Cancer Risk Assessment of Glycidol
Evaluation of a Multiplicative Risk Model for Genotoxic Compounds

Jenny Aasa

Academic dissertation for the Degree of Doctor of Philosophy in Environmental Chemistry at Stockholm University to be publicly defended on Thursday 14 June 2018 at 10.00 in Nordenskiöldsalen, Geovetenskapens hus, Svante Arrhenius väg 12.

Abstract
Humans are exposed to chemicals in everyday life, both from the environment and from endogenous processes. Some compounds constitute a risk for cancer development. One such compound is glycidol, which is genotoxic and an animal carcinogen. It is the model compound of this work, partly due to its presence in food. Glycidol, often together with 3-monochloropropene-1,2-diol (3-MCPD), occurs in the form of esters particularly in refined cooking oils, which are used in a variety of food products. The esters are hydrolyzed in the gastrointestinal tract to form glycidol (and 3-MCPD).

The aim of the thesis has been to evaluate an approach for cancer risk estimation of genotoxic carcinogens based on a multiplicative (relative) risk model and genotoxic potency. Further, the aim was to estimate the cancer risk for exposure to glycidol via food. Measurement of the internal doses (concentration × time) of glycidol in the studied biological systems, including humans, has been crucial. Glycidol is electrophilic and forms adducts with nucleophilic sites in proteins and DNA. The doses of glycidol were quantified by mass spectrometry: in vivo from adduct levels to hemoglobin (Hb); in vitro from adducts to co(II)alamin.

The first part of the thesis concerns the genotoxic potency (genotoxic response per internal dose) of glycidol, measured in vitro by mutation studies and in vivo by micronuclei as a biomarker for genotoxicity (short-term studies in mice). The results were compared to that of ionizing radiation, used as a standard, to estimate the relative genotoxic potency of glycidol: 10 and 15 rad-equiv.·mMh from mutations and micronuclei, respectively. No induction of micronuclei was observed for the related compound 3-MCPD.

Tumor incidence from published carcinogenicity studies of glycidol in mice and rats, together with the measured in vivo doses, was evaluated with the relative cancer risk model. A good agreement between predicted and observed tumor incidence was shown, and no significant difference of the obtained cancer risk coefficients (risk per dose) between mice (5.1 % per mMh) and rats (5.4 % per mMh) was observed. The overall results support that the relative risk coefficient (β) is independent of sex, tumor site, and species, and indicated that it can be transferred also to humans. The doubling dose, expressed as 1/β, is the dose that is required to double the background tumor incidence. The mean of the doubling doses from mice and rats (19 mMh) was assumed valid for risk estimation for humans. Transfer of β of glycidol to rad-equiv. via its relative genotoxic potency showed a risk coefficient in agreement with the relative cancer risk coefficient of ionizing radiation.

In the final work, the lifetime (70 years) in vivo doses of glycidol were calculated from measured Hb adduct levels in blood from 50 children and 12 adults, and compared to the doubling dose. A fivefold variation was observed in the in vivo doses. The estimated lifetime excess cancer risk from glycidol exceeds 1/1000. This is much higher than what is considered as an acceptable risk.

To conclude, the multiplicative (relative) risk model together with relative genotoxic potency is promising to use in an approach for cancer risk estimation and in line with 3R (reduce-refine-replace) initiatives.

Keywords: glycidol, 3-monochloropropene-1,2-diol (3-MCPD), genotoxicity, mutations, micronuclei, hemoglobin adducts, in vivo dose, multiplicative risk model, cancer risk assessment, human cancer risk.

Stockholm 2018
http://urn.kb.se/resolve?urn=urn:nbn:se:diva-155073


Department of Environmental Science and Analytical Chemistry

Stockholm University, 106 91 Stockholm
Cancer Risk Assessment of Glycidol

Evaluation of a Multiplicative Risk Model for Genotoxic Compounds

Jenny Aasa
Till Pontus och Alfred
List of publications

The thesis is based on the following papers, which are referred to by their Roman numerals throughout the text in the thesis. Reprints were made with permission from the publishers.

I. Quantification of the mutagenic potency and repair of glycidol-induced lesions
   J. Aasa, D. Vare, H.V. Motwani, D. Jenssen and M. Törnqvist
   *Mutation Research* 805 (2016), 38-45

II. The genotoxic potency of glycidol established from micronucleus frequency and hemoglobin adduct levels in mice
    J. Aasa, L. Abramsson-Zetterberg, H. Carlsson and M. Törnqvist
    *Food and Chemical Toxicology* 100 (2017), 168–174

III. Measurement of micronuclei and internal dose in mice demonstrates that 3-monochloropropane-1,2-diol (3-MCPD) has no genotoxic potency *in vivo*
    J. Aasa, M. Törnqvist and L. Abramsson-Zetterberg
    *Food and Chemical Toxicology* 109 (2017), 414–420

IV. Cancer risk estimation of glycidol based on rodent carcinogenic studies, a multiplicative risk model and *in vivo* dosimetry
    J. Aasa, F. Granath and M. Törnqvist
    *Submitted manuscript* (2018)

V. Internal dose of glycidol in children and estimation of associated cancer risk
    J. Aasa, E. Vryonidis, L. Abramsson-Zetterberg and M. Törnqvist
    *Manuscript* (2018)
Author contribution to the papers

I  The author has performed the cell cultivation, the mutagenicity and survival studies, internal dose measurements, and the major part of the writing.

II The author has taken part in planning of the study, performed the laboratory work required for the estimation of the $\textit{in vivo}$ doses, and been responsible for the major part of the writing.

III The author has taken part in planning of the study, performed the laboratory work required for the estimation the $\textit{in vivo}$ doses, and been responsible for the major part of the writing.

IV The author has collected all data for modelling of the cancer risk, planned the design of the $\textit{in vivo}$ studies, performed all laboratory work of the blood samples, and been responsible for the major part of the writing.

V The author has been responsible for the laboratory work, all calculations and made the major part of the writing.
## Contents

1. Introduction and aims ........................................................................7  
   1.1 Aims of the thesis ........................................................................8  
2. Background ........................................................................................11  
   2.1 Tumor development .....................................................................11  
   2.2 Lifetime cancer risk .....................................................................12  
   2.3 Classification of carcinogens .......................................................13  
   2.4 Genotoxic compounds in food .....................................................14  
   2.5 Test protocols for genotoxicity .....................................................16  
3. Risk assessment of genotoxic carcinogens in food ............................17  
   3.1 Margin of Exposure .....................................................................18  
      3.1.1 Benchmark dose approach ...................................................18  
      3.1.2 T25 approach ......................................................................19 a  
   3.2 Threshold of Toxicological Concern (TTC) ...................................20  
4. Model compounds .............................................................................21  
   4.1 Glycidol ......................................................................................21  
      4.1.1 ADME properties ...............................................................21  
      4.1.2 Toxicity ..............................................................................22  
   4.2 3-Monochloropropane-1,2-diol (3-MCPD) .......................................22  
      4.2.1 ADME properties ...............................................................23  
      4.2.2 Toxicity ..............................................................................25  
   4.3 Formation and occurrence in food .................................................25  
5. Methods ............................................................................................29  
   5.1 Genotoxicity in vitro ....................................................................29  
      5.1.1 Mutagenicity .................................................................29  
      5.1.2 DNA damage and repair .....................................................30  
   5.2 Genotoxicity in vivo .....................................................................33  
      5.2.1 Induction of micronuclei in vivo .........................................33  
      5.2.2 Short-term in vivo micronucleus test ....................................34  
   5.3 Dosimetry of electrophilic compounds ........................................36  
      5.3.1 Reactivity and adduct formation ........................................36  
      5.3.2 Cob(I)alamin as a tool for in vitro dosimetry .....................37  
      5.3.3 Hemoglobin adducts used for in vivo dosimetry ..................38  
      5.3.4 Internal exposure dose (AUC) calculation .........................41
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADME</td>
<td>absorption, distribution, metabolism, excretion</td>
</tr>
<tr>
<td>ADU</td>
<td>alkaline DNA unwinding</td>
</tr>
<tr>
<td>4-Ani</td>
<td>4-amino-1,8-naphthalimide</td>
</tr>
<tr>
<td>AraC</td>
<td>cytosine arabinoside</td>
</tr>
<tr>
<td>AUC</td>
<td>area under the concentration-time curve</td>
</tr>
<tr>
<td>BER</td>
<td>base excision repair</td>
</tr>
<tr>
<td>BMD</td>
<td>benchmark dose</td>
</tr>
<tr>
<td>b.w.</td>
<td>bodyweight</td>
</tr>
<tr>
<td>Cbl(I)</td>
<td>cob(I)alamin</td>
</tr>
<tr>
<td>CMR</td>
<td>carcinogenic, mutagenic or toxic to reproduction</td>
</tr>
<tr>
<td>CHO</td>
<td>Chinese hamster ovary cells</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>diHOPrVal</td>
<td>N-(2,3-dihydroxypropyl)-valine</td>
</tr>
<tr>
<td>dsDNA</td>
<td>double stranded DNA</td>
</tr>
<tr>
<td>EFSA</td>
<td>European Food Safety Authority</td>
</tr>
<tr>
<td>EH</td>
<td>epoxide hydrolase</td>
</tr>
<tr>
<td>EPA</td>
<td>Environmental Protection Agency</td>
</tr>
<tr>
<td>ERCC1</td>
<td>excision repair cross-complementation protein 1</td>
</tr>
<tr>
<td>ESI</td>
<td>electrospray ionization</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agriculture Organization</td>
</tr>
<tr>
<td>FITC</td>
<td>fluorescein isothiocyanate</td>
</tr>
<tr>
<td>FTH</td>
<td>fluorescein thiohydantoin</td>
</tr>
<tr>
<td>GI</td>
<td>gastrointestinal</td>
</tr>
<tr>
<td>GSH</td>
<td>glutathione</td>
</tr>
<tr>
<td>HAZT</td>
<td>hypoxanthine, azaserine, thymidine</td>
</tr>
<tr>
<td>Hb</td>
<td>hemoglobin</td>
</tr>
<tr>
<td>HPRT</td>
<td>hypoxanthine-guanine phosphoribosyltransferase</td>
</tr>
<tr>
<td>HR</td>
<td>homologous recombination</td>
</tr>
<tr>
<td>HRMS</td>
<td>high resolution mass spectrometry</td>
</tr>
<tr>
<td>IARC</td>
<td>International Agency for Research on Cancer</td>
</tr>
<tr>
<td>JECFA</td>
<td>Joint FAO/WHO Expert Committee on Food Additives</td>
</tr>
<tr>
<td>LC/MS/MS</td>
<td>liquid chromatography mass spectrometry</td>
</tr>
<tr>
<td>LD₅₀</td>
<td>lethal dose killing 50% of test subjects/cells</td>
</tr>
<tr>
<td>LOQ</td>
<td>limit of quantification</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>3-MCPD</td>
<td>3-monochloropropane-1,2-diol</td>
</tr>
<tr>
<td>MN</td>
<td>micronucleus</td>
</tr>
<tr>
<td>MOE</td>
<td>margin of exposure</td>
</tr>
<tr>
<td>MRM</td>
<td>multireaction monitoring</td>
</tr>
<tr>
<td>NCE</td>
<td>normochromatic erythrocyte</td>
</tr>
<tr>
<td>NER</td>
<td>nucleotide excision repair</td>
</tr>
<tr>
<td>NTP</td>
<td>National Toxicology Program</td>
</tr>
<tr>
<td>PARP</td>
<td>poly(ADP-ribose) polymerase</td>
</tr>
<tr>
<td>PCE</td>
<td>polychromatic erythrocyte</td>
</tr>
<tr>
<td>PFPITC</td>
<td>pentafluorophenyl isothiocyanate</td>
</tr>
<tr>
<td>PRM</td>
<td>parallel reaction monitoring</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>SB</td>
<td>strand break</td>
</tr>
<tr>
<td>ssDNA</td>
<td>single stranded DNA</td>
</tr>
<tr>
<td>SPE</td>
<td>solid phase extraction</td>
</tr>
<tr>
<td>TTC</td>
<td>threshold of toxicological concern</td>
</tr>
<tr>
<td>XRCC1</td>
<td>X-ray repair cross-complementing protein 1</td>
</tr>
<tr>
<td>UV</td>
<td>ultra violet</td>
</tr>
<tr>
<td>V_d</td>
<td>volume of distribution</td>
</tr>
</tbody>
</table>
1. Introduction and aims

Exposures to genotoxic and carcinogenic compounds are continuously ongoing from many sources, for example through the diet, at work places and from materials in our homes. Efforts to mitigate these exposures are important. Often the exposures occur without awareness of the risk\textsuperscript{1}. This may be due to formation of carcinogenic compounds through for example cooking, via metabolism from a non-toxic precursor, or other endogenous processes (like oxidative stress and lipid peroxidation). All these processes contribute to the exposome, which is the sum of the total exposures to chemical compounds throughout life for a person (Wild, 2005).

A central part of the risk assessment procedure concerns the exposure assessment, which for compounds in food usually is based on estimations from occurrence of the compound in the food products and intake data, giving only rough estimates. A more true assessment should be obtained if the \textit{in vivo} doses of the compounds were considered. Data from rodent carcinogenicity studies are often the basis for estimation of cancer risk in humans. Extrapolating from animals to humans is associated with major uncertainties due to differences between species in pharmacokinetics. Also, the usually high doses administered in animal studies may result in difficulties in the extrapolations, as high doses often are associated with additional effects not observed at the lower doses relevant to human exposures. In addition, an ethical aspect of using a large number of animals has to be considered.

There is a need for reduction and replacement of the cost- and time-consuming carcinogenicity studies in animals. Initiatives are ongoing to use data from genotoxicity assays for cancer risk assessment (c.f. MacGregor et al., 2015a/b). One approach, developed by our group at Stockholm University, is based on a model used for projection of cancer risk for ionizing radiation, namely \textit{“the multiplicative (relative) risk model”}. This model provides a relative risk coefficient that reflects the genotoxic potency per internal dose of the carcinogen (Granath et al., 1999). The model is

\textsuperscript{1} The meaning of risk has been defined as “The probability of an adverse effect in an organism, system, or (sub)population caused under specified circumstances by exposure to an agent” (WHO, 2004). Is usually expressed as a percentage or a quotient, e.g. per year.
evaluated through comparison with data from genotoxicity tests in vitro or in vivo, where also the internal dose is measured and compared with the genotoxic response by ionizing radiation (used as a standard agent). As genotoxic compounds in general are electrophilic and difficult to measure in free form, the internal dose is measured as adducts (the electrophile is trapped by nucleophiles). Applied nucleophiles are cob(I)alamin for trapping of the electrophile in vitro and the N-terminal valine in hemoglobin (Hb) in vivo.

So far, three compounds, namely butadiene, acrylamide and ethylene oxide have been used for the evaluation of this approach (Fred et al., 2008; Törnqvist et al., 2008, Granath et al., 1999). The overall aim of this thesis work has been to further evaluate the approach for cancer risk estimation with an extended battery of assays, for the genotoxic compound glycidol.

1.1 Aims of the thesis

The specific aims (Figure 1) for the evaluation of the approach for cancer risk estimation (“the multiplicative (relative) risk model”) have been to:

1. Estimate the genotoxic potency (genotoxic response per internal dose) of glycidol from the “HPRT mutation test” (in vitro) and the in vivo micronucleus (MN) test in mice (short-term studies), with simultaneous measurement of the internal doses (in mMh) of glycidol.

2. Compare the genotoxic potencies of glycidol obtained in vitro and in vivo to that of ionizing radiation, used as a standard agent, to generate the relative genotoxic potency of glycidol (expressed as rad-equ./mMh).

3. Evaluate the “relative cancer risk model” (a mathematical model) for glycidol, based on previously published rodent carcinogenicity studies, in combination with internal dose measurements obtained from in vivo studies (mice and rats) performed in the present work.

4. Evaluate the applicability of relative genotoxic potency from in vitro and in vivo short-term studies with the relative risk model, and compare the risk estimate to that of ionizing radiation (as a standard agent).

5. Measure the internal doses of glycidol in humans and estimate the cancer risk from exposure to glycidol.
Figure 1. The aims of the thesis have been to evaluate a cancer risk model (the multiplicative (relative) risk model) for glycidol, based on published carcinogenicity studies and genotoxic potency, and to estimate human cancer risk from glycidol exposure. The numbers refer to the numbered list in the text above. MN: micronuclei.
2. Background

2.1 Tumor development

Development of cancer is a multistep process occurring over decades, with a requirement of several mutations for tumor formation (Vogelstein and Kinzler, 1993; Knudson, 1985). According to Martincorena et al. (2017) 1-10 mutations are required depending on tumor site. This explains the dramatic increase in the number of different forms of cancer incidence at older ages, with about a doubling every fifth year after the age of 25 (Miller, 1980). The dependency of age for cancer incidence is illustrated in Figure 2.

