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Assessment Factors for **Genotoxic Carcinogens**

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J.A. de Heer et al.

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Colophon

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Synopsis

Assessment Factors for Genotoxic Carcinogens

RIVM is working on methods to assess as accurately as possible whether substances may be hazardous to human health. This can, for example, be done with a risk assessment which calculates what the maximum exposure for these substances should be.

In risk assessments uncertainties are taken into account to prevent underestimating the risk. For example, there can be uncertainties for the effect in humans when only information from animals is available. Another uncertainty is that not all humans respond to a substance in the same way.

There are different ways to take into account uncertainties in a risk assessment. One method is by applying assessment factors. For a newer method, RIVM investigated whether the effect of DNA-damaging substances can vary between different species of animals, between animals and humans, and between different humans. If that is the case, it is recommended to apply assessment factors to cover these differences.

The mentioned differences were indeed found, as shown in scientific literature. RIVM therefore recommends using assessment factors with the new method for the risk assessment of DNA damaging substances. However, determining the values of these assessment factors is complex. This can be addressed through discussions with international researchers.

Keywords: deterministic method, probabilistic method, assessment factors, DNA-damaging substances, risk assessment, cancer

Publiekssamenvatting

Assessment factoren voor genotoxische carcinogenen

Het RIVM werkt aan methoden om zo goed mogelijk te kunnen beoordelen of stoffen schadelijke effecten hebben op de gezondheid van mensen. Dat kan bijvoorbeeld met een risicobeoordeling die berekent wat de blootstelling aan dit soort stoffen maximaal mag zijn.

In een risicobeoordeling wordt rekening gehouden met onzekerheden om te voorkomen dat de kans op gezondheidseffecten te laag wordt ingeschat. Zo kunnen er onzekerheden zijn over effecten van stoffen bij mensen, omdat er alleen informatie is over de effecten bij dieren. Een andere onzekerheid is dat niet alle mensen op dezelfde manier op een stof reageren.

Er zijn verschillende manieren om rekening te houden met onzekerheden in een risicobeoordeling. Eén daarvan is het gebruik van assessment factoren. Voor een nieuwe methode onderzocht het RIVM of het effect van stoffen die het DNA beschadigen, kan verschillen tussen verschillende soorten dieren, tussen dieren en mensen en tussen mensen onderling. Als dat zo is, wordt aanbevolen om voor deze verschillen assessment factoren te gebruiken.

De genoemde verschillen blijken er inderdaad te zijn, zo blijkt uit de wetenschappelijke literatuur. Het RIVM adviseert daarom om deze assessment factoren te gebruiken in de nieuwe methode voor risicobeoordeling voor DNA-beschadigende stoffen. Het is nog wel ingewikkeld om te bepalen hoe groot de assessment factoren zouden moeten zijn. Meer discussie met internationale onderzoekers is daarvoor nodig.

Kernwoorden: deterministische methode, probabilistische methode, assessment factoren, DNA-beschadigende stoffen, risicobeoordeling, kanker

Contents

Summary

RIVM supports the use of probabilistic methods for risk assessment of chemicals as this will result in more realistic estimates of health risks. To apply probabilistic risk assessment of genotoxic carcinogens it is necessary to know the uncertainties which need to be taken into account. The goal of this report is to answer the question if it would be required to correct for intraspecies and interspecies differences for genotoxic carcinogens in probabilistic risk assessment. The scope of the current report is limited to assessment factors for intraspecies and interspecies differences. Other possible assessment factors, such as mixture assessment factors, high-to-low risk extrapolation and adjustments for experimental exposure duration will not be discussed.

To answer the research question, guidances of different regulatory bodies were screened to give an overview of default assessment factors, currently used in risk assessment of non-genotoxic and genotoxic carcinogens. In addition, literature searches were conducted to collect qualitative information about intraspecies and interspecies differences for genotoxic carcinogens.

Different methods for risk assessment are available for genotoxic and non-genotoxic carcinogens. For non-genotoxic carcinogens, the highest dose that does not show an adverse effect or the Benchmark dose is used as a Point of Departure (PoD) and is converted into a limit value expressing a safe dose or acceptable/tolerable risk. An assessment factor accounting for interspecies and intraspecies differences is often applied when human data is not available. For genotoxic carcinogens, linear extrapolation and the Margin of Exposure (MoE)-approach are generally applied. In linear extrapolation the high risk (incidence) from studies is extrapolated to a low risk with a high level of conservatism, but this does not explicitly correct for intraspecies and interspecies differences. The MoE includes a factor for possible differences in susceptibility, but with only limited correction for high to low risk. Both methods have margins between the PoD and the "acceptable" exposure of four or five orders of magnitude. However, in both cases, the scientific substantiation for those margins is incomplete. In addition, both of these methods are deterministic, as point estimates are used as PoD and for uncertainties. An alternative to deterministic risk assessment is the probabilistic approach, which is the preferred approach of RIVM. In the probabilistic approach the PoD is linearly extrapolated to the acceptable risk level, but the uncertainties in each parameter are accounted for by using distributions rather than point estimates, making it a less conservative approach. This raises the question whether it is necessary to consider intraspecies and interspecies differences when following a probabilistic approach in the quantitative hazard characterization of genotoxic carcinogens.

Intraspecies differences to genotoxic carcinogens are influenced by increased susceptibility during early life and differences between humans in DNA repair and in toxicokinetics. In literature reviews of animal data it was shown that juvenile animals were more susceptible to

developing cancers compared to adults. Although a full assessment of children's cancer risks is not feasible, early life sensitivity was reported in cases of exposure of humans to carcinogenic substances. Differences in DNA repair are due to individual differences in DNA repair capacity as well as age, circadian rhythm, lifestyle and dietary factors. Nevertheless, there are also indications that the DNA repair capacity can be directly influenced by substances. Similarly, there is evidence that the susceptibility to genotoxic carcinogens in humans is dependent on the toxicokinetics. This can be due to intrinsic differences or substance-specific differences in toxicokinetics.

For interspecies differences two aspects underlying these differences were presented in this report; toxicokinetics and DNA repair. In literature many examples were found where absorption and metabolism of a compound varied between species. As a result of this variation, the internal exposure to the active metabolite can be higher or lower depending on the species. Regarding DNA repair, there are indications that long-lived species have higher DNA repair activity compared to short-lived species. This is relevant, as a large part of the toxicity studies used in risk assessment are conducted in (short-lived) rats or mice.

