of human macrophages.

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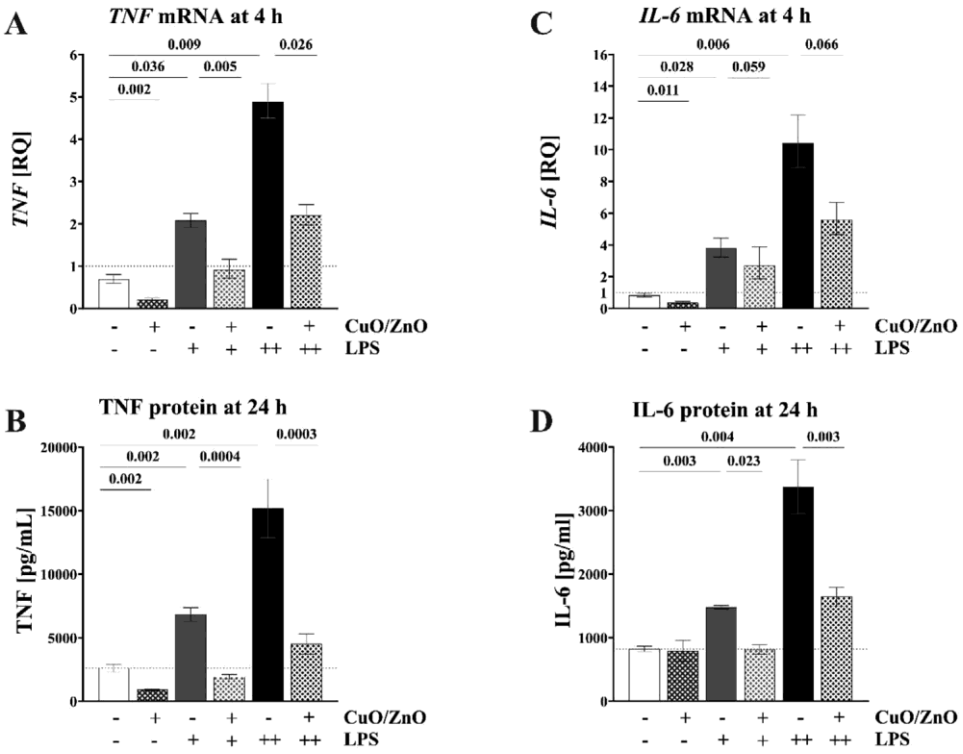


Figure 3. Effects of CuO/ZnO particles on THP-1 macrophages. A) *TNF* mRNA expression after 4 h exposure to 2 μg mL^{-1} CuO/ZnO particles, measured using RT-qPCR. B) TNF protein levels after 24 h of exposure to 2 μg mL^{-1} CuO/ZnO particles, measured using ECLIA. C) *IL-6* mRNA expression after 4 h of exposure to 2 μg mL^{-1} CuO/ZnO particles, measured using RT-qPCR. D) IL-6 protein levels after 24 h of exposure to 2 μg mL^{-1} CuO/ZnO particles, measured using ECLIA. LPS concentrations used: + for 2 ng mL^{-1} and ++ for 20 ng mL^{-1}.

Zn and Cu, as essential trace elements, are crucial for optimal innate immune functions. Nutritional deficiency of either metal compromises neutrophils and macrophages and leads to increased susceptibility to bacterial infection.[84–89] Macrophage functions can also be inhibited by excess supplementation of Cu[90] or Zn.[84] In contrast, moderate doses of Cu or Zn ions can be utilized by phagocytes as "contact killing" agents against intracellular bacteria, or they limit Zn availability to pathogens by sequestering it away from them, concepts known as "nutritional immunity."[85,91–95]

On the other hand, inhaled Cu and Zn may act as nutrients and enzyme cofactors for lung bacteria, thus promoting their growth.[85,94,96,97] In previous reports, it has been suggested that inhalation of another metal, iron, may lead to the release of free iron in the lung, which may act as a nutrient for pneumococci in the lung.[98] This context was discussed as a reason that occupations that entail exposure to iron fumes or dust have been reported to carry an increased risk of infection.[98] Moreover, a mild steel welding fume, which contained 12% iron and 3.8% manganese, has been shown, in vitro and in a mouse model, to increase the host-expressed platelet-activating factor receptor (PAFR) protein expression, and thereby to increase PAFR-dependent pneumococcal adhesion and infection of lower airway cells.[99]

In the current in vitro cell culture study, we used 2 μg mL^{-1} CuO/ZnO particles. To establish a relationship between short-term and/or long-term lung exposure doses in occupational exposure scenarios, and appropriate in vitro exposure doses is not trivial. The deposition and clearance rates of inhaled nanoparticles in the respiratory system with its bifurcate tree structure are very complex to quantify, and can also vary depending on the location.[100,55] Furthermore, there are naturally many limitations of in vivo experiments in volunteers and patients for ethical reasons,[100] which necessitates indirect research approaches for realistic dose estimations. According to the computational multiple-path particle dosimetry (MPPD) model (www.ara.com/mppd), the pulmonary deposition of metal particles after a short-term (6 h) exposure of healthy individuals to occupationally relevant Zn- and Cu-containing welding fume at a controlled average fume concentration of 2.5 mg m^{-3} was approximately 1 mg.[49] Recently, in a review dealing with modeled lung deposition and retention of welding fume particles in occupational scenarios in comparison to in vitro doses, a year of occupational exposure to an occupational exposure limit (OEL) of 5 mg m^{-3} was suggested to result in alveolar retention exceeding the cell dose following the highest concentration (100 μg mL^{-1}) tested in vitro.[55] Furthermore, real-life occupational lung doses over both short and long-term exposure were found comparable to cell doses exerting toxic effects on human bronchial epithelial cells in vitro.[55] On the other hand, we selected with 2 μg mL^{-1} a dose with no significant toxic effects on macrophages in vitro, considering that no significant death of lung immune cells is known among welders. The non-toxicity of 2 μg mL^{-1} Zn- and Cu-containing particles is confirmed by investigations

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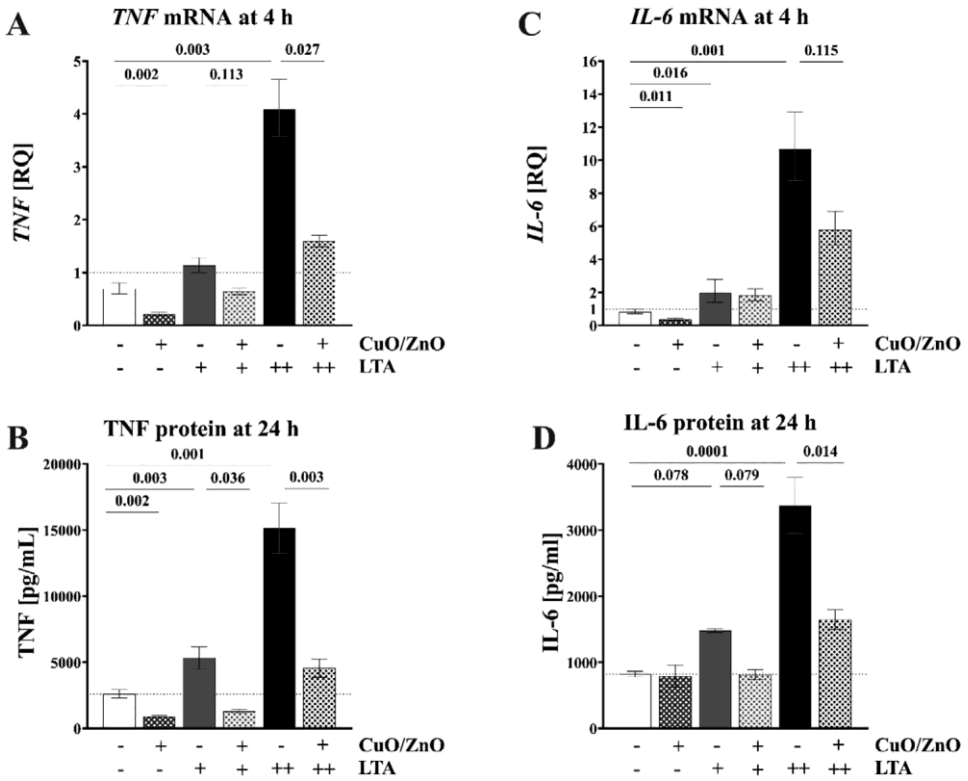


Figure 4. Effects of CuO/ZnO particles on THP-1 macrophages. A) *TNF* mRNA expression after 4 h of exposure to 2 μg mL^{-1} CuO/ZnO particles, measured using RT-qPCR. B) TNF protein levels after 24 h of exposure to 2 μg mL^{-1} CuO/ZnO particles, measured using ECLIA. C) *IL-6* mRNA expression after 4 h of exposure to 2 μg mL^{-1} CuO/ZnO particles, measured using RT-qPCR. D) IL-6 protein levels after 24 h of exposure to 2 μg mL^{-1} CuO/ZnO particles, measured using ECLIA. LTA concentrations used: + for 100 ng mL^{-1} and ++ for 1000 ng mL^{-1}.

using precision-cut lung slices (PCLS) derived from mice, rats, and humans, which demonstrated no toxicity at 0.1 and 1 μg mL^{-1} Zn- and Cu-containing welding fume particle concentrations and concentration-dependent toxicity at 10 and 100 μg mL^{-1}.[101] Based on these assumptions, the dose of 2 μg mL^{-1} Zn/Cu metal particles is a realistic dose, particularly for long-term metal workers and welders. In future studies, dose-response investigations over several time points will have to be considered. The current study paves the way for targeted investigations of the immunotoxicity of occupationally relevant individual metals or combinations thereof.

The use of existing and new chemicals and the rapid development of engineered nanomaterials (or nanoscale materials) require careful toxicity and safety screenings under environmentally relevant exposure conditions.[102–108] As valuable tools for chemical hazard and risk assessments, new approach methodologies (NAMs) have emerged. The use of these in silico, in chemico, in vitro, and ex vivo methodologies aims to replace animal testing whenever possible,[109–111] in accordance with the 3R principle (replacement, reduction, and refinement of animal experiments).[112] NAMs are increasingly recognized and accepted for chemical risk assessment by regulatory agencies worldwide.[113] In addition to chemical risk assessment, NAMs could be used as supplementary data resources for the determination of maximum workplace concentrations in the working environment.[114] Upon inhalation of atmospheric metal fume particles, macrophages are an essential part of the first line of defense in the lung. As macrophages are sensitive to particle-induced toxicity, they represent ideal targets for immunological and toxicological in vitro studies.[115] The current study presents an in vitro model consisting of human THP-1-derived macrophages exposed to bacterial PAMPs such as LPS, LTA, or PGN in the absence or presence of xenobiotics, such as occupationally relevant metal (nano-)particles. Methodically, this pathogen-macrophage infection model enables future investigations of potential immunosuppressive effects of inhaled xenobiotics at workplaces. Our non-animal model may also extend or complement the macrophage cytokine release assay[116] or other existing NAMs.[112,117,118]

4. Conclusions

The results of the current study show that macrophage TNF and IL-6 mRNA and protein responses to the PAMPs LPS, LTA, or PGN are significantly inhibited in the presence of 2 μg/mL welding-fume derived CuO/ZnO particles. Thereby, innate immunity to bacterial pneumonia may be weakened because human macrophages are part of the lung's first-line immune defense,[51–54] and the cytokines TNF and IL-6, which they release (when uninhibited), are essential for defense against bacterial lung pathogens, such as *Streptococcus pneumoniae*.[71–76,78–81,119,120] Since we observed similar results for

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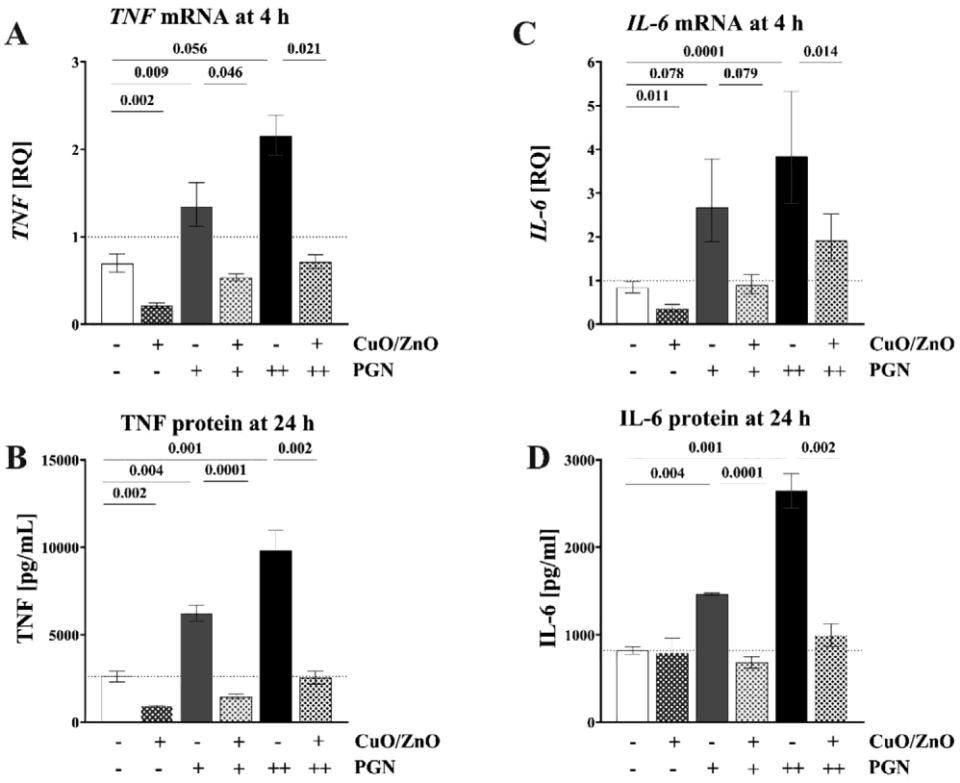


Figure 5. Effects of CuO/ZnO particles on THP-1 macrophages. A) *TNF* mRNA expression after 4 h of exposure to 2 μg mL^{-1} CuO/ZnO particles, measured using RT-qPCR. B) TNF protein levels after 24 h of exposure to 2 μg mL^{-1} CuO/ZnO particles, measured using ECLIA. C) *IL-6* mRNA expression after 4 h of exposure to 2 μg mL^{-1} CuO/ZnO particles, measured using RT-qPCR. D) IL-6 protein levels after 24 h of exposure to 2 μg mL^{-1} CuO/ZnO particles, measured using ECLIA. PGN concentrations used: + for 1000 ng mL^{-1} and ++ for 3162 ng mL^{-1}.

bacterial PAMPs from Gram-positive bacteria (LTA, PGN) and Gram-negative bacteria (LPS, PGN), these results may apply to a broad spectrum of bacteria involved in human pneumonia. The interference of CuO/ZnO particles with the innate immune system's ability to combat lung pathogens provides potential mechanistic explanations for the increased susceptibility to severe or even lethal pneumonia observed in some metal workers.[14–21] The elevated CuO/ZnO doses for the immuno-toxicological effects may mainly occur in the lungs of metal workers after occupational long-term metal fume exposure, which is indeed held responsible for increased severe pneumoniae and even death rates among long-term metal workers.[16,21] Studies by the group of Antonini also showed that chromium in stainless steel welding fumes led to significant lung injury, delayed bacterial clearance, and altered immune responses in mice models.[82,83] Future research will need to explore, possibly by including our in vitro model approach and already existing NAMs, whether the suppression of anti-bacterial lung immunity is also applicable to other metal fume components.

