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Cancer risk estimation of glycidol based on rodent carcinogenicity studies, a multiplicative risk model and *in vivo* dosimetry

Jenny Aasa[a,*], Fredrik Granath[b], Margareta Törnqvist[a,**]

[a] Department of Environmental Science and Analytical Chemistry, Stockholm University, Sweden
[b] Department of Medicine, Solna, Clinical Epidemiology Division, Karolinska Institute, Stockholm, Sweden

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ABSTRACT

Here we evaluate a multiplicative (relative) risk model for improved cancer risk estimation of genotoxic compounds. According to this model, cancer risk is proportional to the background tumor incidence and to the internal dose of the genotoxic compound. Furthermore, the relative risk coefficient per internal dose is considered to be approximately the same across tumor sites, sex, and species.

In the present study, we demonstrate that the relative risk model is valid for cancer risk estimation of glycidol, a common food contaminant. Published tumor data from glycidol carcinogenicity studies in mice and rats were evaluated in combination with internal dose estimates from hemoglobin adduct measurements in blood from mice and rats treated with glycidol in short-term studies. A good agreement between predicted and observed tumor incidence in responding sites was demonstrated in the animals, supporting a relative risk coefficient that is independent of tumor site, sex, and species. There was no significant difference between the risk coefficients for mice (5.1% per mMh) and rats (5.4% per mMh) when considering internal doses of glycidol. Altogether, this mechanism-based risk model gives a reliable risk coefficient, which then was extrapolated to humans considering internal dose, and background cancer incidence.

1. Introduction

The risk for cancer is dependent on interactions between intrinsic factors and exposure to environmental risk factors, including diet, occupational exposures, smoking, and air pollution (NTP, 2016). Hereditary mutations, and particularly spontaneous mutations induced during DNA replication have been discussed lately as important intrinsic factors (Tomasetti et al., 2017; Tomasetti and Vogelstein, 2015). Electrophilic compounds produced endogenously during normal metabolism should also be considered as intrinsic cancer risk factors and are indeed included in the concept of the exposome - the sum of all exposures to an individual over a lifetime (Rappaport, 2016; Wild, 2005).

Avoiding all cancer risk factors in everyday life is not possible, but a reduction of certain exposures would be beneficial to human health. The human diet contains many undesirable chemicals, including genotoxic compounds formed during food-processing (c.f. e.g. Jägerstad and Skog, 2005; Chaundhry et al., 2006). One well known example is acrylamide, formed during preparation of food at high temperatures (Tareke et al., 2002). Acrylamide is metabolized to the genotoxic and carcinogenic metabolite glycidamide (Beland et al., 2015; Vikström et al., 2011; Sumner et al., 1992). Another example of contaminants in food are glycidyl fatty acid esters, which are compounds that occur in, for example, heat-processed edible oils. The ester bonds are hydrolyzed in the gastrointestinal tract (Appel et al., 2013), resulting in the genotoxic and carcinogenic compound glycidol (IARC, 2000). In the present study, glycidol is used as a model compound for the evaluation of an approach for cancer risk estimation.

Cancer risk estimates for a compound can be obtained by different approaches but are normally based on data from standardized rodent carcinogenicity studies, whereby at least 50 animals of each sex at each dose level (≥ 3 levels plus control) are used for the test species, mouse and rat (OECD, 2009). For genotoxic carcinogens in food, linear extrapolations from high dose-response data are used, e.g. as in an additive model used by the U.S. Environmental Protection Agency, EPA (2005a). In 2005, the European Food Safety Authority (EFSA) proposed a harmonized approach for genotoxic carcinogens. The recommendation was to apply the Margin of Exposure (MOE) approach, where a

Abbreviations: AUC, area under the concentration-time curve; b.w., body weight; diHOPrVal, N-(2,3-dihydroxypropyl)valine; FITC, fluorescein isothiocyanate; FTH, fluorescein thiohydantoin; Hb, hemoglobin; HRMS, high-resolution mass spectrometry

* Corresponding author. Stockholm University, SE-10691, Stockholm, Sweden.
** Corresponding author. Stockholm University, SE-10691, Stockholm, Sweden.
E-mail addresses: jenny.aasa@aces.su.se (J. Aasa), margareta.tornqvist@aces.su.se (M. Törnqvist).

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benchmark dose at the lower bound (BMDL) is compared to human intake estimates (EFSA, 2005). Although uncertainties in the extrapolation of risks from high exposure doses in animals to lower exposure doses in humans are accounted for, the derived MOE only gives a rough estimate intended for risk management purposes. For compounds where dose-response data are insufficient for BMDL derivation, the carcinogenic potency index T25 is recommended (EFSA, 2005), which has previously been applied for glycidol (EFSA, 2016).