Therefore, it is concluded that there are indications of intraspecies and interspecies differences for genotoxic carcinogens. This finding sufficiently substantiates the use of these assessment factors in a probabilistic risk assessment. The current report only provides qualitative arguments to include adjustment for intra- and interspecies differences in the risk assessment of genotoxic carcinogens. It would be very helpful to have further discussions with fellow risk assessors on this topic, to eventually have estimations of appropriate assessment factors for intraspecies and intraspecies differences. These will result in more realistic estimates of the health risk posed by genotoxic carcinogens.

1 Introduction

RIVM supports the use of probabilistic methods for risk assessment (Slob et al., 2014; Bokkers et al., 2017). To apply probabilistic risk assessment of genotoxic carcinogens it is necessary to know which uncertainties need to be taken into account.
The goal of this report is to answer the question if it would be required to correct for intraspecies and interspecies differences for genotoxic carcinogens in probabilistic risk assessment. The scope of the current report is limited to assessment factors for intraspecies and interspecies differences. Other possible assessment factors, such as mixture assessment factors, high-to-low risk extrapolation and adjustments for experimental exposure duration will not be discussed. In addition, differences between sexes are not considered in the current report as it is part of study protocols to investigate sex differences and include either both sexes or the most sensitive sex in the study.

The next chapter, describes the focus of this report. Further, an overview of default assessment factors, currently used in risk assessment, is given in chapter 3. In chapter 4, the results of the literature search for intraspecies differences are summarized, followed by the summary of interspecies differences in chapter 5. In chapter 6, the findings are discussed, conclusions are drawn and recommendations are made.

2 Methods

In this report an overview of the origin and use of assessment factors to account for intraspecies and interspecies differences in the evaluation of genotoxic and non-genotoxic substances by regulatory bodies is given, based on guidances of regulatory bodies.
In addition, literature searches were conducted to collect qualitative information about intraspecies and interspecies differences for genotoxic carcinogens. Altogether, early life sensitivity, toxicokinetics and DNA repair were identified as factors which could possibly influence susceptibility of animals and humans. It is expected that there are more factors associated with intraspecies and interspecies differences. The present study focusses on these three factors as they were most thoroughly researched.

3 Overview risk assessment and assessment factors

Risk assessment of chemicals generally includes hazard identification, hazard characterization, exposure assessment and risk characterization (Renwick, 2004). Hazard identification is carried out based on data obtained from *in vitro* studies, *in vivo* studies in animals and epidemiological studies in human populations (RIVM, 2014). In the hazard characterization, the dose response relationship of a relevant effect is assessed (Renwick, 2004). For a carcinogenic endpoint the dose response is usually an increase in tumor incidence in animals. From the dose response it is possible to derive a dose which is associated with a certain increase in tumor incidence compared to the background incidence (RIVM, 2014). In the risk assessment of carcinogenic substances a distinction is made between genotoxic and non-genotoxic carcinogens. Genotoxic carcinogens are substances that may cause cancer via DNA damage. Non-genotoxic carcinogens may cause cancer by indirect mechanisms, such as cell proliferation, cytotoxicity, epigenetic changes or hormonal effects (Nohmi, 2018).

3.1 Non-genotoxic carcinogens

For non-genotoxic carcinogens, a dose corresponding to a negligible or tolerable risk is used as a Point of Departure (PoD) and is converted into a limit value corresponding to an acceptable or tolerable risk (US EPA, 2005a; IPCS, 2020). An assessment factor accounting for interspecies and intraspecies differences, often a factor 100, is frequently applied when human data is not available (EFSA, 2012; IPCS, 2020). The factor applied for interspecies differences accounts for the difference in sensitivity to develop effects between the test species and the species of interest, usually humans, and the uncertainty around this difference. In other words, a dose corresponding to a defined risk in the typical or average animal, which is extrapolated to an equipotent dose in the typical human by applying the interspecies factor. Differences in sensitivity may be caused by interspecies differences in body weight related to metabolic rate (allometric scaling), kinetics and dynamics. The intraspecies factor is applied to account for differences in sensitivity (i.e. variability) between humans and the uncertainty around the human variability. By including the intraspecies factor it is assumed that the obtained (equipotent) limit value also covers sensitive groups in the human population.

3.1.1 *History of default assessment factors for intraspecies and interspecies differences*

The 100-fold assessment factor was first developed as a margin of safety between the level of a chemical in the diet of test animals and that in human diet by Lehman and Fitzhugh (1954) and later adopted by JECFA and JMPR as an adequate assessment factor assuming that the human being is at most 10 times more sensitive than the test animal and that sensitive individuals within the human population are 10 times more sensitive compared to the mean or typical human (WHO EHC 70, 1987; Figure 1). In 1993 Renwick proposes a scheme to allow toxicokinetic and mechanistic data to be incorporated quantitatively into

the risk assessment by the subdivision of each 10-fold factor in a toxicokinetic and a toxicodynamic factor. Later the International Programme on Chemical Safety (IPCS) slightly amended this scheme (IPCS, 2005).

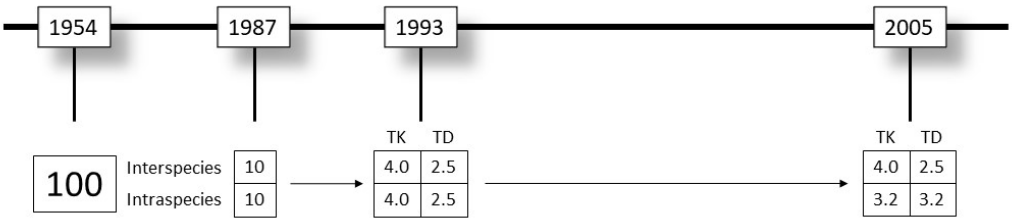

Figure 1 History of default assessment factor accounting for intraspecies and interspecies differences and allowing for toxicokinetic (TK) and toxicodynamic (TD) data to be incorporated quantitively into the risk assessment (Lehman & Fitzhugh, 1954; WHO EHC 70, 1987; Renwick, 1993; IPCS, 2020).