To avoid the dampening of the human lung immune responses to bacterial lung infections, and possibly any unintended nutritional effects[15,18,85,94,96–98] on pathogenic lung bacteria, by CuO/ZnO fumes at working places, our results emphasize the need for careful consideration of preventive occupational health guidelines, including suction devices and respirators at the working place, and compliance to occupational exposure limits for ZnO and CuO. Other research groups even recommend a re-evaluation of occupational exposure limits for ZnO.[45,46] Moreover, our results emphasize that the current vaccine recommendations for long-term metal workers are helpful in protecting them from the adverse health impacts of metal fume exposures.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

CuO/ZnO particles, immunosuppressive effects on macrophages, metal (nano)-particle exposure, welding and metal fumes, workplace safety and new approach methodologies (NAM)

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[1] M. G. Riccelli, M. Goldoni, D. Poli, P. Mozzoni, D. Cavallo, M. Corradi, *Int. J. Environ. Res. Public Health* **2020**, *17*, 2552.
[2] N. Hadrup, V. Zhernovkov, N. R. Jacobsen, C. Voss, M. Strunz, M. Ansari, H. B. Schiller, S. Halappanavar, S. S. Poulsen, B. Kholodenko, T. Stoeger, A. T. Saber, U. Vogel, *Small* **2020**, *16*, e1907476.
[3] E. Ibfelt, J. P. Bonde, J. Hansen, *Occup. Environ. Med.* **2010**, *67*, 772.
[4] T. Wittczak, J. Walusiak, C. Palczynski, *Med. Pr.* **2009**, *60*, 201.
[5] S. De Matteis, *Curr. Opin. Allergy Clin. Immunol.* **2022**, *22*, 73.
[6] D. H. Koh, J. I. Kim, K. H. Kim, S. W. Yoo, G. Korea Welders Cohort, *Occup. Med.* **2015**, *65*, 72.
[7] A. Erdely, R. Salmen-Muniz, A. Liston, T. Hulderman, P. C. Zeidler-Erdely, J. M. Antonini, P. P. Simeonova, *Toxicology* **2011**, *287*, 153.
[8] P. C. Zeidler-Erdely, A. Erdely, J. M. Antonini, *J. Immunotoxicol.* **2012**, *9*, 411.
[9] E. Szucs-Somlyo, J. Lehel, K. Majlinger, F. Toth, A. Jerzsele, C. Kovago, *Sci. Rep.* **2023**, *13*, 22239.
[10] S. J. Sferlazza, W. S. Beckett, *Am. Rev. Respir. Dis.* **1991**, *143*, 1134.
[11] J. M. Antonini, *Crit. Rev. Toxicol.* **2003**, *33*, 61.
[12] T. Storaas, J. P. Zock, A. E. Morano, M. Holm, E. Bjornsson, B. Forsberg, T. Gislason, C. Janson, D. Norback, E. Omenaas, V. Schlunssen, K. Toren, C. Svanes, *Eur. Respir. J.* **2015**, *46*, 1290.
[13] W. S. Cho, R. Duffin, S. E. Howie, C. J. Scotton, W. A. Wallace, W. Macnee, M. Bradley, I. L. Megson, K. Donaldson, *Part. Fibre Toxicol.* **2011**, *8*, 27.
[14] K. T. Palmer, J. Poole, J. G. Ayres, J. Mann, P. S. Burge, D. Coggon, *Am. J. Epidemiol.* **2003**, *157*, 227.
[15] D. Coggon, H. Inskip, P. Winter, B. Pannett, *Lancet* **1994**, *344*, 41.
[16] A. Wong, T. J. Marrie, S. Garg, J. D. Kellner, G. J. Tyrrell, S. Group, *Int. J. Infect. Dis.* **2010**, *14*, e796.
[17] K. T. Palmer, M. Cosgrove, *Occup. Environ. Med.* **2012**, *69*, 932.
[18] D. Coggon, K. T. Palmer, *Thorax* **2016**, *71*, 581.
[19] K. Torén, P. D. Blanc, R. Naidoo, N. Murgia, L. Stockfelt, L. Schiöler, *Int. Arch. Occup. Environ. Health* **2022**, *95*, 1797.
[20] K. T. Palmer, P. Cullinan, S. Rice, T. Brown, D. Coggon, *Thorax* **2009**, *64*, 983.
[21] K. Toren, P. D. Blanc, R. N. Naidoo, N. Murgia, I. Qvarfordt, O. Aspevall, A. Dahlman-Hoglund, L. Schioler, *Occup. Environ. Med.* **2020**, *77*, 57.
[22] M. Riccò, P. Ferraro, S. Zaffina, V. Camisa, F. Marchesi, D. Gori, *Vaccines* **2023**, *11*, 1495.
[23] J. Vinshia, C. Suja, P. K. U. Maheshwari, *Int. J. Curr. Microbiol. Appl. Sci.* **2019**, *8*, 1989.
[24] A. Sinclair, X. Xie, M. Teltscher, N. Dendukuri, *J. Clin. Microbiol.* **2013**, *51*, 2303.
[25] G. Höffken, M. Halank, A. Gillissen, *Med. Klin.* **2004**, *99*, 362.
[26] P. S. Ewig, in *Die Intensivmedizin* (Eds: G. Marx, E. Muhl, K. Zacharowski, S. Zeuzem), Springer, Berlin **2015**, pp 781–802.
[27] DOH, Pneumonia vaccination for employees exposed to welding and metal fume, https://www.hse.gov.uk/pubns/eis44.pdf (accessed: October 2024).
[28] RKI, Vaccination against pneumococcus. https://www.rki.de/SharedDocs/FAQ/Impfen/Pneumokokken/FAQ-Liste_Pneumokokken_Impfen.html (accessed: October 2024).
[29] NHS, Pneumococcal vaccine. https://www.nhs.uk/conditions/vaccinations/pneumococcal-vaccination/ (accessed: October 2024).
[30] STIKO, Empfehlungen der Ständigen Impfkommission (STIKO) am RKI Stand: Ständigen Impfkommission (STIKO), (accessed: October 2024).
[31] K. Torén, R. N. Naidoo, P. D. Blanc, *Am. J. Ind. Med.* **2022**, *65*, 517.
[32] RKI, Wissenschaftliche Begründung für die Aktualisierung der Empfehlungen zur Indikationsimpfung gegen Pneumokokken für Risikogruppen. https://edoc.rki.de/bitstream/handle/176904/2418/29jz7roBl62Xc.pdf?sequence=1&isAllowed=y (accessed: October: 2024).
[33] B. Clarke, K. Ghandi, *Small* **2023**, *19*, 2302864.
[34] U. Reisgen, C. Geffers, K. Willms, M. Angerhausen, K. Deckert, S. Hof, F. Prenger, J. Wisniewski, in *Brazing, High Temperature Brazing and Diffusion Bonding*, DVS-Berichte, Düsseldorf **2013**, pp. 272–277, https://publications.rwth-aachen.de/record/209050?ln=en.
[35] J. M. Fine, T. Gordon, L. C. Chen, P. Kinney, G. Falcone, W. S. Beckett, *J. Occup. Environ. Med.* **1997**, *39*, 722.
[36] W. G. Kuschner, A. D'Alessandro, H. Wong, P. D. Blanc, *Environ. Res.* **1997**, *75*, 7.
[37] J. Borak, H. Cohen, T. A. Hethmon, *AIHAJ* **2000**, *61*, 832.
[38] C. Vogelmeier, G. Konig, K. Bencze, G. Fruhmann, *Chest* **1987**, *92*, 946.
[39] W. G. Kuschner, A. D'Alessandro, S. F. Wintermeyer, H. Wong, H. A. Boushey, P. D. Blanc, *J. Invest. Med.* **1995**, *43*, 371.
[40] L. Hartmann, M. Bauer, J. Bertram, M. Gube, K. Lenz, U. Reisgen, T. Schettgen, T. Kraus, P. Brand, *Int. J. Hyg. Environ. Health* **2014**, *217*, 160.
[41] R. Baumann, S. Joraslafsky, A. Markert, I. Rack, S. Davatgarbenam, V. Kossack, B. Gerhards, T. Kraus, P. Brand, M. Gube, *Toxicology* **2016**, *373*, 63.
[42] E. Dewald, M. Gube, R. Baumann, J. Bertram, V. Kossack, K. Lenz, U. Reisgen, T. Kraus, P. Brand, *J. Occup. Environ. Med.* **2015**, *57*, 845.
[43] A. Markert, R. Baumann, B. Gerhards, M. Gube, V. Kossack, T. Kraus, P. Brand, *J. Occup. Environ. Med.* **2016**, *58*, 127.
[44] R. Baumann, M. Gube, A. Markert, S. Davatgarbenam, V. Kossack, B. Gerhards, T. Kraus, P. Brand, *J. Exposure Sci. Environ. Epidemiol.* **2018**, *28*, 84.
[45] C. Monse, O. Hagemeyer, M. Raulf, B. Jettkant, V. van Kampen, B. Kendzia, V. Gering, G. Kappert, T. Weiss, N. Ulrich, E. M. Marek, J. Bunger, T. Bruning, R. Merget, *Part. Fibre Toxicol.* **2018**, *15*, 8.
[46] C. Monsé, R. Merget, J. Bünger, D. Pallapies, T. Brüning, *Arch. Toxicol.* **2023**, *97*, 2813.
[47] J. Y. Kim, J. C. Chen, P. D. Boyce, D. C. Christiani, *Occup. Environ. Med.* **2005**, *62*, 157.
[48] C. G. Ohlson, P. Berg, I. L. Bryngelsson, K. Elihn, Y. Ngo, H. Westberg, B. Sjogren, *Inhalation Toxicol.* **2010**, *22*, 1083.
[49] R. Baumann, P. Brand, A. Chaker, A. Markert, I. Rack, S. Davatgarbenam, S. Joraslafsky, B. Gerhards, T. Kraus, M. Gube, *Nanotoxicology* **2018**, *12*, 1130.
[50] S. Lanone, F. Rogerieux, J. Geys, A. Dupont, E. Maillot-Marechal, J. Boczkowski, G. Lacroix, P. Hoet, *Part. Fibre Toxicol.* **2009**, *6*, 14.
[51] V. Kumar, *Front. Immunol.* **2020**, *11*, 1722.
[52] A. Ardain, M. J. Marakalala, A. Leslie, *Immunology* **2020**, *159*, 245.
[53] I. Gopallawa, R. Dehinwal, V. Bhatia, V. Gujar, N. Chirmule, *Front. Immunol.* **2023**, *14*, 1119564.
[54] D. E. Zazara, I. Belios, J. Lücke, T. Zhang, A. D. Giannou, D. E. Zazara, I. Belios, J. Lücke, T. Zhang, A. D. Giannou, *Semin. Immunopathol.* **2022**, *44*, 827.
[55] S. McCarrick, H. L. Karlsson, U. Carlander, *Arch. Toxicol.* **2022**, *96*, 969.
[56] C. Cilloniz, C. Dominedo, A. Nicolini, A. Torres, *Microorganisms* **2019**, *7*, 49.
[57] J. Dissemond, *J. Dtsch. Dermatol. Ges.* **2009**, *7*, 544.

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[58] N. W. J. Schröder, S. Morath, C. Alexander, L. Hamann, T. Hartung, U. Zähringer, U. B. Göbel, J. R. Weber, R. R. Schumann, *J. Biol. Chem.* **2003**, *278*, 15587.
[59] S. H. Han, J. H. Kim, M. Martin, S. M. Michalek, M. H. Nahm, *Infect. Immun.* **2003**, *71*, 5541.
[60] W. Chanput, J. Mes, R. A. Vreeburg, H. F. Savelkoul, H. J. Wichers, *Food Funct.* **2010**, *1*, 254.
[61] F. J. Yuan, Y. S. Zhang, Y. Wei, C. Zou, L. Chen, L. Huang, Z. Liu, *Asian Pac. J. Cancer Prev.* **2011**, *12*, 3361.
[62] V. F. Lazarev, I. V. Guzhova, B. A. Margulis, *Pharmaceutics* **2020**, *12*, 416.
[63] V. Voitkevich, *Welding Fumes: Formation, Properties and Biological Effects*, Woodhead Publishing Limited, Sawston, UK **1995**.
[64] K. Srinivasan, V. Balasubramanian, *J. Iron Steel Res. Int.* **2011**, *18*, 72.
[65] M. Oprya, S. Kiro, A. Worobiec, B. Horemans, L. Darchuk, V. Novakovic, A. Ennan, R. Van Grieken, *J. Aerosol Sci.* **2012**, *45*, 50.
[66] A. Hariri, A. M. Leman, M. Z. M. Yusof, *Adv. Mater. Res.* **2013**, *701*, 382.
[67] P. D. Blanc, H. A. Boushey, H. Wong, S. F. Wintermeyer, M. S. Bernstein, *Am. Rev. Respir. Dis.* **1993**, *147*, 134.
[68] P. Blanc, H. Wong, M. S. Bernstein, H. A. Boushey, *Ann. Intern. Med.* **1991**, *114*, 930.
[69] K. T. Palmer, R. M. McNeill Love, J. R. Poole, D. Coggon, A. J. Frew, C. H. Linaker, J. K. Shute, *Eur. Respir. J.* **2006**, *27*, 366.
[70] I. Clark, *Cytokine Growth Factor Rev.* **2007**, *18*, 335.
[71] T. v. d. Poll, C. V. Keogh, W. A. Buurman, S. F. Lowry, *Am. J. Respir. Crit. Care Med.* **2012**, *155*, 603.
[72] D.-G. Jeong, J.-H. Seo, S.-H. Heo, Y.-K. Choi, E.-S. Jeong, *Lab. Anim. Res.* **2015**, *31*, 78.
[73] J.-H. Lee, L. D. Sorbo, A. A. Khine, J. d. Azavedo, D. E. Low, D. Bell, S. Uhlig, A. S. Slutsky, H. Zhang, *Am J. Respir. Crit. Care Med.* **2012**, *168*, 1462.
[74] S. N. Harbour, D. F. DiToro, S. J. Witte, C. L. Zindl, M. Gao, T. R. Schoeb, G. W. Jones, S. A. Jones, R. D. Hatton, C. T. Weaver, *Sci. Immunol.* **2020**, *5*, eaaw2262.
[75] T. van der Poll, C. V. Keogh, X. Guirao, W. A. Buurman, M. Kopf, S. F. Lowry, *J. Infect. Dis.* **1997**, *176*, 439.
[76] S. Nishat, L. M. Wuescher, R. G. Worth, *Infect. Immun.* **2018**, *86*, https://doi.org/10.1128/iai.00186-18.
[77] A. Nakane, M. Okamoto, M. Asano, M. Kohanawa, T. Minagawa, *Infect. Immun.* **1995**, *63*, 1165.
[78] N. N. Andersen, T. Jess, *World J. Gastroenterol.* **2014**, *20*, 16014.
[79] L. P. McLean, R. K. Cross, *Expert Rev. Gastroenterol. Hepatol.* **2014**, *8*, 223.
[80] J. Li, Z. Zhang, X. Wu, J. Zhou, D. Meng, P. Zhu, *Front. Pharmacol.* **2021**, *12*, 746396.
[81] T. Koritala, V. Pattan, R. Tirupathi, A. A. Rabaan, A. Al Mutair, S. Alhumaid, R. Adhikari, K. Deepika, N. K. Jain, V. Bansal, A. Tekin, S. Zec, A. Lal, S. A. Khan, J. P. D. Garces, O. M. Abu Saleh, S. R. Surani, R. Kashyap, *Infez. Med.* **2021**, *29*, 495.
[82] J. M. Antonini, M. D. Taylor, L. Millecchia, A. R. Bebout, J. R. Roberts, *Toxicol. Appl. Pharmacol.* **2004**, *200*, 206.
[83] J. M. Antonini, J. R. Roberts, *J. Immunotoxicol.* **2007**, *4*, 117.
[84] S. L. Stafford, N. J. Bokil, M. E. Achard, R. Kapetanovic, M. A. Schembri, A. G. McEwan, M. J. Sweet, *Biosci. Rep.* **2013**, *33*, e00049.
[85] K. Y. Djoko, C. L. Ong, M. J. Walker, A. G. McEwan, *J. Biol. Chem.* **2015**, *290*, 18954.
[86] D. Skrajnowska, B. Bobrowska-Korczak, *Nutrients* **2019**, *11*, 2273.
[87] A. S. Prasad, *Mol. Med.* **2008**, *14*, 353.
[88] L. Rink, H. Haase, *Trends Immunol.* **2007**, *28*, 1.
[89] H. Gao, W. Dai, L. Zhao, J. Min, F. Wang, *J. Immunol. Res.* **2018**, *2018*, 6872621.
[90] F. Cheng, G. Peng, Y. Lu, K. Wang, Q. Ju, Y. Ju, M. Ouyang, *Front. Oncol.* **2022**, *12*, 1019153.
[91] M. Vincent, R. E. Duval, P. Hartemann, M. Engels-Deutsch, *J. Appl. Microbiol.* **2018**, *124*, 1032.
[92] A. J. Monteith, E. P. Skaar, *Trends Endocrinol. Metab.* **2021**, *32*, 916.
[93] H. Haase, L. Rink, *Metallomics* **2014**, *6*, 1175.
[94] C. Healy, N. Munoz-Wolf, J. Strydom, L. Faherty, N. C. Williams, S. Kenny, S. C. Donnelly, S. M. Cloonan, *Respir. Res.* **2021**, *22*, 133.
[95] C. C. Murdoch, E. P. Skaar, *Nat. Rev. Microbiol.* **2022**, *20*, 657.
[96] A. N. Besold, E. M. Culbertson, V. C. Culotta, *J. Biol. Inorg. Chem.* **2016**, *21*, 137.
[97] S. K. Kunutsor, A. Voutilainen, J. A. Laukkanen, *Lung* **2022**, *201*, 79.
[98] K. Palmer, D. Coggon, *Occup. Environ. Med.* **1997**, *54*, 529.
[99] R. Suri, J. Periselneris, S. Lanone, P. C. Zeidler-Erdely, G. Melton, K. T. Palmer, P. Andujar, J. M. Antonini, V. Cohignac, A. Erdely, R. J. Jose, I. Mudway, J. Brown, J. Grigg, *J. Allergy Clin. Immunol.* **2016**, *137*, 527.
[100] H. Qiao, W. Liu, H. Gu, D. Wang, Y. Wang, S. Zhang, *J. Nanomater.* **2015**, *2015*, 394507.
[101] J. Krabbe, A. Esser, S. Kanzler, T. Braunschweig, S. Kintsler, J. Spillner, T. Schroder, S. Kalverkamp, G. Balakirski, B. Gerhards, A. D. Rieg, T. Kraus, P. Brand, C. Martin, *J. Trace Elem. Med. Biol.* **2018**, *49*, 192.
[102] B. Clarke, K. Ghandi, *Small* **2023**, *19*, e2302864.
[103] B. Fadeel, A. A. Keller, *Small* **2024**, *20*, e2310540.
[104] S. Halappanavar, P. Nymark, H. F. Krug, M. J. D. Clift, B. Rothen-Rutishauser, U. Vogel, *Small* **2021**, *17*, e2007628.
[105] M. H. Kathawala, S. Xiong, M. Richards, K. W. Ng, S. George, S. C. Loo, *Small* **2013**, *9*, 1504.
[106] Y. Kohl, M. Muller, M. Fink, M. Mamier, S. Furtauer, R. Drexel, C. Herrmann, S. Dahnhardt-Pfeiffer, R. Hornberger, M. I. Arz, C. Metzger, S. Wagner, S. Sangerlaub, H. Briesen, F. Meier, T. Krebs, *Small* **2023**, *19*, e2207207.
[107] S. Liu, T. Xia, *Small* **2020**, *16*, e2000603.
[108] L. Rahman, A. Williams, K. Gelda, J. Nikota, D. Wu, U. Vogel, S. Halappanavar, *Small* **2020**, *16*, e2000272.
[109] ECHA, in *New Approach Methodologies in Regulatory Science-Proc. of a Scientific Workshop*, European Chemicals Agency, Helsinki, **2016**, https://op.europa.eu/en/publication-detail/-/publication/4c2ad7eb-9ad0-11e6-868c-01aa75ed71a1/language-en.
[110] EPA, US Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, https://www.epa.gov/sites/default/files/2018-06/documents/epa_alt_strat_plan_6-20-18_clean_final.pdf (accessed: October 2024) **2018**.
[111] EPA, *List of Alternative Test Methods and Strategies (or New Approach Methodologies [NAMs])*, EPA, Lenexa **2018**, https://www.epa.gov/sites/default/files/2021-02/documents/nams_list_second_update_2-4-21_final.pdf (accessed: October 2024).
[112] K. Ciura, E. Moschini, M. Ste˛pnik, T. Serchi, A. Gutleb, K. Jarzyn´ska, K. Jagiello, T. Puzyn, *Small* **2024**, *20*, 2305581.
[113] S. T. Parish, M. Aschner, W. Casey, M. Corvaro, M. R. Embry, S. Fitzpatrick, D. Kidd, N. C. Kleinstreuer, B. S. Lima, R. S. Settivari, D. C. Wolf, D. Yamazaki, A. Boobis, *Regul. Toxicol. Pharmacol.* **2020**, *112*, 104592.
[114] DFG, New Approach Methods (NAMs) in the Sciencebased Derivation of Limit Values - Current and Future Role, https://www.dfg.de/resource/blob/289682/9c91e87decafc9dd9d69783490f9e40c/stellungnahme-nams-grenzwerte-en-data.pdf (accessed: June **2024**).
[115] M. Wiemann, A. Vennemann, U. G. Sauer, K. Wiench, L. Ma-Hock, R. Landsiedel, *J. Nanobiotechnol.* **2016**, *14*, 16.
[116] OECD, Detailed Review Paper on In Vitro Test Addressing Immunotoxicity With a Focus on Immunosuppression. https://one.oecd

