Exclusive genotoxicity data for glycidol, to be used for cancer risk estimation

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Background

A preferable quantitative approach for genotoxic dose-response relationships is to measure the internal dose (area under the time-concentration curve, AUC) causing genotoxicity. This would give accurate extrapolations between biological systems and comparisons of chemicals, and also more reliable cancer risk estimations. We have developed an approach for cancer risk estimation, based on a relative cancer risk model, AUC of the genotoxic agent, and relative genotoxic potency.

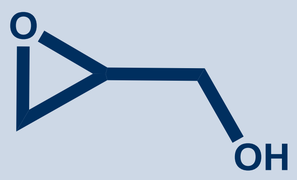


Figure 1. *Glycidol*

The model compound, glycidol (**Figure 1**), is a genotoxic compound of concern as it may end up in the diet. It is classified by IARC as probably carcinogenic to humans (group 2A) [1].

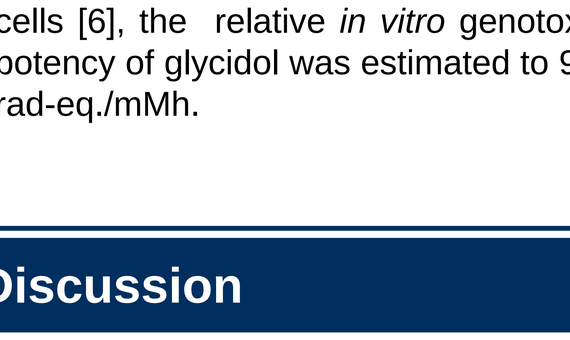
Aim

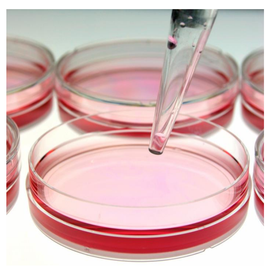
- To generate genotoxicity data for glycidol in both *in vitro* and *in vivo* systems.
- Measure the internal doses of glycidol in the experimental systems used.
- Generate relative genotoxic potencies by comparing the genotoxic effect per dose unit of glycidol to γ- and β-radiation (standard).

In vitro mutagenicity methods

HPRT mutation assay [2]

- Chinese hamster ovary cells were treated with glycidol for 1 h.
- Mutated cells were selected using the toxic guanine equivalent 6-thioguanine.
- The mutation frequency was estimated after staining of mutated clones with methylene blue.





AUC *in vitro* from Cbl(I)adducts

Glycidol stability in the cell exposure medium was measured during 4 h as cob(I)alamin (Cbl(I)) adducts [3] (**Figure 2**).

Figure 2. *Glycidol stability, measured as cob(I)alamin adducts (alkylcobalamin).*

Genotoxic dose-response in cells by glycidol

AUC of glycidol in the cell exposure medium

- No significant disappearance of glycidol was observed in the medium, observed from Gly-Cbl-adduct measurements. Thus, the AUC of glycidol (mM x h) in the medium during treatment is the initial concentrations times the cell treatment time (1 h).

Mutagenic potency by glycidol

- Glycidol induced mutations of the *hprt*-gene in Chinese hamster ovary cells, observed as a linear dose-dependent response (**Figure 4**).
- Using γ-radiation as a standard in the cells [6], the relative *in vitro* genotoxic potency of glycidol was estimated to 9.5 rad-eq./mMh.

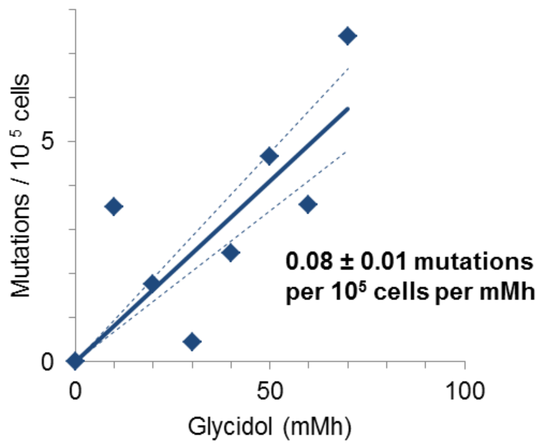


Figure 4. *Induced mutation frequency per 100 000 cells, following exposure to glycidol.*

Short-term *in vivo* genotoxicity methods

Micronucleus assay

- Male BalbC mice were administered glycidol intraperitoneally (0, 30, 60, 90, 120 mg/kg).
- Peripheral blood was taken from the orbital plexus, 45 h after dosing.
- The frequency of micronucleated young erythrocytes (polychromatic erytrocytes, PCE), fMNPCE, was monitored with a flow cytometer equipped with dual lasers [4].

$$AUC = \frac{A}{k_{val}}$$

Equation 1. *AUC estimation from adduct level (A) and in vitro reaction rate constant (k_{val}).*