3.1.2 *Current assessment factors*

Currently, the choice of the numerical value of an assessment factor depends on the quantity and quality of available data. When adequate data exists, derivation of chemical specific adjustment factors to describe intraspecies and interspecies differences in either toxicokinetics or toxicodynamics is preferred to reliance on the default 10-fold assessment factors for each (IPCS, 2005; 2020). In 2012, EFSA published a guidance on default assessment factors to be used in risk assessment in the absence of available data for oral exposure, which is in line with the proposed approach by the IPCS in 2005 and is still in practice today.

The default assessment factors as used by ECHA are published in the Guidance on information requirements and chemical safety (ECHA, 2012a) and slightly deviate from the IPCS and EFSA assessment factors. Similarly to the other assessments, ECHA uses an assessment factor of 2.5 to account for the interspecies differences in toxicodynamics for oral, dermal and inhalation exposure, whereas a species-specific assessment factor is used to correct for differences in metabolic rate per body weight for the different test animals (Table 1). This correction for differences in metabolic rate per body weight is called allometric scaling and is based on the assumption that the effects of toxicological relevance are driven by the basal metabolic rate which affects various physiological processes in the body and therefore the elimination of chemicals. Allometric scaling is used with both oral and dermal exposure, but not with inhalation exposure (ECHA, 2012a).

To account for intraspecies differences, ECHA (2012a) uses a default value of 10 for the general population and a factor 5 for workers for oral, dermal and inhalation exposure (Table 1). However, for inhalation exposure, workers are considered to be in a state of elevated activity with higher respiratory rates compared to the general population which has to be compensated for. It is additionally noted that the default assessment factor of 10 for intraspecies variation is not always covering for very young children and it was recommended to consider a higher intraspecies assessment factor for children when there are indications of

early life sensitivity and there are deficiencies in the data on such effects in young animals (ECHA, 2012a).

Table 1 Default assessment factors as used by ECHA for interspecies and intraspecies differences (ECHA, 2012a).

Aspect	**Assessment factor**
Interspecies scaling to humans (oral & dermal exposure)	1.4 (dog) – 7 (mouse)
Interspecies toxicodynamics (oral, dermal & inhalation exposure)	2.5
Intraspecies (oral & dermal exposure)	5 (worker) 10 (general population)

The approach is slightly different when route-to-route extrapolation is conducted. ECHA argues that allometric scaling should not be applied in cases where the data is already scaled according to the allometric principle, such as where doses in experimental animals are expressed as concentrations (e.g., in mg/m³ air, ppm in diet, or mg/L in the drinking water) (ECHA, 2012a). This is usually the case when a route-to-route extrapolation is used on oral/dermal data to evaluate for inhalation exposure.

This approach as set by ECHA is currently followed nationally in the Netherlands for setting indicative environmental risk limits for assessing whether substances in water (fresh and marine), soil and air may be harmful to people (RIVM, 2015).

3.2 Genotoxic carcinogens

For genotoxic carcinogens, the steps of evaluating the risk differ between the regulatory frameworks. When the aim is to provide human reference values for enforcement and risk management purposes, a PoD is derived and converted to a limit value for genotoxic carcinogens. The extrapolation steps taken to derive a limit value are generally different from the steps described above considering non-genotoxic carcinogens. Levels below the limit value are considered not to impose an unacceptable risk (are of low concern) for carcinogenic effects in the general population following lifelong exposure (RIVM, 2020). In the environmental policy of the Netherlands, the risk levels in for chemicals are set at a cancer incidence of 10^{-4} for lifetime exposure as the individual Maximum Permissible Risk (MPR) and at a cancer incidence of 10^{-6} for lifetime exposure as the individual Negligible Risk (NR) (VROM, 1988; RIVM; 1991). Currently several agencies, such as the European Chemicals Agency (ECHA), United States Environmental Protection Agency (US-EPA), the German Federal Institute for Risk Assessment (BfR) and National Institute for Public Health and the Environment (RIVM), use the deterministic, linear extrapolation approach as the standard approach for setting an acceptable risk level. With this approach the PoD, which is the dose level resulting in the preset risk level (e.g. 5% or 10% extra risk), is linearly extrapolated to the acceptable or tolerable risk level (of e.g. 1 per 100,000 or 1 per million). In contrast to the approach for non-genotoxic carcinogens, no assessment factors accounting for interspecies and intraspecies differences are used. According to the guidance document on

information requirements and chemical safety assessment of ECHA, assessment factors accounting for intraspecies and interspecies differences are considered not necessary as it is reasoned that the linear model is sufficiently conservative to also cover the differences in susceptibility (ECHA, 2012a). It is noted however, that ECHA does perform a correction for allometric scaling for oral and dermal exposure (ECHA, 2012a). This correction is not performed in the case of inhalation exposure as it is assumed to be covered by the differences in respiratory rates between species (ECHA, 2012a).

It is mentioned in the guidelines for carcinogen risk assessment of US EPA that one can additionally adjust for the potential for early-life exposure to make a greater contribution to cancers appearing later in life for toxins with a mutagenic mode of action, but only when there is case-specific information available (US EPA, 2005a). In these cases, in the absence of early-life studies on a specific chemical under consideration, a default assessment factor of 10 is applied for exposures before 2 years of age and a factor 3 for exposures between 2 and 16 years of age.

Another approach for evaluation the risk of genotoxic carcinogens is the Margin of Exposure (MoE) approach, preferred by European Food Safety Authority (EFSA) and Health Canada (RIVM, 2020). In this approach the margin between the PoD and the estimated level of human exposure is calculated (EFSA, 2005). The size of this MoE indicates the priority of exposure reduction, but this approach does not result in a risk estimate. To indicate low concern, EFSA proposes a minimal MoE of 10,000 between the $BMDL_{10}$ derived from an animal experiment and the human exposure. The rationale provided for this minimal MoE is: a 100-fold factor to take into account intraspecies and interspecies differences (analogous to the use of a 100-fold assessment factor for non-genotoxic substances). A further 100-fold factor is considered to take into account additional uncertainties related to human differences in cell cycle control and DNA repair, and uncertainties related to the dose-response relationship below the PoD corresponding to 10% extra cancer risk (EFSA, 2005; Barlow, 2006).

