



Linking nanoparticle exposure to pulmonary fibrosis and mortality:

Evaluating the key messages of Song et. al.

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A study published in the forthcoming issue of the European Respiratory Journal (ERJ), has for the first time claimed a concrete link between exposure to nanoparticles in adhesive paint and development of severe pulmonary fibrosis in a group of young female workers; two of whom went on to suffer fatal lung failure.

In this special SAFENANO Feature, we attempt to break down & present concisely the evidence reported in this case, including some of the key aspects of the workplace investigated, the materials used, and the symptoms presenting in the workers who became ill. In addition, we consider whether Song and his Beijing-based team of researchers are justified in making a causal link between occupational nanoparticle exposure and the resulting illness seen in the workers.

Through this summary of the paper's findings and key issues, SAFENANO hope that you as the reader will be assisted in fully considering the evidence presented, and feel somewhat more able to form their own balanced conclusions about its ability to link nanoparticle exposure to such catastrophic health effects.

The Workplace

Beginning with an overview of the conditions in which the workers were employed, it is immediately clear that the occupational hygiene of the workplace reported was, at best, extremely poor.

The workspace

The room in which the employees were working was 70m², with 1 door and no windows. The room housed one machine, which was used to air spray materials, heat and dry boards. No mention of any specific ventilation system (separate to that which may be on the machine used) was made. Although it was the only source of ventilation in the room the door was routinely kept closed due to the cold outside temperatures.

Ventilation

A gas exhaust system was present on the machine used in the industrial paint spraying process; however the exhaust on the machine itself had been reported as broken for 5 months prior to the hospitalisation of the workers. There is no further description of the exhaust system or how it works.

Based on the above, the researchers concluded the gas flow within the workplace indoor air would be very slow, or even quiescent.

The Industrial Process

The workplace was reported to be a print plant, in which a machine was used to coat polystyrene boards (organic glass) with an ivory white coating mixture of polyacrylic ester. The composition of this coating mixture and its characterisation (based on that performed by the researchers) is discussed further within The Materials section.

The coating mixture was loaded by hand at room temperature into the open bottom pan of the machine using a spoon. From here, it was sprayed at a pressure of 100-120kPa onto polystyrene boards of 0.5-1m² via 3 atomising spray nozzles. All handling of the boards (including clipping into place, heating, and handling) was undertaken by the workers manually.



The boards were then heated and dried at 75-100°C, and any smoke produced during this process was in theory to be removed by the exhaust system (which as mentioned previously was non-functional). The finished coated boards were used in the painting and decorating industry.

On average, 6kg of coating mixture and 5000m² of the board were used per day.

PPE available and in use

No PPE was used on a routine basis by the workers. Occasionally, cotton gauze masks were utilised. Although the decision-making process behind this intermittent use was not described, the researchers note that the workers reported regular presence of 'flocculi produced during air spraying, which caused itching on their faces and arms'.

The Employees

Eight workers were employed in the paint plant in total – 7 females and 1 male. The 7 females (all of whom presented with symptoms) had been employed for a period of between 5 & 12 months, and the 1 male (who was asymptomatic) had been employed for only 3 months.

The female workers were between 18 and 47 years of age (average 29). Two were single, and 5 married. Their working history (although self-reported and not extensively documented in the paper) was house work or farm work related.

Prior to the onset of the industrial related illness, all had (self) reported to be in generally good health, were non-smokers and claimed not to have suffered any knowing prior occupational exposure to hazardous materials.

The Materials in use

The material in use is referred to as a 'polyacrylate-based adhesive paint' within the paper. The authors report that this was described as 'polyacrylic ester' by the paste producer (who is not named). No discussion on the process by which this paste was synthesised was given/available.

In their evaluation, the researchers examined both this paste, and accumulated dust particles collected from the intake of the gas exhauster.

The chemical composition of the paste was investigated using gas chromatography and mass spectroscopy. Those chemicals identified to be within the composition are listed within Box 1.

Further information on the chemical composition, activity and known health effects of each of the chemicals listed may be located via the links in Box 1. There was no discussion of the possible mixture toxicity of the chemicals within the paste in the context of the occupational exposure.

