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# Toxicity mechanisms of cobalt oxide particles (Co<sub>3</sub>O<sub>4</sub>P) on human lung cells: impact of solubilization

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The wide use of cobalt in industrial applications, including the nuclear industry, could lead to accidental or chronic occupational exposure mainly by inhalation. Cobalt may be encountered in industry in a variety of chemical forms, including metal dusts, oxides (Co<sub>3</sub>O<sub>4</sub>), and soluble salts and could also be present in fragments of irradiated fuel. <sup>60</sup>Co is an important activation product produced in nuclear power plants. The potential toxicity of poorly soluble cobalt (II, III) oxide particles (Co<sub>3</sub>O<sub>4</sub>P) is a consequence of the long retention time (years) in lungs of the gamma-ray emitting radionuclide. Lung damage may be expected from both chronic radiation exposure, and chemical toxicity of Co.

As a first approach to evaluate the chemical toxicity of cobalt particles, stable <sup>59</sup>Co<sub>3</sub>O<sub>4</sub> particles were used. We have demonstrated that cobalt oxide particles were readily endocytosed by the BEAS-2B human lung cells via the clathrin-dependent pathway. Despite their very low solubility in the culture medium, cobalt particles were partially solubilized at low pH within lysosomes, leading to the release of cobalt ions. Using ion beam microanalysis, solubilized cobalt was detected within the cytoplasm and the nucleus. As expected with these low-solubility particles, the intracellular solubilized cobalt content was low compared with the intracellular particulate cobalt content in the parts-per-thousand range or below. However, this minute fraction of intracellular solubilized cobalt was found to be responsible for the overall toxicity [1].

The genotoxic potential of poorly soluble Co<sub>3</sub>O<sub>4</sub>P was then investigated in the BEAS-2B cell line, which exhibits the highest homology in gene expression pattern with primary non

tumor lung cells. Cytokinesis-block micronucleus assay and comet assay revealed that  $\text{Co}_3\text{O}_4\text{P}$  had a genotoxic potential following exposure at non cytotoxic concentrations. Indeed, enhanced micronuclei formation and both primary and oxidative DNA damage were noted following exposure to increasing concentrations of  $\text{Co}_3\text{O}_4\text{P}$ . The scoring of  $\gamma$ -H2Ax foci demonstrated that  $\text{Co}_3\text{O}_4\text{P}$  were able to generate double DNA strand breaks. The involvement of oxidative stress was confirmed by the inhibition of foci formation in the presence of the ROS scavenger N-acetylcysteine. Comparison of the effects induced by  $\text{Co}_3\text{O}_4\text{P}$  with those triggered by  $\text{Co}^{2+}$ , indicated that the genotoxicity of  $\text{Co}_3\text{O}_4\text{P}$  was not solely due to the released soluble fraction [2].

To go further, a project aiming at broadening the therapeutic offer in case of accidental contamination with insoluble cobalt particles was recently undertaken. This project, dealing with  $^{60}\text{Co}_3\text{O}_4$ , uses a transversal approach ranging from chemistry (to select ligands able to enhance particle solubilization), to decorporation studies in rodents. Preliminary results obtained in the framework of this collaborative project will be presented.

## References

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Results presented here were obtained in the frame of the EDF-CEA collaboration.