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org/document/env/cbc/mono%282022%2916/en/pdf (accessed: June 2024).

[117] Z. Wu, P. Shi, H. K. Lim, Y. Ma, M. I. Setyawati, D. Bitounis, P. Demokritou, K. W. Ng, C. Y. Tay, *Small* **2020**, *16*, e2000963.

[118] D. Boraschi, A. Alijagic, M. Auguste, F. Barbero, E. Ferrari, S. Hernadi, C. Mayall, S. Michelini, N. I. Navarro Pacheco, A. Prinelli, E. Swart, B. J. Swartzwelter, N. G. Bastus, L. Canesi, D. Drobne, A. Duschl, M. A. Ewart, J. Horejs-Hoeck, P. Italiani, B. Kemmerling, P. Kille, P. Prochazkova, V. F. Puntes, D. J. Spurgeon, C. Svendsen, C. J. Wilde, A. Pinsino, *Small* **2020**, *16*, e2000598.

[119] A. Banerjee, F. Stevenaert, K. Pande, E. Haghjoo, S. Antonenko, D. M. Gorman, M. Sathe, T. K. McClanahan, R. Pierce, S. P. Turner, M. E. Bigler, J. H. Phillips, P. G. Heyworth, *Infect. Immun.* **2010**, *78*, 1353.

[120] X. Gou, W. Xu, Y. Liu, Y. Peng, W. Xu, Y. Yin, X. Zhang, *Microbiol. Spectrum* **2022**, *10*, e0204921.

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Inflammatory Responses to Zn/Cu-Containing Welding Fume in Human Alveolar Epithelial and Macrophage Cell Lines, with MIP-1β/CCL4 as a Much More Sensitive Macrophage Activation Marker than IL-8 and TNF-α

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Abstract: Zinc (Zn)- and copper (Cu)-containing welding fumes elevate inflammatory markers (CRP, TNF-α, IL-6, IL-8) in healthy individuals and welders. Zn- and Cu-containing nanoparticles are toxic to human macrophages. Therefore, ZnO exposure limits are under discussion. In this study, the effects of Zn/Cu-containing welding fume suspensions on A549 alveolar epithelial cells (exposure concentrations: 0.01/0.1/1/10/100 µg/mL) and THP-1 macrophages (additionally 0.001 µg/mL) were investigated over a period of 48 h. Effects on apoptosis, cytotoxicity, genotoxicity, superoxide dismutase (SOD) activity, and cytokine levels (IL-6, IL-8, MIP-1β/CCL4, TNF-α) were evaluated. Welding fume exposure increased SOD activity, and it increased Annexin-V binding and cytotoxicity effects starting at 10 µg/mL in A549 cells and particularly in THP-1 macrophages. A549 cells showed increased IL-6 at 10 and 100 µg/mL, and significant IL-8 release occurred at 10 µg/mL for A549 and 0.1 µg/mL for macrophages. Exposed macrophages released TNF-α at 1 µg/mL after 24 and 48 h and MIP-1β/CCL4 at 0.01 µg/mL after 6 h and at 0.001 µg/mL after 48 h. No genotoxic effects were detected. MIP-1β/CCL4 is a sensitive new biomarker for human macrophages exposed to Zn/Cu-containing welding fumes. The findings suggest that Zn/Cu particles affect lung cells already at doses below current occupational thresholds.

Keywords: welding and metal fumes; zinc (Zn); copper (Cu); metal (nano)particle exposure; nanotoxicology; oxidative stress; inflammatory response; cytokines (IL-6; IL-8; MIP-1β/CCL4; TNF-α); early-stage marker; occupational health

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1. Introduction

Welding, a versatile method used to join metallic components, is crucial in manufacturing. But it exposes workers to welding fumes containing a variety of gases and single or agglomerated particles with diameters ranging from 10 to 400 nm [1]. These fumes can be inhaled [2], leading to inflammatory responses in the respiratory system and systemic subclinical inflammatory effects [3,4]. Epidemiological studies have shown that welders have an increased risk of both systemic and pulmonary diseases, including cardiovascular diseases, occupational asthma, chronic obstructive pulmonary disease (COPD), chronic

bronchitis, and pneumonia [3–6]. Welders experience more frequent, longer-lasting, and severe respiratory infections [3]. Exposure to fumes containing metals like nickel, chromium, manganese, and iron has been associated with increased soluble cytokines, such as IL-8, in post-shift nasal lavage fluid (NALF) [7]. Significant systemic increases of CRP and especially IL-6 were reported after short-term exposures to welding fumes [8]. Of note, IL-6 is a strong risk marker [9] and a mediator for future cardiovascular diseases [10].

In modern joining technology, especially in the automotive industry, an increasing number of welding fumes contain zinc (Zn) and copper (Cu) [11]. Controlled short-term Zn exposure caused increased levels of TNF-α, IL-6, and IL-8 in bronchoalveolar lavage (BAL) fluid of exposed welders [12] and increased levels of TNF-α and IL-8 in BAL of exposed healthy subjects [13]. Healthy individuals showed increased systemic IL-6 levels after ZnO exposure [14]. Moreover, we have shown that controlled short-term exposure of healthy individuals with a Zn/Cu-containing welding fume caused significantly increased levels of C-reactive protein (CRP) and serum amyloid A (SAA) in nasal mucosal lining fluid [15] that preceded and accompanied increased systemic levels of IL-6 at 10 h, and CRP and SAA at 29 h after exposure compared to unexposed control individuals [15–18]. Repeated controlled exposures to Zn- and Cu-containing welding fumes of healthy individuals for 6 h on 4 consecutive days showed a persistent systemic CRP increase, suggesting that long-term exposure to these metals may lead to prolonged subclinical inflammation [19].

Pollutants and metal fumes reach the exposed subject primarily via the airways. Alveolar macrophages are key players in lung inflammation and are involved in the defense against pathogenic microbes and inhaled pollutants. In a previous study, we have shown that the toxicological effects of different ambient (urban traffic or rural) PM10 samples containing different trace metals caused distinct dose-dependent toxicological responses in human alveolar epithelial cells (A549) and murine macrophages (RAW264.7) [20]. Furthermore, we have shown that exposure of THP-1 macrophages in vitro to Zn/Cu-containing particle suspensions leads to an increased expression of lncRNAs [21] and a reduced response to bacterial products [22]. In another in vitro study, Cu- and Zn-based nanoparticles were identified to be the most toxic among 24 tested different metal-based nanoparticles for A549 cells and THP-1 macrophages, based on the cytotoxicity MTT and Neutral Red assays [23]. Moreover, exposure of macrophages to ZnO nanoparticles decreased lysosomal stability and increased cell death [24]. In addition, Zn/Cu-particles have been found to negatively impact lung function in isolated perfused mouse lungs (IPLs) [25].

Several studies have investigated oxidative stress effects of welding fume and metal oxide (nano)particles, using the antioxidant enzyme superoxide dismutase (SOD) as an indicator. A study performed in Turkey on welders revealed a significant decrease in SOD activity compared to controls [26]. The effects of ZnO nanoparticles on male Wistar rats demonstrated a significant reduction in SOD activity and gene expression in the liver [27]. Additionally, macrophages exposed in vitro to CuO nanoparticles underwent cell death through the misfolding of Cu/Zn superoxide dismutase 1 (SOD1) [28].

To further investigate the cellular responses of A549 alveolar epithelial cells and PMA-differentiated THP-1 macrophages in vitro to a Zn/Cu-containing welding fume suspension in this study, we examined the concentration- and time-dependent effects on cell toxicity via viability, cell death through apoptosis, cellular stress as indicated by SOD activity levels, and immunoregulatory effects through selected secreted cytokines. The cytokines IL-6, TNF-α, and the chemokine IL-8 are central proinflammatory mediators and are also involved in metal fume fever [12]. IL-6 is a main mediator for the orchestration of the acute-phase reaction [29]. Levels of IL-6, TNF-α, and IL-8 were elevated in the bronchoalveolar lavage (BAL) of individuals exposed to Zn oxide particles [13]. Moreover, IL-6 was identified as a sensitive and early systemic biomarker for exposure to metal

fumes containing Zn and Cu [17]. Therefore, we focused on IL-6, TNF, and IL-8 secretion of lung epithelial cells, and macrophages exposed to Zn/Cu-containing metal particles. These key inflammatory cytokines were also shown to be regulated in lung epithelial cells and macrophages exposed to a particular matter [20]. The chemokine MIP-1β/CCL4, secreted by macrophages [30], is involved in macrophage activation [31] and chronic inflammation [32]. Therefore, MIP-1β/CCL4 was selected to investigate the cell response to further particle exposure. The presented in vitro study was designed to achieve a comprehensive understanding of the mechanisms underlying the (immuno-)toxicological and inflammatory effects of welding fumes across a wide concentration range in epithelial cells (A549) and monocyte-derived macrophages (THP-1). Moreover, this approach may help to identify potential biomarkers for risk assessment in the context of occupational health surveillance.

2. Results

2.1. Cytotoxicity

To evaluate the cytotoxic impact of Zn/Cu-containing welding fume exposure on human alveolar lung epithelial A549 cells and THP-1 macrophages, the uptake of trypan blue was determined. The viabilities of both exposed cell lines are presented at different concentrations at various time points (6 h, 24 h, and 48 h) (Figure 1).

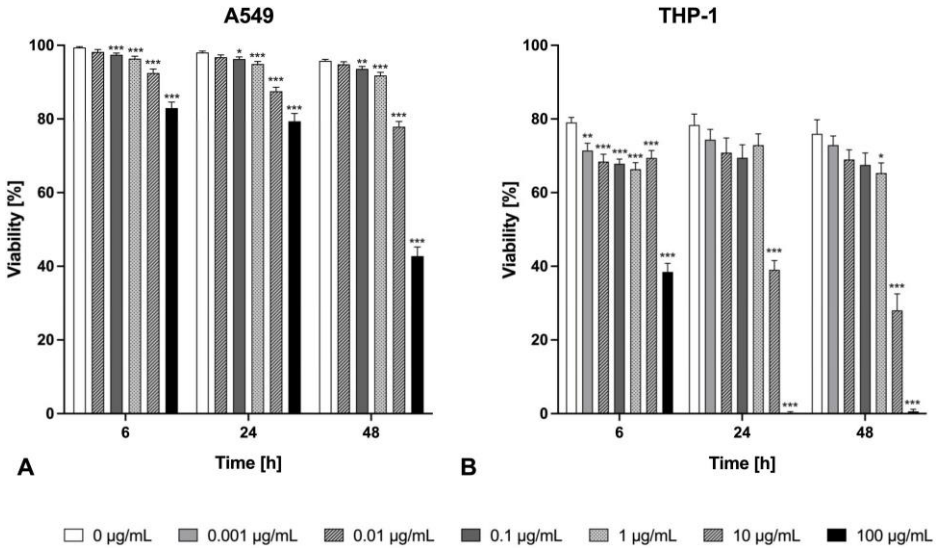


Figure 1. Assessment of cell viability by trypan blue exclusion test of A549 epithelial cells (**A**) and THP-1 macrophages (**B**) after exposure to Zn/Cu-containing welding fume particles. Viability of different particle concentrations (0–100 µg/mL) over the exposure time (6 h, 24 h, and 48 h). Each measuring point shows the mean ± SEM for $n = 15$ (A549) or $n = 12$ (THP-1). (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$.