In the present study we evaluate a multiplicative (relative) risk model for its applicability to data from carcinogenicity studies of genotoxic compounds with data for glycidol. This is the risk model that is used for quantitative extrapolation of tumor induction by ionizing radiation (BEIR, 2006). The model has previously been further developed by our research group for application to genotoxic carcinogens, aiming to obtain more reliable cancer risk estimations of genotoxic compounds (Granath et al., 1999). According to this model, the cancer risk increment is proportional to the internal dose (*in vivo* dose) of the genotoxic compound and the baseline (background) cancer incidence in the target tissue of the studied species. The internal dose of the genotoxic agent is defined as the area under the concentration-time curve (AUC, expressed in e.g. mMh), reflecting the pharmacokinetics of the compound. Background cancer incidence in different sites, measured from control animals or estimated in human populations, could be anticipated to reflect background mutations (partly from replication errors during stem cell divisions) and their interaction with growth-promotive factors (Granath et al., 1999).

From the relative risk model, an estimate of the relative cancer risk coefficient (β) is obtained, expressed as risk per internal dose. This coefficient is assumed to be approximately independent of tumor site, sex, and species (Granath et al., 1999). The relative risk could also be expressed as the doubling dose (1/β) of the carcinogen, which is the dose that doubles the lifetime cumulative hazard; this implies that it approximately leads to a doubling of lifetime risk if the background tumor incidence is low. The applicability of the relative risk model to data from carcinogenicity studies of genotoxic compounds has so far been validated for a few compounds. In these evaluations the number of tumors in exposed animals predicted with the relative risk model was shown to correlate well with observed tumor incidence in responding sites, irrespectively of tumor site or species (Fred et al., 2008; Törnqvist et al., 2008; Granath et al., 1999). The model has also been successfully evaluated and validated with data from mutagenicity tests *in vitro* (expressed as relative mutagenic potency) for two of these compounds (Fred et al., 2008; Granath et al., 1999). Thus, the evaluations support that the cancer risk coefficient obtained with this model is a measure of the genotoxic potency of the studied compound. These evaluations strongly indicate that application of the relative risk model for cancer risk assessment of genotoxic chemicals also facilitates the use of *in vitro* genotoxicity data for assessment of the relative cancer risk coefficients for such chemicals.

In the present work we have further evaluated the relative risk model for its applicability to genotoxic chemicals using data from published carcinogenicity studies of glycidol in mice and rats (Irwin et al., 1996; NTP, 1990). To obtain accurate estimates of the risk coefficient, the AUCs per exposure dose of glycidol in mouse and rat are obtained from short-term exposures performed at similar conditions as in the carcinogenicity studies. Hemoglobin (Hb) adduct levels measured in the exposed animals are used for calculation of the AUC's. Finally, the risk coefficient is transferred to human exposures.

2. Materials and methods

2.1. In vivo dosimetry of glycidol

Male and female B6C3F1 mice (approximately 10 weeks) and Sprague Dawley rats (approximately 8 weeks) were obtained from Envigo, Venray (Netherlands). The animals were housed in a controlled facility with standard diet and tap water *ad libitum*. The ethical application was approved by the Ethical committee on animal experiments, Swedish Board of Agriculture, license number S7-15. Three animals per dose group were administered glycidol (96%; Acros Organics, Geel, Belgium; CAS 556-52-5; 74.08 g/mol) (10 mL/kg), dissolved in water, at identical dose levels as in the published 2-year carcinogenicity studies (Irwin et al., 1996; NTP, 1990): 25 and 50 mg/kg (mice) and 37.5 and 75 mg/kg (rats), by gavage once daily for five consecutive days. Blood collection from each animal was performed at one occasion in EDTA-coated tubes three days after the final dosing day (eight days after first exposure) by sampling from the tail vein (rats) or from the orbital plexus during anesthesia with isoflurane and oxygen (mice). The blood samples were stored at −20 °C until preparation and analysis of Hb adducts, used as a biomarker for internal dose.

2.2. Quantification of hemoglobin adducts

Blood samples from the treated mice and rats were prepared according to the FIRE procedure to measure the adduct levels from glycidol to the N-terminal valine in Hb (Aasa et al., 2017; von Stedingk et al., 2010). This procedure enables the detachment from Hb of the formed adduct, *N*-(2,3-dihydroxypropyl)valine (diHOPrVal), through derivatization with the Edman reagent fluorescein isothiocyanate (FITC), giving the corresponding fluorescein isothiohydantoin (FTH). Quantification of diHOPrVal-FTH and the corresponding ($^{13}C_5$)-substituted internal standard was performed by ultra-pressure liquid chromatography with high resolution mass spectrometry (UPLC/HRMS) after clean-up of the blood samples. The calibration curve, prepared by adding known amounts of diHOPrVal-FTH to derivatized human blood samples, was processed in parallel. See Supplementary Data and Fig. S1 for further details.