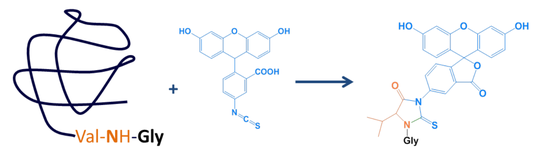


Figure 3. *Glycidol adducts to the N-terminal valines in Hb are deriviatized and detached by FITC, forming Gly-FTH.*

AUC *in vivo* from Hb adducts

- Each blood sample was derivitized with fluoroisothiocyanate (FITC) and cleaned up on SPE columns before analysis of glycidol-fluorescein-thiohydantoins (Gly-FTH) with LC-MS/MS, according to the adduct FI*R*E procedure [5] (**Figure 3**).
- The AUC was estimated from the adduct level (A) and the second order reaction rate constant, k_{val}, obtained from incubations of glycidol in blood (**Equation 1**).

Genotoxic dose-response in mice by glycidol

Increased micronucleus frequency

- Chromosomal damage was induced by glycidol, observed as a dose-dependent increase in the fMNPCE (**Figure 5, Table 1**).
- Using γ- and β-radiation as a standard in mice [4], the relative *in vivo* genotoxic potency of glycidol was estimated to 10 rad-eq./mMh.

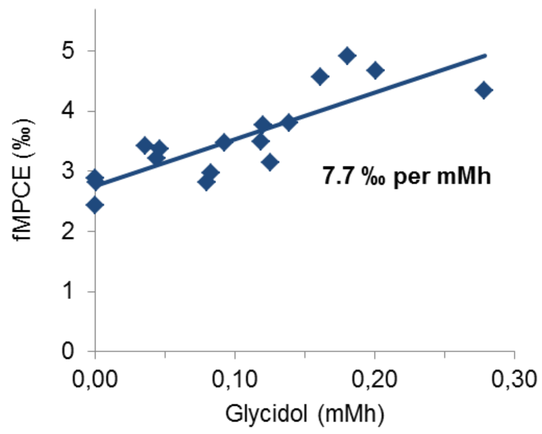


Figure 5. *Induced frequency of micronucleated polychromatic erythrocytes, following exposure to glycidol in mice.*

Administered dose (mg/kg)	fMPCE[b] ± SD (‰)	Gly-Val-FTH ± SD (nmol/g Hb)	AUC[c] ± SD (mMh)
Vehicle	2.71 ± 0.24	0.00 ± 0.00	0.00 ± 0.00
30	3.34 ± 0.10	0.54 ± 0.07	0.04 ± 0.01
60	3.09 ± 0.34	1.09 ± 0.09	0.09 ± 0.01
90	3.69 ± 0.17	1.60 ± 0.14	0.13 ± 0.01
120	4.33 ± 0.69	2.41 ± 0.73	0.19 ± 0.06

Table 1. *Levels of fMNPCE, Hb adducts and estimated AUC, induced by glycidol in mice.0*

Discussion

The realtive genotoxic potencies of glycidol are exclusive data that will be used in an approach for cancer risk estimation using a relative cancer risk model, where estimated cancer risks from prestented results are compared to real cancer incidences [7].

Our group has previously successfully evaluated the relative cancer risk model for a few model compounds, e.g. ethylene oxide, butadiene and acrylamide [7, 8, 9].

We believe that the experimental designs presented here, are attractive alternatives to long-term cancer tests. They fulfil the 3R principles; replacement and/or reduction of animal use, and refinement of the risk assessment as a result of inclusion of AUC and a standard (radiation).

Conclusions

- The genotoxic effect of glycidol has been quantified both *in vitro (*cells) and *in vivo* (mice).
- A relative genotoxic potency of glycidol of ca 10 rad-eq./mMh was obtained in both cells and in mice.
- The presented study designs are in line with the 3R principles, and are also cost- and time efficient.

Acknowledgement

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References: **[1]** IARC vol. 77:469-486 (2000), **[2]** Jenssen *et al.*, Handbook of Mutagenicity Test Procedures, Elsevier, Amsterdam, pp. 269-290 (1984), **[3]** Haglund *et al*., Toxicol. Environ. Chem. 85:81-94 (2003), **[4]** Abramsson-Zetterberg *et al.*, Int. J. Radiat. Biol. 67:29-36 (1995), **[5]** von Stedingk *et al.*, J. Chromatogr. B. Analyt Technol. Biomed. Life. Sci. 878:2483-2490 (2010), **[6]** Silvari *et al.*, Mutat. Res. 580:91-101 (2005), **[7]** Granath *et al*., Risk Anal. 19:309-320 (1990), **[8]** Fred et al., Cancer Res 68:8014-8021, **[9]** Törnqvist et al., J. Agric Food Chem 56:6004-6012

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