The welding fume exposure induced a time- and concentration-dependent cytotoxic effect on human alveolar epithelial cells and macrophages. In direct comparison, the macrophages reacted more sensitively (Figure 1B) than the lung epithelial cells (Figure 1A). The negative controls (0 µg/mL) showed that THP-1 (80.13 ± 1.83%) is less viable at the beginning of exposure than A549 (98.86 ± 0.42%) as a result of the previous macrophage differentiation. Generally, both cell lines already showed a significant decrease in viability at lower particle concentrations (0.001 µg/mL THP-1; 0.1 µg/mL A549). Exposure to 10–100 µg/mL welding fume particle concentrations lead to a viability reduction of 20–56% for A549 cells over the incubation time in comparison to the negative control. Under the same conditions, THP-1 cells showed a viability reduction of 50 to 80% (or higher).

2.2. Apoptosis

During apoptosis, phosphatidylserine (PS) translocates to the surface of apoptotic cells, where it can be bound by annexin V. The apoptotic response of cells to welding fume particles was studied using annexin V staining in live cell imaging, measured by normalized orange fluorescence (Figure 2). Fluorescent signals were quantified at different exposure concentrations for 2 h and 4 h exposure times for A549 epithelial cells and THP-1 macrophages. In A549 cells (Figure 2A), the applied exposure doses from 0.01 to 100 µg/mL did not induce any significant increases in annexin V. The highest increase was observed at the exposure dose of 1 µg/mL for both time points; at 2 h, there was a 1.87-fold increase, and at 4 h, the increase was slightly higher. At the exposure dose of 100 µg/mL, there was also a moderate increase over time; at 4 h, there was a 1.55-fold increase approaching statistical significance ($p = 0.055$). In THP-1 macrophages (Figure 2B), the applied exposure doses from 0.001 to 100 µg/mL caused a dose- and time-dependent increase of the annexin V staining. At lower concentrations (0.001–0.1 µg/mL), fluorescence remained near baseline levels (approximately 15–27% of control) across both time points. However, substantial increases were observed at the highest concentration. The 100 µg/mL treatment induced statistically significant elevations of approximately 3.28-fold at 2 h and 4.29-fold at 4 h compared to the control. The time profile showed that the fluorescence intensity in THP-1 macrophages generally increased with extended exposure, with the 4 h values exceeding the corresponding 2 h measurements for equivalent concentrations.

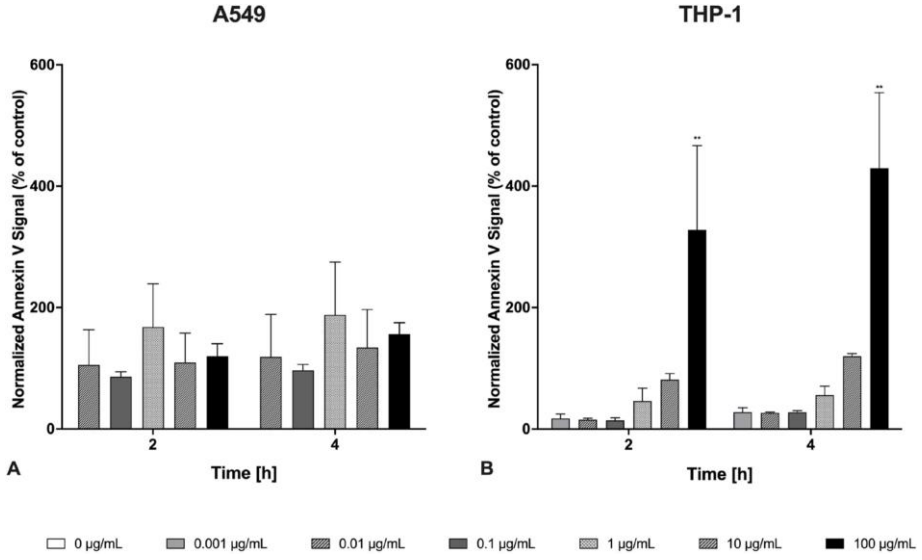


Figure 2. Annexin V staining of A549 epithelial cells (**A**) and THP-1 macrophages (**B**) during exposure to Zn/Cu-containing welding fume particles, evaluated by the Incucyte® live-cell analysis system. The graph shows the annexin V fluorescence signal area (orange) to phase confluence area ratio. Baseline correction was performed by subtracting the non-exposed control values from the exposed cell data at 2 h and 4 h. Data are presented relative to the non-exposed control after 0 h of exposure to varying concentrations of Zn/Cu-containing welding fume particles. Each bar represents the mean ± SEM for $n = 3$. Statistically significant differences between exposed and unexposed cells are indicated with (**) $p < 0.01$.

2.3. Inflammatory Effects

The release of the inflammatory cytokines TNF-α and IL-6, as well as the chemokines MIP-1β/CCL4 and IL-8, were determined in the cell supernatant after 0 h, 6 h, 24 h, and 48 h of welding fume exposure (Figures 3–5). With respect to physiological function and available data about the secretion spectrum of both cell lines, TNF-α and MIP-1β/CCL4 were only investigated for THP-1 macrophages. In contrast, the secretions of IL-6 and IL-8

were quantified for both cell lines. In general, the inflammatory marker secretion showed a clear cell line-, concentration-, and time-dependent cytokine release. As a positive control for the pro-inflammatory response, we used LPS at a relatively high dose of 1000 ng/mL, which resulted in a significant and time-dependent increase in IL-6 and IL-8 secretion in both A549 epithelial cells and THP-1 macrophages, as well as a significant and time-dependent increase in MIP-1β/CCL4 and TNF-α in THP-1 macrophages (Figure A1).

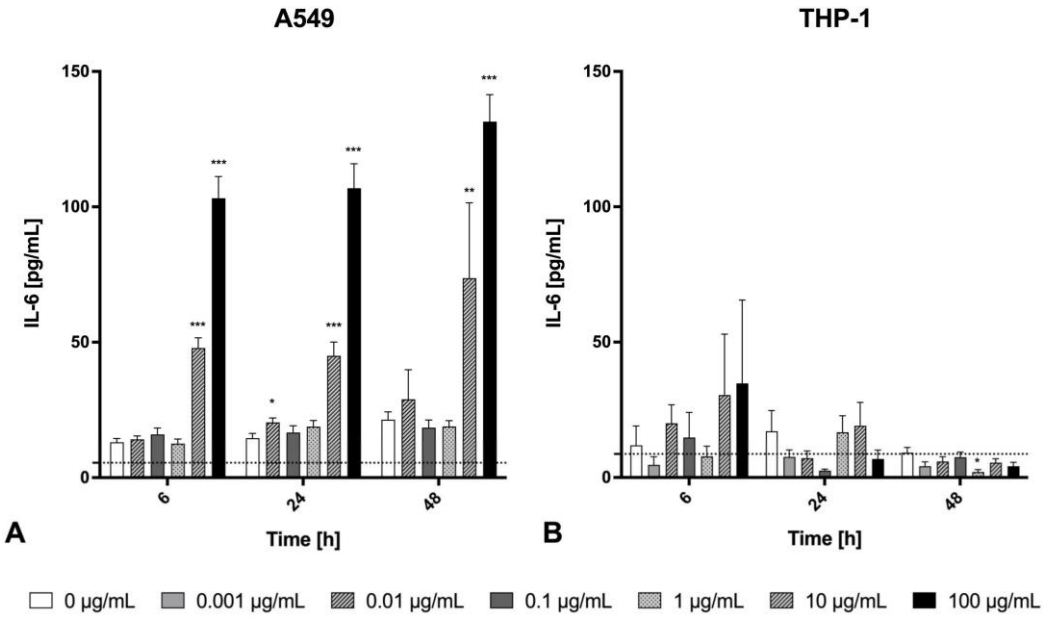


Figure 3. IL-6 release from A549 cells (**A**) or THP-1 macrophages (**B**) after exposure to Zn/Cu-containing welding fume particles at different concentrations (0–100 µg/mL) over time (6 h, 24 h, and 48 h). Each bar shows the mean ± SEM for $n = 15$ (A549) or $n = 12$ (THP-1). Dashed lines show the level of the negative control. Dashed lines show the level of negative control. Statistically significant differences between exposed and unexposed cells (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$.

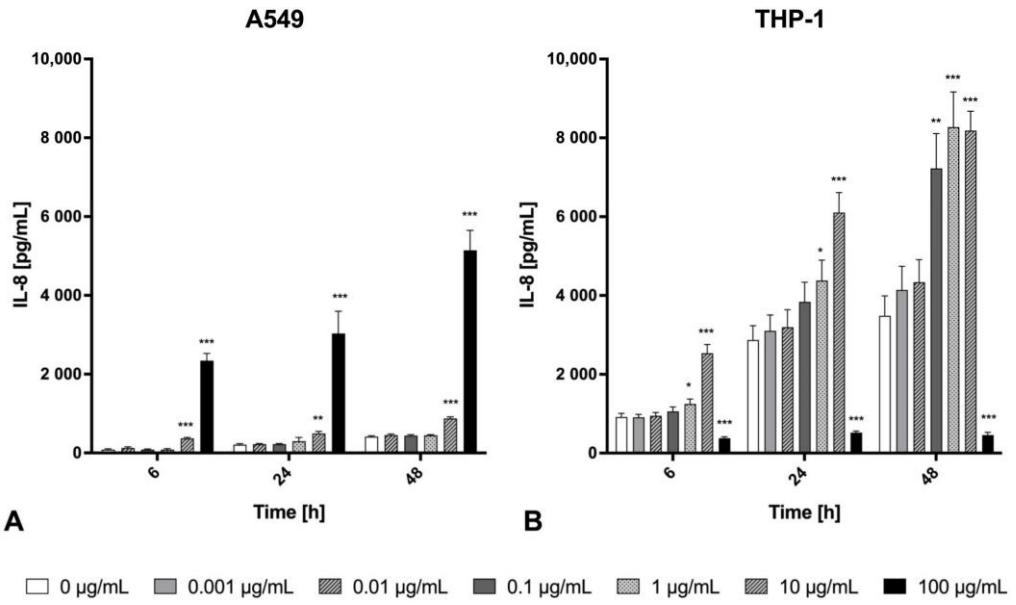


Figure 4. IL-8 release from A549 cells (**A**) or THP-1 macrophages (**B**) after exposure to Zn/Cu-containing welding fume particles at different concentrations (0–100 µg/mL) over time (6 h, 24 h, and 48 h). Each bar shows the mean ± SEM for $n = 15$ (A549) or $n = 12$ (THP-1). Statistically significant differences between exposed and unexposed cells (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$.

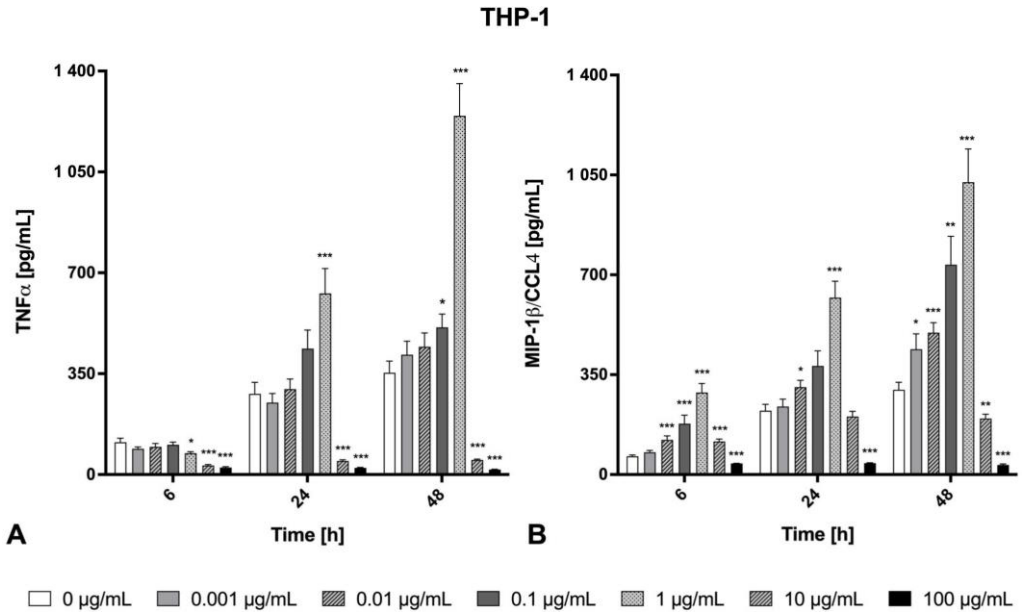


Figure 5. TNF-α (**A**) and MIP-1β/CCL4 (**B**) release from THP-1 macrophages after exposure to Zn/Cu-containing welding fume particles at different concentrations (0–100 µg/mL) over time (6 h, 24 h, and 48 h). Each bar shows the mean ± SEM for n = 15 (A549) or n = 12 (THP-1). Statistically significant differences between exposed and unexposed cells (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$.

The production and release of IL-6 is presented in Figure 3. The exposed alveolar epithelial cells (A549) showed a clear concentration- and time-dependent increase in IL-6 secretion compared to the control (Figure 3A). The maximal IL-6 releases in A549 cells were already detected after 6 h at a high particle concentration of 10 µg/mL. In comparison, no significant changes in secreted IL-6 levels were found for exposed THP-1 macrophages compared to the control (Figure 3B). Possible interferences of cytotoxicity could be determined for THP-1 macrophages (but not A549 cells) after treatment with concentrations > 10 µg/mL at 24 h and 48 h, which is made evident by the stagnated or decreased trend in IL-6 concentrations (Figure 3B).

Lung epithelial cells and macrophages showed increased IL-8 secretion in a time- and concentration-dependent manner (Figure 4). The levels of IL-8 secreted by macrophages increased approximately 30-fold after 48 h of exposure to 1 µg/mL particles compared to unexposed control at the same time point, reaching 8300 pg/mL. At the highest particle concentration (100 µg/mL), the IL-8 secretion showed a significant cytotoxicity-based decrease in THP-1 cells. In comparison, the alveolar epithelial A549 cells demonstrated an approximately 1.6–20-fold increase in IL-8 compared to unexposed control at the same time point, with a maximum of 5140 pg/mL (at 100 µg/mL after 48 h). THP-1 macrophages showed increased TNF-α as well as MIP-1β/CCL4 secretion after exposure to Zn/Cu-containing welding fume suspension in a time- and concentration-dependent manner (Figure 5). Regarding the decreased IL-6, TNF-α, and MIP-1β/CCL4 levels at 10 and/or 100 µg/mL fume particle exposure concentration at 24 h and 48 h (Figures 3 and 5), the cytotoxicity values (depicted in Figure 1) will have to be considered. At lower concentrations, THP-1 macrophages secreted high concentrations of TNF-α. At the exposure concentration of 1 µg/mL, the first significant increases of TNF-α could be detected at 24 h and reached a high release of 1244.4 ± 112.2 pg/mL after an incubation period of 48 h. In comparison to TNF-α, the chemokine MIP-1β/CCL4 was significantly released by THP-1 macrophages already at an earlier time point (6 h) and at a lower exposure concentration of 0.01 µg/mL. Furthermore, THP-1 macrophages significantly released MIP-1β/CCL4 at 0.001 µg/mL of the Zn/Cu-containing welding fume suspension after 48 h exposure time.

2.4. Genotoxicity

The genotoxicity of Zn/Cu-containing welding fumes was assessed using the umu-test with Salmonella typhimurium TA 1535 [pSK 1002]. The umuC gene is part of the cell's SOS repair system that counteracts damage to the bacterial genetic material.

The test was carried out both with and without S9 microsomal rat liver extract for the metabolic activation of potential genotoxins. For all exposure concentrations of the test material, the growth factors (GFs) were in the range of 0.83–1.24 (Table 1), and thus, the bacterial growth inhibition was clearly less than 50%, which is the prerequisite for the validity of the test result. The induction ratios (IRs) were from 0.88 to 1.24 (Table 1), indicating that the welding fumes did not induce genotoxicity for any of the welding fume suspension concentrations. Thus, no genotoxic effects of the Zn/Cu-containing welding fume were detected.

Table 1. Results of genotoxicity testing using the umu-test for different concentrations of Zn/Cu-containing welding fume particles (0–100 µg/mL). Each value represents the mean ± SEM of one independent experiment in triplicate. The following terminology denotes specific assay parameters: Dilution Factor for Induction (DLi value), Induction Ratio (IR), and Growth Factor (GF).