2.3. Calculation of the internal dose

The internal doses of glycidol, expressed as AUC, in the treated mice and rats were calculated from the measured diHOPrVal adduct levels (A), using the second-order reaction rate constant (k_{val}) for glycidol binding to the N-terminal valine in Hb. The rate constant, k_{val}, was determined by incubation of glycidol in pooled blood from both sexes of mice and of rats, respectively, from the same strains as in the *in vivo* dosimetry study. The procedure was similar to the previously described experiment with glycidol and blood from mice (Aasa et al., 2017). Glycidol at five different concentrations (in duplicates) was incubated in lysed blood from respective species for 1 h (at 37 °C), followed by derivatization with FITC. Following work-up and analysis of the samples, the second-order rate constant (k_{val}) given for mouse and rat, respectively, was derived from the linear slopes of Hb adduct levels versus the glycidol AUC *in vitro* ($r^2 > 0.91$). The unit [pmol/g Hb per μMh] is used for k_{val} when the adduct level (A) is expressed as [pmol/g Hb] and the AUC as [μMh]. See Supplementary Data for details of the procedure.

The decrease in adduct level due to elimination of Hb (erythrocyte lifetime) during the exposure period in the short-term animal exposure was considered by adjusting A by multiplying by a factor (dependent on exposure and sampling time and erythrocyte lifetime) of 1.1 (mice) or 1.07 (rats), denoted species-specific factor in Equation (1) (cf. Granath et al., 1992). The daily adduct level increment (a) was then obtained by dividing by five (days of administration) according to Equation (1). The daily AUC's (AUC_{day}) were then calculated using Equation (2).

$$a\ (pmol/g\ Hb\ per\ day) = \frac{A\ (pmol/g\ Hb) \times species\ specific\ factor}{5\ (\mathrm{day})} \quad (1)$$

$$AUC_{day}(\mu Mh/day) = \frac{a\ (pmol/g\ Hb\ per\ day)}{k_{val}\ (pmol/g\ Hb/\mu Mh)} \quad (2)$$

For projection to the lifetime AUC ($AUC_{lifetime}$) in the carcinogenicity studies, the AUC per daily administered dose (mg/kg per day) (that

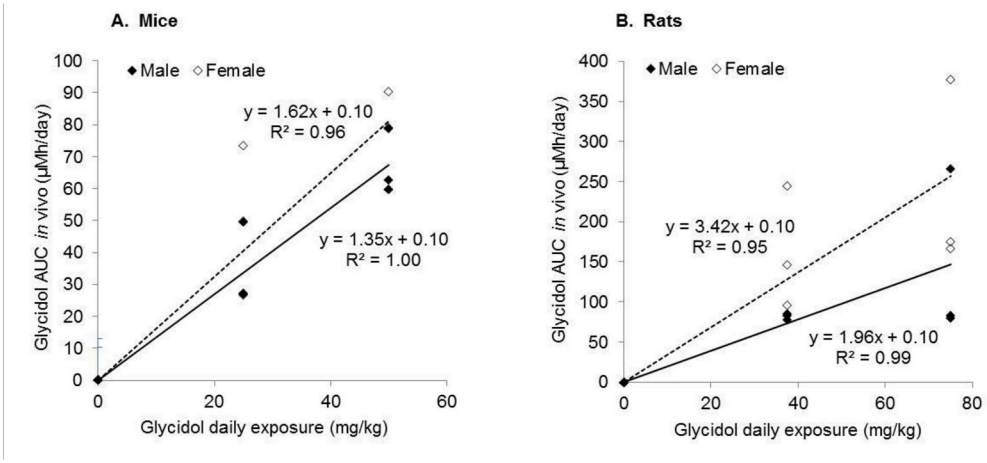

Fig. 1. Internal doses (AUC) of glycidol, calculated from Hb adduct levels in blood from both sexes of mice (**A**) and rats (**B**), after treatment with glycidol by gavage for five consecutive days. Three animals per dose level and for the controls were used. For the AUC calculations the measured adduct levels were adjusted for the erythrocyte lifetime of each species. The slopes of the linear regressions correspond to the mean AUC per administered dose (note different scales on the y-axis for the two species).

is AUC_{day}) obtained from the short-term exposure studies were multiplied by the number of days in the carcinogenicity studies, that is 5 days per week and 103 weeks for the different exposure doses (Irwin et al., 1996; NTP, 1990).

