労災疾病臨床研究事業費補助金

インジウム等レアメタルによる職業性疾患予防および病態解明のための疫学研究および動物実験研究

平成 26 年度　総括・分担研究報告書

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はじめに

インジウムは希少金属であり、亜鉛精錬の副産物として産出される。薄膜化した際の高い導電性と透明性により液晶ディスプレイのほか，低融点合金，ボンディング用途，ヒューズ，歯科用合金，化合物半導体，電池材料，太陽電池など広く用いられ、2013年度のインジウムの国内需要は1990年代後半に比べて約10倍に増大し、インジウムの約90%がインジウム・スズ酸化物(Indium tin oxide ： ITO)として用いられている。

インジウムの健康影響は1990年代半ばまで毒性情報が極めて少なかったためにインジウム取り扱い作業者のインジウムの安全性についての配慮は乏しく､インジウムは”安全な金属”として認識されていた。しかし､2001年に世界で初めてITOの吸入に起因すると考えられる間質性肺炎の死亡例が我が国で発生し、さらに、動物実験では肺障害性や肺発がん性が報告され、インジウムの肺炎惹起性が明らかになってきた。

一方、IGZO等に含まれるレアメタルであるガリウム(Ga)や他のレアメタルの毒性情報は非常に乏しく、インジウムの例から、化学形態により毒性に大きな差があり、他のレアメタルとの複合曝露による毒性の修飾が懸念される。CIGSやIGZOは規制対象物質になっていないため、事業所ではこれらの物質の毒性情報がないために安全だと認識されており、正しい毒性情報の発信が喫緊の課題である。これら先端製品材料の毒性を明らかにし、適切な管理を実施することは、日本の労働衛生研究者・行政職の責務である。さらに、この研究のための労働者コホートは、世界中で我々のみが維持している。

本研究の目的は（１）インジウム(In)以外のレアメタルによる健康障害を明らかにすること、（２）インジウムによる呼吸器障害についての知見を深めることである。

インジウムによる呼吸器障害に関しては、我々が継続的に実施している世界唯一のインジウムコホート研究から、高濃度曝露群では肺気腫性変化が優位に強く進行することが明らかになった。さらに、ITOの動物実験では肺発がん性が証明されたことより、インジウム作業者での発がんの可能性が危惧される。このため、気腫性変化と肺発がんに至るメカニズムの解明は重要な課題であり、動物実験や疫学研究によりその一端を明らかにしていく。

日本産業衛生学会は、血清Inの生物学的許容値として3 μg/ Lを勧告したが、この値は時間断面研究に基づく数値で追跡研究が欠落していることから、質的に十分な情報に基づいているとはいえず、長期観察による検証が必要である。

安衛法の改正においては、金属インジウム曝露作業者に関する科学的な情報が欠落していることから、金属インジウム曝露作業は法規制から外れた。今回、金属インジウム作業者の健康調査結果により安衛法のなかで対象物質として金属インジウムが追加される可能性がある。さらに、健康調査とともに作業環境および個人曝露濃度測定を実施することより、管理濃度策定へ期待できる。

一方、インジウム以外の化合物半導体・太陽電池等先端産業で使用されているレアメタルに関する健康影響情報は皆無に近く、今般の研究で職業性疾患発生の可能性の有無が明らかになれば、レアメタルによる未知の職業性疾病の発生の未然防止対策の策定が可能になり、インジウムでの失敗を繰り返さないことが期待できる。

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インジウム等レアメタルによる職業性疾患予防および病態解明のための
疫学研究および動物実験研究
総括研究報告書

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研究要旨
平成26年度は疫学調査、環境調査を実施し、動物実験では種々の化学形態のインジウム化合物の肺障害について検討を行った。
疫学調査ではインジウム製造工場、リサイクル工場および金属インジウム合金製造工場に勤務する作業者に健康調査を施行した。インジウム製造工場1か所では作業環境測定調査を施行した。本年度の調査では、肺がんを含む発がん疾患は認めなかった。疫学調査の結果から血清インジウム濃度や間質性肺炎のバイオマーカーであるKL-6値は低下してきているが、依然として量影響関係は示している。一方、肺機能検査の拘束性/閉塞性障害の所見や血清インジウム濃度高値者の胸部CT所見の改善は乏しい。過去に曝露した難溶性化合物のITOは今も肺内に残存し、肺内炎症は持続している可能性や血清インジウム濃度高値者の不可逆性の変化が示唆されるため、今後の継続健診で経過観察する必要がある。また、高温溶解作業の金属インジウム曝露者は、ITO等のインジウム化合物と同様の影響を認めたため、健康影響に定期的に評価する必要がある。
作業環境調査では、就業期間1年以内の新規作業者では血清インジウム濃度高値を認める作業者もあり、更なる作業環境管理上の工学的な対策を行うとともに、作業管理として作業のやり方・姿勢、使用工具の見直しなどの対策も行い、個人曝露濃度を下げる改善が必要である。電動ファン付き呼吸用保護具の使用により肺へのインジウム取り込み量はかなり減少していることが示唆された。
動物実験においては、ITO ターゲット廃材の粉砕から酸化インジウム製造のリサイクル工程で精製される水酸化インジウムとITOターゲット研削粉(ITO)、酸化インジウム(In_2O_3)の肺障害についてラットを用いて比較検討を行った。その結果、水酸化インジウム粒子の反復経気道投与によって、ITO粒子やIn_2O_3粒子に比べて肺障害は強く発現した。ITOやIn_2O_3粒子に加えて水酸化インジウム粒子の投与によっても肺障害が引き起こされることが明らかになった。インジウムの化学形態による肺障害の差はあるものの、各インジウム化合物は肺障害を引き起こすため、インジウム化合物の曝露や取り扱いには十分注意を払う必要がある。

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A.研究目的

レアメタルであるインジウムの曝露による死亡事例が2001年に世界で初めてわが国で発生し、その後の疫学研究によりインジウム化合物吸入と肺障害の因果関係を確立した。一方、主要なインジウム化合物であるITOの動物発がん実験より肺発がん性が明らかになり、今後のインジウム作業者での肺がん発生が非常に危惧される。さらに、これらのインジウム作業者の一部は、IGZO等の化合物半導体、CIGS等の非シリコン系太陽電池等の新素材の開発や生産を行っているため、種々のレアメタルに複合曝露されており、早急にレアメタルの安全性評価が必要である。

本年は疫学調査では、インジウム労働者コホートのインジウム製造工場、リサイクル工場および金属インジウム合金製造工場に勤務する作業者に健康調査を施行し、インジウム製造工場1か所では作業環境測定調査を施行した。さらに、動物実験ではインジウムのライフサイクルで製造されるITOターゲット材の研削粉、酸化インジウム（In_2O_3）、水酸化インジウムをラットの気管内に反復投与し、肺障害について比較検討を行った。

B.研究方法

Ⅰ　疫学調査

ITO 等インジウム化合物製造工場 3 工場（A 工場、B 工場、C 工場）とリサイクル工場（D 工場）、11 の金属インジウム合金製造工場作業者の健康調査を実施した。

曝露指標として血清インジウム濃度(In-S)測定および曝露歴、影響指標として呼吸器系自覚症状、胸部高分解能 CT（HRCT）撮影、スパイロメトリー、一酸化炭素肺拡散能試験、肺間質性肺炎マーカーである KL-6、SP-D 等を測定した。また、健康調査票にて年齢、性別、身長、喫煙歴を調査した。HRCT 撮影は、A 工場、B 工場のみ施行した。

Ⅱ　ITO等インジウム化合物製造工場の作業環境測定

インジウムを対象とした作業環境測定、粒径を考慮したインジウム定点濃度測定、作業中における防じんマスク内側とマスク外側(個人曝露濃度測定)のインジウム濃度測定、マスクの漏れ率測定等を実施した。作業環境中のインジウム濃度測定は「インジウム・スズ酸化物等の取扱い作業による健康障害防止に関する技術指針 平成 22 年 12 月 22 日」に定められている「空気中の ITO 等の濃度の測定方法について」に従い実施した。

Ⅲ　動物実験

被験物質としてITO研削粉（ITO）　酸化インジウム（In_2O_3）　水酸化インジウム（$In(OH)_3$）の3物質を用い、実験動物としてWistar rat（♂、8週齢）を用いた。実験群はITO群、酸化インジウム（In_2O_3）群、水酸化インジウム（$In(OH)_3$）群、対照群（蒸留水）の4群、各群36匹で構成し、合計144匹のラットを用いた。各被験物質を蒸留水に懸濁し、週2回、計5回、2週間にわたって反復投与した。各群最終投与日の翌日（0週）、1週、2週、3週目にラットを各群9匹ずつ安楽死させた。各評価時点のラットの肺、血液中のインジウム測定、肺の病理学的変化について評価した。

C.研究成果

Ⅰ　疫学調査

A工場の結果

健診受診者のうち4名がIn-S≧3μg/Lの高値であった。2014年度と比して大きな変化はなかった。KL-6は、4名が高値（＞500U/ml）であった、そのうち3名は、In-Sも高値であった。肺機能検査結果は、2014年と比べて、大きな変化はなかった。

B工場の結果

In-Sは、健診者全員が1μg/L未満、かつ間質性肺炎マーカー（KL-6、SP-D）も全員正常範囲だった。過去のIn-S高値者は、ゆるやかに低下傾向で、KL-6もIn-S＜3μg/lになる前にすでに低下し、現在は正常範囲内で安定していた。肺機能検査（努力性肺活量、一秒率ともに）も全員正常範囲で、かつ変化を認めなかった。胸部CTの結果は、多くの者が異常を認めなかった。胸部CTの有所見者も2008年と比べて変化がなかった。

C工場の結果

In-Sは、ゆるやかに低下傾向だった。間質性肺炎マーカー（KL-6、SP-D）も、緩やかに低下傾向だった。KL-6およびSP-Dの有所見者1名を除いて、すべてが正常範囲だった。肺機能検査（努力性肺活量、一秒率とも）は、全員正常範囲だった。肺拡散能は、多くの者が変化なしで、1名は低値でゆるやかに低下傾向であった。胸部CTの結果は、初回検査In-S≧10μg/Lの作業者でブラがやや増悪していたり、間質性変化は陳旧化しつつも陰影は改善を認めなかったりした。

D工場の結果

曝露者のIn-Sは、平均値、およびIn-S≧3μg/Lの高値者の値も低下していたが、2011年より上昇している作業者もいた。非曝露者は、全員≦0.1μg/Lであった。就業期間1年の作業者ではIn-S高値者がおり、作業環境、作業環境管理（マスク着用・管理なども）を再度確認する必要があった。In-S高値継続の作業者は、肺内のインジウムのクリアランスが遅いため、経過観察が必要であった。間質性肺炎マーカー（KL-6、SP-D）は、曝露者の全体の平均、および高値者（1-2名を除き）の値は、低下していた。間質性肺炎マーカー高値、かつIn-S高値の作業者は、インジウムの肺からのクリアランスが遅いため、経過観察が必要だった。肺機能検査結果は、全員正常範囲内であった。肺拡散能検査（DLCO）結果は、%DLCOの低値者が2名いた。その2名の%DLCO/VA（肺胞面積）はすべて正常範囲内であった。

金属インジウム合金製造工場の結果

高温溶解工程（1000度以上の高温で溶解を行っている工程）における高濃度曝露者

では ITO 製造工場作業者と同様の影響を認めた。

Ⅱ　ITO等インジウム化合物製造工場の作業環境測定

E工場での作業環境中のインジウム濃度測定を行った。その結果、午前のITO研削作業場の幾何平均値：1.88μg/m^3、第1評価値：12.32μg/m^3、午後の幾何平均値：5.31μg/m^3、第1評価値：25.00μg/m^{3}であり、午前に比べて午後の濃度が高い値を示した。

電動ファン付き呼吸用保護具による曝露状態のインジウム濃度測定（マスク内インジウム濃度）では、マスク内部のインジウム濃度は許容される濃度0.3μg/m^{3}より低い値であることが確認された。

Ⅲ　動物実験

$In(OH)_3$群では投与期間中から観察期間中体重増加が著しく抑制され、ITO群、In_2O_3群および対照群に比べて有意に減少した。肺の相対重量は、各時点の各インジウム投与群で対照群と比べて有意に増加した。各時点のITO群およびIn_2O_3群の相対肺重量は、対照群の約 2 倍で推移した。一方、$In(OH)_3$群では、ITO群およびIn_2O_3群に比べて有意に増加し、経時的に増加していた。血液中インジウム濃度は $In(OH)_3$群では約1000μg/L であり、他のインジウム群に比べて 70 倍～200 倍の高値であった。肺インジウム量は緩やかに経時的に減少した。肺の病理学的評価では、ITO 群、In_2O_3 群、$In(OH)_3$ 群で肺の炎症性変化を主体とする病変が観察され、特に、$In(OH)_3$ 群では肺病変の程度がITO群およびIn_2O_3群に比べて重度であった。

D. 考察

健康影響調査の結果に関しては、作業環境管理・作業管理が改善されたため、作業環境中のインジウム濃度が低下し、作業者の血清インジウム濃度が低下してきている。その結果、これまでの影響指標として明瞭な量反応関係、量影響関係を示していた間質性肺炎のバイオマーカーのKL-6値も同時に低下してきているが、依然として量影響関係は示している。一方、肺機能検査の拘束性/閉塞性障害の所見やIn-S高値者の胸部CT所見の改善は乏しい。これらの結果は、これまでに曝露した難溶性化合物のITOは今も肺内に残存し、肺内炎症は持続している可能性や血清インジウム濃度高値者の不可逆性の変化が示唆される。今後の継続健診で経過観察する必要がある。また、就業期間1年以内の新規作業者では血清インジウム濃度高値を認める作業者もあり、特にマスク着用などの作業環境管理を定期的に確認する必要がある。

作業環境測定結果では、作業環境中インジウム濃度は、午前より午後の測定時の方が高い結果であった。午前中にインジウムに曝露される作業が多く行われたことに因ると推測される。作業環境管理上の工学的な対策の他、作業のやり方や姿勢、使用工具の見直しなど作業管理上の対策も行い、曝露濃度を下げる改善が必要である。

電動ファン付き呼吸用保護具による曝露状態の濃度測定（マスク内インジウム濃度）結果について概ね、マスク内部のインジウム濃度は許容される濃度 0.3μg/m^3 より低い値であることが確認された。電動ファン付き呼吸用保護具の使用により肺へのインジウム取り込み量はかなり減少していることを示唆するものと考えられる。また、マスクフィッティングテスターによる大気中の粉じんを用いたマスク面体の漏れ率測

定結果では、全て 1%以下と良好な装着を示した。このような電動ファン付き呼吸用保護具を適正に装着することにより健康影響の未然防止が期待できる。

しかし、マスクの面体と顔面との接触面におけるインジウムの付着量は、ブランクである午前の作業前にも 1.13～2.75μg が検出されたものの、午前の作業終了時は 8.92～13.68μg、午後の作業終了時は 10.73～19.74μg と作業後には付着量が増加することが確認された。マスク面体について、毎使用時の確実な清拭が重要と考えられる。

ラットを用いた気管内投与によって、ITO や In_2O_3 だけでなく、$In(OH)_3$ においても肺障害が引き起こされ、さらに ITO や In_2O_3 に比べて肺障害性は著しく強いことが明らかになった。今回、ITO、In_2O_3 および $In(OH)_3$ は同じモル濃度のインジウム化合物を投与したにも係わらず、$In(OH)_3$ 群で毒性は強く発現した。毒性発現の強さは血液中インジウム濃度および粒子径に関連している可能性があり、高濃度の血液中インジウム濃度が観察された理由の一つとしては ITO や In_2O_3 に比べて $In(OH)_3$ の粒子径が非常に微細であることが考えられる。アメリカ NTP のインジウムリンの吸入曝露および日本における ITO 研削粉の吸入曝露実験において肺の発がん性が認められていることより、インジウムが発がん性に寄与していると推測され、他のインジウム化合物の発がん性の可能性は否定できない。今後、水酸化インジウムを含めたインジウム化合物の発がん性の評価が必要であると考える。

E. 結論

本年度の健康調査では、肺がんを含む発がん疾患は認めなかった。しかし、血清インジウム濃度や KL-6 値は低下してきているが、依然として量影響関係は示し、さらに、肺機能検査の拘束性/閉塞性障害の所見や血清インジウム濃度高値者の胸部 CT 所見の改善は乏しいため、さらに継続した経過観察をする必要がある。さらに、高温溶解作業の金属インジウム曝露者は、ITO 等インジウム化合物と同様の呼吸器影響を認めたため、健康影響に定期的に評価する必要がある。インジウム化合物の動物実験結果より $In(OH)_3$ の急性肺障害が認められたため、ITO や In_2O_3 に加えて、$In(OH)_3$ の健康影響に注意を払う必要があると考える。

F. 健康危険情報

- 追跡調査の結果から、インジウム作業者の血清 KL-6 は低下しているが、依然として量影響関係を示した。
- 高温溶解作業の金属インジウム曝露者は、血清インジウム濃度と KL-6 が上昇した。
- 肺機能検査の拘束性/閉塞性障害の所見や血清中のインジウム濃度高値者の胸部 CT 所見の改善は乏しい。
- 就業期間 1 年未満の新規就業者において血清中のインジウム濃度が高値である作業者が認められる。
- 水酸化インジウムの吸入により急性肺障害が惹起される。

G. 知的所有の取得状況

1. 特許取得　なし
2. 実用新案登録　なし

インジウム曝露者の疫学研究

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研究要旨

本研究期間中に 4 つのインジウム製造工場およびリサイクル工場に勤務する作業者に健康調査を施行した。1 つインジウム製造工場で作業環境測定調査を施行した。本年度の調査では、肺がんを含む発がん疾患は認めなかった。追跡調査では、作業環境管理・作業管理が改善され、インジウムの曝露濃度が改善されてきていることから、これまでの影響指標として明瞭な量反応関係、量影響関係を示していた間質性肺炎のバイオマーカーである KL-6 値も同時に低下してきているが、いまなお量影響関係は示している。一方、肺機能検査の拘束性/閉塞性障害の所見や血清中のインジウム濃度（In-S）高値者の胸部 CT 所見の改善は乏しい。それは、これまでに曝露した難溶性化合物のインジウム・スズ酸化物（ITO）は今も肺内に残存し、肺内炎症は持続している可能性や In-S 高値者の不可逆性の変化が示唆される。今後の継続健診で経過観察する必要がある。新規作業者（就業期間 1 年）で、In-S 高値を認める作業者もあり、更なる、作業環境管理上の工学的な対策を行うとともに、作業管理として作業のやり方・姿勢、使用工具の見直しなどの対策も行い、個人曝露濃度を下げる改善が必要である。電動ファン付き呼吸用保護具の使用により肺へのインジウム取り込み量はかなり減少していることが示唆された。また、高温溶解作業の金属インジウム曝露者は、金属インジウム以外のインジウム化合物と同様の影響を認めたため、健康影響に定期的に評価する必要がある。

A. 研究目的

インジウム肺は、2010 年 12 月に厚生労働省から「インジウム・スズ酸化物（ITO）等の取扱い作業による健康障害防止に関する技術指針」により間質性肺炎や続発性慢性閉塞性肺疾患の発症防止目的の健診施行を通達された新しい職業性肺疾患である。

その背景は、1998 年に間質性肺炎を発症し、2001 年にその間質性肺炎に併発した両側性気胸を発症し死亡した症例は、インジウムに起因する間質性肺炎として世界で初めてわが国で発生し、日本産業衛生学会英文誌 *Journal of Occupational Health* (Homma *et al.* 2003) に公表された。我々はこの症例発生の情報を得た後、日本の主要 ITO 製造工場の 3 社 4 工場で実施した断面疫学調査をおこない、血清中のインジウム濃度（In-S）を曝露指標とし、血清中の間質性肺炎のバイオマーカーである KL-6 等を影響指標とし、非常に明確な量・影響関係、量・反応関係を示した(Hamaguchi *et al.*2007)。その後、3 社 4 工場以外のインジウム取扱工場で疫学調査を実施し、3 社 4 工場での観察結果は再現し、インジウムによる間質性肺障害の因果関連を世界で初めて確立し（Nakano *et al.*2009)、現在もそのフィールドを維持継続している。

さらには、ITOの長期曝露動物実験では肺発がん性が証明されたこと(Nagano *et al.* 2011)より、インジウム作業者での発がんの可能性が危惧される。観察開始からまだ１０年余りのために評価できていない、肺がんを含む未知の慢性健康影響などの評価は重要な課題である。

また、金属インジウム曝露作業者は、科学的な情報が欠落していることから、安衛法の改正においては、法規制から外れた。

① ITO等インジウム化合物製造・リサイクル作業者の慢性影響の評価
② 金属インジウム取扱作業者の健康影響の評価

B. 研究方法

① ITO等インジウム化合物製造・リサイクル作業者の疫学調査

1. ITO等インジウム化合物製造に関わる作業者
   A工場：平成27年1月29日実施
   B工場：平成27年2月6日実施
   C工場：平成27年2月6日実施
2. リサイクル業務に関わる作業者
   D工場：平成26年12月12日実施

疫学調査は、曝露指標として血清 In 濃度(In-S)測定および曝露歴、影響指標として呼吸器系自覚症状、胸部高分解能 CT（HRCT）撮影、スパイロメトリー、一酸化炭素肺拡散能試験、KL-6、SP-D 等を測定した。また、健康調査票にて年齢、性別、身長、喫煙歴を調査した。HRCT 撮影は、A 工場、B 工場のみ施行した。

② 金属インジウム取扱作業者の疫学調査

2011年から2013年に、11の金属イ

ンジウム合金製造工場に勤務する141名に実施した。本年度は、日本産業衛生学会英文誌*Journal of Occupational Health*にその結果が公表された。(別添)

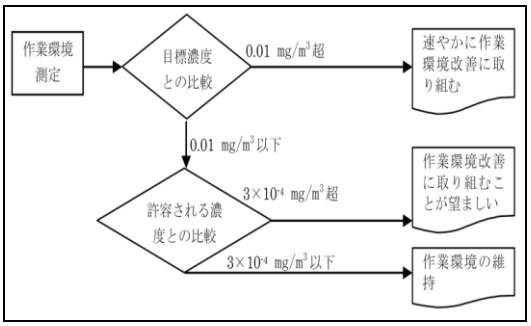
3. ITO等インジウム化合物製造工場の作業環境測定
E工場：平成27年2月17日実施

インジウムを対象とした作業環境測定、粒径を考慮したインジウム定点濃度測定、作業中における防じんマスク内側とマスク外側(個人曝露濃度測定)等を実施した。

a) インジウム作業環境測定

作業環境中のインジウム濃度測定を「インジウム・スズ酸化物等の取扱い作業による健康障害防止に関する技術指針 平成22年12月22日」に定められている「空気中のITO等の濃度の測定方法について」に従い実施した。測定はミニポンプΣ3(柴田科学社製)へ PM-4 NMPS-254 型分粒装置(柴田科学社製)を装着して行った。分粒特性は50%カット粒径4μm(流量2.0L/分)で、4μm 以下（吸入性インジウム濃度：肺に沈着しやすいインジウム濃度）と4μm以上の2段階に分けて捕集した。ろ紙は混合セルロース・エステルメンブレンフィルターNo225-1930 25mm(SKC 社製)を使用した。サンプリング時間は原則として継続した30分間とした。また、評価も技術指針に因った。すなわち、表1より、作業環境測定のA測定の吸入性インジウム濃度を用いて第1評価値を計算するとともに、あわせてB測定値を各々、目標濃度や許容される濃度と比較した。

表1　評価基準



作業環境測定結果：第Ⅰ評価値又はB測定値
目標濃度　0.01mg/m^3=10μg/m^3
許容される濃度　3×10^{-4}mg/m^3=0.3μg/m^3

b)インジウム含有製品の取扱い者についてのマスク内側およびマスク外側(個人曝露濃度測定)のインジウム濃度測定

作業者襟元に捕集部(分粒装置)、腰にポンプを装着し、シフト作業中連続的に捕集し、吸入性インジウム濃度を測定した。装置は以下を用いた。またマスク内についても同様に測定した。さらに、午前の作業開始前、作業終了後、午後の作業終了後に使用していたマスク内側をアルコール綿にて清拭し、マスク内側へのインジウム付着の有無を確認した。

・ミニポンプΣ3型 柴田科学社製

・PM4 NMPS-254 型分粒装置　柴田科学社製

c)マスク漏れ率測定

主な作業者について、マスクフィッティングテスター MT-03 型 柴田科学社製にて、午前の作業開始時、作業終了時、午後の作業開始時、作業終了時の面体からのマスク漏れ率測定を実施した。

インジウム濃度分析方法

a)フィルター

試料は4分の1量を用いた。硝酸4ml および過酸化水素水 1ml を添加して、マイクロ波試料分解装置 Multiwave3000(Anton Paar 製)で前処理を行った。前処理液は PTFE 0.45μm フィルター(ADVANTEC 製)でろ過した。ろ過した前処理液を適当濃度に希釈して、ICP-MS(Agilent 7500ce)でインジウム濃度を測定した。

b) アルコール綿(脱脂綿)

試料は細切した一部を用いた。1試料につき2重測定を行い、各約 0.1g を用いた。各試料に硝酸 4ml および過酸化水素水 1ml を添加して、マイクロ波試料分解装置 Multiwave3000(Anton Paar 製)で前処理を行った。前処理液は PTFE 0.45μm フィルター(ADVANTEC 製)でろ過した。ろ過した前処理液を適当濃度に希釈して、ICP-MS(Agilent 7500ce)でインジウム濃度を測定した。

(倫理面での配慮)

インジウムコホート研究は、「疫学研究に関する倫理指針」に基づき研究計画書を作成し、慶應大学医学部倫理委員会の承認のもとで継続実施した。本研究は、すべての対象者から同意を取得したうえで実施した。

C. 研究結果

① ITO等インジウム化合物製造・リサイクル作業者の疫学調査

A 工場の結果

健診受診者のうち 4 名が In-S≧3μg/L の高値であった。2014 年度と比して大きな変化はなかった。KL-6 は、4名が高値(＞500U/ml)であった、そのうち3名は、In-S も高値であった。肺機能検査結果は、2014 年と比べて、大きな変化はなかった。(資料1参照)

B 工場の結果

In-S は、健診者全員が 1μg/L 未満、かつ KL-6、SP-D も全員正常範囲だった。過去の In-S 高値者は、ゆるやかに低下傾向で、KL-6 も In-S＜3μg/l になる前にすでに低下し、現在は正常範囲内で安定していた。肺機能検査（努力性肺活量、一秒率ともに）も全員正常範囲で、かつ変化を認めなかった。肺拡散能が著明に低下している者がいるが、測定不良で参考値であった。胸部 CT の結果は、多くの者が異常を認めなかった。胸部 CT の有所見者も 2008 年と比べて変化がなかった。肝腫瘤疑い（1 名）を認めたが、精査にて

肝血管腫と診断された。(資料 2 参照)

C 工場の結果

In-S は、ゆるやかに低下傾向だった。間質性肺炎マーカー(KL-6、SP-D)も、緩やかに低下傾向だった。KL-6 および SP-D の有所見者 1 名を除いて、すべてが正常範囲だった。肺機能検査(努力性肺活量、一秒率とも)は、全員正常範囲だった。肺拡散能は、多くの者が変化なしで、1 名は低値でゆるやかに低下傾向であった。胸部 CT の結果は、初回検査 In-S≧10μg/L の作業者でブラがやや増悪していたり、間質性変化は陳旧化しつつも陰影は改善を認めなかったりした。(資料 3 参照)

D 工場の結果

曝露者の In-S は、平均値、および In-S ≧3μg/L の高値者の値も低下していたが、2011 年より上昇している作業者もいた。非曝露者は、全員≤0.1μg/L であった。就業期間 1 年の作業者で、In-S 高値がおり、作業環境、作業環境管理(マスク着用・管理なども)を再度確認する必要があった。In-S 高値継続の作業者は、肺内のインジウムのクリアランスが遅いため、経過観察が必要であった。間質性肺炎マーカー(KL-6、SP-D)は、曝露者の全体の平均、および高値者(1-2 名を除き)の値は、低下していた。間質性肺炎マーカー高値、かつ In-S 高値の作業者は、インジウムの肺からのクリアランスが遅いため、経過観察が必要だった。肺機能検査結果は、全員正常範囲内であった。肺拡散能検査(DLCO)結果は、%DLCO の低値者が 2 名いた。その 2 名の%DLCO/VA(肺胞面積)はすべて正常範囲内であった。(資料 4 参照)