	Without Metabolic Activation				**With Metabolic Activation**			
	D_{Li}	**IR**	**GF**	**Genotoxicity**	**D_{Li}**	**IR**	**GF**	**Genotoxicity**
Blank (Assay medium)	100	1.11 ± 0.23	0.99 ± 0.1	-	100	1.03 ± 0.11	0.97 ± 0.06	-
Welding fume [µg/mL]								
0.001	100	1.06 ± 0.08	1.01 ± 0.11	-	100	0.88 ± 0.03	1.09 ± 0.05	-
0.01	100	1.06 ± 0.08	1.24 ± 0.11	-	100	1.06 ± 0.06	1.10 ± 0.06	-
0.1	100	1.0 ± 0.08	1.12 ± 0.13	-	100	1.0 ± 0.05	1.16 ± 0.03	-
1.0	100	1.07 ± 0.10	1.07 ± 0.11	-	100	0.89 ± 0.17	1.13 ± 0.04	-
10	100	1.24 ± 0.05	0.95 ± 0.11	-	100	1.07 ± 0.05	0.89 ± 0.08	-
100	100	1.05 ± 0.06	1.15 ± 0.15	-	100	1.24 ± 0.12	0.83 ± 0.11	-

2.5. Oxidative Stress

The activity of the defense enzyme SOD was measured as an indirect method to analyze whether exposure to welding fumes results in the formation of reactive oxygen species, which could lead to oxidative stress in the cells. In A549 epithelial cells, a significant to highly significant increase in SOD activity was observed following treatment with concentrations ranging from 1 to 100 µg/mL at 6 h. The highest enzyme activity recorded for these cells was approximately 2 µmol/min/10^6 cells after 48 h of exposure to 10 µg/mL fume particles (Figure 6A). In contrast, THP-1 macrophages exhibited a different response. When exposed to the highest concentration for 6 h, SOD activity was found to be 2-fold higher compared to A549 under the same conditions. The highest enzyme activity recorded for macrophages was about 4 µmol/min/10^6 cells after 24 h of exposure to 100 µg/mL fume particles (Figure 6B). For both cell lines, the increases in SOD activity were most consistent at the 6 h time point (Figure 6). Regarding the lower SOD activity values at the 100 µg/mL fume particle exposure concentration at 48 h (Figure 6), the cytotoxicity values (depicted in Figure 1) will have to be considered.

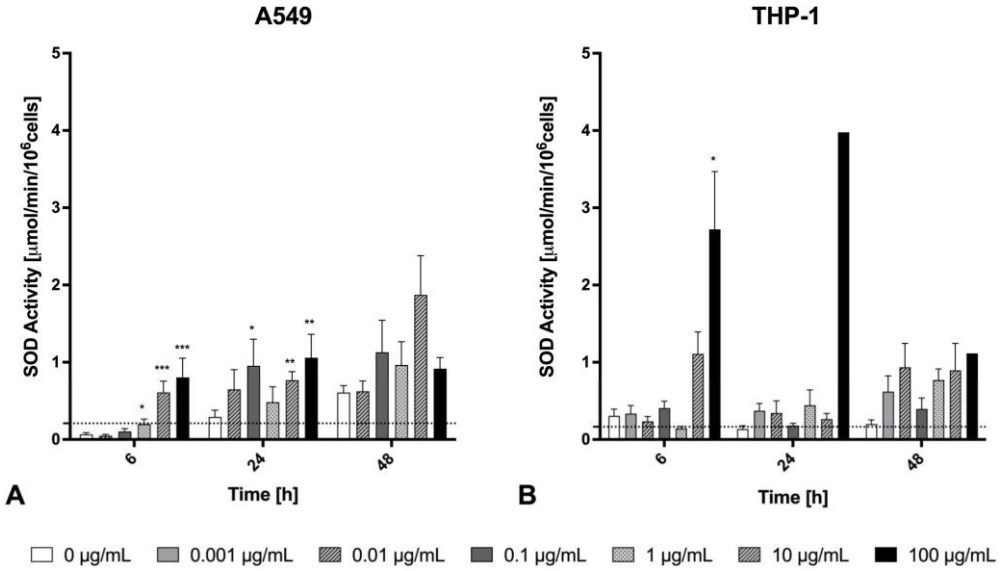


Figure 6. SOD activity in A549 cells (**A**) or THP-1 macrophages (**B**) after exposure to Zn/Cu-containing welding fume particles at various concentrations (0–100 µg/mL) over time (6 h, 24 h, and 48 h). Each bar shows the mean ± SEM for $n = 4$. Dashed lines show the level of the negative control. Statistically significant differences between exposed and unexposed conditions are highlighted as follows: (*) $p < 0.05$, (**) $p < 0.01$, and (***) $p < 0.001$.

3. Discussion

Toxic airborne ultrafine metal particles are typically inhaled in the airways and may cause lung inflammation and cytotoxicity. Two essential cell types in the lung are alveolar epithelial cells and alveolar macrophages. Therefore, we tested the toxicological and inflammatory effects of suspensions of the occupationally relevant Zn/Cu-containing welding fume on human A549 alveolar epithelial cells and PMA-differentiated human THP-1 macrophages in vitro in comparable experimental settings. Our study demonstrates differential (time- and concentration-dependent) toxicological and inflammatory effects of Zn/Cu-containing welding fume in both cell types: (i) We found, to the best of our knowledge for the first time, that THP-1 macrophages significantly secreted MIP-1β/CCL4 at an exposure concentration of 0.01 µg/mL after 6 h and 0.001 µg/mL after 48 h, thereby suggesting MIP-1β/CCL4 as a highly sensitive inflammatory biomarker for human macrophages after Cu- and Zn-containing welding fume exposure. (ii) THP-1 macrophage exposure to 1 µg/mL welding fume particles caused significantly increased IL-8 and TNF-α concentrations in the supernatant at 6 and 24 h, respectively. THP-1 macrophage exposure with a 10-fold lower concentration (0.1 µg/mL) caused significantly increased IL-8 and TNF-α concentrations in the supernatant after 48 h. (iii) A549 typically do not secrete TNF-α and MIP-1β/CCL4, and significant increases of IL-6 and IL-8 were determined in A549 supernatants only at high exposure concentrations of 10 µg/mL and particularly 100 µg/mL. (iv) Overall, THP-1 macrophages reacted more sensitively to the Zn/Cu-containing welding fume suspension exposure than A549 cells, as indicated by earlier and/or more pronounced increases of SOD activity, apoptosis rates measured by annexin V staining and cell cytotoxicity percentages. Notably, while A549 cells proliferate during exposure, THP-1 macrophages do not, which may contribute to the observed heightened sensitivity of THP-1 macrophages to Zn/Cu-containing welding fume suspensions. (v) No genotoxic effect of the Zn/Cu-containing welding fume could be detected.

Using relatively high, constant bolus doses of manufactured nanoparticles during the exposure period is common practice in in vitro particle research. Some representative examples are ZnO and CuO particles on lung epithelial cells and macrophages

(0.1 to 330 µg/mL) [23], ZnO nanoparticles on macrophages (36 µg/mL) [24], and metal particles containing high levels of Zn and Cu on endothelial cells (0.1 to 50 µg/mL) [33]. In our study, we used a wide concentration range of Zn/Cu exposure doses from 1 ng/mL to 0.1 mg/mL in order to extend the typically tested dose ranges, especially at lower exposure concentrations. In order to relate these in vitro values to real-world occupational conditions, the authors of McCarrick et al. (2022) developed a methodology [34] based on the computational Multiple-Path Particle Dosimetry (MPPD) model (www.ara.com/mppd; accessed on 1 March 2025). The MPPD model is used to simulate the behavior of aerosols in the respiratory system and to calculate nasal, tracheobronchial, and pulmonary deposition of inhaled (nano)particles on the basis of particle properties such as particle diameter and mass concentration, under the consideration of mucociliary and macrophage-mediated clearance. To enable direct comparison with in vitro studies, the authors of McCarrick et al. (2022) converted deposited mass into deposition per surface area, dividing tracheobronchial deposits by 3220 cm^2 of airway surface area and alveolar deposits by 790,000 cm^2 of alveolar surface. The general OEL used in the regulation of Zn welding fumes corresponds to 5 mg/m^3 according to IARC [35], which translates to 0.89 $\mu g/cm^2$ for tracheobronchial deposition (TBD) and 0.017 $\mu g/cm^2$ for alveolar deposition (ALD) after 6 h of exposure.

To compare these values to cytotoxic doses from in vitro cell cultures, this concentration-to-area ratio approach is used for the cell culture Petri dishes, each containing adherent cells in 5 mL of medium on 21 cm^2 of growth surface. For example, a particle concentration of 0.1 µg/mL means that we have (in 5 mL) a total of 0.5 µg of particles, which, divided by the 21 cm^2 growth area, results in 0.024 $\mu g/cm^2$. This concentration is 2.7% of 6h-TBD and 1.41-fold that of 6h-ALD. Thus, 0.1 µg/mL (corresponding to 0.024 $\mu g/cm^2$) is less than the 6h-TBD but above the 6h-ALD and close to the 1 week-ALD (Table A1). Based on this methodology, the concentrations of 0.001, 0.01, 0.1, 1, 10, and 100 µg/mL in the cell culture medium applied in the current in vitro study correspond to concentration-to-area ratios of around 0.00024, 0.0024, 0.024, 0.24, 2.4, and 24 $\mu g/cm^2$, respectively. A detailed comparison with exposure classification is shown in Table A1.

When using A549 cells as a model system for lung research, the type of culture medium influences the outcome of toxicity assays. Although it is still common to use DMEM as a culture medium for A549, e.g., [36–38], Cooper et al. (2016) demonstrated that long-term cultures of A549 cells in Ham's F-12K medium for up to 25 days had a transcriptomic profile more similar to primary alveolar type II (ATII) cells compared to A549 cells cultured in DMEM [39]. In addition, A549 cells cultured in DMEM medium showed increased crowding and piling up compared to Ham's F-12K only after continuous long-term cultures of up to 25 days but not after short-term cultures [39]. Consistent with this, we did not observe any crowding or piling up of A549 cells in DMEM in our short-term cultures of up to 2 days. Future studies on the toxicity of Cu and/or Zn particles in A549 lung epithelial cells might consider long-term cultures in Ham's F-12K medium to further verify the physiological relevance of previous results (e.g., this study, Lanone et al. (2009), [23] and Karlsson et al. (2008) [40]). Interestingly, cells exposed to selenium in Ham's F-12K medium for 1–2 days showed an even higher sensitivity to selenite toxicity than cells cultured in DMEM [41].

When interpreting the SOD data, one should consider that SOD activities do not necessarily reflect oxidative stress. One of the main functions of SODs is to convert superoxide radicals to hydrogen peroxide and molecular oxygen [42]. The SOD activity kit used in this study is designed to detect the total catalytic activity of Cu,Zn-SOD (SOD1), Mn-SOD (SOD2), and Cu,Zn-SOD (SOD3). As the names indicate, SOD1 and SOD3 contain Cu and Zn. Considering that the main intracellular SOD is Cu,Zn-SOD, thus having both Cu and Zn as co-factors, a direct effect of Cu/Zn-addition to cells on SOD activity may

be suggested. For example, oral Zn supplementation increased both gene expression and catalytic activity of SOD in overweight patients [43]. Therefore, the Zn/Cu concentration-dependent increase in SOD activity in the current study may indicate a higher protection of the cells from oxidative stress at medium Zn/Cu concentrations. In contrast, decreased SOD activity levels could—in view of the cytotoxicity data—indicate increased oxidative stress at higher metal concentrations. In a recent study, high concentrations of CuO nanoparticles (25 µg/mL) have been reported to cause SOD1 misfolding in macrophages, exacerbating oxidative stress and proteasomal dysfunction [28]. In order to further elucidate the oxidative stress status of the Zn/Cu-exposed cells, future studies should incorporate the direct detection of ROS production (for example, via the DCFH assay [44] or other fluorescence-based probes) in order to be able to correlate SOD activity with ROS levels [45]. Furthermore, one should consider that SOD1 does not only act as an antioxidant but also activates nuclear gene transcription [46].

This study found significantly elevated chemokine MIP-1β/CCL4 secretion by THP-1 macrophages after Zn/Cu-containing welding fume exposure. The higher exposure concentration of 10 µg/mL and especially 100 µg/mL showed inflammatory effects in A549 cells but caused stronger cytotoxic effects in THP-1 macrophages after 24 h to 48 h, confirming a higher sensitivity of THP-1 macrophages towards Zn/Cu-exposure in comparison to A549 cells. The secretion of MIP-1β/CCL4 by THP-1 macrophages was also observed in response to a low concentration of 0.001 µg/mL Zn/Cu-containing welding fume suspension. In contrast, TNF-α secretion by THP-1 macrophages was only induced at a concentration approximately 100-fold higher at 0.1 µg/mL. This finding underlines that MIP-1β/CCL4 is a highly sensitive biomarker for macrophages, as its original name indicates. The study did not identify the lowest effective concentration of Zn/Cu-containing welding fume required to induce MIP-1β/CCL4 secretion in THP-1 cells, as no lower concentrations than 0.001 µg/mL were tested. However, it is unlikely that clearly lower concentrations than 0.001 µg/mL could cause MIP-1β/CCL4 release, as exposure to 0.001 µg/mL metal particles caused detectable significantly elevated levels of secreted MIP-1β/CCL4 only after 48 h.

From a regulatory perspective, this study demonstrated the sensitivity of this human-relevant in vitro biomarker MIP-1β/CCL4, suggesting its further validation for integration into standardized macrophage-based New Approach Method (NAM) assays for nanomaterial risk assessment. Future applications of MIP-1β/CCL4 as macrophage-specific sensitive biomarkers could help safety assessment for emerging materials by enabling parallel screening of inflammatory potential and mechanistic toxicity across human cell systems, complementing existing NAMs focused on acute cytotoxicity. Integrating such sensitive in vitro models into regulatory decision-making would accelerate evidence-based updates to exposure guidelines while reducing animal testing burdens. This is not only useful for high-production volume metals such as Cu and Zn, but the proven ability to detect biological activity at environmentally relevant concentrations may establish this approach as a fundamental methodology for next-generation risk assessment frameworks that balance industrial innovation with worker health protection.

MIP-1β/CCL4 affects immune and non-immune cells by binding them to its main receptor, CCR5. MIP-1β/CCL4 is particularly known as one of the major HIV-suppressive factors [31], and increased systemic MIP-1β/CCL4 levels have been suggested as potential biomarkers of severe COVID-19 [47]. MIP-1β/CCL4 secretion by THP-1 monocytes was detected after 24 h of exposure to nickel sulfate (100 µg/mL) and cobalt sulfate (130 µg/mL) [48]. While these metal concentrations are relatively high, the specificity of MIP-1β/CCL4 induction in response to metals suggests its utility as a biomarker. Notably, MIP-1β/CCL4 production is mechanistically linked to canonical inflammatory path-

ways, such as TLR4-MyD88 signaling and downstream NF-κB/AP-1 activation, which are shared between lipid-mediated metabolic inflammation and metal-induced stress responses [49,50]. For instance, induction of MIP-1β/CCL4 occurs when THP-1 cells are co-stimulated with TNF-α and palmitate, a process dependent on clathrin-mediated endocytosis and MAPK/NF-κB signaling [49]. This suggests that MIP-1β/CCL4 production may result from direct metal stimulation and secondary inflammatory signaling cascades. Moreover, MIP-1β/CCL4's functional role in recruiting monocytes to sites of inflammation underscores its potential biological relevance as a marker of macrophage activation during metal exposure [50,51]. Significantly increased MIP-1β/CCL4, found in bronchoalveolar lavage fluid (BALF) of smokers affected by chronic bronchitis compared to smoking or non-smoking healthy control groups, showed a moderate negative correlation between MIP-1β/CCL4 levels and forced expiratory volume in one-second values ($p = -0.64$, $p = 0.035$) of chronic bronchitis [32]. This suggests that MIP-1β/CCL4 might be a lung inflammation biomarker and exerts pro-inflammatory and immune-modulatory effects. Current biomonitoring approaches under investigation for metal workers exposed to welding fumes or metal particles primarily focus on measuring urinary or blood concentrations of toxic metals (e.g., chromium, manganese, nickel) [52] and oxidative stress biomarkers like 8-oxo-7,8-dihydroguanosine (8-oxoGuo) and 3-nitrotyrosine, which reflect RNA/DNA damage from reactive oxygen species generated by metal exposure [53] and standard inflammatory markers [54–56]. Given its role as a chemokine produced during macrophage-mediated inflammatory responses, MIP-1β/CCL4 could complement existing biomarkers by assessing chronic inflammation risks [57,58]. This approach could help to recognize inflammatory pathologies in high-risk occupations at an early stage.

