Introduction

Pharmaceutical companies regularly generate and receive requests for assessment of potential hazards of drugs and intermediates. It is a part of many regulations for pharmaceutical development and good manufacturing practices designed to protect workers from occupational exposure.

Trans-1,4-dibromobut-2-ene (Fig. 1) is a commercially available pharmaceutical intermediate (IM). It is registered by ECHA. Under REACH/GHS regulations pharmaceutical IMs are considered equal to other industrial chemicals. For IMs, health hazard assessment has been traditionally done by animal experiments and laboratory tests. This is increasingly replaced by alternative methods considering animal welfare and resources. It would be especially attractive, if some of the health endpoints could be predicted by grouping and read-across. Since IMs are usually chemically reactive manufacturing components, they may pose a pronounced occupational risk for manufacturing workers.

Physico-Chemical Properties

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Target</th>
<th>Surrogate</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical</td>
<td>Trans-1,4-dibromobut-2-ene</td>
<td>Trans-1,4-dichlorobut-2-ene</td>
<td>(1)</td>
</tr>
<tr>
<td>Group</td>
<td>Alkylating agent</td>
<td>Alkylating agent</td>
<td>(2)</td>
</tr>
<tr>
<td>Alkylating activity (by S&lt;sub&gt;N&lt;/sub&gt;-1 reactivity)</td>
<td>54</td>
<td>0.29</td>
<td>(2)</td>
</tr>
<tr>
<td>Leaving group potency</td>
<td>Br » Cl</td>
<td>Cl » Br</td>
<td>(2)</td>
</tr>
<tr>
<td>Molecular Formula</td>
<td>C&lt;sub&gt;4&lt;/sub&gt;H&lt;sub&gt;6&lt;/sub&gt;Br&lt;sub&gt;2&lt;/sub&gt;</td>
<td>C&lt;sub&gt;4&lt;/sub&gt;H&lt;sub&gt;6&lt;/sub&gt;Cl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>(1)</td>
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<tr>
<td>Molecular weight</td>
<td>212.9</td>
<td>125</td>
<td>(1)</td>
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<tr>
<td>CAS Number</td>
<td>821-06-7</td>
<td>110-57-6</td>
<td>(1)</td>
</tr>
<tr>
<td>EINECS Number</td>
<td>212-472-7</td>
<td>203-779-7</td>
<td>(1)</td>
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<tr>
<td>Description</td>
<td>White to light yellow crystal powder</td>
<td>Colorless liquid with a distinct odor</td>
<td>(1)</td>
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<tr>
<td>REACH</td>
<td>Registered as intermediate</td>
<td>Registered as intermediate</td>
<td>(1)</td>
</tr>
</tbody>
</table>

Results From Acute Toxicity, QSAR and Genotoxicity

- Both, trans-1,4-dibromobut-2-ene and trans-1,4-dichlorobut-2-ene are corrosive to skin.
- They may cause immediate irritation to the skin with a low density of 3.93 mg/ml.
- Air of surrogate leads to degenerative organ changes by inhalation and should be considered by read-across for the target.
- QSAR studies confirmed the likely mutagenicity attributed to Br and Cl alkylating groups.
- Both, target and surrogate are mutagens (but not clastogen as demonstrated in the surrogate).
- Mutagenic activity has been shown to be greater in trans-1,4-dichlorobut-2-ene, probably because of the higher alkylating activity of the Br leaving group. The mechanism is attributed to nucleophilic substitution (S<sub>N</sub>-1 reactivity).

Results From Carcinogenicity Studies

- Rat carcinogenicity studies were conducted by inhalation of the (corrosive) surrogate trans-1,4-dichlorobut-2-ene.
- Incidence of benign nasal tumors were increased in all dose groups, incidence of malignant nasal tumors only in the highest dose group. Mortality was greater in the highest dose group.
- Whereas all rats were necropsied, histopathology was only done with respiratory tract, cervical lymphnodes and brain tissues. Only nasal tumors were classified.
- Rats are known for their susceptibility to nasal tumors.
- In the mouse study with different routes of administration, there were no tumors, except for some injection site sarcomas.
- Following the precautionary principle, carcinogenicity of the surrogate and the target are suspected by read-across.

Read-across Results

The two genotoxic alkylating agents analysed in this read-across study appear structurally very similar. Consequently, read-across and classification for possible carcinogenicity of the target appears adequate. However, it cannot be argued definitely, if the observed nasal tumors were triggered by the mutagenicity or by the corrosivity of the surrogate or by rat-specific susceptibility of mucous membranes in the airways.

Open Questions

- Are genotoxic alkylating agents a separate group/category of chemicals with similar properties (S<sub>N</sub>-1 reactivity) for the purpose of read-across (Searle-1) (6)
- Why is the target agent classified as H350: May cause cancer (read-across), whereas the surrogate agent trans-1,4-dichlorobut-2-ene is not classified for carcinogenicity, although the carcinogenicity studies have been conducted with the surrogate? Does the higher leaving group potency of Br have an impact?

References

4. Van Duuren BL, Goldschmidt BM and Seidman I: Cancer Res 35, pp. 2553-2557, 1975