E 工場の結果

表 2　インジウム作業環境測定結果

定点番号	開始	終了	吸入性粉じん中In濃度(μg/m3)	作業内容 幾何平均値、評価値等	総粉じん中In濃度(μg/m^3)	吸入性粉じん中In/総粉じん中Inの割合(%)
A①	9:00	11:32	0.27		0.46	59.9
②			0.44	ITO研削作業場	0.51	86.7
③			0.87		1.47	58.9
④			1.79		3.00	59.8
⑤			2.76		3.82	72.5
⑥			1.25	幾何平均値:1.88	1.30	96.3
⑦			2.08	幾何標準偏差:3.14	3.46	60.1
⑧			9.32	第1評価値:12.32	28.80	32.4
⑨			3.24	参考:第2評価値:3.61)	4.61	70.4
⑩			3.49	(μg/m^3)	4.97	70.2
⑪			3.71		5.12	72.5
⑫			1.56		1.62	96.4
⑬			1.92		2.53	75.8
⑭			4.32		6.31	68.5
B①	10:30	10:40	11.15	平面研削盤作業者位置	64.98	17.2
B②	10:50	11:00	14.02	グラインダー作業者位置	41.20	34.0
A①	13:00	15:00	7.77		12.01	64.7
②			6.57	ITO研削作業場	9.25	71.0
③			7.11		12.60	56.5
④			6.79		8.00	84.8
⑤			7.08		10.75	65.9
⑥			9.54	幾何平均値:5.31	10.58	90.2
⑦			24.24	幾何標準偏差:2.57	26.96	89.9
⑧			3.59	第1評価値:25.00	4.31	83.3
⑨			3.33	参考:第2評価値:8.27	3.70	90.1
⑩			3.14	(μg/m^3)	3.49	90.2
⑪			2.93		3.23	90.8
⑫			3.89		4.49	86.6
⑬			5.42		8.33	65.1
⑭			1.51		2.13	70.7
B①	13:30	13:40	24.24	平面研削盤作業者位置	835.05	2.9
B②	14:00	14:10	2.31	平面研削盤作業者位置	101.34	2.3

A①	10:28	11:28	0.70	インジウム未使用研削作業場	0.76	91.7
②			0.16	幾何平均値 :0.35	0.20	83.0
③			0.09	幾何標準偏差:3.16	0.67	14.1
④			0.94	第1評価値: 2.34	1.12	83.8
⑤			0.69	参考:第2評価値:0.68)	1.42	48.8
⑥			0.30	(μg/m^3)	0.36	83.0
B①	10:35	10:45	0.78	平面研削盤作業者位置	0.99	78.9

表 4　マスクの面体と顔面との接触面におけるインジウム付着量測定結果

測定時	In付着量(μg)	マスクの種類
午前作業前	1.99	興研製サカエ式電動ファンBL−100U型
	1.13	重松製作所製 AP-S11PV
	2.75	興研製サカエ式電動ファンBL−1005型
午前作業終了時	8.92	興研製サカエ式電動ファンBL−100U型
	13.68	重松製作所製 AP-S11PV
	12.59	興研製サカエ式電動ファンBL−1005型
午後作業終了時	10.73	興研製サカエ式電動ファンBL−100U型
	19.74	重松製作所製 AP-S11PV
	18.03	興研製サカエ式電動ファンBL−1005型

D. 考察

健康影響の結果について

作業環境管理・作業管理が改善され、インジウムの曝露濃度が改善されてきている。その結果、これまでの影響指標として明瞭な量反応関係、量影響関係を示していた間質性肺炎のバイオマーカーである KL-6 値も同時に低下してきているが、いまなお量影響関係は示している。

表 3　マスク内側と外側のインジウム濃度測定およびマスクの漏れ率測定結果

作業内容	使用したマスクの種類		マスク内外のIn濃度測定結果					マスクフィッティングテスターによるマスク漏れ率測定結果（%)(括弧内はPAPR停止時)	
	PAPR	フィルター捕集効率(%)	作業時刻	マスク	In濃度 ($\mu g/m^3$)	In濃度/許容される濃度	内／外 (%)	作業開始時	作業終了時
ITO研磨、切断	興研製サカエ式電動ファンBL－100U型	99.97	午前	外	1.34	4.5	0.75	0.04	0.06
				内	0.01	0.0		(-)	(2.38)
	興研製サカエ式電動ファンBL－100U型	99.97	午後	外	1.41	4.7	2.13	0.06	0.05
				内	0.03	0.1		(2.89)	(1.70)
研削、グラインダー	重松製作所製 AP-S11PV	99.9	午前	外	6.34	21.1	0.47	0.09	0.04
				内	0.03	0.1		(-)	(-)
	重松製作所製 AP-S11PV	99.9	午後	外	3.94	13.1	1.53	0.26	0.13
				内	0.06	0.2		(2.89)	(1.70)
研削、研磨	興研製サカエ式電動ファンBL－1005型	95.0	午前	外	276.8	922.6	0.38	0.77	0.19
				内	1.06	3.5		(-)	(-)
	興研製サカエ式電動ファンBL－1005型	95.0	午後	外	38.68	128.9	0.13	0.91	0.15
				内	0.05	0.2		(-)	(-)

一方、肺機能検査の拘束性/閉塞性障害の所見や In-S 高値者の胸部 CT 所見の改善は乏しい。それは、これまでに曝露した難溶性化合物の ITO は今も肺内に残存し、動物実験でも低濃度慢性曝露で発がん性（肺がん）を認めることから（Nagano *et al.* 2011)、肺内炎症は持続している可能性や In-S 高値者の不可逆性の変化が示唆される。今後の継続健診で経過観察する必要がある。また、新規作業者には、就業期間 1 年で、In-S 高値を認める作業者もあり、特にマスク着用などの作業環境管理を定期的に確認する必要がある。

金属インジウム合金製造工場では、高温溶解作業は、高濃度曝露で、ITO 製造工場に勤務する作業者と同様影響を認めた。

作業環境測定結果と個人ばく露濃度（マスク外の濃度）の測定結果について

1 工場の結果では、作業環境測定は、午前より午後の方がインジウム濃度が高い結果であった。しかし、2 名の個人曝露濃度は午前中の方が高い傾向にあった。これはグラインダー

作業等の直接インジウムに曝露する可能性が高い作業が、午前中の方に多く行われたことに因ると推測される。作業環境管理上の工学的な対策の他、作業のやり方や姿勢、使用工具の見直しなど作業管理上の対策も行い、曝露濃度を下げる改善が必要である。

電動ファン付き呼吸用保護具による曝露状態の濃度測定（マスク内インジウム濃度）結果について 1 名の午前中以外は、マスク内部のインジウム濃度は許容される濃度 0.3μｇ/m^3 より低い値であることが確認された。電動ファン付き呼吸用保護具の使用により肺へのインジウム取り込み量はかなり減少していることを示唆する。また、マスクフィッティングテスターによる大気中の粉じんを用いたマスク面体の漏れ率測定結果では、全て 1%以下と良好な装着を示した。このような電動ファン付き呼吸用保護具を適正に装着することにより健康影響を少なくすることができればと思われる。

しかし、マスクの面体と顔面との接触面におけるインジウムの付着量は、ブランクである午前の作業前にも 1.13～2.75μｇが検出されたものの、午前の作業終了時は 8.92～13.68μg、午後の作業終了時は 10.73～19.74μｇと作業後には付着量が増加することが確認された。マスク面体について、毎使用時の確実な清拭が重要と考えられる。

E. 結論

本年度の調査では、肺がんを含む発がん疾患は認めなかった。さらに継続した経過観察をする必要がある。高温溶解作業の金属インジウム曝露者は、金属インジウム以外のインジウム化合物と同様の影響を認めたため、健康影響に定期的に評価する必要がある。

G. 主な発表論文等

［雑誌論文］（計 2 件）

① Nakano M, Tanaka A, Hirata M, Iwasawa S, Omae K. Pulmonary effects in workers exposed to indium metal: a cross-sectional study. J Occup Health. 2015. (in press)（査読あり）

② Nakano M, Omae K, Uchida K, Michikawa T, Yoshioka N, Hirata M, Tanaka A. Five-year cohort study: emphysematous progression of indium-exposed workers. Chest. 2014;146:1166-75. doi: 10.1378/chest.13-2484.（査読あり）

［学会発表］

① 大前和幸、中野真規子、岩澤聡子、田中昭代、平田美由紀、田中茂、宮内博幸、東久保一朗、川澄八重子。インジウム：吸入性粉塵個人曝露濃度と生物学的モニタリング指標の関係　許容濃度は提案可能か？　第 42 回産

業中毒・生物学的モニタリング
研究会. 2014.10.25-26.長野.

H. 知的所有権の取得状況

1. 特許取得　なし
2. 実用新案登録　なし

A工場
インジウム健診報告

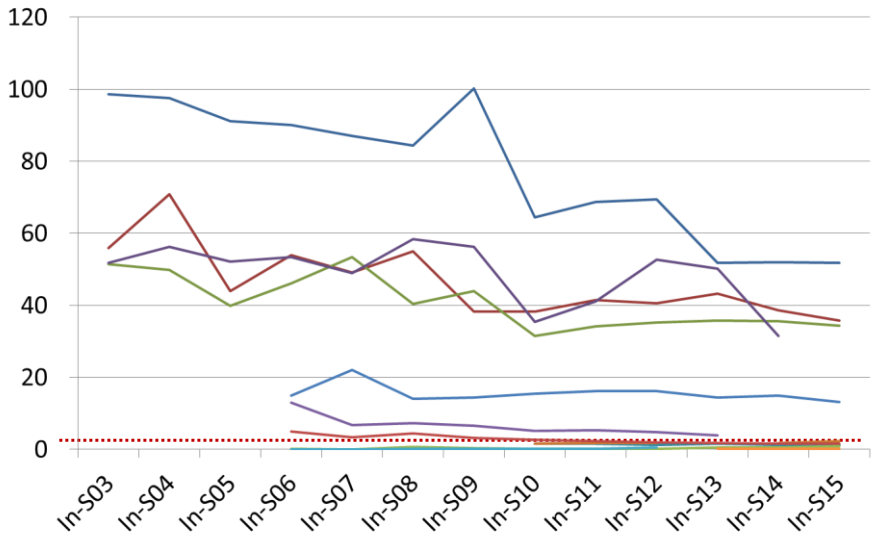
検診日 2015年 1月29日

	人数	内訳
A工場	9名	新規 0名、欠席 0名 受診者数（2006年）10名 （2010年）16名 （2011年）13名 （2012年）11名 （2013年）10名 （2014年）10名

検診内容

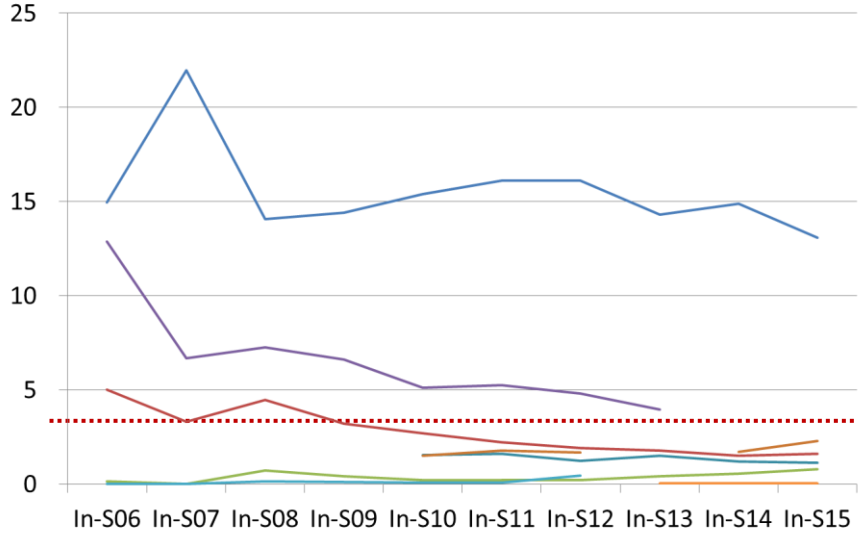
- 問診
- 採血 血清インジウム濃度
  間質性肺炎マーカー(KL-6,SP-D,SP-A)
- 肺機能検査
  肺活量、努力性肺活量、肺拡散能

血清インジウム濃度（<3）の推移

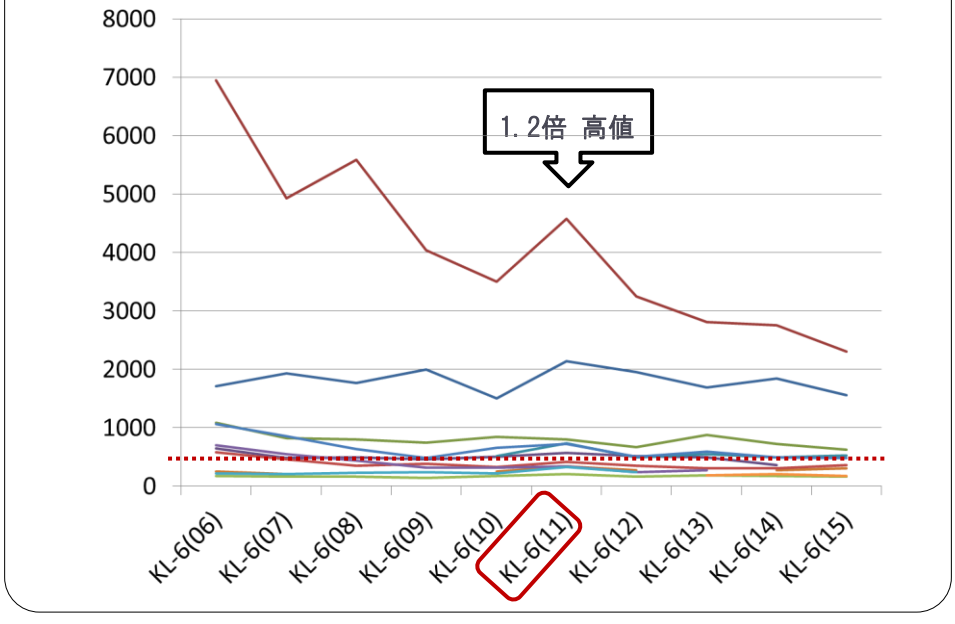


血清インジウム濃度（<3）の推移

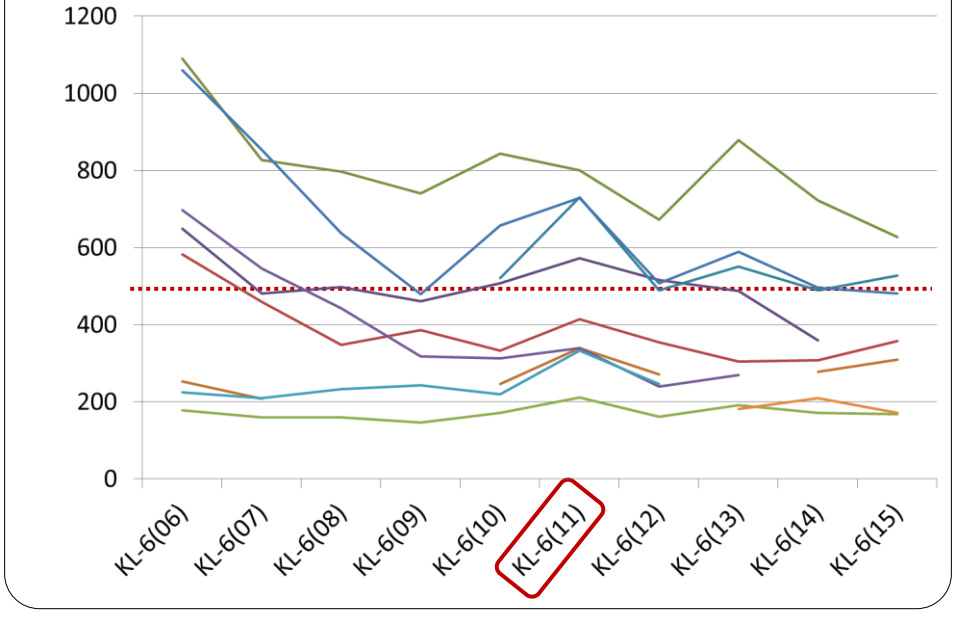
2006年以降にIn-S測定開始した方



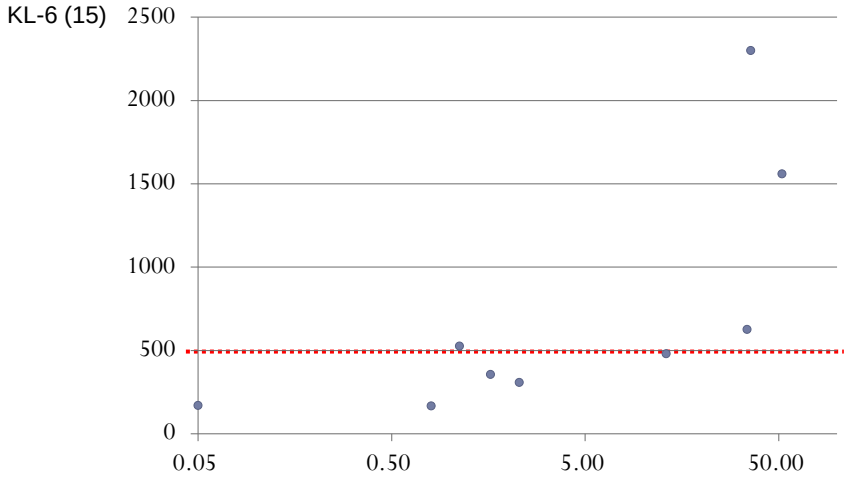
血清KL-6（＜500）の推移１



血清KL-6（＜500）の推移２



2015年1月のIn-SとKL-6との関係



まとめ

In-S

- ４名：高値
- 2014年と比べて、大きな変化はありません。
- A氏は、2014年と比べると低下傾向です。
- 作業環境管理、作業管理、適切なマスクを着用・管理し、健診にて経過観察しましょう。

KL-6

- ４名：高値（うち３名　In-S高値）
- 2014年と比べて、大きな変化はありません。健診にて経過観察しましょう。

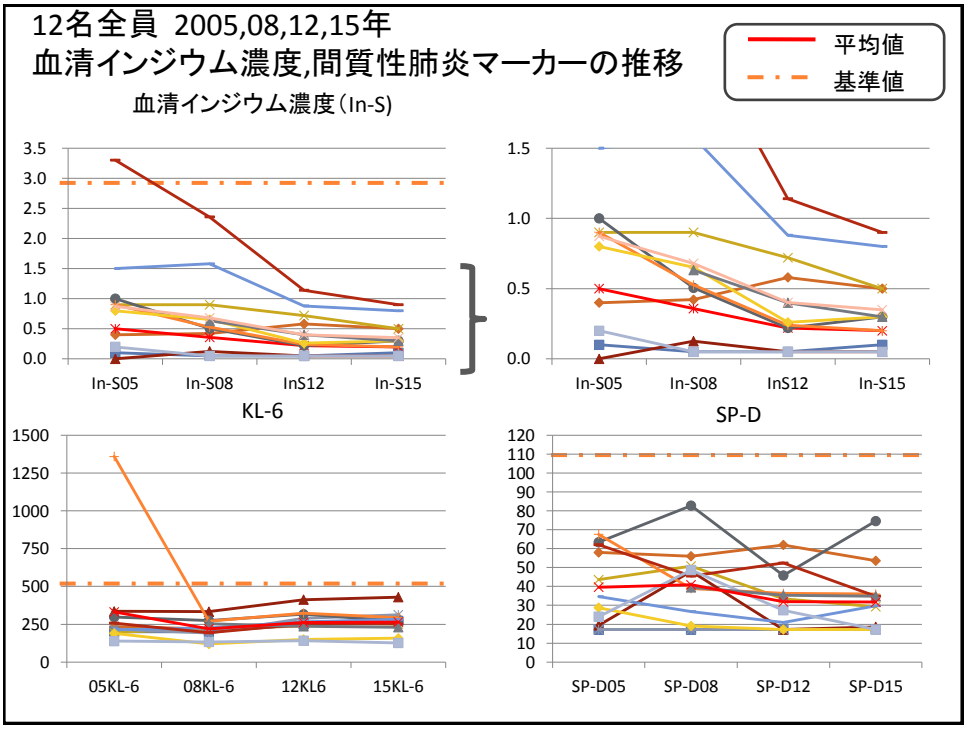
肺機能検査

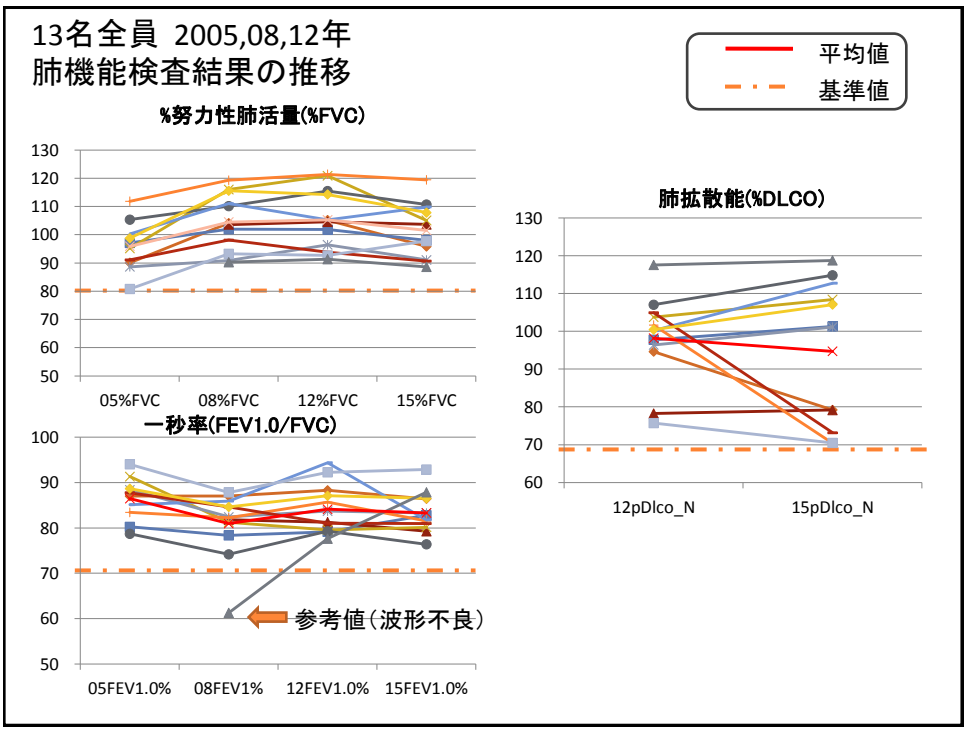
- 2014年と比べて、大きな変化はありません。

B工場
インジウム健診結果2015

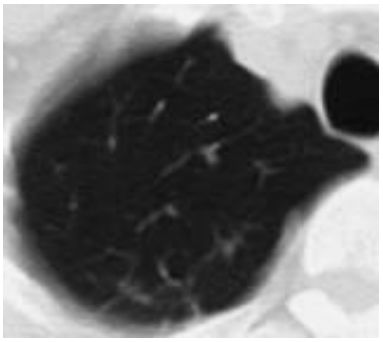
2015年2月6日　検診概要（12名）

検診年	健診内容/備考
2005年	24名
2008年	26名
2012年	13名
2015年	12名（全員：現在インジウム曝露者） 健診内容 健康調査票（自覚/他覚症状, 喫煙歴） 血清インジウム濃度（In-S） KL-6, SP-D, 他 肺機能検査（%FVC, FEV_1%、肺拡散能） 胸部CT


12名全員 2005,08,12,15年
血清インジウム濃度,間質性肺炎マーカーの推移
平均値
基準値
血清インジウム濃度（In-S)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
In-S05
In-S08
InS12
In-S15
1.5
1.0
0.5
0.0
In-S05
In-S08
InS12
In-S15
KL-6
1500
1250
1000
750
500
250
0
05KL-6
08KL-6
12KL6
15KL-6
SP-D
120
110
100
90
80
70
60
50
40
30
20
10
0
SP-D05
SP-D08
SP-D12
SP-D15


13名全員 2005,08,12年
肺機能検査結果の推移
平均値
基準値
%努力性肺活量(%FVC)
130
120
110
100
90
80
70
60
50
05%FVC
08%FVC
12%FVC
15%FVC
肺拡散能(%DLCO)
130
120
110
100
90
80
70
60
12pDlco_N
15pDlco_N
一秒率(FEV1.0/FVC)
100
90
80
70
60
50
参考値（波形不良）
05FEV1.0%
08FEV1%
12FEV1.0%
15FEV1.0%

胸部CT　肺野の所見者



右上葉に線状粒状影あり、
2008年と変化なし

まとめ

- In-Sは全員が1μg/L未満、KL-6、SP-Dも全員正常範囲。
- 過去高値者のIn-Sは、ゆるやかに低下傾向。
- 間質性肺炎マーカー（KL-6）も、すでに低下し、現在は正常範囲内で横ばい。
- 肺機能検査

✓ 努力性肺活量、一秒率ともに全員正常範囲、かつ変化なし。

✓ 肺拡散能が著明に低下している者いるが、測定不良で参考値のため、経過観察をしてください。

- 胸部CT

✓ 多くが異常なし。有所見者も2008年と比べて変化なく、経過観察をしてください。

✓ 肝腫瘤疑い（1名）→精査にて異常なし

最後に

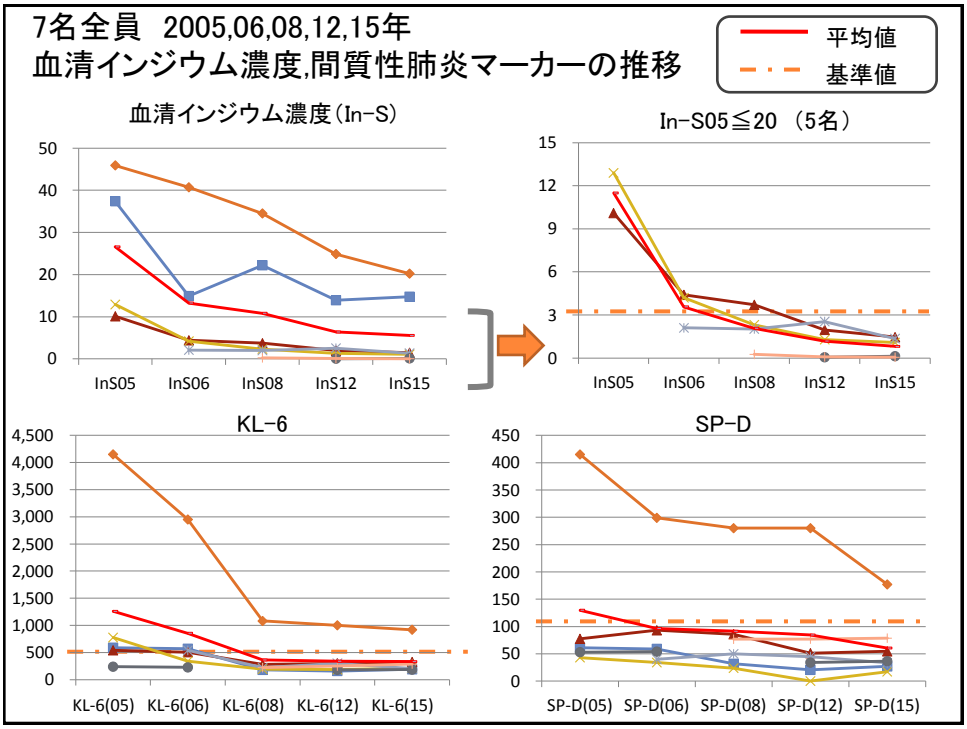
すべての健診者へ、禁煙をお勧めします.

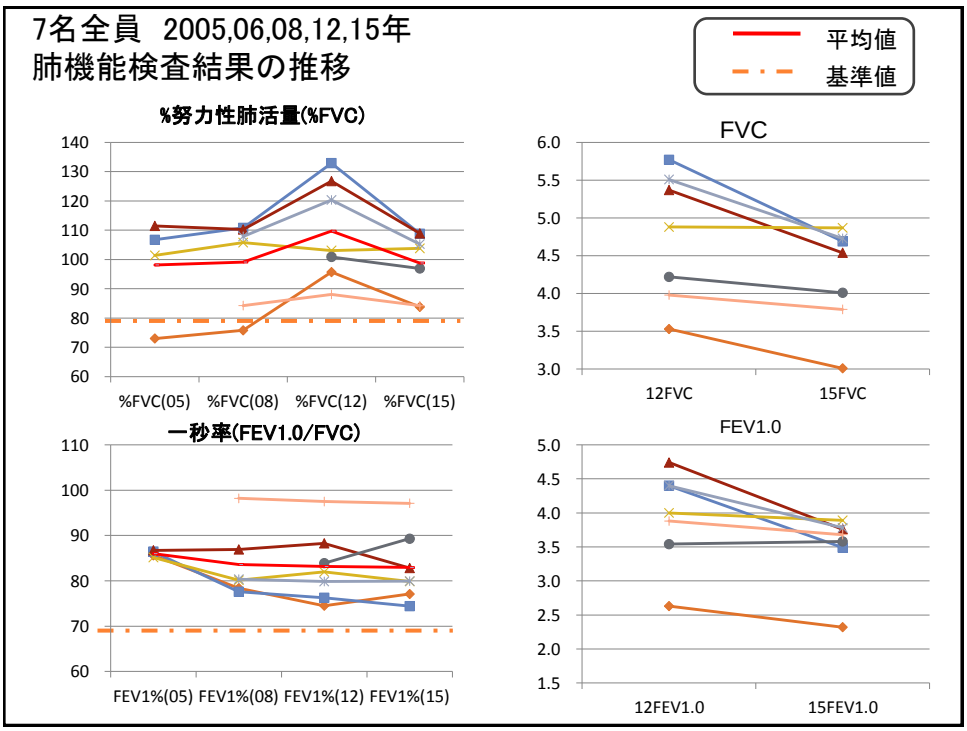
今後も経過観察が必要です.

