Regulatory Toxicology and Pharmacology 108 (2019) 104444

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Review

Classification or non-classification of substances with positive tumor findings in animal studies: Guidance by the German MAK commission

Britta Laube[1], Sandra Michaelsen[1], Veronika Meischner[1], Andrea Hartwig[b], Bernd Epe[c], Michael Schwarz[d,*]

[1] Scientific Secretariat of the Senate Commission on the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK Commission), Institute for Applied Biosciences, Karlsruhe Institute of Technology (KIT), Department of Food Chemistry and Toxicology, Karlsruhe, Germany
[b] Food Chemistry and Toxicology, Institute of Applied Bioscience, Karlsruhe Institute of Technology (KIT), Adenauerring 20a, 76131, Karlsruhe, Germany
[c] Institute of Pharmacy and Biochemistry, University of Mainz, Staudingerweg 5, D-55099, Mainz, Germany
[d] Dept. of Experimental and Clinical Pharmacology and Toxicology, Dept. Toxicology, Eberhard Karls University, Wilhelmstr. 56, 72074, Tübingen, Germany

ARTICLE INFO

Keywords:
Carcinogenicity
Animal tumor studies
Human relevance
Species-specific tumors
Mechanism of tumorigenesis
Organ-specific tumors
Maximally tolerated dose
Abbreviations: CC
Carcinogen category
MTD
Maximally tolerated dose
ROS
Reactive nitrogen species
PPARα
Peroxisome proliferator activated receptor α
ROS
Reactive oxygen species

ABSTRACT

One of the important tasks of the German Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (known as the MAK Commission) is in the evaluation of a potential for carcinogenicity of hazardous substances at the workplace. Often, this evaluation is critically based on data on carcinogenic responses seen in animal studies and, if positive tumor responses have been observed, this will mostly lead to a classification of the substance under investigation into one of the classes for carcinogens. However, there are cases where it can be demonstrated with a very high degree of confidence that the tumor findings in the experimental animals are not relevant for humans at the workplace and, therefore, the MAK Commission will not classify the respective substance into one of the classes for carcinogens. This paper will summarize the general criteria used by the MAK Commission for the categorization into “carcinogen” and “non-carcinogen” and compare this procedure with those used by other national and international organizations.

1. Introduction

The German MAK commission has established five categories for the classification of carcinogenic chemicals in the work area. Generally, substances that have been found to be human carcinogens are classified in Category 1, while those substances that have proven to be carcinogenic in animal studies are classified in Category 2. Suspected carcinogens are classified in Category 3. Up to 1990 the Commission classified substances according to their carcinogenic hazard for man. Since the Commission has a long tradition of evaluating the mechanism of action as part of its assessment procedure, two additional Categories (4 and 5) of carcinogens were introduced in the late 1990s to account for findings related to the mechanism of carcinogenicity and the dose- and time-dependence of the carcinogenic effect (Greim, 1999, 2000; 2006a; Neumann et al., 1998). These new Categories include substances with carcinogenic properties for which there is sufficient data available to allow for an assessment of their carcinogenic potency. Substances classified in Category 4 are known to act via non-genotoxic mechanisms and genotoxic effects play no or only a minor role at levels that do not exceed the MAK and BAT values. Under these circumstances, the substance is not expected to add to the cancer risk of humans.Classification is based in addition to the results of the carcinogenicity studies on findings on the (primary) mechanism of action, for example an increase in cell proliferation, delayed apoptosis or impaired differentiation. Both the classification category and the MAK and BAT values take into account the wide range of mechanisms that may contribute to carcinogenicity and their characteristic dose–time–response relationships. Carcinogens, which are genotoxic, but of weak potency at and below

* Corresponding author.
E-mail address: michael.schwarz@uni-tuebingen.de (M. Schwarz).

https://doi.org/10.1016/j.yrtph.2019.104444
Received 18 April 2019; Received in revised form 10 August 2019; Accepted 14 August 2019
Available online 18 August 2019

the MAK and BAT values, are classified in Category 5. A more precise definition of each of the categories can be found in the Annex.

