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Review report



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Comments to the NTP draft report on the inhalation studies on cobalt metal powder 'NTP TR 581'

1. Introduction

On September 19th the NTP published the draft version of the 'NTP TECHNICAL REPORT ON THE TOXICOLOGY STUDIES OF COBALT METAL, (CAS NO. 7440-48-4) IN F344/N RATS AND B6C3F1/N MICE AND TOXICOLOGY AND CARCINOGENESIS STUDIES OF COBALT METAL IN F344/NTac RATS AND B6C3F1/N MICE (INHALATION STUDIES)'.

This report contains the results of sub-acute (2-week), subchronic (90-day) and chronic (2 year) inhalation studies in rats and mice, and of a genotoxicity screening in bacterial reverse mutation assay and mouse in vivo micronucleus assay.

2. Observations in the report

- The lung is the target organ for Co metal powder via the inhalation route
- Local effects observed in the lung are consistent over the various exposure durations and in the two animal species with neoplastic lesions being preceded by and co-occurring with non-neoplastic lesions, indicative of sequential events in the pathogenesis:
 - 2-week studies show local effects of tissue damage and onset of inflammation and fibrosis (irreversible damage) in the airways
 - 90-day studies show serious local effects in both upper and lower airways including pulmonary alveolar proteinosis, bronchiolar epithelial hyperplasia and chronic inflammation.
 - 2-year studies show concentration dependent increase of alveolar and bronchiolar adenoma and carcinoma, increase in point mutations (T-G) in the Kras gene in tumor tissue, pulmonary alveolar proteinosis and chronic inflammation
- There is evidence of systemic uptake of Co in both rats and mice through:
 - Observed urinary excretion
 - Co distribution to other organs is proven: Lung>Liver>Kidney>Femur>Heart>Serum>Blood~Testes
 - Reproductive organ weight is reduced, sperm count and motility is affected
- Dose dependent systemic carcinogenesis is observed exclusively in male rats (2-year exposure) with affected organs:

- Adrenal medulla: pheochromocytoma, benign and malignant
- Pancreas: pancreatic isle adenoma and carcinoma
- Kidney: some incidence of tubular adenoma and carcinoma at the highest concentration
- Female rats show a slight increase in mononuclear cell leukemia
- Genetic toxicology assessment occurred at 3 levels:
 - Ames test (bacterial reverse mutation): positive in TA98 without S9 but negative with S9
 - Mouse in vivo micronucleus test: negative
 - Mutation analysis of selected genes in observed tumors: T-G mutation in Kras gene observed
- Genetic toxicology results indicate a mechanism of oxidative stress (threshold effect) and no direct interaction of Co with DNA. Oxidative stress in combination with reduced DNA repair (known for Co) can lead to mutations as observed in the Kras gene.

3. Points of critique – Comments

- Choice of animal species, more precisely the rat strain:
 - NTP has chosen to replace the F344/N rat strain with the F344/NTac rat strain for carcinogenicity studies for reasons of too many spontaneous malignancies and other idiopathic incidences in the F344/N strain (King-Herbert et al). The current 2-year inhalation study is the first study conducted with the new strain by NTP by which no historical control data exist. Hence no interpretation on the natural background incidence of spontaneous tumors is possible.
- Systemic carcinogenesis of Co seems to be species and gender dependent
 - Pheochromocytoma most expressed in male rats, absent in mice (male & female)
 - Pancreatic isle tumors are exclusively observed in male rats, not in female rats nor in mice (male & female)
 - Kidney tumors observed only in the high dose group
- It has not been investigated whether these organs are target organs for Co, except for the kidney
- Incidences of pheochromocytoma in the adrenal medulla has been correlated to conditions of severe respiratory stress after exposure to particulate matter inducing endocrine disturbances and hyperproliferative adrenal medulla cells. These effects are most likely not exposure related. (Ozaki et al)
- In absence of historical control data for the new rat strain, it is well known that F344/N rats show a gender dependent spontaneous background incidence of adrenal medullar tumors (pheochromocytoma) and pancreatic isle tumors (Kuroiwa et al, Haseman et al).
- The 'first incidence' of the systemic tumors in males is not different from the spontaneous incidence in the control group. This is contraindicative for an exposure related systemic tumor inducing effect. This is clearly different from the lung tumor incidence where there is a earlier incidence of the local tumors upon Co exposure.

4. Conclusion

- In a chronic exposure set-up there is clear evidence of a local carcinogenicity in the lung induced by Co in both rats and mice.
- There is evidence of a mode-of-action for Co to induce lung tumors through the induction of oxidative stress (threshold effect).
- The evidence to prove systemic carcinogenicity is weak and not interpretable without a strong data set of historical control incidences with information on species and gender dependencies.

5. References

Haseman et al. Effect of Diet and Animal Care/Housing Protocols on BodyWeight, Survival, Tumor Incidences, and Nephropathy Severity of F344 Rats in Chronic Studies, *Toxicologic Pathology*, 31:674-681, 2003

King-Herbert et al. Commentary: Update on Animal Models for NTP Studies, *Toxicologic Pathology*, 38: 180-181, 2010

Kuroiwa et al. Transition of historical control data for high incidence tumors in f344 rats. *Journal of toxicologic pathology* 26:2 2013 Jun pg 227-30

Ozaki et al, Association of adrenal pheochromocytoma and lung pathology in inhalation studies with particulate compounds in the male F344 rat--the National Toxicology Program experience, *Toxicol Pathol.* 2002 Mar-Apr;30(2):263-70.