C工場
インジウム健診結果2015

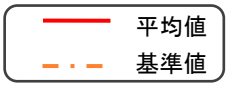
2015年2月6日　検診概要（7名）

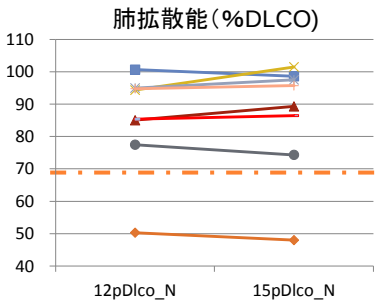
検診年	健診内容/備考
2005年	11名
2008年	28名
2012年	7名
2015年	7名（うち6名：過去インジウム曝露者） 健診内容 健康調査票（自覚/他覚症状, 喫煙歴） 血清インジウム濃度（In-S） KL-6, SP-D, 他 肺機能検査（%FVC, FEV_1%、肺拡散能） 胸部CT

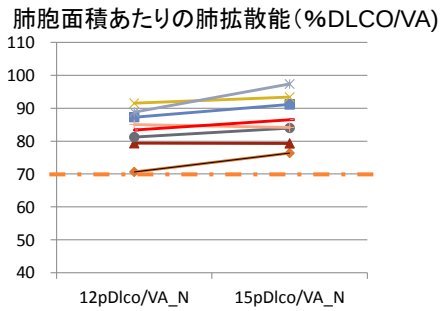

7名全員　2005,06,08,12,15年
血清インジウム濃度,間質性肺炎マーカーの推移
平均値
基準値
血清インジウム濃度（In-S）
50
40
30
20
10
0
InS05
InS06
InS08
InS12
InS15
In-S05≦20　（5名）
15
12
9
6
3
0
InS05
InS06
InS08
InS12
InS15
KL-6
4,500
4,000
3,500
3,000
2,500
2,000
1,500
1,000
500
0
KL-6(05)
KL-6(06)
KL-6(08)
KL-6(12)
KL-6(15)
SP-D
450
400
350
300
250
200
150
100
50
0
SP-D(05)
SP-D(06)
SP-D(08)
SP-D(12)
SP-D(15)


7名全員　2005,06,08,12,15年
肺機能検査結果の推移
平均値
基準値
%努力性肺活量(%FVC)
140
130
120
110
100
90
80
70
60
%FVC(05)
%FVC(08)
%FVC(12)
%FVC(15)
FVC
6.0
5.5
5.0
4.5
4.0
3.5
3.0
12FVC
15FVC
一秒率(FEV1.0/FVC)
110
100
90
80
70
60
FEV1%(05)
FEV1%(08)
FEV1%(12)
FEV1%(15)
FEV1.0
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
12FEV1.0
15FEV1.0

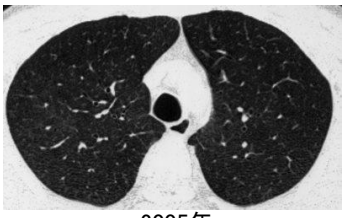
7名全員　2005,06,08,12,15年
肺機能検査結果の推移



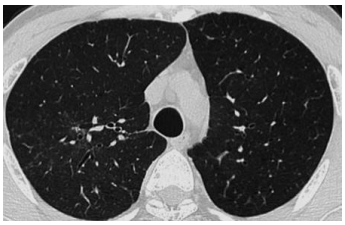




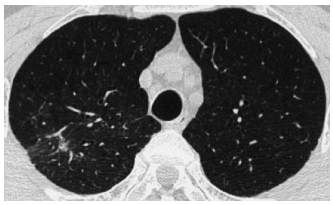
CT所見進行者の例 1
上肺野



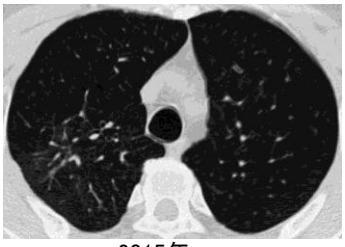
2005年



2008年

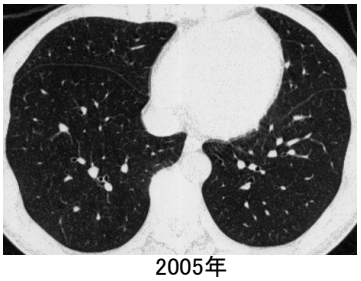


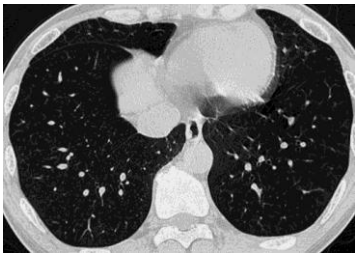
2012年

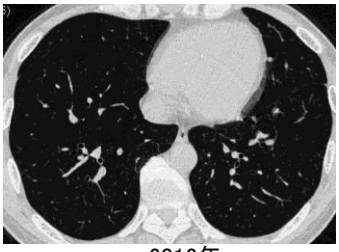


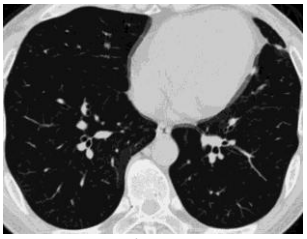
2015年

CT所見進行者の例 1
下肺野

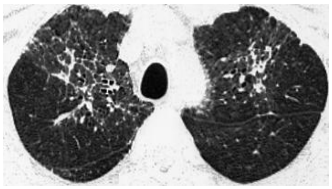

2005年

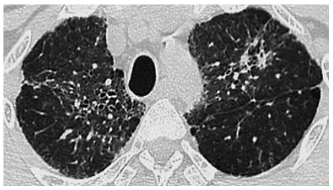

2008年

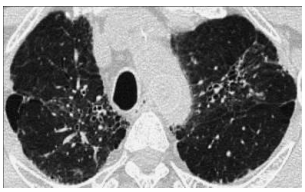

2012年

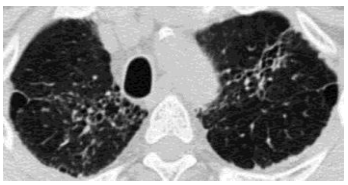

2015年

CT所見進行者の例 2
上肺野

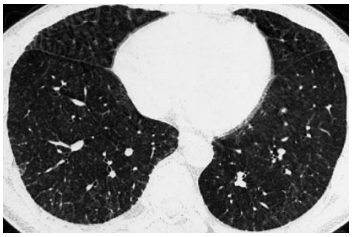

2005年


2008年

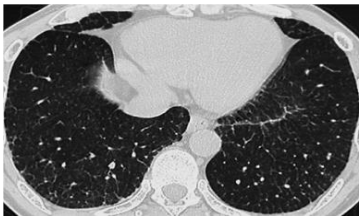

2012年


2015年

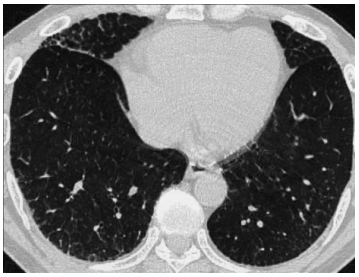
CT所見進行者の例 2
の下肺野



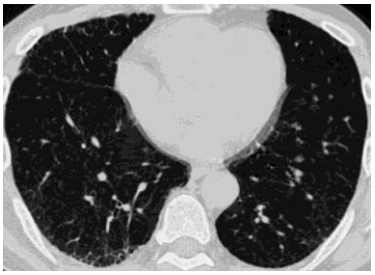
2005年



2008年

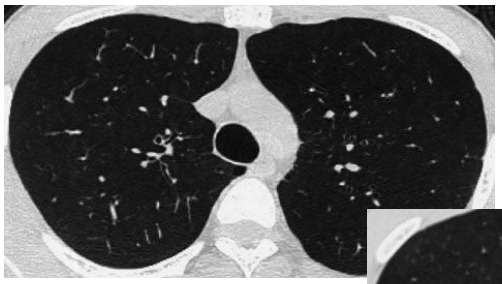


2012年

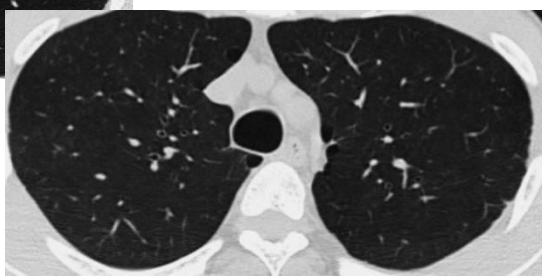


2015年

CT所見進行者の例 3(気腫)
上肺野



2012年



2015年

まとめ

- In-Sは、ゆるやかに低下傾向
- 間質性肺炎マーカー（KL-6、SP-D）も、緩やかに低下傾向。
- KL-6,およびSP-Dの有所見者（高値者）1名を除いて、すべてが正常範囲
- 肺機能検査

✓努力性肺活量、一秒率ともに全員正常範囲。

✓変化なし、あるいはゆるやかに低下傾向。

- 胸部CT

✓初回検査In-S10μg/L以上の方：ブラやや増悪あり、間質性変化陳旧化しつつも陰影は改善はなし。

最後に

すべての健診者へ、禁煙をお勧めします.

今後も経過観察が必要です.

D工場
インジウム健診結果2014

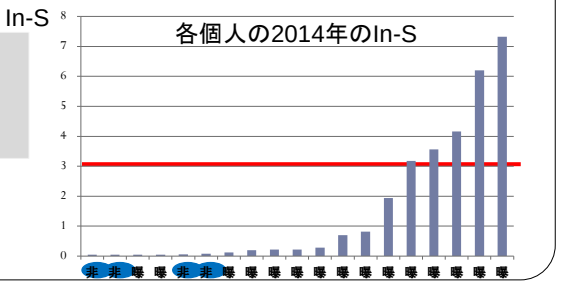
2014年12月12日　検診概要（19名）

検診年	健診内容/備考
2005年	4名
2008年	5名
2011年	12名
2014年	19名（うち4名：インジウム曝露なし） 健診内容 健康調査票（自覚/他覚症状, 喫煙歴） 血清インジウム濃度（In-S） KL-6, SP-D, 他 肺機能検査（%FVC, FEV_1%、肺拡散能）

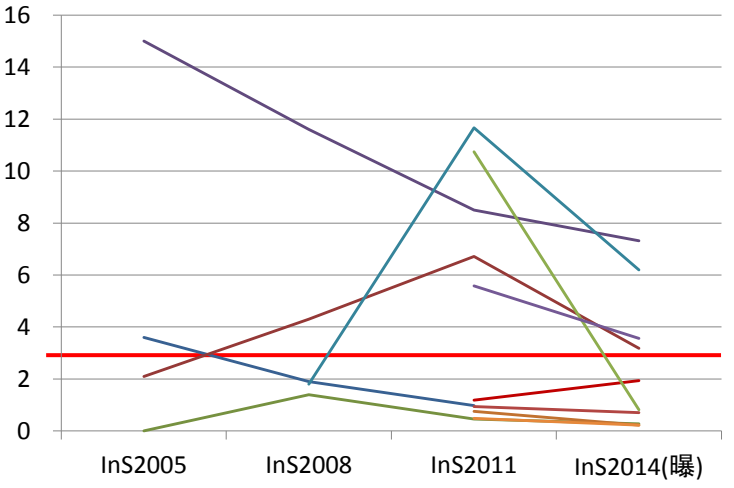
血清インジウム濃度（In-S)の 推移

	インジウム曝露群					非曝露群		
年度	N	算術平均	所見率	最小値	最大値	N	算術平均	所見率
2014	15	1.9	33%	<0.1	7.3	4	≤0.1	0%
2011	12	5.3	50%	0.5	15.1	-		
2008	5	4.2	40%	1.4	11.6	-		
2005	4	5.2	50%	<0.1	15.0	-		

生物学的許容値
In-S＜3 μg/L
（5/15名：33%）

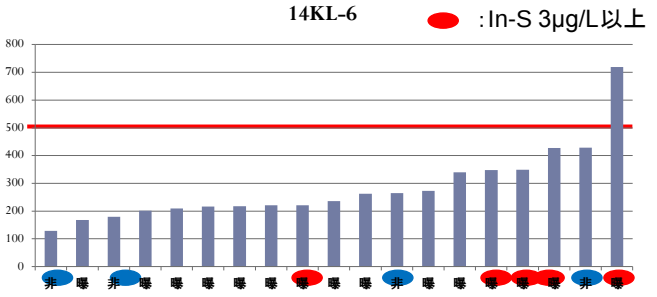


曝露群のIn-Sの比較（2005-14年）

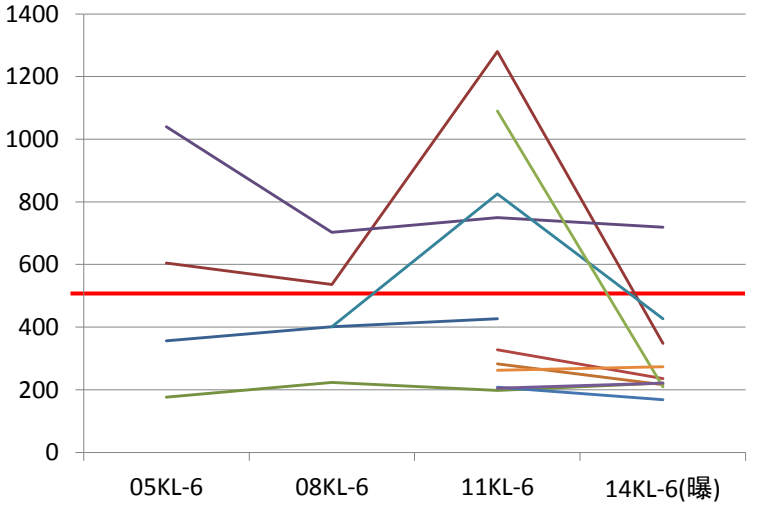


KL-6 の 結果と推移

	インジウム曝露群					非曝露群		
KL-6 (<500)	N	幾何平均	所見率	最小値	最大値	N	幾何平均	所見率
2014	15	273	6.7%	168	719	4	227	0%
2011	12	446	42%	198	1280	-		
2008	5	423	40%	223	703	-		
2005	4	446	50%	176	1040	-		



曝露群のKL-6 の 結果と推移

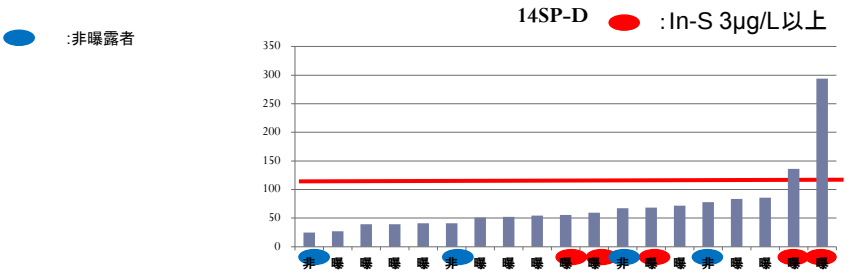


SP-Dの結果と推移

＊：2008.10月測定法変更

	インジウム曝露群					非曝露群		
SP-D（<110）	N	幾何平均	所見率	最小値	最大値	N	幾何平均	所見率
2014	15	63.7	13%	27.2	294	4	47.9	0%
2011*	12	73.3	25%	36.8	205	-		
2008#	3	124.2	67%	93.9	166	-		

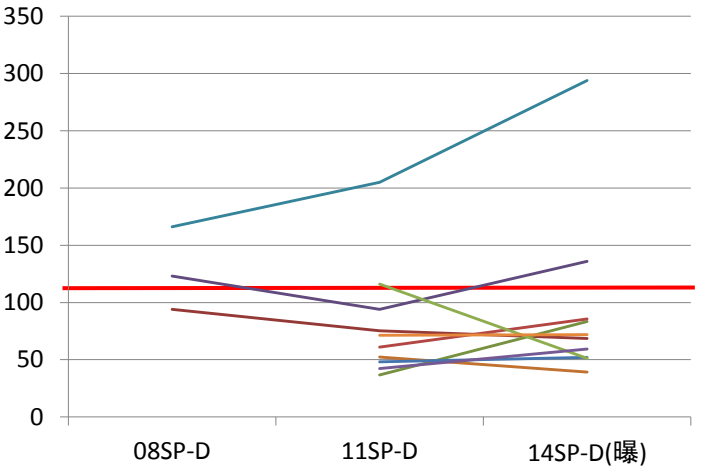
#：2008/7/24：採血



曝露群のSP-Dの結果と推移

#：2008/7/24：採血

＊：2008.10月：測定法変更

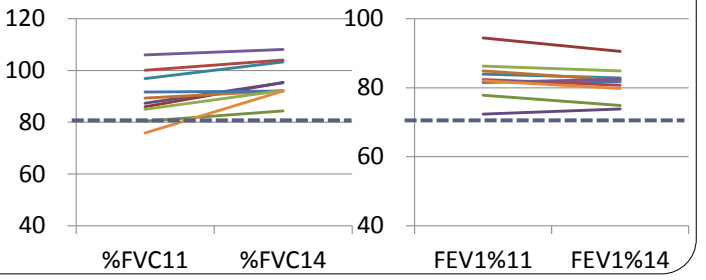


肺機能検査の結果と推移

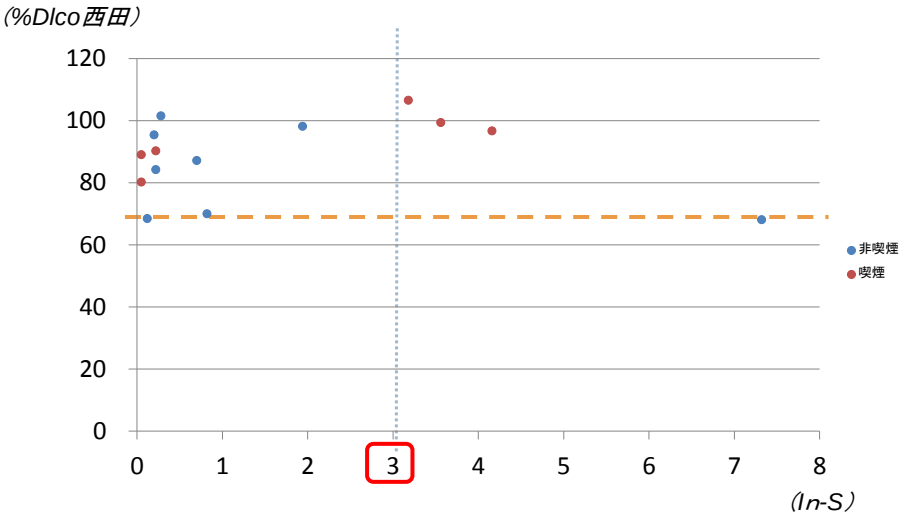
＊：%肺活量（%VC)は、ともに正常範囲

%FVC（実測値/予測値）	算術平均	所見者数(所見率)	最小値	最大値
2014	98.1	0%	84.3	122.7
2011	89.7	（2名*）16.7%	75.9	106

$FEV_{1.0}$/FVC（一秒率/努力性肺活量）	算術平均	所見者数(所見率)	最小値	最大値
2014	81.1	0%	73.9	90.5
2011	81.4	（1名）8.3%	66.2	94.4



2014年の%DLcoとln-Sの散布図



まとめ

- In-S
- 曝露者は、全体の平均、および高値者の値も低下している。
- 非曝露者は、全員≤0.1μg/Lであった。
- 2011年より上昇している方は、作業環境、作業環境管理（マスク着用・管理なども）を再度確認してください。
- 就業期間1年方で、高値の方は、作業環境、作業環境管理（マスク着用・管理なども）を再度確認してください。
- In-S高値者継続検診者は、クリアランスが遅いため、経過観察が必要。

- KL-6、SP-D
- 曝露者の全体の平均、および高値者（1-2名を除き）の値は、低下している。
- 高値者、かつIn-S高値は、Inの肺からのクリアランスが遅いため、経過観察が必要。

- 肺機能検査
- すべて正常範囲内であった。

- 肺拡散能検査（DLCO）
- %DLCOの低値者は、2名いたが、%DLCO/VA（肺胞面積）はすべて正常範囲内であった。経過観察をしてください。

最後に

すべての健診者へ、禁煙をお勧めします.

今後も経過観察が必要です.

ラットを用いた水酸化インジウム、ITO、酸化インジウムの肺毒性評価

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研究要旨
インジウムリサイクル工場ではITOターゲット廃材の粉砕から酸化インジウム製造のリサイクル工程で水酸化インジウムが精製される。しかし、経気道性に体内に侵入した水酸化インジウム（$In(OH)_3$）の肺障害に関する知見は皆無である。そこで、ITOターゲット研削粉や酸化インジウムと同様に $In(OH)_3$ が肺障害を引き起こすかどうかを検討するために、$In(OH)_3$、ITOターゲット研削粉（ITO）、酸化インジウム（In_2O_3）をラットの気管内に反復投与を行い、肺毒性の発現につい比較検討した。$In(OH)_3$ 群では投与期間中から観察期間中体重増加が著しく抑制され、ITO群、In_2O_3 群および対照群に比べて有意に減少した。肺の相対重量は、各時点の各インジウム投与群で対照群と比べて有意に増加した。各時点のITO群および In_2O_3 群の相対肺重量は、対照群の約2倍で推移した。一方、$In(OH)_3$ 群では、ITO群および In_2O_3 群に比べて有意に増加し、経時的に増加していた。血液中インジウム濃度は $In(OH)_3$ 群では約1000μg/Lであり、他のインジウム群に比べて70倍～200倍の高値であった。肺インジウム量は緩やかに経時的に減少した。肺の病理学的評価では、ITO群、In_2O_3 群、$In(OH)_3$ 群で肺の炎症性変化を主体とする病変が観察され、特に、$In(OH)_3$ 群では肺病変の程度がITO群および In_2O_3 群に比べて重度であった。以上の結果から、水酸化インジウム粒子の反復経気道投与によって、ITO粒子や In_2O_3 粒子に比べて肺障害は顕著に発現した。ITO や In_2O_3 粒子だけでなく、水酸化インジウム粒子の投与によっても肺障害が引き起こされることが明らかになった。インジウムの化学形態による肺障害の差はあるものの、各インジウム化合物は肺障害を引き起こすため、インジウム化合物の曝露や取り扱いには十分注意を払う必要がある。

A. 研究目的

インジウム（In）は亜鉛の精錬の副産物として回収されるレアメタルであり、日本のインジウム需要は世界最大である。国内インジウム需要の約 90%がインジウム・スズ酸化物（Indium tin-oxide; ITO）のターゲット材としてノート型パソコン、液晶テレビやプラズマテレビのフラットディスプレイの透明導電膜に用いられている。ITO は酸化インジウムと酸化スズの焼結体であり、その需要が拡大しており、ITO の主成分であるインジウムが希少金属であることから、 日本では ITO ターゲットの廃材を回収して、In として精錬、リサイクルの割合が増加し、2013 年度ではインジウムの使用量の約 60%がリサイクルによって供給されている。

インジウムは 1990 年代半ばまで毒性情報が極めて少なかったためにインジウム取り扱い作業者のインジウムの安全性についての配慮は乏しく、インジウムは”安全な金属”として認識されていた。しかし、2001 年に世界で初めて ITO の吸入に起因すると考えられる間質性肺炎の死亡例が我が国で発生し、さらに、動物実験では肺障害性や肺発がん性が報告され、インジウムの肺炎惹起性が明らかになってきた。

今まで、インジウム吸入による死亡例が ITO ターゲット材の研削作業に携わっていたことから、ITO やその原材料である酸化インジウムの毒性に着目した動物実験を行い、ITO 研削粉や酸化インジウムの吸入によって肺障害が発現することを報告してきた。一方、インジウム作業者の健康調査の結果から、インジウムリサイクル工場作業者の間では短期間で血清中インジウム濃度の上昇が観察されている。インジウムリサイクル工場では ITO ターゲット廃材の粉砕から酸化インジウム製造のリサイクル工程で水酸化インジウムが精製される。現在までに、水酸化インジウムの生体影響に関する報告はない。そこで、今回、ITO ターゲット研削粉や酸化インジウムと同様に水酸化インジウムが肺障害を引き起こすかどうかを検討するために、ITO ターゲット研削粉、酸化インジウムや水酸化インジウムをラットの気管内に反復投与を行い、肺毒性の発現について評価を行った。

B. 実験方法

被験物質として ITO 研削粉（ITO） 酸化インジウム（In_2O_3） 水酸化インジウム（$In(OH)_3$） の 3 物質を用いた。これら 3 物質は水に不溶性である。ITO 研削粉は走査電子顕微鏡（SEM）および画像解析装置を用い、In_2O_3 および $In(OH)_3$ は BET 比表面積から平均粒子径を求めた。

実験動物として Wistar rat（♂、8 週齢）を用いた。実験群は ITO 群、酸化インジウム（In_2O_3）群、水酸化インジウム（$In(OH)_3$）群、対照群（蒸留水）の 4 群、各群 36 匹で構成し、合計 144 匹のラットを用いた。実験開始時の平均体重は 290.4±1.4（平均±標準誤差）であり、各群間で有意な差はなかった。 1 回投与量はインジウムとして 10mg /kg（ITO として 13.4mg、In_2O_3 として 12.0mg、$In(OH)_3$ として 14.4mg）を用いた。各被験物質を蒸留水に懸濁し、週 2 回、計 5 回、2 週間にわたって反復投与した。 各群最終投与日の翌日（0 週）、1 週、2 週、3 週目にラットを各群 9 匹ずつ安楽死させた。後大静脈より血液を採取後、各評価時点のラットの血液中のインジウム測定を行った。各時点の肺の病理学的変化について評価した。肺病変の程度は-から３+までの５段階（-：none, ±:slight, +: mild, 2+:moderate, 3+:severe）で評価した。

（倫理面への配慮）
本研究は、研究機関等における動物実験等の実施に関する基本指針、九州大学動物実験規則および九州大学動物実験規則細則に基づき、動物実験計画を作成し、九州大学大学院医学研究院等動物実験委員会の承認を得て行われた。

C. 結果

各粒子の平均粒子径は ITO；0.95μm、In_2O_3；0.14μm、$In(OH)_3$；0.02μm であった。各群の総投与量はインジウム量として ITO 群 15.6 ± 0.2 mg(平均±平均誤差)、In_2O_3 群 15.6 ± 0.2 mg、$In(OH)_3$群 15.1±0.2 mg であった(Fig.1)。投与期間および観察期間中 $In(OH)_3$群ではラットが著しく衰弱し、観察期間中 2 匹が死亡した。各群の評価数は $In(OH)_3$ 群を除いた各群は 36 匹、$In(OH)_3$群では 2 週目 8 匹、3 週目 8 匹であり、合計 34 匹のラットの評価を行った。他の群では衰弱や死亡は観察されなかった。

Fig.2 に各群の体重推移を示している $In(OH)_3$ 群では投与期間中から観察期間中著しい体重増加の抑制が観察され、対照群に比べて有意に低下していた。ITO 群および In_2O_3 群では観察期間中および観察期間中は対照群と比べて対照群と有意な差は認められなかった。

Fig.3 に各群の相対肺重量の推移を示している。各時点の各インジウム投与群の相対肺重量は対照群に比べて有意に増加し、さらに、$In(OH)_3$群では ITO および In_2O_3 群に比べて各評価時点で有意に増加していた。

Fig.4 に 0 週から 3 週の各時点の血液中のインジウム濃度を示している。各インジウム投与群では経時的にインジウム濃度が上昇し、$In(OH)_3$ 群では ITO 群および In_2O_3 群に比べて各時点で有意に増加し、約 70 倍から 200 倍であり、さらに、In_2O_3 群では ITO 群に比べて各時点で有意に上昇し、2～4 倍の高値を示した。3 週時の各群の血液中のインジウム濃度は $In(OH)_3$ 群:1137.6±167.8μg/L(平均±平均誤差)、ITO 群：6.5±1.3μg/L, In_2O_3 群：13.8±2.0μg/L であり、対照群では定量下限以下であった。

Fig.5 に肺中のインジウム濃度の推移を示している。各群とも経時的に肺インジウム量は徐々に減少した。投与終了直後の各群の肺インジウムの沈着率は $In(OH)_3$ 群：67.8±8.8%（平均±平均誤差)、ITO 群：24.7±3.9%、In_2O_3群：25.0 ±5.1%であった。

Table 1 に各群の肺病変の程度を示している。肺の炎症性変化、線維性増殖、肺胞内への壊死片を含む滲出液の沈着などの病変が発現し (Fig.6)、これらの病変の程度は $In(OH)_3$群では ITO および In_2O_3 群に比べて重度に発現し、これらの病変は 3 週目まで増悪した。

D. 考察

ラットを用いた気管内投与によって、ITO や In_2O_3 だけでなく、$In(OH)_3$においても肺障害が引き起こされ、さらに ITO や In_2O_3 に比べて肺障害性は著しく強いことが明らかになった。

今回、ITO、In_2O_3 および $In(OH)_3$ は同じモル濃度のインジウム化合物を投与したにも係わらず、$In(OH)_3$ 群で毒性は強く発現した。毒性発現の強さは血液中インジウム濃度および粒子径に関連している可能性があると考えられる。$In(OH)_3$群の血液中インジウム濃度は約 1000μg/L と非常に高濃度であり、観察期間中に $In(OH)_3$ 群では 2 匹死亡した。ヒトの死亡例での血清中インジウム濃度が約 300μg/L であったことから、短期間での血清中インジウム

濃度の急激な増加が死亡に至るほどの衰弱を引き起こしたものと考えられる。高濃度の血液中インジウム濃度が観察された理由の一つとしてはITOやIn_2O_3に比べて$In(OH)_3$の粒子径が非常に微細であることが考えられる。これらインジウム化合物は不溶性ではあるが、$In(OH)_3$群の血液中インジウム濃度はITOおよびIn_2O_3群の約70倍から200倍と高濃度であった。金属微粒子（MMD=16nm）をラットに曝露した実験では肺胞Ⅰ型細胞の細胞質内に金粒子の存在が確認されたことや肺の炎症時などの血管透過性が亢進している場合には、明らかにナノ粒子の肺胞壁通過が起こっていることから、体内で極わずかに溶解したインジウムと超微細な$In(OH)_3$粒子が血中に移行し、高濃度の血液中インジウム濃度に寄与している可能性が考えられた。