The development of a tumor proceeds in three stages (Oliveira, 2016). First, the mutation is induced, following exposure to a genotoxic agent, acting as an *initiator*. Second, the presence of a *promoter*, an agent that stimulates and accelerates the transformation process, is required for further development of the tumor. Exposure to the promoter needs to last for a long time period (months to years) to be effective. Removing the promoter causes tumor development to stop. Lastly, exposure to a *progressor* enables the cells to rapidly divide and invade normal tissue. Progression is an irreversible process. Complete carcinogens (as ionizing radiation) exhibit all three properties of an initiator, promoter, and progressor.

![Figure 2. Total cancer incidence at different age groups of both sexes (U.S. population). Modified from data from the National Cancer Institute (2017).](image)
The size of the human diploid genome is approximately $6 \times 10^9$ base pairs. The DNA polymerases make about 1 mistake per 100 000 nucleotides during replication (Loeb, 2001; Thomas et al., 1991), which results in about 60 000 errors every time a cell divides. Fortunately, the DNA repair enzymes correct the majority of the errors. A background mutation rate of about $2.5 \times 10^{-8}$ per nucleotide per generation has been estimated in humans (Nachman and Crowell, 2000).

### 2.2 Lifetime cancer risk

Cancer is one of the leading causes of death worldwide, with 8.8 million deaths in 2015 (WHO, 2017). In Sweden, the annual number of people diagnosed with cancer has doubled since 1970. One important factor for the increased incidence\(^2\) is an aging population. After correction for changes in the size of the Swedish population (ca. 8 million to 9.6 million) and aging the increased incidence is still high, about 40 % from 1970 to 2013 (Cancerfonden, 2015). Looking at 28 different types of cancers worldwide, a total increase of diagnosed cancers of 5 % was observed from 1990 to 2013, with a very large spread between different cancer forms (Fitzmaurice et al., 2015). The most common cancer forms in Sweden and worldwide are summarized in **Figure 3** together with the lifetime risk (U.S. data) for being diagnosed with any of the different cancer forms.

Many factors are associated with an increased cancer risk, where lifestyle may be considered an important factor. About 40 % of all cancers could be assumed to be prevented by changing lifestyle (avoiding risk factors), such as stop smoking or eating healthier (WHO, 2007). There is an ongoing debate about which factors contribute most to the lifetime risk of cancer. Tomasetti and Vogelstein have published two well cited studies, where they observed a strong correlation ($r > 0.8$) between lifetime cancer risk in several tissues and the total number of stem cell divisions in those tissues. They suggested that the majority contribution (about two thirds) to cancers is due to intrinsic factors, such as from “random mutations” (Tomasetti et al., 2017; Tomasetti and Vogelstein, 2015). The results by Tomasetti are perceived as controversial by many researchers. Wu et al. (2016) made a re-analysis of their data and concluded that a correlation analysis cannot distinguish between intrinsic and environmental factors, and that the majority of the cancer incidence is dependent on the environmental factors.

---

\(^2\)Cancer incidence: number of cancer cases in a population (often given per 100 000) during a specified time frame (typically: per year).
Well known examples of epidemiological results showing strong correlations between an environmental factor and cancer are; smoking, giving lung cancer, and UV radiation, giving skin cancer. Large geographical differences in cancer incidence rates also indicate the importance of environmental factors (Fitzmaurice et al., 2015). Another study supporting that the environmental factors give the major contribution to cancers are from studies with more than 10 000 monozygotic and dizygotic twins (Lichtenstein et al., 2000). Also from studies of immigrant populations it have been demonstrated that the environment influences the type of cancer, as observed for Hawaiian immigrants from Japan, where the rate of stomach cancer decreases over time simultaneously as the rates for breast and prostate cancers increase over time (Peto, 2001). It is likely that there is a strong combined effect of all exposures during the lifetime and of intrinsic factors for the development of cancer (Rappaport, 2016). The origin of cancer types where no known association with environmental factor occurs is particularly difficult to understand.

* Excluding malignant melanoma and basal cell cancer.

Figure 3. The most common cancer forms and their prevalence\(^3\) (%) and lifetime risk (from U.S. data) in brackets (%), observed in Sweden and worldwide (Cancerfonden, 2017; National Cancer Institute, 2017).

2.3 Classification of carcinogens

The initial intention of the introduction of classification systems was to raise warning flags for chemicals that require further evaluations (Boobis et al., 2016). Usually, the classifications of carcinogens are not used for risk estimations as exposures are not considered. Focus is on the hazard identification, preferably from human epidemiological data if available, but much more often from animal studies. Different systems for classification of carcinogens occur, where common lists are presented by, for example the International Agency for Research on Cancer (IARC) and the American

---

\(3\) Cancer prevalence: the proportion of a population that has cancer at a given time.
Conference of Governmental Industrial Hygienists (ACGIH). The different classifications applied by IARC, based on the weight of the results from available studies of the hazard are shown in Table 1.

Table 1. IARC classification system of environmental factors. Number of compounds/factors until 18 April 2018 (IARC homepage, 2018).

<table>
<thead>
<tr>
<th>Group</th>
<th>Classification</th>
<th>No. of agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carcinogenic to humans</td>
<td>120</td>
</tr>
<tr>
<td>2A</td>
<td>Probably carcinogenic to humans</td>
<td>82</td>
</tr>
<tr>
<td>2B</td>
<td>Possibly carcinogenic to humans</td>
<td>299</td>
</tr>
<tr>
<td>3</td>
<td>Not classifiable as to its carcinogenicity to humans</td>
<td>502</td>
</tr>
<tr>
<td>4</td>
<td>Probably not carcinogenic to humans</td>
<td>1a</td>
</tr>
</tbody>
</table>

*Caprolactam: monomer that occurs in the manufacture of nylon 6.

2.4 Genotoxic compounds in food

Food may contain genotoxic compounds, either as residues or as contaminants that are induced during processes as cooking at high temperatures. In addition, a compound *per se* may not constitute a problem but may generate genotoxic compounds *in vivo* through metabolism. Residues from pesticides are regulated carefully and are not accepted to be present in food. Process-induced compounds have to be handled differently as these exposures are generally not possible to avoid. Reduction of exposures via food can be obtained if avoiding grilling and cooking at high temperatures. Authorities usually perform risk estimations for harmful compounds based on toxicity and carcinogenicity studies in animals (hazard identification and characterization) and data on human intake. Research in our group at Stockholm University has included genotoxic compounds in food. These are presented in Table 2 together with risk estimations from the European Food Safety Authority (EFSA). Note that the reported estimated exposure levels are rough values that may vary depending on eating habits, but also depending on available analytical techniques used for quantification.

A well-known heat-induced compound is acrylamide, which has been detected to occur in food by our research group; Tareke et al. (2002). It forms during cooking at high temperatures of food such as potatoes, cereals and bread, and also in coffee (EFSA, 2015; Rosén and Hellenäs, 2002), and is classified by the IARC as probably carcinogenic to humans, Group 2A (IARC, 1994). Acrylamide is metabolized *in vivo* by CYP2E1 to the genotoxic metabolite glycidamide. Another well-known heat-induced
compound is benzo(a)pyrene (BaP) which is classified as carcinogenic to humans, Group 1 (IARC, 2012). BaP belongs to a class of compounds known as polycyclic aromatic hydrocarbons (PAH). Often the major exposure sources of PAHs and BaP comes from barbequed and smoked meat and other roasted foods. One large part of the exposure to the general population also comes from inhalation of polluted air from incomplete combustion of coal, wood heating and from cars (Boström et al., 2002).

The model compounds investigated in this thesis, glycidol (Group 2A; IARC, 2000) and 3-monochloropropane-1,2-diol, 3-MCPD (Group 2B; IARC, 2013) occur simultaneously in processed (at high temperatures) cooking oils and in foods containing these oils (EFSA, 2016a). The compounds are bound as esters, which are hydrolyzed in the gastrointestinal tract resulting in exposures to glycidol and 3-MCPD. The compounds are described in detail in Chapter 4.

Table 2. Compounds present in different food products and their risk estimations. All numbers have been extracted from reports by the European Food Safety Authority (EFSA). See Chapter 3 for explanations of BMDL, T25 and MOE.

<table>
<thead>
<tr>
<th>Food product</th>
<th>Median exposure&lt;sup&gt;d&lt;/sup&gt; μg/kg b.w./day</th>
<th>BMDL&lt;sub&gt;10&lt;/sub&gt; mg/kg b.w. per day</th>
<th>MOE&lt;sup&gt;g&lt;/sup&gt;, median – high</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrylamide&lt;sup&gt;a&lt;/sup&gt;</td>
<td>potatoes, cookies, bread, coffee etc.</td>
<td>0.4 – 1.9</td>
<td>0.05 – 0.20</td>
</tr>
<tr>
<td>Benzo(a)pyrene&lt;sup&gt;b&lt;/sup&gt;</td>
<td>fried/grilled food, cereals</td>
<td>0.003 – 0.006</td>
<td>0.05 – 0.20</td>
</tr>
<tr>
<td>Glycidol esters&lt;sup&gt;c&lt;/sup&gt;</td>
<td>refined cooking oils, cookies, cereals, infant formula</td>
<td>0.2 – 0.7</td>
<td>10.2 (T25)</td>
</tr>
<tr>
<td>3-MCPD esters&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td>0.3 – 0.9</td>
<td>0.077</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data from the EFSA, 2015, <sup>b</sup> Data from the EFSA, 2008, <sup>c</sup> Data from the EFSA, 2016a, <sup>d</sup> Mean consumers, all age groups, <sup>e</sup> BMDL<sub>10</sub>: benchmark dose at lower bound (10 %), <sup>f</sup> T25: carcinogenic potency index, <sup>g</sup> MOE: margin of exposure, calculated from the ratio between the critical effect dose in animals and a measured or estimated exposure (intake) in humans, <sup>h</sup> TDI: tolerable daily intake.
2.5 Test protocols for genotoxicity

There is a high correlation between genotoxic effects and cancer, which makes the study of genotoxicity relevant for the estimation of cancer risk. Investigation of the genotoxicity of a compound is performed either in vitro or in vivo. Many test protocols are available. Two types of genetic toxicology studies are considered to be particularly important; those which investigate irreversible changes of the DNA, such as mutations that are transferred to the next generation, and those that investigate reversible effects of the DNA, such as mechanistic studies, i.e. formation of strand breaks and DNA adducts (OECD, 2016).

Testing of a compound is often based on a combination of several tests (a test battery) in order to cover different endpoints relevant for human risk, such as gene mutations, chromosomal damage, and aneuploidy⁴ (OECD, 2016). It is beyond this thesis work to cover all different available genotoxicity tests. Focus will be on the specific methodologies underlying the studies in this thesis; in vitro mutations (HPRT) and in vivo micronuclei, described in Chapter 5.

---

⁴ Aneuploidy: abnormal number of chromosomes in a cell.
3. Risk assessment of genotoxic carcinogens in food

The general process for risk assessment includes several steps, as illustrated in Figure 4. The risk characterization for carcinogenic compounds is based on data from epidemiological studies (observations in humans), animal cancer studies, and/or short-term genotoxicity studies in vitro or in vivo. Every study type has its drawbacks and limitations. Human cancer data are rarely available. Also, a delay of several decades may occur between the specific exposure that causes genotoxic damage and the effect (cancer). A problem with carcinogenicity studies in animals and short-term genotoxicity studies is generally the use of high doses, which are not relevant for human exposures. This implies uncertainties for extrapolation from high doses (in animals) to low doses (in humans) and also for interspecies extrapolations regarding pharmacokinetics.

This chapter briefly summarizes commonly applied risk assessment approaches for carcinogenic compounds in food, primarily based on animal cancer studies.

Figure 4. Scheme of the conventional risk assessment process. For cancer risk estimation data from genotoxicity studies and carcinogenicity studies are collected.
3.1 Margin of Exposure

The margin of exposure (MOE) describes a ratio between a critical effect dose in animals and a measured or estimated exposure (intake) in humans (Equation 1). The critical effect dose, named the reference point or point of departure (POD), is derived from dose-response curves from rodent carcinogenicity studies through different methods, such as the benchmark dose approach (BMD) and the T25 approach, further described below. The BMD approach considers the full dose-response curve, whereas the T25 approach derives from a single point estimate. Estimation of human exposures should be based on long-term intake data (EFSA, 2005).

In general, a MOE of 10 000 or higher, if it is based on BMDL_{10}^5, is considered to be of low concern. The number (10 000) is based on two factors of 10 for inter- and intraspecies differences, respectively plus an additional factor of 100, which considers uncertainties related to variabilities in the cell cycle control and DNA repair and uncertainties of the shape of the dose-response curve below the benchmark dose (SCHER, 2009). A T25 would be 2.5 times the BMDL_{10} assuming a linear dose-response. A MOE based on T25 is therefore considered of low concern at 25 000 or higher (Dybing et al., 2008). The magnitude of a MOE calculated for different compounds can be used from a management perspective for prioritization of compounds. The MOE approach is recommended by the EFSA for substances that are classified as both genotoxic and carcinogenic (EFSA, 2005).

\[
MOE = \frac{POD}{Exposure}
\]  

(1)

3.1.1 Benchmark dose approach

A benchmark dose (BMD) can be defined as a dose that corresponds to a low but measurable change in response (EFSA, 2017). All experimental data are considered in the curve fitting of the dose-response relationship, which makes it a more advanced model in contrast to derivation of a NOAEL (no observed adverse effect level) value, which is highly dependent on the dose settings in the study (Figure 5). From the fitted curve a pre-defined critical effect level (benchmark response, BMR), often 5 % or 10 % increase compared to the background response, determines the BMD. From confidence intervals (95 %) of the plotted data, a lower and upper BMD (BMDL and BMDU) can be derived, where the BMDL_{10} commonly is used

---

5 BMDL_{10}: benchmark dose lower confidence limit 10 %, which represents an estimate of the lowest dose which is 95 % certain to cause an increase of 10 % cancer incidence.
for calculation of the MOE. The BMD approach is recommended for risk assessment of carcinogens by the U.S. Environmental Protection Agency, EPA and the EFSA (U.S. EPA, 2012; EFSA, 2005).

3.1.2 T25 approach

When the experimental dose-response data are not sufficient for the BMD approach, the simpler T25 approach may be applied, which only considers the lowest tumor incidence data showing a statistically significant response (Figure 5) (EFSA, 2005). The T25 has been defined as “the chronic dose rate in mg/kg b.w. per day which will give 25 % of the animals tumors at a specific tissue site, after correction of spontaneous incidence, within the standard life time of that species”, and can be used as an index of carcinogenicity (Dybing et al., 1997). The approach was originally evaluated for 110 carcinogens, where the calculated T25 indexes were compared to the corresponding TD_{50} values for the same tumor sites giving a correlation coefficient of 0.96 (p < 0.0001) (Dybing et al., 1997).

---

* TD_{50} is the daily lifetime dose in mg/kg b.w. per day which induces tumors in 50 % of the studied animals, used as a carcinogenic potency measure (Gold et al., 1984).
The T25 is determined by linear extrapolation, from the significant lowest induced tumor frequency to the dose at which a 25% increase in incidence is expected (normalizing the induced tumor frequencies to 25%), according to Equation 2. The T25 approach has been applied for the studied compound in this thesis, glycidol (EFSA, 2016a) and is discussed further in Chapter 9.

\[ T25 = \text{Dose (mg/kg/day)} \times \frac{25\%}{\text{Frequency at dose (\%)}} \]  
\[ (2) \]

### 3.2 Threshold of Toxicological Concern (TTC)

When there are insufficient experimental data on toxicity (genotoxicity/carcinogenicity) for a compound to meet the requirements for a quantitative risk assessment, a read across approach may be performed, where toxicological data from other compounds with related sub-structures are used. Structural alerts (Figure 6) can indicate possible genotoxicity and/or carcinogenicity and are accordingly called genotoxicity/carcinogenicity alerts (Cartus and Schrenk, 2017; Kroes et al., 2004). One such approach is the Threshold of Toxicological Concern (TTC) approach.

The TTC approach is based on established exposure threshold values, below which there is very low probability of a risk to human health. From an evaluation of dose-response data for 730 compounds a threshold dose of 0.15 μg/day (0.0025 μg/kg/day) has been derived, which gave 86–97% probability that any risk would be less than a lifetime risk of 1/10^6 if the intake is below the threshold value and the chemical is a genotoxic carcinogen (Kroes et al., 2004). If a human exposure is below the derived TTC value, the likelihood for an adverse effect is low. The TTC approach is used for risk assessment or as a prioritization tool by the U.S. Food and Drug Administration (FDA) for food contact materials and by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2002) and the EFSA for flavoring compounds (EFSA, 2016b).

