ESRF	**Experiment title: Environmental Mn compounds distribution and speciation in dopaminergic cells in relationship to Parkinson's disease aetiology**	**Experiment number**: MD-635
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Report:

Abstract

Manganese is a neurotoxic element that leads to severe neurological disorders when excessive exposure occurs, mainly in occupational settings, but that can also lead to more subtle neurological deficits, especially to the dopaminergic system, for lower exposure conditions. Mn exists in a variety of chemical species in the environment but the influence of Mn speciation on its neurotoxicity has not been fully evaluated yet. In this study we compared the cytotoxicity towards dopamine producing cells of environmental Mn compounds with a diversity of physico-chemical forms: inorganic compounds of distinct oxidation states and solubility ($MnCl_2$, $MnSO_4$, Mn_2O_3); and organic compounds MMT, a gasoline additive, maneb, a Mn-dithiocarbamate fungicide. We observed that maneb exhibited the highest toxicity, followed by $MnCl_2$, $MnSO_4$ and MMT which resulted in a similar intermediate toxicity, the less toxic compounds being the insoluble compound Mn_2O_3. We combined micro-SXRF (Synchrotron X-Ray Fluorescence) for imaging and micro-XANES (X-ray Absorption Near Edge Structure) to determine Mn oxidation state at the single cell level. Mn_2O_3 entered readily the cell but remained in its initial state as Mn(III) particles within the cytoplasm. The lack of toxicity of Mn_2O_3 can be explained by its insolubility. For all the other compounds, Mn(II) was observed and was located mainly into the Golgi apparatus, probably for detoxification purposes via exocytosis. Organic compounds MMT and maneb were degraded releasing Mn and behaved similarly to the soluble Mn(II) inorganic

compounds. Maneb cytotoxicity was higher probably because of the combined toxicity due to both Mn and dithiocarbamate residues. Overall these results raise the concern about environmental exposure to Mn since, either inhalation of Mn combustion products, or ingestion of contaminated food and drinking water will end up in neurotoxic soluble and bioavailable Mn species.

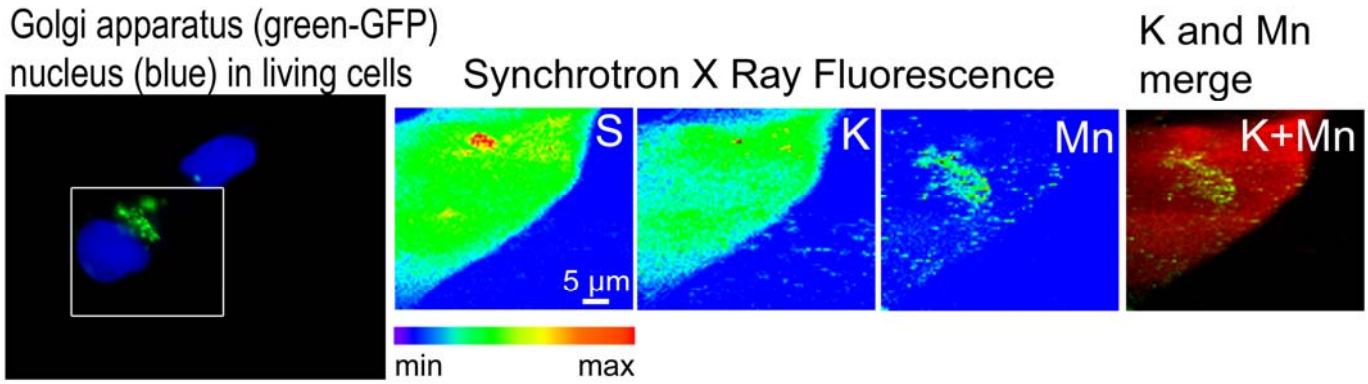

Fig. Co-localization of Mn and GA in PC12 cells exposed to 500 µM $MnCl_2$ during 24 h. Left panel shows the fluorescence of GA labelled with GFP proteins (green) and nucleus using Hoechst stain (blue) into living PC12 cells prior to cryofixation. White square indicates the analyzed area by micro-SXRF (48 µm x 40.5 µm). Elemental maps (S, K and Mn) are presented in right panels, and the merged image of K (red) and Mn (green) distributions, scale bar 5 µm.

Carmona A, Roudeau S, Perrin L, Veronesi G, Ortega R. Environmental manganese compounds accumulate as Mn(II) within the Golgi apparatus of dopamine cells: relationship between speciation, subcellular distribution, and cytotoxicity. Metallomics. 2014. **DOI: 10.1039/C4MT00012A**

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