投与終了直後と3週目の血液中インジウム濃度は有意な差はないが、3週目で上昇したことより、血清中インジウム濃度は少なくても投与終了後3週間までは増加するものと考えられた。以前の研究では、インジウムヒ素やインジウムリン投与終了後8週目では投与終了直後に比べて血清中インジウム濃度が減少していたことより、インジウム化合物の曝露が無くなった後も血中濃度はしばらく上昇し、ある時点で、減少に転じると考えられる。このことは職業性曝露における血清中インジウム濃度のモニタリングでは、曝露中止後も血清中インジウム濃度が上昇する可能性があることを考慮する必要があると考えられた。

肺相対重量がITO群やIn_2O_3　群では3週目まで横ばいであり、さらに、$In(OH)_3$群では経時的に増加していたことより、肺の炎症は少なくても3週目までは持続していると考えられた。$In(OH)_3$群では肺胞内にマクロファージの壊死片を含む浸出液の著明な沈着が観察された。このことは肺胞マクロファージが$In(OH)_3$の微粒子を貪食したのちインジウムが肺胞マクロファージに対して障害性が強いために肺胞マクロファージの細胞質が崩壊し、肺胞内に沈着したものと推測される。さらに、肺胞マクロファージか崩壊する際にNOが放出され、肺胞上皮細胞が持続的に障害されていた可能性が考えられる。粒子径と肺障害性に関しては、2種類の二酸化チタン（粒子径が21nm以下と250nm）をラットの気管内に投与した場合に粒子径が21nm以下の超微細二酸化チタン粒子が粒子径250nm粒子に比べて肺の炎症性病変が強く発現することが報告されている。 このことより、インジウム化合物の粒子径と肺障害性には強い関連性があると考えられた。

今回用いたインジウム化合物は不溶性であるが、肺や体内組織中での溶解性や体内分布はほとんど解明されていない。インジウム化合物の毒性発現の機序の解明には肺を含む臓器中インジウム濃度を測定することによるインジウムの代謝についての検討が必要だと考える。現在までに、種々のインジウム化合物の毒性実験が行われ、肺障害性や肺発がん性が報告されている。発がん性に関しては化合物半導体であるインジウムリン(InP)とITOの吸入曝露実験で発がん性が報告されている。米国ではInPのラットとマウスを用いた吸入曝露実験が実施され、ラットおよびマウスで肺発がん性が報告された。その結果を受けて、国際がん研究機関（IARC）では「ヒトに対しておそらく発がん性がある」とされるグループ2Aと評価した。さらに、日本においてはITO研削粉のラットとマウスを用いた吸入曝露実験が行われ、最低濃度の0.1 mg/m^{3}より肺の細気管支・肺胞上皮腺腫と細気管支・肺胞上皮癌の発生が雌雄のラットで観察され、悪性・良性腫瘍発生率は

曝露濃度依存性に増加した。一方、マウスでは明らかな肺腫瘍発生の増加は観察されなかった。これらの結果より、ITOの吸入曝露実験でラットでは肺発がん性が明らかになった。

アメリカ NTP のインジウムリンの吸入曝露および日本における ITO 研削粉の吸入曝露実験において肺の発がん性が認められていることより、インジウムが発がん性に寄与していると推測され、他のインジウム化合物の発がん性の可能性は否定できない。今後、水酸化インジウムを含めたインジウム化合物の発がん性の評価が必要であると考える。

E. 結論

今回の結果より、$In(OH)_3$の全身性障害や肺障害はITOおよびIn_2O_3に比べて非常に強いことが明らかになった。インジウムのリサイクル工程では$In(OH)_3$が精製されることより、$In(OH)_3$の取り扱いには格段の注意を払う必要があると考えられた。

F. 研究発表

1.論文発表

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2. Akiyo Tanaka, Miyuki Hirata, Nagisa Matsumura, Kazunori Koga, Masaharu Shiratani and Yutaka Kiyohara, Comparative study on the pulmonary toxicity of indium hydroxide, indium-tin oxide, and indium oxide following intratracheal instillations into the lungs of rats. MRS Sympodium Proceedings/MRS Online Proceeding Library, Vol 1723, 2015 CAMBRIDGE UNIVERSITY PRESS DOI:http://dx.doi.org/10.1557/opl./2015.21

2. 学会発表

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G. 知的所有権の取得状況

1. 特許取得　なし
2. 実用新案登録　なし

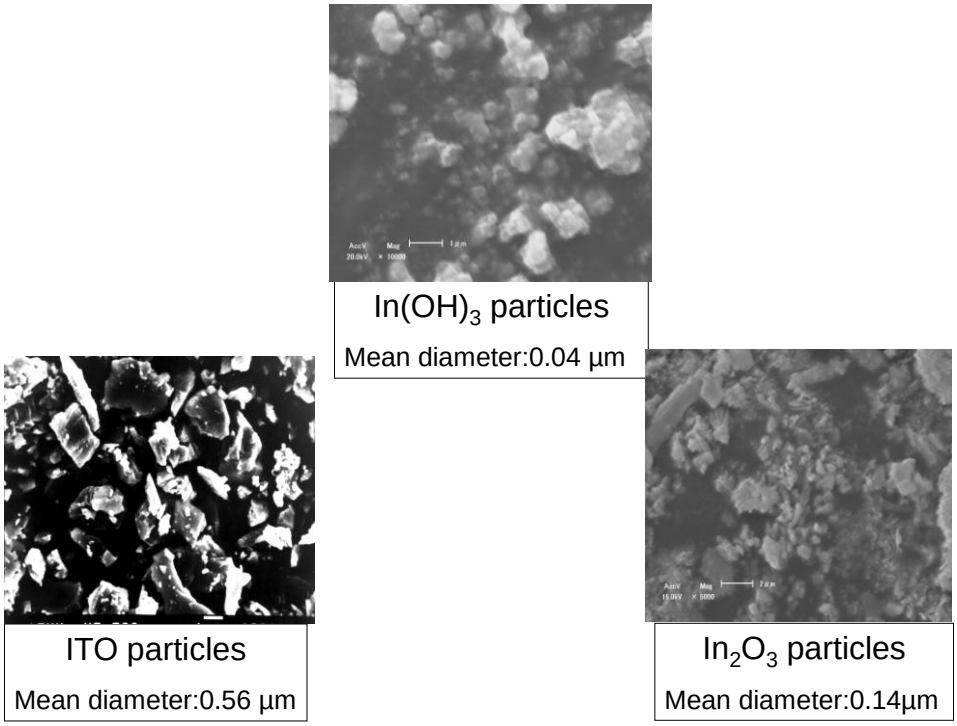


Fig.1 Scanning electron micrographs of $In(OH)_3$, ITO and In_2O_3 particles

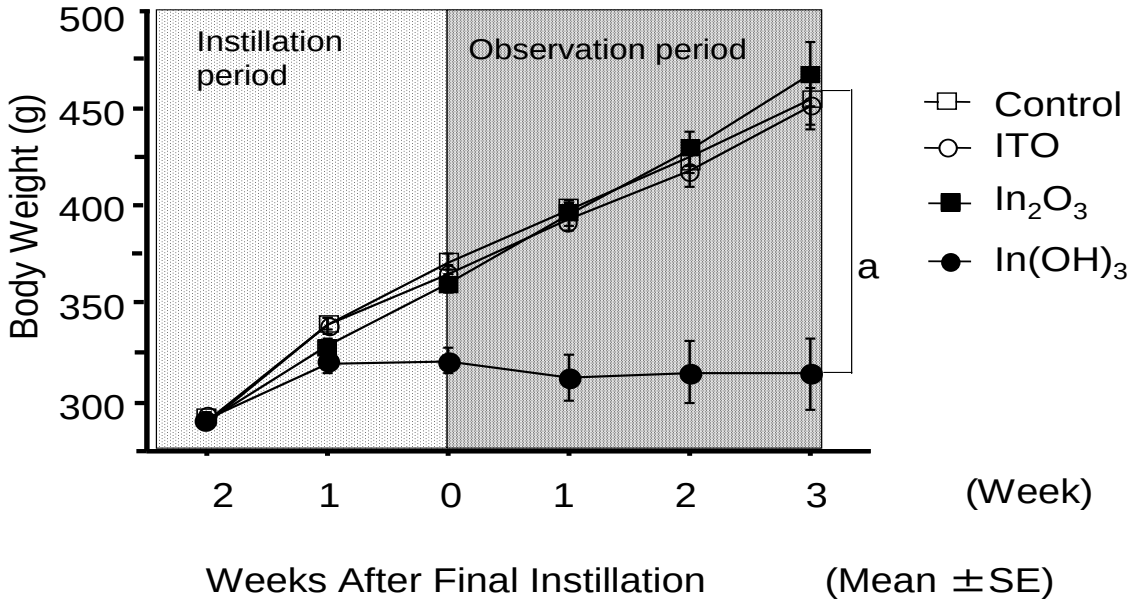
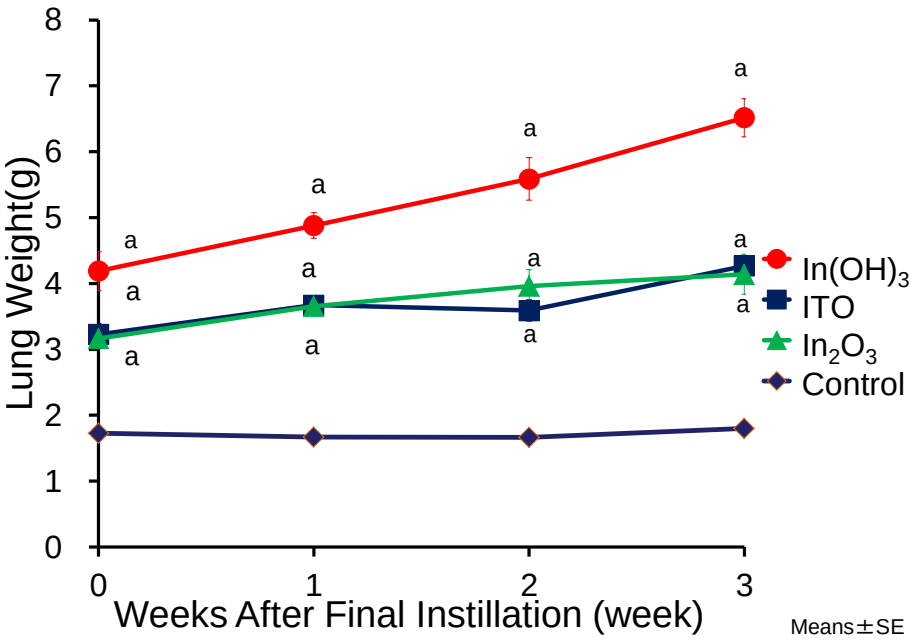


Fig.2 Changes in body weight gain during instillation and observation period. The results shown are mean±SE. Significant differences are indicated by a ($P<0.05$).

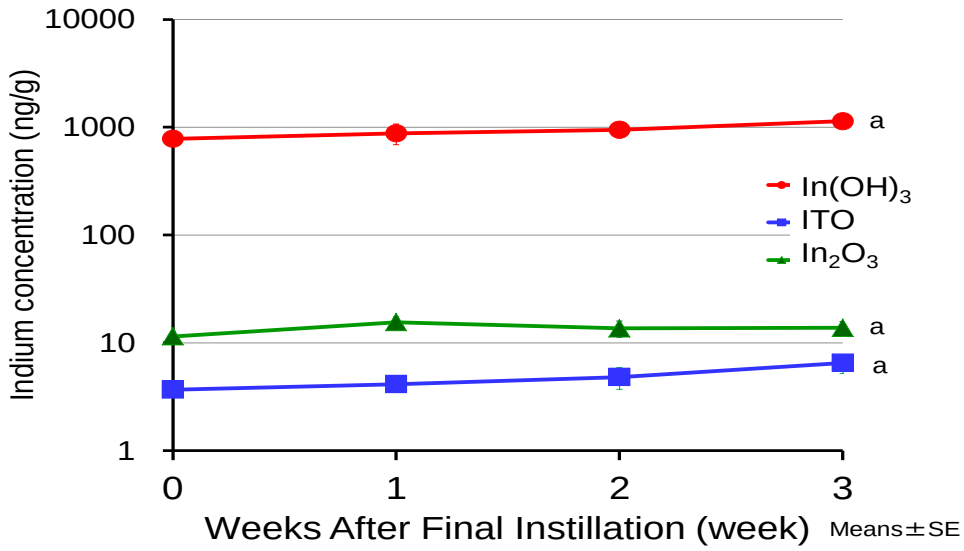


a:Significantly different from the control group at each time point.

Fig.3 Changes in relative lung weights from the final instillation. The results shown are mean±SD of rats euthanized at each time point

a: Significantly different from the control group($P<0.05$)

b: Significantly different from the ITO or the In_2O_3 group($P<0.05$)



a:Significantly different from the control group at each time point.

Fig.4 Change in blood indium concentration from the final instillation of $In(OH)_3$, ITO and In_2O_3.

a: Significantly different from the control group ($P<0.05$)

b: Significantly different from the ITO or the In_2O_3 group ($P<0.05$)

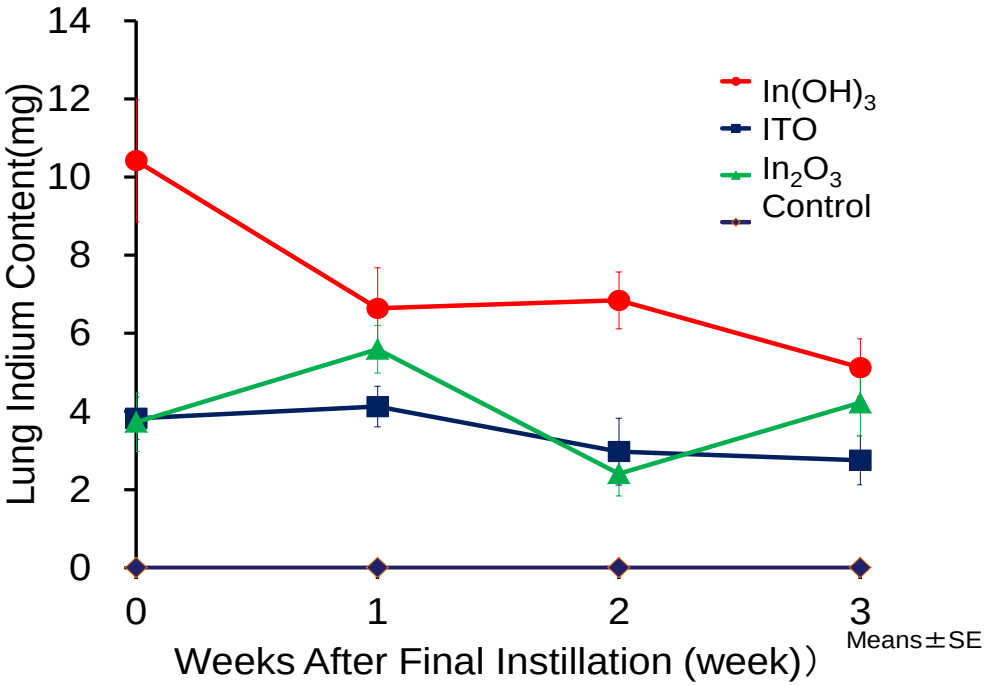
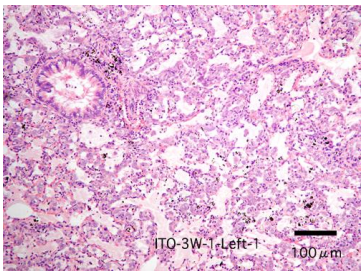
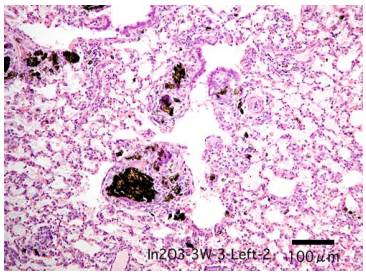


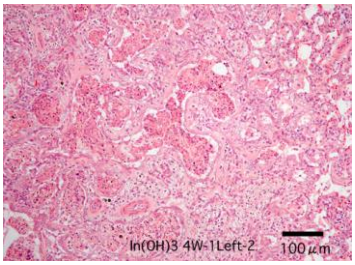
Fig.5 Change of indium content in the lungs after final instillation of $In(OH)_3$, ITO and In_2O_3.



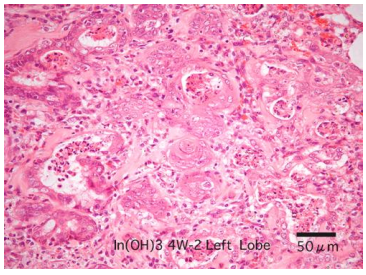
Slight inflammation and deposition of ITO particles at 3 weeks in the ITO group



Slight inflammation and deposition of In_2O_3 particles at 3 weeks in the of In_2O_3 group



Severe inflammation and interstitial fibrotic proliferation at 3 weeks in the $In(OH)_3$ group



Squamous cell metaplasia of the alveolar epithelium and inflammation at 3 weeks in the $In(OH)_3$ group

Fig.6 Pathological changes in the lung in the $In(OH)_3$, ITO and In_2O_3 groups.

Table 1 Pathological changes in the rats lung after intratracheal instillation of $In(OH)_3$, ITO, In_2O_3 .

Pathological changes	Group	Weeks after final instillation (W)			
		0	1	2	3
Inflammatory response with diffuse hyperplasia of bronchiolo-alveolar epithelium	ITO	+	+	+	+
	In_2O_3	+	+	+	+
	$In(OH)_3$	3+	3 +	3 +	3 +
	Control	±	±	±	±
Interstitial fibrotic proliferation	ITO	-	-	-	-
	In_2O_3	-	-	-	-
	$In(OH)_3$	+	2 +	2+	3 +
	Control	-	-	-	-
Exudation	ITO	±	2 +	+	+
	In_2O_3	±	±	+	+
	$In(OH)_3$	2 +	3+	3+	3+
	Control	-	-	-	-

The severity of the lung lesions was evaluated by five grades:
－, negative; ±, slight; ＋, mild; 2＋, moderate; 3＋, severe

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
特になし							

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Nakano M, Omae K, Uchida K, Michikawa T, Yoshioka N, Hirata M, Tanaka A	Five-year cohort study : emphysematous progression of indium-exposed workers	Chest	146	1166-1175	2014
Tanaka A, Hirata M, Matsumura N, Kiyohara Y	Tissue distribution of indium after repeated intratracheal instillations of indium-tin oxide into the lungs of hamsters	J Occup Health	57	189-192	2015
Tanaka A, Hirata M, Matsumura N, Koga K, Shiratani M, Kiyohara Y	Comparative study on the pulmonary toxicity of indium hydroxide, indium-tin oxide, and indium oxide following intratracheal instillations into the lungs of rats.	MRS Sympodium Proceedings /MRS Online Proceeding Library、CAMBRIDGE UNIVERSITY PRESS	Vol 1723	DOI:http://dx.doi.org/10.1557/opl.2015.21	2015
Nakano M, Tanaka A, Hirata M, Iwasawa S, Omae K	Pulmonary effects in workers exposed to indium metal: a cross-sectional study	J Occup Health	in press		
Amano T, Sarinont T, Koga K, Hirata M, Tanaka A, Shiratani M	Synthesis of indium-containing nanoparticles in aqueous suspension using plasmas in water for evaluating their kinetics in living body	Journal of nanoscience and nanotechnology	in press		

Iwasawa S, Nakano M, Miyauchi H, Tanaka S, Kawasumi Y, Higashikubo I, Tanaka A, Hirata M, Omae K	Personal indium exposure concentration in respirable dusts and serum indium level	J Occup Health	in press		

CHEST

Five-Year Cohort Study
Emphysematous Progression of Indium-Exposed Workers

Makiko Nakano, MD, PhD; Kazuyuki Omae, MD, PhD; Kazuhiko Uchida, MD, PhD; Takehiro Michikawa, MD, PhD; Noriyuki Yoshioka, DVM; Miyuki Hirata, PhD; and Akiyo Tanaka, DVM, PhD

BACKGROUND: Dose-dependent adverse lung effects due to indium exposure have been reported in a cross-sectional study. This is a 5-year longitudinal cohort study of indium-exposed and unexposed workers, assessing indium exposure levels and its clinical lung effects.

METHODS: From 2008 to 2011, a 5-year follow-up study was conducted on 40 unexposed and 240 workers formerly or currently exposed to indium at 11 factories. Indium exposure was assessed by serum indium (In-S) (μg/L). Lung effects were assessed by subjective symptoms, serum biomarkers, spirometry, and chest high-resolution CT scan. Effect biomarkers used were Krebs von den Lungen and surfactant protein D.

RESULTS: Mean values of In-S, Krebs von den Lungen, and surfactant protein D among the workers exposed to indium at baseline declined during the 5-year follow-up by 29.8%, 27.2%, and 27.5%, respectively. Of the exposed subjects with In-S levels > 20 μg/L, 26.3% experienced emphysematous progression on high-resolution CT scan. Ninety percent (18 of 20) of workers with emphysematous progression during follow-up were current smokers at baseline, and a trend of increasing incidence of emphysematous progression at higher In-S levels was observed among the smokers ($P = .005$). Emphysematous changes among subjects with In-S levels > 20 μg/L were likely to progress, after adjusting for age, mean duration since initial indium exposure, and smoking history (OR = 10.49, 95% CI = 1.54-71.36).

CONCLUSIONS: Long-term adverse effects on emphysematous changes were observed. The results suggest workers exposed to indium with In-S levels > 20 μg/L should be immediately removed from exposure.

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ABBREVIATIONS: HRCT = high-resolution CT; In-S = serum indium; ITO = indium-tin oxide; KL-6 = Krebs von den Lungen; SP-D = surfactant protein D

AFFILIATIONS: From the Department of Preventive Medicine and Public Health (Drs Nakano, Omae, Uchida, Michikawa, and Yoshioka), School of Medicine, Keio University, Tokyo; Environmental Epidemiology Section (Dr Michikawa), Center for Environmental Health Sciences, National Institute for Environmental Studies, Tsukuba; and Environmental Medicine (Drs Hirata and Tanaka), Graduate School of Medical Science, Kyushu University, Fukuoka, Japan.

FUNDING/SUPPORT: This study was supported by Grants-in-aid for Scientific Research (Project Nos. 15390191, 17390179, 20249039, and 23249033) from the Ministry of Education, Culture, Sports, Science and Technology of Japan [2003-4, 2005-6, 2008-10, and 2011] and in part by donations for research in preventive and environmental medicine from two of the surveyed companies.

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DOI: 10.1378/chest.13-2484

Indium is a rare metal used in the form of indium-tin oxide (ITO) as the electrode in flat panel displays. Japan is the largest consumer of indium accounting for 85% of global demand.[1] To our knowledge, the first (and fatal) case of indium-related lung disease (indium lung) was reported in Japan in 2003.[2] As of 2011,[3] seven more cases have been reported in Japan,[4,5] two in the United States,[6] and one in China.[7] In Japan, a study of indium-exposed workers was conducted at the ITO-processing factory where, to our knowledge, the first case was reported,[8] as well as a multicenter study of indium-exposed and unexposed workers in other ITO-processing and ITO-recycling plants.[9] The multicenter cross-sectional cohort study was later expanded and found dose-dependent adverse lung effects due to indium exposure.[10] The harmful effect of indium was further brought to light by a 2-year ITO inhalation experiment, revealing ITO as a lung carcinogen in rats.[11] Based on these findings, in 2010, the Japanese Ministry of Health, Labor and Welfare established a prevention guideline for ITO-processing workers[12] and, in 2013, added indium to the list of substances regulated by the Ordinance on Prevention of Hazards due to Specified Chemical Substances.[13,14]

The long-term effects of indium exposure on the lungs remain largely unknown. This is a 5-year follow-up study of the largest cohort of indium-exposed and unexposed workers. Our objective is to assess the association between exposure levels of indium and its clinical effects on the lungs.

Materials and Methods

This study was approved by the ethical committee of the School of Medicine at Keio University (approval numbers 15-46 and 20110268). Written informed consent was obtained from all subjects.

Study Design and Subjects

In comparison with the multicenter baseline study conducted at 12 factories and one research laboratory between 2003 and 2006,[10] this longitudinal study added workers from an additional factory and removed cohort members from three factories due to logistics. The resulting dataset covered 11 plants, including 383 exposed and 159 unexposed workers. Approximately 5 years after the baseline study (2003-2006), we conducted a follow-up study at these 11 plants between 2008 and 2011, involving 247 exposed and 63 unexposed workers (follow-up rates 64.5% and 39.6%). Among the subjects with high baseline serum indium (In-S) levels ($\geq$ 20 μg/L), the follow-up rate was 82.6% (Fig 1).

At the 5-year follow-up, in accordance with the baseline study, a medical interview, questionnaire, blood test, spirometry, and high-resolution CT (HRCT) scan examination of the lungs were all conducted on 240 exposed and 40 unexposed subjects, excluding seven subjects with undetermined exposure duration and 23 unexposed workers exposed during follow-up. One unexposed and 10 exposed workers were excluded from final analysis of the lung function test results due to inadequate test maneuvers or a medical history of surgical lung resection. Of the 280 subjects, baseline HRCT scans for 207 workers were obtained, allowing direct comparison with the follow-up scans. Thirty-five unexposed and 172 exposed workers had HRCTs from both the baseline and follow-up.

Categorization of exposed workers into currently or formerly exposed groups was based on their exposure status at baseline. Job history was based on the job records at the plants, or if unavailable, based on physician's interview about occupational history.

Exposure Indexes

In-S (μg/L) was measured by inductively coupled plasma mass spectrometry at the Center of Advanced Instrumental Analysis, Kyushu University.[9] In-S below the detection limit (0.1 μg/L) was ascribed an arbitrary value of 0.05 μg/L for statistical analysis.

Effect Indexes and Confounding Factors

Medical examinations conducted at the 5-year follow-up were the same as those at the baseline study. Serum Krebs von den Lungen-6 (KL-6) (EIDIA Co, Ltd)[15,16] and serum surfactant protein D (SP-D) (Yamasa Corporation)[17] were used as biomarkers for interstitial changes in the lungs and evaluated at a major commercial clinical laboratory (Special Reference Laboratory).

Spirometry was performed using electronic spirometers (HI-701 or HI-801; CHEST M.I. Inc) based on American Thoracic Society guidelines. Age and height-adjusted predicted values of FVC and FEV_1 were determined by sex, using the regression formula recommended by the Japanese Respiratory Society[18]; and percentages of predicted FVC and FEV_1 were calculated.

At nine of the plants, HRCT scanning was performed in a specially assembled vehicle, using the same multislice CT scanner as the baseline study[10] at 120 kV, 200 mA, and a slice thickness of 1 mm. For the other two plants, HRCT scanning was performed at nearby hospitals with a helical or multislice CT scanner. All HRCT scans were carried out at three lung levels (the upper, middle, and lower lung fields) as recommended by the Japanese Respiratory Society. The same technique was used in the baseline study.[10]

In accordance with the Japanese Respiratory Society guideline for the diagnosis and management of COPD,[19] interstitial changes, including interlobular septal thickening, ground-glass appearance, and nodular infiltrate, as well as emphysematous changes in the upper, middle, and lower bilateral lung fields[20] were jointly assessed by a Japan Radiologic Society-certified radiologist and a Japanese Respiratory Society-certified pulmonologist. The two experts assessed all the scans together, comparing side by side the clearly defined lung fields on the baseline and follow-up HRCT scans for each subject. Emphysematous change was defined as an emergence of a new or enlarged low attenuation area on any one of the six HRCT scan slices. Worsening of the follow-up CT scan compared with the baseline CT scan was labeled "progression of interstitial changes" or "progression of emphysematous changes," and an improvement or no change was labeled "no progression."

Using the Japanese version[21] of the American Thoracic Society-Division of Lung Disease questionnaire[22] and supplementary questions, the following were investigated: respiratory symptoms, smoking history, and confounding factors including sex, age, medical history, and history of exposure to other materials.

Statistical Analysis

Nonnormally distributed data were transformed to an approximately normal distribution before analysis. The Student *t* test or the Mann-Whitney *U* test was used to compare continuous variables between exposed and unexposed groups. The χ^2 test or Fisher exact method was used to compare proportions, prevalence, or incidence.

