COMPARISON OF NICKEL TOXICITY IN AIR-LIQUID INTERFACE MODELS OF HUMAN AND RAT BRONCHIAL EPITHELIAL CELLS

NiPERA
NICKEL HEALTH AND ENVIRONMENTAL SCIENCES

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INTRODUCTION

Ni is used in many applications, from stainless steel to batteries. Historically, Ni ore refining has been associated with increase in lung and nasal cancers[2].

Robust databases of human and animal data are available for Ni and Ni compounds to support refined risk assessments[2,3]. However, uncertainties exist about the comparability of toxicity between soluble and insoluble Ni compounds, and between animals and humans.

3D respiratory cell models with ALI exposures reduce animal use, are more realistic *in vitro* test systems that mimic *in vivo* inhalation, and allow the correlation of responses in animals to human tissues, potentially bridging any toxicity gap.

This pilot study using two Ni compounds in rat and human MucilAir models was conducted to address the rat-to-human toxicity comparability gap and investigate the differential sensitivity to toxicity triggered by the two Ni compounds.

METHODS

$NiSO_4.6H_2O$ (22% Ni); Ni_3S_2 (73% Ni)

Vitrocell® system used for cell exposures. MucilAir models in PET (-HF) or polycarbonate (-RF) membranes were purchased from Epithelix (Geneva, Switzerland). 24 h after the 6 h exposures, TEER, LDH and IL-6 were measured.

Test atmospheres of $NiSO_4.6H_2O$ solution and Ni_3S_2 dry powder were generated using ultrasonic mesh nebulizer in a stream of compressed dry air (10.7 l/min) and turntable dust feeder, respectively.

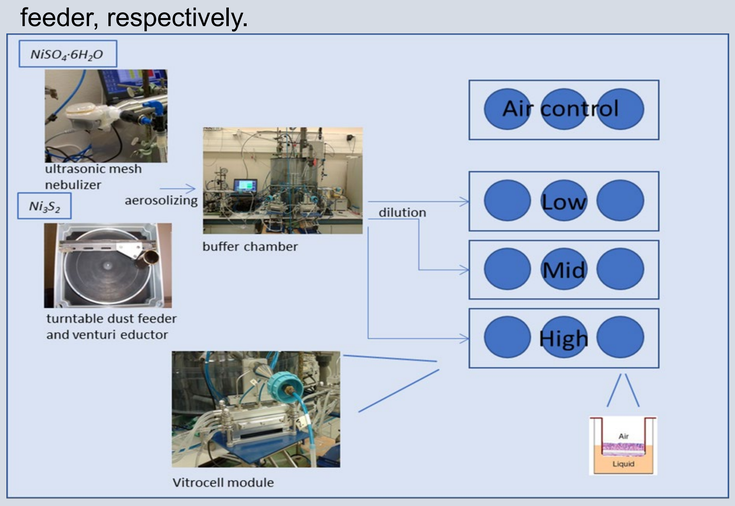

FIGURE 1. SCHEMATIC OF THE SETUP FOR AIR EXPOSURES

ABSTRACT

Nickel (Ni) compounds are indirect genotoxic carcinogens with threshold mode-of-action. Both Ni subsulfide (Ni_3S_2) and Ni sulfate hexahydrate ($NiSO_4.6H_2O$) are classified as human carcinogens, but only Ni_3S_2 induces tumors in rodents. To study the carcinogenic mode of action of both Ni compounds, we conducted studies in Air-Liquid Interface (ALI) models of rat and human bronchial epithelial cells. Rat (MucilAir-RF) and human (MucilAir-HF) MucilAir models were exposed to Ni_3S_2 and $NiSO_4.6H_2O$ via droplet or air exposures, and transepithelial electrical resistance [TEER] (membrane integrity), lactate dehydrogenase [LDH] (cytotoxicity) and IL-6 (inflammation) were measured.

In the droplet exposures, $NiSO_4.6H_2O$ caused more cytotoxicity than Ni_3S_2 in MucilAir-RF (data not shown). Both compounds decreased membrane integrity and increased inflammatory cytokine IL-6 in a concentration-dependent manner.

In the air exposures, both compounds decreased membrane integrity, increased cytotoxicity, and inflammatory cytokine production. At comparable mg/m^3 levels, $NiSO_4.6H_2O$ caused a greater decrease in TEER and a greater increase in inflammatory IL-6 than Ni_3S_2. These results are consistent with *in vivo* rodent studies, where at equal mg/m^3 or mg Ni/m^3 exposure, $NiSO_4$ triggered a higher increase in the expression of an inflammatory cytokine than Ni_3S_2[1]. Higher intracellular levels of Ni_3S_2 than NiSO4, particularly in the nucleus, were observed, in agreement with previous cell studies.