2.4. Glycidol data from carcinogenicity studies

Published data from NTP (Irwin et al., 1996; NTP, 1990) on glycidol-induced neoplasms in dose groups of 50 female and 50 male F344 rats and B6C3F1 mice were used for evaluation of the applicability of the relative cancer risk model to glycidol. In those carcinogenicity studies the animals were administered water (control) or glycidol via gavage, 5 days per week for 103 weeks at dose levels of 37.5 and 75 mg/kg (rats) and of 25 and 50 mg/kg (mice). Different types of neoplasms were observed in rats (13 types) and mice (10 types); see Supplementary Tables S1–S2 for details.

2.5. Multiplicative cancer risk model

We assume that the observed relative frequencies of tumours in the published animal carcinogenicity studies represent the cumulative risk over two years. If $S_i(t)$ denotes the survival function for cancer in site i, and $\lambda_i(t)$ is the corresponding hazard function among unexposed, the cumulative risk up to time t, among unexposed animals, $P_i(0)$, can be expressed as:

$$P_i(0) = 1 - S_i(t) = 1 - e^{-\alpha_i} \quad (3)$$

where α_i *is the cumulative hazard function* $\int_0^t \lambda_i(u)du$

According to the multiplicative risk model, that is the excess relative risk model, animals exposed to a daily dose D have the cumulative hazard function:

$$\beta D \int_0^t \lambda_i(u)du \quad (4)$$

and the cumulative risk among animals exposed to a daily dose D, $P_i(D)$, will thus be:

$$P_i(D) = 1 - e^{-\alpha_i(1+\beta D)} \quad (5)$$

The observed number of glycidol-responding neoplasms, assumed to be binomially distributed, was fitted to the proposed model by non-linear regression model, by the maximum likelihood method using PROC NLIN in SAS 9.4 (SAS Institute Inc., Cary, NC, USA). A common relative risk coefficient per dose unit, β, was estimated for males and females, and separately for rats and mice. The relative risk is expressed as the doubling dose ($1/\beta$), which is the dose that leads to a cumulative hazard of $2\alpha_i$. The evaluation was performed based both on administered dose (mg/kg) and on AUC (mMh), derived from Hb adducts in blood, acting as a surrogate measurement for target dose of glycidol (assuming an even distribution of glycidol throughout the body that would mean approximately the same dose in blood as in the target tissues). The predicted number of animals with neoplasms was estimated as $N_i(D) \times P_i(D)$, where $N_i(D)$ is the number animals examined for site i and dose D and was compared to the corresponding observed neoplasms.

3. Results

3.1. In vivo dosimetry of glycidol in short-term exposure studies

The measured Hb adduct levels (and AUC) increased with increasing exposure dose for both mice and rats with large individual variations at each dose level (Fig. 1). This variation is independent of the analytical precision (cf. Supplementary Figs. S1 and S2) and is most probably due to inter-individual differences in dosing and toxicokinetics. Higher levels of Hb adducts per administered dose were observed in rats compared to mice ($p < 0.001$). The data do not contradict an assumption of linearity, which would imply no saturation of the metabolism of glycidol at the administered doses. The internal doses (AUC) of glycidol in the exposed animals were calculated from the measured Hb adduct levels and the second-order reaction rate constant, k_{val} (Equations (1) and (2)), for respective species. The second-order rate constant, k_{val}, for formation of the adducts from glycidol to Hb was determined *in vitro* to be 19.3 (18.3–20.2, 95% CI) pmol/g per µMh and 23.7 (20.8–26.6, 95% CI) pmol/g per µMh for mice and rats, respectively (see Supplementary Fig. S2). In the *in vivo* short-term exposure experiments, no statistically significant differences in the adduct levels (or AUC) were observed between the sexes of either species exposed to glycidol (Table 1, Fig. 1). Therefore, the mean AUC_{day} per administered dose (of both sexes) was calculated and used in the further evaluation of the risk model. The mean AUC_{day} per administered dose observed in rats, 2.9 ± 1.6 µMh/day per mg/kg, was about twice that observed in mice, 1.6 ± 0.6 µMh/day per mg/kg ($p < 0.01$).

3.2. Evaluation of the multiplicative risk model

The evaluation of the applicability of the relative (multiplicative) risk model (Equation (5)) to the tumor data from published carcinogenicity studies (Irwin et al., 1996; NTP, 1990) was performed by using the cumulative dose over a lifetime (D) expressed both as administered dose (mg/kg) and internal dose (mMh). The internal dose estimates were derived from Hb adduct levels obtained from the performed short-term exposure studies with glycidol in mice and rats. The risk

Table 1
Measured Hb adduct levels and corresponding daily internal doses (AUC) in mice and rats treated with glycidol by gavage for five consecutive days. The mean values were calculated from three animals per dose level.