Both the linear extrapolation and the MoE-approach come to margins between the PoD and the "acceptable" exposure of four or five orders of magnitude. However, in both cases, the scientific substantiation for those margins is incomplete. In short, the linear extrapolation approach focuses on low (extrapolated) risk, but does not explicitly correct for intraspecies and interspecies differences. On the other hand the MoE includes assessment factors for intraspecies and interspecies differences, but has only a limited correction for high to low risk. Clearly, both of these approaches have their merits and limitations.

Table 2 Default assessment factors used for linear extrapolation and the margin of exposure approach (ECHA, 2012a; EFSA, 2005), applied to a PoD corresponding to 10% extra cancer risk. Additionally to the standard linear extrapolation approach, ECHA may use allometric scaling for oral and dermal exposure and US-EPA may use an assessment factor for early life exposure in the linear extrapolation (US EPA, 2005a).

	Linear extrapolation	**Margin of exposure**
Low risk extrapolation	10.000-100.000	-
Interspecies	-	10
Intraspecies	-	10
Additional uncertainties	-	100
Total factor	**10.000-100.000**	**10,000**

3.3 Probabilistic risk assessment

The assessment factors described above are all currently applied in deterministic methods of risk assessment. In a deterministic risk assessment point estimates are used to account for uncertainties. An alternative to deterministic risk assessment is the probabilistic approach. In a probabilistic approach the PoD is extrapolated to the acceptable low risk level, but the uncertainties in each step of the extrapolation are accounted for by using distributions rather than point estimates (ECHA, 2012b). Consequently, the risk characterization can be quantitatively evaluated and translated into an estimate of the cancer risk in the form of an uncertainty range. The probabilistic approach is considered the preferred approach of RIVM (Slob et al., 2014; Bokkers et al., 2017). It is also considered the highest tier analysis in the assessment of uncertainties which appear critical to the outcome of the chemical risk assessment by ECHA (ECHA, 2012b). Ideally, the probabilistic approach includes a full analysis of both variability and uncertainty. However, such analysis requires a large amount of data and time (Bokkers et al., 2017). As an alternative APROBA-Plus has been developed, a simplified method in which the uncertainties, but not the variability in all parameters of the assessment can be accounted for (Bokkers et al., 2017).

APROBA-Plus includes default assessment factors in accordance with the IPCS guidance document (2017) on probabilistic hazard characterization. In this document it is suggested to take into account interspecies differences for oral exposure by applying allometric scaling to adjust for body size differences and a (maximum) assessment factor of 3 for remaining differences in toxicokinetics and dynamics. For inhalation exposure IPCS suggests different types of body size assessment factors for particles and gases in combination with the assessment factor of 3 for remaining differences in toxicokinetics and dynamics.

4 Intraspecies differences

Responses to chemicals can vary widely among individuals and between population groups. It is also likely that the carcinogenic process as induced by chemicals is not the same across individuals. In the literature search, various review articles were found discussing intraspecies variation and the susceptibility for developing cancer. In these reviews possible mechanisms were introduced that explain the differences. The differences in susceptibility for developing cancer could be explained by for instance age, DNA repair capacity and differences in absorption, distribution, metabolism, and elimination of chemicals. For both DNA repair capacity and toxicokinetics these differences can be intrinsic or substance specific. In this chapter the influence of early life sensitivity, DNA repair capacity and toxicokinetics on the differences in response to genotoxic carcinogens is discussed.

4.1 Early life sensitivity

Although cancer is a disease most commonly associated with aging, exposure to substances early in life can result in the development of cancer (US EPA, 2005b). Many tumors develop in both the old and the young, however, children are more susceptible to a number of tumors. Where adults generally develop more carcinomas, childhood is associated with more embryonic cell tumors. The most common cancers of children are leukemias, brain and other nervous system tumors, lymphomas, bone cancers, soft tissue sarcomas, kidney cancers, eye cancers and adrenal gland cancers, whereas skin, prostate, breast, lung and colorectal cancers are most common in adults (US EPA, 2005b). The differences between childhood and adult cancers suggest the importance of evaluating the impacts of maternal exposures during pregnancy as well as exposures to children (US EPA, 2005b).

Guidance values derived for genotoxic carcinogens are usually applicable to lifetime exposure. However, the underlying data is almost always based on adult exposure only. The relative rarity in the incidence of childhood cancers and the lack of animal testing guidelines with perinatal exposure impede a full assessment of children’s cancer risks (US EPA, 2005b). Ginsberg (2003) performed a review of juvenile animal bioassay data in comparison to adult animal data for various carcinogens. The cancer incidence from short exposure studies early in life can be as high as, and in some cases higher than, the cancer incidence from longer exposures during adult life. This indicates early life sensitivity to some carcinogens (Ginsberg, 2003). Evidence of childhood cancer in humans occurring from chemical exposures is limited. The pharmacological use of diethylstilbesterol (DES) during pregnancy to prevent miscarriages was associated with an increased incidence of adenocarcinomas in the vagina of the offspring exposed *in utero*, which was not seen in the exposed mother animals (US EPA, 2005b).

Evaluating the available data on the effect of early-life exposures, the US EPA made a crude estimation that exposure to mutagenic chemicals

in the first 2 years of life could assumed to be 10 times as potent as exposure in adulthood and a 3-fold adjustment factor is thought to cover the increased in risk for exposures between 2 and 16 years of age (US EPA, 2005b). This increased potency of carcinogens during development is the result of increased susceptibility due to the mode of action of genotoxic carcinogens (Ginsberg, 2003). Genotoxic carcinogens are generally more effective in rapidly dividing tissues as the increased frequency increases the opportunities for interaction and due to there being less time for DNA repair between cell divisions and thus greater chance of fixation of the damage as a mutation. This was confirmed by showing that fetal tissues are more sensitive for the induction of micronuclei from mutagenic chemicals than maternal tissues in *in vivo* transplacental micronuclei assays in fetal or neonatal mice (Hayashi et al., 2000). Additionally, some embryonic cells, such as brain cells, lack key DNA repair enzymes (Felter et al., 2011). During development there is greater cell division in tissues that are normally quiescent during adulthood, such as the brain, thus leading to greater sensitivity in these tissues, which is supported by evidence in clinical and epidemiological data (Ginsberg, 2003). Also, early life sensitivity can be due to the induction of developmental abnormalities that can result in a predisposition to carcinogenic effects later in life (Felter et al., 2011; Prins et al., 2015). For instance, stem cell programming during early life results in increased sensitivity to endocrine disrupting chemicals which in turn could lead to aberrant stem cell reprogramming which can contribute to an increased cancer risk as an adult (Prins et al., 2015). An example of an endocrine disrupting chemical that is associated with cancer due to developmental exposure is dioxin (Birnbaum and Fenton, 2003).