THE MUCILAIR MODELS REPRODUCED THE PATTERN OF TOXICITY OBSERVED WITH THESE TWO Ni COMPOUNDS *IN VIVO*.

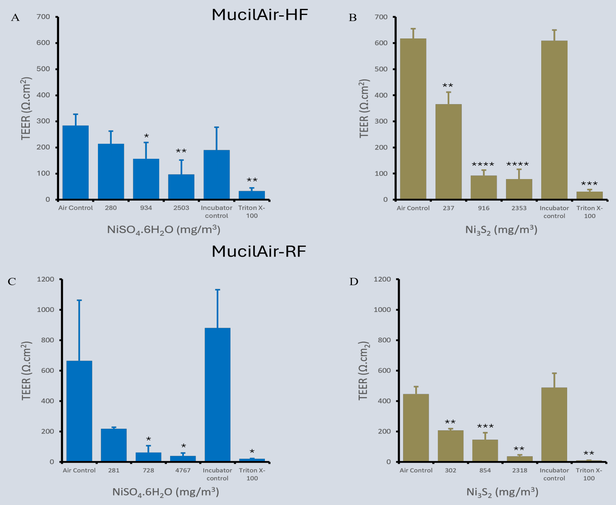

FIGURE 3. MEMBRANE INTEGRITY AFTER AIR EXPOSURE TO $NiSO_4.6H_2O$ AND Ni_3S_2

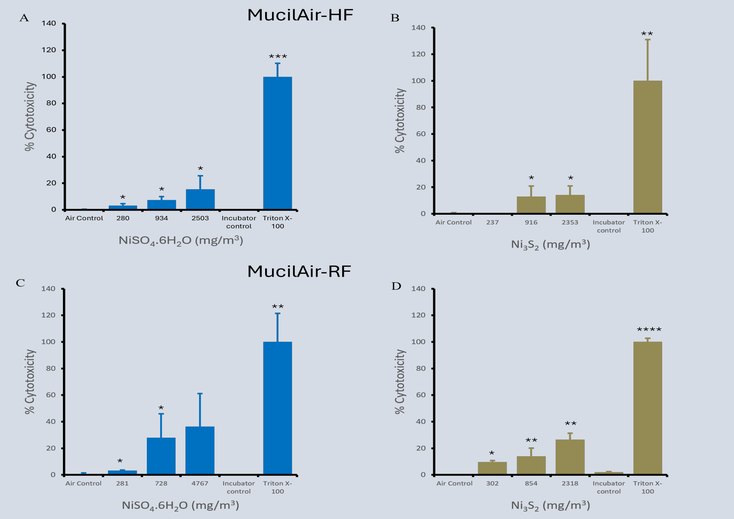

FIGURE 4. CYTOTOXICITY OF $NiSO_4.6H_2O$ AND Ni_3S_2 AFTER AIR EXPOSURE

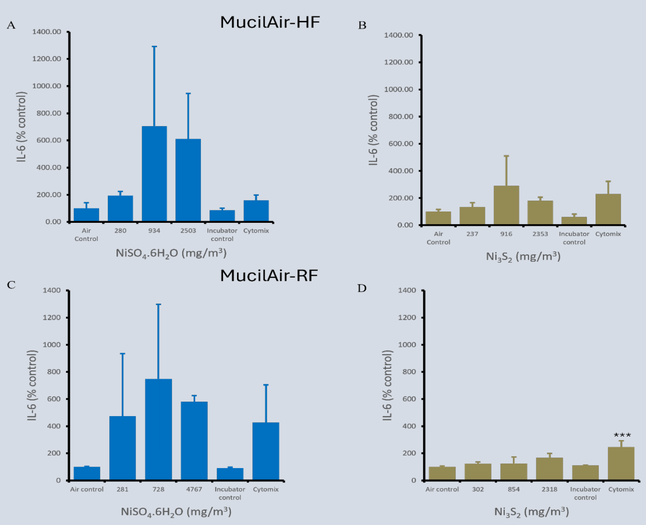

FIGURE 5. IL-6 AS A MEASURE OF INFLAMMATORY RESPONSE TO $NiSO_4.6H_2O$ AND Ni_3S_2

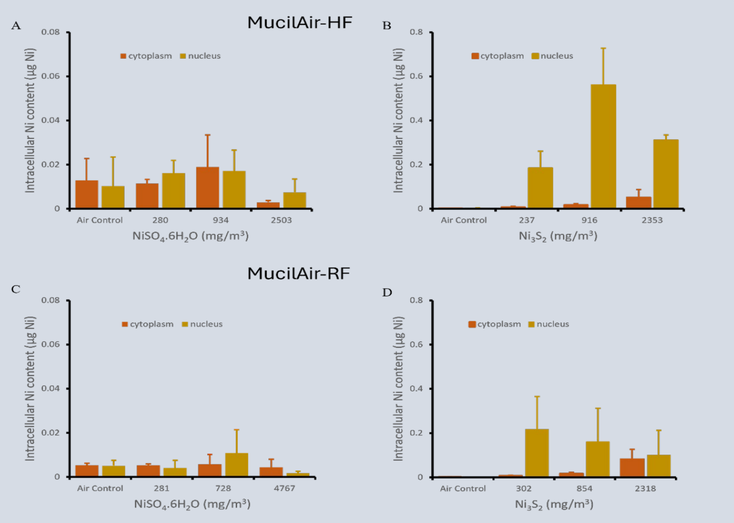

FIGURE 6. INTRACELLULAR Ni CONTENT AFTER AIR EXPOSURES TO $NiSO_4.6H_2O$ AND Ni_3S_2

RESULTS

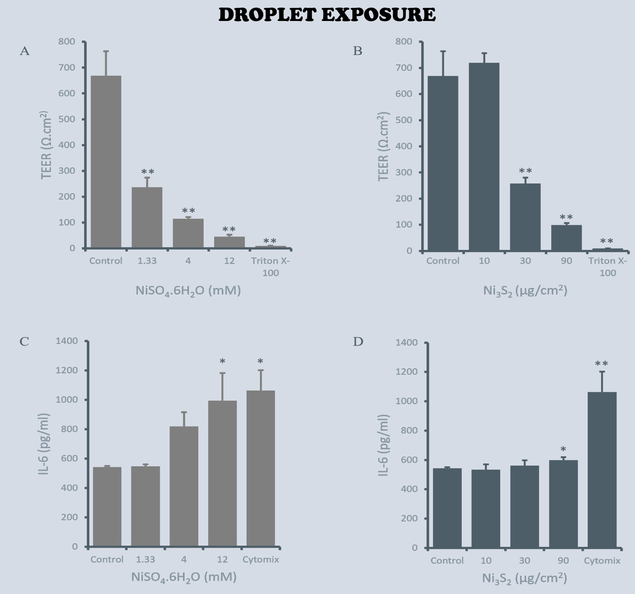

FIGURE 2. TEER & IL-6 RESPONSE AFTER DROPLET EXPOSURE TO $NiSO_4.6H_2O$ AND Ni_3S_2

TABLE 1. CORRELATION BETWEEN TARGET AND ACTUAL AIR EXPOSURE CONCENTRATIONS

MucilAir origin	Batch nr	Ni exposure	Measurement method	Actual concentration (mg/m³) Low [mg Ni/m³]	Mid [mg Ni/m³]	High [mg Ni/m³]	Target conc. (mg/m³)
Rat	RF-MD0025	$NiSO_4.6H_2O$	APS	281 [62]	728 [160]	4767 [1049]	489, 1468, 4407
Human	HF-MD059401	$NiSO_4.6H_2O$	APS	280 [62]	934 [205]	2503 [551]	
Human	HF- MD059401	Ni_3S_2	APS	237 [173]	916 [669]	2352 [1717]	297, 890, 2670
Rat	RF-MucilAir Rat	Ni_3S_2	Cascade Impactor	302 [220]	854 [623]	2318 [1692]	

