Establishing an adequate margin of protection for non-genotoxic carcinogens

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Disclosure Statement

- Member of several science advisory boards (public and private sector) [non-remunerated]
- Member/chair of several national and international scientific advisory committees
- I have no financial interests in the subject matter of the meeting
Health protection goals

- The Acceptable Daily Intake (ADI)/Tolerable Daily Intake is an estimate of the amount of a chemical, expressed on a body weight basis, to which an individual can be exposed daily over a lifetime without appreciable health risk. It is derived on the basis of all the known facts at the time of the evaluation.
- It is intended to cover all durations of exposure greater than acute (over 24 h or less) and all life-stages
- It is based on the most sensitive, relevant adverse effect observed in studies of the chemical
**Hazard and Risk Assessment**

Reference value (e.g. ADI) \[\text{[RV]} = \text{POD}/\text{UF}\]

**Hazard ID**
**Hazard characterisation**

**Uncertainty factor**

**Exposure assessment**

**Risk characterisation**

**Exposure of toxicity**

**Risk**
What is the NOAEL

- “The NOAEL is the highest level of a test substance that does not cause any observed and statistically significant adverse effects compared with the controls.” (OECD, 2012)
- “…it is assumed that a NOAEL is an estimate of that threshold, and is associated with zero risk” (Braakhuis et al, 2018)
Quantitative Adverse Outcome Pathway (AOP)

Dose-MIE (KE1)

Exposure

ADME/TK → KE1 → KE2 → KE3 → AO

Adverse Outcome Pathway
Risk Assessment: MOA for Renal Carcinogenicity

Is the weight of evidence sufficient to establish a mode of action (MOA)?

- GSH- and downstream metabolites found in urine
- Accumulation of radiolabel in kidney; inhibition by probenecid
- Evidence from studies with analogous compounds
- Consistent time-and dose-dependent histopathological and clinical chemical evidence for toxicity
- Proliferation observed within 7 days; dose and time-dependent hyperplasia

Is the weight of evidence sufficient to establish a mode of action (MOA)?

- GSH conjugation and further metabolism to Cys conjugates
- Active uptake of Cys conjugate by proximal convoluted tubule
- Metabolism to thiols by C-S lyase
- Renal cytotoxicity
- Renal cell regenerative proliferation

Chlorothalonil

- Classify as a carcinogen?
- Manage risk of renal toxicity?

Increased renal adenoma and carcinoma

- YES
Nephrotoxicity of Chlorothalonil in Rats

- **28-day study**: Increased renal weight, LOAEL 80 mg/kg bw

- **90-day study**: Increased BUN (NOAEL 40 mg/kg bw), increased renal weight (LOAEL 40 mg/kg bw), renal hyperplasia (LOAEL 40 mg/kg bw), karyomegaly in kidneys (LOAEL 40 mg/kg bw)

- **90-day study**: increased renal weight (NOAEL 1.5 mg/kg bw), hyperplasia of the epithelium of proximal convoluted tubules (NOAEL 10 mg/kg bw)

- **2-year study**: Increased BUN and serum creatinine (NOAEL 3.8 mg/kg bw), increased renal weight (NOAEL 3.8 mg/kg bw), hyperplasia of the epithelium of proximal convoluted tubules (NOAEL 1.8 mg/kg bw)
## Carcinogenicity of Chlorothalonil in Rats

### Renal adenomas + carcinomas

<table>
<thead>
<tr>
<th>Dose (mg/kg bw per day)</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>116 week study</strong></td>
<td></td>
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<tr>
<td>0 (control)</td>
<td>0/60</td>
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</tr>
<tr>
<td>40</td>
<td>7/60</td>
<td>3/60</td>
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<tr>
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<td>7/60</td>
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<tr>
<td>175</td>
<td>19/60</td>
<td>23/60</td>
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<td><strong>2-year study</strong></td>
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<tr>
<td>0 (control)</td>
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<td>1.8</td>
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<tr>
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<td>1/54</td>
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<tr>
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<td>4/54</td>
<td>0/53</td>
</tr>
<tr>
<td>175</td>
<td>23/55</td>
<td>32/55</td>
</tr>
</tbody>
</table>

**Overall NOAEL:** 3.8 mg/kg bw per day
Hazard Characterisation of Chlorothalonil

- JMPR concluded that it is unlikely that chlorothalonil is genotoxic.
- JMPR concluded that the formation of kidney tumours was the result of prolonged renal cytotoxicity and regenerative cell proliferation, and is consistent with a threshold phenomenon.
- JMPR established an ADI for chlorothalonil of 0-0.02 mg/kg bw based on a NOAEL of 1.8 mg/kg bw per day identified on the basis of kidney toxicity observed in long-term studies of toxicity in rats and using a safety factor of 100.
- This ADI provides a margin of 200 for the induction of renal tumours in rats (NOAEL 3.8 mg/kg bw per day).
- The Meeting concluded that, based on the MOA, while it is plausible that humans are less sensitive to the renal effects of chlorothalonil, it was not possible to dismiss relevance to humans on quantitative grounds, nor was it possible to quantify any difference in sensitivity.
- However, given the species differences in the β-lyase bioactivation pathway, the ADI is likely to be conservative.
Comparison of NOAELs and BMDLs for non-genotoxic carcinogens

Braakhuis et al, 2018
Is the weight of evidence sufficient to establish a mode of action (MOA)?

**YES**

Cadmium:
MOA for nephrotoxicity

- **Thiol group inactivation**
- **Cytotoxicity**
- **Proximal tubular damage**
- **Low mol wt proteinuria (e.g. B2M)**
- **Accumulation in kidney**
- **Cadmium**
- **Nephrotoxicity**
Renal carcinogenicity of cadmium

Waalkes et al, 1999

Noble (NBL/Cr) rats received CdCl$_2$ in drinking water for 102 weeks

Renal tumours (%)
Renal hyperplasia (%)
Severity of hyperplasia (score x 10)

N ~ 29

p = 0.01 (trend test)

p < 0.001 (trend test)

p < 0.0001 (trend test)
CSAF for interindividual variability in TK for Cd$^{2+}$

$CSAF = \frac{95\text{th Percentile (BMD)}}{\text{Median (BMD)}} = 3.9 \quad \rightarrow \quad \text{TWI} = 2.5 \text{ Cd } \mu g/kg \text{ bw}$

From EFSA, 2009/11
Derivation of reference values

**Toxic effect**

**NOAEL/BMDL**

**Test species**

**Response**

**Dose**

**Sensitive human**

**Average human**

**RV**

**UF**

**NOAEL/BMDL**

**0**

**0.1**

**1**

**10**

**100**

**1000**
Cancer is often secondary to primary toxicity, i.e. through a non-genotoxic mode of action

In such cases, early key events are reversible and will occur some time before the development of a carcinogenic response, at lower doses

Where possible, risk assessment should be based on mechanistic considerations rather than statistically-based defaults

Hence a risk-based approach, in which health-based protection is based on the most sensitive relevant endpoint, will be more than adequate to prevent cancer risk from a non-genotoxic mode of action