Controlled short-term inhalation of Zn- and Cu-containing welding fumes increases the systemic acute-phase mediator IL-6 and the acute-phase proteins CRP and SAA [16–18,59]. CRP is a risk marker for future cardiovascular diseases (CVDs) [9], and IL-6 and SAA are regarded as risk markers and even mediators for CVD [9,60–62]. Furthermore, epidemiological studies show that long-term welders have a higher risk of CVD [5,63]. Recent proteomic analyses of welders' sera reveal dose-dependent changes in CD84 and FGF23, proteins implicated in vascular inflammation and cardiac remodeling, even at exposures below current occupational limits (1–5 mg/m^3) [64,65]. Of note, hypertensive patients with high MIP-1β/CCL4 quartiles face elevated cerebrocardiovascular risks [66]. Elevated systemic levels of MIP-1β/CCL4 correlate with an increased risk of stroke and cardiovascular events [67]. Experimental models further show that MIP-1β/CCL4 inhibition attenuates atherosclerotic plaque development by reducing macrophage infiltration and stabilizing vulnerable lesions, highlighting its therapeutic potential [67]. Overall, future studies should investigate whether elevated MIP-1β/CCL4 levels can be detected in serum, airway secretions, and/or exhaled breath condensate of metal workers, including welders. In this context, it is important to consider that in real-world scenarios, the metal workers being tested should not have any infections that could cause a similar increase in biomarkers.

IL-6 activates macrophages and T cells and is a potent acute-phase mediator, which causes the liver to release acute phase proteins like CRP and SAA [68]. This study found that A549 alveolar epithelial cells secrete high levels of IL-6 after exposure to Zn/Cu-containing welding fumes. This possibly provides a potential link to the previous findings of elevated systemic IL-6 (and CRP and SAA) levels in healthy individuals after controlled Zn/Cu-containing welding fume exposure [17]. The chemokine IL-8 recruits leucocytes, particularly neutrophils, to inflammatory sites. In our study, we found its release by both cell types, A549 and THP-1 macrophages, with the levels secreted by the macrophages being higher than those secreted by lung epithelial cells. This may be due to the macrophages'

ability to internalize foreign particles. The fact that THP-1 cells were overall more sensitive than A549 cells in the current study is consistent with previous reports: A549 cells are known to be more resistant to exposure to nanomaterials [40]. In addition, THP-1 macrophages reacted more sensitively than A549 cells to CuO and ZnO particles for both single or mixed metal particles [23].

4. Materials and Methods

4.1. Welding Fume Generation

The welding process described in the study involves metal inert gas (MIG) soldering, a technique commonly used in vehicle manufacturing for joining galvanized steel. This process utilizes a low-alloy copper wire as the filler material, which is applied to hot-dip galvanized steel substrates. The base metal used is DX51D + Z275 (1.0226), classified under EN 10346 standards [69], while the filler material is CuSi3Mn (Bercoweld S3, Bedra, Germany), composed of 96% copper, 1% manganese, and 3% silicon, conforming to ISO 24373 specifications [70] with a diameter of 1.2 mm. Argon serves as the shielding gas during the soldering process, which operates in pulsed arc mode to ensure precise control of heat input and material deposition. The fumes generated during this process consist of ultrafine particles with a modal diameter of approximately 122 ± 1.46 nm. Detailed information regarding the welding process and materials can be found in research conducted by Hartmann et al. in 2014 [56].

4.2. Welding Fume Characterization via Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

The composition of the welding fume was analyzed using inductively coupled plasma mass spectrometry (ICP-MS). A mixture of 50 mg of welding fume, 1 mL Rhodium-ICP-Standard solution, 7 mL hydrogen peroxide, and 5 mL aqua regia (75% (*v*/*v*) HCl, 25% (*v*/*v*) HNO_3) was digested for 1 h in the microwave. For measurement, the digested welding fume was diluted 1:10 with aqua bidest. Standards for elements aluminum, arsenic, barium, beryllium, boron, cadmium, calcium, cobalt, copper, chromium, iron, lead, lithium, magnesium, manganese, molybdenum, nickel, phosphorus, rubidium, selenium, strontium, tellurium, thallium, tin, vanadium, and zinc were used for quantification. The main elemental metal components of the welding fume are 8% copper oxide and 70.2% zinc oxide. Additionally, the welding fume contains a remaining part that shows traces of iron, manganese, calcium, phosphorus, and others in varying amounts. A proportion of the weight of the remaining welding fume particles may be due to the presence of oxygen from metal oxides. We refer to it as Zn- and Cu-containing welding fume or Zn/Cu-containing welding fume because it mainly consists of large amounts of zinc and significant portions of copper.

4.3. Preparation of the Welding Fume Extracts and Exposure Solutions

The welding fume was collected as a dark grey powder and subsequently extracted in an assay medium using sonication for 30 min to produce a stock exposure solution of 1000 µg/mL. Following the sonication process, a 1:10 dilution series was prepared to yield five (A549) or six (THP-1) particle extracts, each at final concentrations of 100, 10, 1, 0.1, 0.01, and 0.001 µg/mL (only THP-1). The preparation and dilution of the extract solutions were conducted under strict sterile and standardized conditions. The resulting welding fume exposure solutions were aliquoted and stored at −20 °C until usage.

4.4. Controlled Experimental In Vitro Exposure to Welding Fumes Containing Zinc and Copper on A549 Lung Epithelial Cells and THP-1 Macrophages

4.4.1. Cell Culture and Culture Conditions

The A549 cell line represents a human alveolar basal epithelial cell line derived from a type II-like lung carcinoma. It was obtained from the cell line service (CLS, Eppelheim, Germany). Unless specified, the cell culture reagents were obtained from Gibco (Thermo Fisher Scientific Inc., Waltham, MA, USA), ensuring consistent results. The cell line was cultured as a monolayer in Dulbecco's Modified Eagle Medium (DMEM) cell culture medium containing GlutaMAX, 10% (*v*/*v*) FBS (Invitrogen, Darmstadt, Germany), 1% (*v*/*v*) penicillin (100 IU/mL) and streptomycin (100 µg/mL). The monocytic cell line THP-1 has been isolated from the blood of an acute monocytic leukemia patient (male). The cell line was cultured as suspension cells in Roswell Park Memorial Institute (RPMI 1640) cell culture medium containing GlutaMAX, 10% (*v*/*v*) FBS (Invitrogen, Darmstadt, Germany), 1% (*v*/*v*) penicillin (100 IU/mL), and streptomycin (100 µg/mL). The monocytes were differentiated into macrophages by culturing the cells in the above-mentioned culture medium spiked with 200 nM phorbol 12-myristate 13-acetate (PMA; Sigma-Aldrich GmbH, Steinheim, Germany) and 0.1% (*v*/*v*) dimethyl sulfoxide (DMSO; Sigma-Aldrich GmbH, Steinheim, Germany). All cell lines were grown in sterile plastic flasks (175 cm^2, Greiner Bio-One, Frickenhausen, Germany) in a humidified 5% CO_2 atmosphere at 37 °C.

4.4.2. Experimental In Vitro Exposure Procedure and Sample Preparation

Cells were seeded at a density of 5×10^5 cells (A549) and 2.4×10^6 (THP-1) in 5 mL respective cell culture medium per cell culture dish (60 mm; Greiner Bio-One, Frickenhausen, Germany) and incubated at 37 °C in a humidified atmosphere containing 5% CO_2 for 48 h. After this time, the culture or differentiation medium was removed to eliminate non-adherent cells and washed thrice with prewarmed phosphate-buffered saline (PBS). Only vital adherent and differentiated (THP-1) cells were incubated with assay medium (cell culture medium with reduced FBS to 1% (*v*/*v*), without phenol red indicator and with 200 mM L-glutamine instead of GlutaMAX) containing 0.001 (only THP-1), 0.01, 0.1, 1, 10 and 100 µg/mL welding fume for 0 h, 6 h, 24 h, and 48 h. The lowest concentration of 0.001 µg/mL was only used for THP-1 cells, given the internal preliminary study results concerning the high sensitivity of this cell line to the particles. Non-exposed cells were incubated with assay media only and used as a negative control. An assay medium containing 1 µg/mL Lipopolysaccharides (LPS) from Escherichia coli 0111:B4 (Sigma-Aldrich GmbH, Steinheim, Germany) was used as a positive control. After every incubation period (0 h, 6 h, 24 h, and 48 h), 2 mL of cell culture supernatants were collected to isolate the extracellular protein fraction, and an additional 20 µL were collected for viability testing. The 2 mL of supernatant was centrifuged (100× *g*, 5 min), transferred to aliquots, and frozen immediately at −80 °C to determine later on the cytokine and chemokine amount secreted by the cells.

4.4.3. Cytotoxicity by Assessing the Cell Viability

The cytotoxicity of Zn/Cu-containing welding fume was evaluated by staining the cells with trypan blue to distinguish between viable and nonviable cells. After the experimental exposure, 20 µL of detached cells in solution were mixed with 20 µL trypan blue solution and transferred to a Neubauer counting chamber. Viable cells appear light, while nonviable cells appear dark blue under the microscope due to trypan blue permeating the cell membrane and dyeing the cytosol dark blue. The number of viable and nonviable cells was determined for each sample, and the percentage ratio was then calculated to assess cell viability.

4.4.4. Cytokines and Chemokines Detection Cell Culture Supernatant

The levels of secreted TNF-α (K151QWD), IL-8 (K151RAD), and MIP-1β/CCL4 (K151NRD) in the cell culture supernatants were measured using the electrochemiluminescence immunoassays (V-Plex) from Meso Scale Discovery (Meso Scale Diagnostic, Rockville, MD, USA). The levels of secreted IL-6 were analyzed using an enzyme-linked immunosorbent assay (ELISA) BD OptEIA™ Human IL-6 Set (555220; BD Biosciences, Franklin Lakes, NJ, USA). All assays were conducted following the instructions provided by the manufacturers.

4.4.5. Genotoxicity Assessment Due to Exposure to Welding Fumes Containing Zinc and Copper

The reporter gene assay umu-test is a genotoxicity test for detecting chemical mutagens and carcinogens [71]. The test system is based on the induction by alkylating agents of SOS functions coupled to a reporter gene in the umuC lacZ fusion plasmid. For the investigation, the test strain *Salmonella typhimurium* TA1535/pSK1002 was used for the investigation. The genotoxicity activities were determined by measuring the β-galactosidase activity. The test performance was realized according to DIN 38415-3:1996-12 [72]. Deviating from ISO 13829 [73], the samples were extracted in a 100% assay medium without antibiotics and introduced into the test system. The extent of induction of the SOS repair system was indicated by β-galactosidase units, which result in the induction rate (IR). According to the standard (ISO 13829), a sample was supposed to be genotoxic if this induction rate exceeds 1.5. For the welding fume sample, the first dilution with an induction rate below 1.5 and a growth factor exceeding 0.5 is presented (Lowest ineffective dilution: DLI-value).

4.4.6. Annexin V Detection Using the Sartorius Incucyte® SX5 Live-Cell Analysis System for Live-Cell Imaging

The THP-1 cells were seeded in a 96-well plate (Sarstedt, Nümbrecht, Germany) at a density of 2.5×10^4 cells per well and were then incubated for 48 h in RPMI 1640 with GlutaMAX (Gibco, Waltham, MA, USA) medium containing 10% (*v*/*v*) fetal bovine serum (FBS; PAN-Biotech, Aidenbach, Germany), 1% (*v*/*v*) penicillin and streptomycin (Gibco, Waltham, MA, USA), and 200 nM phorbol 12-myristate-13-acetate (PMA; Sigma-Aldrich, Saint Louis, MO, USA) to allow for differentiation and attachment at 37 °C, 5% CO_2, and 95% humidity. Furthermore, A549 cells were seeded in a 96-well plate (Sarstedt, Nümbrecht, Germany) and were grown in DMEM with GlutaMAX (Gibco, Waltham, MA, USA) containing 10% (*v*/*v*) FBS (PAN-Biotech, Aidenbach, Germany) and 1% (*v*/*v*) penicillin and streptomycin (Gibco, Waltham, MA, USA). These cells were then incubated for 48 h to allow attachment and the formation of a cell monolayer. After the 48 h incubation period, the cells were washed three times with sterile PBS (Gibco, Waltham, MA, USA). Subsequently, each well was spiked with 5 µM of annexin V inert dye (4759; Sartorius, Goettingen, Germany). The control samples remained unexposed, while the treated cells were exposed to varying concentrations (0.001, 0.1, 1, 10, and 100 µg/mL) of Zn/Cu-containing welding fumes for 72 h (37 °C, 5% CO_2, 95% humidity) in the Sartorius Incucyte® SX5 Live-Cell analysis system for live-cell imaging. The concentrations of the exposure treatments corresponded to those indicated in the text and the figures. Images from the scan interval were analyzed using the Sartorius IC Incucyte® software 2023A. The generated data was further processed and visualized using GraphPad Prism 10.0.0 (GraphPad Software, Boston, MA, USA).

4.4.7. Determination of Oxidative Stress via Superoxide Dismutase (SOD)

Commercial colorimetric assays determined superoxide dismutase after cell disruption in the cell extract. Superoxide dismutase was performed using the Superoxide Dismu-

tase Assay Kit by Sigma-Aldrich (19160; Sigma Aldrich, Steinheim, Germany) and was performed according to the manufacturer's instructions.

4.5. Statistical Analysis

Statistical analyses were performed using GraphPad Prism 10.0.0 (GraphPad Software, Boston, MA, USA) and MedCalc 23.0.5 for Windows (MedCalc Software 23.0.5, Ostende, Belgium). Results are presented as mean values ± SEM. For each incubation time point (2 h, 4 h, 6 h, 24 h, and/or 48 h), data from cell cultures exposed to welding fumes were compared to the non-exposed control cultures. Two statistical tests were used: the Student's *t*-test for normally distributed data and the Mann–Whitney U test for non-parametric data. Both tests were two-sided tests to account for potential differences in either direction. Statistical significance was defined as $p \leq 0.05$.

5. Conclusions

The increasing industrial use of Zn and Cu across sectors ranging from welding technologies to nanotechnology applications highlights the importance of assessing the safety of occupational exposure to metal fumes, as exposure to those particles is associated with potential health risks. The toxicological analyses in this in vitro study indicated that exposure to Zn/Cu-containing welding fumes caused cell-specific cytotoxicity, apoptosis, increased SOD activity, and inflammatory cytokine responses. A distinct secretion of MIP-1β/CCL4 by THP-1 macrophages occurred at low Zn/Cu-containing welding fume suspension concentrations, down to 0.001 µg/mL, at a concentration when none of the other tested cytokine was observed. This suggests that MIP-1β/CCL4 could be a highly sensitive biomarker for exposure to metal-containing particles and that it may be useful to validate its integration into standardized macrophage-based NAM assays for sensitive safety assessment of emerging (nanoscale) materials. The chemokine MIP-1β/CCL4, along with IL-8 and TNF-α secreted by THP-1 cells as well as IL-6 and IL-8 produced by A549 cells at higher concentrations, contributes to local lung inflammation and potential systemic effects and may increase cardiovascular risk in chronically exposed individuals. The release of IL-6 by A549 cells may stimulate acute phase protein production in the liver, explaining previously observed elevated systemic inflammatory markers in welders. The differential cellular responses highlight the importance of studying multiple cell types when assessing occupational and environmental exposures.