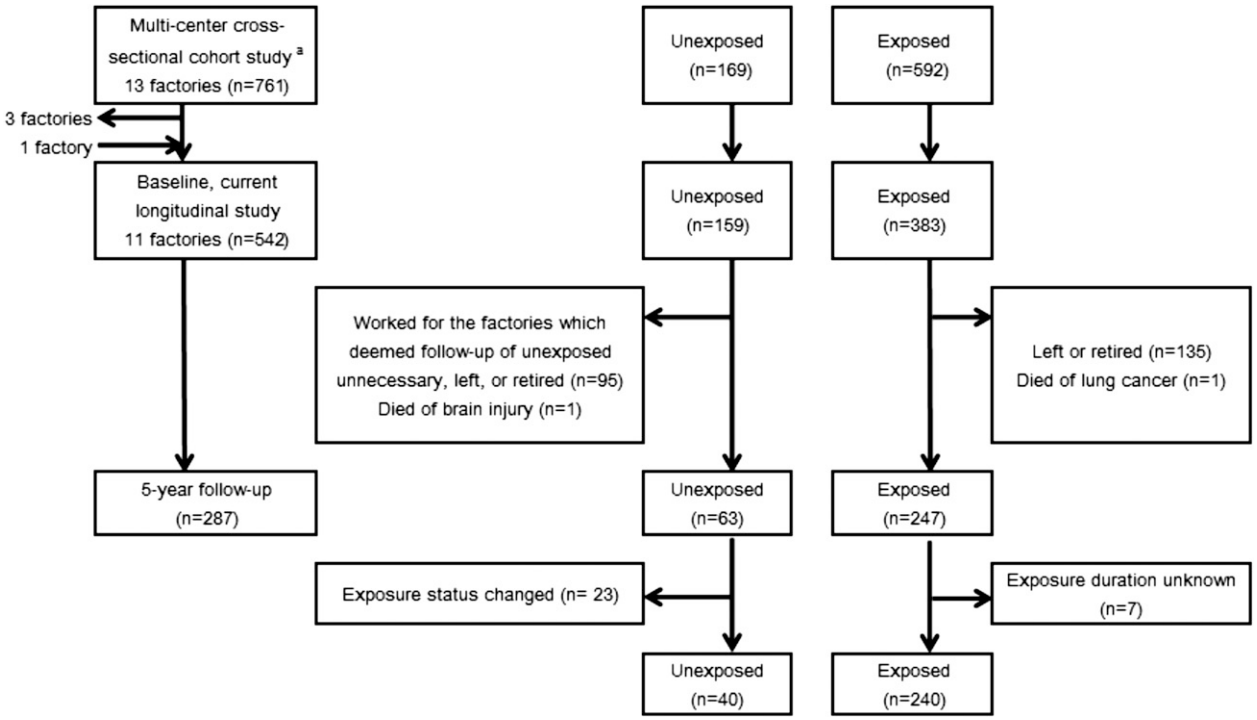

Figure 1 – *Study population. The flow of the subjects.* [a]*Nakano et al.*[10]

Based on the classification criteria of In-S adopted in our baseline study,[10] the exposed subjects were stratified into six In-S categories: In-S level $<$ 1.0 μg/L, 1.0 to 2.9 μg/L, 3.0 to 4.9 μg/L, 5.0 to 9.9 μg/L, 10.0 to 19.9 μg/L, and $>$ 20.0 μg/L. These six categories were used to assess the risk of indium exposure on the effect variables, as well as their dose-response relationship.

Mean values of 5-year differences in biomarkers and lung functions among the exposed subjects were stratified by the aforementioned six In-S categories and compared with the unexposed subjects using the Dunnett test. Incidence of abnormalities (change from normal to abnormal values) by exposure group for the biomarkers, lung function, and HRCT scan progression was analyzed, using the following cutoff for abnormal values: KL-6 $\geq$ 500 U/mL, SP-D $\geq$ 110 ng/mL, $FEV_1/FVC < 70\%$, $\%FVC < 80\%$, and $\%FEV_1 < 80\%$. Test for trend in the In-S categories was performed using the Cochran-Armitage test for categorical data.

Based on the test for trend in the In-S categories, as well as the analysis of exposure and effect indexes with respect to HRCT scan progression, the relationship between In-S and HRCT scan progression was further analyzed, using a logistic regression model. Adjusted variables were age, mean duration since initial indium exposure, and smoking.

Statistical significance was assessed by two-tailed analysis with $P < .05$. All statistical analyses were performed using SPSS, version 19 (IBM) and JMP, version 10.0.2 (SAS Institute Inc).

Results

Table 1 shows the characteristics of the study subjects and the pulmonary effects of indium at baseline and at 5-year follow-up. The mean duration since first indium exposure was 5.5 years for the currently exposed group at baseline and 12.1 years for the formerly exposed. The currently exposed subjects were younger than the unexposed workers ($P < .05$). No difference in the proportion of male subjects and smoking history was observed between the exposed and unexposed subjects.

At baseline, the mean values for KL-6, SP-D, and pulmonary symptoms in the exposed group were significantly higher than in the unexposed group. For the mean values of pulmonary function test results, no difference was observed between the two groups.

At follow-up, the mean values of In-S, KL-6, and SP-D among the currently exposed workers declined from baseline by 29.8%, 27.2%, and 27.5%; those among the formerly exposed declined by 39.4%, 24.7%, and 21.9%, respectively. The significant difference observed at baseline in KL-6 between the unexposed and the exposed groups disappeared at follow-up. Mean values of FEV_1/FVC, %FVC, and $\%FEV_1$ in the exposed group slightly decreased during follow-up.

Table 2 shows the 5-year differences and incidence of abnormal values in serum biomarkers and lung function, stratified by In-S levels. Those with baseline In-S levels above 3 μg/L displayed a notable 5-year decline in In-S. With increasing baseline In-S levels,

TABLE 1] Characteristics, Exposure Levels, Biomarkers of Effect, and Lung Function of Unexposed and Indium-Exposed Subjects at Baseline and at 5-Year Follow-up

	Baseline Study			Follow-up Study		
Characteristics	Unexposed (n = 40)	Currently Exposed (n = 207)	Formerly Exposed (n = 33)	Unexposed (n = 40)	Currently Exposed (n = 207)	Formerly Exposed (n = 33)
Age (SD), y	44.5 (10.5)	36.8[a] (10.5)	40.4 (8.8)	49.9 (10.6)	41.9[a] (11.9)	45.2 (8.4)
Male, %	90.0	90.8	97.0	...	...	...
In-S (range), μg/L	0.4 (<0.1, 1.5)	10.4[a] (<0.1, 117)	17.5[a] (<0.1, 83.3)	0.1 (<0.1, 0.5)	7.3[a] (<0.1, 132)	10.6[a] (<0.1, 64.4)
Mean duration (SD), y						
Since initial indium exposure	...	5.5 (0.1, 38.6)	12.1[a] (0.6, 23.8)	...	10.5 (3.8, 44.3)	16.8[a] (5.5, 27.7)
Since exposure cessation	...	...	5.2 (0.2, 16.8)	...	3.7 (0.3, 13.1)	9.7[a] (0.3, 20.9)
Smoking, No. (%)						
Never smokers	13 (32.5)	65 (31.4)	7 (21.2)	12 (30.0)	60 (29.0)	5 (15.2)
Ex-smokers	11 (27.5)	24 (11.6)	6 (18.2)	15 (37.5)	50 (24.2)	10 (30.3)
Current smokers	16 (40.0)	118 (57.0)	20 (60.6)	13 (32.5)	97 (46.9)	18 (54.5)
Exposure status at follow-up, No. (%)						
Currently exposed	...	...	...	...	158 (76.3)	6 (18.2)
Formerly exposed	...	...	...	...	49 (23.7)	27 (81.8)
Cough or sputum, No./total (%)	6/40 (15.0)	69/206 (33.5)	10/32 (31.3)	5/40 (12.5)	21/202 (10.4)	3/32 (9.4)
Biomarkers of effect, geometric mean (geometric SD)						
KL-6, U/mL	245.8 (1.5)	382.4[a] (2.1)	373.9[b] (2.2)	229 (1.5)	278.2 (1.7)	281.7 (1.7)
SP-D, ng/mL	42.4 (1.7)	62.9[a] (2.0)	80.7[a] (2.3)	39.9 (1.8)	45.6 (2.0)	63.0[a] (2.0)
Lung function, % (SD)						
FEV_1/FVC	80.7 (6.7)	82.8 (6.0)	82.2 (5.0)	80.3 (6.2)	82.0 (6.2)	80.9 (5.2)
%FVC	100.1 (12.8)	100.0 (12.3)	99.3 (12.5)	100.5 (12.7)	99.2 (11.8)	98.2 (10.6)
$\%FEV_1$	93.7 (12.9)	94.5 (11.8)	93.9 (11.4)	94.9 (12.8)	94.1 (12.3)	92.3 (10.5)

In-S = serum indium; KL-6 = Krebs von den Lungen; SP-D = surfactant protein D.

[a] $P < .05$ by Student t test, χ^2 test, or Fisher exact method with respect to unexposed subjects.

[b] $P < .01$ by Student t test, χ^2 test, or Fisher exact method with respect to unexposed subjects.

TABLE 2] Five-Year Differences and Incidence of Abnormal Values in Biomarkers and Lung Function, Stratified by In-S Categories

Findings	Unexposed (n = 40)	Exposed Workers by In-S Categories (N = 240)						*P* Value for Trend
		< 1.0 (n = 70)	1.0-2.9 (n = 43)	3.0-4.9 (n = 20)	5.0-9.9 (n = 34)	10.0-19.9 (n = 35)	≥ 20.0 (n = 38)	
In-S								
Mean difference (range), μg/L	−0.28 (−1.5, 0.2)	0.26 (−0.5, 5.6)	0.50 (−1.8, 8.3)	−0.86 (−4.0, 5.6)	−2.12 (−7.6, 8.0)	5.66 (−15.0, 4.9)	16.38[a] (−78.9, 32.4)	...
KL-6								
Mean difference (SD), U/mL	−19.2 (84.3)	−36.7 (152.5)	−28.5 (269.6)	−105.4 (126.7)	−169.5 (213.7)	−300.7[b] (396.5)	−747.3[a] (1036.3)	...
Incidence of abnormal values,[c] No. (%)	1/40 (2.5)	0/70 (0.0)	2/43 (4.7)	0/20 (0.0)	0/34 (0.0)	2/35 (5.7)	0/38 (0.0)	.909
Currently exposed at baseline	...	0/65 (0.0)	2/36 (5.6)	0/19 (0.0)	0/29 (0.0)	2/28 (7.1)	0/30 (0.0)	.781
Formerly exposed at baseline	...	0/5 (0.0)	0/7 (0.0)	0/1 (0.0)	0/5 (0.0)	0/7 (0.0)	0/8 (0.0)	.444
SP-D								
Mean difference, ng/mL (SD)	−0.42 (20.1)	−8.14 (18.5)	−14.13 (31.2)	−19.53 (21.2)	−19.69 (19.4)	−25.17[a] (37.4)	−54.92[a] (63.6)	...
Incidence of abnormal values,[c] No. (%)	2/40 (5.0)	0/70 (0.0)	0/43 (0.0)	0/20 (0.0)	1/34 (2.9)	0/35 (0.0)	0/38 (0.0)	.246
Currently exposed at baseline	...	0/65 (0.0)	0/36 (0.0)	0/19 (0.0)	1/29 (3.4)	0/28 (0.0)	0/30 (0.0)	.296
Formerly exposed at baseline	...	0/5 (0.0)	0/7 (0.0)	0/1 (0.0)	0/5 (0.0)	0/7 (0.0)	0/8 (0.0)	.276
FEV_1/FVC								
Mean difference, % (SD)	−0.37 (2.93)	−0.69 (3.65)	−0.18 (3.70)	−0.82 (2.42)	−1.06 (2.95)	−0.56 (3.93)	−2.26 (5.07)	...
Incidence of abnormal values,[c] No. (%)	1/39 (2.6)	1/69 (1.4)	0/42 (0.0)	0/20 (0.0)	2/33 (6.1)	0/33 (0.0)	4/33 (12.1)	.034
%FVC								
Mean difference (SD), %	0.39 (6.73)	−0.41 (7.96)	−1.65 (5.82)	−0.28 (6.89)	−3.58[b] (5.52)	−0.84 (5.22)	1.81 (4.77)	...
Incidence of abnormal values,[c] No. (%)	0/39 (0.0)	0/69 (0.0)	1/42 (2.4)	0/20 (0.0)	1/33 (3.0)	0/33 (0.0)	0/33 (0.0)	.796

(Continued)

TABLE 2 *(continued)*

Findings	Unexposed (n = 40)	Exposed Workers by In-S Categories (N = 240)						*P* Value for Trend
		< 1.0 (n = 70)	1.0-2.9 (n = 43)	3.0-4.9 (n = 20)	5.0-9.9 (n = 34)	10.0-19.9 (n = 35)	≥ 20.0 (n = 38)	
$\%FEV_1$								
Mean difference (SD), %	1.11 (5.69)	0.46 (6.39)	−0.82 (5.58)	−0.27 (6.40)	−3.63 [a] (4.68)	−0.37 (4.81)	0.17 (6.92)	...
Incidence of abnormal values,[c] No. (%)	0/39 (0.0)	0/69 (0.0)	1/42 (2.4)	0/20 (0.0)	1/33 (3.0)	2/33 (6.1)	2/33 (6.1)	.012

See Table 1 legend for expansion of abbreviations.
[a] $P < .05$ by Dunnett test (for categorized In-S) with respect to unexposed subjects.
[b] $P < .01$ by Dunnett test (for categorized In-S) with respect to unexposed subjects.
[c] Cutoff for abnormal values: KL-6 ≥ 500 U/mL, SP-D ≥ 110 ng/mL, $FEV_1/FVC < 70\%$, $\%FVC < 80\%$, $\%FEV_1 < 80\%$.

increasing incidence of abnormal lung functions (FEV_1/FVC and $\%FEV_1$) was observed ($P < .05$).

Table 3 shows the incidence of progression on HRCT scan findings during 5-year follow-up stratified by In-S categories. Incidences of emphysematous progression in unexposed and exposed workers were two of 35 (5.7%) and 20 of 172 (11.6%). Dose-dependent increase of the incidence was observed (*P* for trend = .002). Among the exposed subjects with In-S levels above 20 μg/L, 26.3% experienced emphysematous progression, and the crude OR was 5.89 (95% CI = 1.19-29.17). Of 20 exposed workers with emphysematous progression, 18 were ex- or current smokers at baseline. A statistically significant trend of increasing incidence was observed for smokers ($P = .005$). When exposed to high levels of indium (In-S ≥ 20 μg/L), nine of 31 smokers (29.0%) experienced emphysematous progression, compared with one of seven (14.3%) never smokers. Meanwhile, incidence of interstitial progression was two of 35 (5.7%) among the unexposed and 10 of 172 (5.8%) among the exposed workers. No statistically significant trend was observed.

Table 4 compares, within the exposed workers, the baseline indium exposure levels, serum biomarkers, and lung functions between those with and without HRCT scan progression. Compared with those without an emphysematous deterioration, those who experienced emphysematous progression exhibited significantly higher In-S levels, were older in age, had higher KL-6 levels, and lower $\%FEV_1$. Regarding those with and without progression in interstitial changes, there was no statistical difference in In-S and in KL-6, while those with interstitial changes had statistically higher SP-D levels ($P < .01$) and lower FEV_1/FVC ($P < .05$).

Discussion

The 5-year follow-up study of indium-exposed and unexposed workers revealed a long-term effect of indium on the lungs in the form of HRCT scan progression in emphysematous changes, despite a significant decline in In-S likely due to the workplace improvements enforced after our baseline study. The dose-response trend between In-S levels and incidence of emphysematous progression indicated that indium inhalation is a serious risk factor for emphysematous progression, especially among the highly exposed workers (In-S ≥ 20 μg/L). Due to the very slow clearance of hardly soluble indium particles from the lungs,[11,23,24] both the currently and formerly exposed workers were

TABLE 3] Incidence of Progression of HRCT Scan Findings During 5-Year Follow-up, Stratified by In-S Categories

Findings	Unexposed (n = 35)	Exposed Workers by In-S Categories (N = 172)						*P* Value for Trend
		< 1.0 (n = 27)	1.0-2.9 (n = 36)	3.0-4.9 (n = 16)	5.0-9.9 (n = 23)	10.0-19.9 (n = 32)	≥ 20.0 (n = 38)	
Emphysematous progression, incidence rate (%)								
All subjects, No./total (%)	2/35 (5.7)	2/27 (7.4)	1/36 (2.8)	0/16 (0.0)	3/23 (13.0)	4/32 (12.5)	10/38 (26.3)	.002
Crude OR (95% CI)	1	1.32 (0.17, 10.03)	0.47 (0.04, 5.45)	1.37 (0.22, 8.75)		2.36 (0.40, 13.85)	5.89 (1.19, 29.17)	...
Adjusted OR (95% CI)	1	1.65 (0.20, 13.96)	0.78 (0.06, 10.13)	1.86 (0.24, 14.45)		3.48 (0.48, 25.08)	10.49 (1.54, 71.36)	...
Currently exposed at baseline	...	2/23 (8.7)	1/29 (3.4)	0/15 (0.0)	2/18 (11.1)	4/25 (16.0)	10/30 (33.3)	< .001
Formerly exposed at baseline	...	0/4 (0.0)	0/7 (0.0)	0/1 (0.0)	1/5 (20.0)	0/7 (0.0)	0/8 (0.0)	.690
Ex-/current smokers at baseline	2/24 (8.3)	1/16 (6.3)	1/23 (4.3)	0/16 (0.0)	3/18 (16.7)	4/24 (16.7)	9/31 (29.0)	.005
Never smokers at baseline	0/11 (0.0)	1/11 (9.1)	0/13 (0.0)	0/0 (0.0)	0/5 (0.0)	0/8 (0.0)	1/7 (14.3)	.506
Interstitial progression, incidence rate (%)								
All subjects, No./total (%)	2/35 (5.7)	2/27 (7.4)	3/36 (8.3)	0/16 (0.0)	0/23 (0.0)	3/32 (9.4)	2/38 (5.3)	.852
Crude OR (95%CI)	1	1.32 (0.17, 10.03)	1.50 (0.24, 9.57)	0.73 (0.12, 4.57)			0.92 (0.12, 6.88)	...
Adjusted OR (95% CI)	1	1.20 (0.13, 10.72)	2.09 (0.25, 17.14)	1.68 (0.20, 14.27)			2.58 (0.24, 27.90)	...
Currently exposed at baseline	...	2/23 (8.7)	2/29 (6.9)	0/15 (0.0)	0/18 (0.0)	2/25 (8.0)	2/30 (6.7)	.911
Formerly exposed at baseline	...	0/4 (0.0)	1/7 (14.3)	0/1 (0.0)	0/5 (0.0)	1/7 (14.3)	0/8 (0.0)	.928
Ex-/current smokers at baseline	1/24 (4.2)	1/16 (6.3)	0/23 (0.0)	0/16 (0.0)	0/18 (0.0)	2/24 (8.3)	2/31 (6.5)	.465
Never smokers at baseline	1/11 (9.1)	1/11 (9.1)	3/13 (23.1)	0/0 (0.0)	0/5 (0.0)	1/8 (12.5)	0/7 (0.0)	.516

Adjusted OR: adjusted variables are age, mean duration since initial indium exposure, and smoking history at baseline. In-S categories with zero incidence were combined to assess the ORs using logistic regression models. See Table 1 legend for expansion of abbreviation.

TABLE 4] Exposure and Effect Indexes at Baseline Among Exposed Workers With or Without Progression of HRCT Scan Findings During Follow-up Period

Characteristics	Emphysematous Changes		Interstitial Changes	
	Progressed	No Progression	Progressed	No Progression
Total subjects, No.	20	152	10	162
In-S (SD), μg/L	34.4[a] (36.0)	12.4 (17.0)	13.3 (16.6)	15.0 (21.5)
Mean duration since initial indium exposure (SD), y	9.0 (5.8)	6.8 (6.3)	7.0 (4.9)	7.1 (6.3)
Age (SD), y	45.1[a] (11.7)	37.8 (12.0)	52.7[b] (11.7)	37.8 (11.6)
Smoking status, No. (%)				
Never smokers	2 (10.0)	42 (27.6)	5 (50.0)	39 (24.1)
Smokers	18 (90.0)	110 (72.4)	5 (50.0)	123 (75.9)
Ex-smokers	2 (10.0)	21 (13.8)	2 (20.0)	21 (13.0)
Current smokers	16 (80.0)	89 (58.6)	3 (30.0)	102 (63.0)
Cough or sputum, No./total (%)	4/20 (20.0)	50/150 (33.3)	1/10 (10.0)	53/160 (33.1)
Biomarkers, geometric mean (geometric SD)				
KL-6, U/mL	651.6[b] (2.3)	405.4 (2.1)	518.3 (1.6)	423.4 (2.2)
SP-D, ng/mL	100.0 (1.9)	73.0 (2.0)	132.4[b] (1.8)	73.2 (2.0)
Lung function, mean (SD)				
FEV_1/FVC	79.0 (6.8)	82.4 (5.2)	78.1[a] (7.8)	82.3 (5.2)
%FVC	94.5 (10.5)	99.8 (11.7)	94.6 (9.5)	99.6 (11.7)
%FEV_1	86.6[a] (9.7)	94.5 (11.9)	87.0 (10.1)	94.0 (11.9)

See Table 1 legend for expansion of abbreviations.
[a]$P < .05$ by Student *t* test, Mann-Whitney *U* test, χ^2 test, or Fisher exact method, with respect to subjects with no progression.
[b]$P < .01$ by Student t test, Mann-Whitney U test, χ^2 test, or Fisher exact method, with respect to subjects with no progression.

being continually exposed to indium by the particles in their lungs, even after the reduction of occupational exposures to indium. Indium particles in the lungs perpetuated the phagocytosis and phagolysosomal acidification[25] cycle by the alveolar macrophages. The proteases released by the macrophages and the cytotoxicity of indium may have promoted macrophage-mediated elastolysis, which is known to cause inflammation and destruction of the lung parenchyma,[26] leading to emphysematous deterioration.

Smoking is a major risk factor of COPD,[27] and the current study showed that smoking is also an important effect modifier of the risk of emphysematous progression among indium-exposed workers. Due to the small number of emphysematous progression in the unexposed workers, we could not assess the interaction between indium exposure and smoking using the logistic regression model. However, in addition to the statistically significant dose-response trend of the incidence among smokers, the incidence ratio of emphysematous progression between smokers and nonsmokers among the highly exposed workers (In-S $\geq$ 20) was 2.03 (95% CI = 0.31-13.5) (data not shown).

In contrast to the emphysematous progression, the incidence rate of progression in interstitial changes was not detected, although a causal relationship between indium exposure and interstitial changes has been previously identified.[10] KL-6 is known to increase in the active phase of interstitial pneumonia and decrease in the inactive phase.[16] In the current study, KL-6 among the indium-exposed subjects declined by 25% after the baseline study and subsequent intervention. These results imply that once indium exposure is eliminated or drastically reduced, KL-6 may significantly decline, although not to the level of the unexposed. Despite no statistically significant exposure-response relationship for interstitial changes across the categories of In-S, 10 exposed workers did have progression of interstitial change, of which four also experienced emphysematous progression. When restricted to In-S $\geq$ 10 μg/L, three of five had both interstitial and emphysematous progression. This raises the concern that interstitial changes and emphysematous changes coexist among highly exposed workers with interstitial progression.

The response rate of the unexposed group was low (40 of 159, 25.2%). Of the 119 workers who contribute to the low response rate, 23 were removed from the analysis because they switched to an exposed environment during follow-up. The remaining 96 unexposed workers lost to follow-up were due to the decision made by a few factories that further follow-up of the unexposed workers was unnecessary. This suggests that self-selection on ill health was unlikely, but the implication of selection bias remains a substantial limitation of the study.

The 5-year follow-up rate was also low at 57.2%. Subanalysis showed that the baseline mean In-S of the subjects lost to follow-up was significantly lower than that of the workers retained at follow-up (2.6 μg/L vs 9.1 μg/L). Additionally, 73 of the 280 followed-up subjects had no baseline HRCT scans. It is not possible to conclude whether including those lost to follow-up and those without baseline scans would have lowered or increased the risk reported in the current study.

Some of the unexposed workers exhibited measurable In-S up to 1.5 μg/L at baseline. The possibility of contamination of unexposed subjects' workplace and exposure misclassification remains.

While a 5-year assessment is insufficient to predict clinical outcomes after longer periods, the current study results provide significant rationale to immediately remove highly exposed workers (In-S $\geq$ 20 μg/L) from indium exposure. The increased prevalence of progression was also evident at lower In-S levels and suggest that an In-S level between 5 μg/L and 19.9 μg/L is a warning sign for action. A larger sample size may have provided the statistical power to lower the preventive recommendation below 20 μg/L. Additionally, high levels of biomarker and abnormal lung function warrant attention as potential predictors of emphysematous changes.

The agenda for future research includes monitoring new clinical cases of indium lung, following further progression of emphysematous and interstitial changes in the current cohort, and studying the occurrence of lung cancer.

Conclusions

This 5-year cohort study of indium workers suggests that In-S burden is a risk factor for progression of emphysematous changes, particularly in smokers, and persists after In-S decreases. The study results provide the basis to immediately remove indium workers with In-S above 20 μg/L from indium exposure and monitor their lung conditions, as well as to consider In-S levels between 5 μg/L and 19.9 μg/L as a warning sign for action.

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Brief Report

Tissue distribution of indium after repeated intratracheal instillations of indium-tin oxide into the lungs of hamsters

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Abstract: Tissue distribution of indium after repeated intratracheal instillations of indium-tin oxide into the lungs of hamsters: Akiyo TANAKA, *et al*. Department of Environmental Medicine, Graduate School of Medical Sciences, Kyushu University—Objectives: The aim of this study was to analyze the tissue distribution of indium after intratracheally instilling indium-tin oxide (ITO) into the lungs of hamsters. **Methods:** Male Syrian hamsters received an intratracheal dose of 3 mg/kg or 6 mg/kg of ITO particles containing 2.2 mg/kg or 4.5 mg/kg of indium, twice weekly for 8 weeks. In parallel, control hamsters received only an intratracheal dose of distilled water. A subset of hamsters was euthanized periodically throughout the study from 8 up to 78 weeks after the final instillation. The distribution of indium in the lungs, liver, kidneys and spleen, as well as pathological changes in the liver, kidneys, and spleen, was determined. **Results:** The contents of indium in the lungs in the two ITO groups gradually decreased over the 78-week observation period, with elimination half-lives of approximately 142 weeks for the 3 mg/kg ITO group and 124 weeks for the 6 mg/kg ITO. The indium concentrations in the liver, kidneys, and spleen gradually increased throughout the observation period. Although foci of the lesions were observed histopathologically in the extrapulmonary organs among the two ITO groups, the control group showed similar lesions. **Conclusions:** The results clearly demonstrate that the clearance of indium from the body is extremely slow after intratracheal instillation in hamsters.
(J Occup Health 2015; 57: 189–192)

Key words: Hamsters, Indium, Indium-tin oxide, Intratracheal instillation, Lung clearance, Tissue indium concentration

Indium is an essential rare metal that is commonly used in the electronics industry; the use of indium compounds, most notably indium-tin oxide (ITO), has risen sharply since the 1990s[1]. Several case reports and epidemiological studies of workers exposed to ITO have heightened awareness of the potential hazards of occupational exposure to this metal[2–4]. Although evidence of pulmonary lesions has been reported after respiratory ITO exposure in animals and humans[2–7], it is not clear whether indium can further distribute throughout the body. In our previous study, pulmonary toxicity in hamsters was demonstrated over a four-month period of repeated intratracheal instillation of 3 mg/kg or 6 mg/kg doses of ITO (2.2 mg/kg or 4.5 mg/kg as indium, respectively)[6]. The serum indium concentrations among the two test groups gradually increased throughout the observation period, and the severity of pulmonary pathologies increased over time. The present study evaluated the long-term peripheral organ distribution of indium from 8 to 78 weeks after repeated intratracheal instillation of ITO into the lungs of hamsters as reported in our previous study[6].

Materials and Methods

ITO particles were prepared as previously reported[6] and were obtained by donation from a corporate source. All animal studies were conducted in accordance with the Guidelines for Animal Experiments in the Graduate School of Medical Sciences, Kyushu University, and in compliance with Law No. 105 and Notification No. 6 of the Government of Japan. Eighty-seven 6-week-old male Syrian hamsters were purchased from the colony of Japan SLC Inc. (Shizuoka, Japan) to be used in this study. The animals were housed in a specific pathogen-free environment at the Laboratory of Animal Experiments in the Graduate School of Medical Sciences, Kyushu University. The lighting, food supply and drinking water were maintained under the same conditions as described previously[6]. Intratracheal instillation of ITO began after a 2-week acclimatization period when

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the animals were 8 weeks old.

The hamsters were randomly divided into 3 groups: a control group (n=29), a 3 mg/kg ITO treatment group (2.2 mg/kg as indium; n=29) and a 6 mg/kg ITO treatment group (4.5 mg/kg as indium; n=29). There was no significant difference in body weight among the groups at the start of the study. A vehicle or test agent was instilled into the trachea of ether-anesthetized hamsters twice weekly over 8 weeks for a total of 16 doses. The control group received 1.0 m*l*/kg of distilled water. Six to eight surviving hamsters in each group were euthanized using carbon dioxide gas and autopsied at 8, 16, 40 or 78 weeks after their final dose. The indium concentrations were measured in the organs of the ITO-treated hamsters, as were the total organ weights, and histopathological examination of the liver, kidneys and spleen was also completed for all hamsters. To measure lung indium content, one apical lobe was soaked in 10 m*l* of 68% ultrapure nitric acid (TAMAPURE-AA-100, Tama Chemicals Co., Ltd., Kawasaki, Kanagawa, Japan) overnight, and 0.1 m*l* of lung soak solution, 0.02 g of liver tissue, 0.1 g of kidney, 0.1 g of spleen tissue or 1 m*l* of serum was digested with 6 m*l* of 68% ultrapure nitric acid (TAMAPURE-AA-100, Tama Chemicals Co., Ltd., Kawasaki, Kanagawa, Japan) and 0.5 m*l* of 35% ultrapure hydrogen peroxide (TAMAPURE-AA-100, Tama Chemicals Co., Ltd., Kawasaki, Kanagawa, Japan) using a microwave digestion apparatus (Multiwave 3000, PerkinElmer, Yokohama, Japan). Digested samples were then diluted into ultrapure water for a total volume of 20 m*l* and injected into an inductively coupled plasma mass spectrometer (ICP-MS, Agilent 7500ce, Agilent Technologies, Tokyo, Japan) at the Center of Advanced Instrumental Analysis, Kyushu University. Rhodium was used as an internal standard for indium measurements. The lower limit of quantitative detection for indium was 0.04 μg/g for the lungs, 0.005 μg/g for the liver, 0.001 μg/g for the kidneys and 0.001 μg/g for the spleen. In the cases where the indium concentrations were below the limit of detection, a value equal to one-half of the limit of detection was used for statistical calculations. The distribution of indium in the lungs was calculated as the concentration of indium in lung tissue.