However, it is not a general feature for all genotoxic substances to show an increased potency for inducing cancer during early life exposure. RIVM (2014) investigated the potential of various substances to induce DNA mutations or chromosomal damage and the overall tumor incidence. They found that the increased susceptibility to develop mutagenic effects when exposure occurred early in life is dependent on the specific mechanism of action of the substance. Benzo(a)pyrene showed increased mutant frequencies in the treated mice at the earlier life stage while acrylamide that causes DNA breaks by DNA adducts showed no increased potential after early-life exposure (RIVM, 2014).

An example of a carcinogenic substance showing an increased potency after early life exposure is arsenic. The population of a large town in Chile was exposed to high levels of arsenic due to a contaminated water supply. The exposure started in the beginning of 1958 and ended with the introduction of water filtration in 1970. The cohort of individuals exposed to arsenic early in life (in utero and/or childhood exposure) was later found to have significantly increased incidences of bladder, laryngeal, lung, kidney and liver cancers along with chronic obstructive pulmonary disorders and circulatory diseases (Boekelheide et al., 2012; Bommarito and Fry, 2016). Also in other parts of the world, in people who were exposed to high levels of arsenic via drinking water prenatally or as infants, exposure has been associated with a wide range of negative health outcomes, including cancers of the skin, bladder, liver and lung compared to lower levels of arsenic exposure (Bommarito and

Fry, 2016). These poisoning incidents with exposure to arsenic *in utero* or during early childhood show unique health outcomes compared to exposures occurring during adulthood (Bommarito and Fry, 2016). The results were confirmed in an *in vivo* study using mouse models. Following an exposure to 42.5 or 85 ppm $NaAsO_2$, from gestational day 8-18, offspring developed cancer at an increased incidence compared to controls and compared to tests using mouse models without prenatal exposure (Bommarito and Fry, 2016). Additionally, arsenic was associated with epigenomic alterations leading to sustained reprogramming of cellular behavior which may underlie the carcinogenic effects and the latency of effects of arsenic associated cancers (Bommarito and Fry, 2016). Compared to humans, the effects seen in the animal models occur at higher concentrations and it seems that rodents are relatively resistant to the effects of arsenic exposure (see section on interspecies variation).

In conclusion, there is evidence that suggests that the susceptibility to carcinogenic agents is higher in the early stages of life, such as infancy and childhood, but also prenatal (US EPA, 2005b). During development there is more frequent cell division, also in tissues that are quiescent during adulthood and due to the high frequency less time in-between for DNA repair, resulting in a greater chance of fixation of the DNA damage (Ginsberg, 2003; US EPA, 2005b; Felter et al., 2011). Also, early life sensitivity can be due to the induction of developmental abnormalities that can result in a predisposition to carcinogenic effects later in life (Felter et al., 2011; Prins et al., 2015). It is expected that these and other differences between children and adults may influence which types of cancer are caused and/or the risk that cancer develops.

4.2 DNA Repair

One of the reasons for the increased susceptibility in early life was stated to be the reduced time and capacity for DNA repair (US EPA, 2005b). Reduced DNA repair efficiency is associated with increased cancer risk (Cheong et al., 022). DNA damage is an important factor for initiation of tumorogenic changes in a cell and the further process of cancer development (Slaga et al., 1988). DNA repair mechanisms can reverse DNA damage caused by genotoxic carcinogens and unrepaired DNA damage could lead to carcinogenesis due to the accumulation of mutations (Chatterjee and Walker, 2017; Cheong et al., 2022).

The study by Mohrenweiser and Jones (1998) describes the research that has been done on the intraspecies variation in DNA repair capacity. An *in vitro* exposure assay showed interindividual differences in DNA repair capacity in lymphocytes by exposure to X-ray radiation and strand break inducing agents. The authors note that the variation measured is independent of previous exposure of the individual and independent of the variation in ability to metabolize DNA damaging agents as these were *in vitro* experiments. These reported differences in DNA repair capacity also result in differences in cancer incidence. It was shown that it is more likely that individuals of a cancer cohort have reduced DNA repair capacity compared to the control individuals and thus reduced DNA repair capacity is associated with a statistically significant risk factor for cancer (Mohrenweiser and Jones, 1998;

Benhamou and Sarasin, 2000; Berwick and Vineis, 2000; Nagel et al., 2014). For instance, lung cancer risk is increased after passive exposure to tobacco smoke in individuals with a reduced DNA repair capacity (Cheong et al., 2022).

There are various DNA repair pathways that are employed dependent on the type of damage. The majority of physical and chemical carcinogens produce bulky DNA lesions which are exclusively repaired by the nucleotide excision repair (NER) pathway (Benhamou and Sarasin, 2000). Patients with the rare autosomal recessive disorder xeroderma pigmentosum (XP) have a NER capacity that is only 1-2% of the normal (Mohrenweiser and Jones, 1998; Benhamou and Sarasin, 2000). This results in severe photosensitivity and an 1000-4000-fold increased risk of skin cancer due to their inability to repair UV-induced DNA damage (Benhamou and Sarasin, 2000; Cheong et al., 2022). Individual DNA repair capacity is not only based on genetic variation, but is also affected by age, circadian rhythm, lifestyle and dietary factors (Cheong et al., 2022). With increasing age the DNA repair capacity declines. The rate of DNA repair in lymphocytes irradiated with UV-light decreases approximately 30% between the ages of 20 to 90 years (Cheong et al., 2022). Another study found the rates of repair to be similar in most individuals, but attributed the decreases in rate of DNA repair to a subset of older individuals with repair deficient lymphocytes (Cheong et al., 2022). The likely explanation is that the age dependent changes in DNA repair capacity depend on the cell type, DNA repair pathway and the health status of the study participants (Cheong et al., 2022). Besides the age-dependent sensitivity to arsenic that was discussed earlier, arsenic exposure also leads to alterations in DNA repair capacity (Cheong et al., 2022).

