# Quantification of hemoglobin adducts as a measure of exposure dose in an *in vivo* genotoxicity study implies reliability in risk assessment

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## Background

The model compound, glycidol (**Figure 1**), is a genotoxic compound, which is classified by IARC as probably carcinogenic to humans (group 2A)[1]. It is of concern for human exposures, as glycidyl fatty acid esters has shown to be present in e.g. cooking oils[2].

Here we present an approach for the determination of the genotoxic potency of glycidol in mice, by monitoring of micronuclei and measurement of the internal doses (AUC, area under the time-concentration curve) from Hb adducts.

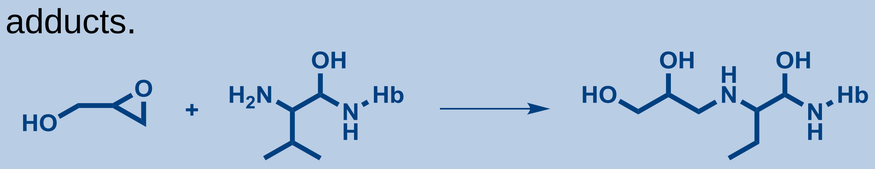



***Figure 1.*** *Glycidol forms adducts at the N-terminal valine in hemoglobin (Hb).*

## *In vivo* micronucleus test & Hb adduct measurement

- 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 erythrocytes, PCE), fMPCE, was monitored with a flow cytometer equipped with dual lasers[3].
- 180000 cells were counted per sample.

- Blood samples were handled according to the adduct FI*R*E procedure[4] (**Figure 2**).

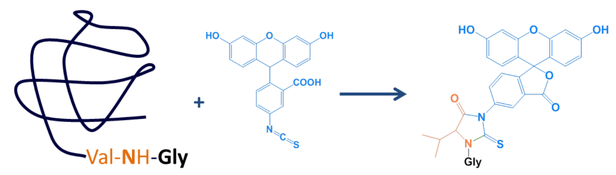



***Figure 2.*** *Glycidol adducts to the N-terminal valines in Hb are derivatized and detached by fluorescein isothiocyanate (FITC), forming Gly-Val-FTH, which is quantified by LC-MS/MS.*

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

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

## 1. Glycidol formes Hb adducts in mice

- Glycidol showed a dose-dependent formation of adducts to the N-terminal valine in Hb in mice (**Figure 3**).
- The slope of the linear fit is equivalent to the formation of the number of moles of adduct per gram Hb per administered dose.

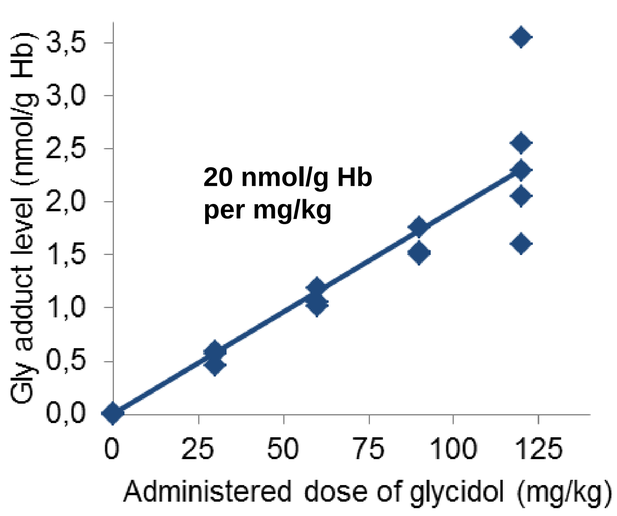



***Figure 3.*** *Hb adduct levels by glycidol in intraperitoneally dosed mice.*

## 2. AUC of glycidol in mice

- Calculation of the AUC for glycidol (**Equation 1**) requires knowledge about the second-order reaction rate constant, $k_{val}$.
- The rate constant, $k_{val}$, was obtained from incubations of glycidol in mouse blood and corresponds to the linear fit in **Figure 4**.

