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論文題目	In vitro and in vivo investigations of lead-induced changes in membrane protein expression and assessment of risks associated with children’s exposure to environmental lead （インビトロおよびインビボによる膜タンパク発現の鉛誘導変化研究と子供の環境中鉛曝露に関するリスクアセスメント）		
（論文内容の要旨） The role of some membrane proteins in Pb-induced impairment of intellectual function and a method for the assessment of blood Pb concentrations arising from non-dietary ingestion using modified exposure equations are proposed in this thesis. This thesis consists of six chapters. The background information and research motivation and objectives are presented in Chapter 1. Pb is a known neurotoxicant and low levels of Pb have been shown to be associated with intellectual impairment in children. However, the mechanisms of action of Pb that eventually lead to cognitive effects are not yet fully understood. Particularly in developing countries, data on the extent of Pb poisoning are scarce. Blood Pb concentrations are used as reference in describing health risks associated with exposure to Pb. However, routine PbB monitoring is costly. Translating environmental Pb concentrations to PbB levels may prove to be an alternative method. Chapters 2 and 3 deal with the <i>in vitro</i> studies. The effects of Pb on the expression of selected membrane proteins in neuronal PC12-cells are discussed in Chapter 2. The results from Western blot analysis of membrane proteins expression and real-time reverse-transcriptase PCR evaluation of mRNA expression suggest that heme oxygenase 2 (HMOX2) and Ras-related protein RAB-1B (RAB1B) may be involved in the mechanisms of toxic action of Pb on neuronal cells. The results of the cell viability assay and measurement of neurite length, which are presented in Chapter 3, showed that the range of Pb concentrations that caused changes in the expression of HMOX2 and RAB1B failed to cause overt cytotoxicity or disruption in neurite outgrowth. Taken together, these results suggest that the down-regulation of HMOX2 and RAB1B may not be related to general cell health but probably to specific neuronal function. Both HMOX2 and RAB1B have been reportedly linked to neuronal processes related to learning and memory. HMOX2 is involved in the synthesis of CO, a putative neurotransmitter with role in long-term potentiation (LTP), a physiological model for learning and memory. RAB1B is thought to be a memory related gene and has been implicated in a mechanism for long-term information storage. Chapter 4 is about the <i>in vivo</i> studies. Adult and young Sprague-Dawley rats were treated with Pb and then blood and brain tissues were collected and analyzed for Pb concentration. The effects of Pb on the expression of HMOX2 and RAB1B in the whole brain of the animals were also evaluated. Dose-dependent increase in blood and brain Pb			

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concentrations were observed. Pb caused a reduction in RAB1B expression in the brain of Pb treated young rats, however a dose-dependent pattern was not evident. The decrease was apparent only up to 10 mg/kg-BW, and the response of the 100 mg/kg-BW group was comparable to the control. It is proposed that the response of the animals to lower doses may be different from those caused by higher doses. An analysis of the changes in RAB1B expression relative to blood Pb (PbB) concentration showed an ED50 of 6.69 µg/dL, a value lower than the 10 µg/dL level of concern for Pb poisoning in children. In Chapter 5, the assessment of the exposure of preschool children in Metro Manila, Philippines to playground Pb is presented. The exposure of children to Pb from the playgrounds was evaluated by analyzing the Pb content of hand wipe samples collected from the children after playing in the playgrounds. The potential contributions of the playground Pb to PbB levels were estimated using modified equations for estimating intake and PbB. The average PbB level in children that may arise from exposure to Pb from various sources was also estimated. Although the playgrounds are located in the same school, the Pb concentrations measured in the hand wipe samples varied. The hand-Pb concentrations did not correspond to soil-Pb levels, indicating that other factors such as paint in the play sets contribute to children's exposure. The assessments revealed that the contribution of playground Pb to PbB levels was minimal, and that Pb in blood of children in Metro Manila mainly comes from food sources. An average of 10.82 µg/dL PbB was estimated from various sources of Pb exposure, suggesting that children in Metro Manila are at risk from the adverse effects of Pb in intellectual function. Finally, Chapter 6 provides a summary of the findings in the various sub-studies as well as recommendations for further studies. The results of the <i>in vitro</i> studies which implicate HMOX2 and RAB1B in Pb neurotoxicity can provide fresh insight into the mechanisms of action of Pb that eventually result in intellectual impairment. The <i>in vivo</i> studies showed that RAB1B expression is reduced in Pb-treated young rats and the estimated ED50 was lower than the 10 µg/dL level of concern for Pb poisoning in children. The assessment of exposure of preschool children in Metro Manila, Philippines to environmental Pb revealed that playground Pb is not a major contributor to PbB in children, and that food sources account for the majority of Pb in the children's blood.			

(論文審査の結果の要旨)

本論文は、鉛の神経細胞に対する影響反応を *in vitro* 試験においては、ヘム・オキシゲナーゼ 2 (HMOX2) と Ras タンパクに関係するタンパク RAB-1b (RAB1B) に注目することで、また *in vivo* 試験においては若年ラットの脳中 RAB1B 濃度と鉛曝露量との関係に注目することによって明らかにする手法を開発したものである。また、フィリピンのマニラにおける子供を例として、主たる鉛曝露源を、手に付着した鉛量や食品中鉛濃度データから明らかにする方法を確立したものであり、鉛に関して、*in vitro* 実験、*in vivo* 実験、フィールド調査という総合的な観点からの健康リスク評価方法の確立を目標に研究した成果についてまとめたものであり、得られた主な成果は次のとおりである。

1. *in vitro* 実験において、神経細胞 PC-12 に鉛を曝露した結果、タンパク質量の変化あるいは mRNA の発現・修飾という観点から、学習や記憶機能に関係していると考えられている、ヘム・オキシゲナーゼ 2 (HMOX2) と Ras タンパクに関係したタンパク質 RAB 1b (RAB1B) が、減少する場合は観測された。その結果、HMOX2 や RAB1B などの膜タンパク量の変化が神経毒性の指標として使用できる可能性を示した。

2. *in vivo* 実験により、若齢および成体ラットの血中と脳中の鉛濃度は、鉛摂取量の増加に従って増加すること、一般に若齢ラット組織中濃度の方が成体ラットよりも高いこと、血中鉛濃度と脳中鉛濃度には正の相関があることを示した。若齢ラットの脳においては鉛曝露によって RAB1B の発現量が減少したが、成体ラットにおいてはそのような傾向は認められないこと、若齢ラットの脳における RAB1B の発現に対する半数効果量 ED50 は $6.69 \mu\text{g/dL}$ 程度であることを明らかにした。

3. マレーシアの首都マニラにおける子供の鉛摂取量は、校庭で 30 分間遊んだことによって手に付着する鉛曝露によっては、典型的なマニラの子供の血中鉛濃度の 3%ほどにしかならず、他の鉛曝露源についても検討した結果、マニラの子供達はおおよそ $10.82 \mu\text{g/dL}$ の血中鉛濃度を持ち、その 88%は食品に由来することを示した。

以上のように、本論文は、*in vivo*, *in vitro*, フィールド調査による、鉛の神経毒性の総合的評価方法を提案するものであり、他の物質による神経系への総合的リスク評価方法の確立に対しても大きく貢献するものと判断され、学術上、實際上寄与するところが少なくない。よって、本論文は博士（工学）の学位論文として価値あるものと認める。また、平成 24 年 8 月 23 日、論文内容とそれに関連した事項について試問を行って、申請者が博士後期課程学位取得基準を満たしていることを確認し、合格と認めた。