![Figure 6](image.png)

**Figure 6.** Examples of structural alerts for genotoxicity.
4. Model compounds

4.1 Glycidol

Glycidol (2,3-epoxy-1-propanol, CAS no. 556-52-5) (Figure 7) is a low-molecular weight organic chemical compound (Mw 74.08 g/mol), which contains both an epoxide and an alcohol functional group. Glycidol is used as an intermediate during pharmaceutical production, for synthesis of compounds like glycerol, glycidyl ethers, esters and amines (IARC, 2000). It is also present as a food process compound, in refined cooking oils, in the form of esters (glycidyl fatty acid esters), further discussed below.

4.1.1 ADME properties

Glycidol is a water-soluble compound that is absorbed from the gastrointestinal (GI) tract. It has been estimated that about 90 % of the glycidol dose is absorbed after oral (p.o) administration to rats (Nomeir et al., 1995). Comparable bioavailability of glycidol has been observed in rats administered free glycidol or glycidyl fatty acid esters that are hydrolyzed in the gastrointestinal tract (Appel et al., 2013). Glycidyl fatty acid esters are rapidly hydrolyzed by gut lipases to form glycidol, as observed in an in vitro gastrointestinal model (Frank et al., 2013).

Glycidol is metabolically hydrolyzed by epoxide hydrolase (EH) to glycerol, according to studies in vitro and in vivo (rats, mice, humans). Also, conjugation of glycidol with glutathione has been observed. The conjugate is further metabolized and excreted in urine as a mercapturic acid metabolite (Jones, 1975, Patel et al., 1980, Eckert et al., 2011) (Figure 8). The major excretion for glycidol goes via the urine, where 40–48 % of the radioactive dose has been recovered in rats treated with 14C-labelled glycidol (Nomeir et al., 1995).

Figure 7. Chemical structures of glycidol (left) and 3-monochloropropane-1,2-diol (3-MCPD) (right).
The rate of elimination of glycidol is about the same in rats and monkeys, but after oral administration of glycidol or glycidyl esters the AUC and \( C_{\text{max}} \) was \( \leq 50 \% \) in the monkeys compared to the rats (Wakabayashi et al., 2012). These differences after oral administration suggest that the GI environment in each species is important for the bioavailability. The lower pH in the stomach of monkeys (2.8–4.8) compared to rats (3.8–5.0) may affect the hydrolysis rate and may be one explanation to the species differences (Wakabayashi et al., 2012).

4.1.2 Toxicity
Glycidol is classified as a CMR (carcinogenic, mutagenic, toxic to reproduction) substance (ECHA, 2012). It is strongly toxic to testes and to the brain in rats and mice. Also signs of kidney toxicity in both species and lymphoid necrosis of the thymus in rats was observed at high doses (NTP, 1990). Glycidol also exhibits reproductive and developmental toxicity, observed in rodents (summarized by IARC, 2000).

Glycidol is a known animal carcinogen and has been extensively evaluated in many different \textit{in vitro} and \textit{in vivo} genotoxicity tests and carcinogenicity studies by the National Toxicology Program (NTP, 1990). IARC has classified glycidol as probably carcinogenic to humans (Group 2A) (IARC, 2000). Glycidol is positive in several \textit{in vitro} genotoxicity tests in bacteria (Ames) and mammalian cells (Paper I; Ikeda et al., 2012; El Ramy et al. 2007; NTP, 1990; Thompson et al., 1981). Available genotoxicity tests \textit{in vivo} are more limited. Two studies present positive results for induction of micronuclei in intraperitoneally treated mice (Paper II; NTP, 1990), and another study demonstrates negative results for orally treated mice (Ikeda et al., 2012). The different routes of exposure have been discussed as an explanation to the different outcomes.

4.2 3-Monochloropropane-1,2-diol (3-MCPD)
3-Monochloropropane-1,2-diol (3-MCPD, CAS no. 96-24-2) (Mw 110.54 g/mol) belongs to a class of compounds called chloropropanols (2-monochloropropane-1,3-diol, 1,3-dichloropropan-2-ol and 2,3-dichloropropan-1-ol). It is structurally similar to glycidol but lacks the epoxide function (\textbf{Figure 7}). It is used as a raw material in the synthesis of pharmaceuticals but also as a sterilant for rat control (summarized by IARC, 2013). 3-MCPD is also present as a contaminant in soya sauces (reviewed by Lee and Khor, 2015) and in refined cooking oils (as esters), similar to glycidol.
4.2.1 ADME properties

As for glycidol, 3-MCPD is released from esters (in food) through hydrolysis in the gastrointestinal tract. The bioavailability for 3-MCPD released from esters has been shown to be similar to free compound (86 %) in orally treated rats, and complete hydrolysis of the esters is assumed (Abraham et al., 2013).

The biotransformation of 3-MCPD is illustrated in Figure 8. The major pathway in mammals goes via the hepatic enzymes alcohol- and aldehyde dehydrogenases forming β-chlorolactic acid which is further metabolized to oxalic acid, a nephrotoxic metabolite (reviewed by Lynch et al., 1998). It has been suggested that the bacterial enzyme halohydrin dehalogenase can dehalogenate 3-MCPD, giving glycidol (Van Den Wijngaard et al., 1989). However, this pathway is not supported by in vivo studies (Gao et al., 2017; Lynch et al., 1998). Recently, Gao et al. tentatively identified eight additional metabolites (in rats), based on accurate masses and fragmentation pattern of the ions using LC/MS/MS. These metabolites have been formed from direct conjugations of 3-MCPD via glucuronidation, acetylation, sulfonation and addition of amino acids (Gao et al., 2017).

It has also been discussed if glycidol can be converted to 3-MCPD due to the presence of hydrochloric acid in the stomach. This was assumed in rats treated with repeated oral doses of glycidol (100 mg/kg), where the 3-MCPD metabolite β-chlorolactic acid was observed in the urine (Jones and O’Brien, 1980). However, this may be questioned as the sample preparation was performed with the addition of strong hydrochloric acid (10 M), and thus the formation of 3-MCPD could be an artefact. Insignificant levels of β-chlorolactic acid recovered in the urine were observed in another study with rats, treated with single oral and intravenous doses of glycidol (37.5 mg/kg and 75 mg/kg) (Nomeir et al., 1995). Also, a study applying a gastrointestinal model did not support any conversion of glycidol to 3-MCPD (Frank et al., 2013). On the other hand, in a study where rats were treated with a single oral dose of glycidol (37.5 mg/kg) 3-MCPD could be detected in serum samples (Onami et al., 2015). No analysis of the urine was performed in the latter study, which makes it difficult to compare the results.
Figure 8. Tentative biotransformation scheme of glycidol and 3-MCPD. Major excretion via the urine; mercapturic acid pathway for glycidol and β-chlorolactic pathway for 3-MCPD (dashed). NAD⁺ Nicotinamide adenine dinucleotide, *Bacterial enzyme.
4.2.2 Toxicity

The kidneys are the main target of 3-MCPD toxicity. The toxicity is believed to be due to inhibition of the glycolysis by metabolites associated with the β-chlorolactic acid pathway (summarized by JECFA, 2002). Also, 3-MCPD is toxic to male fertility by reducing sperm motility. The mechanism is ascribed to inhibition of the spermatozoan glycolysis by metabolites of 3-MCPD (summarized by IARC, 2013).

3-MCPD has been classified by IARC as possibly carcinogenic to humans (Group 2B) (IARC, 2013). The general view is that 3-MCPD is positive with regard to genotoxicity in vitro. However, both positive and negative results have been reported in several in vitro genotoxicity tests in bacteria and cells. Published in vivo genotoxicity tests show negative results (Paper III; El Ramy et al. 2007; Robjohns et al., 2003).

4.3 Formation and occurrence in food

Fatty acid esters of both glycidol and chloropropanols (like 3-MCPD) have been found as process-induced contaminants in refined vegetable oils. The formation during the processing of oils occurs during the deodorization step, where the oils are heated at high temperatures (> 200 °C) to remove volatile components responsible for e.g. odor (Craft et al., 2013; Zelinková, 2006). High levels of the esters have been found particularly in palm oils (Cheng et al., 2017; EFSA, 2016a; MacMahon et al., 2013).

The level of glycidyl fatty acid esters correlates well with the levels of monoacylglycerols and in particular diacylglycerols in the oils (Craft et al., 2012; Destaillats et al., 2012a). A proposed mechanism for the formation of glycidyl esters from diacylglycerols is shown in Figure 9A. Chloropropanol fatty acid esters have been proposed to be formed through a reaction between triacylglycerols, the predominant lipid (90-95%) in edible oils, and chlorine. The chlorine can originate from decomposed chlorine-containing compounds, such as pesticides or salts (MgCl₂, FeCl₂) in the crude oil material (Destaillats et al., 2012b; Nagy et al., 2011) (Figure 9B).
It has been shown that oils high in glycidyl fatty acid esters are susceptible to degradation at high temperatures (frying). In studies where refined palm oil (ca. 10 mg glycidyl esters per kg) was used for frying of potatoes, both time- and temperature-dependent degradation of the esters were observed (Aniolowska and Kita, 2016; 2015). Using refined sunflower oil, containing less glycidyl esters and 3-MCPD esters (ca. 0.5 mg/kg), no change in the contents was observed during frying over time (Dingel and Matissek, 2015). One factor that seems to play a role for the content of particularly 3-MCPD esters during frying is the amount of salt (NaCl) added. In a study with fried chicken soaked in salt solutions containing 1, 3 or 5 % of NaCl significantly increased concentrations of 3-MCPD esters were detected with the higher levels of salt (Wong et al., 2017).

Both glycidyl fatty acid esters and 3-MCPD esters may also be formed in food during cooking independent of added oils. In a study where different types of ground meat (pork, beef, chicken) were fried at gas fire (150 °C and 250 °C) or charcoal grilled (350–600 °C) with no oil added, elevated levels of glycidyl fatty acid esters were detected dependent on both the temperature and cooking time (Inagaki et al., 2016). The highest concentrations were detected in the meat after charcoal grilling, with levels varying between 1–2 μg/g meat. This implies that exposure to glycidyl fatty acid esters may originate from other components of the diet than edible oils.
The formation of fatty acid esters of glycidol and 3-MCPD during processing of vegetable fats and oils is of concern to health as they are proposed to be subject to complete hydrolysis in the gastrointestinal tract, which generates exposure to free glycidol and 3-MCPD \textit{in vivo} (Abraham et al., 2013; Appel et al., 2013; Frank et al., 2013). In 2009, the German Federal Institute of Risk Assessment (BfR) raised concern that glycidol may be released from glycidyl fatty acid esters present in infant formulas (BfR, 2009). Efforts to mitigate the esters in these products have been undertaken since then, and a clear decrease was observed for bound glycidol in infant formulas from 2009 to 2010 (Weißhaar, 2011). In 2015 a study was presented where a further decrease could be observed for particularly 3-MCPD (Wöhrlin et al., 2015). Several studies have presented levels of glycidyl esters and 3-MCPD esters in different food products. A few examples are presented in Figure 10 (from data by the EFSA, 2016a). Knowledge about the levels in food can be used for estimation of human exposures to perform risk assessments.

\textbf{Figure 10.} Mean abundances of free 3-MCPD and glycidol in different food products: (A) Different oils and fats. (B) Food products containing oils and fats. Adapted from data from the EFSA (2016a).
5. Methods

5.1 Genotoxicity *in vitro*

5.1.1 Mutagenicity

The HPRT test is applied for studying of gene mutations in mammalian cells. The test makes use of the *hprt* gene as a model gene. The *hprt* gene codes for the enzyme hypoxanthine-guanine phosphoribosyl transferase (HPRT), which catalyzes the process of recycling the purine bases hypoxanthine and guanine by condensation with 5’-phosphoribosyl-1-pyrophosphate, PRPP (transforms phosphoribose groups), known as the salvage pathway (Stout and Caskey, 1985). Coupling of the purine bases to PRPP results in nucleotides, which are building blocks of the DNA and RNA. The function of HPRT for the formation of guanosine monophosphate (GMP) is illustrated in Figure 11A. The process is similar for hypoxanthine, giving inosine monophosphate.

![Figure 11A](image)

**Figure 11.** (A) Example of the function of HPRT, catalyzing the synthesis of guanosine monophosphate (GMP) from guanine and 5’-phosphoribosyl-1-pyrophosphate (PRPP). The synthesis continues to guanosine triphosphate (GTP), used for DNA synthesis. (B) If 6-thioguanine, the toxic guanine equivalent, is added to the cell cultivation media *in vitro*, it will be incorporated in the DNA, which leads to cell death. Cells with a mutation in the *hprt* gene will survive and the pathway in (A) can proceed as usual.
A variety of cell lines can be used in the HPRT test, for example Chinese hamster ovary (CHO) cells, L5178Y mouse lymphoma cells or TK6 human lymphoblastoid cells (OECD, 2016). In the HPRT test, a forward mutation in the hprt gene alters its function. When the toxic equivalent to guanine, 6-thioguanine is added to the cell cultivation medium it is incorporated in the DNA which leads to cell death (Figure 11B). Cells with a mutated hprt gene will survive. 6-Thioguanine can therefore be used for selection of mutants (Jenssen, 1984). To reduce spontaneous (background) forward mutants, a selection of cells with a functioning HPRT enzyme is performed prior to treatment with the genotoxic compound (glycidol in the present work). The selection is enabled by the addition of a solution of Hypoxanthine-Azaserine-Thymidine (HAzT), where azaserine inhibits de novo synthesis of nucleotides. Cells with a functioning HPRT can incorporate hypoxanthine and thymidine from the HAzT solution, whereas cells with a non-functioning HPRT die. The work-flow of the HPRT test in cultivated cells is briefly illustrated in Figure 12.

Figure 12. General procedure of the HPRT test, applied for glycidol in Paper I. The cell cultivation is performed at 37 °C and 5 % CO₂. The read-out of cytotoxicity (day 11) and mutagenicity (day 18) is done through manual counting of the stained colonies.

5.1.2 DNA damage and repair

Exposure to genotoxic agents may form DNA adducts, i.e. covalent modifications of DNA bases. A result of unsuccessful repair of a DNA damage (adduct) may be manifested mutations. Another effect may be chromosomal aberrations, which are changes in the structure or number of chromosomes. Repair is a response to DNA damage, as briefly described below, and which has been investigated in Paper I. Different categories of DNA repair pathways occur and are depending on the type of lesion. They are active either before or after replication (Figure 13).
Repair pathways before replication

Base excision repair (BER) and nucleotide excision repair (NER) are common repair processes taking care of damages before replication (Bjergbæk, 2012; reviewed by Jenssen et al., 2002). BER is activated by specific DNA glycosylases that removes single damaged bases, resulting in an apurinic or apyrimidinic (AP) site, followed by cleavage of the phosphodiester bonds by AP lyases/endonucleases. Filling of the gap (by DNA polymerases) and ligation (by ligases) is further processed via either the short patch: one nucleotide is synthesized and used for filling, or the long patch: 2-10 nucleotides are synthesized and used for filling (Sancar et al., 2004). NER is initiated by bulky adducts (by UV). A nuclease complex (several polypeptides) recognizes a distortion of the DNA helix and removes the damaged nucleotides (about 30 base pairs) followed by gap filling and ligation. Another repair pathway is the reversal repair (RR), like alkylguanine-DNA-alkyltransferase, which transfers alkyl groups from the O6-position of guanine to a cysteine present in the transferase, rendering an unmodified guanine. Unlike the other described repair pathways above the transferase is consumed during the process (Pegg et al., 1995).

Repair pathways after replication

Repair of double strand breaks may proceed via the homologous recombination (HR), where DNA synthesis of an invading strand is achieved by taking advantage of the sister chromatid as a template. Either the process advances through formation of a Holliday junction where a physical exchange of DNA strands occurs or through the “Synthesis Dependent Strand Annealing”, where exchange of DNA information is performed without physical movement of the strands. Another pathway involved in double strand break repair is the non-homologous end-joining pathway (NHEJ), where a protein complex binds to the two ends of the double strand break and recruits a ligase to seal the ends. NHEJ is sometimes associated with loss of nucleotides which leads to aberrations (Hoeijmakers, 2001). The translesion synthesis system (TLS) is a group of polymerases that synthesizes DNA past a lesion. Depending on the recruited polymerase the pathway is error-free or error-prone (Lehner and Jinks-Robertson, 2009). Errors occurring during replication can also be taken care of by the mismatch repair (MMR), where mispaired nucleotides are removed by degradation of the erroneous daughter strand past the mismatch, followed by re-synthesis of the excised part (Hoeijmakers, 2001).
Figure 13. Illustration of the repair pathways involved in DNA damage. The DNA damage (adduct) may be repaired prior to replication by BER, NER and RR. Unrepaired lesions result in a stalled replication and recruitment of other repair systems. Some of the post-replication pathways may be both error-free and error-prone, where the latter results in either mutations (HPRT test in the thesis) or aberrations (micronucleus test in the thesis). Repair pathways in bold have been studied in the thesis. (Modified from an illustration by D. Jenssen, SU.)

