Studies in Experimental Animals

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Office of the Report on Carcinogens

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Outline

Overview of studies in experimental animals

Study quality assessment

Cancer assessment
  – Inhalation tumors in lung and other sites
  – Injection site tumors
## Carcinogenicity Studies

<table>
<thead>
<tr>
<th>Cobalt form (# studies)</th>
<th>Species</th>
<th>Route of exposure</th>
<th>Reference (10 pub.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Water soluble</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cobalt sulfate heptahydrate (2)</td>
<td>Rat ♂♀</td>
<td>Inhalation</td>
<td>NTP 1998</td>
</tr>
<tr>
<td></td>
<td>Mouse ♂♀</td>
<td></td>
<td>NTP 1998</td>
</tr>
<tr>
<td>Cobalt chloride (1)</td>
<td>Rat ♂♀</td>
<td>Injection – SC</td>
<td>Shabaan et al. 1977</td>
</tr>
<tr>
<td></td>
<td>Rat ♂</td>
<td>Injection – IR</td>
<td></td>
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<tr>
<td></td>
<td>Rat ♂♀</td>
<td>Injection – IM</td>
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<tr>
<td></td>
<td>Rat ♂♀</td>
<td>Injection – IT</td>
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<tr>
<td></td>
<td>Rat ♂</td>
<td>Injection – SC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rat ♂♀</td>
<td>Injection – IM</td>
<td></td>
</tr>
<tr>
<td>Cobalt metal (6)</td>
<td>Rat ♂♀</td>
<td>Inhalation</td>
<td>NTP 2014</td>
</tr>
<tr>
<td></td>
<td>Mouse ♂♀</td>
<td>Inhalation</td>
<td>NTP 2014</td>
</tr>
<tr>
<td></td>
<td>Rat ♂</td>
<td>Injection – IR</td>
<td>Jasmin &amp; Riopelle 1976</td>
</tr>
<tr>
<td></td>
<td>Rat ♂♀</td>
<td>Injection – IM</td>
<td>Heath 1956</td>
</tr>
<tr>
<td></td>
<td>Rat ♂</td>
<td>Injection – IT</td>
<td>Heath &amp; Daniel 1962</td>
</tr>
<tr>
<td>Cobalt nanoparticles (1)</td>
<td>Rat ♂</td>
<td>Injection – IM</td>
<td>Hansen et al. 2006</td>
</tr>
<tr>
<td></td>
<td>Rat ♂♀</td>
<td>Injection – SC</td>
<td></td>
</tr>
<tr>
<td>Poorly water soluble</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cobalt(II) oxide (6)</td>
<td>Hamster ♂</td>
<td>Inhalation</td>
<td>Wehner et al. 1977</td>
</tr>
<tr>
<td></td>
<td>Rat ♂♀</td>
<td>Intratracheal instillation</td>
<td>Steinhoff &amp; Mohr 1991</td>
</tr>
<tr>
<td></td>
<td>Rat ♂♀</td>
<td>Injection – IP</td>
<td>Steinhoff &amp; Mohr 1991</td>
</tr>
<tr>
<td></td>
<td>Rat ♂</td>
<td>Injection – SC</td>
<td>Steinhoff &amp; Mohr 1991</td>
</tr>
<tr>
<td></td>
<td>Rat ♂♀</td>
<td>Injection – IM</td>
<td>Gilman &amp; Ruckerbauer 1962</td>
</tr>
<tr>
<td></td>
<td>Mouse ♂♀</td>
<td>Injection – IM</td>
<td>Gilman &amp; Ruckerbauer 1962</td>
</tr>
<tr>
<td>Cobalt sulfide (1)</td>
<td>Rat ♂♀</td>
<td>Injection – IR</td>
<td>Jasmin &amp; Riopelle 1976</td>
</tr>
</tbody>
</table>

IM = intramuscular, IP = intraperitoneal, IR = intrarenal, IT = intrathoracic, SC = subcutaneous.
Carcinogenicity Study Quality Assessment

Study design/population

Exposure conditions

Outcome assessment and measurement

Confounding

Analysis and reporting

Sensitivity

Overall study utility: high, moderate, low, inadequate

Responses for questions:
- Low/minimal concern
- Some concern
- Major concern
- Critical concern
- No information
## Overall study utility