	Daily dose	Hb adduct level		Internal dose[a]			
	mg/kg	Mean ± SD (nmol/g Hb)		Mean ± SD (μMh/day)		Mean ± SD (μMh per mg/kg)	
		Female	Male	Female	Male	Female	Male
Mice	0	0.01 ± 0	0.01 ± 0	0.1 ± 0	0.1 ± 0	n.a.	n.a.
	25	4.4 ± 2.1	3.0 ± 1.1	50.1 ± 23.3	34.5 ± 12.9	2.0 ± 0.9	1.4 ± 0.5
	50	6.7 ± 1.4	5.9 ± 0.9	76.4 ± 15.4	67.1 ± 10.4	1.5 ± 0.3	1.3 ± 0.2
Rats	0	0.01 ± 0	0.01 ± 0	0.1 ± 0	0.1 ± 0	n.a.	n.a.
	37.5	18.0 ± 8.4	9.1 ± 0.42	162 ± 76	81.8 ± 3.8	4.3 ± 2.0	2.2 ± 0.1
	75	26.5 ± 13.2	15.8 ± 11.8	240 ± 119	143 ± 107	3.2 ± 1.6	1.9 ± 1.4

[a] Measured Hb adduct levels adjusted for elimination due to erythrocyte lifetime (× 1.1 for mice and × 1.07 for rats) for calculation of the internal dose according to Equations (1) and (2), n.a.: not applicable.

Table 2
Comparison of the relative cancer risk of glycidol, expressed as β per dose (per mg/kg/day or per mMh, corresponding to the lifetime dose) or expressed as doubling dose, 1/β (mMh). The parameters were calculated from data from carcinogenicity studies in mice and rats and measurements of the internal dose of glycidol (per administered dose) in short-term exposure studies.

	Mean relative risk coefficient (β) [95% CI]		Mean doubling dose (1/β) [95% CI]
	Relative risk increase per mg/kg/day (%)	Relative risk increase per mMh[a] (%)	mMh
Mice	4.1 [2.3–6.0]	5.1 [2.8–7.4]	19.6 [13.5–35.5]
Rats	8.0 [4.9–11.2]	5.4 [3.3–7.5]	18.6 [13.4–30.5]

[a] The AUC per lifetime administered dose (mg/kg/day), calculated from the short-term *in vivo* studies, was used to transfer to the % risk increase per mMh, 1.6 μMh per mg/kg/day (mean for female and male mice) and 2.9 μMh per mg/kg/day (mean from female and male rats).

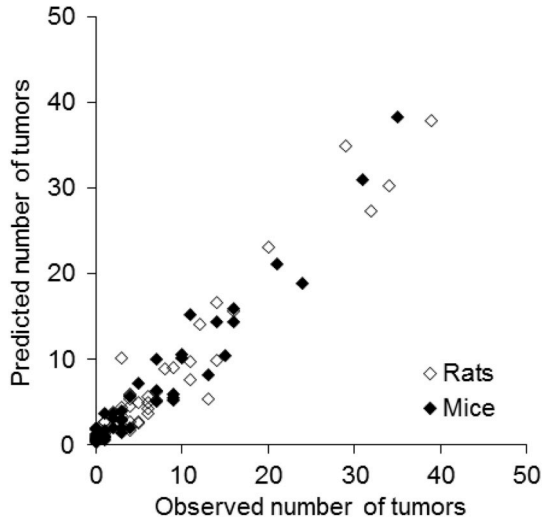

Fig. 2. Predicted versus observed incidence of tumors in mice and rats applying the relative risk model (section 2.5), using the observed tumor incidence in treatment-responding sites reported in the 2-year carcinogenicity studies of glycidol (Irwin et al., 1996; NTP, 1990). Analysis of the residuals reveals no serious lack of fit for the suggested model. The scaled deviance for both mice and rats was 1.22, compared to the expected value of unity. The scaled deviances for mice and rats separately were 1.12 and 1.32, respectively.

coefficient obtained from these species was expressed as risk per lifetime dose (per mg/kg or per mMh) (Table 2). The evaluation showed that a common relative risk coefficient (β) could be derived across all responding tumor sites, doses, and sexes in mice and rats, respectively. A significant difference between the species was observed when the risk coefficient was calculated per administered dose, compared to when it was calculated per internal dose (Table 2). The mean doubling doses (1/β), expressed as lifetime AUC's were not statistically significant different for the species: 19.6 mMh for mice and 18.6 mMh for rats. The applicability of the relative risk model to tumor data for glycidol is illustrated in Fig. 2, showing that a good agreement between the observed and predicted number of tumors in responding sites in glycidol-treated mice and rats was obtained.