In conclusion, there is evidence that there are differences in DNA repair capacity in humans due to genetic differences or other intrinsic factors and also due to exposure to chemicals. Both factors could influence the susceptibility to genotoxic carcinogens.

4.3 Toxicokinetics

Once a genotoxic carcinogen is ingested or inhaled, the internal exposure is influenced by the absorption, distribution, metabolism and excretion of the specific chemical. The three examples described below show that these intraspecies differences in toxicokinetics can result in differences in cancer susceptibility.

DNA adducts are considered relevant biomarkers of carcinogen exposure and tumor incidence in experimental animals. Polycyclic aromatic hydrocarbons (PAHs) present in tobacco smoke are associated with DNA adducts in the larynx (Badawi et al., 1996; Dickey et al., 1997). The adduct levels are dependent on the expression of cytochrome P450 which can vary over 40-fold in the case of enzyme P450 1A2 in different individuals (Badawi et al., 1996; Guengerich et al., 1999; Lamba et al., 2012). Additionally, it has been shown that the intraspecies differences in DNA adduct formation is increased in persons that lack the Glutathione S-Transferase M1 gene which is important in the detoxification pathway of PAH (Dickey et al., 1997; Norppa et al., 2004).

Thus, individual differences in metabolism of PAH could explain the differences in DNA adduct formation.

Also the toxicity of arsenic is associated with individual differences in metabolism. Inorganic arsenic is methylated to methylarsonic acid (MMA) and dimethylarsinic acid (DMA) in most mammalian species, which are more rapidly excreted than inorganic arsenic. There are large intraspecies differences in the methylation of arsenic and thus in the excretion (Vahter et al., 1999). Additionally, Hattis et al. (1987) showed in an analysis of individual measurements that there are also significant intraspecies differences in other toxicokinetic parameters for arsenic. Thus, the variation in sensitivity towards arsenic toxicity could be due to the variation in toxicokinetics of arsenic (Vahter et al., 1999).

Another well-known example is acetaldehyde, the first metabolite of ethanol oxidation. Chronic ethanol consumption is a strong risk factor for the development of liver cancer and cancers of the aerodigestive tract, large intestines and female breasts. However, specific polymorphisms or mutations of genes involved in the generation of acetaldehyde and detoxifying enzymes result in an increased cancer risk. For instance, even a small amount of alcohol leads to high concentrations of acetaldehyde in Japanese, Koreans or Chinese people due to an approximate 40% increase in the dehydrogenase allele which codes for an ALDH2 enzyme with little activity (Seitz and Stickel, 2010). This results in a significantly increased risk for the upper aerodigestive tract cancer and colorectal cancer. Similarly, an increased risk for cancer of the upper aerodigestive tract, liver, colon and female breast is seen in Caucasians due to an allele that encodes for an enzyme that produces 2.5 times more acetaldehyde (Seitz and Stickel, 2010).

Thus, there is evidence that the susceptibility to genotoxic carcinogens in humans is dependent on the toxicokinetics, which can be due to intrinsic differences or substance specific differences in toxicokinetics.

5 Interspecies differences

In the past many scientists have tried to compare carcinogenic effects of chemicals in different animal species, most often in rats and mice (Crouch, 1983; Gold et al., 1989; Lin et al., 1995). Concordance is one of the methods to show interspecies agreement. This is the percentage of chemicals that are classified in the same way in different species with respect to carcinogenicity (Gold et al., 1989). Using a database of 392 carcinogens, Gold et al. (1989) found a concordance of 76%. This is comparable to the concordance of around 75% found by Lin et al. (1995) using the national toxicology program database with 297 carcinogens. However, groups of scientists have also noted that it is unlikely that results of these studies are close to reality (Piegorsch et al., 1992; Lin et al., 1995; Gold et al., 1998). Piegorsch et al, (1992) argue that true concordance is underestimated at low potencies. Whereas Gold et al. (1998) consider it likely that reality is overestimated, meaning that the true concordance would be lower. It should be noted that it is very difficult to compare different toxicity studies, as the study design of different studies will not be identical. It is therefore the question if different toxicity studies can be compared in this way. In this chapter we will dive a bit deeper into interspecies differences and the possible underlying mechanisms, specifically for genotoxic carcinogens. In the literature search, no general reviews about interspecies differences in toxicokinetics or DNA repair for genotoxic carcinogens were identified. However, many articles about specific genotoxic carcinogens and possible differences in toxicokinetics were found. Further, several articles studied the link between DNA repair and life span of species.

5.1 DNA repair

DNA damage is an important factor for initiation of tumorogenic changes in a cell and the further process of cancer development (Slaga et al., 1988). DNA repair mechanisms can reverse DNA damage caused by genotoxic carcinogens (Chatterjee and Walker, 2017). A reduced DNA repair efficiency is associated with increased cancer risk (Cheong et al., 2022). Unrepaired DNA damage could lead to carcinogenesis due to the accumulation of mutations (Cheong et al., 2022). Cortopassi and Wang (1996) showed that different animal species have different rates of DNA repair. The highest rate was observed in humans and gorillas and the lowest rate in mice and rats (around 5-fold lower). It has to be noted that there was some variance in results from different laboratories, however the authors conclude that the results were still in agreement. In addition, the DNA repair rate seemed to correlate with life span of the species (Coropassi and Wang, 1996). More recently, the study of MacRae et al. (2015) tried to show the correlation of DNA repair activity with life span using gene expression data. The study found that expression of genes involved in DNA repair were upregulated in humans and naked mole rat, long lived species, compared to mice (MacRae et al., 2015). It was difficult to find specific examples of genotoxic carcinogens which elicit interspecies differences in DNA repair. DNA repair is measured after exposure to various substances, however this is

often done as a marker of the genotoxic potential of the compound (Martelli et al., 2003; Yoshimi et al., 1988; Holme and Soderlund, 1985; Kornbrust and Barfknecht, 1984). In those studies, DNA adduct formation is often not measured and therefore it is not possible to conclude if there is a difference in DNA repair or a difference in toxicity i.e. DNA adduct formation.

Overall, there are indications that DNA repair rate correlates with life span and species differences, which can influence the susceptibility to genotoxic carcinogens.