In the work presented in Paper I, the repair pathways BER, NER and HR were investigated for involvement of glycidol-induced adducts. Different cell lines (CHO) with defect repair systems were used. If a repair defect cell line treated with glycidol generates more strand breaks compared to treated wild type cells, that specific studied repair pathway is important for the glycidol-induced lesions. Detection of strand breaks was performed with the alkaline DNA unwinding technique (ADU). The basis for this technique is the assumption that the amount of single-stranded DNA in alkali treated cells correlates to the number of strand breaks in the genome (Erixon and Ahnström, 1979). Briefly, the DNA was labelled with $^3$H-thymidine (incorporated in the DNA) prior to exposure to glycidol. After unwinding of the DNA using NaOH and sonication, the single stranded DNA (ssDNA) and double stranded DNA (dsDNA) were separated using hydroxylapatite chromatography (ion chromatography). dsDNA binds more tightly to the stationary phase compared to ssDNA, due to double negative charges. Elution was performed with potassium phosphate buffers of different ionic strengths. The analysis was performed by scintillation counting of the $^3$H-thymidine labeled DNA strands. The ratio between ssDNA and dsDNA represents the number of strand breaks per cell. The results are further discussed in Chapter 6 and Paper I.
5.2 Genotoxicity *in vivo*

5.2.1 Induction of micronuclei *in vivo*

A common method for the study of genotoxicity *in vivo* is the short-term *in vivo* micronucleus (MN) test. A MN is a small, extranuclear body resulting from fragments of the whole chromosome, which is not properly attached to the spindle apparatus during cell division (Figure 14). The frequency of MN increases following exposure to genotoxic compounds and can be used as a biomarker of chromosomal instability, i.e. genetic changes.

![Figure 14](image)  
**Figure 14.** Formation of a micronucleated cell after exposure to a genotoxic compound, Cpd (A). During the cell division chromosome fragment(s) or whole chromosome(s) lag behind (B-C), which results in the extranuclear body, i.e. the micronucleus (D).

MN can be monitored in all tissue with cell divisions, but is often investigated in young erythrocytes in the bone marrow or in peripheral blood. Young immature (polychromatic) erythrocytes (PCE) are developed from erythroblasts in the bone marrow. During the maturation step to PCE the main nucleus is expelled. The bone marrow PCEs then migrates to the peripheral circulation where they mature to normochromatic erythrocytes (NCE) without RNA and DNA. RNA is still left in the cytoplasm of PCEs, which makes monitoring and discrimination from NCEs relatively simple (through RNA staining). If a MN is part of the erythrocyte (PCE or NCE) DNA is present and will be possible to discriminate from other cells through DNA specific staining.

The history of methodologies for the detection of MN stretches more than 100 years back in time. The first description of a MN was done in the shift around the 19th/20th centuries by the hematologists W. Howell and J. Jolly who found small inclusions in erythrocytes from cats and rats. They called
these inclusions for Howell-Jolly bodies (reviewed by Sears et al., 2012). Since then, different methods for detection of MN in erythrocytes in vivo have developed. The predecessor of the methods used today was developed during the 1970s when Schmid and co-workers set up the procedure for monitoring of MN in bone marrow (Schmid et al., 1975). Discrimination between NCE and PCE was performed through staining with May-Grünwald Giemsa and microscopy scoring.

One important milestone was the automation of the analysis of MN using flow cytometry instead of manual microscopy scoring. Hutter and Stöhr were the pioneers in this field, who developed a flow cytometry method where fluorescent dyes for DNA and proteins were used to discriminate normal erythrocytes (non-nucleated) from micronucleated erythrocytes in bone marrow (Hutter and Stöhr, 1982). The sensitive dual-laser flow cytometry technique, used in this thesis work, was introduced in 1992 by Grawé and co-workers. Specific staining for DNA and RNA enable discrimination of PCE from NCE, with or without MN at the same analysis run (Grawé et al., 1992). A dot plot showing the different regions of interest based on DNA and RNA content is illustrated from a fictive flow cytometry analysis in Figure 15.

5.2.2 Short-term in vivo micronucleus test

In the studies of this thesis glycidol and 3-MCPD have been investigated using the short-term in vivo MN test in male and female BalbC mice, respectively. Below follows a brief discussion of the applied procedure. The results are further discussed in Chapter 7 and in Paper II-III.

![Figure 15](image-url)

**Figure 15.** Schematic flow cytometry dot plot showing regions of interest for the separation of cells based on their content of RNA and DNA. In a real sample usually several 100 000 cells are analyzed, where each dot corresponds to one cell.
The animals were dosed with glycidol or 3-MCPD via intraperitoneal (i.p.) injection. One reason for this administration route and not the oral route, which is more relevant to humans, is to ensure complete availability to the systemic circulation. Also, i.p. administration avoids potential hydrolysis in the GI tract and other first-pass effect that could occur after oral administration. Smaller standard deviations between mice within the same dose group are generally observed when treating the animals intraperitoneally compared to orally (L. Abramsson-Zetterberg, personal communication). The ability to observe small variations is important when treating the animals with weak genotoxic compounds (where the expected response is small). Also, little spread in data between animals gives more certain results and enables fewer animals per dose group, which is in line with 3R (reduce – replace – refine).

At 45 hours after administration, peripheral blood was taken from the orbital plexus of the animals, and prepared for analysis of MN by purification, fixation, and staining with fluorescent dyes (Abramsson-Zetterberg et al., 1996, 1995; Grawé et al., 1993; 1992). The DNA was stained with Hoechst 33342 and the RNA with thiazole orange. The flow cytometer was equipped with argon lasers (488 nm) and UV (350 nm) which enabled simultaneous detection of DNA and RNA in a large number of cells in a short time. For glycidol and 3-MCPD about 100 000 – 200 000 PCE’s were counted per animal, and dot plots as in Figure 15 were obtained. The large number of counted cells increases the sensitivity and the statistical power compared to the manual microscopy-based method (ca. 2000 – 4000 PCE).

The number of PCE in relation to the total number of erythrocytes (PCE + NCE) is used as a measure of dose dependent bone marrow toxicity. Genotoxicity is expressed as the frequency of MPCE (fMPCE), where the number of MPCEs is related to the total number of PCE. Information about the DNA content in the cells can also be extracted. An aneugenic\(^7\) effect of the studied compound results in a high mean DNA content, due to presence of intact chromosomes in the micronuclei (Grawé et al., 1994). Clastogenic\(^8\) compounds break the chromosome into fragments and therefore the mean DNA content in the micronuclei is not as high.

---

\(^7\) Aneugenic compounds cause abnormal number of chromosomes in the daughter cells. This is due to a non-functioning spindle apparatus, which fails to separate the chromosomes.

\(^8\) Clastogenic compounds cause disruption or breakage of chromosomes.
5.3 Dosimetry of electrophilic compounds

5.3.1 Reactivity and adduct formation
Electrophilic compounds are difficult to analyze in their free forms due to their reactivity. The reactivity results in formation of reaction products (adducts) with nucleophilic sites (e.g. N, O and S) in biomacromolecules.

The nucleophilic strength \( (n) \) of the sites is one determinant of the reaction rate. The reactivity commonly increases with increasing \( n \)-values in the order O \( (n \sim 2) \) < N \( (n \sim 4) \) < S \( (n \sim 6) \). The pKa of the nucleophilic atoms is another factor affecting the reactivity as well as steric factors, particularly of bulky electrophiles. Figure 16 highlights some nucleophilic sites in amino acids in hemoglobin (Hb) and in the DNA bases.

The half-life in vivo of reactive compounds may vary from seconds up to hours, depending on their reactivity and rate of metabolism. Therefore, detection of the corresponding stable adducts offers a tool for the measurement of internal doses of such reactive compounds. Biomacromolecules that have been used as monitor molecules in vivo are DNA and the blood proteins Hb and serum albumin (SA). The high levels in blood and the known lifetimes of Hb (ca. 120–150 mg/mL, ca. 4 months in humans) and SA (ca. 30 mg/mL, ca. 26 days of half-life in humans) make these molecules suitable for monitoring of electrophilic compounds. It is more difficult to use DNA as a monitor molecule due to the low levels in the blood (ca. 0.005-0.008 mg/mL) and the activity of repair enzymes removing DNA adducts. The rate of formation of adducts to DNA and Hb from exposure to genotoxic compounds are correlated, as observed in animals (c.f. Segerbäck, 1985). Therefore, the internal dose of genotoxic compounds could be measured by protein adducts instead of DNA adducts, even though it may seem more appropriate to measure adducts to DNA, the target for genotoxic response (Törnqvist et al., 2002; Osterman-Golkar et al., 1976). In this thesis Hb adducts of the model compounds (glycidol and 3-MCPD) have been measured, as described below.
Figure 16. Examples of amino acids and the DNA bases with some nucleophilic sites (in red) for adduct formation and their corresponding pKa values.

5.3.2 Cob(I)alamin as a tool for in vitro dosimetry

A nucleophilic agent can be used for trapping of short-lived electrophiles in a solution (in vitro) through the formation of adducts, to enable measurement of the concentration/dose of the electrophilic compound. In this thesis work cob(I)alamin has been applied as a tool for trapping of glycidol in cell cultivation medium, with the aim to calculate internal doses in experiments with cells (Paper I). Cob(I)alamin is the reduced form of cobalamin (vitamin B₁₂) and referred to as a “supernucleophile” due to its high nucleophilic strength \( (n = 10) \) compared to other nucleophiles like thiosulphate \( (n \sim 6) \) and aniline \( (n \sim 4) \) (Haglund et al., 2003; Schrauzer et al., 1969).

The applied procedure for trapping of electrophilic compounds in vitro with cob(I)alamin was initially developed by Haglund et al. (2006; 2003). First, cob(III)alamin is reduced to cob(I)alamin by the reducing agent sodium borohydride (NaBH₄) in an inert environment (bubbling of argon gas in the solution) and in the dark for ca. 10 min. The reaction is catalyzed by cobalt(II) nitrate. The subsequent reaction (ca. 20 min) is initiated by the addition of the electrophile (glycidol in the present work) to the solution generating an alkyl cobalamin, which is analyzed with LC/MS/MS. The procedure is briefly illustrated in Figure 17.
5.3.3 Hemoglobin adducts used for in vivo dosimetry

Hemoglobin (Hb) is a blood protein that in adult humans consists of four folded polypeptide chains: two α-chains and two β-chains containing 141 and 146 amino acids, respectively. Major sites for adduct formation in Hb for alkylating agents, such as simple epoxides, are the sulfur in cysteine, the ring nitrogens of histidine and the terminal amino group in valine (in both α and β subunits) (Figure 16). The high abundance of Hb in blood and its well defined life span, where the stable adducts follow the life span of the erythrocytes in Hb, make Hb adducts useful as biomarkers for both acute and chronic exposures.

A brief historical review of the development of the Hb adduct methods at Stockholm University is illustrated in Figure 18. Already in the mid-1970s the dosimetry of alkylating agents in mice was investigated through isolation of chromatographically separated radiolabelled histidine adducts, measured by scintillation counting (Osterman-Golkar et al., 1976). In a work by Calleman et al. the analytical tool was developed further for GC/MS, for analysis of histidine adducts of ethylene oxide in occupationally exposed workers (Calleman et al., 1978). Later a faster and more sensitive method based on a modified Edman degradation was developed (Törnqvist et al.,...
Here a detachment of a modified (with adduct) N-terminal valine in Hb was achieved using a fluorinated Edman reagent, pentafluorophenyl isothiocyanate (PFPITC) for derivatization, which gave an improved sensitivity in the GC/MS analysis. The so-called N-alkyl Edman method made it possible to quantify very low adduct levels, i.e. background levels of ca. 1-2 pmol/g Hb, using GC/MS/MS, and has been the method of choice for a long time.

Along with the increased use of the LC/MS technology further development of the Hb adduct method was conducted to achieve faster procedures. In 2010 von Stedingk et al. published a protocol for a new modified Edman method referred to as the “adduct FIRE procedure” (von Stedingk et al., 2010). This procedure is adapted for LC/MS/MS analysis. The use of LC/MS/MS allows more easy analysis of high-molecular weight compounds and non-volatile compounds compared to GC/MS/MS. Several alternative derivatization reagents have been tested, where fluorescein isothiocyanate (FITC) was chosen based on superior sensitivity for LC/MS analysis (Rydberg et al., 2009). The FIRE procedure has been applied in this thesis work and is schematically illustrated in Figure 19.
Glycidol and 3-MCPD both form the Hb adduct \( N\)-(2,3-dihydroxypropyl)-valine (diHOPrVal) (Figure 20). In the studies presented in Paper II and Paper III Hb adducts from glycidol or 3-MCPD, respectively, were quantified in blood from treated mice with the aim to correlate micronuclei formation to internal dose. In Paper IV the Hb adduct levels from glycidol were quantified in mice and rats to be used for more accurate estimation of the relative cancer risk coefficient of glycidol in the animals. Finally, in Paper V calculation of the internal dose of glycidol in humans from measured diHOPrVal adducts in blood samples was performed, with the aim to estimate the cancer risk from glycidol in food.

Figure 19. General description of the different steps of the FIRE procedure used in the thesis work for measurement of Hb adducts. SPE: solid phase extraction

Figure 20. Reaction between glycidol or 3-MCPD and \( N\)-terminal valine in Hb generates the adduct \( N\)-(2,3-dihydroxypropyl)-valine (diHOPrVal). Addition of the reagent fluorescein isothiocyanate (FITC) detaches the modified \( N\)-terminal valine. Acidification leads to ring closure which results in the analyte, diHOPrVal-FTH (FTH, fluorescein thiohydantoin). Inserted is the crystal structure\(^9\) of one \( \alpha\)-helix of Hb with the heme group (center) and the 2,3-dihydroxypropyl adduct attached to the \( N\)-terminus of valine (top right corner).

\(^9\) Structure kindly provided by Dr. Daniel Mucs (Swetox, Sweden).
5.3.4 Internal exposure dose (AUC) calculation

The internal dose in the studied system can be expressed as the concentration of the compound over time, measured as the area under the concentration-time curve (AUC, with the unit \( M \times h \), Figure 21). The AUC *in vivo* reflects the net effect of absorption, distribution, metabolism and excretion of the compound. Measurement of the AUC is important for accurate determination of genotoxic potencies (response per internal dose) of electrophilic/genotoxic compounds.

![Figure 21](image.png)

**Figure 21.** The internal dose is expressed as area under the concentration-time curve (AUC), schematically illustrated here. The AUC is equivalent to a net effect of all kinetic reactions of a compound.

The AUC can be calculated from a measured adduct level \( A \) if the second-order reaction rate constant \( k_Y \) for adduct formation to a specific site \( Y \) is known (Ehrenberg et al., 1983). When \( k_Y \) denotes the rate constant for formation of Hb adducts the unit is given as mol/g per Mh (or \( L \times g^{\frac{1}{1}} \times h^{-1} \)) (g denotes the gram of Hb). Equation 3 illustrates a simple case when an adduct level to Hb is measured immediately or a short time after an acute exposure.

During a chronic (continuous) exposure, Hb adducts will accumulate until a steady state level \( (A_{ss}) \) has been reached. This is due to a simultaneous formation of adducts and elimination of adducts following the erythrocyte life span \( (t_{er}) \). A steady state occurs when the exposure period has exceeded the life span of erythrocytes and the daily adduct level increment \( (a) \) could be calculated according to Equation 4 (Törnqvist et al., 2002; Granath et al., 1992).

\[
\begin{align*}
AUC &= \frac{\text{Adduct level (A)}}{\text{Rate constant (}k_Y\text{)}} \\
A(t) &= a \times t \left(1 - \frac{t}{2t_{er}}\right); \quad A_{ss} = A(t \geq t_{er}) = a \frac{t_{er}}{2}
\end{align*}
\]

Equations 3 and 4.
5.4 LC/MS/MS

The analyses of adducts to cobalamin and to Hb in the present work have been performed with LC/MS/MS. The chromatographic separation was obtained using reversed phase columns (C18) with the mobile phases running in gradient mode, from low to high organic solvent concentration. Two different LC/MS/MS systems have been applied. In Paper I-II a quadrupole linear ion trap mass spectrometer (Sciex API3200) was used in multireaction monitoring (MRM) mode. In Paper III-V ultra-pressure liquid chromatography (UPLC) connected to a high resolution mass spectrometer (HRMS) (Thermo Fisher Scientific, Q Exactive™ HF Hybrid Quadrupole-Orbitrap™) operating in parallel reaction monitoring (PRM) mode was applied. Electrospray in positive ionization mode (ESI+) was applied for both instruments. A major advantage of using HRMS is the possibility to obtain accurate masses. The application of HRMS in this thesis work facilitated the analysis of Hb adducts appearing at very low levels, as the \( m/z \) of the targeted ion could be monitored with very high specificity and at a low LOQ (limit of quantification).