4. Discussion

We have evaluated a relative (multiplicative) cancer risk model for application to the genotoxic compound glycidol, using data from published carcinogenicity studies where background tumor incidence in responding sites and the internal doses (from short-term exposure studies) from glycidol exposure in the studied species (mice and rats) were included in the evaluation.

4.1. Selection of tumor sites

As discussed in our first evaluation on the model (Granath et al., 1999), we limit the study to responding sites, even though neoplasms are observed in other sites in control animals. The spectrum of responding tumor sites are rather similar for the studied genotoxic compounds within one strain of animal (present study; Granath et al., 1999; Fred et al., 2008, Törnqvist et al., 2008). In the glycidol carcinogenicity studies most of the neoplasms that occur in non-responding sites have low incidence and would likely not affect the relative risk coefficient (β) if included in the calculations. There are also non-responding sites with high background incidence. In rats, these correspond to e.g. neoplasms in the testes and leukemia (males) and in the pituitary gland (females) (NTP, 1990). In mice the sites with high incidence are mainly the pituitary gland and lymphoma, both in females (NTP, 1990). These strains have been developed to be sensitive, and likely have a genetic predisposition for the development of tumors of this kind and therefore no treatment-related effect is observed. The high background tumor incidence in Leydig cells tumors and mononuclear cell leukemia is for instance one reason for the switch from F344 rats to Sprague Dawley rats in the carcinogenicity studies, decided in 2006 (Maronpot et al., 2016).

4.2. Internal doses as a basis for the relative cancer risk coefficient

The ultimate, although difficult, dose measurements would be in target organs for the derivation of cancer risk estimates in those tissues. A low-molecular mass compound like glycidol can be assumed to be distributed rather evenly throughout the body, and the internal dose in blood inferred from Hb adduct levels can be used as an estimate for

Scheme 1. Calculation of the daily intake of glycidol in humans, corresponding to the doubling dose.

(a) $DD = 19.1\ mMh$

$$\frac{19.1\ mMh}{4.3\ \mu Mh\ per\ mg/kg} = 4442\ mg/kg$$

(b) $$DD_{day} = \frac{4442\ mg/kg}{365\ days/year \times 70\ years} = 0.174\ mg/kg\ per\ day$$

(a) The lifetime doubling dose (DD) was obtained from the mean from mice and rats (19.1 mMh). The corresponding lifetime intake of 4442 mg/kg b.w. is calculated from the relation of the AUC per intake of glycidol from humans (4.3 μMh per mg/kg b.w.).
(b) The daily intake of glycidol is calculated from the lifetime intake, corresponding to the DD, assuming a lifetime of 70 years.

Scheme 1. Calculation of the daily intake of glycidol in humans, corresponding to the doubling dose.

doses of the reactive compound in the target tissues. This has been demonstrated for the structurally similar epoxide ethylene oxide, which gave about equivalent doses (AUC) in several organs and in the blood in mice, as inferred from levels of adducts to DNA and Hb (Segerbäck, 1985). A quantitative risk estimate for glycidol, as the relative risk coefficient (β), for all responding tumor sites is therefore estimated based on internal dose measured in blood.

In the present evaluation of the applicability of the relative risk model to data from published carcinogenicity studies of glycidol, a common β was derived across all responding tumor sites and both sexes for mice and rats administered different doses of glycidol. An approximately two-fold higher lifetime relative risk coefficient per administered dose was indicated for rats compared to mice. This variation between the species was to a large extent explained by the observed differences (statistically significant) of the mean of internal doses per administered doses of glycidol (both sexes): mice ca. 1.6 μMh per mg/kg per day; and rats ca. 2.9 μMh per mg/kg per day ($p < 0.01$). These internal doses reflect the pharmacokinetics of the species, with slower detoxification of glycidol in rats compared to mice.

Recalculation of the relative risk coefficients per internal dose (mMh) instead of administered dose resulted in a non-significant difference between the species; 5.1% per mMh (mice) and 5.4% per mMh (rats) (Table 2), illustrating the importance of *in vivo* dosimetry for a more accurate estimation of β. A larger number of animals and more dose groups in an *in vivo* dosimetry study could give a further improvement of the precision in the estimation of internal doses and β, but could only give a small adjustment of the risk estimate and not change the overall conclusion. Altogether, the obtained relative risk coefficients per internal dose indicate that the probability for tumor development due to glycidol exposure is approximately the same for mice and rats, and the adequate agreement between predicted and observed tumor incidence, for the responding sites demonstrated in Fig. 2, supports the assumption of a common β per internal dose.