5.2 Toxicokinetics

Once a genotoxic carcinogen is ingested or inhaled, the internal exposure is influenced by the absorption, distribution, metabolism and excretion of the specific chemical. In risk assessment it is broadly accepted that species specific toxicokinetics can cause interspecies differences for (non-genotoxic) substances (EFSA, 2012; IPCS, 2020). In scientific literature no reviews about possible interspecies differences in toxicokinetics for genotoxic carcinogens were found. However, many examples can be found of individual substances for which interspecies differences in metabolism might influence the genotoxic potential of the chemical, i.e. 1,3-butadiene, acrylamide, heterocyclic amines, nitrosamines, inorganic arsenic and ochratoxin.

1,3-Butadiene (BD) can be metabolised to several genotoxic metabolites, of which some have a greater genotoxic potency than others (Swenberg et al., 2011; Kirman et al., 2010; Arce et al., 1990). Mice very efficiently oxidise BD to 1,2-epoxy-3-butene, the second most genotoxic metabolite and subsequently to 1,2:3,4-diepoxybutane, the most genotoxic metabolite (Kirman et al., 2010). Studies showed that these metabolites are formed to a lesser extent in rats. In addition, it was found that rats and humans have a higher ability to detoxify these metabolites, resulting in lower blood and tissue levels (Kirman et al., 2010; Jackson et al., 2000). When studying protein adducts after inhalation exposure to BD, it was found that mice are about 50 times more sensitive than rats and about 200 times more than humans (Swenberg et al., 2011). That rats are less sensitive to the effects of BD compared to mice is also confirmed in long-term carcinogenicity studies (Swenberg et al., 2011; Kirman et al., 2010; Arce et al., 1990). Metabolism is therefore an important determinant of BD exerted carcinogenicity.

In case of acrylamide, the metabolite glycidamide is most likely responsible for the carcinogenic effects (Kopp and Dekant, 2009; Paulsson et al., 2001). In rodents the contribution of glycidamide or glycidamide derived metabolites to total metabolites is higher compared to humans (Kopp et al., 2009). In other studies it was found that 51% of the metabolites identified in mice were glycidamide or glycidamide derived metabolites (Sumner et al., 1999). In rat studies percentages of 30 and 41% were observed (Fennel et al., 2005; Sumner et al., 2003). In a study in humans only 14% of the metabolites were identified as glycidamide or glycidamide derived metabolites (Fennel et al., 2005). Even though acrylamide induced tumours in mice and rats in

carcinogenicity studies, most epidemiological studies were not able to find this association (Gargas et al., 2009; Hansen et al., 2010). It is however noted that it is in general more difficult to find effects in epidemiological studies compared to toxicity studies in experimental animals. In scientific literature, the major focus for the interspecies differences of acrylamide is on the metabolism (Kopp et al., 2009; Paulsson et al., 2001).

The heterocyclic amines, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), can also be metabolised to several metabolites, of which only some are genotoxic. It was shown that human hepatocytes were very efficient in metabolising PhIP and MelQx with P450 1A2 compared to rat hepatocytes, as 75% of the dose was metabolised in human hepatocytes and the majority of the dose was excreted unchanged in rat hepatocytes after 24h (Nauwelaers et al., 2011). In addition, there seems to be interspecies differences in regioselectivity with respect to the P450 1A2 metabolism, where in human hepatocytes this enzyme mainly catalyzes N-oxidation which activates the genotoxic carcinogens, but in rat hepatocytes this enzyme catalyzed oxidation at the heterocyclic rings, which detoxifies PhIP and MelQx, a pathway which is negligible in human hepatocytes. Also Langouët et al. (2002) and Turesky et al. (2002) found that human hepatocytes were significantly more efficient than rat hepatocytes in metabolising PhIP to genotoxic metabolites and agree that the detoxifying pathway mediated by P450 1A2 seen in rat hepatocytes is negligible in human hepatocytes. However, it has to be noted that these authors observed that PhIP is also detoxified in human hepatocytes, but to a lesser extent and by other enzymes than P450 1A2 (Langouët et al., 2002; Turesky et al., 2002). For MelQx, Turesky et al. (2002) found that P450 1A2 is involved in a detoxifying pathway by oxidation of MelQx. The metabolite formed is the major detoxication product excreted in human urine, however it is not formed by rat P450 1A2 (Turesky et al., 2002). When DNA adduct formation is studied, the interspecies differences are confirmed as a 100-fold higher DNA adduct formation was seen in human hepatocytes, compared to rat hepatocytes after PhIP and MelQx exposure (Nauwelaers et al., 2011). The authors note that the differences in metabolism may partly explain the interspecies differences in DNA adduct formation (Nauwelaers et al., 2011).

In addition to scientific literature, also some risk assessments have already shown that interspecies differences in toxicokinetics do occur for genotoxic carcinogens and can influence the risk assessment.
In three recent EFSA opinions about the genotoxic carcinogens nitrosamines and inorganic arsenic and possible genotoxic carcinogen ochratoxin A, interspecies differences in toxicokinetics were acknowledged and taken into account in the risk assessment (EFSA, 2024; EFSA, 2023; EFSA, 2020).
For nitrosamines EFSA observed differences in absorption, distribution, metabolism and excretion. EFSA noticed that oral bioavailability for dimethylnitrosamine (NDMA), one of the nitrosamines, varied greatly between several animal species. In hamsters and rats oral bioavailability was low (8-10%), whereas in monkeys, pigs and dogs oral bioavailability ranged from 49 to 93% (EFSA, 2023). Furthermore,

interspecies differences were observed when the NDMA metabolism in several tissues was compared. For example, the human colonic epithelial is able to metabolise NDMA, whereas this is not the case for the intestine of rodent. In addition, in liver microsomes, NDMA-N-demethylases activity was threefold higher in hamsters compared to rats. This finding is in line with results of the AMES test, where hamster liver preparations had a higher capacity to generate mutagenic metabolites compared to rat liver preparations. Also, in liver slices from Syrian golden hamsters, formation of N7-alkylguanine adducts was greater than for rats, humans, monkeys and trout (in decreasing order). These differences were identified as possible reasons why the main target tissues in rodents are not consistently identified in epidemiological studies in humans (EFSA, 2023). It is again noted that it is in general more difficult to find effects in epidemiological studies compared to toxicity studies in experimental animals.