Samples of the liver, kidneys and spleen were fixed in 10% neutral buffered formalin and processed in paraffin for histopathological examination, which included an evaluation of the severity of lesions as reported in a previous study[6]. Briefly, histopathological findings in these organs were scored as present or absent; if they were absent, findings were expressed as 0. In the case of lesions, the severity of each of the lesions was graded on a 4-point scale ranging from slight to severe. Slight lesions were expressed as 1, mild lesions were expressed as 2, moderate lesions were expressed as 3, and severe lesions were expressed as 4.

For the statistical analyses of organ weights and indium content in the lung, indium concentrations in the liver, kidneys and spleen and score of the severity of the lesions, one-way analysis of variance followed by a Fischer's least significant difference test was applied. In all the statistical comparisons, a p value of <0.05 was considered to represent a significant difference.

Results

Over the course of 8 weeks, hamsters received mean indium doses of 4.8 ± 0.4 mg (mean ± SD) and 9.5 ± 0.9 mg per animal in the 3 mg/kg and 6 mg/kg ITO treatment groups, respectively. The data collected in the course of the study for body weight, lung weight, mortality, serum indium concentration and lung lesions were presented in our previous study[6]. The liver, kidney and spleen weights were not significantly different throughout the course of the study among the two ITO-treated groups and the control group. The indium content in the lungs decreased slowly from 8 to 78 weeks in both ITO-treated groups (Table 1). Although the concentrations of indium in the lung for the 6 mg/kg ITO group were slightly higher at 78 weeks than at 40 weeks, the clearance of indium from the lungs fit a biphasic exponential rate

Table 1. Indium content in the lungs after the final instillation of ITO

Organ	Group	Weeks after final instillation (weeks)			
		8	16	40	78
In (mg/whole lung)	ITO 3 mg/kg	3.850 ± 1.425 (8)[a]	3.183 ± 0.709 (8)	3.219 ± 0.440 (7)	2.587 ± 0.443 (6)
	ITO 6 mg/kg	6.633 ± 1.595 (8)[b]	4.404 ± 1.750 (7)	3.690 ± 1.040 (7)	4.078 ± 0.636 (7)[b]
% of In dose	ITO 3 mg/kg	82.6 ± 30.5	69.8 ± 18.1	66.5 ± 7.7	56.3 ± 8.3
	ITO 6 mg/kg	64.5 ± 17.1	49.7 ± 18.9	39.4 ± 11.3[b]	44.6 ± 7.3[b]

The results are shown as means ± SD. a: The number of hamsters examined. b: Statistically different between the ITO 3 mg/kg group and the ITO 6 mg/kg group.

model for both ITO-treated groups. The elimination half-life of indium from the lungs was 142 weeks in the 3 mg/kg ITO group and 124 weeks in the 6 mg/kg ITO group. Indium was not detected in the lungs of the control group hamsters at any time during the observation period.

The indium concentrations in the serum, which were reported in our previous study[6], liver, kidneys and spleen gradually increased during the observation period, with the concentrations at least 7-fold higher at 78 weeks than at 8 weeks for both ITO treatment groups. However, the accumulation ratio of indium in the liver, kidneys and spleen for the total dose instilled was very low; that is, it was less than 2% at 78 weeks (Table 2). No indium was detected in the organs of control group hamsters at any point during the observation period. There was one renal adenocarcinoma in the ITO 3 mg/kg group at 16 weeks and one cavernous hemangioma of the liver in the ITO 6 mg/kg group at 78 weeks. The pathological evaluations revealed some lesions in the organs that increased in severity as the study progressed. There were no significant differences between the ITO-treated groups and the control group (data not shown).

Discussion

In this study, the long-term tissue distribution of indium was assessed after repeated intratracheal administration of ITO in hamsters. This is the first study of long-term indium distribution following respiratory exposure. Indium was found to be absorbed and retained in the lungs for a long time. The half-life of indium elimination from the lungs was more than two years and was similar for the two different levels of ITO dosing. Although the indium accumulation ratio in the liver, kidneys and spleen for the total instillation dose was very low and quantity of indium excretion in feces and urine was not clear, indium accumulation in these organs indicated that translocation from the lungs occurred, but there was no gradual elimination from these organs during the observation period. These results are consistent with our previous study in which it was reported that ITO-induced lung lesions and that serum indium levels increased significantly after exposure[6]. It may be that the low solubility of ITO particles leads to long-term deposition in the lungs.

To date, a few studies have assessed lung indium levels among workers who handle indium or its derivatives occupationally. These studies have reported indium levels of up to 31.2 μg/g among recycling workers[8] or 29.3 μg/g in an ITO-handling worker[9]. Furthermore, although there is some data assessing indium levels in peripheral organs at a single point in time[7, 10], the data do not clarify whether there was long-term absorption of indium in tissue. In the present study, it was demonstrated that indium was significantly absorbed in peripheral organs after respiratory exposure and that the absorption continued to increase long after ITO instillation. Interestingly, Yamazaki *et al.*[11] reported a gradual decrease in serum indium concentrations after the cessation of dosing with indium arsenide (InAs) or indium phosphide (InP). It may be that serum and peripheral organ absorption depend upon the species of indium compound to which the animals are exposed. Further clarification will be needed to elucidate a trend between absorption or excretion and the properties of various indium compounds.

Table 2. Indium concentrations in the liver, kidneys and spleen after the final instillation of ITO

Organ	Group	Weeks after final instillation (weeks)			
		8	16	40	78
Liver	ITO 3 mg/kg	0.538 ± 0.163 (8)[a]	1.055 ± 0.455 (7)	3.094 ± 1.222 (7)	8.370 ± 2.504 (6)
(μg In/g)	ITO 6 mg/kg	1.130 ± 0.573 (8)[b]	1.293 ± 0.869 (7)	7.181 ± 5.165 (7)	14.420 ± 3.199 (7)[b]
Kidney	ITO 3 mg/kg	1.435 ± 0.177 (8)	1.423 ± 0.249 (7)	4.684 ± 0.949 (7)	9.362 ± 3.879 (6)
(μg In/g)	ITO 6 mg/kg	2.210 ± 1.110 (8)	3.177 ± 0.997 (7)[b]	6.639 ± 2.419 (7)	17.773 ± 7.236 (7)[b]
Spleen	ITO 3 mg/kg	0.612 ± 0.260 (8)	0.822 ± 0.382 (7)	1.617 ± 0.337 (7)	2.910 ± 1.217 (6)
(μg In/g)	ITO 6 mg/kg	1.067 ± 0.477 (8)[b]	1.083 ± 0.792 (7)	2.071 ± 0.397 (7)[b]	5.682 ± 3.832 (7)
Serum[c]	ITO 3 mg/kg	0.060 ± 0.019 (8)	0.070 ± 0.029 (8)	0.184 ± 0.062 (7)	0.237 ± 0.127 (6)
(μg In/m*l*)	ITO 6 mg/kg	0.080 ± 0.022 (8)	0.087 ± 0.018 (8)	0.213 ± 0.089 (7)	0.436 ± 0.149 (7)[b]
% of In dose in the liver,	ITO 3 mg/kg	0.1 ± 0.0	0.2 ± 0.1	0.7 ± 0.2	1.7 ± 0.4
kidneys, and spleen	ITO 6 mg/kg	0.1 ± 0.0	0.1 ± 0.1	0.7 ± 0.5	1.4 ± 0.3

The results are shown as means ± SD. a: The number of hamsters examined. b: Statistically different between the ITO 3 mg/kg group and the ITO 6 mg/kg group. c: Data from this study were shown as a part of our previous study[6].

The degree of lesions observed in the liver, kidneys or spleen was not significantly different between the two dose levels of ITO and was similar to that of lesions observed in the same organs of the control animals in this study. This finding is consistent with a previous report that no exposure-related organ lesions were observed outside the lungs in mice and rats[7]. It is conceivable that the toxic effect on extrapulmonary tissues is relatively weak with the instillation doses delivered herein.

In conclusion, the present study presents the first real evidence that indium is eliminated from the lungs very slowly and does accumulate in extrapulmonary organs over a long period of time after respiratory exposure to ITO.

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Comparative Study on the Pulmonary Toxicity of Indium Hydroxide, Indium-Tin Oxide, and Indium Oxide Following Intratracheal Instillations into the Lungs of Rats

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ABSTRACT

We studied the pulmonary toxicity of indium hydroxide ($In(OH)_3$), which is produced during a recycling process of indium-tin oxide (ITO), in comparison with that of ITO or indium oxide (In_2O_3), two raw materials of flat panel displays. One hundred and forty-four male Wistar rats were intratracheally given equivalent doses of 10 mg/kg indium as $In(OH)_3$, ITO, or In_2O_3 particles, twice a week, for a total of 5 times for 2 weeks. Control rats were given distilled water as a vehicle. After 3 weeks, these rats were serially euthanized, and toxicological effects were determined. Body weight gain was significantly suppressed in the $In(OH)_3$-treated rats compared to that in the control group, but not in the ITO- or In_2O_3-treated rats. Relative lung weights in all the indium-treated groups significantly increased compared to those in the control group throughout the observation period. Furthermore, lung weights in the $In(OH)_3$ group were significantly higher than those in either the ITO or In_2O_3 group. Blood indium levels in the $In(OH)_3$-treated rats were much higher, 70- to 200-fold, than those in the In_2O_3- or ITO-treated rats at each time point. Although the lung indium content decreased gradually during the observation periods, the content in the $In(OH)_3$ group was significantly higher than that in either the ITO or In_2O_3 group. A histopathological analysis revealed foci indicating a slight to severe pulmonary inflammatory response, including exudation to alveolar spaces, were present in all the indium-treated groups. Interstitial fibrotic proliferation was seen only in the $In(OH)_3$-treated rats. The severity of these lesions in the $In(OH)_3$-treated rats was greater than that in either the ITO- or In_2O_3-treated rats.

The results of our study clearly demonstrated that $In(OH)_3$ particles caused severe pulmonary toxicity when repeated intratracheal instillations were performed in rats. Furthermore, the toxic potency of $In(OH)_3$ in the lung was much higher than that of ITO and In_2O_3. Accordingly, the toxicity of $In(OH)_3$ particles should be considered in addition to that of ITO and

In_2O_3 particles when indium exposure occurs.

INTRODUCTION

Indium hydroxide is produced during the recycling process of indium-tin oxide (ITO) target, which is a sintered alloy containing a large portion of indium oxide (In_2O_3) and a small portion of tin oxide, and is mostly used for thin-film coatings of liquid-crystal displays (LCDs) or mobile phone displays. In 2013, the indium demand for ITO was reported to have been 90% of the total indium demand in Japan, and ITO production is expected to increase significantly owing to the increasing need for its use in electronic devices, such as LCDs [1]. Due to this increased production of ITO, recovery of indium from ITO may increase.

Since the mid-1990s, data have become available indicating that indium compounds can be toxic to animals [2]. In 2003, the first case of interstitial pneumonia caused by occupational exposure to ITO was reported [3]. In our previous study, which has been the only study regarding the chronic toxicity of ITO or In_2O_3, we demonstrated chronic lung toxicity of ITO or In_2O_3 particles in hamsters after repeated intratracheal instillations [4].

On the other hand, indium is recovered from the ITO target remainder by sputtering to a thin-film coating. About 60% of the indium consumed in 2013 was supplied by recycling of waste ITO target or other indium products in Japan [1]. Indium hydroxide is an intermediate product of the recycling process, and In_2O_3, which is a raw material of ITO, is generated in the next step of the recycling process. In this study, we evaluated the comparative lung toxicity of $In(OH)_3$, ITO, and In_2O_3 when administered repeatedly into the trachea of rats. These indium compounds were administered at the equivalent dose of 10 mg/kg of indium.

MATERIALS AND METHODS

Test materials: $In(OH)_3$ and ITO particles were obtained from a company. ITO contained 74.4% (wt%) indium and 7.8% tin, with the remainder being oxygen, and $In(OH)_3$ was 99.9% pure. Indium oxide, over 99.99% pure, was purchased from Katayama Chemicals, Osaka, Japan. The mean diameters of $In(OH)_3$, ITO, and In_2O_3 particles were 0.04, 0.56, and 0.14 μm, respectively.
Animals: One hundred forty-four male Wistar rats aged 6 weeks were purchased from the colony of Kyudo Co. (Tosu, Japan) and housed within a conventional laboratory room at the Graduate School of Medical Sciences, Kyushu University. Intratracheal instillations were started once the rats reached the age of 8 weeks.
Intratracheal instillation of materials: Each material was suspended in 1.0 mL/kg body weight

distilled water and instilled into the trachea of rats anesthetized with ether, twice a week, for a total of 5 times during for 2 weeks. Each instillation per animal performed at a dose of 10 mg/kg body weight indium. The control rats received 1.0 mL/kg body weight distilled water only. All the rats were randomized into 4 groups, with each group consisting of 36 rats.

Toxicological evaluations: All the surviving rats in each group were euthanized by exposure to carbon dioxide gas at 0, 1, 2, or 3 weeks after the final instillation and were then autopsied. Although blood indium concentrations were measured in all the rats, lung indium concentrations were measured in 5 rats and pulmonary pathological evaluation was performed in 4 rats of each group. Indium concentrations in digested lung or blood samples were measured using an inductively coupled plasma mass spectrometer (ICP-MS, Agilent 7500ce, Agilent Technologies, Tokyo, Japan) at the Center of Advanced Instrumental Analysis, Kyushu University. Lung indium content was determined as the concentration of indium in lung tissue.

Histopathological findings in the lungs were scored as present or absent; a score of 0 was recorded if findings were absent. In the case of lesions, the severity of each of the lesion was graded as slight to severe on a 4-item scale.

These experiments were conducted according to the Guidelines for Animal Experiments at the Graduate School of Medical Sciences, Kyushu University and under the Law (No. 105) and Notification (No. 6) of the Government of Japan.

RESULTS

The mean total indium dosage per animal (mean ± S.E.) was 15.1 ± 0.2 mg in the $In(OH)_3$ group, 15.6 ± 0.2 mg in the ITO group, and 15.6 ± 0.2 mg in the In_2O_3 group. No rats died during the observation period, except 2 that died due to emaciation in the $In(OH)_3$ group. Finally, 36 animals in the ITO, In_2O_3, and the control groups and 34 animals in the $In(OH)_3$ group were examined. Changes in the body weights of the surviving rats in each group at each time point during the instillation and observation periods are shown in Fig. 1. The body weight of the $In(OH)_3$ group did not increase from the start of the instillation period and was extremely suppressed during the observation period. Trends in body weight change were significantly different ($P < 0.05$) between the $In(OH)_3$ group and the ITO- or In_2O_3

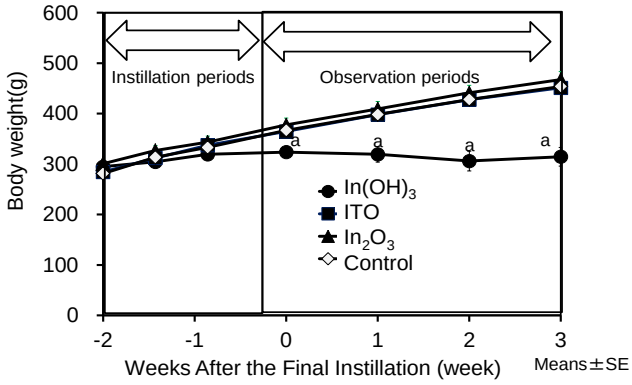

Fig.1 Changes in body weight gain among the $In(OH)_3$, ITO and In_2O_3 groups during the instillation and observation periods.
a: Significantly different from the control group.

group, or the control group between weeks 0 and 3 after the final instillation. In the ITO and In_2O_3 groups, the trend in body weight change was similar to that observed in the control group throughout the observation period.

Changes in lung weight during the observation period are shown in Fig. 2. The lung weights in the $In(OH)_3$-, ITO and In_2O_3 groups were significantly greater than that in the control group at each corresponding time point. Moreover, the $In(OH)_3$ group showed significantly greater lung weight than that shown by the ITO and In_2O_3 groups at each corresponding time point.

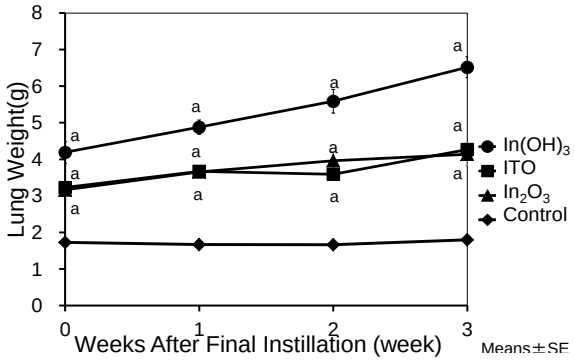

Fig.2 Changes in lung weight from the final instillation of $In(OH)_3$, ITO and In_2O_3 .
a:Significantly different from the control.

Blood indium concentrations during the observation period are shown in Fig. 3. The blood indium concentration gradually increased from week 0 to 3 following the final instillation in all the indium-treated groups. Furthermore, blood indium levels in the $In(OH)_3$ group were 70- or 200-fold higher than those in the In_2O_3 or ITO group, respectively, throughout the observation period. At the end of the observation period, blood indium levels were 1137.6 ± 167.8 ng/g (mean ± SE) in the $In(OH)_3$ group, 6.5 ± 1.3 ng/g in the ITO group, and 13.8 ± 2.0 ng/g in the In_2O_3 group. The differences in the blood indium levels between the $In(OH)_3$, ITO, and In_2O_3 groups at each time point were significant. Indium was not detected in the blood of the animals in the control group at any time point.

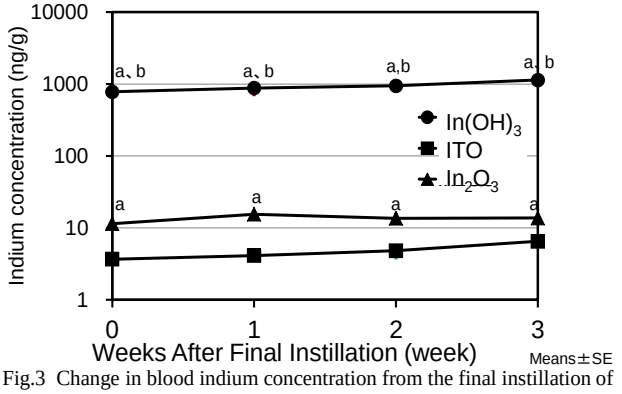

Fig.3 Change in blood indium concentration from the final instillation of $In(OH)_3$, ITO and In_2O_3.
a: Significantly different from the ITO group.
b: Significantly different from the In_2O_3 group.

The indium content in the lungs during the observation period is shown in Fig. 4. Although the lung indium content for the In_2O_3 group was higher at week 1or 3 than that at week 0, it gradually decreased from week 0 to 3 in both the $In(OH)_3$ and ITO groups. Indium was not detected in the lungs from the control group at any time during the observation period.

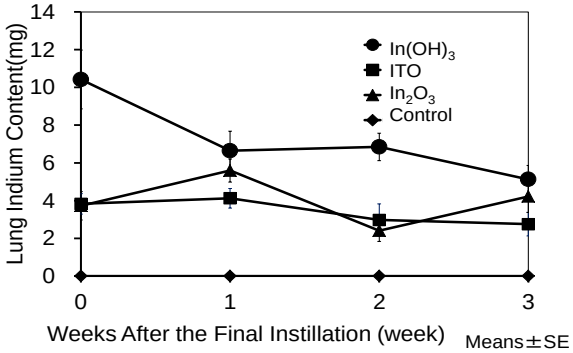

Fig.4 Change of indium content in the lungs after the final instillation of $In(OH)_3$, ITO and In_2O_3.

The severity of the pathologic change in the lungs is shown in Table 1. Diffuse foci of slight to severe inflammation were present in all indium-treated rats during the observation periods. Slight to severe exudation was seen within the alveolar spaces in the indium-treated groups. Mild to moderate interstitial fibrotic proliferation was apparent from weeks 0 to 3 in the $In(OH)_3$ group. Although a slight inflammatory response was observed in the control group, neither interstitial fibrosis nor exudation was evident.

Table 1 Pathological changes in the rats lung after intratracheal instillation of $In(OH)_3$, ITO and In_2O_3 .

Pathological Changes	Group	Weeks after final instillation(Wk)			
		0	1	2	3
Inflammatory response	$In(OH)_3$	3+	3+	3+	3+
	ITO	+	+	+	+
	In_2O_3	+	+	+	+
	Control	±	±	±	±
Interstitial fibrotic proliferation	$In(OH)_3$	+	2+	3+	3+
	ITO	–	–	–	–
	In_2O_3	–	–	–	–
	Control	–	–	–	–
Exudation	$In(OH)_3$	2+	3+	3+	3+
	ITO	±	2+	+	+
	In_2O_3	±	±	+	+
	Control	–	–	–	–

The severity of the lung lesions was evaluated by five grades:
-:negative; ±: slight; +: mild; 2+: moderate; 3+: severe

DISCUSSION

In this study, the acute toxic effect of $In(OH)_3$ was assessed after repeated intratracheal instillation in rats. This is the first study of acute pulmonary toxicity of $In(OH)_3$ following respiratory exposure. Of the three indium compounds evaluated in this study, $In(OH)_3$ had the greatest degree of systemic toxicity, causing significant decreases in body weight and 2 deaths during the instillation period. $In(OH)_3$ was also the most toxic compound for the lung, causing significant increases in lung weight and severe manifestation of lung lesions. On the other hand, when the same indium dosage as that of $In(OH)_3$, ITO, or In_2O_3 was instilled, the blood indium level in the $In(OH)_3$-treated rats was much higher than that in either the ITO- or In_2O_3-treated rats. A high concentration of blood indium in the $In(OH)_3$-treated rats may be correlated with the severe toxicological damage in $In(OH)_3$-treated rats. Furthermore, it was thought that the fact that $In(OH)_3$ particles had the smallest diameter might contribute to a more severe toxic profile among the three indium compounds. Although there was no additional exposure to these indium compounds after the final instillation, blood indium levels of three indium-treated rats gradually increased up to the end of the observation period. This finding was consistent with the results of Tanaka et al.[4] who reported that serum indium levels among ITO- or In_2O_3-treated hamsters increased gradually until week 78 week following the final instillation. Since $In(OH)_3$, ITO, or In_2O_3 particles are insoluble in water [5], these findings would seem to be related to the low solubility of these particles within the lung. Due to the slow clearance of these indium compound particles from the lung, it is suspected that the long-term persistence of these particles within the lung resulted in continuous damage to the alveolar epithelial cells. On the other hands, it is thought that the pulmonary indium content was not stable at early observation periods because

inhaled indium compounds might be removed by mucociliary transport from the trachea and swallowed into the stomach just after intratracheal instillations of indium compounds. Further clarification is needed regarding the pulmonary clearance of these particles and the influence of particle size on the development of lung damage.

To date, the evidence of lung disorders has been reported after respiratory ITO exposure in animals and humans [2,6]. Although a few studies have reported pulmonary disorders among indium recycling workers [7,8], it is not clear whether these recycling workers inhaled $In(OH)_3$ particles, or if they were exposed to several kinds of indium compounds.

In conclusion, the acute pulmonary toxicity of $In(OH)_3$ particles was confirmed when repeated intratracheal instillations of $In(OH)_3$ were performed in rats, and the toxic potency of $In(OH)_3$ was much higher than that of either ITO or In_2O_3. These findings suggest a need to pay much greater attention to the danger of human exposure to indium compounds.

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Abstract

Objectives: Indium was added to the list of substances regulated by the Ordinance on Prevention of Hazards due to Specified Chemical Substances (OPHSCS) in 2013. Indium metal (IM), however, is not regulated by the OPHSCS due to insufficient information on pulmonary effects following exposure.

Methods: From 2011 to 2013, a cross-sectional study was conducted on 141 IM-exposed workers at 11 factories. Subjective symptoms were assessed, including levels of serum biomarkers, spirometry readings and total and diffuse lung capacity. Krebs von den Lungen-6 (KL-6) and surfactant protein D (SP-D) were selected as biomarkers of interstitial pneumonia. Indium serum concentration (In-S) and personal air sampling data were used to estimate exposure. Subjects were categorized into 5 groups based on occupation and type of exposure: smelting, soldering, dental technician, bonding and other.

Results: The highest level of In-S was 25.4 μg/l, and the mean In-S level was significantly higher in the smelting group than in other groups. In the smelting group, the prevalence of increased In-S levels was 9.1%, while that of abnormal KL-6 was 15.2%. A significant dose-effect relationship was observed between the In-S and KL-6 levels. No marked differences were observed between any of the groups in SP-D values, pulmonary symptoms, or pulmonary function test results. A total of 31% of the subjects worked in an environment with IM levels exceeding 0.3 $\mu g/m^3$, which requires a protective mask to be worn.

45 *Conclusions*: For workers exposed to IM, work environments should be monitored,
46 appropriate protective masks should be worn, and medical monitoring should be conducted
47 according to the OPHSCS.

Key words

Indium metal, Indium concentration, KL-6, SP-D, Interstitial pneumonia, Cross-sectional study

Abbreviations

IM, indium metal

In-S, serum indium concentration

ITO, indium-tin oxide

In-A, 8-hour time-weighted average personal indium concentration in respirable dust fractions

KL-6, Krebs von den Lungen-6

SP-D, surfactant protein D

OPHSCS, Ordinance on Prevention of Hazards due to Specified Chemical Substances

Introduction

Indium lung disease is a newly described occupational lung disease that affects workers exposed to indium compounds, such as indium tin oxide (ITO), which is used to manufacture electrodes to produce flat-panel displays, and indium oxide, indium hydroxide, and indium chloride, which are involved in the production or reclamation of ITO. The Japanese Society of Occupational Health recommended an exposure limit for indium and indium compounds of 3.0 μg/l[1)] based on monitoring of the dose-effect relationship between the level of serum indium (In-S; exposure index) and the serum biomarker of interstitial pneumonia (Krebs von den Lungen, KL-6) associated with adverse pulmonary effects[2-4)] in 2007. An inhalation experiment conducted in rats over 2 years identified ITO with an indium concentration of 0.01 mg/m^3 as a lung carcinogen[5)]. Based on these findings, the Japanese Ministry of Health, Labour and Welfare established prevention guidelines for workers exposed to ITO and other indium compounds in 2010[6)]. In addition, indium compounds were added to the list of substances regulated by the Ordinance on Prevention of Hazards due to Specified Chemical Substances (OPHSCS) in 2013[7, 8)]. Under the OPHSCS, employers at indium-processing factories are required to measure indium concentrations in the respirable dust fraction at their sites and to conduct health checks twice a year. These health checks consisted of a review of job career and working conditions; a review of past medical history, including pulmonary symptoms of coughing, sputum, dyspnea, cyanosis and clubbed fingers; evaluation of current

pulmonary symptoms, including coughing, sputum, dyspnea, cyanosis, and clubbed fingers; and measurement of In-S and KL-6 values of workers. In addition, X-Ray or computed tomography of the chest was conducted at the start and end of employment. However, due to insufficient information on the pulmonary effects of indium metal (IM) exposure, IM and indium alloys are not listed in the OPHSCS. To our knowledge, no studies have been conducted in IM-processing factories.

Here, we determined whether or not IM exposure induces adverse pulmonary effects similar to the effects of non-IM indium compounds. We measured In-S and pulmonary effects of IM exposure and evaluated the relationship between IM exposure and markers of pulmonary effects at IM-processing factories.

Methods

This study was approved by the Ethics Committee of the School of Medicine at Keio University (approval number 20110268). Written informed consent was obtained from all subjects.

Study design and subjects

This multicenter study was conducted at 11 IM-processing factories, including 2 dental technician shops, 1 electric contact plant, 1 indium alloy target manufacturing plant, 3 lead-free solder manufacturing plants using an alloy containing less than 10% indium, 3 dental manufacturing plants using an alloy containing less than 25% indium, and 1 indium-free target plate bonding plant using 100% indium as an adhesive material. This study was conducted from 2011 to 2013. There were 142 subjects, and the proportions of subjects enrolled were dependent on the size of each factory and ranged from 2 to 41. One of the subjects was excluded from the statistical analysis due to a history of exposure to non-IM indium compounds.

Study subjects were categorized into five groups, as follows: high-temperature (≥1000°C) alloy smelting workers (smelting workers), soldering workers, dental technicians, bonding workers and other workers.

