Ruth Danzeisen
(on behalf of the Cobalt Development Institute)

Nov 24, 2015

Oral comments on the revised RoC Monograph on Cobalt for the NTP Board of Scientific Counselors Meeting on Dec 2, 2015

Dear Dr. White,

We thank you for the opportunity to comment on the revised RoC Monograph on Cobalt.

Differences in oral versus inhalation bioavailability

The written public comment made by Dr. Neuwirth reflects our view, and we support his submission that the revised Monograph, as it is currently written, is unhelpful to risk assessors needing to address oral exposures to cobalt compounds.

As stated by Dr. Neuwirth, it would be helpful to elaborate on the differences in oral vs respiratory bioavailability for cobalt compounds that are insoluble in water and poorly soluble in low pH (gastric phase) fluids. This is important not only in environmental cleanup decisions where the oral ingestion pathway is considered the larger source of exposure, but also for bioavailability considerations of non-respirable airborne chemicals, since inhalable particulates are swallowed and absorbed in the gastro-intestinal tract (this was already mentioned by Dr. Neuwirth). In addition, cobalt is part of the human diet, with daily intakes of 20 – 40 µg Co/day. The current Cobalt Monograph could be misconstrued as indicating that these normal dietary intakes represent a risk to humans, when instead there is emerging evidence that cobalt is essential to the intestinal microbiome of humans (Chen et al. 2014, Shafquat et al. 2014).

Table 7-1. “Comparison of chemical and biological properties of cobalt metal and cobalt compounds”

We would also like to echo the previous public comment in requesting an amendment to table 7-1.

This table misrepresents the *in vivo* bioavailability of the poorly soluble (in all fluids) cobalt compounds, and it incorrectly groups two very different cobalt oxides (CoO and Co_3O_4) in one group. According to our knowledge and data on these compounds, CoO is moderately to highly bioaccessible in all biological fluids, and it meets the criteria for classification for the following endpoints: acute toxicity by inhalation Cat 2, acute oral toxicity Cat 3, and skin sensitization Cat 1. Co_3O_4 is not classifiable for any of these endpoints, and has a very low bioaccessibility in all biological fluids, as summarized below:

Summary of the bioelution behavior of the cobalt compounds reported in table 7-1, expressed as % release:

Gastric Fluid

Substance	Incubation duration	Cobalt % release
$CoSO_4$ heptahydrate	2 hours	99.7%
$CoCl_2$ hexahydrate	2 hours	86%
Co metal particle	2 hours	79%
Co monoxide	2 hours	55.2%
Tricobalt tetraoxide	2 hours	0%

Lysosomal Fluid

Substance	Incubation duration	Cobalt % release
$CoSO_4$ heptahydrate	2 hours	78.8%
$CoCl_2$ hexahydrate	2 hours	89.2%
Co metal particle	Average of 2 and 5 hours	92.4%
Co monoxide	Average of 2 and 5 hours	87.8%
Tricobalt tetraoxide	5 hours	1.5%

It is clear that Co_3O_4 is different in many aspects from the other compounds in that table, and we believe that it is unhelpful to present the two cobalt oxides in a shared column in table 7-1. We suggest that the two compounds need to be represented by a column each, as drafted below:

Endpoint	**$CoCl_2$**	**$CoSO_4$**	**Co metal particles**	**CoO**	**Co_3O_4**
<u>Bioaccessibility</u> Lysosome Gastric Cellular uptake Cytotoxicity ROS HIF1 stabilization DNA repair inhibition Genotoxicity in vitro Genotoxicity in vivo				 + + **?** + (CDI) + (CDI) **?** ND - (*) ND	 - - + (#) + (#) + (^) + (?) ND - (*) ND
Animal carcinogenicity Lung Other Injection site				 + ND +	 ND ND ND

(*) = based on Kirkland 2015
(#) = based on studies with sub-micron size particles
(^) = based on studies with nano particles
? = no data known to CDI, please complete if data known to NTP
(?) = please indicate the particle size, or source of data; we were unable to find the reference in the revised monograph
+ (CDI) = unpublished CDI data

Assessment of systemic cancers by comparison to the historic controls

Due to the lack of a dose-response in the systemic tumors observed in the cobalt metal inhalation study, the interpretation of these tumors relies heavily on a comparison with the historic controls. The cobalt exposed animals were compared as one group with the historic control database. In the document “National Toxicology Program Response to the Peer-Review Report” it is not explained that the historic control dataset is extremely limited for the rat strain used in the cobalt study. This particular strain (F344/NTac) was used in only four NTP cancer assays (TR 583, Bromodichloroacetic Acid, drinking water study; TR-587, Tetrabromobisphenol A, gavage study; TR-585 Green Tea Extract in F344/NTac Rats, gavage study; and cobalt metal), out of 590 NTP cancer bioassays in total. According to the NTP website (http://ntp.niehs.nih.gov/results/pubs/longterm/reports/longterm/index.html), the F344/NTac strain was used in only one inhalation cancer study, the study with cobalt metal.

It would be helpful to explain in the Response to the Peer-Review Report or in the revised Cobalt Monograph what the historic control database was (considering the few studies carried out at NTP with this strain; does the control database stem from Taconic labs data, or from NTP?), and why this strain was only used for such a limited number of studies, especially its one-time-only use in an inhalation study.

We thank you again for the possibility to make an oral comment on the revised Cobalt Monograph, with best regards,

Ruth Danzeisen
(on behalf of the Cobalt Development Institute)

References

Chen, Y., N. Qin, J. Guo, G. Qian, D. Fang, D. Shi, M. Xu, F. Yang, Z. He, J. D. Van Nostrand, T. Yuan, Y. Deng, J. Zhou, and L. Li. 2014. "Functional gene arrays-based analysis of fecal microbiomes in patients with liver cirrhosis." *BMC Genomics* 15:753. doi: 10.1186/1471-2164-15-753.

Shafquat, A., R. Joice, S. L. Simmons, and C. Huttenhower. 2014. "Functional and phylogenetic assembly of microbial communities in the human microbiome." *Trends Microbiol* 22 (5):261-6. doi: 10.1016/j.tim.2014.01.011.