Box 1: Chemical Composition of the Paste

- [butanoic acid](#),
- [butyl ester](#),
- [N-butyl ether](#),
- [Acetic Acid](#),
- [Toluene](#),
- [di-tert-butyl peroxide](#),
- [1-butanol](#),
- [Acetic acid ethenyl ester](#),
- [Isopropyl Alcohol](#), and
- Ethylene dioxide*

Ethylene Dioxide is occasionally used in error to describe ethylene oxide, but it also occasionally is used as a synonym for 1,4 dioxane, a carcinogenic solvent. Diethylene oxide is more commonly used as a synonym. More information on 1,4 dioxane is available [here](#).



The paper's authors reported that 'Electron microscopy of both the paste and the dust particles found nanoparticles, 30 nm in diameter'. There is no specific reference in the paper as to how the particles within were confirmed as being polyacrylate. The nanoparticles are however described as 'polyacrylate nanoparticles' for the rest of the paper.

So what is polyacrylate to paint?...

The paper states that 'polyacrylate is widely used as an adhesive in the building, print and decoration fields, and has often been regarded as low toxicity' (no reference for this statement is provided). Further information on the use of polyacrylate within the coatings and paints industry is provided in Box 2, in a passage quoted Polyurethanes: coatings, adhesives and sealants by Ulrich Meier-Westhues.

...and why would it contain nanoparticles?

The authors discuss utilisation of functionalised nanoparticles (altered to change their surface chemistry from hydrophilic to organophilic) as additives to the organic resin. The resultant organic-inorganic hybrid makes materials stronger and more resistant to abrasion. Nanoparticulate additives reported to be used in this way from recent literature include TiO₂, zinc oxide, silver clusters, and silicon-containing polyacrylate nanoparticles (which the authors note are now widely used).

Box 2: Polyacrylate Dispersions in Paint

Passage from: Polyurethanes: coatings, adhesives and sealants, by Ulrich Meier-Westhues, in European coatings tech files, Vincentz Network GmbH & Co KG, 2007

'Hydroxyfunctional polyacrylate dispersions present an important class of compounds in the formulation of aqueous polyurethane coatings. Polyacrylate dispersions are differentiated into primary and secondary dispersions. Primary dispersions are produced directly in water via emulsion polymerisation of acrylate monomers with the use of emulsifiers. They are characterised by low molecular weights and are usually solvent-free.

Secondary dispersions on the other hand are prepared via polymerisation of the corresponding monomers in a suitable solvent. Following this step, a neutralising amine and water are added to this organic solution, whereby a phase reversal takes place with the formation of a dispersion. Secondary dispersions are characterised by moderate molecular weights and can contain up to 10% co solvent.

Polyacrylate secondary dispersions can be used to formulate aqueous two-component polyurethane coating systems that yield high quality paint films. In particular, properties such as gloss, chemical resistance and mechanical strength are at levels comparable to films yielded by solvent-borne two-component polyurethane coatings.'

The Health Effects Recorded

In the paper, it is clear that the researchers undertook a thorough clinical examination of the patients, via various investigatory techniques and tests. Some of the key findings for each system are outlined below:

Table 1: Key Health Effects Recorded

System Investigated	Findings / Observations
<i>Preliminary/Overall observations</i>	All patients presented with shortness of breath on exertion, symptoms consistent with pleural fluid (fluid in the lungs) (which was confirmed by ultrasound), & a rash with intense itching on their faces, hands and forearms. In addition, 5 of the 7 patients were found to have pericardial effusions (fluid around the heart), and 4/7 had hypoxaemia (low blood oxygen).
<i>Liver</i>	Alanine aminotransferase and aspartate aminotransferase levels were found to be elevated in several patients, both being associated with liver damage.
<i>Urine</i>	In all 7 patients urine tests were found to be normal.



