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I implore you to oppose the Copper World mine's requested air pollution permit. The proposed permit doesn't offer safeguards to the environment or air as it is now written. At the very least, you should mandate that the Copper World mine receive a Class I permit. To ensure that the mine is always accountable for reducing its air pollution and conserving the environment, without exception, a Class I permit is required. The attached pdf discusses the health impacts of copper mining on mammalian lungs.

In case there is any doubt in your mind about the dangers of copper mining.

Excerpt from: Gaun, S., Ali, S.A., Singh, P. *et al.* Melatonin ameliorates chronic copper-induced lung injury. *Environ Sci Pollut Res* **30**, 24949–24962 (2023). https://doi.org/10.1007/s11356-022-19930-4.

The references cited can be found in the original paper.

Copper is an important trace element involved in various biological activities like antioxidant mechanism, neurotransmitter biosynthesis, mitochondrial respiration, heme synthesis and iron absorption and also plays a significant role as a cofactor for enzymes like superoxide dismutase-(SOD)-1, ceruloplasmin, lysyl oxidase and cytochrome c oxidase (Barceloux 1999; Kim et al. 2008; Lutsenko et al. 2019). Copper is absorbed into intestinal cells through copper transporter-(CTR)-1, 2 and is stored majorly in the liver, though it is highly concentrated in the brain, liver and kidney and is excreted mostly through bile. And the efflux of copper is encoded with ATP7A and ATP7B genes respectively (Gaetke and Chow 2003; Scheiber et al. 2013). Over accumulation of copper in the body leads to copper toxicity, through contaminated drinking water and fortuitous intake of copper-contaminated foods, resulting in several pathological conditions like Wilson's disease (WD), Menke's disease (MD), hemolytic anemia, gastrointestinal bleeding and diarrhoea (Scheiber et al. 2013). In addition, accumulated levels of copper are reported to involve in the progression of neurodegenerative diseases (Mathys and White 2017; Scheiber et al. 2013). Growing with the evidences, the increased use of copper as an additive in inks, skin products, cooking, semiconductor devices etc. promotes serious health issues. As during its application in several manufacturing processes, the metal particles get retained in the respiratory airways (Kim et al. 2019). Existing evidences have reported that the progressive deposition of copper in lungs is linked with time and dose, whereas liver is reported to be the most vulnerable organ against copper toxicity (Benson et al. 2000; Kumar et al. 2015; Patwa and Flora 2020; Zhang et al. 2021). In compliance, the deposition of copper in the lungs attains more focus as lungs are the major health concern of the population due to the inhaled particles, released from industries (Sebio et al. 2019). Recent evidences demonstrated that excess copper leads to alter various cellular and biological pathways inducing oxidative radicals (Janssen et al. 2018; Padrilah et al. 2017; Zhang et al. 2021). The generated oxidative radicals are characteristic of augmented tissue injury. Similarly, studies have found that copper could induce oxidative stress, autophagy and apoptosis via excessive generation of reactive oxygen species (ROS) (Liu et al. 2021; Zhang et al. 2021). ROS-mediated oxidative stress results in the initiation of several inflammatory responses (Gaetke et al. 2014; Yang et al. 2020). Researchers have also

found that copper induces several pro-inflammatory cytokines like interleukin-(IL)-1β and tumor necrosis factor-(TNF)-α (Patwa and Flora 2020). The existing literature findings also suggest that copper acts as an anti-inflammatory agent but this certainly remains largely unexplained (Hussain et al. 2019; Walker and Keats 1976). Nevertheless, the stimulation of inflammatory responses is thought to be helpful against the invading foreign particles but the persistent release of inflammatory mediators governs the tissue injury via activating nuclear factor kappa B (NF-κB) pathway, inflammatory cytokines like TNF-α and IL-6, mitogen-activated protein kinases (MAPKs) and apoptosis (Gaetke et al. 2014). The transcription of immune and inflammatory response genes get involved in the metal toxicity including copper (Patwa and Flora 2020; Yang et al. 2020). Similarly, several preclinical studies have demonstrated that excessive generation of ROS damages lung tissue irreversibly (Benson et al. 2000; Zhang et al. 2021).

Pulmonary inflammation is a chronic disease that involves the permanent scarring of alveolar sacs, which eventually results in declined lung function (Ali et al. 2021b; Janssen et al. 2018; Padrilah et al. 2017). A recent study revealed that imbalanced copper homeostasis leads to generate ROS triggering tissue fibrosis (Janssen et al. 2018; Zhang et al. 2021). Nonetheless, diverse mechanisms are involved in the progression of pulmonary dysfunctions. In particular, the activated inflammatory responses stimulate pleiotropic factors like transforming growth factor (TGF-β), smad signalling and α-smooth muscle actin (α-SMA), including several other fibrotic markers, indicative of continuous exposure of copper that could be a cause of resultant pulmonary fibrosis (Lai et al. 2018). Moreover, lung injury is characterized by the excessive proliferation of extracellular matrix components in tissue matrix (Gérard et al. 2010; Wynn 2011). Another study reported the elevated levels of cytokines in the bronchoalveolar lavage fluid (BALF) after inhalation of copper nanoparticles which resulted in perivasculitis and alveolitis (Assad et al. 2018). However, to date, no efficient therapies are available to treat the respiratory dysfunctions. And the current available agents only provide symptomatic relief without reversing the pathological conditions. For instance, several metal chelators like D-penicillamine, triethylenetetramine (trien) and meso-2,3-dimercaptosuccinic acid (DMSA or succimer) are in effect to treat and chelate the metals from the body via increasing their excretion (Cao et al. 2015). And the reduction of oxidative stress and inflammation has been shown to ameliorate pulmonary injury and toxicity. In addition, earlier studies found that the use of antioxidants prevented the lung injury and pulmonary fibrosis (Ali et al. 2021a; Pooladanda et al. 2019).

Melatonin (N-acetyl-5-methoxytryptamine) is an amphiphilic tryptophan-derived indoleamine (Cipolla-Neto and Amaral 2018). Primarily, secreted from the pineal gland, apart from this, it is also secreted from various other sources like retina, gut, skin, platelets

and bone marrow (Claustrat and Leston 2015). Melatonin is also known as “the hormone of darkness” as its synthesis and secretion are related to darkness during night time. The primary role of melatonin is to regulate the circadian rhythm; apart from this, it also exhibits various biological properties such as antioxidant, anti-inflammatory and free radical scavenger (Esposito et al. 2019; Hosseinzadeh et al. 2018). Various studies evidenced that melatonin shows anti-fibrotic effects on different organs like the heart, lung, liver and kidney by modulating various signalling cascades (Arslan et al. 2002; Che et al. 2020; Chen et al. 2011). In particular, its direct free radical scavenging property has been found as a key mechanism of protection. Additionally, Yu et al. demonstrated the anti-inflammatory effect of melatonin by maintaining a balance between pro- and anti-inflammatory cytokines (Yu et al. 2017). Melatonin treatment inhibited the fibrotic genes like fibronectin, TGF-β and connective tissue growth factor (CTGF) in diabetic cardiomyopathy mediated via NLRP3 inflammasome (Che et al. 2020). Several other studies have supported that melatonin exerts antioxidant properties by inhibiting lipid peroxidation in cells and overcome bleomycin (BLM)-induced pulmonary fibrosis (Arslan et al. 2002; Yildirim et al. 2006). To the best of our knowledge, no study has reported the beneficial effects of melatonin in animals challenged with copper. Therefore, the current study was designed to evaluate the protective effects of melatonin against tissue oxidative stress and inflammation in chronic copper-induced lung injury in rats.