For inorganic arsenic, EFSA concluded that the toxicokinetics, including the metabolism, in experimental animals and humans differed to such extent that toxicity studies in animals were not suitable for use in the human risk assessment (EFSA, 2024). The most important difference is the methylation of inorganic arsenic. Rodents, rabbits and dogs have a high ability to methylate inorganic arsenic and mainly excrete dimethylated arsenic, whereas marmoset monkeys do not methylate inorganic arsenic (EFSA, 2024). Rats also have a high methylation capacity, but dimethylated arsenic is taken up by erythrocytes and therefore excreted to a lesser extent. In contrast, humans excrete more monomethylated arsenic. An *in vitro* experiment showed that primary rat hepatocytes had a higher methylation capacity than primary human hepatocytes and keratinocytes (EFSA, 2024).

Also for ochratoxin A, EFSA observed interspecies differences in toxicokinetics for ochratoxin A (EFSA, 2020). The plasma protein binding of ochratoxin A differs between animal species and humans. In general, only free ochratoxin A can be filtrated in the kidney and excreted. In humans, plasma protein binding is higher, which results in a longer half-life in humans compared to for example pigs and rodents, of several weeks versus several days respectively. In case of repeated exposure the longer half-life will lead to increased accumulation. EFSA discussed if an additional assessment factor was needed to account for the toxicokinetic interspecies differences between humans and experimental animals. Eventually EFSA decided that the default assessment factor for toxicokinetics of 4 was sufficient (EFSA, 2020), in contrast to the previous EFSA opinion where the assessment factor for toxicokinetics was raised to 6 (EFSA, 2006). Note that the previous EFSA opinion only covered a non-genotoxic endpoint, in contrast with the most recent opinion where ochratoxin A was considered possibly genotoxic.

In addition to EFSA opinions, several Committee for Risk Assessment (RAC) reports for classification and labelling are available in which interspecies differences in toxicokinetics were acknowledged. However, as the observed differences did not influence the hazard assessment conducted these were only shortly described. For cobalt, RAC observed that the results of the in vivo mutagenicity studies were different between mice and rats, as all studies in rats were negative and all

studies in mice positive (RAC, 2017). In the RAC report it is noted that the species differences could not be explained. In the part of the opinion about toxicokinetics it was described that there were interspecies differences for the absorption of soluble cobalt substances (1 to 2% in cows; 4 to 5% in guinea pigs; 13 to 34% in rats), clearance rates (10- to 20-fold higher in rodents) and translocation rates (3- to 10-fold differences) (RAC, 2017). For 2,2-bis(bromomethyl)propane-1,3-diol, RAC acknowledges that there are clear interspecies differences in toxicokinetics (RAC, 2018). Specifically, the glucuronidation of 2,2-bis(bromomethyl)propane-1,3-diol was 150-fold higher in rodent cells compared to human hepatocytes (RAC, 2018).

Overall, species depended variations in toxicokinetics were observed for several substances which may affect their genotoxic potential.

6 Conclusions

RIVM supports the use of probabilistic methods for risk assessment (Slob et al., 2014; Bokkers et al., 2017). To apply probabilistic risk assessment to genotoxic carcinogens it is necessary to know which uncertainties need to be taken into account. The goal of this report is to answer the question whether it would be required to correct for intraspecies and interspecies differences for genotoxic carcinogens in probabilistic risk assessment.

Causes of intraspecies differences are increased susceptibility during early life, differences between humans in DNA repair and in toxicokinetics. In literature reviews of animal data it was shown that juvenile animals were more susceptible in developing tumors compared to adults. Although a full assessment of children's cancer risks is not feasible, early life sensitivity was reported in human cases of exposure to carcinogenic substances.
Differences in DNA repair are due to individual differences in DNA repair capacity as well as age, circadian rhythm, lifestyle and dietary factors. There are also indications that the DNA repair capacity can be influenced by substances directly. Similarly, for toxicokinetics there are individual substance specific differences in metabolization and excretion of genotoxic carcinogens.

Two causes for interspecies differences were presented in this report: toxicokinetics and DNA repair. In literature we found many examples of species dependent variation in absorption and metabolism of a substance. As a result of this variation, the internal exposure to the active parent substance/ metabolite can be higher or lower depending on the species.
Regarding DNA repair, there are indications that long-lived species have higher DNA repair activity compared to short-lived species. This is relevant, as a large part of the toxicity studies used in risk assessment are conducted in (short-lived) rats or mice. It is noted that many other aspects may influence the risk of cancer (Vincze et al., 2021) and the differences in DNA repair does not necessarily equal a difference in cancer susceptibility.

It is concluded that there are indications of intraspecies and interspecies differences for genotoxic carcinogens, which sufficiently supports the use of assessment factors for these differences in a risk assessment.

This report provides qualitative arguments to include adjustment for intra- and interspecies differences in the risk assessment of genotoxic carcinogens. This will result in more realistic estimates of the health risk that genotoxic carcinogens pose as compared to the currently used approaches.
Empirical inter- and intraspecies assessment factors (or distributions thereof) (Baird et al., 1996; Bokkers & Slob, 2007; Hattis & Lynch, 2007; IPCS, 2017; Schneider et al. 2004; Vermeire et al., 1999) have been applied for the assessment of genotoxic carcinogens (Jang et al. 2023; Slob et al., 2014) and have been included in guidance of

deterministic risk assessment methods (IPCS, 2017). However, the underlying historical data generally related to non-genotoxic substances, and thus may not be appropriate to set assessment factors for the purpose of risk assessment of genotoxic chemicals.
In the present report appropriate quantitative assessment factors for intraspecies and intraspecies differences are not proposed. This would require an extensive analysis of the available data to get insight in the quantitative differences in, for instance, early life susceptibility, DNA repair and toxicokinetics with respect to genotoxic chemicals. However, based on such an analysis quantitative inter- and intraspecies assessment factors for genotoxic chemicals might be proposed. To achieve this goal it would be very helpful to establish a group of experts, to discuss the data using the process of expert knowledge elicitation, and propose appropriate assessment factors for various inter- and intraspecies differences to be applied in the probabilistic risk assessment method. The data collection and analysis and establishing assessment factors will require investment in resources, however better-founded assessment factor values would lead to more accurate risk assessment of genotoxic carcinogens. Well-founded assessment factors would increase its acceptance among toxicologists and risk managers. In order to encourage the use of these factors in the risk assessment, established assessment factors could be included in a future update of the IPCS (2017) guidance.

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