京都大学	博士（都市環境工学）	氏名	TAN SHIH WEI
論文題目	Toxicity Evaluation of Gallium- and Indium-Related Chemicals by Using Freshwater Amphipod (*Hyalella azteca*) and Human Cultured Cells （淡水性ヨコエビおよびヒト培養細胞を用いたガリウムとインジウム化合物の毒性評価）		

（論文内容の要旨）

The rapid development of emerging technologies has been relevant to technology-critical elements of concern. Among those elements, gallium (Ga) and indium (*In*) are important raw materials in semiconductors and optoelectronic industries, and the enhanced production of the two metals has increased the distribution of Ga and In in the environment through the industrial manufacturing processes, especially in discharging of sewage in water bodies. Also, the workers engaged in the indium tin oxide (ITO) production line and e-waste recycling progresses are potentially exposed to Ga- and In-containing dusts. Occupational inhalation exposure to *In*-containing dusts (*e.g.*, ITO, In_2O_3) has been demonstrated to cause indium lung disease. Although there has been progress in investigating and understanding the interaction of Ga and In with biological systems, much remains to be learned about their interaction with other Fe-dependent and Fe-independent processes.

In Chapter 1, chemical transformation, bioavailability, short-term and long-term toxic effects of ion species and insoluble hydroxide/oxide chemicals of Ga and In in the aquatic environment were introduced. In Chapter 2, we focused on exploring the interaction of Ga and In with biological systems, which was either Fe-dependent or Fe-independent processes. Moreover, the potential biological factors and/or modes of action related to chronic human health such as indium lung disease, senescence, and carcinogenicity were also investigated.

The research purpose of Chapter 1 aims at investigating the effects of aqueous chemical transformation on the bioavailability, toxicity (acute and chronic) and potential impacts of Ga- and In-related chemicals, including In(III), citrate-In(III), Ga(III), citrate-Ga(III), $In(OH)_{3(s)}$, $In_2O_{3(s)}$ and $Ga_2O_{3(s)}$, by using freshwater amphipod (*Hyalella azteca*) *in vivo* bioassays. The present study provides new insight into the aquatic toxicity of Ga- and In-related chemicals that have not previously been evaluated in epibenthic freshwater amphipod. Our results proposed that the lower levels of In(III) and Ga(III) exposure, the higher toxic effects would be induced due to hydrolysis in higher concentrations. Furthermore, the use of metal chelator such as citrate could affect hydrolysis of both Ga(III) and In(III), thereby increasing their bioavailability and toxicity to *H. azteca*. We also investigated that the hydrolysis products of In(III), Ga- and In-based hydroxide/oxide chemicals may have lethal and sublethal effects, which appeared to be affected by environmental factors (*e.g.*, water temperature) relating to locomotor and feeding behaviors.

京都大学	博士（都市環境工学）	氏名	TAN SHIH WEI

Since the workers engaged in In and Ga processing are potentially exposed to In- and Ga-containing aerosols through inhalation, which have known to increase serum indium levels and the risk of indium lung disease (interstitial pneumonia and pulmonary fibrosis) and lung cancer. The latency period (month to years) of indium lung disease is relatively shorter than other occupational lung disease such as silicosis and asbestosis. So far, the entire pathogenesis of indium lung disease and factors affecting the latency period in this disease remain elusive, the available evidence indicates that the pathogenesis is closely associated with dissolved and accumulated indium in the body. The main modes of toxic action of Ga and In to humans could be categorized into two types, Fe-dependent and Fe-independent pathways. However, there is still a large gap in knowledge about the relationship between the exposure of Ga- and In-related chemicals, pathogenic mechanisms, and chronic health effects. Therefore, the research purpose of Chapter 2 aims to investigate the biological factors that potentially associated with chronic health impacts of occupational exposure to Ga- and In-related chemicals and the progression of indium lung disease.

Chapter 2 provides new insight into the potential role of In-induced cellular senescence in the pathological progression of indium lung disease. In recent years, the aging-related lung diseases have been identified to be associated with alterations in lung function, increased susceptibility to acute and chronic lung diseases, such as obstructive and fibrotic lung disease. The hallmarks of cellular aging include genome-based failures (genomic instability, telomere attrition, epigenetic alterations), signaling dysfunction (deregulated nutrient sensing, altered intercellular communication), organelle compromise (mitochondrial dysfunction, loss of proteostasis), and cell phynotypic changes (stem cell exhaustion, cellular senescence). Our results demonstrated that In(III) and Ga(III) could induce mitochondrial abnormalities and cellular senescence based on Fe deficiency stress. Other toxic outcomes regarding Fe-independent pathways also indicated the two metals may disturb proteasome homeostasis and cause DNA damage. These hallmarks of cellular aging appeared to accelerate cellular senescence processes, and eventually affect the rate of pathological progression of indium lung disease.

氏　名	TAN SHIH WEI

（論文審査の結果の要旨）

レアメタルの一種であるガリウムおよびインジウムは、ウェハー、太陽電池、光検出器、発光ダイオード、フラットパネルディスプレイ画面、タッチスクリーンなどに用いられ、その世界生産量は年々増加している。すでに、これら元素による土壌汚染や水質汚染が報告されているが、その毒性や生態系への影響は不明な点が多い。

本論文では、ガリウムおよびインジウム化合物の生態毒性とヒトへの健康影響を明らかにすることを目的として、淡水性ヨコエビの *Hyalella azteca* とヒト培養細胞株を用いて様々な毒性試験を行なった。

水環境中の底生生物である淡水性ヨコエビについては、急性および慢性曝露実験を行い、生物濃縮、生存率、成長阻害、性分化への影響、および繁殖への影響を解析した。慢性曝露実験においては、ガリウムイオンおよびインジウムイオンの 0.5 mg/L の曝露において、顕著な生存率の低下、性分化の遅延、繁殖率の低下など、様々な影響を示した。

一方、ヒト培養細胞にガリウムイオンおよびインジウムイオンを曝露すると、いずれも細胞周期の G2 期における停止を誘導し、これに伴い核の肥大や細胞の巨大化を引き起こすことを明らかにした。また、鉄イオンの添加により、これら毒性影響が緩和されることを発見し、ガリウムイオンやインジウムイオンが、細胞内の鉄のホメオスタシスを破綻させることにより、その毒性影響を発揮することを明らかにした。さらに、ガリウム、インジウムのミトコンドリアの呼吸鎖複合体に対する影響も詳細に解析し、ミトコンドリア膜電位の低下や呼吸鎖複合体ⅡおよびⅣの阻害が生じることを発見し、この現象が、鉄ホメオスタシス破綻によるヘム代謝の異常によることを示唆した。

また、ガリウムやインジウムによる鉄に依存しない毒性経路として、プロテアソームの阻害や DNA 損傷などを明らかにした。

本研究により、ガリウムおよびインジウムの淡水性ヨコエビとヒト細胞に対する毒性影響とそのメカニズムの一端が解明され、今後これらレアメタルの環境基準を設定する際に有用な知見が得られた。また、産業医学上重要なインジウム肺の誘導メカニズム解明に向けて重要なヒントが得られた。これにより、本論文は博士（工学）の学位論文として価値あるものと認める。また、令和 4 年 2 月 22 日、論文内容とそれに関連した事項について試問を行って、申請者が博士後期課程学位取得基準を満たしていることを確認し、合格と認めた。

なお、本論文は、京都大学学位規程第１４条第２項に該当するものと判断し、公表に際しては、(令和 5 年 12 月 30 日までの間)当該論文の全文に代えてその内容を要約したものとすることを認める。