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Appendix A

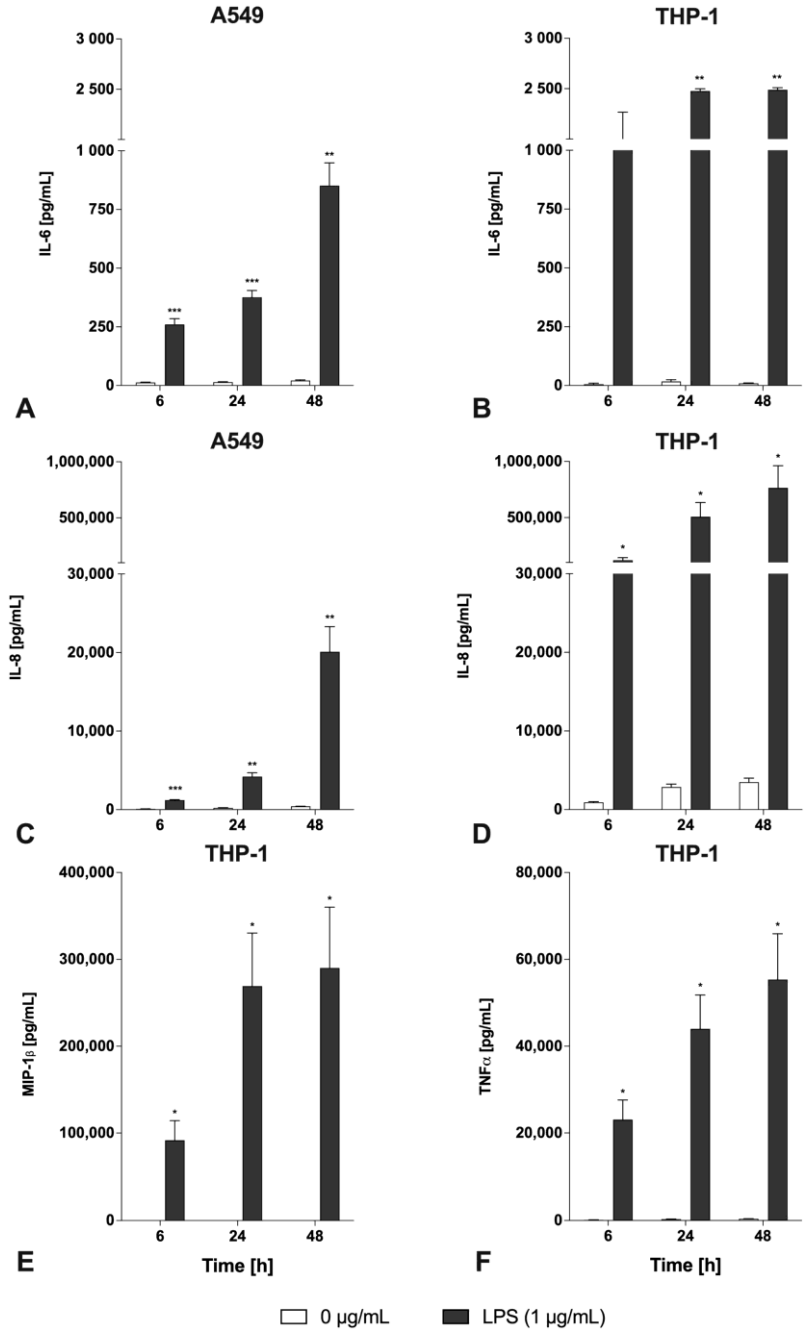


Figure A1. Cytokine release from A549 epithelial cells and THP-1 macrophages after 6 h, 24 h, and 48 h exposure to 1 µg/mL LPS as a positive control for a pro-inflammatory response. Each bar shows the mean ± SEM of at least 3 independent experiments. (**A**) IL-6 of A549. (**B**) IL-6 of THP-1 macrophages. (**C**) IL-8 of A549. (**D**) IL-8 of THP-1 macrophages. (**E**) MIP-1β/CCL4 of THP-1 macrophages. (**F**) TNF-α of THP-1 macrophages. Statistically significant differences between exposed and unexposed cells (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$.

Appendix B

Table A1. Based on McCarrick et al. (2022) [34], the table highlights the differences between in vitro concentrations (0.00024–24 µg/cm²) and human inhalation data for the occupational exposure level (OEL) of 5 mg/m³ welding fume, corresponding to 0.89 µg/cm² for tracheobronchial deposition (TBD) and 0.017 µg/cm² for alveolar deposition (ALD) after 6 h of exposure (short-term exposure). One week of this exposure was calculated to 0.102 µg/cm² TBD and 0.083 µg/cm² ALD, and one year to 1.15 µg/cm² TBD and 2.85 µg/cm² ALD (long-term exposure). This enables a comparison of in vitro and realistic occupational exposure data.

		In Vitro vs. Human Inhalation		In Vitro vs. Human Inhalation		Exposure Classification
In Vitro [µg/mL]	**In Vitro [µg/cm²]**	**1-Week TBD (0.102 µg/cm²)**	**1-Week ALD (0.083 µg/cm²)**	**1-Year TBD (1.15 µg/cm²)**	**1-Year ALD (2.85 µg/cm²)**	
-	-	0.102	0.083	1.15	2.85	OEL 6 h as baseline (5 mg/m³)
0.001	0.00024	0.24%	0.29%	0.021%	0.0084%	Realistic short-term exposure
0.01	0.0024	2.4%	2.9%	0.21%	0.084%	Realistic short-term exposure
0.1	0.024	23.5%	28.9%	2.1%	0.84%	Upper limit of short-term exposure
1	0.24	2.35-fold	2.89-fold	20.9%	8.4%	Most representative for long-term exposure
10	2.4	23.5-fold	28.9-fold	2.09-fold	16%	Exceeds occupational thresholds
100	24	235-fold	289-fold	20.9-fold	8.4-fold	Exceeds occupational thresholds

References

1. Ali, N.; Mattsson, K.; Rissler, J.; Karlsson, H.M.; Svensson, C.R.; Gudmundsson, A.; Lindh, C.H.; Jonsson, B.A.; Cedervall, T.; Karedal, M. Analysis of nanoparticle-protein coronas formed in vitro between nanosized welding particles and nasal lavage proteins. *Nanotoxicology* **2016**, *10*, 226–234. [CrossRef] [PubMed]
2. Oberdorster, G.; Oberdorster, E.; Oberdorster, J. Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. *Environ. Health Perspect.* **2005**, *113*, 823–839. [CrossRef] [PubMed]
3. Antonini, J.M. Health effects of welding. *Crit. Rev. Toxicol.* **2003**, *33*, 61–103. [CrossRef]
4. Antonini, J.M.; Lewis, A.B.; Roberts, J.R.; Whaley, D.A. Pulmonary effects of welding fumes: Review of worker and experimental animal studies. *Am. J. Ind. Med.* **2003**, *43*, 350–360. [CrossRef]
5. Ibfelt, E.; Bonde, J.P.; Hansen, J. Exposure to metal welding fume particles and risk for cardiovascular disease in Denmark: A prospective cohort study. *Occup. Environ. Med.* **2010**, *67*, 772–777. [CrossRef]
6. Zeidler-Erdely, P.C.; Kashon, M.L.; Battelli, L.A.; Young, S.H.; Erdely, A.; Roberts, J.R.; Reynolds, S.H.; Antonini, J.M. Pulmonary inflammation and tumor induction in lung tumor susceptible A/J and resistant C57BL/6J mice exposed to welding fume. *Part. Fibre Toxicol.* **2008**, *5*, 12. [CrossRef]
7. Raulf, M.; Weiss, T.; Lotz, A.; Lehnert, M.; Hoffmeyer, F.; Liebers, V.; Van Gelder, R.; Udo Kafferlein, H.; Hartwig, A.; Pesch, B.; et al. Analysis of inflammatory markers and metals in nasal lavage fluid of welders. *J. Toxicol. Environ. Health A* **2016**, *79*, 1144–1157. [CrossRef] [PubMed]
8. Ohlson, C.G.; Berg, P.; Bryngelsson, I.L.; Elihn, K.; Ngo, Y.; Westberg, H.; Sjogren, B. Inflammatory markers and exposure to occupational air pollutants. *Inhal. Toxicol.* **2010**, *22*, 1083–1090. [CrossRef]
9. Ridker, P.M.; Rifai, N.; Stampfer, M.J.; Hennekens, C.H. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation* **2000**, *101*, 1767–1772. [CrossRef]

10. Lim, G.B. Coronary artery disease: IL-6 signaling linked with CHD. *Nat. Rev. Cardiol.* **2012**, *9*, 313. [CrossRef]
11. Reisgen, U.; Geffers, C.; Willms, K.; Angerhausen, M.; Deckert, K.; Hof, S.; Prenger, F.; Wisniewski, J. Low-energy thermal joining with zinc and tin base solder for application in vehicle construction. In *Brazing, High Temperature Brazing and Diffusion Bonding*; DVS Media: Düsseldorf, Germany, 2013; pp. 272–277.
12. Blanc, P.D.; Boushey, H.A.; Wong, H.; Wintermeyer, S.F.; Bernstein, M.S. Cytokines in metal fume fever. *Am. Rev. Respir. Dis.* **1993**, *147*, 134–138. [CrossRef] [PubMed]
13. Kuschner, W.G.; D'Alessandro, A.; Wong, H.; Blanc, P.D. Early pulmonary cytokine responses to zinc oxide fume inhalation. *Environ. Res.* **1997**, *75*, 7–11. [CrossRef]
14. Fine, J.M.; Gordon, T.; Chen, L.C.; Kinney, P.; Falcone, G.; Beckett, W.S. Metal fume fever: Characterization of clinical and plasma IL-6 responses in controlled human exposures to zinc oxide fume at and below the threshold limit value. *J. Occup. Environ. Med.* **1997**, *39*, 722–726. [CrossRef]
15. Baumann, R.; Brand, P.; Chaker, A.; Markert, A.; Rack, I.; Davatgarbenam, S.; Joraslafsky, S.; Gerhards, B.; Kraus, T.; Gube, M. Human nasal mucosal C-reactive protein responses after inhalation of ultrafine welding fume particles: Positive correlation to systemic C-reactive protein responses. *Nanotoxicology* **2018**, *12*, 1130–1147. [CrossRef]
16. Baumann, R.; Gube, M.; Markert, A.; Davatgarbenam, S.; Kossack, V.; Gerhards, B.; Kraus, T.; Brand, P. Systemic serum amyloid A as a biomarker for exposure to zinc and/or copper-containing metal fumes. *J. Expo. Sci. Environ. Epidemiol.* **2018**, *28*, 84–91. [CrossRef]
17. Baumann, R.; Joraslafsky, S.; Markert, A.; Rack, I.; Davatgarbenam, S.; Kossack, V.; Gerhards, B.; Kraus, T.; Brand, P.; Gube, M. IL-6, a central acute-phase mediator, as an early biomarker for exposure to zinc-based metal fumes. *Toxicology* **2016**, *373*, 63–73. [CrossRef]
18. Markert, A.; Baumann, R.; Gerhards, B.; Gube, M.; Kossack, V.; Kraus, T.; Brand, P. Single and Combined Exposure to Zinc- and Copper-Containing Welding Fumes Lead to Asymptomatic Systemic Inflammation. *J. Occup. Environ. Med.* **2016**, *58*, 127–132. [CrossRef]
19. Krabbe, J.; Beilmann, V.; Gerhards, B.; Markert, A.; Thomas, K.; Kraus, T.; Brand, P. The Effects of Repeated Exposure to Zinc- and Copper-Containing Welding Fumes on Healthy Volunteers. *J. Occup. Environ. Med.* **2019**, *61*, 8–15. [CrossRef]
20. Michael, S.; Montag, M.; Dott, W. Pro-inflammatory effects and oxidative stress in lung macrophages and epithelial cells induced by ambient particulate matter. *Environ. Pollut.* **2013**, *183*, 19–29. [CrossRef]
21. Scheurer, T.; Steffens, J.; Markert, A.; Du Marchie Sarvaas, M.; Roderburg, C.; Rink, L.; Tacke, F.; Luedde, T.; Kraus, T.; Baumann, R. The human long noncoding RNAs CoroMarker, MALAT1, CDR1as, and LINC00460 in whole blood of individuals after controlled short-term exposure with ultrafine metal fume particles at workplace conditions, and in human macrophages in vitro. *J. Occup. Med. Toxicol.* **2022**, *17*, 15. [CrossRef]
22. Steffens, J.; Michael, S.; Kuth, K.; Hollert, H.; Du Marchie Sarvaas, M.; Nesic, A.; Kraus, T.; Baumann, R. Occupationally Relevant Zinc- and Copper-Containing Metal Fumes Inhibit Human THP-1 Macrophage TNF and IL-6 Responses to Bacterial Stimuli. *Glob. Chall.* **2024**, 2400302. [CrossRef]
23. Lanone, S.; Rogerieux, F.; Geys, J.; Dupont, A.; Maillot-Marechal, E.; Boczkowski, J.; Lacroix, G.; Hoet, P. Comparative toxicity of 24 manufactured nanoparticles in human alveolar epithelial and macrophage cell lines. *Part. Fibre Toxicol.* **2009**, *6*, 14. [CrossRef]
24. Cho, W.S.; Duffin, R.; Howie, S.E.; Scotton, C.J.; Wallace, W.A.; Macnee, W.; Bradley, M.; Megson, I.L.; Donaldson, K. Progressive severe lung injury by zinc oxide nanoparticles; the role of Zn2+ dissolution inside lysosomes. *Part. Fibre Toxicol.* **2011**, *8*, 27. [CrossRef]
25. Krabbe, J.; Kraus, T.; Krabbe, H.; Martin, C.; Ziegler, P. Welding Fume Instillation in Isolated Perfused Mouse Lungs-Effects of Zinc- and Copper-Containing Welding Fumes. *Int. J. Mol. Sci.* **2022**, *23*, 9052. [CrossRef]
26. Tokac, D.; Anlar, H.G.; Bacanli, M.; Dilsiz, S.A.; Iritas, S.; Basaran, N. Oxidative stress status of Turkish welders. *Toxicol. Ind. Health* **2020**, *36*, 263–271. [CrossRef]
27. Bayat, M.; Daei, S.; Ziamajidi, N.; Abbasalipourkabir, R.; Nourian, A. The protective effects of vitamins A, C, and E on zinc oxide nanoparticles (ZnO NPs)-induced liver oxidative stress in male Wistar rats. *Drug Chem. Toxicol.* **2023**, *46*, 209–218. [CrossRef]
28. Gupta, G.; Cappellini, F.; Farcal, L.; Gornati, R.; Bernardini, G.; Fadeel, B. Copper oxide nanoparticles trigger macrophage cell death with misfolding of Cu/Zn superoxide dismutase 1 (SOD1). *Part. Fibre Toxicol.* **2022**, *19*, 33. [CrossRef]
29. Castell, J.V.; Gomez-Lechon, M.J.; David, M.; Andus, T.; Geiger, T.; Trullenque, R.; Fabra, R.; Heinrich, P.C. Interleukin-6 is the major regulator of acute phase protein synthesis in adult human hepatocytes. *FEBS Lett.* **1989**, *242*, 237–239. [CrossRef]
30. Wolpe, S.D.; Davatelis, G.; Sherry, B.; Beutler, B.; Hesse, D.G.; Nguyen, H.T.; Moldawer, L.L.; Nathan, C.F.; Lowry, S.F.; Cerami, A. Macrophages secrete a novel heparin-binding protein with inflammatory and neutrophil chemokinetic properties. *J. Exp. Med.* **1988**, *167*, 570–581. [CrossRef]
31. Menten, P.; Wuyts, A.; Van Damme, J. Macrophage inflammatory protein-1. *Cytokine Growth Factor Rev.* **2002**, *13*, 455–481. [CrossRef]