Further support for a common risk coefficient β is given from previous evaluations of the applicability of the model for other simple genotoxic epoxides/epoxide metabolites; ethylene oxide (Granath et al., 1999), 1,3-butadiene (Fred et al., 2008), and acrylamide (Törnqvist et al., 2008), where approximately the same estimates of β per internal dose were obtained for different tumor sites, sexes and species (mice and/or rats) for respective compound. The evaluations throughout have shown solid results. Furthermore, in the evaluations of the carcinogenicity studies of ethylene and 1,3-butadiene, the risk coefficient was in agreement with relative genotoxic potency per AUC (compared with ionizing radiation) from *in vitro* genotoxicity studies. For glycidol, the relative genotoxic potency (from *in vitro* and *in vivo* experiments) has been compared with the relative cancer risk coefficient in a preliminary evaluation, which indicated a good agreement (Aasa, 2018).

4.3. Human cancer risk

A common β per internal dose (AUC) in mice and rats supports the transfer of the risk coefficient to other species, as humans. Therefore, the mean doubling dose of glycidol (19.1 mMh, c.f. Table 2) for mice and rats is assumed to be the best estimate of the cancer risk coefficient of glycidol also for humans. Translation of the doubling dose to the risk coefficient per exposure (intake) dose for humans requires that the relation between AUC and intake of glycidol in humans is known. In a 4-week exposure study, recently published by Abraham et al. (2018), 11 human individuals received a daily portion of palm fat equivalent to a mean daily dose of glycidol of 4.2 μg/kg body weight. The Hb adduct levels (diHOPrVal) were monitored from blood samples using the FIRE procedure. The relation between the internal and administered dose of glycidol was calculated to 4.3 μMh per mg/kg using the rate constant for diHOPrVal adduct formation (k_{val}) from glycidol with human Hb, from our studies (Aasa et al., 2019). In Supplementary Table S3 the relation between the internal and administered dose of glycidol for several species, obtained in published studies, are given, showing a difference between the studied species up to ca. 8-fold. As this total variation also includes differences between the methods and laboratories (and without inter-calibrations), the data indicate that the interspecies variation in the detoxification of glycidol and the resulting ratio between AUC and administered dose is not very large.

Thus, the doubling dose of 19.1 mMh (1/β) of glycidol is corresponding to a lifetime intake of ca. 4400 mg/kg in humans, according to the relation between internal and administered dose of glycidol. This means that a mean daily intake of glycidol of ca. 0.17 mg/kg throughout a lifetime of 70 years is estimated to double the background cancer incidence in the population. The calculations are illustrated in Scheme 1 below. A lifetime cancer risk of $1/10^5$ is often used as a figure for acceptable risk in the population. The daily intake associated with a lifetime risk level of $1/10^5$ was further calculated to 0.40 μg/day (70 kg) using Equation (5) (Section 2.5). In this calculation the total background cancer incidence in humans, 30%, is used as a conservative approximate of P^0 (P^0 for the Swedish population below 75 years; Cancerfonden, 2017). At low doses and at low background tumor incidence Equation (5) may be approximated with Equation (6), which gives a simplified illustration of the risk model. The approximation using Equation (6) in this case ($\Delta P = 1/10^5$ and $P^0 = 30\%$) would give a deviation of only 20% of corresponding daily intake of glycidol compared to the exact calculation.

$$\Delta P \approx P^0 \times \beta \times D \quad (6)$$

There is only one other quantitative cancer risk estimation of glycidol found in the literature which allows a comparison. The California Environmental Protection Agency (C. EPA) has performed a risk

estimation for glycidol and arrived at a no significant risk level (NSRL) for humans of 0.54 μg glycidol per day considering a lifetime cancer risk of $1/10^5$ (OEHHA, 2010). The risk estimation by C. EPA is based on the same carcinogenicity studies as in the present evaluation study but estimated by using an additive model. The cancer risk due to exposure to glycidol measured in humans (children) is further discussed in a recently published paper by Aasa et al. (2019).