<table>
<thead>
<tr>
<th>Overall Utility</th>
<th>Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>NTP 1998 (rat and mouse)</td>
</tr>
<tr>
<td></td>
<td>NTP 2014 (rat and mouse)</td>
</tr>
<tr>
<td>Moderate</td>
<td>Hansen <em>et al.</em> 2006</td>
</tr>
<tr>
<td></td>
<td>Steinhoff &amp; Mohr 1991 (3 routes)</td>
</tr>
<tr>
<td></td>
<td>Wehner <em>et al.</em> 1977</td>
</tr>
<tr>
<td>Low</td>
<td>Gilman &amp; Ruckerbauer 1962 (rat and mouse)</td>
</tr>
<tr>
<td></td>
<td>Heath 1956</td>
</tr>
<tr>
<td></td>
<td>Heath &amp; Daniel 1962</td>
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<td></td>
<td>Jasmin &amp; Riopelle 1976</td>
</tr>
<tr>
<td></td>
<td>Shabaan <em>et al.</em> 1977</td>
</tr>
</tbody>
</table>

- All carcinogenicity studies were considered to have some utility for cancer hazard evaluation

- High utility: NTP studies were most informative
  - Sufficient number of animals
  - Both sexes
  - Lifetime treatment
  - Three dose levels
  - Untreated controls

- In general, major limitation in studies with low or moderate utility was low sensitivity
  - Due to, e.g., short duration, single dose, few animals
  - Little concern that duration or single dose would decrease confidence in a positive finding
Different types of cobalt compounds caused lung neoplasms after exposure in rodents

<table>
<thead>
<tr>
<th>Cobalt form</th>
<th>RAT</th>
<th>MOUSE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Water soluble (CoSO$_4$$ \cdot $7H$_2$O)</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Metal</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Poorly water soluble (CoO)</td>
<td>✔</td>
<td>✔</td>
</tr>
</tbody>
</table>

NT = not tested.

Source: Behl et al. 2014
Cobalt induces alveolar/bronchiolar adenoma or carcinoma

Inhalation Exposure

**CKE** = Cystic keratinizing epithelioma.

**RAT**
- Cobalt sulfate-7H₂O
- Cobalt metal
  - CKE ♀
  - Hyperplasia ♂♀
  - Inflammation ♂♀

**MOUSE**
- Cobalt sulfate-7H₂O
- Cobalt metal
  - Hyperplasia ♂♀
  - Inflammation ♂♀

Individual comparisons: *P < 0.05  ***P < 0.001.
Trend-test P-value < 0.001 for all except cobalt sulfate in male rats (P = 0.032).
Intratracheal/Inhalation Exposure

Cobalt(II) oxide

• Steinhoff & Mohr 1991
  – Intratracheal instillation treatment
    • Male and female rats
    • 0, 2, or 10 mg/kg bw
  – Significant increases in lung neoplasms (alveolar/bronchiolar adenoma or carcinoma) in male rats; non-significant increases were observed in females

• Wehner et al. 1977
  – Inhalation treatment
    • Male hamsters
    • 10 mg/m³
  – No lung tumors
  – Pneumoconiosis, as evidenced by a variety of lesions
  – Poor survival (thus limited sensitivity)
  – Hamsters less sensitive model (than rats or mice) for detecting lung tumors
Inhalation exposure to cobalt metal caused significant increases in tumor incidence in rat tissues other than lung.

<table>
<thead>
<tr>
<th>Tissue (Neoplasm)</th>
<th>Findings (Increased incidence)</th>
</tr>
</thead>
</table>
| **Pancreas**     | Male rat: significant increase for top two doses; $P_{trend} = 0.002$  
| (Islet-cell adenoma or carcinoma, combined)  | Female rat: findings equivocal (non-significant increase) |
| **Hematopoietic system** | Female rat: significant increases in all exposed groups; increased incidence exceeded historical control for all routes |
| (Mononuclear-cell leukemia, MCL) | |
| **Kidney**     | Male rat: findings equivocal (non-significant increase); rare tumor; $P_{trend} = 0.023$ (standard and extended evaluation) |
| (Renal tubule adenoma or carcinoma, combined) | |

Source: NTP 2014
Inhalation Exposure: Other Site

Cobalt also induced adrenal gland neoplasms: Benign and malignant pheochromocytoma

• Cobalt metal (NTP 2014)
  – Male and female rats
    • Significant increases in tumor incidence for top two doses, $P < 0.001$
    • $P_{\text{trend}} < 0.001$

• Cobalt sulfate heptahydrate (NTP 1998)
  – Female rats
    • Significant increase in tumor incidence for top dose, $P < 0.001$
    • $P_{\text{trend}} < 0.001$
  – Male rats
    • For mid-dose exposure, incidence significantly greater ($P < 0.05$) than chamber control and exceeded historical control range