32. Capelli, A.; Di Stefano, A.; Gnemmi, I.; Balbo, P.; Cerutti, C.G.; Balbi, B.; Lusuardi, M.; Donner, C.F. Increased MCP-1 and MIP-1beta in bronchoalveolar lavage fluid of chronic bronchitics. *Eur. Respir. J.* **1999**, *14*, 160–165. [CrossRef]
33. Elfsmark, L.; Ekstrand-Hammarstrom, B.; Forsgren, N.; Lejon, C.; Hagglund, L.; Wingfors, H. Characterization of toxicological effects of complex nano-sized metal particles using in vitro human cell and whole blood model systems. *J. Appl. Toxicol.* **2022**, *42*, 203–215. [CrossRef]
34. McCarrick, S.; Karlsson, H.L.; Carlander, U. Modelled lung deposition and retention of welding fume particles in occupational scenarios: A comparison to doses used in vitro. *Arch. Toxicol.* **2022**, *96*, 969–985. [CrossRef]
35. IARC. *Welding, Molybdenum Trioxide, and Indium Tin Oxide*; International Agency for Research on Cancer: Lyon, France, 2018.
36. Uski, O.J.; Rankin, G.D.; Wingfors, H.; Magnusson, R.; Boman, C.; Muala, A.; Blomberg, A.; Bosson, J.; Sandstrom, T. In vitro toxicity evaluation in A549 cells of diesel particulate matter from two different particle sampling systems and several resuspension media. *J. Appl. Toxicol.* **2024**, *44*, 1269–1278. [CrossRef]
37. Kumarathasan, P.; Nazemof, N.; Blais, E.; Syama, K.P.; Breznan, D.; Dirieh, Y.; Aoki, H.; Phanse, S.; Tayabali, A.; Babu, M. In Vitro Exposure of A549 and J774A.1 Cells to SiO_2 and TiO_2 Nanoforms and Related Cellular- and Molecular-Level Effects: Application of Proteomics. *J. Proteome Res.* **2025**, *24*, 1672–1687. [CrossRef]
38. Nazemof, N.; Breznan, D.; Dirieh, Y.; Blais, E.; Johnston, L.J.; Tayabali, A.F.; Gomes, J.; Kumarathasan, P. Cytotoxic Potencies of Zinc Oxide Nanoforms in A549 and J774 Cells. *Nanomaterials* **2024**, *14*, 1601. [CrossRef]
39. Cooper, J.R.; Abdullatif, M.B.; Burnett, E.C.; Kempsell, K.E.; Conforti, F.; Tolley, H.; Collins, J.E.; Davies, D.E. Long Term Culture of the A549 Cancer Cell Line Promotes Multilamellar Body Formation and Differentiation towards an Alveolar Type II Pneumocyte Phenotype. *PLoS ONE* **2016**, *11*, e0164438. [CrossRef]
40. Karlsson, H.L.; Cronholm, P.; Gustafsson, J.; Moller, L. Copper oxide nanoparticles are highly toxic: A comparison between metal oxide nanoparticles and carbon nanotubes. *Chem. Res. Toxicol.* **2008**, *21*, 1726–1732. [CrossRef]
41. Arodin Selenius, L.; Wallenberg Lundgren, M.; Jawad, R.; Danielsson, O.; Bjornstedt, M. The Cell Culture Medium Affects Growth, Phenotype Expression and the Response to Selenium Cytotoxicity in A549 and HepG2 Cells. *Antioxidants* **2019**, *8*, 130. [CrossRef]
42. McCord, J.M.; Fridovich, I. Superoxide dismutase: An enzymic function for erythrocuprein (hemocuprein). *J. Biol. Chem.* **1969**, *244*, 6049–6055. [CrossRef]
43. Nazem, M.R.; Asadi, M.; Jabbari, N.; Allameh, A. Effects of zinc supplementation on superoxide dismutase activity and gene expression, and metabolic parameters in overweight type 2 diabetes patients: A randomized, double-blind, controlled trial. *Clin. Biochem.* **2019**, *69*, 15–20. [CrossRef]
44. Aranda, A.; Sequedo, L.; Tolosa, L.; Quintas, G.; Burello, E.; Castell, J.V.; Gombau, L. Dichloro-dihydro-fluorescein diacetate (DCFH-DA) assay: A quantitative method for oxidative stress assessment of nanoparticle-treated cells. *Toxicol. Vitr.* **2013**, *27*, 954–963. [CrossRef]
45. Murphy, M.P.; Bayir, H.; Belousov, V.; Chang, C.J.; Davies, K.J.A.; Davies, M.J.; Dick, T.P.; Finkel, T.; Forman, H.J.; Janssen-Heininger, Y.; et al. Guidelines for measuring reactive oxygen species and oxidative damage in cells and in vivo. *Nat. Metab.* **2022**, *4*, 651–662. [CrossRef]
46. Eleutherio, E.C.A.; Silva Magalhaes, R.S.; de Araujo Brasil, A.; Monteiro Neto, J.R.; de Holanda Paranhos, L. SOD1, more than just an antioxidant. *Arch. Biochem. Biophys.* **2021**, *697*, 108701. [CrossRef]
47. Grishaeva, A.; Ponezheva, Z.; Chanyshev, M.; Ploskireva, A.; Usenko, D.; Tsvetkova, N.; Omarova, K.; Pshenichnaya, N. MIP-1a and MIP-1b in serum as potential markers of the severe course COVID-19. *Int. J. Infect. Dis.* **2022**, *116*, S44. [CrossRef]
48. Hirota, M.; Moro, O. MIP-1beta, a novel biomarker for in vitro sensitization test using human monocytic cell line. *Toxicol. Vitr.* **2006**, *20*, 736–742. [CrossRef]
49. Sindhu, S.; Kochumon, S.; Shenouda, S.; Wilson, A.; Al-Mulla, F.; Ahmad, R. The Cooperative Induction of CCL4 in Human Monocytic Cells by TNF-α and Palmitate Requires MyD88 and Involves MAPK/NF-κB Signaling Pathways. *Int. J. Mol. Sci.* **2019**, *20*, 4658. [CrossRef]
50. Kochumon, S.; Wilson, A.; Chandy, B.; Shenouda, S.; Tuomilehto, J.; Sindhu, S.; Ahmad, R. Palmitate Activates CCL4 Expression in Human Monocytic Cells via TLR4/MyD88 Dependent Activation of NF-κB/MAPK/ PI3K Signaling Systems. *Cell. Physiol. Biochem.* **2018**, *46*, 953–964. [CrossRef]
51. Ahmad, R.; Kochumon, S.; Chandy, B.; Shenouda, S.; Koshy, M.; Hasan, A.; Arefanian, H.; Al-Mulla, F.; Sindhu, S. TNF-α Drives the CCL4 Expression in Human Monocytic Cells: Involvement of the SAPK/JNK and NF-κB Signaling Pathways. *Cell. Physiol. Biochem.* **2019**, *52*, 908–921. [CrossRef]
52. Bocca, B.; Battistini, B. Biomarkers of exposure and effect in human biomonitoring of metal-based nanomaterials: Their use in primary prevention and health surveillance. *Nanotoxicology* **2024**, *18*, 1–35. [CrossRef]
53. Buonaurio, F.; Astolfi, M.L.; Pigini, D.; Tranfo, G.; Canepari, S.; Pietroiusti, A.; D'Alessandro, I.; Sisto, R. Oxidative Stress Biomarkers in Urine of Metal Carpentry Workers Can Be Diagnostic for Occupational Exposure to Low Level of Welding Fumes from Associated Metals. *Cancers* **2021**, *13*, 3167. [CrossRef]

54. Corradi, M.; Gergelova, P.; Mutti, A. Use of exhaled breath condensate to investigate occupational lung diseases. *Curr. Opin. Allergy Clin. Immunol.* **2010**, *10*, 93–98. [CrossRef]
55. Palmer, K.T.; McNeill Love, R.M.; Poole, J.R.; Coggon, D.; Frew, A.J.; Linaker, C.H.; Shute, J.K. Inflammatory responses to the occupational inhalation of metal fume. *Eur. Respir. J.* **2006**, *27*, 366–373. [CrossRef]
56. Hartmann, L.; Bauer, M.; Bertram, J.; Gube, M.; Lenz, K.; Reisgen, U.; Schettgen, T.; Kraus, T.; Brand, P. Assessment of the biological effects of welding fumes emitted from metal inert gas welding processes of aluminium and zinc-plated materials in humans. *Int. J. Hyg. Environ. Health* **2014**, *217*, 160–168. [CrossRef]
57. Koh, D.H.; Kim, J.I.; Kim, K.H.; Yoo, S.W.; Korea Welders Cohort, G. Welding fume exposure and chronic obstructive pulmonary disease in welders. *Occup. Med.* **2015**, *65*, 72–77. [CrossRef]
58. Grahn, K.; Gustavsson, P.; Andersson, T.; Linden, A.; Hemmingsson, T.; Selander, J.; Wiebert, P. Occupational exposure to particles and increased risk of developing chronic obstructive pulmonary disease (COPD): A population-based cohort study in Stockholm, Sweden. *Environ. Res.* **2021**, *200*, 111739. [CrossRef]
59. Krabbe, J.; Esser, A.; Kanzler, S.; Braunschweig, T.; Kintsler, S.; Spillner, J.; Schroder, T.; Kalverkamp, S.; Balakirski, G.; Gerhards, B.; et al. The effects of zinc- and copper-containing welding fumes on murine, rat and human precision-cut lung slices. *J. Trace Elem. Med. Biol.* **2018**, *49*, 192–201. [CrossRef]
60. Johnson, B.D.; Kip, K.E.; Marroquin, O.C.; Ridker, P.M.; Kelsey, S.F.; Shaw, L.J.; Pepine, C.J.; Sharaf, B.; Bairey Merz, C.N.; Sopko, G.; et al. Serum amyloid A as a predictor of coronary artery disease and cardiovascular outcome in women: The National Heart, Lung, and Blood Institute-Sponsored Women's Ischemia Syndrome Evaluation (WISE). *Circulation* **2004**, *109*, 726–732. [CrossRef]
61. Dong, Z.; Wu, T.; Qin, W.; An, C.; Wang, Z.; Zhang, M.; Zhang, Y.; Zhang, C.; An, F. Serum amyloid A directly accelerates the progression of atherosclerosis in apolipoprotein E-deficient mice. *Mol. Med.* **2011**, *17*, 1357–1364. [CrossRef]
62. Thompson, J.C.; Jayne, C.; Thompson, J.; Wilson, P.G.; Yoder, M.H.; Webb, N.; Tannock, L.R. A brief elevation of serum amyloid A is sufficient to increase atherosclerosis. *J. Lipid Res.* **2015**, *56*, 286–293. [CrossRef]
63. Taj, T.; Gliga, A.R.; Hedmer, M.; Wahlberg, K.; Assarsson, E.; Lundh, T.; Tinnerberg, H.; Albin, M.; Broberg, K. Effect of welding fumes on the cardiovascular system: A six-year longitudinal study. *Scand. J. Work Environ. Health* **2021**, *47*, 52–61. [CrossRef] [PubMed]
64. GESTIS. Institut fur Arbeitsschutz der Deutschen Gesetzlichen Unfallversicherung IFA GESTIS International Limit Values. Available online: https://ilv.ifa.dguv.de/substances (accessed on 1 March 2025).
65. Dauter, U.M.; Gliga, A.R.; Albin, M.; Broberg, K. Longitudinal changes in cardiovascular disease-related proteins in welders. *Int. Arch. Occup. Environ. Health* **2024**, *97*, 803–812. [CrossRef] [PubMed]
66. Tatara, Y.; Ohishi, M.; Yamamoto, K.; Shiota, A.; Hayashi, N.; Iwamoto, Y.; Takeda, M.; Takagi, T.; Katsuya, T.; Ogihara, T.; et al. Macrophage inflammatory protein-1beta induced cell adhesion with increased intracellular reactive oxygen species. *J. Mol. Cell. Cardiol.* **2009**, *47*, 104–111. [CrossRef] [PubMed]
67. Chang, T.-T.; Yang, H.-Y.; Chen, C.; Chen, J.-W. CCL4 Inhibition in Atherosclerosis: Effects on Plaque Stability, Endothelial Cell Adhesiveness, and Macrophages Activation. *Int. J. Mol. Sci.* **2020**, *21*, 6567. [CrossRef]
68. Zhang, Y.; Zhang, J.; Sheng, H.; Li, H.; Wang, R. Acute phase reactant serum amyloid A in inflammation and other diseases. *Adv. Clin. Chem.* **2019**, *90*, 25–80. [CrossRef]
69. *German Version EN 10346:2015*; Continuously Hot-Dip Coated Steel Flat Products for Cold Forming—Technical Delivery Conditions. DINMedia: Berlin, Germany, 2015. Available online: https://www.dinmedia.de/de/norm/din-en-10346/226007287 (accessed on 1 March 2025).
70. *German version EN ISO 24373:2018*; Welding Consumables—Solid Wires and Rods For Fusion Welding of Copper and Copper Alloys—Classification (ISO 24373:2018). DINMedia: Berlin, Germany, 2018. Available online: https://www.dinmedia.de/de/norm/din-en-iso-24373/295232973 (accessed on 1 March 2025).
71. Oda, Y.; Nakamura, S.; Oki, I.; Kato, T.; Shinagawa, H. Evaluation of the new system (umu-test) for the detection of environmental mutagens and carcinogens. *Mutat. Res. Environ. Mutagen. Relat. Subj.* **1985**, *147*, 219–229. [CrossRef]
72. *DIN 38415-3:1996-12*; German Standard Methods for the Examination of Water, Waste Water and Sludge—Sub-Animal Testing (Group T)—Part 3: Determination of the Genotype Potential of Water and Waste Water Components with the Umu-Test (T 3). DINMedia: Berlin, Germany, 1996. Available online: https://www.dinmedia.de/de/norm/din-38415-3/2902871 (accessed on 1 March 2025).
73. *ISO 13829:2000-03*; Water Quality—Determination of the Genotoxicity of Water and Waste Water Using the Umu-Test. DINMedia: Berlin, Germany, 2000. Available online: https://www.dinmedia.de/de/norm/iso-13829/33008417 (accessed on 1 March 2025).

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Hamburg, im Mai 2025

Erklärung § 5 Abs. 1 zur Datenaufbewahrung

Hiermit erkläre ich, dass die dieser Dissertation zu Grunde liegenden Originaldaten im Institut für Arbeits-, Sozial- und Umweltmedizin des Universitätsklinikums Aachen hinterlegt sind.

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Jan Steffens, M.Sc.

Erklärung gemäß § 5 Abs. (1) und (2), und § 11 Abs. (3) 12. der Promotionsordnung

Hiermit erkläre ich, **Jan Steffens,** an Eides statt, dass ich den wesentlichen Anteil an der Publikation:

Steffens, J., Michael, S., Kuth, K., Hollert, H., Du Marchie Sarvaas, M., Nesic, A., Kraus, T., & Baumann, R. (2025). Occupationally relevant zinc- and copper- containing metal fumes inhibit human THP-1 macrophage TNF and IL-6 responses to bacterial stimuli. *Global Challenges, 9*(5), 2400302. https://doi.org/10.1002/gch2.202400302

geleistet habe.

Die Anteile an der Arbeit waren wie folgt:

	Steffens, J.	Michael, S.	Kuth, K.	Hollert, H.	Du Marchie Sarvaas, M.	Nesic, A.	Kraus, T.	Bauman n, R.	Summe (%)
Konzeption	30	5	5		5		5	50	100
Formale Analyse (Statistik)	80							20	100
Untersuchung (Experimente)	100								100
Datenerhebung/-aufbereitung	90				10				100
Interpretation der Daten	70							30	100
Visualisierung der Daten	70							30	100
Verfassung des Manuskripts	**100**								100
Korrektur des Manuskripts	25	5				5	5	60	100
Betreuung/Supervision				5			15	80	100
Projektadministration	10			5			20	65	100
Bereitstellung von Mitteln							60	40	100

Aus diesem wesentlichen Anteil ergibt sich selbstverständlich die Stellung als Erstautor.

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Jan Steffens, M. Sc.

Als Doktorvater bestätige ich die Angaben von Jan Steffens.

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Prof. Ralf Baumann, Ph.D.

Ich schließe mich der Erklärung von Privatdozent Dr. Ralf Baumann als Koautor*in an.

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Erklärung gemäß § 5 Abs. (1) und (2), und § 11 Abs. (3) 12. der Promotionsordnung

Hiermit erkläre ich, **Jan Steffens,** an Eides statt, dass ich den wesentlichen Anteil an der Publikation:

Steffens, J., Kuth, K., Kraus, T., Dott, W., Michael, S., & Baumann, R. (2025). Inflammatory responses to Zn/Cu-containing welding fume in human alveolar epithelial and macrophage cell lines, with MIP-1β/CCL4 as a much more sensitive macrophage activation marker than IL-8 and TNF-α. International Journal of Molecular Sciences, 26(8), 3843. https://doi.org/10.3390/ijms26083843

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	Steffens, J.	Kuth, K.	Kraus, T.	Dott, W.	Michael, S.	Baumann, R.	Summe (%)
Konzeption	5	5	15	5	35	35	100
Formale Analyse (Statistik)	70	10			10	10	100
Untersuchung (Experimente)	60	30			10		100
Datenerhebung/-aufbereitung	60	30			5	5	100
Interpretation der Daten	60	5			15	20	100
Visualisierung der Daten	70	30					100
Verfassung des Manuskripts	**100**						100
Korrektur des Manuskripts	40	5			15	40	100
Betreuung/Supervision			10	5	40	45	100
Projektadministration	10		20	10	30	30	100
Bereitstellen von Mitteln			30	25	20	25	100

Aus diesem wesentlichen Anteil ergibt sich selbstverständlich die Stellung als Erstautor.

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