Actual air concentrations were 50-60% of target concentrations for $NiSO_4.6H_2O$ and ≥80% for Ni_3S_2.

MMAD ~3.58 µm & GSD ~1.60 $NiSO_4.6H_2O$, & 3.15 µm & 1.60 for Ni_3S_2.

CONCLUSIONS

The rat MucilAir model was more sensitive to the effects of the Ni compounds than the human MucilAir model, with $NiSO_4.6H_2O$ generally having greater effects than Ni_3S_2. Both models yielded results consistent with the toxicity of the two Ni compounds observed *in vivo,* and support the bioavailability model of Ni carcinogenesis.

This pilot study shows that the rat and human MucilAir models are viable for studying the toxicity of Ni compounds. Studies with these models can help bridge the gap between the animal and human mechanisms of toxicity of Ni compounds.

1. Efremenko AY, Campbell JL, Dodd DE, Oller AR, Clewell HJ 3rd. Time- and concentration-dependent genomic responses of the rat airway to inhaled nickel sulfate. Environ Mol Mutagen. 2017 Oct;58(8):607-618. doi: 10.1002/em.22139.
2. Buxton S, Garman E, Heim KE, Lyons-Darden T, Schlekat CE, Taylor MD, et al. Concise review of nickel human health toxicology and ecotoxicology. Inorganics. 2019;7(7).
3. Oller AR, Buxton S, March TH, Benson JM. Comparative pulmonary and genotoxic responses to inhaled nickel subsulfide and nickel sulfate in F344 rats. J Appl Toxicol. 2022;2022(October):1–18.