4.4. Comparison of relative and additive risk models and transfer across species

The risk coefficients for glycidol estimated by the approach used by C. EPA and by the relative risk model used by us, respectively, would not be expected to yield so similar values considering the large differences in the applied models. Fundamental differences are that the additive risk model used by C. EPA uses linear extrapolation of the dose-response data for the most sensitive species (rat) and without considering background tumor incidence or internal doses. Potential species differences were considered through allometric scaling $(b.w._{human}/b.w._{animal})^{1/3}$. Furthermore, a difference is that in the additive model the "dose per day" is used, compared to the relative model that uses the "lifetime dose". Thus, the factors that are considered in our relative risk model and not in an additive model are: 1) the background tumor incidence; 2) the relation between administered and internal dose (for consideration of pharmacokinetics in the extrapolation between exposure doses and between species); and, 3) the estimated lifetime dose; (c.f. Törnqvist and Ehrenberg., 1992; Paulsson et al., 2001). The additive model is for instance used by the U.S. Environmental Protection Agency for estimations of cancer risk in humans and for regulations of carcinogens (U.S. EPA, 2005a). For a comparison of the risk coefficients estimated with the risk models, there are only a few earlier evaluations performed with the relative risk model. The evaluations with the relative risk model indicated in the order of 10 times higher estimate for ethylene oxide (Granath et al., 1999), and about 3 times higher estimate for acrylamide (Törnqvist et al., 2008), respectively, than corresponding estimates by U.S. EPA.

The suggestion to project to human cancer risk using background risk relies on the observation of an approximately equal excess relative risk coefficient between tumour sites seen among A-bomb survivors (Pierce et al., 1996; UNSCEAR, 2000). Furthermore, evaluations of carcinogenicity test data for ionizing radiation suggest a common relative risk coefficient which is approximately the same in responding sites in several strains of mice (Storer et al., 1988) and is approximately the same for mice, dogs and humans (Granath et al., 1999).

The background risk for cancer is assumed to depend on background mutations interacting with "background promotion" (conditions that stimulate growth and clonal expansion). Background mutations could be inherited and/or caused by mutagenic factors from endogenous and exogenous sources, and could also originate from spontaneous DNA replication errors, referred to as "random mutations" by Tomasetti and co-workers (Tomasetti et al., 2017; Tomasetti and Vogelstein, 2015). In the relative risk model, the background cancer incidence is assumed to give a rough estimate of background conditions interacting with the exogenous genotoxic factor, like glycidol in the present study, to develop cancer (cf. Granath et al., 1999).

As the strains of mice and rats, which are commonly used in carcinogenicity studies, have been developed to be sensitive for tumor development, the background incidence for certain types of tumors are high in these strains (e.g. in the liver). Using an additive risk model can result in overestimations in the species-extrapolation of a risk coefficient to humans, in general for populations with lower background incidence than the test species. This was observed by Kuo et al. (2002) who compared cancer risk predictions by either an additive risk model or a relative risk model (without consideration of internal doses) for about 100 tumor types in mice and rats. In general, extrapolating risks from species with high background rates to species with low background rates using the additive risk model resulted in overpredictions, whereas the relative risk model matched the observed tumor incidence better (Kuo et al., 2002). This supports that a relative risk model is preferable because it considers the background incidence of the studied species, which the model is further improved if internal doses are considered.

Background tumor incidence of different cancer types vary between human populations, likely related to lifestyle. Another support for the relative cancer risk models is a study by Korobitsyn (2011). He developed a relative (multiplicative) cancer risk model for cross-population extrapolations, considering population specific health and demographic parameters in the studied Russian population. Compared to an additive risk model, the relative risk model enabled calculations of risks for cancer development at different ages for males and females in different subpopulations. This approach is similar as for cancer risk projection of ionizing radiation using a relative risk model, where population specific background cancer rates and dose rates at different ages are considered in the calculations of the lifetime attributable risk (LAR) from the excess relative risk coefficient (ERR) for ionizing radiation. This is required to adjust for the high exposure doses of ionizing radiation underlying the ERR, obtained from survivors of the Hiroshima/Nagasaki A-bombings (ICRP, 2007; BEIR, 2006).

The experience from ionizing radiation should be further explored for improvement of species-extrapolation to humans of risk estimates for chemicals, as e.g. have been discussed by the U.S. EPA for age-dependent susceptibility (U.S. EPA, 2005b; Barton et al., 2005). Application of a relative risk model to chemical genotoxic carcinogens would give possibilities to further refine the transfer of the cancer risk coefficient to different human populations.

5. Conclusions

We have demonstrated the applicability of the multiplicative (relative) risk model to data from glycidol carcinogenicity studies. A good agreement between observed and predicted tumor incidence was shown in glycidol-exposed mice and rats, indicating a relative risk coefficient per internal dose that is approximately independent of tumor site, sex, and species. Further, internal dose measurements of the genotoxic compound improve the accuracy of the risk estimation and enable a more reliable extrapolation between exposure doses and between species to a risk coefficient for humans.

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Appendix A. Supplementary data

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