5.5 The rad-equivalence approach

The rad-equivalence approach (or relative genotoxic potency) was early suggested as an approach for risk estimation of chemical carcinogens (Ehrenberg, 1980). The approach refers to the number of rads\(^{10}\) (dose unit of ionizing radiation) giving the same genotoxic response (or risk) as a unit of a chemical dose, e.g. mMh (Equation 5) (Granath et al., 1999; Ehrenberg, 1996). Ionizing radiation serves as a reference standard, as its carcinogenic potency in humans is well known from studies in A-bomb survivors from Hiroshima Nagasaki (Pierce et al., 1996).

\[
\text{Rad - equ.} = \frac{\text{Compound (genotox/mMh)}}{\text{Radiation (genotox/ rad)}} = \text{rad/mMh} \tag{5}
\]

The rad-equivalence has been calculated for glycidol based on induction of HPRT mutations (Paper I) and micronuclei (Paper II). Historical values of the genotoxic effect per dose unit of ionizing radiation in these systems have been applied for the calculations (HPRT: Silvari et al., 2005; MN: Abramsson-Zetterberg et al., 1995). The results for glycidol were further used for comparison with other genotoxic compounds and for comparison

\(^{10}\) 100 rad = 1 Gray (Gy): absorbed energy (joule) per kg matter.
with the cancer risk coefficient obtained with the relative risk model, which is further discussed in Chapter 10.

### 5.6 The multiplicative risk model

The multiplicative (relative) risk model (Equation 6) is the model accepted for projection of cancer risk for ionizing radiation (BEIR, 2006). The model has been further developed and evaluated for genotoxic compounds, namely ethylene oxide (Granath et al., 1999), acrylamide (Törnqvist et al., 2008), butadiene (Fred et al., 2008) and now also glycidol, discussed in Chapter 8 and Paper IV. The model describes the probability ($P$) for neoplasms in a target tissue ($i$) of mice and rats exposed to target dose, AUC ($D$). The background incidence ($P^0$) represents the cumulative risk for neoplasm formation in the target tissue among unexposed subjects and ($\beta$) is the relative risk coefficient per dose unit. Equation 6 can be approximated by Equation 7 at low doses/low induced tumor incidence. The relative risk coefficient is assumed to be approximately independent of species, sex, and tumor site, as observed for ionizing radiation and a few genotoxic compounds. The relative risk coefficient could be seen as a measure of the cancer initiating ability which interacts with background conditions in the development of cancer. The background cancer incidence is a rough measure of background conditions promoting cancer development. From the relative risk coefficient, the doubling dose ($1/\beta$) can be calculated, representing the dose giving a doubling of risk compared to the background risk.

$$P_i(D) = 1 - e^{P_i^0(1+\beta D)}$$  \hspace{1cm} (6)

$$\Delta P = P^0 \times \beta \times D$$  \hspace{1cm} (7)

Measurement of the dose in the target tissue is difficult. An even distribution of the genotoxic agent throughout the body is assumed for a compound like glycidol, and the internal dose can then be inferred from Hb adduct measurements in blood, as a surrogate for target dose. This assumption has shown to be valid for the related compound ethylene oxide, which showed approximately the same doses in blood (from Hb adducts) and in different organs, measured from DNA adducts (Segerbäck, 1985).

One aim of the thesis was to evaluate the relative risk model based on relative genotoxic potency for glycidol, obtained from the *in vitro* and *in vivo* genotoxicity tests (Chapter 10).
6. *In vitro* genotoxicity of glycidol (Paper I)

The primary aim of the study in Paper I was to quantify the mutagenic potency of glycidol in mammalian cells. Also, the involved repair mechanism(s) of the glycidol induced DNA lesions have been investigated.

### 6.1 *In vitro* dosimetry

To enable a quantitative evaluation of the mutagenicity of glycidol the doses, in the meaning of AUC, of glycidol were measured in the cell medium in incubations corresponding to the treatment of cells. This was performed by trapping glycidol with reduced cobalamin (cob(I)alamin), according to the method described in Chapter 5. The formed alkyl cobalamin was measured by LC/MS/MS. According to this measurement the concentration of glycidol during 1–4 hours of incubation remained essentially unchanged. Therefore, glycidol was assumed to be stable during the 1-hour cell treatment time at the used concentrations (cf. Figure 2, Paper I). Thus, the AUC of glycidol was thereby equal to the initial concentration in the medium times the cell treatment time (mM × h).

### 6.2 Cell survival and mutagenicity

Cytotoxicity and mutagenic potency of glycidol were investigated in wild-type and base excision repair (BER) deficient Chinese hamster ovary (CHO) cells. Due to a defect *XRCC1*-gene in the BER deficient cell line, these cells are more sensitive to DNA damaging agents, such as radiation and electrophilic compounds (Thompson, 1991). The induction of mutations was measured by the HPRT mutation test, described in Chapter 5. The cells were treated with glycidol for one hour. Cytotoxicity was observed at the highest concentrations of glycidol in both cell lines, with a pronounced effect in the BER deficient cells compared to wild-type cells (Figure 22A). Glycidol also induced mutations in both cell lines, but due to the enhanced cytotoxicity in the BER deficient cells a limited dataset was available for the evaluation of the mutagenicity in these cells. The mutagenic potency in wild-type cells was $0.08 \pm 0.01$ mutations/10$^5$ cells per mMh (Figure 22B).
6.3 Relative genotoxic potency in vitro

The mutagenic potency of glycidol was further transformed to “relative genotoxic potency” by using the rad-equivalence approach (Chapter 5). The mutagenic potency of glycidol, $0.08 \pm 0.01$ mutations/$10^5$ cells per mMh, was compared to that of ionizing radiation ($\gamma$), also derived from experiments in CHO cells, $0.0083 \pm 0.0034$ mutations/$10^5$ cells per rad (Silvari et al., 2005). The mean relative genotoxic potency of glycidol was estimated to $9.5$ rad-equ./mMh. This value is lower than those for ethylene oxide and glycidamide, two epoxides that have been evaluated for mutagenicity in the same system. The relative genotoxic (mutagenic) potencies of these epoxides are approximately $40$ rad-equ./mMh (ethylene oxide) and $400 \pm 300$ rad-equ./mMh (glycidamide), respectively (Silvari et al., 2005; Kolman et al., 2002; 1988).

6.4 DNA repair

The use of BER deficient cells in the cytotoxicity and mutation studies indicated that BER is involved in the repair of glycidol-induced lesions. In order to get further insight into the mechanism(s) behind the mutagenic effect an investigation was conducted with repair deficient cells. More mechanistic studies would be required to get the whole picture, but that was not within the scope of this work.
In studies with cells exposed to glycidol and analyzed by the alkaline DNA unwinding (ADU) technique (Chapter 5), an increased level of strand breaks and a delayed time for repair were observed in repair deficient cells compared to wild type cells. The largest effect was observed in BER (XRCC1 protein) and NER (ERCC1 protein) deficient cells, indicating that these repair pathways may be important for the repair of glycidol-induced lesions (c.f. Figure 4 in Paper I).

PARP-1 (poly-(ADP-ribose)-polymerase 1) is a protein that has been suggested to have a role in the BER pathway, where it binds to a nick in the DNA strand break. This leads to a rapid assembly of the XRCC1 protein to the lesion and thereby induces repair. In experiments with cells exposed to glycidol and an inhibitor of the PARP-1 (4-amino-1,8 naphthalimide; 4-ANI) further increases of strand breaks and further delayed repair times were observed in BER deficient cells and wild-type cells treated with glycidol (Figure 23A). This confirmed that the BER pathway is of importance in the repair of the glycidol-induced lesions.

From similar experiments, but with specific inhibition of the NER pathway by a mixture of cytosine arabinoside, AraC (inhibition of the DNA polymerase) and hydroxyurea, HU (decrease of the nucleotide pool), it was concluded that NER is not involved in the repair of glycidol-induced lesions (Figure 23B). This discrepancy from the assumed NER-dependency (above) can be explained by the fact that overlaps occur in DNA repair processes, where proteins participate in more than one pathway (Bjergbæk, 2012).

Figure 23. (A) Increased levels of strand breaks (SB) in glycidol-treated BER deficient cells compared to wild type (wt) cells with/without PARP1-inhibition by 4-ANI indicate a pathway dependent on BER/PARP1 for repair. (B) No difference in the level of glycidol-induced strand breaks (SB) in wild-type (wt) cells with or without inhibition of NER by AraC/HU indicates that the NER pathway is not involved in the repair (originally published in Paper I, Figure 5-6).
Apart from being active in NER the ERCC1 protein can also act in a complex with the protein XPF, which is active in double strand break repair (Ahmad et al., 2008). Glycidol has been demonstrated to delay the replication fork elongation (cf. Figure 7 in Paper I), which means that lesions escape excision repair pathways and proceed to replication, likely followed by triggering of specific strand break repair mechanisms, such as the ERCC1-XPF complex. It is therefore likely that the increased level of strand breaks observed in the ERCC1 deficient cells are due to the role of this complex instead of NER.

6.5 Conclusions from *in vitro* genotoxicity studies of glycidol

From the studies of mutations and DNA repair it can be concluded that glycidol is a genotoxic compound. From the mutation studies a quantitative measure (as rad-equ. per mMh) was obtained that has been further compared with the relative risk coefficient from carcinogenicity studies of glycidol and used for risk estimation, as discussed in Chapter 10. The qualitative results from the studies of DNA repair indicate a complex pattern, where multiple pathways seem to be involved in the repair of glycidol-induced lesions. Here the BER pathway was indicated to be involved, in agreement with earlier studies of the epoxide glycidamide (Johansson et al., 2005).
7. *In vivo* genotoxicity of glycidol and 3-MCPD (Paper II–III)

As esters of glycidol and 3-MCPD (3-monochloropropane-1,2-diol) are formed simultaneously in processing of cooking oils it was of interest to investigate their respective genotoxic potencies. In Paper II and Paper III the aims were to quantify the induction of micronuclei (MN) in mice per *in vivo* dose of glycidol and 3-MCPD, respectively.

7.1 Micronucleus test

Male and female mice (BalbC) were administered glycidol or 3-MCPD by a single intraperitoneal injection, respectively. The dose ranges were similar to those used in genotoxicity studies *in vivo* presented by others (3-MCPD: Robjohns et al, 2003; glycidol: NTP, 1990), and below their LD₅₀ (Table 3).

**Table 3.** Treatment details of mice (BalbC) administered glycidol and 3-MCPD.

<table>
<thead>
<tr>
<th></th>
<th>Sex</th>
<th>Dose level (mg/kg b.w.)</th>
<th>Administration i.p. (μL/g b.w.)</th>
<th>Number of animals per dose level</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glycidol</strong></td>
<td>male</td>
<td>0, 30, 60, 90, 120</td>
<td>10</td>
<td>3 (5 at 120 mg/kg)</td>
</tr>
<tr>
<td><strong>3-MCPD</strong></td>
<td>female</td>
<td>0, 50, 75, 100, 125</td>
<td>15</td>
<td>4 (5 at 100, 125 mg/kg)</td>
</tr>
</tbody>
</table>

*a Vehicle control, PBS. *b Vehicle control, 0.9 % saline.

The genotoxicity was monitored based on the induction (frequency) of MN in young (polychromatic) erythrocytes, PCE (fMPCE) in peripheral blood, taken from the orbital plexus at 45 h after dosing according to the method described in Chapter 5. Analysis was performed with dual-laser flow cytometry. These studies showed a significant dose-dependent induction of the MN for mice treated with glycidol, where the *in vivo* dose was calculated from measured Hb adducts (see Chapter 7.2), giving a genotoxic potency of 12 ‰ per mMh.
Figure 24. Induction of MPCE in peripheral blood in mice by glycidol and 3-MCPD. A significant induction per in vivo dose (AUC) was observed for glycidol (p < 0.001), but not for 3-MCPD (modified from Figure 5B in Paper III).

No induction of MN was observed in the mice treated with 3-MCPD (Figure 24, Table 4). Genotoxic concern, in terms of induction of MPCE, should therefore only be due to glycidol at simultaneous exposure to the compounds.

7.2 In vivo dosimetry

The levels of the formed Hb adduct, N-(2,3-dihydroxypropyl)-valine in blood from mice treated with glycidol or 3-MCPD were analyzed according to the FIRE procedure (Chapter 5). The adduct level in the glycidol-treated mice was about 500 times higher than in the 3-MCPD-treated mice when compared at equal administered amount (Table 4, Figure 25). A large difference between the compounds was also reflected in the rate constants, \( k_{\text{val}} \), where glycidol had about 1200-fold higher reactivity than 3-MCPD towards the N-terminal valine in hemoglobin (Table 4). The in vivo doses (AUC), were calculated from the Hb adduct levels and \( k_{\text{val}} \), according to Equation 3 (Chapter 5).

Hence, glycidol is more prone to form Hb adducts (19.4 pmol/g Hb per \( \mu \text{Mh} \)) compared to 3-MCPD (0.016 pmol/g Hb per \( \mu \text{Mh} \)) (c.f. Paper I and Paper II). The large difference in reactivity could be an underlying explanation to the observed differences in genotoxicity and is likely explained by the chemical structures of glycidol and 3-MCPD (Figure 7), where the epoxide function in glycidol is more reactive compared to the alkyl-chloride in 3-MCPD.
Figure 25. Hemoglobin adduct levels in blood from mice treated with glycidol and 3-MCPD. The levels due to glycidol exposure are about 500 times higher compared to 3-MCPD (note the different scales on the y-axis).

Table 4. Induction of micronuclei (fMPCE) and in vivo dose (AUC) of glycidol and 3-MCPD in mice (originally published in Paper III). The AUC is calculated from the Hb adduct levels and $k_{val}$.

<table>
<thead>
<tr>
<th></th>
<th>$k_{val}$ (in vitro)</th>
<th>Hb adduct increment (in vivo)</th>
<th>AUC increment (in vivo)</th>
<th>fMPCE increment (in vivo)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pmol/g per μMh</td>
<td>pmol/g per mg/kg</td>
<td>μMh per mg/kg</td>
<td>% per mg/kg (95% CI)</td>
</tr>
<tr>
<td>3-MCPD</td>
<td>0.016</td>
<td>0.035</td>
<td>2.15</td>
<td>no induction*</td>
</tr>
<tr>
<td>Glycidol</td>
<td>19.4</td>
<td>19.4</td>
<td>0.98</td>
<td>0.013 (0.007 – 0.018)</td>
</tr>
</tbody>
</table>

7.3 Relative genotoxic potency of glycidol in vivo

The relative genotoxic potency of glycidol for the induction of MN was calculated to be 15 rad-eq./mMh, using the rad-equivalence approach (Chapter 5): the genotoxic potency of glycidol (12 %/mMh) was compared to the genotoxic potency for ionizing radiation, obtained from studies of mice exposed to low doses of β- and γ-radiation; 0.77 %/rad (Abramsson-Zetterberg et al., 1995). The relative genotoxic potency of glycidol has been used for comparison with the risk coefficient obtained from carcinogenicity studies of glycidol, further discussed in Chapter 10.
The genotoxic potencies of other epoxides; ethylene oxide and propylene oxide for induction of MN in mice (bone marrow PCEs) are approximately 65 rad-equ./mMh and 230 rad-equ./mMh, respectively. These estimated figures have been calculated from data by Farooqi et al. (1993) and with preliminary dose estimates. Although a direct comparison to glycidol cannot be made with complete accuracy as the methods for the analysis of Hb adducts and for induction of micronuclei differ between the compounds, the data indicate that glycidol is less potent than these epoxides in terms of inducing MN.
8. Evaluation of the multiplicative risk model for glycidol (Paper IV)

The evaluation of the applicability of the multiplicative (relative) risk model for glycidol has been divided into two parts. In the first part (Paper IV) (Figure 26, Part 1) the applicability of the model was tested, using data from published rodent carcinogenicity studies and \textit{in vivo} dosimetry. In the second part (only in the summary of the thesis), the applicability of relative genotoxic potency data as a risk coefficient in the relative risk model was evaluated (Figure 26, Part 2) and is discussed in Chapter 10.