Blood	In all 7 patients White blood Cell & Red Blood counts were found to be normal 5/7 patients had monocytosis (often associated with chronic inflammation), and neutropenia (lack of a certain type of white blood cell).
Kidney	Kidney Function was in general found to be within normal limits. However 6/7 had lowered levels of protein in the blood (hypoproteinemia) after 7 months of observation, a symptom often associated with kidney problems.
Immunological, viral and bacterial tests	In all 7 patients, immunological tests showed little abnormality, virology examinations showed no sign of viral infection, and bacterial investigation was also unable to identify any potential infection that could be linked to the symptoms exhibited.
Lungs	<p>Evaluation of lung function revealed small airway injury and restrictive ventilation dysfunction in all workers, and in 3/7 severe lung damage was reported.</p> <p>Imaging of the lungs confirmed pleural effusions, pulmonary interstitial inflammation and pulmonary fibrosis in all 7 patients..</p> <p>Re-examination at 7 months found that in the majority of patients this pulmonary interstitial fibrosis developed very slowly, but that in the two patients who later died rapid progressive pulmonary interstitial fibrosis was underway, and pleural calcification was also seen in one of the two.</p> <p>Evaluation of the pleural fluid (via thoracentesis) showed an amber exudate, which is commonly associated with chemical or physical irritation, however no cancerous cells were found.</p> <p>All patients had swelling and congestion of the tunica mucosa bronchiorum – the inner coating of the bronchi</p> <p>Inflammatory processes in the lungs were also supported by:</p> <ol style="list-style-type: none"> 1. lung biopsy, which showing aggregations of inflammatory cells within the lung 2. biopsies of the pleura and fluid in the chest, which showed fibrinous and inflammatory cells alongside haemorrhage and granulomas 3. Video assisted thorascopic surgery (VATS) conducted in 2 patients, which showed fibrinous thickening of the pleural membrane & emphysema-like alterations (amongst other symptoms).

Nanoparticles found within the workers' bodies...

According to the study, TEM analysis of lung tissue, pleural fluid, and bronchio-alveolar lavage fluid samples identified round nanoparticles 30nm in diameter.

Within the pleural fluid, these were 'wrapped up in a fibrinous structure' which the authors speculate may have originated from cytolysis in the fluid. In cells from the lung epithelium analysed, the NPs were seen to lodge in the cytoplasm and caryoplasm of cells. These cells exhibited morphologies characteristic of those undergoing apoptosis. In addition, the researchers noted that there was a massive aggregation of NPs around intravascular red blood cells in the pulmonary interstitial tissue.

No TEM images of the nanoparticles identified were provided in the paper on which to base further comment.



Patient outcomes

In all 7 patients, the clinical symptoms were unpleasant and similarly severe in nature. However, the final outcome varied between those affected.

Two patients suffered a pneumothorax (collapsed lung), but recovered quickly following treatment. Likewise, the dermal symptoms were treated successfully. After 20 months both still suffered from shortness breath and pleural effusions and slow progressive fibrosis.

The pleural effusions in two patients were so fast in development that 'continuous closed drainage of the thoracic cavity was necessary'. 18 and 21 months following onset of the symptoms respectively, the two patients went on to undergo emergency VATS, but suffered severe pneumothorax followed by respiratory failure soon after, resulting in death.

The final three patients, although not specifically discussed, were stable at the completion of the study.

Key Issues & Knowledge Gaps

So, given the evidence presented, what are the problems or knowledge gaps with the paper? Song and his team provide a good start to consideration of this, via the limitations noted within the paper's discussion section. Amongst these, they note that:

- *the description of the workers occupational and personal health histories is limited by the absence of environmental monitoring data of the workplace*
- *timely environmental monitoring of the workplace was lacking, and accurate concentrations of the polyacrylate nanoparticles that the workers were probably exposed to remain unknown*
- *the exact composition of the nanoparticles identified is still unknown. This is a data-gap which in the eyes of many will be a fundamental one.*

Having read the paper in detail, observations from myself and the rest of the SAFENANO team have highlighted the following additional issues, outstanding questions & data gaps:

