In Silico and Read-Across Mutagenicity and Carcinogenicity Assessment to Close Data Gaps for the Pharmaceutical Intermediate Trans-1,4-dibromobut-2-ene

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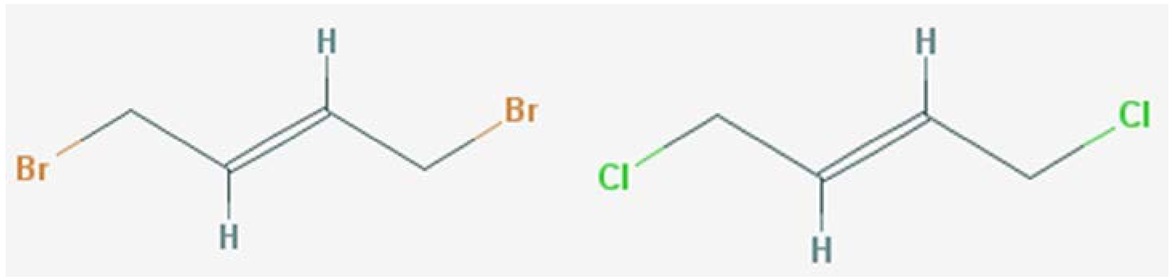

Figure 1. Structures of the target (left) and surrogate (right)

Physico-Chemical Properties			
	Target	**Surrogate**	**Ref.**
Chemical	Trans-1,4-dibromobut-2-ene	Trans-1,4-dichlorobut-2-ene	(1)
Group	**Alkylating agent**	Alkylating agent	(2)
Alkylating activity (by S_N-1 reactivity)	54	0.29	(2)
Leaving group potency	Br >> Cl	Cl << Br	(2)
Molecular Formula	C4H6Br2	C4H6Cl2	(1)
Molecular weight	213.9	125	(1)
CAS Number **EINECS Number**	821-06-7 212-472-7	110-57-6 203-779-7	(1)
Description	White to light yellow crystal **powder**	Colorless **liquid** with a distinct odor	(1)
REACH	Registered as intermediate	Registered as intermediate	(1)

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.

Acute Toxicity				
	Species	**Target**	**Surrogate**	**Ref.**
Acute oral	Rat, male	75 mg/kg **GHS: H301**	414 mg/kg **GHS: H301**	(1)
Acute inhalation	Rat	Read-across **GHS: H330**	LC = 3.93 mg/L air, degenerative changes in liver and other organs **GHS: H331**	(1)
Skin irritation	Rabbit	Irritant, corrosive **GHS: H314**	Corrosive **GHS: H314**	(1)
LLNA (GLP)	Mouse	Skin sensitization **GHS: H317**	n.a.	(1)

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
- A relatively low dose of 3.93 mg/L air of surragate 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-dibromobut-2-ene, probably because of the higher alkylating activity of the Br leaving group. The mechanism is attributed to nucleophilic substitution (S_N-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 there 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

QSAR and Genotoxicity				
	Strains/Species	**Target**	**Surrogate**	Ref.
DEREK Nexus 4.1.0 (knowledge based) **Sarah Nexus 1.2.0 (statistical)** **Lhasa Ltd. Leeds UK**	QSAR *In silico*	DEREK: plausible (3) Sarah: positive (1)	DEREK: plausible (3) Sarah: positive (4)	NVS report Dec-2015
In vitro **Ames test**	TA 100 without S9	P: 700 revertants/µmol N: 0 revertants/µmol	P: 9 revertants/µmol N: 1 revertants/µmol	(2)
In vitro **Ames test**	TA1535,1537, 1538 TA100 and 98 without S9	n.a. positive positive	Negative n.a. n.a.	(1)
In vitro **Ames test**	Saccharomyces cerevisiae D4 With S9	n.a.	+/- positive	(1)
SLRL (point mutations on X chrom,)	Drosophila melanogaster	Positive	n.a.	(3)
In vivo **Sister chromatid exchange assay**	Rat, male, i.p. administration of 40 mg/kg	n.a.	(positive) weak mutagen	(1)
In vivo **Micronucleus test**	Rat, by inhalation of 0.1, 1.0 or 10 ppm	n.a.	Not clastogenic nor aneugenic	(3)

Carcinogenicity				
	Species, route	**Target**	**Surrogate**	Ref.
Carcinogenicity study **(duration 537 days)**	Mouse ICR/HA Swiss, female **Skin**, 3 times/week, 1 mg in 0.1 ml acetone **s.c.**, weekly, 0.05 mg in 0.05 ml tricaprylin **i.p.**, weekly, 0.05 mg in 0.05 ml tricaprylin	n.a.	No tumors at admin. site 3/30 with local sarcoma 2/30 with local sarcoma No increased incidence of any other tumors.	(4)
Carcinogenicity study (duration 24 months for 0.5 ppm; 7 months at 5 ppm and 5 months at 2.5 ppm followed by 12 months recovery)	CD®Rats, 140 males and 140 females, 6 hr/d, 5 d/week by inhalation Histopathology of respiratory tract, cervical lymphnodes and brain.	n.a.	0.5 ppm: Malignant nasal tumors in m↑, benign tumors in m and f ↑ 5/2.5 ppm: Mortality m and f ↑ Malignant nasal tumors↑	(5)
Carcinogenicity study **(duration 24 months, 19 months treatment)**	CD®Rats, 128-160 males per dose group of 0, 0.1, 0.3 or 1.0 ppm, 6 hr/d, 5 d/week by inhalation,	n.a. **GHS:** H350: May cause cancer	Benign nasal tumors ↑ in all dose groups, malignant nasal tumors↑ in 1.0 ppm group. Histopath. of respiratory tract, cervical lymphnodes, brain. **GHS**: none (data lacking)	(3)

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_N-1 reactivity) for the purpose of read-across (Seurat-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

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