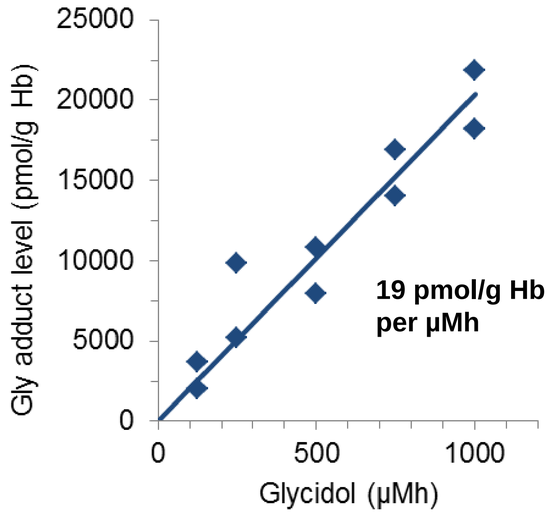
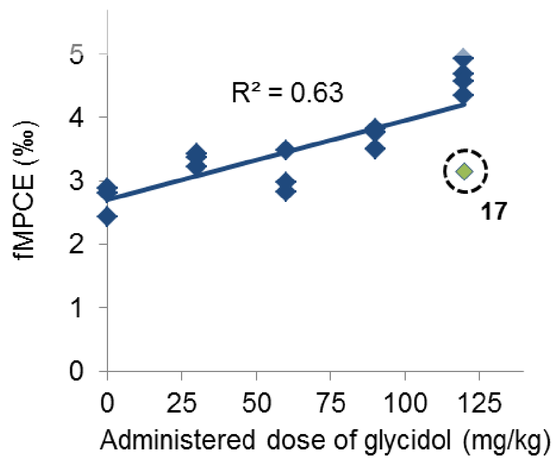



***Figure 4.*** *Hb adduct levels by glycidol from incubations in blood from mice.*

## 3. Glycidol induces micronuclei in mice

- Chromosomal damage was induced by glycidol, observed as a dose-dependent increase in the fMPCE (**Figure 5**).
- The mouse no. 17 showed a lower induced level of micronuclei compared to the other mice in the same dose group (**Figure 5a**). This could be explained by a lower AUC of glycidol in this mouse (**Figure 5b**).



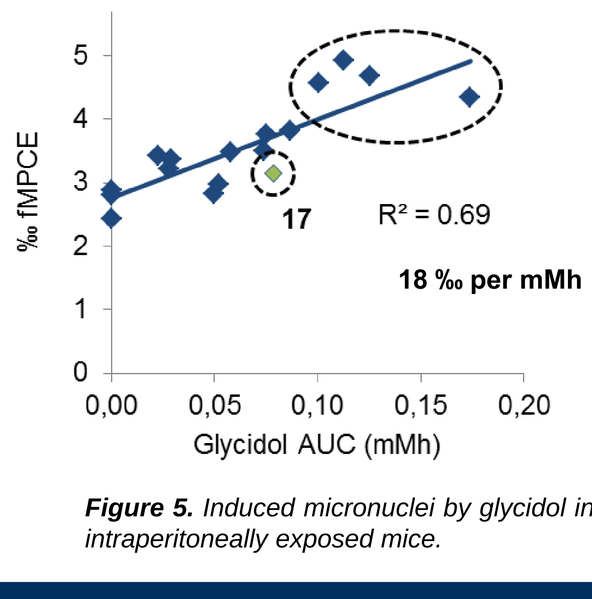




***Figure 5.*** *Induced micronuclei by glycidol in intraperitoneally exposed mice.*

## 4. Relative genotoxic potency of glycidol

- The genotoxic potency of glycidol, measured by fMPCE, was 18 ‰ per mMh.
- Using γ- and β-radiation ($^{137}Cs$) as a standard for quantification of genotoxic potency in mice[3], the relative *in vivo* genotoxic potency (rad-eq./mMh) of glycidol was estimated to 23 rad-equivalences per mMh.

**Relative genotoxic potency of glycidol:**
**23 rad-eq./mMh**

## Discussion

The approach that we present here has several strenghts:

- Variations between animals, exposures and metabolism are corrected for when measuring Hb adducts/AUC, and therefore this approach gives more accurate dose-response relationships compared to the use of administered dose.
- The use of flow cytometry with two lasers in addition to counting of a large number of cells (ca 200000) for the monitoring of micronuclei is much more sensitive compared to the commonly manually measured 2000 cells.
- Use of a standard (ionizing radiation), with a known genotoxic and carcinogenic potency.

The obtained relative genotoxic potency of glycidol will be compared with the risk coefficient obtained from published cancer tests (using a relative cancer risk model[5]) for evaluation of the usefulness of risk coefficients from short term tests.

## Conclusions

- The relative genotoxic potency of glycidol with regard to fMPCE *in vivo* (mice), has been quantified to 23 rad-eq./mMh.
- This approach enables reliable quantitative estimates for dose-response relationships for genotoxic compounds.
- The approach is attractive from a 3R principles perspective.

**Acknowledgement**

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**References**: **[1]** IARC vol. 77:469-486 (2000), **[2]** Kuhlmann *et al.*, Eur. J. Lipid Sci. Technol. 113, 335-344 (2011), **[3]** Abramsson-Zetterberg *et al.*, Int. J. Radiat. Biol. 67:29-36 (1995), **[4]** von Stedingk *et al.*, J. Chromatogr. B. Analyt Technol. Biomed. Life. Sci. 878:2483-2490 (2010), **[5]** Granath *et al.*, Risk Anal. 19:309-320 (1990)

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