- *The lack of any real data on the actual exposures the workers were subjected to is a major hampering issue in determining the cause of the illness*
- *Likewise, the lack of any detailed data on the amounts (mass, volume, concentration) of the nanoparticles within the paste mixture, dust sample and subsequent biological samples is a hurdle to the validity of the conclusions drawn.*
- *The a lack of adequate characterisation on the nanoparticles identified within the paste, dust and tissue samples forms a critical gap in the data in relation to of deciphering the cause of the health effects observed.*
- *Bar the statement that they were 30nm in diameter; no evidence is presented to confirm that the same NP type was identified in the dust, paste or biological sample and there is little consideration of whether the particles identified may be polyacrylic particles, an inorganic additive to the paste, perhaps (within the dust and biological samples) combustion derived particles, or even something totally different. We just don't know, and this remains one of the study's key data gaps.*
- *Despite the complex mixture of components to the paste revealed by chemical analysis, and historical reporting of acute onset of illness relating to exposure to such complex*



chemical mixtures, particularly in the presence of heat, no specific consideration or investigation of potential mixture toxicity of those chemicals within the paste is provided;

- *Clinical observations and testing undertaken during the study were clearly detailed; however the authors' reporting lacks key information in places. For example, the 'Outcomes of the Patients' section details the immediate and longer term outcomes for only 5 of the 7 original patients.*
- *In drawing their conclusions, the authors at times use comparison between the case at hand and multiple types of nanomaterial (despite widely varying physico-chemical properties which in some cases bear no relation to the spherical 30nm NPs within the paste, dust and biological samples), and testing system (in vitro and in vivo tests are used for comparison).*
- *There is minimal discussion of the pre-existing knowledge on polyacrylate nanoparticles. This is particularly relevant as there are a number of journal papers available which consider such nanoparticles and their biological effects in relation to both medical and industrial applications.*

However, despite some fairly key data gaps (some of which may be attributed to the paper's clinical rather than occupational health approach to the incident's investigation), at the end of the day, the importance of this study cannot be denied, particularly in relation to preventing further incidents of such poor occupational health, and its contribution to expanding the as yet small knowledge base on nanoparticle-related health effects.

To read the study in full, click here to access the [European Respiratory Journal online](#).

Postscript

More on the study's outcomes and what they mean may be sourced from SAFENANO guest expert Dr Andrew Maynard, who has provided a fascinating three-part opinion piece on the paper. This features not only a summary of the key issues highlighted together with his thoughts on these, but also those of key nanotoxicologists from around the globe. Included is input from:

Ken Donaldson - Professor of Respiratory Toxicology specializing in workplace lung diseases, University of Edinburgh, UK. Professor Donaldson is one of the world's leading authorities on the health impacts of inhaling airborne nanoparticles. His group at the University of Edinburgh have led research into the potential health impacts of inhaling carbon nanotubes and other nanomaterials.

Gunter Oberdorster - Professor of environmental medicine, Rochester University, USA. Prof. Oberdorster is considered by many to be the father of modern toxicology research into the potential health impacts of inhaling nanoparticles. His research group at the University of Rochester has led global research in this area for over two decades.

Vicki Stone - Professor of toxicology, Edinburgh Napier University, SAFENANO's Director of Toxicology & Editor of the journal *Nanotoxicology*). Prof. Stone is a foremost expert on the mechanisms by which nanoparticles potentially interact with the body and cause harm.

Kristen Kulinowski - a Director of the International Council On Nanotechnology (ICON) at Rice University, and a global leader in developing safe and responsible nanotechnology applications.

Rob Aitken - Director of Strategic Consulting, IOM UK & Director of SAFENANO. Dr Aitken has a wealth of experience addressing workplace safety and health. He is a leading international expert in developing safe practices for working with engineered nanomaterials - including nanoparticles.

Anthony Seaton - a distinguished clinical physician, specializing in occupational health, and a highly regarded expert on the potential health impacts of inhaling airborne nanoparticles. He is currently emeritus professor in the Department of Environmental and Occupational Medicine at the University of Aberdeen.

To access the blogs, select a link from below:

- [New study seeks to link seven cases of occupational lung disease with nanoparticles and nanotechnology](#) - Andrew provides an overview of the study and introduction to the issues.
- [Is nanotechnology poised for the ride of its life?](#) - Andrew's own thoughts on the paper.
- [Nanoparticle exposure and occupational lung disease](#) - Six expert perspectives on the clinical study.