\textbf{Figure 26. Part 1} shows briefly the work-flow where the multiplicative (relative) risk model and \textit{in vivo} dosimetry are used for the derivation of the doubling dose (doubling of the background risk for tumor induction) from published rodent carcinogenicity studies. \textbf{Part 2} shows the work-flow where the application of relative genotoxic potency (Q) (from mutagenicity studies in cells or \textit{in vivo} short-term studies) is evaluated for the derivation of relative risk, which is compared with $\beta$ obtained from carcinogenicity studies in Part I.
8.1 Carcinogenicity data

The carcinogenic potency of glycidol has previously been investigated in both sexes of F433 rats and B6C3F1 mice, administered glycidol or vehicle (water) via gavage five times per week for 103 weeks (Irwin et al., 1996; NTP, 1990). The mice were administered 25 and 50 mg/kg b.w. and the rats 37.5 and 75 mg/kg b.w. The number of neoplasms observed in both strains as compiled in these published studies were used for cancer risk estimation applying the relative risk model, as described here and in Paper IV.

8.2 Internal dose of glycidol

The internal \((in \, vivo)\) doses of glycidol were not measured in the treated animals in the published carcinogenicity studies. Therefore, a study where glycidol was administered by gavage to Sprague Dawley rats and B6C3F1 mice was conducted at similar dose levels and dosing regimen as in the carcinogenicity studies (Table 5). The formed Hb adduct, \(N\)-(2,3-dihydroxypropyl)-valine was quantified in blood from the animals using the FIRE procedure with UPLC/MS/MS analysis (Chapter 5). The \(in \, vivo\) doses were calculated from the Hb adduct levels and the second-order reaction rate constant, \(k_{\text{val}}\) (Equation 3, Chapter 5), determined for each species in \(in \, vitro\) experiments. About equal rate constants were obtained for glycidol in both species (Table 5) and were also about the same as that obtained in mouse blood of a different strain (Chapter 7, Paper II).

The \(in \, vivo\) doses in the rats were approximately double compared to the mice \((p < 0.01)\), indicating species differences in the pharmacokinetics (Table 5). There were no statistically significant differences between the sexes of any species, c.f. Figure 1 in Paper IV.

\textbf{Table 5}. Treatment details (gavage) of rats and mice (both sexes) and results on Hb adduct levels and the corresponding \(in \, vivo\) doses (AUC) of glycidol.

<table>
<thead>
<tr>
<th></th>
<th>Dose level(^a) (mg/kg b.w.)</th>
<th>(k_{\text{val}} (in , vitro)) (pmol/g Hb per (\mu)Mh)</th>
<th>Hb adduct level per administered dose (pmol/g Hb per mg/kg)</th>
<th>AUC per administered dose ((\mu)Mh per mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sprague Dawley</td>
<td>0(^b), 37.5, 75 1 \times 5 days</td>
<td>23.7</td>
<td>68.8 ± 37.9</td>
<td>2.9 ± 1.6</td>
</tr>
<tr>
<td>B6C3F1</td>
<td>0(^b), 25, 50 1 \times 5 days</td>
<td>19.3</td>
<td>29.3 ± 10.4</td>
<td>1.6 ± 0.6</td>
</tr>
</tbody>
</table>

\(^a\) Three animals per dose level, 10 mL per kg b.w. \(^b\) Vehicle control, tap water.
8.3 Relative risk coefficients and doubling doses

The tumor incidence data of glycidol from mice and rats was evaluated with the multiplicative (relative) risk model. The obtained relative risk coefficient (β) per administered dose was independent of tumor site and sex but differed by a factor of 2.5 between the species; mice (4.1 % per mg/kg) and rats (8.0 % per mg/kg). This difference could to a large extent be explained by the difference in the \textit{in vivo} doses between the species. When the relative risk coefficients were based on \textit{in vivo} dose approximately the same mean estimates for mice (5.1 % per mMh) and rats (5.4 % per mMh) were obtained (\textbf{Table 6}). This indicated that the risk coefficient also is independent of species when internal dose is considered. The results supported a good agreement between predicted and observed tumor incidence (\textbf{Figure 27}).

The relative risk coefficient may also be expressed as the lifetime doubling dose (1/β) that is the dose of the studied compound required to double the background cancer incidence. The lifetime doubling doses for glycidol (mean of ca. 19 mMh from mice and rats) are somewhat higher than the values obtained for ethylene oxide (mean of ca. 11 mMh from mice and rats), which is another simple epoxide that has been evaluated with the relative risk model (\textbf{Table 6}). This means that glycidol is a somewhat less potent carcinogen than ethylene oxide.

\textbf{Table 6.} Relative cancer risk coefficients and corresponding lifetime doubling doses of glycidol obtained for mice and rats using the relative risk model. The doubling doses for ethylene oxide are used for comparison.

\begin{center}
\begin{tabular}{|l|c|c|c|c|}
\hline
 & \textbf{Relative risk coefficient (β)} & \multicolumn{2}{|c|}{\textbf{Mean doubling dose (mMh)}} \\
 & of glycidol & \% per mg/kg & \% per mMh & Glycidol & Ethylene oxide$^a$ \\
\hline
\textbf{Mice} & & & & & \\
4.1 & 5.1 & 19.6 & 11.3 \\
\textbf{Rats} & & & & & \\
8.0 & 5.4 & 18.6 & 10.9 \\
\hline
\end{tabular}
\end{center}

$^a$From Granath et al., 1999.
Figure 27. A good agreement was observed between the number of predicted versus observed number of tumors in mice and rats treated with glycidol, obtained with the relative risk model. The data points correspond to a combination of number of tumors at different sexes, doses and target tissues for respective species (Paper IV).
9. Human cancer risk from glycidol exposure (Paper IV–V)

The final aim of the thesis work was to estimate cancer risk in humans due to glycidol exposure. This chapter discusses the applied approach, based on the results obtained with the multiplicative (relative) risk model (Chapter 8 and Paper IV) and from monitoring of the internal doses in human blood samples (Paper V).

9.1 Assumptions for human risk estimation

There are a few assumptions that have been made in the present work for the estimation of cancer risk to humans due to glycidol exposure. First, there are several precursors (exposures) that potentially may form the $N$-(2,3-dihydroxypropyl)-valine (dihOPrVal) adduct, illustrated in Figure 28 (Paper IV; c.f. Hindsø Landin et al., 2000). 3-MCPD, often occurring simultaneously as glycidol in food, has shown about 1200 times slower rate of adduct formation compared to glycidol (Chapter 7.2) and could only give a negligible contribution to the adduct level. There is an indication that glycidol can be formed upon heating of carbohydrate-rich food (from anhydro sugars) from experiments with rats fed with heated food (Hindsø Landin et al., 2000). Another potential precursor is allyl alcohol, found in food such as garlic (Lemar et al., 2005), which could form glycidol through metabolic oxidation (Hindsø Landin et al., 2000). Glyceraldehyde (produced endogenously) and glycidaldehyde (potentially produced endogenously) could also theoretically form the diHOPrVal adduct, but would require reduction after binding to hemoglobin as a Schiff base. The assumption has been made that the quantified adduct levels only originates from glycidol exposure, which means that an overestimation of the internal doses of glycidol cannot be excluded.

Second, the relation between intake of glycidol and the internal doses (AUC) in humans has not yet been determined, although an intervention study has been performed and will be published (personal communication with B. Monien, German Federal Institute for Risk Assessment, BfR).
Paper IV (Chapter 8) glycidol was administered to mice and rats to obtain the ratio between AUC and administered dose (Table 7).

**Figure 28.** Examples of a few possible precursors to the Hb adduct \(N-(2,3\text{-dihydroxypropyl})\)-valine.

In general, the use of rats is a better model compared to mice for estimation of the pharmacokinetics in humans. There are two other published studies where the AUC has been calculated after oral administration of glycidol to rats (Honda et al., 2014; Wakabayashi et al., 2012) (Table 7). In the study by Wakabayashi et al. monkeys were also administered glycidol (p.o). Overall, the difference between the obtained AUC per administered dose in the three species (mice, rats, monkeys) across the different studies is not large (ca. 5-fold). In this thesis work it was assumed that the pharmacokinetics of glycidol in humans are the same as for rats measured in Paper IV (Chapter 8) and for the monkeys measured with another method by Wakabayashi et al. The relation (AUC per exposure dose) was therefore assumed to be 2.9 μMh per mg/kg for humans.

**Table 7.** Relation between orally administered glycidol and internal dose (AUC per mg/kg) in different species. In yellow are the studies of this thesis (Paper IV).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sex</th>
<th>Matrix</th>
<th>Analyte</th>
<th>AUC per adm. dose (μMh per mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse(^a)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B6C3F1</td>
<td>mixed</td>
<td>erythrocytes</td>
<td>Hb adduct</td>
<td>1.6</td>
</tr>
<tr>
<td>Rats(^a)</td>
<td></td>
<td>mixed</td>
<td>erythrocytes</td>
<td>2.9</td>
</tr>
<tr>
<td>Monkeys(^b)</td>
<td></td>
<td></td>
<td>free glycidol</td>
<td>2.9</td>
</tr>
<tr>
<td>Cynomolgus</td>
<td>male</td>
<td>plasma</td>
<td>free glycidol</td>
<td>2.9</td>
</tr>
<tr>
<td>Rats(^b)</td>
<td></td>
<td>male</td>
<td>free glycidol</td>
<td>5.8</td>
</tr>
<tr>
<td>Rats(^c)</td>
<td></td>
<td>female</td>
<td>Hb adduct</td>
<td>8.2</td>
</tr>
</tbody>
</table>

\(^a\) Obtained in Paper IV, \(^b\) Calculated from Wakabayashi et al. 2012, \(^c\) Calculated from Honda et al., 2014, \(^d\) Sprague Dawley.
9.2 Estimation of human cancer risk from doubling doses

From the evaluation of rodent carcinogenicity data of glycidol with the relative risk model, the estimated relative risk coefficient ($\beta$) per internal dose was shown to be independent of species, sex, and tumor site (Paper IV, Chapter 8), indicating that it can be transferred across species. Therefore, the lifetime doubling dose ($1/\beta$) of glycidol obtained from the rodent studies could be used for cancer risk estimation in humans.

The mean of the obtained lifetime doubling doses (19 mMh) of glycidol from mice and rats corresponds to a lifetime intake in humans of ca. 6600 mg/kg, assuming the relation between intake and internal dose in humans to be the same as for rats (2.9 μMh per mg/kg), as discussed above (Table 7).

This lifetime intake would imply that a daily intake of ca. 0.25 mg/kg throughout life (70 years) would double the background cancer incidence. The background incidence is assumed to be ca. 30 % for all cancer types, which is in line with the reported cancer incidence from Cancerfonden, that at least 1/3 of the Swedish population will develop cancer over a lifetime (Cancerfonden, 2017). Using these figures, the intake of glycidol associated with a risk level of $1/10^5$ was calculated to be 0.60 μg/day (0.0086 μg/kg/day) as demonstrated in Box 1 using Equation 7 (Chapter 5). This intake is equivalent to a daily intake of for example 21 g of crisp breads, or 6 g of chocolate cake, or 4 g of cookies (using glycidol occurrence data of glycidol, from esters, by the EFSA, 2016a). The figure is much lower than the intake values calculated from measured Hb adduct levels as well as the values estimated by the EFSA and by the Swedish National Food Agency (NFA) (Table 8, Chapter 9.4). This would mean that the corresponding lifetime risk in humans exceeds the acceptable risk level $1/10^5$.

9.3 Internal doses in human subjects

The internal doses (AUC) of glycidol were calculated from the quantified levels of the Hb adduct $N$-(2,3-dihydroxypropyl)-valine in blood samples from children (50 subjects) and adults (12 subjects), with the assumption that the measured adduct exclusively originated from glycidol.

The measured adduct levels were considered to correspond to steady state adduct levels ($A_{ss}$). The daily adduct increment ($a$) was calculated from $A_{ss}$.
and the erythrocyte lifetime in humans (\(t_e\)), assumed to be 126 days (Equation 4, Chapter 5). The daily and lifetime AUC and intakes were further calculated by assuming the relation between the AUC and exposure dose of glycidol to be the same in humans as for rats (2.9 \(\mu\)Mh per mg/kg), as discussed above (Table 7).

9.4 Estimation of human cancer risk based on measured internal doses

For the estimation of cancer risk in humans from animal studies, the internal doses (AUC) in humans are of importance. The mean daily intake of glycidol has been estimated from occurrence values in food and food frequency questionnaires to be 0.2 \(\mu\)g/kg in adults and 0.6 \(\mu\)g/kg in children (EFSA, 2016a) or 0.1 \(\mu\)g/kg in adults (NFA, 2017). The corresponding estimated lifetime (70 years) AUC values are much lower than the lifetime AUC in children and in non-smoking adults, calculated from measured Hb adducts in Paper V (Table 8).

In Box 2 an example of the calculation of the relative risk increment for an intake of 1 \(\mu\)g glycidol/kg per day throughout life is given. A background cancer incidence of 30% was assumed (Cancerfonden, 2017). The resulting relative risk increment of 0.00117 per \(\mu\)g glycidol per kg per day is equivalent to 117 additional cancer cases in a population of 100 000 at the given exposure conditions.
The AUC measured from Hb adducts in both children and adults correspond to higher intakes of glycidol per day (>1 μg/kg per day), and accordingly to higher risk. However, overestimations of the relative risk increments cannot be excluded as other exposures may have contributed to the quantified Hb adduct levels.

Table 8. The daily intake and lifetime in vivo dose (AUC) of glycidol in children and adults, and the corresponding estimated lifetime excess cancer risk in a population of 100,000. The values have been derived from present measurement of Hb adduct levels (row 1–4) or from glycidol occurrence values in food in combination with food frequency questionnaires (row 5–7).

<table>
<thead>
<tr>
<th></th>
<th>Daily intake (μg/kg/day) Mean [min – max]</th>
<th>Lifetime AUC (μMh)</th>
<th>Lifetime excess cancer risk (cases per 100,000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Reference value</td>
<td>1</td>
<td>74</td>
</tr>
<tr>
<td>2</td>
<td>Children &lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.6 [1.0 – 4.3]</td>
<td>117 [71 – 322]</td>
</tr>
<tr>
<td>3</td>
<td>Adults (non-smokers) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.2 [1.4 – 3.0]</td>
<td>164 [100 – 224]</td>
</tr>
<tr>
<td>4</td>
<td>Adults (smokers) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.1 [3.9 – 6.8]</td>
<td>374 [290 – 503]</td>
</tr>
<tr>
<td>5</td>
<td>Adults (EFSA) &lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.2 [0.2 – 0.3]</td>
<td>15 [15 – 22]</td>
</tr>
<tr>
<td>6</td>
<td>Children (EFSA) &lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.6 [0.4 – 0.9]</td>
<td>45 [30 – 67]</td>
</tr>
<tr>
<td>7</td>
<td>Adults (NFA) &lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.1</td>
<td>7.4</td>
</tr>
</tbody>
</table>

<sup>a</sup>Paper V, <sup>b</sup>EFSA (2016a), <sup>c</sup>NFA (2017).
9.5 Comparison of estimations of human cancer risk of glycidol

9.5.1 No significant risk level
The Office of Environmental Health Hazard Assessment (OEHHA) at the California Environmental Protection Agency (C.EPA) has calculated the risk from exposure to glycidol based on the same carcinogenicity studies as have been used in the study in Paper IV (Chapter 8). In their approach the lowest administered dose inducing tumors in rodents was used for the calculation of a corresponding human cancer potency estimate to $1.3 \ (\text{mg/kg/day})^{-1}$, including scaling $(\text{b.w. human}/\text{b.w. animal})^{1/3}$. The no significant risk level (NSRL) was calculated to be 0.54 μg/day (0.0077 μg/kg/day), based on the most sensitive tumor site and by considering a lifetime cancer risk of $1/10^5$ according to Equation 8 (C.EPA, 2010). This figure is almost the same to the intake estimated from the doubling dose: 0.60 μg/day (0.0086 μg/kg/day) (Paper IV, Chapter 9.2). Accordingly, the intake values in Table 8 are orders of magnitude higher compared to these limit values for acceptable risk.

$$NSRL = \frac{1/10^5 \times 70 \ \text{kg}}{1.3 \ (\text{mg/kg/day})^{-1}} = 0.54 \ \mu g/day$$  \hspace{1cm} (8)

9.5.2 MOE based on T25
In the report by the EFSA (2016a) the cancer potency index, T25$^{11}$ (Chapter 5) was derived for glycidol from data from the published carcinogenicity studies (Irwin et al., 1996; NTP, 1990), as shown in Box 3. T25 was derived to be 10.2 mg/kg/day. The MOE (margin of exposure) for glycidol was estimated by dividing the T25 with the chronic mean and high (95th percentile) exposure levels from dietary surveys for each studied age group, summarized in Table 9. As children are estimated to be more exposed than adults their MOEs are smaller. For all children in the high exposure group and for more than half of the surveys regarding children in the mean exposure group, the MOE is below the critical value 25 000 (EFSA, 2016a). Where infants are fed exclusively by supplement to breast feeding (infant formula) the MOE is very low in both the mean and high exposure group. This is of concern as infants in general are more sensitive compared to older children and adults. However, many uncertainties are associated with the reported MOE values derived from both the limited (few doses) carcinogenicity studies and the T25 approach itself.