It is unclear whether this is a direct systemic effect of cobalt exposure or an indirect secondary response to lung damage (e.g., chronic inflammation).
Significant increase in tumor incidence at injection site

### Injection Site Tumors in Rats

- **Relevance to humans (e.g., hip replacement)**
- **Injection study results provide supporting evidence of carcinogenicity of cobalt**
  - Consistency of tumor types
  - Similar findings for different forms of cobalt
  - Evidence that tumors are due to carcinogenic properties of cobalt and not just a reaction to physical implant (Hansen *et al.* 2006)
    - Cobalt metal (nanoparticle) and nickel implants induced tumors in rats
    - Titanium dioxide, silicon dioxide, and PVC implants did not induce tumors, although they have the same physical characteristics (i.e., surface to volume ratio)

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**Table: **

<table>
<thead>
<tr>
<th>Injection site tumors</th>
<th>Form of Cobalt</th>
<th>Water soluble</th>
<th>Metal</th>
<th>Poorly water soluble</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sarcoma (all types)</td>
<td>SC</td>
<td>IM</td>
<td>IM, SC, IP</td>
<td></td>
</tr>
<tr>
<td>Histiocytoma</td>
<td></td>
<td>IM, IT</td>
<td>SC, IP</td>
<td></td>
</tr>
</tbody>
</table>

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**Form of Cobalt:**
- Water soluble: CoCl₂
- Metal: Co metal/nano
- Poorly water soluble: CoO
Exposure to cobalt causes carcinogenic effects in experimental animals

<table>
<thead>
<tr>
<th>Site</th>
<th>Water soluble (CoSO₄•7H₂O; CoCl₂)</th>
<th>Cobalt metal</th>
<th>Poorly water soluble (CoO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>Rat ♂♀ Mouse ♂♀</td>
<td>Rat ♂♀</td>
<td>Rat ♂</td>
</tr>
<tr>
<td>Pancreas</td>
<td></td>
<td>Rat ♂♀</td>
<td></td>
</tr>
<tr>
<td>MCL</td>
<td></td>
<td>Rat ♂</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td>Rat ♂</td>
<td></td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>Rat ♂</td>
<td>Rat ♂♀</td>
<td></td>
</tr>
<tr>
<td>Injection site</td>
<td>Rat ♂</td>
<td>Rat ♂♀</td>
<td></td>
</tr>
</tbody>
</table>

( ) = results equivocal
MCL = mononuclear-cell leukemia
Approach for Review

Defer vote on carcinogenicity of cobalt in experimental animals

Evidence from studies of individual cobalt compounds in experimental animals

Mechanistic and other data relevant for considering as a class

Vote on preliminary level of evidence for the class of Cobalt and Certain Cobalt Compounds
Clarifications?
Reviewer questions

• Comment on whether the scientific information from cancer studies in experimental animals for cobalt and certain cobalt compounds is clear, technically correct, and objectively presented.
  – Identify any information that should be added or deleted.

• Comment on whether the approach and assessment of the utility of the animal carcinogenicity studies (study quality and sensitivity) for informing the cancer evaluation is systematic, transparent, objective, and clearly presented (Appendix D, Sections 5.1.2 and 5.2.2).

• Provide any scientific criticisms of NTP’s cancer assessment of the experimental animal studies of exposure to cobalt and certain cobalt compounds and how findings from the scientific evidence across studies were synthesized.
Evaluation on study quality and sensitivity

Quality

- Population of study animals
  - Controls (concurrent, historical)
  - Randomization of dosing groups
- Quality of exposure
  - Chemical purity
  - Dosing regimen
  - Survival, body weight changes
- Quality of endpoint assessment
  - Pathology (necropsy, histology, diagnosis
  - Potential for confounding
- Analysis and reporting
  - Statistical analysis
  - Combination of tumor types

Sensitivity

- Animal model
  - Source
  - Species
  - Strain
  - Sex
- Statistical power
  - number of animals in control or test groups
  - Survival effects (high mortality)
- Study duration
  - Adequate to detect effect, at least one year but better near lifespan of 2 years
Carcinogenicity study quality assessment

Evaluation of studies for overall utility

• Assessment
  – Potential for bias (limitations)
  – Sensitivity

• Ratings
  – High
    • Low/minimal concerns for most potential bias
  – Moderate
    • Low/minimal concern or some concerns for most potential bias
  – Low
    • Major concerns for several potential biases
  – Inadequate
    • Critical concern for some potential bias