All subjects underwent a health check, which consisted of a medical interview,

115 questionnaire, blood test, spirometry examination and evaluation of total lung capacity (TLC)
116 and diffuse lung capacity for carbon monoxide (DLCO). To investigate the relationship
117 between the levels of In-S and serum biomarkers of interstitial pneumonia (KL-6 or
118 surfactant protein D [SP-D]), subjects were divided into currently and formerly exposed
119 workers according to their exposure status. Job history was based on records at the plants or,
120 if unavailable, on findings from the interview regarding occupational history.
121
122 *Exposure indices*
123 In-S (μg/L) was measured by inductively coupled plasma mass spectrometry (ICP-MS) at the
124 Center of Advanced Instrumental Analysis, Kyushu University[3, 6)] or the Japan Industrial
125 Safety and Health Association[6)]. In-S below the detection limit (0.1 μg/l) was ascribed an
126 arbitrary value of 0.05 μg/l for statistical analysis.
127
128 *Effect indices and confounding factors*
129 KL-6 (EIDIA Co., Ltd., Tokyo, Japan)[9, 10)] and SP-D (Yamasa Corporation, Tokyo, Japan)[11)]
130 were used as biomarkers for assessing interstitial changes in the lungs and were evaluated at a
131 major commercial clinical laboratory (Special Reference Laboratory, Tokyo, Japan). SP-D
132 levels were not measured in 26 subjects due to logistics.
133 Spirometry was performed using an electronic spirometer (HI-801; Chest MI, Tokyo,

Japan) based on the standards of the Japanese Respiratory Society[12]. TLC was determined by helium dilution lung volume tests, and DLCO was determined by single breath diffusing capacity tests performed using a portable compact machine (EasyOne Pro®, ndd Medical Technologies, Zurich, Switzerland) based on the standards of the American Thoracic Society/European Respiratory Society[13]. TLC and DLCO were not measured in 12 workers due to rib fractures (n=1) or lack of measurement at the factory due to logistics (n=11). Spirometry was not evaluated in 5 workers due to rib fracture (n=1) or lack of measurement at the factory due to logistics (n=4). Age- and height-adjusted predicted values of vital capacity (VC), forced vital capacity (FVC), and forced expiratory volume in one second ($FEV_{1.0}$) were determined by sex, using the regression formula recommended by the Japanese Respiratory Society[14]. TLC was determined by sex, using reference values generated from the third National Health and Nutrition Examination Survey (NHANES III)[15]. DLCO and DLCO/VA were determined by sex, using the regression formula of Nishida for Japanese subjects[16]. Predictions were calculated for VC, FVC, $FEV_{1.0}$, TLC, DLCO and DLCO/VA.

Respiratory symptoms, smoking history and confounding factors of sex, age, medical history and history of exposure to indium metal and other materials were investigated using the Japanese version[17] of the American Thoracic Society Division of Lung Disease questionnaire[18] and supplementary questions.

153 *Personal indium exposure concentration in respirable dust fraction*

154 Eight-hour time-weighted average personal indium concentrations in respirable dust fractions
155 (In-A) were measured in 35 study subjects using respirable dust samplers (GS-3 Respirable
156 Dust Cyclone; SKC Inc., Eighty Four, PA, USA) and ICP-MS (Agilent 7500i; Agilent
157 Technologies, Santa Clara, CA, USA) by the Japan Industrial Safety and Health Association
158 according to guidelines[6)] at approximately the same time as the health checks. An In-A level
159 below the detection limit (0.006 μg/m^3) was ascribed an arbitrary value of 0.006 μg/m^3 for
160 statistical analysis.

161

162 *Statistical analysis*

163 Non-normally distributed data for KL-6 and SP-D were log-transformed to an approximately
164 normal distribution before analysis. In-S and values of lung function were not
165 log-transformed before analysis. Differences between occupational groups were assessed
166 using one-way analysis of variance (ANOVA) for KL-6, SP-D, and values of lung function or
167 the Kruskal-Wallis test (non-normal distribution) for age, exposure duration, time since last
168 exposure and In-S. The Chi-square test was used to compare the proportion and prevalence of
169 sex, smoking habits, exposure status, pulmonary symptoms, increased In-S levels and
170 abnormal KL-6 and SP-D levels. A single regression model was used to evaluate the
171 dose-effect relationship between In-S and KL-6 or SP-D levels by exposure status and

between In-A and KL-6 or S-D levels.

Based on the adopted reference value of In-S[4], subjects were classified as either In-S <3.0 μg/l or ≥3.0 μg/l. In-S ≥3.0 μg/l was used to assess the risk of indium exposure on the effect variables. The prevalence of abnormalities for biomarkers and lung function was analyzed using the following cutoffs for abnormal values: KL-6 ≥500 U/ml, SP-D ≥110 ng/ml, $FEV_{1.0}$/FVC <70%, %VC <80%, %FVC <80%, $\%FEV_{1.0}$ <80%, %TLC <80%, %DLCO <70% and %DLCO/VA <70%.

The reference value of In-A was set as 10 $\mu g/m^3$ based on the target indium concentration in respirable dust for immediately improved workplace environments or as ≥0.3 $\mu g/m^3$ based on the acceptable exposure limits calculated according to the exposure concentration found to be potentially carcinogenic in rats, as established in the technical guidelines of the Japanese Ministry of Health, Labour and Welfare[6].

Statistical significance was assessed by two-tailed analysis, with P <0.05. All statistical analyses were performed using SPSS version 19 (IBM Corp., Armonk, NY, USA).

187

188 **Results**

189 Tables 1 and 2 show the characteristics of study subjects and the pulmonary effects for each
190 group. The mean age of the subjects was 40.9 years, and 88.7% were male. The mean
191 duration from the start to end of indium exposure for all workers was 7.5 years. For currently
192 exposed workers, the duration of indium exposure was calculated from the start to the time of
193 the health check. The proportion of formerly exposed workers who were no longer
194 experiencing indium exposure at the time of the health check was 24.2% in the smelting
195 group, 31.3% in the bonding group, 40.0% in the "other" group and 0% in the remaining two
196 groups. No marked differences in smoking history or age were observed among groups. The
197 highest level of In-S in the smelting group was 25.4 μg/l, and the highest level of In-S among
198 all other groups, excluding the smelting group, was less than 1.0 μg/l, with In-S levels below
199 the detection limit being found in 82% of workers (89/108). The In-S level in the smelting
200 group was significantly higher than that in all other groups (P <0.001). The KL-6 level in
201 the smelting group was also significantly higher than that in the other groups (P <0.001).
202 However, no marked differences were observed in the SP-D level, pulmonary symptoms or
203 pulmonary function test results among all groups.

204 For each group, the respective proportions of subjects with abnormal levels of In-S,
205 KL-6 and SP-D stratified by occupation type were as follows: 9.1%, 15.2% and 21.2%
206 (smelting); 0.0%, 0.0%, and 0.0% (soldering); 0.0%, 0.0%, and 0.0% (dental technicians);

207 0.0%, 0.0%, and 6.7% (bonding); and 0.0%, 4.0%, and 6.3% (other). The proportion of
208 increased In-S and abnormal KL-6 levels were significantly higher in the smelting group than
209 in the other groups (*P*=0.040 and *P*=0.034, respectively).

210 Figure 1 shows a scattergram comparing the levels of In-S and KL-6 or SP-D by
211 exposure status (currently and formerly exposed workers). In currently exposed workers,
212 significant increases in KL-6 levels were observed with increasing In-S levels (*P* <0.001).
213 Dose-effect relationships between In-S and SP-D levels in currently and formerly exposed
214 workers (*P*=0.018 and 0.014, respectively) were also observed. However, in formerly
215 exposed workers, no significant dose-effect relationship between In-S and KL-6 levels was
216 observed (*P*=0.192).

217 The Mean In-A level (n=35) was 15.93 μg/m^3, with values ranging from <0.006
218 (undetectable) to 510.28 μg/m^3 and differing significantly between groups (*P*=0.006). In-A in
219 the smelting group (mean, 68.36 μg/m^3; standard deviation, 178.75 μg/m^3; range,
220 0.12-510.28 μg/m^3) represented the highest level of exposure to respirable indium dust
221 among groups. The proportions of workers with In-A levels exceeding 10 μg/m^3 (target
222 indium concentration criteria requiring immediate improvement of work environments) in
223 each group were as follows: 25% (smelting), 0% (soldering), 0% (dental technicians), 0%
224 (bonding) and 0% (other). The proportions of workers with In-A levels exceeding 0.3 μg/m^3
225 (acceptable exposure concentration limit not requiring an appropriate mask) in each group

were as follows: 63% (smelting), 14% (soldering), 20% (dental technicians), 17% (bonding), and 33% (other).

Figure 2 shows a scattergram comparing In-A and KL-6 or SP-D levels by occupation groups in currently exposed workers. Although increasing In-A levels were associated with increasing KL-6 and SP-D levels, the relationships between these parameters were not significant (P=0.687 and P=0.657, respectively).

Discussion

In currently exposed workers, a dose-effect relationship between the levels of In-S and KL-6 was observed. In particular, in the smelting group, the level of In-S increased to over 20 μg/l, which is a risk factor for interstitial pneumonia and progression of emphysematous changes[2-4, 19]. Workers in the smelting group were involved in constructing indium alloys with palladium, gold, silver and other metals. Although the melting point of indium is 157°C, the dissolution temperature in the melting process is dependent on the other mixed metals in the alloy and exceeds 1000°C. Although exposure to indium metal at room temperature is generally not harmful to workers, indium melts at 157°C, and indium vapor is generated at higher temperature[20]. The vapor is cooled down in air and ultimately becomes airborne respirable particles. These respirable particles might contribute to increases in In-A and In-S levels.

In the smelting group, 25% of workers required immediate improvement to their work environment according to prevention guidelines[6]. In addition, regardless of occupational group, approximately 31% of workers exposed to IM exceeding 0.3 μg/m^3 were required to wear a protective mask according to prevention guidelines[6]. Based on these results, for workers exposed to IM, periodical monitoring of the work environment including monitoring of whether or not they wear an appropriate protective mask and medical monitoring is required.

253 In this study, IM-exposed workers who were working without improvement of the
254 workplace environment or use of a protective mask were investigated. The level of exposure
255 to respirable indium dust might be directly reflected in the amount of dust inhaled in the
256 lungs. Although an increase in In-A levels resulted in an increase in KL-6 and SP-D levels,
257 this change was not significant. The In-A levels were considered to be low, with only a small
258 proportion of subjects having levels in excess of 10 μg/m^3. A metric of cumulative exposure
259 might have a preference for In-S levels over In-A levels.

260 Although hamsters exposed to indium oxide[21)] and workers formerly exposed to indium
261 compounds have been found to have elevated In-S levels for a prolonged period of time,[4, 19)]
262 the dose-effect relationship between In-S and KL-6 levels was not significant in the formerly
263 exposed workers in the present study. Clearance of the indium burden on the lungs may be
264 more rapid following the inhalation of mist containing indium oxide[22)] than that of dust
265 containing ITO or indium oxide at room temperature. In addition, the amount of indium
266 inhaled into the lungs by IM-exposed workers in this study might be lower than that by
267 ITO-exposed workers observed in previous studies[4, 19)].

268 Full evaluation with high-resolution computed tomography (HRCT) of the chest was
269 not conducted in the present study. However, one IM-exposed worker with high In-S levels
270 (≥20 μg/l) visited a hospital and underwent chest HRCT, which showed interlobular septal
271 thickening and a mild reticular shadow. IM-exposed workers with high In-S levels might

therefore suffer adverse effects that are similar to those of workers with noted exposure to indium compounds at ITO-processing factories[2-4].

Due to the cross-sectional nature of our study, a longitudinal observational study is also required. We recommend that future studies monitor the lung conditions of workers following the reduction of occupational exposure to IM.

Conclusions

We observed a dose-effect relationship between In-S and KL-6 levels in workers currently exposed to IM. The results of this study indicate that workers exposed to IM require monitoring of their work environment, appropriate protective masks and ongoing medical checks according to the OPHSCS to prevent indium lung disease.

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Table 1. Study subjects by occupational group

Prev, prevalence; SD, standard deviation; In-S, serum indium; yr, year; exposure duration, duration from start to end of indium exposure or to time of health check

P-value by one-way analysis of variance, Kruskal-Wallis test or chi-square test among all groups

Table 2. Effective markers by occupational group

Prev, prevalence; SD, standard deviation; KL-6, Krebs von den Lungen-6; SP-D, surfactant protein D; VC, vital capacity; FVC, forced vital capacity; $FEV_{1.0}$, forced expiratory volume in one second; TLC, total lung capacity; DLCO, diffusing lung capacity for carbon monoxide; VA, alveolar volume. *P*-value by one-way analysis of variance, Kruskal-Wallis test or chi-square test among all groups. %VC, %FVC, $\%FEV_{1.0}$ and $FEV_{1.0}$/FVC measured in the smelting (n=32), soldering (n=37), dental technician (n=5), bonding (n=14) and other groups (n=48) (total, n=136). %TLC, %DLCO and %DLCO/VA measured in the smelting (n=32), soldering (n=37), dental technician (n=5), bonding (n=10) and other groups (n=45) (total, n=129).

Figure 1. Dose-effect relationships between In-S and biomarkers by exposure status

1a) and 1b) Scattergrams between In-S and KL-6 or SP-D in currently exposed workers. 1c) and 1d) Scattergrams between In-S and KL-6 or SP-D in formerly exposed workers. Cut-off values: KL-6, 500 U/ml, and SP-D, 110 U/ml. A single regression model was used to evaluate the dose-effect relationship between In-S and KL-6 or SP-D levels.

Figure 2. Dose-effect relationships between In-A and biomarkers by occupational group

2a) Scattergram between In-A and KL-6 levels in currently exposed workers. 2b) Scattergram between In-A and SP-D levels in currently exposed workers. Target concentration for indium according to prevention guidelines: 10 µg/m^3. Acceptable exposure limit for concentration of indium according to prevention guidelines: 0.3 µg/m^3. A single regression model was used to evaluate the dose-effect relationship between In-A and KL-6 or S-D levels.

Table 1. Study subjects by occupational group

Occupational group	Smelting (n=33)	Soldering (n=37)	Dental technician (n=5)	Bonding (n=16)	Other (n=50)	*P*
Age (yr), mean (SD)	39.5 (14.5)	38.7 (11.3)	41.6 (15.7)	39.5 (13.1)	43.9 (11.5)	0.334
Male, n (%)	33 (100.0)	29 (78.4)	2 (40.0)	16 (100.0)	45 (90.0)	<0.001
Exposure duration (yr), mean (range)	7.9 (0.08-33.2)	10.2 (0.83-34.8)	16.1 (0.75-32.7)	5.4 (0.25-17.8)	4.9 (0.33-36.2)	0.001
Time since last exposure (yr), mean (range)	6.6 (0.83-29.6)	-	-	9.7 (4.67-22.7)	6.4 (1.17-12.6)	0.122
In-S (μg/l), mean (range)	2.2 (0.1>-25.4)	0.1 (0.1>-0.4)	0.1 (0.1>-0.5)	0.1 (0.1>-0.5)	0.1 (0.1>-0.9)	<0.001
Smoking habit, n (%)						0.438
Nonsmokers	10 (30.3)	16 (43.2)	2 (40.0)	3 (18.8)	15 (30.0)	
Ex-smokers	10 (30.3)	6 (16.2)	2 (40.0)	8 (50.0)	15 (30.0)	
Current smokers	13 (39.4)	15 (40.5)	1 (20.0)	5 (31.3)	20 (40.0)	
Exposure status, n (%)						<0.001
Currently exposed	25 (75.8)	37 (100.0)	5 (100.0)	11 (68.8)	30 (60.0)	
Formerly exposed	8 (24.2)	0 (0.0)	0 (0.0)	5 (31.3)	20 (40.0)	

Prev, prevalence; SD, standard deviation; In-S, serum indium; yr, year; exposure duration, duration from start to end of indium exposure or to time of health check.

P-value by one-way analysis of variance, Kruskal-Wallis test, or chi-square test among all groups.

Table 2. Effective markers by occupational group

Occupational group	Smelting (n=33)	Soldering (n=37)	Dental technician (n=5)	Bonding (n=16)	Other (n=50)	*P*
In-S ≥3.0 μg/l, n (%)	3/33 (9.1)	0/37 (0.0)	0/5 (0.0)	0/16 (0.0)	0/50 (0.0)	0.040
Cough or sputum, n (%)	2/33 (6.1)	3/37 (8.1)	0/5 (0.0)	0/15 (0.0)	3/50 (6.0)	0.805
Biomarkers						
KL-6 (U/ml), GM (GSD)	322.0 (1.7)	216.5 (1.3)	181.0 (1.2)	237.6 (1.4)	261.8 (1.4)	<0.001
KL-6 ≥500 U/ml, n (%)	5/33 (15.2)	0/37 (0.0)	0/5 (0.0)	0/16 (0.0)	2/50 (4.0)	0.034
SP-D (ng/ml), GM (GSD)	55.7 (2.2)	38.1 (1.8)	28.4 (1.5)	43.0 (2.0)	41.5 (1.7)	0.105
SP-D ≥110 ng/ml, n (%)	7/33 (21.2)	0/14 (0.0)	0/5 (0.0)	1/15 (6.7)	3/48 (6.3)	0.094
Lung function, mean (SD)						
%VC	104.8 (12.5)	109.7 (11.3)	105.8 (13.0)	111.1 (12.7)	105.1 (12.2)	0.224
%FVC	101.8 (11.5)	108.3 (11.8)	103.4 (12.0)	107.9 (9.9)	103.0 (11.9)	0.112
%$FEV_{1.0}$	97.0 (12.8)	103.0 (12.6)	99.5 (19.0)	103.0 (10.4)	99.7 (11.6)	0.314
$FEV_{1.0}$/FVC	82.7 (5.8)	82.4 (5.4)	82.4 (11.3)	82.8 (4.6)	83.5 (7.0)	0.951
%TLC	94.8 (8.8)	94.2 (8.6)	94.3 (7.9)	98.6 (9.7)	94.2 (10.1)	0.743
%DLCO	96.9 (13.4)	89.9 (12.8)	95.8 (11.6)	94.9 (11.4)	91.5 (15.2)	0.260
%DLCO/VA	93.6 (9.5)	87.0 (14.1)	91.2 (13.5)	87.3 (12.0)	90.6 (14.2)	0.294

Prev, prevalence; SD, standard deviation; KL-6, Krebs von den Lungen-6; SP-D, surfactant protein D; VC, vital capacity; FVC, forced vital capacity; $FEV_{1.0}$, forced expiratory volume in one second; TLC, total lung capacity; DLCO, diffusing lung capacity for carbon monoxide; VA, alveolar volume.

P-value by one-way analysis of variance, Kruskal-Wallis test, or chi-square test among all groups. %VC, %FVC, $\%FEV_{1.0}$ and $FEV_{1.0}$/FVC measured in the smelting (n=32), soldering (n=37), dental technician (n=5), bonding (n=14) and other groups (n=48) (total, n=136). %TLC, %DLCO and %DLCO/VA measured in the smelting (n=32), soldering (n=37), dental technician (n=5), bonding (n=10) and other groups (n=45) (total, n=129).

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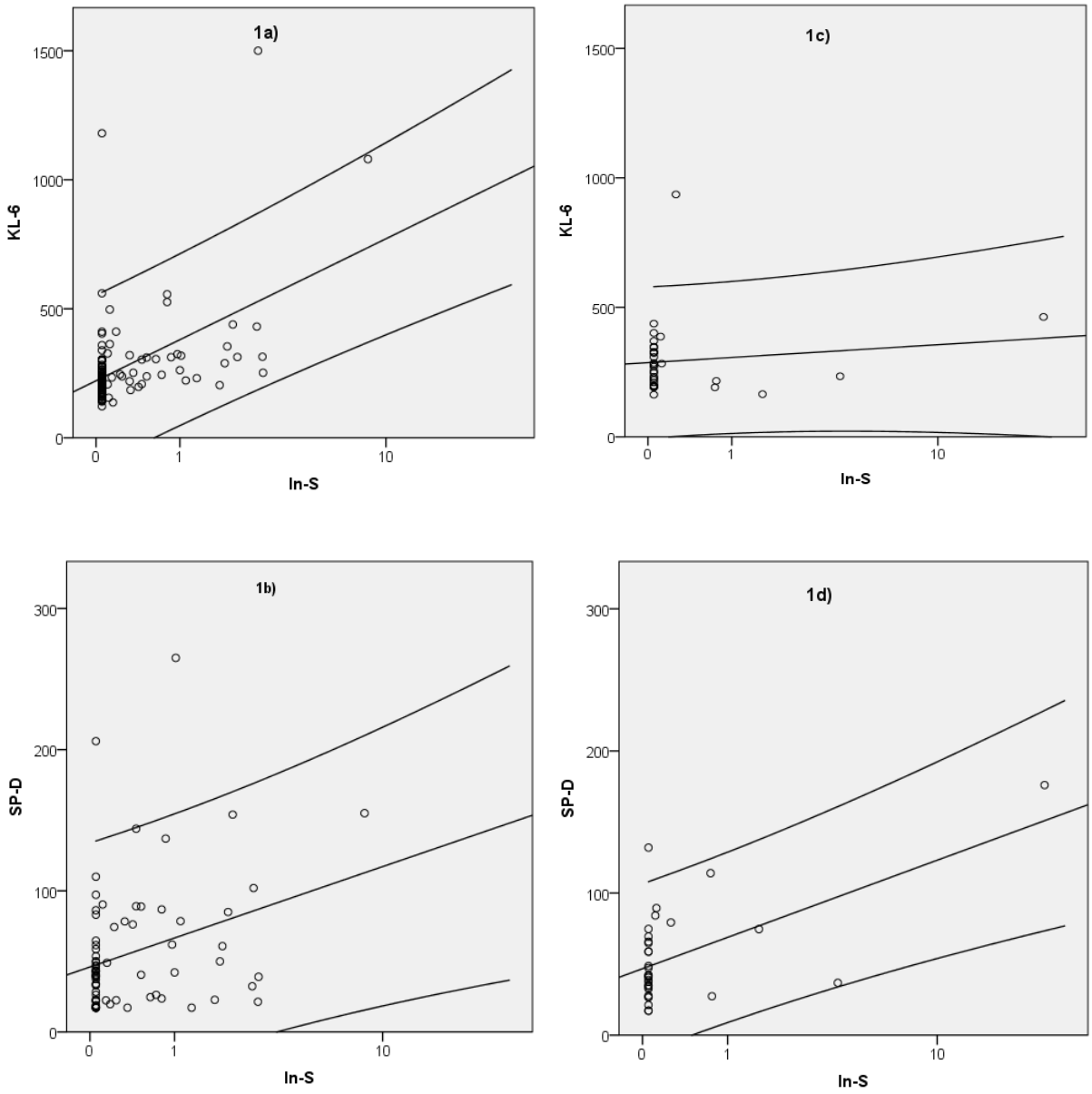
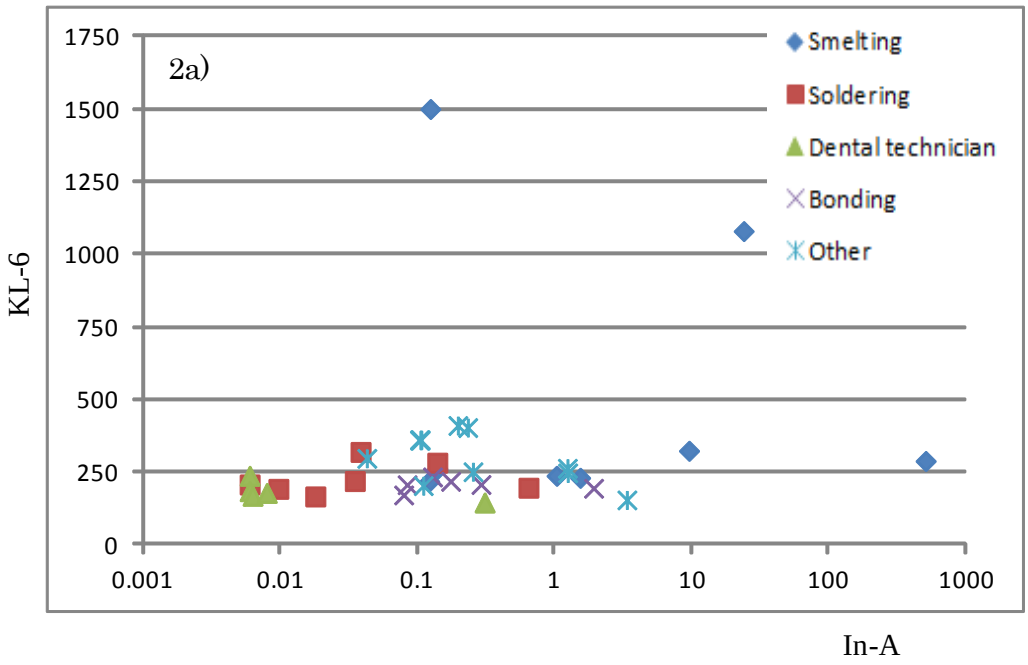
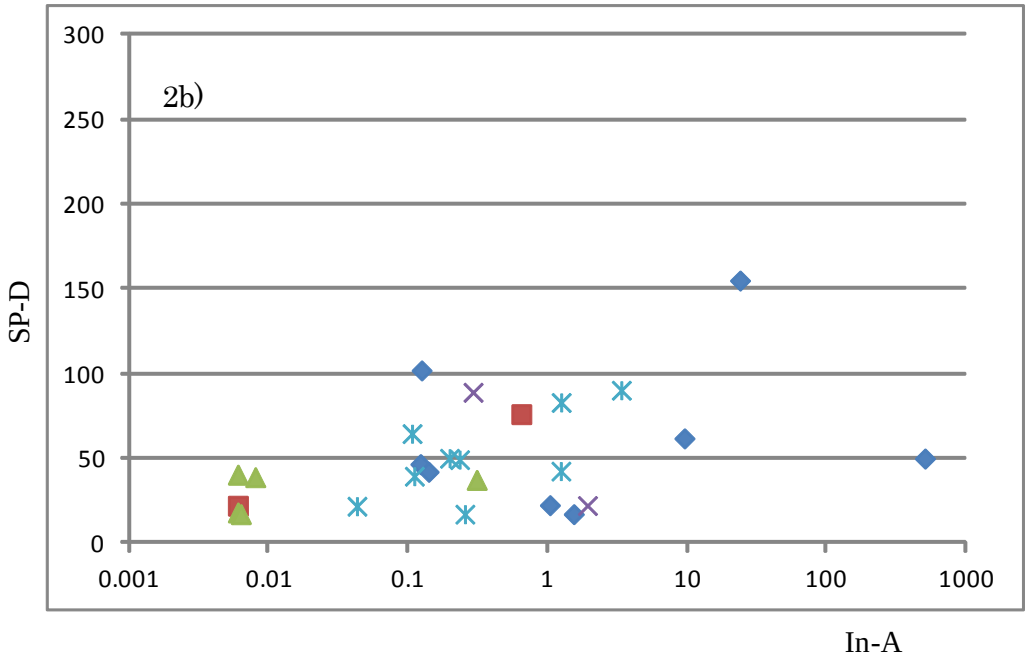
Figure 1. Dose-effect relationships between In-S and biomarkers by exposure status



1a) and 1b) Scattergrams between In-S and KL-6, or SP-D in currently exposed worekrs. 1c) and 1d) Scattergrams between In-S and KL-6, or SP-D in formerly exposed workers. Cut-off values: KL-6, 500 U/ml and SP-D, 110 U/ml. A single regression model was used to evaluate the dose-effect relationship between In-S and KL-6 or SP-D levels.

Figure 2. Dose-effect relationships between In-A and biomarkers by occupational group





2a) Scattergram between In-A and KL-6 levels in currently exposed workers. 2b) Scattergram between In-A and SP-D levels in currently exposed workers. Target concentration for indium according to prevention guidelines: 10 µg/m^3. Acceptable exposure limit for concentration of indium according to prevention guidelines: 0.3 µg/m^3. A single regression model was used to evaluate the dose-effect relationship between In-A and KL-6 or S-D levels.

Synthesis of indium-containing nanoparticles in aqueous suspension using plasmas in water for evaluating their kinetics in living body

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Nanoparticles have great potential for medical applications such as cancer therapy, whereas their toxic effects on human body are pointed out. To study kinetics and toxicity of nanoparticles in living body, we synthesized indium-containing nanoparticles in aqueous suspension using pulsed electrical discharge plasmas in water, because no indium compounds exist in the living body in the normal situation and hence indium-containing nanoparticles are useful tracer materials for analyzing kinetics of nanoparticles in living body. The mean size of synthesized primary nanoparticles is 7 nm, whereas the mean size of secondary nanoparticles is 315 nm. EDX and XRD analysis reveal that nanoparticles are indium crystalline and indium hydroxide crystalline with the mass ratio of 8:2. Premliminary subcutaneous administration of nanoparticles to mice shows that indium is transported from subcutaneous to blood. These results show that synthesized indium-containing nanoparticles are useful for analyzing kinetics of nanoparticles in living body.

Keywords: nanoparticles, indium, indium hydroxide, discharge plasma in water, nanotoxicology
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1. INTRODUCTION

In recent years, nanoparticles have attracted much attention because of their great potential for applications in medical fields.[1-4] For cancer therapy, nanoparticles enable us to detect cancer cells and to treat cancer cells by delivering drugs or generating heat.[5, 6] However, toxic effects of nanoparticles on human body are pointed out.[7] For instance, we have found that CIGS nanoparticles cause subacute pulmonary toxicity by installing into lung.[8, 9] It is important to study kinetics and toxicity of nanoparticles in living body to prepare safety guidelines of nanoparticle applications in medical fields. Although several studies of kinetics and toxity of nanomaterials such as carbon nanotube, [10] TiO_2 nanoparticles, [11] and gold nanoparticles [12-14] have been reported, very limited nanomaterials are employed for the studies. Sicne the specific physico-chemical properties at the nanoscale are expected to result in increased reactivity with biological systems, kinetics and toxity of various nanomaterials should be examined.