$^{11}$ T25: “the chronic dose rate in mg/kg b.w. per day which will give 25% of the animals tumors at a specific tissue site, after correction of spontaneous incidence, within the standard life time of that species” (Dybing et al., 1997).
Table 9. Comparison of chronic minimum and maximum (95th percentile) exposures of glycidol and the corresponding MOE values in humans at different age groups based on dietary surveys (EFSA, 2016a). A MOE lower than 25 000 is considered as safety concern.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Mean exposure</th>
<th>MOE</th>
<th>P95 exposure</th>
<th>MOE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg/kg/day</td>
<td></td>
<td>µg/kg/day</td>
<td></td>
</tr>
<tr>
<td>infants (formula only)</td>
<td>1.9</td>
<td>5 400</td>
<td>4.9</td>
<td>2 100</td>
</tr>
<tr>
<td>infants</td>
<td>0.4 – 0.8</td>
<td>25 500 – 12 800</td>
<td>1.2 – 2.1</td>
<td>8 500 – 4 900</td>
</tr>
<tr>
<td>toddlers</td>
<td>0.4 – 0.9</td>
<td>25 500 – 11 300</td>
<td>1.0 – 2.0</td>
<td>10 200 – 5 100</td>
</tr>
<tr>
<td>other children</td>
<td>0.3 – 0.9</td>
<td>34 000 – 11 300</td>
<td>0.8 – 1.7</td>
<td>12 800 – 6 000</td>
</tr>
<tr>
<td>adolescents</td>
<td>0.2 – 0.5</td>
<td>51 000 – 20 400</td>
<td>0.4 – 1.1</td>
<td>25 500 – 9 300</td>
</tr>
<tr>
<td>adults</td>
<td>0.1 – 0.3</td>
<td>51 000 – 34 000</td>
<td>0.3 – 0.7</td>
<td>34 000 – 17 000</td>
</tr>
<tr>
<td>elderly</td>
<td>0.1 – 0.3</td>
<td>102 000 – 34 000</td>
<td>0.3 – 0.6</td>
<td>51 000 – 17 000</td>
</tr>
<tr>
<td>very elderly</td>
<td>0.1 – 0.3</td>
<td>102 000 – 34 000</td>
<td>0.2 – 0.7</td>
<td>51 000 – 14 600</td>
</tr>
</tbody>
</table>

a MOE: margin of exposure is a tool for consideration of safety concern, and not to estimate a risk.

9.5.3 Summary of risk estimations of glycidol

The conclusion of all applied approaches for estimation of the cancer risk or safety margins is that exposure to glycidol is of concern to human health taking into account what is considered as an acceptable risk. The risk based on the estimated lifetime exposure of glycidol from measured Hb adducts exceeds 1/1000, whereas an acceptable lifetime risk is considered to be 1 cancer case in a population of 10^5 (WHO, 2001). As glycidol, in the form of esters, is present in a variety of food products it is almost impossible to avoid. Many of the food products are consumed mostly by children (cookies, sweets etc.) who in general are more sensitive compared to adults. There are ongoing efforts to mitigate the use of glycicyl esters in food products (Chapter 4) to reduce the levels of glycidol exposure and the associated cancer risk.
10. General discussion

In recent papers by Tomasetti and co-workers they discuss that there is a strong correlation between cancer incidence and the total number of stem cell divisions, and that about two thirds of all studied cancer types (n = 17–31) are due to “random” replication errors rather than environmental or hereditary mutations (Tomasetti et al., 2017; Tomasetti and Vogelstein, 2015). A cancer caused by mutations due to both replication errors and to environmental exposures may still be preventable, as it generally requires more than one mutation for a cancer to develop (c.f. Vogelstein and Kinzler, 1993). Therefore, prevention and risk assessments of environmental exposures are of importance.

Risk evaluations are usually performed based on data from long-term (2 years) rodent carcinogenicity studies, whereas genotoxicity studies traditionally have been used for identification of hazards and to provide a “yes or no” answer. Using quantitative analysis of data on genotoxic dose-response for cancer risk assessment is of growing interest (MacGregor et al., 2015a/b). Reliable risk assessments using genotoxicity data would result in a reduced need of the long-term carcinogenicity studies, in line with 3R.

There is no agreement of a “best practice” for cancer risk assessment of genotoxic and carcinogenic compounds and therefore different methods are used internationally (EFSA, 2005). The approach for cancer risk estimation evaluated in this thesis, the “multiplicative (relative) risk model” combined with relative genotoxic potency is relevant for the initiatives of replacing carcinogenicity studies with more cost- and time-effective alternatives. The model considers background cancer incidence, assumed to depend on the interaction between induced background mutations and promoting factor(s). The background mutations include what Tomasetti and co-workers describe as DNA replication errors (Tomasetti et al., 2017; Tomasetti and Vogelstein, 2015), but also mutations that are inherited and/or induced by endogenous and unknown exogenous factors. The promoting factors are conditions that favor cell growth and expansion. An exogenous exposure, like glycidol, will interact with the background conditions, which are reflected by the background cancer incidence. This is considered in the relative risk coefficient (risk per dose) that is expressed relative to the background cancer incidence.
10.1 The multiplicative risk model

A central part in the presented approach is monitoring of the internal doses of the genotoxic compound. Internal dose and the background tumor incidence are important parameters in the relative risk model that will improve the accuracy of cancer risk estimations (Equation 6, Chapter 5). The model was shown to be valid for carcinogenicity data of glycidol, as well as for previously evaluated epoxides: the epoxy-metabolites of butadiene, acrylamide/glycidamide and ethylene oxide (Paper IV; Fred et al., 2008; Törnqvist et al., 2008; Granath et al., 1999), where all compounds demonstrate good agreements between predicted and observed tumor incidence. This is illustrated for glycidol in Figure 27 (Chapter 8).

10.1.1 Transfer of risk between populations

The multiplicative (relative) risk model has long been used for projection of cancer risk for ionizing radiation (BEIR, 2006), which demonstrates almost equivalent relative risk coefficients in humans exposed to high doses and a few tested species (Granath et al., 1999). The relative cancer risk coefficients for glycidol and the previously studied compounds (see references in 10.1) have also demonstrated to be approximately independent of species, sex, and tumor site. This implies that that the relative cancer risk coefficients based on internal dose for genotoxic compounds also can be transferred between species and also to different human populations for the estimation of cancer risk.

A standard procedure often used for regulations of carcinogens by e.g. the Environmental Protection Agency in the U.S. is the additive (absolute) risk model (U.S. EPA, 2005), which does not consider background tumor incidence contrary to the relative risk model. This implies that overestimations of the risks may occur when extrapolating from species with high background tumor incidence to humans or other species with lower background incidence. This was demonstrated by Kuo et al. (2002) who compared the additive risk model to the relative risk model for 100 tumor types in mice and rats exposed to different types of carcinogenic compounds. Also in another comparison of the models, the relative risk model was more appropriate for projection of risk of most tumor types from mice treated with ionizing (γ) radiation to humans (survivors of the Nagasaki/Hiroshima A-bomb) (Storer et al., 1988).

There is a difference in cancer incidence between populations in different parts of the world (Figure 29), indicating population dependent background tumor incidence, but also different exposures between populations. In a study where populations in different parts of Russia were compared, the
multiplicative risk model was shown to be applicable for extrapolations of cancer risk at different ages for males and females across populations, considering health and demographic parameters (Korobitsyn, 2011).

### 10.2 Relevance of genotoxicity studies for cancer risk estimation

The recommendation for a full evaluation of the genotoxic profile of a compound includes assays that detect different mechanisms, like gene mutations, chromosomal damage, and aneuploidy (OECD, 2016). The assays employed in this thesis work (\textit{in vitro} mutations and \textit{in vivo} micronuclei) cover all three criteria. For quantitative risk assessment, \textit{in vivo} studies are in general given more weight compared to studies performed \textit{in vitro} as they are considered more relevant to humans, for example due to the presence of a complete metabolic system. It is however important to remember that the outcome of a cancer risk assessment based on genotoxicity studies is dependent on the sensitivity of the applied method and if the test system is relevant for the type of DNA damage that is being investigated.

Accumulation of genetic changes may increase the risk for cancer. Such genetic changes may be mutations and micronuclei. Studied tumors from cancer patients have demonstrated presence of mutational signatures, where a few mutations seem to be specific to certain cancers (Alexandrov et al., 2013; Kandoth et al., 2013). Also, an increased frequency of micronuclei has been shown in patients with tumors, which has led to the hypothesis that micronuclei could be used as a tool for diagnosis of cancer. It is believed that the increase in the micronuclei frequency is correlated with an increased grade of a neoplastic disease, as observed in a study of different grades of oral squamous cell carcinoma (Jadhav et al., 2011). In a study with subjects (n = 6718) from 10 countries, where the frequency of micronuclei was screened in peripheral blood lymphocytes, a significant increase in cancer incidence two decades later was observed in subjects with micronuclei frequencies in the medium or high tertile compared to those in the low tertile (Bonassi et al., 2007). In general, dose-response data from the \textit{in vivo} micronucleus test have demonstrated good correlations to data from rodent carcinogenicity studies (Soeteman-Hernandez et al., 2016). As micronuclei are good biomarkers for chromosom al instability and correlates well to cancer, efforts for risk estimations based on \textit{in vivo} micronuclei tests are reasonable. Particularly useful is the sensitive micronucleus method applied in this thesis, which to my knowledge at present only is used by L. Abramsson-Zetterberg (collaborator at the Swedish National Food Agency).
10.3 Relative genotoxic potency for cancer risk estimation

One important aim of this thesis was to investigate if data from genotoxicity studies can be used for estimation of cancer risk. The genotoxic potency compared to ionizing radiation (the rad-equivalence; relative genotoxic potency, denoted Q) is a good estimate of relative risk and is useful for comparison between compounds and different genotoxicity test systems. Ionizing radiation is considered as a good reference standard due to its well-known risk in humans. Many studies have investigated the carcinogenicity of ionizing radiation among survivors of the Hiroshima/Nagasaki A-bomb. The excess relative risk (ERR)\textsuperscript{12} coefficient of ionizing radiation for humans exposed at high doses has been estimated to ca. 0.4 % per rad (both sexes) for solid tumors (Table 10) (Preston et al., 2012; Pierce et al., 1996).

In this thesis it was shown that the relative risk model was successfully applied to rodent carcinogenicity data of glycidol. In earlier evaluations of the applicability of the risk model it has been indicated that the relative risk coefficient (β) is a measure of the genotoxic potency and thus should be possible to replace by the rad-equivalence (Q) in the risk model. The relative genotoxic potency (rad-eq.) for glycidol, obtained by \textit{in vitro} mutations in mammalian cells (HPRT) and by induction of micronuclei in mice, was 9.5 rad-equ./mMh (Paper I) and 15 rad-equ./mMh (Paper II), respectively.

\textbf{Table 10.} Relative risk coefficients and corresponding relative risks for glycidol, obtained for mice and rats. Ionizing radiation at high doses is used as a reference standard (reviewed by Granath et al., 1999).

<table>
<thead>
<tr>
<th>Relative risk coefficient (% per mMh)</th>
<th>Relative risk (% per rad-equ.)</th>
<th>Relative risk (% per rad)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycidol</td>
<td>Glycidol</td>
<td>Ionizing radiation</td>
</tr>
<tr>
<td>Cancer</td>
<td>Mutations</td>
<td>Micronuclei</td>
</tr>
<tr>
<td>Mice</td>
<td>5.1</td>
<td>0.54</td>
</tr>
<tr>
<td>Rats</td>
<td>5.4</td>
<td>0.57</td>
</tr>
<tr>
<td>Humans</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Mean</td>
<td>5.3</td>
<td>0.55</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Acute doses of radiation (50-200 rad), \textsuperscript{b} from A-bomb survivors.

\textsuperscript{12} The ERR of ionizing radiation is used for the calculation of lifetime attributable risk (LAR), where population specific background cancer rates and dose rates at different ages are considered. The common applied figure of LAR is ca. 5 % per Sievert (ICRP, 2007).
In Box 4 the procedure for the calculation of the relative risk of glycidol is presented. The mean relative risks of ca. 0.55 % per rad and ca. 0.35 % per rad were obtained from in vitro mutations and induction of micronuclei in vivo, respectively, assuming even distribution (see Table 10 for individual values). These figures are in a good agreement with the “true relative risk” observed for ionizing radiation and indicate that the relative risk model with genotoxicity data and internal dose measurements is a useful approach, which gives reliable estimates of the risk.

10.3.1 Relative genotoxic potency of different epoxides

As a comparison to glycidol the epoxides ethylene oxide and glycidamide will be discussed. Their relative genotoxic potencies, obtained from HPRT mutations, are 40 rad-equ./mMh for ethylene oxide (Kolman et al., 2002; 1988) and 400 ± 300 rad-equ. for glycidamide (Silvari et al., 2005), meaning that they have about 4 and 40 times higher mutagenic efficiency in vitro than glycidol, respectively (Table 11).

The difference in the lifetime doubling doses for the evaluated epoxides (Törnqvist et al., 2008; Granath et al., 1999) is lower than the differences of the relative genotoxic potencies (Table 11). Rather small differences in carcinogenic potency are expected as the reactivity is similar for this type of compounds (c.f. Silvari et al., 2005; Ohlén, 2005).

In comparison with the estimated lifetime internal dose the lifetime doubling dose from carcinogenicity studies is used for estimation of cancer risk. Ethylene oxide and glycidamide demonstrate about 2 to 7 times higher risk compared to glycidol. More careful evaluations are required for a more precise conclusion, particularly for glycidamide where a large uncertainty is observed for the mutagenic potency, and as the doubling dose is based on carcinogenicity studies with acrylamide.
Table 11. Comparison of the relative genotoxic potency and the estimated cancer risks of different epoxides.

<table>
<thead>
<tr>
<th></th>
<th>HPRT mutations</th>
<th>Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Relative genotoxic potency</td>
<td>Relative potency</td>
</tr>
<tr>
<td>Glycidol</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Ethylene oxide</td>
<td>40</td>
<td>4</td>
</tr>
<tr>
<td>Glycidamide</td>
<td>400</td>
<td>40</td>
</tr>
</tbody>
</table>

<sup>a</sup> Obtained from the mean of mice and rats (Paper IV; Granath et al., 1999), <sup>b</sup> From acrylamide administered rats (Törnqvist et al., 2008), <sup>c</sup> Estimated value for children, Paper V, <sup>d</sup> Calculated from data by Törnqvist et al., 1996, <sup>e</sup> Calculated from data by Vikström et al., 2011.

10.3.2 Conclusions of relative genotoxic potency

To conclude, despite different mechanisms for formation of measured genotoxicity (HPRT mutations or in vivo micronuclei), the respective estimated relative risk for glycidol, obtained from the two test systems, were comparable. It has been demonstrated in this thesis work that the procedure for derivation of the relative risk from short-term studies is straight-forward, where data on genotoxicity and internal dose are sufficient basis for the calculations. The approach gives the advantages of savings in animal lives and time, as well as reduced costs. Further evaluations with additional model compounds should be conducted to further validate this procedure for cancer risk estimation. This approach should be an attractive choice for risk estimation to be used for regulation of carcinogens. A suggested work-flow is given below (Figure 30). For a new compound with a hypothesized exposure to be introduced it would be sufficient with step 1-2 below. For ongoing exposures step 3-4 should also be included to get a more accurate risk estimation.
Figure 30. Suggested work-flow for estimation of cancer risk of genotoxic compounds based on genotoxicity studies and internal dose measurements. The internal dose in humans can be included if the exposure is ongoing.
11. Future perspectives

The presented approach for estimation of cancer risk with the multiplicative (relative) risk model in combination with data on relative genotoxic potency is promising.

So far carcinogenicity studies of only four compounds have been evaluated with the relative risk model and internal dose measurements. An extended battery of compounds of different classes of reactivity should be evaluated to further test the applicability/validate the approach. For ethylene oxide and glycidol, and butadiene the applicability of relative genotoxic potency (the rad-equivalence) for cancer risk estimation has been evaluated with the model. As acrylamide through its metabolite glycidamide is posing the highest estimated risk of the evaluated compounds it would be particularly important to complement the evaluation of acrylamide/glycidamide.