There are various methods for producing nanoparticles. The methods are categorized into three : condensation from gas, [15-21] chemical synthesis in liquid phase, [22, 23] and solid-state processes such as milling and attrition. [24] The condensation and chemical synthesis are bottom-up processes, whereas solid-state processes are top-down ones. Synthesis of nanoparticles using electrical discharge plasmas in water is a condensation method which has drawn great attention for various technological applications because of a simplicity of apparatus, no need for vacuum equipment, environmental safety, high-throughput and cost-effective procedure to generate high yield of nanoparticles. [25, 26] Synthesis of nanoparticles using electrical discharge plasmas in water offers a simple way to prepare nanoparticles in aqueous suspension, being useful for their administration to animals.

Here we report on synthesis of indium-containing nanoparticles in aqueous suspension using pulsed electrical discharge plasmas in water, because 1) indium and its compunds are widely employed in electronic applications such as tranparent conducting oxcides, 2) there is little infomration of kinetics and toxcity of in indium-containing nanoparticles, and 3) no indium compounds exist in living body and hence indium-containing nanoparticles are useful tracer materials for analysis of kinetics of nanoparticles in living body.

2. EXPERIMENTAL DETAILS

Synthesis of indium-containing nanoparticles using pulsed electrical discharge plasmas in deionized (DI) water was carried out with a device shown in Fig. 1. An indium rod electrode of 3 mm in diameter and an indium plate electrode of 1 mm in thickness were immersed into DI water. The discharges were generated between these electrodes by applying an AC high voltage with a high voltage source (Logy Electoric, LHV-10AC). The discharge voltage and the discharge current were measured with a high voltage probe (Tektronix, P0615A) and a Rogowski coil (U_RD, CTL-28-S90-05Z-1R1-CL1), respectively. The frequency of the applied voltage was 7.6 kHz, and its peak to peak voltage was 15.2 kV. The discharge power density was 5.1 W deduced from voltage/charge Lissajous plots. Optical emissions from the plasma were collected with an optical fiber and were measured with a spectroscope of spectral resolution of 0.38 nm (Ocean Optics, USB2000+). We deduced electron density of the discharge plasmas from the Stark broadening of H_β line. The generation rate of nanoparticles was 42 mg/min. The color of water gradually became brown with the discharges. The large particles were precipitated quickly just after the discharges.

After 3 min discharge, we sampled supernatant of the solution with a pipette and collected nanoparticles desiccating the solution on TEM-meshes and Si substrates. Nanoparticles collected on TEM-meshes and Si substrates were observed by TEM (JEOL, JEM-2010) and SEM/EDX (Hitachi High-Technologies, SU8000), respectively. Size destribution of secondary nanoparticles was measured by the dynamic light scattering method (Otsuka Electronics, ELSZ-0S). [27, 28] To obtain chemical and structural information of nanoparticles, we measured x-ray diffraction (XRD) spectra (Bruker, D8 DISCOVER-KU/I 3kw) of nanoparticles collected on Si substrates.

Synthesized nanoparticles were administrated towards mice for a preliminary examination of kinetics of nanoparticles in living body. Solution of nanoparticles was condensed at the concentration of 10 mg/ml by vaporizing water with heat. Subcutaneous administration was carried out to 13-week-old mice (Crlj:ICR) after 7 weeks acclimation. The dose of administration was 1 mg/10gBW per each mouse. We collected the blood of the mice 10 days after the administration. The collected blood was wet-ashed with nitric acid, and indium content in the blood was measured by ICP-MS (Agilent 7500c).

3. RESULTS AND DISCUSSION

Figure 2 shows an optical emission spectra of plasmas with an exposure time of 0.1 s. Atomic emission lines of indium (325.8 nm, 410.2 nm, and 451.1 nm), [29] oxygen (777 nm), and hydrogen (H_α 656.3 nm and H_β 486.1 nm) exist in the spectra. There also exit weak molecular emission bands in the wavelength ranges of 390-550 nm and 540-588 nm referred to radiation of H_2O and H_2 molecules, respectively. Apart from the Stark broadening, any emission line spontaneously emitted by plasma can be broadened by other mechanisms, and the total broadening of the line profile is due to the combined contribution of all causes. [30] Each mechanism can produce a shift in the energy levels of the emitter atoms and the relative importance of these broadening depends on the plasma conditions. In our case, for plasmas at atmospheric pressure with moderate electron density and temperature, the important sources of broadening of the H_β line are the Stark effect with FWHM of about 7.37 nm, the Doppler effect with FWHM of about 0.006 nm, and the van der Waals broadening with FWHM of about 0.06 nm.[30] The broadenings due to other less important effects are neglected, such as the natural broadening and the resonant broadening. The instrumental broadening has a FWHM of about 1.33 nm and it is an external cause of line broadening. The electron density deduced from the Stark broadening of the H_β line is 2.2×10^{17} cm^{-3}. [30, 31]

Figure 3. (a) shows a typical TEM image of the synthesized nanoparticles and the size destribution of primary nanoparticles. The size destribution is the Gaussian one and the mean size of the primary nanoparticles is 7 nm. The morhporogy of nanoparticles are mostly spherical or distorted spherical. Figure 3. (b) shows the size destribution of secondary nanoparticles. The size destribution is the log-normal one and the mean size of secondary nanoparticles is 315 nm. EDX spectra of the nanoparticles is shown in Fig. 4. The peaks of Indium and oxygen were observed from the spectra, showing that the synthesized nanoparticles contain indium and oxygen.

Further chemical and structural information of the synthesized nanoparticles is obtained from Fig. 5. This figure shows an XRD pattern of the synthesized nanoparticles. The presence of sharp peaks on the XRD patterns is usually connected with a crystal structure in the samples. The peaks of In (JCPDS card No. 85-1409), and $In(OH)_3$ are observed in the XRD pattern. [32, 33] These results indicate that the nanoparticles are indium crystalline (In) and indium hydroxide

($In(OH)_3$) crystalline. The mass ratio of indium nanoparticles to indium hydroxide nanoparticles is 8:2, deduced from the XRD pattern using the normalized relative intensity ratio method.[34, 35]

Although up to now no direct experimental evidence has been obtained indicating a specific mechanism for the formation of nanoparticles in pulsed electrical discharge plasmas in DI water, we can hypothesize that the following processes take place during the discharges.

During the discharge, the indium electrodes are heated, melted at the indium melting point of 156 ^oC, and vaporized. Near the elecrodes, the water is also vaporized as a result of exposure to high temperature. Due to this vaporization of water, we observed many bubbles near the electrodes during the dischrages. The discharge plasmas take place in a gas mixture consisting of the water vapor and indium, with a temperature gradient from a high temperature region near the electrodes to the boiling point of the liquid at the gas mixture-liquid boundary. The gas mixture can contain indium, H_2O, O_2 and H_2. Moreover, H_2O, O_2 and H_2 can be dissociated into OH, O, and H by electron impact in discharge plasmas and thermal decomposition.

Nanoparticles are formed in the gas mixture as a result of the sequential change from nucleation to growth to coalescence to aggregation. The growth of nanoparticles stops as a result of interaction with the cold water surrounding the discharge zone. We measured temperature rise of water during discharges with an electrical themometer. The temperature increases from 25 ^oC to 38 ^oC after 5 min. discharge. Therefore, the average temperature of water surrounding the discharge zone is in this range. Some molecules and atoms originated from water vapor in the gas mixture react with indium and lead to nanoparticles of indium hydroxide ($In(OH)_3$).

Synthesized nanoparticles were administrated towards mice for a preliminary examination of kinetics of nanoparticles in living body. The subcutaneous administration was well tolerated and no adverse effects were observed during the 10 days observation period. The preliminary examination shows that indium of 1.2-1.6 ng/ml is transported from subcutanious to blood in 10 days. These results show that synthesized nanoparticles are useful for analyzing kinetics of nanoparticles in living body.

4. CONCLUSION

We synthesized indium-containing nanoparticles in aqueous suspension using pulsed electrical discharge plasmas in DI water. We obtained the following conclusions.

1) Nanoparticles of 7 nm in primary nanoparticle size and 315 nm in secondary nanoparticle size are produced using pulsed electrical discharge plasmas in water with the indium plate electrode and the indium rod electrode.
2) EDX, and XRD analysis indicate that the synthesized nanoparticles are indium (In) crystalline and indium hydroxide ($In(OH)_3$) crystalline with the mass ratio of 8:2.
3) Synthesized nanoparticles are useful for analyzing kinetics of nanoparticles in living body.

The remaining issues are to reveal the mechanisms of synthesis of nanoparticles using pulsed electrical discharge plasmas in water in detail, and to carry out further experiments to reveal kinetics and toxicity of nanoparticles as a parameter of size of primary and secondary nanoparticles.

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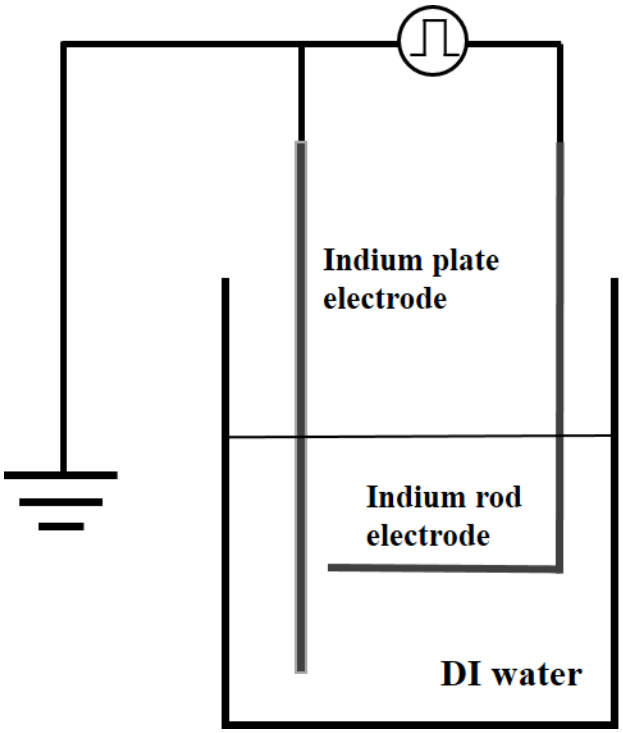

Fig. 1. Schematic of experimental setup for synthesis of indium-containing nanoparticles using pulsed electrical discharge plasmas in DI water.

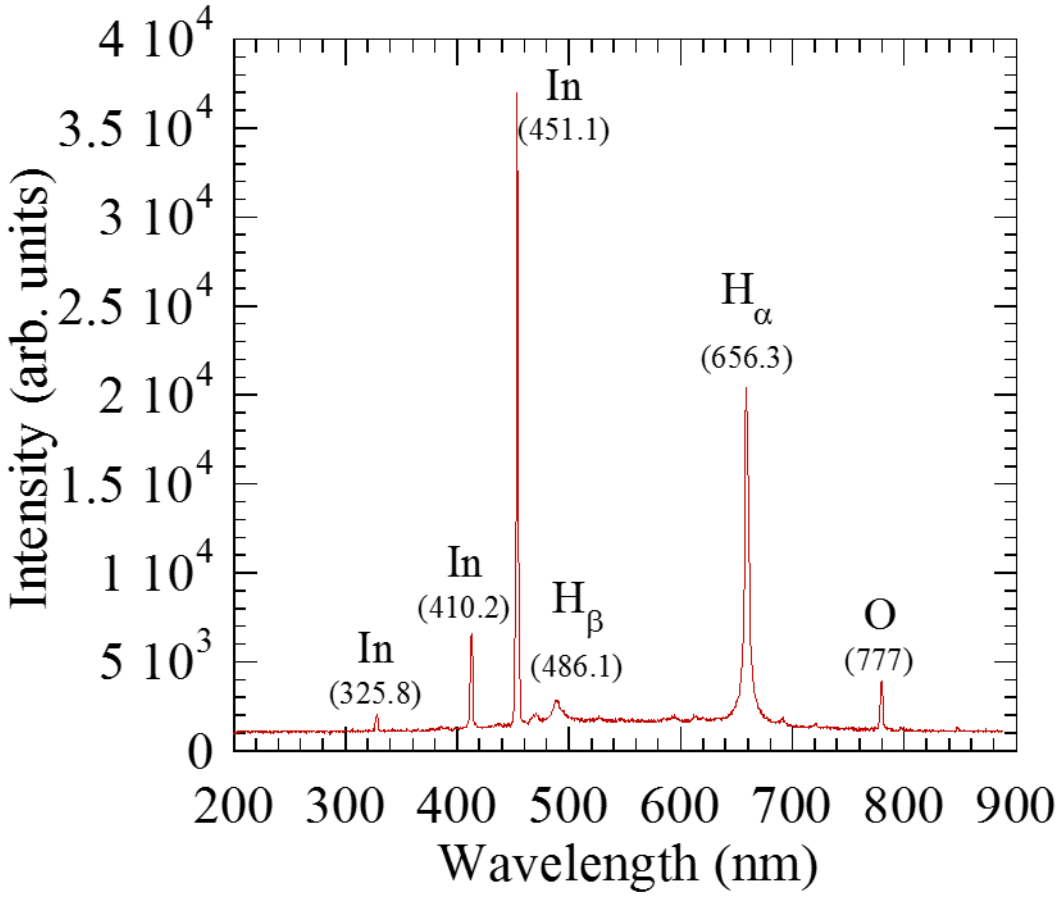

Fig. 2. Optical emission spectrum of pulsed electrical discharge plasma in DI water.

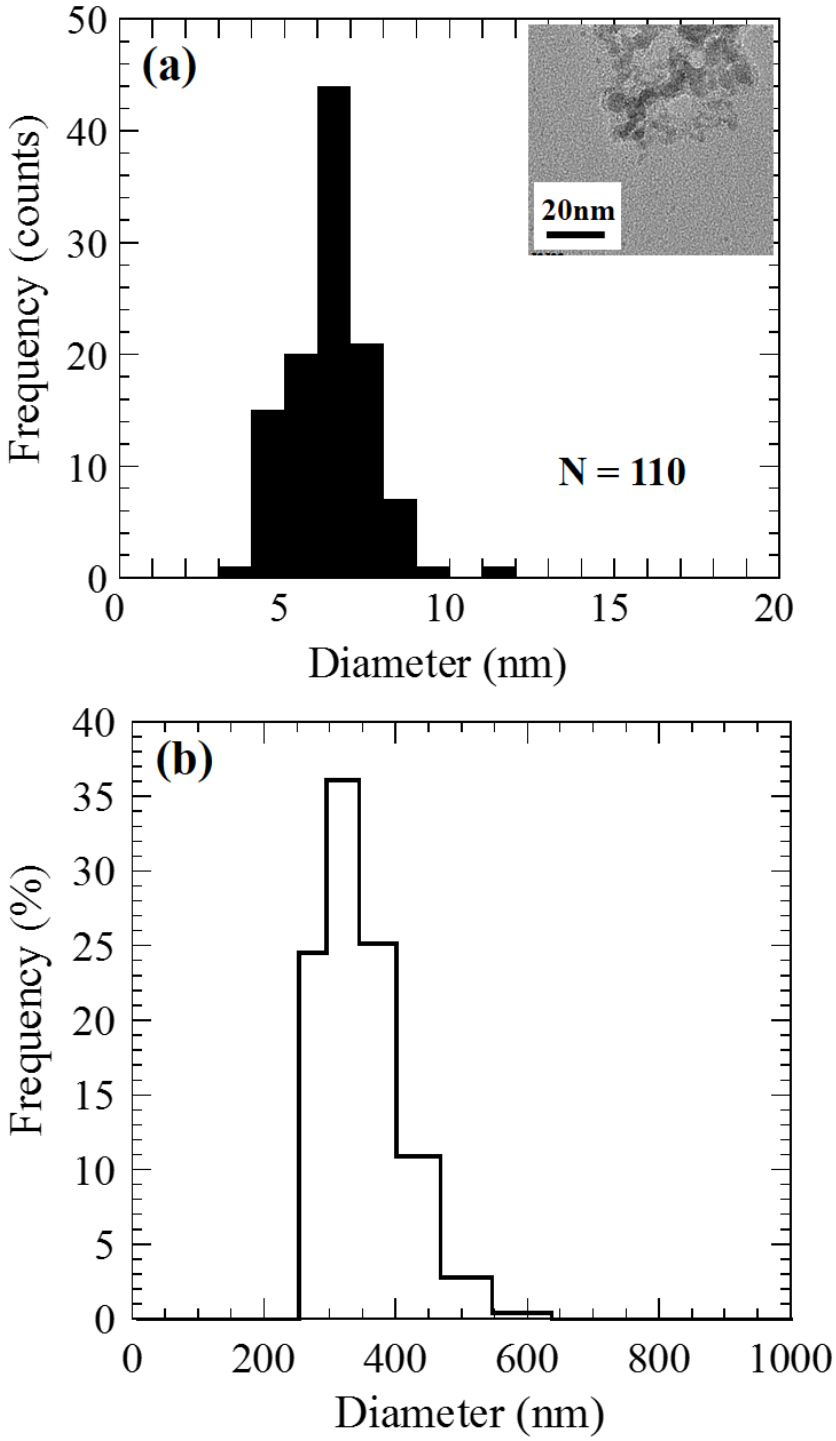

Fig. 3. (a) TEM image of indium-containing nanoparticles and size distribution of primary nanoparticles, and (b) size distribution of secondary nanoparticles.

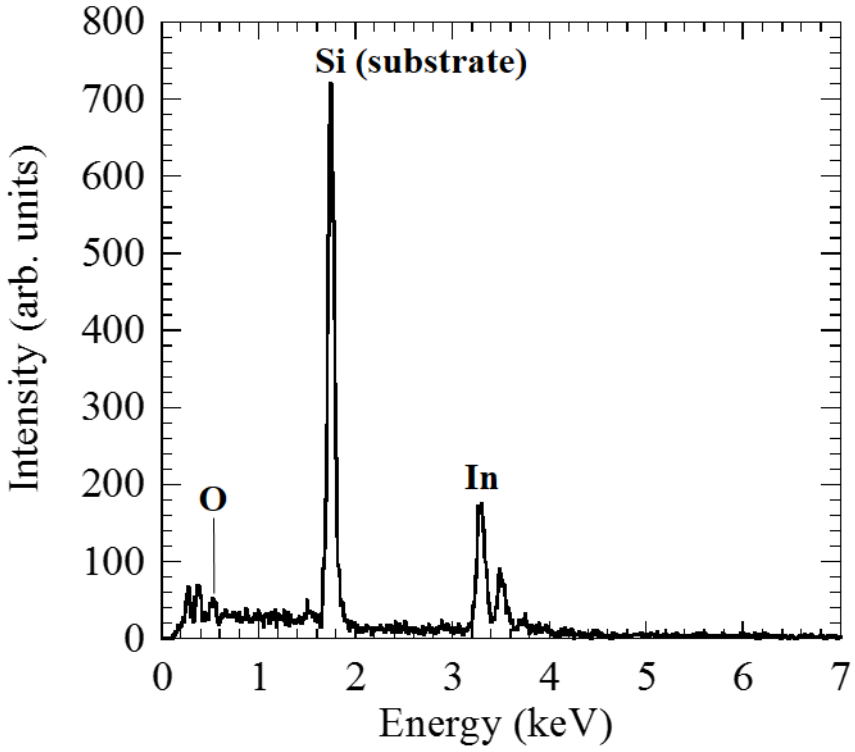

Fig. 4. EDX spectrum of synthesized nanoparticles.

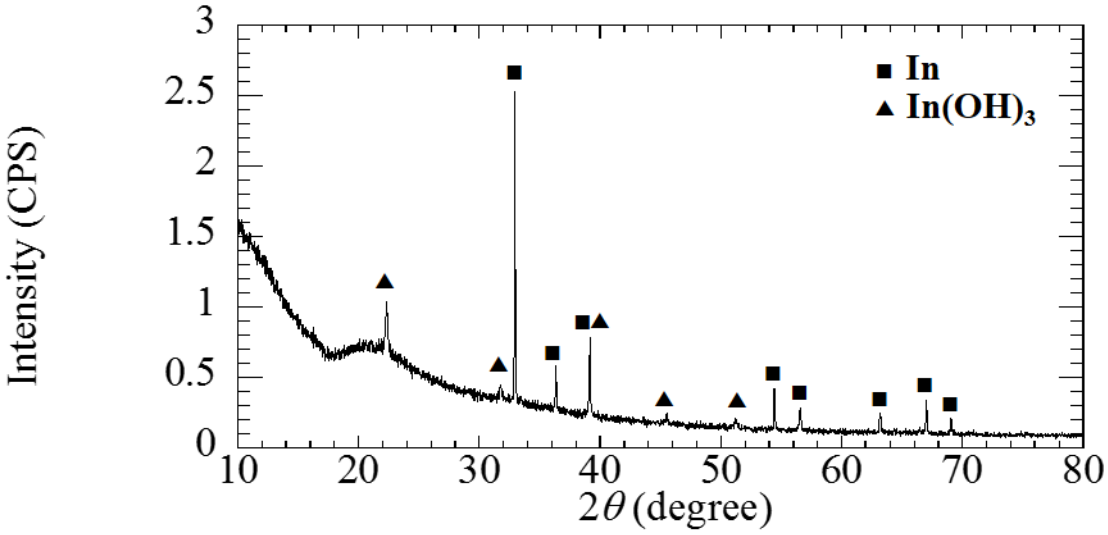

Fig. 5. XRD pattern of synthesized nanoparticles.

In press

Personal indium exposure concentration in respirable dusts and serum indium level

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・Abstract:

OBJECTIVE: Due to very slow clearance rate of indium (In) accumulated in the lungs, it is not easy to estimate the relationship between personal In exposure concentration and In in serum (In-S) reflecting both In in recent exposure and in the lungs. The aim of this study was to clarify the relationship between personal In exposure concentration in respirable dust fraction (In-E) and In-S in workers probably with small amount of In in the lungs.
METHODS: A number of workers studied were 40 from 11 factories. They wore no or ineffective respiratory protective devices, In concentrations in their workplaces were low, and their working environments have not been improved over the past few years. Respirable dust fraction was sampled using a personal respirable dust cyclone. In-E and In-S were measured by ICP-MS.
RESULTS: In-E ranged from 0.039 – 24.0 μg/m^3, and In-S ranged from 0.11 –8.50 μg/L, respectively. Linear regression equation between In-E and In-S was In-S= 0.291 × In-E + 0.456, and correlation coefficient was 0.86 ($p < 0.001$).
CONCLUSION: According to the calculations, In-E corresponding to In-S of 3μg/L is calculated to be 8.74μg/m^3.
(186 word)

・Keywords: no more than 6 keywords should be described.
Keywords: indium, indium exposure concentration, indium in serum

Text:

Introduction

Recent epidemiological studies in Japan revealed that inhalation of hardly soluble indium (In) compounds caused interstitial and emphysematous lung diseases[1-6]. Indium tin oxide and indium phosphide induced lung cancer on rats and/or mice[7, 11].

Based on these findings[2-3], in 2007, the Japan Society for Occupational Health (JSOH) recommended an occupational exposure limit based on biological monitoring (OEL-B) of indium as 3μg/L of indium in serum (In-S).

However, JSOH could not propose occupational exposure limit-mean (OEL-M) in the workplace due to the lack of information about exposure-effect and exposure-response relationships between indium exposure and its effects on the lungs. In this case, there are several reasons. First, there was few information about indium concentration from both personal air sampling and area sampling data in occupational setting. Second, due to very slow clearance rate of In accumulated in the lungs[7), 11), 12)], it has been difficult to estimate the relationship between indium concentration from personal air sampling recently measured and In-S reflecting both In level accumulated in the lungs and recent exposure levels. In any case, it is necessary to elucidate the relation between indium concentration from personal air sampling and indium in serum.

The aim of this study was to clarify the relationship between personal indium exposure concentration in respirable dust fraction (In-E) and In-S in 40 workers in 11 factories.

Subjects

Be based on precedence knowledge, we selected this study group which exposed low and was low-accumulated level. Additionally, this study group also wore no or ineffective respiratory protective devices.

Therefore, we measured In-E at 14 plants involving 54 exposed workers. Unfortunately, we could measure In-S only at 11 plants, including 41 exposed workers. We excluded 23 workers whose In-S below the detection limit (0.1μg/L), because they exposed too small. And we excluded one outlier (In-S: 14.9μg/L, In-E: 2.56μg/m^3), because the In-E was too low to measure exactly. The resulting dataset covered 11 plants, including 40 exposed workers. Exposed workers were exposed to multiple indium compound dusts such as indium metal, ITO, indium oxide.

We informed all study candidates about the health risks of indium exposure and the purpose of the epidemiological study, about obtained their informed consent

before they participated. The study was approved by the Ethical Committee, School of Medicine, Keio University (approval number 20110268).

The subjects were 16 male workers. The mean length of indium exposed 112 mo (range: 12-415). 8 workers were current smoker.

Methods

Respirable fraction of the indium dust in the breathing zone were collected by a personal sampling device (GS-3 Respirable Dust Cyclone・SKC inc) at a flow rate of 2.75liters/min or by a Total and Respirable Dust Sampler (PM4 NWPS-254, Sibata Scientific Technology Ltd.) coupled with a minipump (MP-Σ3,Sibata Scientific Technology Ltd.) at a flow rate of 2liters/min. Due to sampling time ranging from 251 to 483min, we converted the concentration to a conventional 8-hour time-weighted average.

Indium exposure concentration in respirable dust fraction (In-E, μg/m^3) was measured by inductively coupled plasma mass spectrometry (ICP-MS) at the Center of Advanced Instrumental Analysis, Kyushu University and the Japan Industrial Safety and Health Association[3, 10]. Serum indium (In-S, μg/L) was measured by ICP-MS at the Center of Advanced Instrumental Analysis, Kyushu University[3].

Results

In-E ranged from 0.039 to 24.0 μg/m^3, and In-S ranged from 0.11 to 8.50 μg/L, respectively. Figure 1 shows scatter gram about In-E and In-S. In-E on the abscissa is plotted against the In-S on the ordinate. Distribution seems to be linear. Linear regression equation was In-S = 0.291 × In-E + 0.456, and correlation coefficient was 0.86 ($p < 0.001$). In-E corresponding to In-S of 3μg/L is calculated to be 8.74μg/m^3 (95%CI: 6.41-12.5).

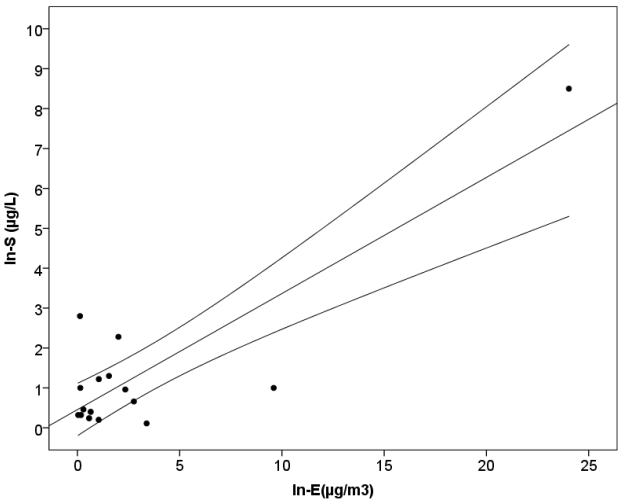

Figure1. Scatter gram about In-E and In-S.

Discussion:

This study clarifies the relationship between personal indium exposure concentration in respirable dust fraction (In-E) and In-S. In-E corresponding to In-S of 3μg/L is calculated to be 8.74μg/m^3 (95%CI: 6.41–12.5). We might recommend an occupational exposure limit-mean (OEL-M) of indium as 10μg/L of indium in respirable dust fraction (In-E).

This study subjects has several strength as following reasons.

First, company had not already taken actions to improve the work environment. Second, this study group also wore no or ineffective respiratory protective devices. Finally, this study group which exposed low and was low-accumulated level. From animal data, accumulated In was very slow clearance rate. In-S was a biological marker of indium as both current and former exposure. Then our study subject clears these problems. Accordingly, we could discuss about current In-E and current In-S.

However, this study has also some limitations.

First, this study was not assessed day-to-day sampling variation of the In-E. This might pose a limitation of the present study. However, amounts of indium consumption were small in each plant and day-to-day variations of workers' task were small. Consequently, the In-S levels in the present study seem to reflect the daily In-E.

Second, In-E corresponding to In-S of 3μg/L is only calculated to be 8.74μg/m^3. However, respirable fraction dust concentration (In-E) on the abscissa is plotted against the In in serum (In-S) on the ordinate. Distribution seems to be linear.

And, In-E was pulled to high 1 example (In-S: 8.5μg/L, In-E: 24.0μg/m^3). However, we can resolve this when samples are collected.

Most importantly, according precedence knowledge, In-S seems to be a currently representative value in terms of slow clearance rate of In. Without knowing this, it's dangerous. OELs should be applied by individuals well-trained and experienced in occupational health[13]. Because OELs do not represent a definitive borderline between safe and hazardous conditions, it is not correct to conclude that working environments above OEL are the direct and sole cause of health impairment in workers, or vice versa[13].

(942words)

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