Including internal dose measurements in the inter-species extrapolations of risk from rodent carcinogenicity studies will give more accurate estimations of risk compared to estimations where only the administered doses are considered. This is particularly important when the genotoxic compound is generated through metabolism and when there are large metabolic differences between species, as in the case of butadiene (Fred et al., 2008). One conclusion from this thesis work is that the procedure to generate data for internal dose calculation should be further refined and standardized.

The performed evaluations of the relative risk model only consider one compound at a time. In real life we are constantly exposed to a variety of compounds during our whole lifetime. How to deal with these cocktail exposures is important, particularly for compounds at high doses. Additive, synergistic, and antagonistic genotoxic responses are possible to investigate utilizing short-term studies. However, the doses of the studied compound in in vivo and in vitro genotoxicity tests are generally orders of magnitude higher than human exposures. It is known that humans are constantly exposed to genotoxic compounds at low doses, as has been illustrated in for instance the recent adductomics work discussed below. New exposures to consider for a risk evaluation may be detected from such work.
In a previous study by our research group the total exposure to different reactive compounds was measured (as Hb adducts) in the same subset of blood samples from children as in the present work (Chapter 9). Twenty-four Hb adducts were measured, with about half having known precursors (Figure 31). In this thesis an improved method for analysis of adducts of glycidol has been applied to the blood samples from the children. With this improved method adducts from additional epoxides have been detected and identified (to be published). There are considerable remaining research efforts to identify the other precursors and, if they are genotoxic, perform risk assessments.

**Figure 31.** An adductome map illustrating relative min-max adduct levels of the respective adduct in blood samples from 51 subjects (children). The x-axis gives the retention time in the MS-analysis and the y-axis indicates the molecular weight of the respective adduct. The levels of the discussed genotoxic and carcinogenic compounds: glycidol, ethylene oxide, and glycidamide are relatively low compared to other reactive compounds. Reprinted with permission from H. Carlsson, J. Aasa, N. Kotova et al., Chemical Research in Toxicology, 2017. Copyright 2017 American Chemical Society.
12. Svensk sammanfattning

Vi är ständigt exponerade för kemikalier i vår vardag, där vissa utgör en risk för cancer genom sina genotoxiska egenskaper. Exponeringen sker från material i våra hem, på arbetsplatser eller i skolor men också via luften och vattnet i vår utomhusmiljö. Kemiska ämnen med genotoxiska egenskaper bildas också naturligt i kroppens metabolism. En stor del av exponeringen kommer genom maten vi åter. Oönskade kemikalier i mat upptäcks ofta genom att födöämnets processats på något sätt, ofta i relation till upphettning. Upphettning är dock nödvändig för att t.ex. förenkla upptag av näringsämnen, förbättra smak och ta bort bakterier. Fokus i detta avhandlingsarbete har varit på den genotoxiska och cancerframkallande substansen glycidol och den relaterade substansen 3-monokloropropan-1,2-diol (3-MCPD). Estrar av dessa substanser har hittats i olika typer av raffinerade matoljor, där palmolja uppvisat de högsta halterna. Dessa oljor används i många olika matprodukter. I mag-tarm kanalen bryts estrarna ner och frigör glycidol och 3-MCPD.

Det övergripande syftet med avhandlingen har varit att utvärdera data från redan publicerade cancerstudier för glycidol med hjälp av en cancerriskmodell, den ”relativa riskmodellen” och att undersöka om resultat från genotoxicitetsstudier av glycidol i celler (in vitro: i provrör) eller korttidsstudier i möss (in vivo: i kroppen) går att använda för att uppskatta cancerrisken med denna modell. Vidare har syftet varit att uppskatta cancerrisk av glycidol i människor. En central del i arbetet har varit att mäta den interna dosen av glycidol i de olika biologiska testsystemen. Glycidol är elektrofil (reaktiv) och reagerar med elektrontäta (nukleofila) atomer i proteiner och DNA vilket resulterar i stabila reaktionsprodukter, s.k. addukter. Kvantifieringen av dos in vivo har gjorts från mätning av addukter till aminosyran valin i hemoglobin (i blod). Dosquantifiering in vitro har gjorts genom att fånga upp glycidol i form av en addukt till nukleofilen kobalamin (vitamin B12) som tillsätts proverna. Detektion och kvantifiering av addukterna har gjorts med masspektrometri.

Den första delen av avhandlingen rör uppskattning av genotoxicitets potens (genotoxicisk respons per intern dos) av glycidol från mutationsstudier i celler (Paper I) och från studier i möss med mätning av mikrokärnor som ett mått på genotoxicitet (Paper II). Den genotoxiska potensen (för glycidol)
jämfördes sedan med den genotoxiska potensen för joniserande strålning i
samma testsystem för att få ett normaliserat värde, vilket gör det lättare att
jämföra substanser och olika testsystem. Joniserande strålning har en känd
cancerrisk i människor och är på så sätt en optimal referens. Substansen 3-
MCPD, testades också i möss men visade ingen genotoxicitet (Paper III).

Nästa steg i avhandlingsarbetet rör utvärdering av tumördata från redan
publicerade cancerstudier för glycidol i möss och råttor, med ”den relativa
riskmodellen”. Då de interna doserna ej var uppmätta i cancerstudierna för
glycidol gjordes en korttidsstudie med dosmätning i möss och råttor i syfte
att kunna relatera intern dos mot tumörfrekvens (Paper IV). Modellen
predikterade antalet tumörer med god överensstämmelse med de observerade
tumörerna i de glycidolbehandlade djuren. Ingen signifikant skillnad i
”cancerrisk per dos” (riskkoefficient) observerades mellan mössen och
råttorna. Detta stödjer idén om att riskkoefficienten är oberoende av
tumörtyp, kön och djurslag, och kan då också gälla för människor.
Riskkoefficienten kan även uttryckas som s.k. livstidsfördubblingsdos, vilket
är den dos av den cancerframkallande substansen som krävs för att fördubbla
bakgrundsincidensen av tumörer. Cancerriskkoefficienten, uppmätt i
djurförsök för glycidol, jämfördes med de värden för normaliserad
genotoxisk potens (jämfört med joniserande strålning) uppmätt från
studierna med celler och mikrokärnor. Den erhållna s.k. relativa risken
indikerade en bra överensstämmelse med vad som observerats för joniserande
strålning.

I den sista delen av avhandlingen uppskattades cancerrisken av glycidol i
människor (Paper V). Denna baserades på uppmätta interna doser av
glycidol i blodprover från 50 barn och 12 vuxna i jämförelse med
livstidsfördubblingsdosen för glycidol (ovan). En femfaldig variation av
glycidol doserna kunde påvisas i de olika individerna.
Cancerriskuppskattningen visade att glycidol ger ett bidrag till
bakgrundsrisken för cancer med mer än ett cancerfall per 1000 personer
(>1/1000) vilket är högre än den livstidsrisk som anses acceptabel.

Sammanfattningsvis har detta avhandlingsarbete visat att cancerrisken för
DNA-skadande kemikalier kan uppskattas enligt en relativ riskmodell,
vilken redan används för joniserande strålning. Modellen ger tillförlitliga
resultat för modellsubstansen glycidol. Avhandlingen stödjer att det är
mötligt att uppskatta cancerrisken (nu studerat för enkla epoxider) från
genotoxicitetsstudier när man inkluderar mätningar av den interna dosen.
Annu fler substanser, företrädesvis av substansklass med annan reaktivitet,
bör testas för att bredda och ytterligare validera denna lovande ansats för
riskuppskattning.

76
Acknowledgements

Tiden går så fort, jag kan inte riktigt förstå att det nästan har gått 5 år sedan jag började på ”Miljökemi”! Det har varit roligt att gå till jobbet, en härlig känsla! Och det beror ju inte enbart på att jag har haft en intressant forskning. Nå, jag skulle nog vilja påstå att det till en stor del beror på att jag har haft så många trevliga kollegor runt mig. Ni!

Först och främst, ett stort och innerligt tack till min handledare Margareta Törnqvist för att jag fick börja i din grupp! Det har varit väldigt inspirerande och lärorikt att få vara en del av din forskning! Jag har uppskattat att du alltid tar dig tid för diskussioner när det behövs, liksom dina kloka synpunkter på allt skrivande. Dessutom har vi haft väldigt roligt tillsammans. Vi har hunnit med några k onferenser, Mexiko (bilden) bl.a. är ett fantastiskt minne!

Margareta och jag kombinerar konferens på IUTOX med sightseeing i Maya-staden Uxmal.

bra samarbete och att du var så snabb med att räkna om siffrorna i det fjärde arbetet så att jag slapp en massa errata! **Siv Österman-Golkar**, tack för bra synpunkter vid genomläsningen av kappan! **Mats Harms-Ringdahl**, ett stort tack för diskussioner om strålning!


**Johan Gustavsson**, bra att du kom så att jag slapp vara ”sista doktoranden i miljökemi”. År övertygad om att du kommer att hålla fanan högt för ämnet! Tiden går undan, kom ihåg att njuta av tiden! **Isabella Karlsson**, jätteroligt att du började hos oss, bara lite synd att det inte blev tidigare. Lycka till med stundande bröllop, bebis och karriär inom akademien! **Jon Martin**, it has been a great pleasure to get to know you, and convenient to have a native English-speaking person next door when it comes to language-issues! **Ioannis Sadikitis**, tack för dina tips för att få inspiration till skrivandet, följde de inte riktigt dock… ;-). **Lisa d’Agostino**, good luck with your research, I hope you enjoy Sweden! Mitt sista år har förgyllts av att vi har haft flera examensarbeteare. Ett särskilt tack till min ”egna”, **Efstathios Vryonidis**, det har varit otroligt roligt att jobba med dig, vi blev ett bra team i mitt sista arbete och jag har lärt mig mycket! Nu håller jag tummarna att du blir kvar! **James Cumming** and **Giulia Martella**, I wish you all the best for the future. It has been fun to have you around! Tack till resten av ACESx också, trevligt med en tradition av regelbundet ”gofika” (även om jag inte har haft möjlighet att delta så ofta). Salsan var riktigt roligt tycker jag!

Ni är ett flertal miljökemister som inte är kvar på universitetet, och jag är väldigt glad att jag hann lära känna er! **Henrik Carlsson**, den perfekta rumsjakmarmaten, förutom när det kommer till temperaturen i rummet förstas. Tur att det har funnits en fleecefilt att ta till ;-) Stort tack för all hjälp med ”the adduct FIRE procedure” och instrumenten. Och kul att vi hann få till ett samarbete! **Emelie Westberg**, du är nog snällaste människa jag känner! Du fick verkligen mig att känna mig välkommen och som en i gänget. **Jessica**
Norrgran Engdahl, många bra diskussioner och glada skratt med dig! Bra att du blev kvar ett tag till här i korridoren! Anna-Karin Dahlberg, stor hjälp när jag skulle ratta kurslab. inom ditt gebit för första gången, tack! Anna Strid, det blev tomt utan dig här, det var roligt att få jobba med dig den korta stund det blev! Birgit Paulsson, det blev tomt utan dig också! Tack för all din hjälp vid kursverksamheten och för trevligt sällskap i Köpenhamn! Johan Fång och Andreas Rydén, det var inte länge vi hann jobba parallellt, men kul så länge det varade!

Tack alla på analytisk kemi! Det var roligt att vara en del av seminarsierien och utbildningsrådet, har lärt mig mycket! Ulrika Nilsson, Roger Westerholm, Conny Östman, trojkan som man kan lita på levererar finurliga ämnen att avhandla vid lunchbordet, tack för alla roliga historier och goda skratt! Kan givetvis säga detsamma om er Lena Elfver och Jonas Rutberg, men också tack för all hjälp med allt från administrativt ”mäste-göra” till bra arrangemang vid olika firanden. Er kan man lita på! Jan Holmbäck, tack för bra info om glycidyl/3-MCPD-estrar! Tack Leopold Ilag, för din Nespressomaskin och Karin Englund, för glasspauser! Tack Gunnar Thorsén för att du har sett till att jag ”regelbundet” fått känna utanför labbet också (nu har jag tid igen)! Bra löppass med bra diskussioner. Men shit vad du är i bra form hela tiden! Thanks to my PhD colleagues at ACESk: Farshid Mashayekhy Rad for always being so friendly and helpful, Francesco Iadaresta, for bringing mozzarella directly from Italy! Alessandro Quaranta, it is fun to listen to you when you are really engaged (football…)! Javier Zurita Perez thanks for side-kicking in the PhD council! Nadezda Kiselova, good luck with your future plans (in Estonia?), Hatem Elmongy, good to see you around sometimes, good luck with your final work at KI! Pedro Sousa thanks for your help with statistics! Hwanmi Lim thanks for good advice before my Korea trip! Ahmed Gamal Ramzi, good luck with your future career! Jonas Fyrestam, reflexerna på rådjuren är oslagbara! Rozanna Avagyan, drottningen av handväskor och Google!

med gråt och skratt om vartannat! Ett särskilt tack till dig Johanna för att du höll mina tankar om att doktorera vid liv, och för ditt förtroende att få stå kvar med en fot i läkemedelsbranschen!

Tack till mamma, pappa och Inger, Monika och Erling förstås, för att ni har tagit hand om våra killar så att jag och Andreas har kunnat rå om varandra på egen hand emellanåt. Kram!

Mitt sista tack är fullt av kärlek och går till mina killar där hemma: min man Andreas och våra älskade söner Pontus och Alfred. Nu kan vi åka på semester igen utan att jag behöver sitta och skriva samtidigt. Ni har stöttat mig toppenbra, puss och kram! ♥♥♥

Andreas, Pontus och Alfred i sina rätta element.

The research of this thesis was funded by the Swedish Research Councils Formas and Vetenskapsrådet, and by Stockholm University.
References


Aniolowska, M. and Kita, A., 2015. The Effect of Type of Oil and Degree of Degradation on Glycidyl Esters Content During the Frying of French Fries. J. Am. Oil Chem. Soc. 92, 1621-1631


BIR (Federal Institute for Risk Assessment), 2009. Initial evaluation of the assessment of levels of glycidol fatty acid esters detected in refined vegetable fats. BfR Opinion No. 007/2009


outmoded and serve neither science nor society. Regul. Toxicol. Pharmacol. 82, 158-166
C.EPA (California Environmental Protection Agency), 2010. Office of Environmental Health Hazard Assessment (OEHHA). No significant risk level (NSRL) for the proposition 65 carcinogen glycidol. California Environmental Protection Agency, USA (16 pp)
Dingel, A. and Matissek, R., 2015. Esters of 3-monochloropropane-1,2-diol and glycidol: no formation by deep frying during large-scale production of potato crisps. European Food Research and Technology 241, 719-723

82


EFSA (European Food Safety Authority), 2005. Opinion of the scientific committee on a request from EFSA related to a harmonized approach for risk assessment of substances which are both genotoxic and carcinogenic. The EFSA Journal, 282, 1-31

EFSA (European Food Safety Authority), 2008. Polycyclic aromatic hydrocarbons in food. Scientific opinion of the panel on contaminants in the food chain. EFSA Journal, 724, 1-114

EFSA (European Food Safety Authority), 2015. Scientific opinion on acrylamide in food. EFSA Journal, 13(6), 4104 (321 pp)

EFSA (European Food Safety Authority), 2016a. Risks for human health related to the presence of 3- and 2-monochloropropanediol (MCPD), and their fatty acid esters, and glycidyl fatty acid esters in food. The EFSA Journal 14(5), 4426 (159 pp)

EFSA (European Food Safety Authority), 2016b. Review of the Threshold of Toxicological Concern (TTC) approach and development of new TTC decision tree. EFSA Supporting Publications, 2016:EN-1006, 1 (55 pp)

EFSA (European Food Safety Authority), 2017. Update: use of the benchmark dose approach in risk assessment. The EFSA Journal 15(1), 4658 (41 pp)


http://monographs.iarc.fr/ENG/Classification


Jones, A.R., 1975. The metabolism of 3-chloro-, 3-bromo- and 3-iodoprop-1,2-diol in rats and mice. Xenobiotica 5, 155-165


Lee, B.Q. and Khor, S.M., 2015. 3-Chloropropane-1,2-diol (3-MCPD) in soy sauce: a review on the formation, reduction, and detection of this potential carcinogen. Comprehensive Reviews in Food Science and Food Safety 14, 48-66


Schrauzer, G.N. and Deutsch, E., 1969. Reactions of cobalt(I) supernucleophiles. The alkylation of vitamin B12s cobaloximes(I), and related compounds. J. Am. Chem. Soc. 91, 3341-3350


Tomasetti, C. and Vogelstein, B., 2015. Cancer etiology. Variation in cancer risk among tissues can be explained by the number of stem cell divisions. Science 347, 78-81