Epidemiological studies are an important source for information on a potential carcinogenic risk associated with a certain exposure. However, such studies are often not available or may be difficult to interpret for various reasons, such as e.g. mixed exposure to more than just one chemical. Therefore, where human data are not available, animal studies on the carcinogenic effect of a substance play an important role in classifying the substance as carcinogenic or non-carcinogenic. However, not all positive tumor tests in animals can be judged as relevant for humans. There are, for example, mechanisms of action that are specific to a certain animal species, but are absent in humans, at least under realistic exposure conditions. This is of particular practical importance for non-genotoxic mechanisms of action, since genotoxic carcinogens are generally considered to be relevant for humans and classified in one of the categories for carcinogens by the German MAK Commission (see above).

The purpose of this paper is to develop a general guidance for use by the MAK Commission to evaluate tumor findings from animal studies on a case-by-case basis, to determine their relevance for humans and to decide whether a substance should be classified in one of the carcinogen categories.

The following key points are identified in this guidance document:

1. Determination of the primary mechanisms of action underlying the carcinogenicity:
   (a) Classification of the mechanisms of action as genotoxic vs. non-genotoxic
   (b) Assessment of the identified mechanisms with respect to their relevance for humans.
2. Characterization of the dose/time–response relationships of tumor formation, including the assessment of their relevance for actual scenarios of human exposure.
3. Assessment of the human relevance of tumors induced only in certain species and animal strains or in organs not found in humans (as an example: forestomach tumors in rats).
4. Evaluation of the human relevance of substance-induced cancer precursors such as adenomas and the characterization of their risk to develop into malignancy.

Examples of substances evaluated by the MAK Commission are provided throughout the paper. In addition, in chapter 7 some instructive cases are discussed that, despite having yielded positive tumor findings in animal studies, were not classified in one of the carcinogen categories by the Commission as well as others classified into Category 4 after thorough analysis and review of experimental findings.

2. Approaches used by other national and international organizations

The CLP criteria for classification used by the European Chemicals Agency (ECHA, 2017), the Preamble published by the IARC (International Agency for Research on Cancer; IARC, 2006) and the guidelines used by the U.S. EPA (U.S. Environmental Protection Agency; U.S. EPA, 2005) all explicitly include the analysis of possible mechanisms of tumor formation in the assessment of carcinogenicity. All organizations emphasize the importance of careful evaluation by expert judgments (ECHA, 2017; IARC, 2006; U.S. EPA, 2005; WHO (Boobis et al., 2006; Sonich-Mullin et al., 2001)).

2.1. SCOEL

The Scientific Committee on Occupational Exposure Limits for Chemical Agents (SCOEL) has classified chemical carcinogens into four groups, namely A, B, C and D, according to the mode of action (Bolt and Huici-Montagud, 2008; SCOEL, 2017). Thus, clearly DNA-reactive genotoxic carcinogens and/or those compounds which have the potential to initiate DNA reactivity are grouped in A or B, depending on the degree of evidence; here, a health-based threshold cannot be established. Group C comprises other DNA-reactive genotoxic carcinogens, which are only weakly genotoxic and their carcinogenicity appears to arise from other mechanisms, such as sustained local tissue damage and increased cell proliferation; here, a “practical” or “apparent” threshold can be established. Finally, chemical agents that are non-DNA reactive and act on the chromosomal level alone in the absence of gene mutations are grouped in D. A respective Mode of Action (MoA) includes the induction of numerical chromosomal aberrations, and given the existence of sufficient data a threshold concentration can be established below which the substance is not considered to be carcinogenic. SCOEL may also conclude that the available toxicokinetic and mechanistic evidence does not provide evidence of any MoA or internal exposure that is of relevance to workers (SCOEL, 2017). The concept of a “mode of action based threshold” for carcinogens was also emphasized within the joint task force between SCOEL and the ECHA Committee for Risk Assessment (RAC) (ECHA/RAC-SCOEL, 2017), and will be applied by ECHA/RAC after the termination of the SCOEL committee in February 2019.

2.2. ECHA

According to the CLP criteria for classification of the European Chemicals Agency, substances which have induced benign and malignant tumors in well performed experimental studies on animals are considered to be proven or suspected human carcinogens unless there is strong evidence that the mechanism of tumor formation is not relevant for humans. Genotoxicity, cytotoxicity with growth stimulation, mitogenesis and immunosuppression are listed as mechanisms of tumor formation with human relevance. If there is unequivocal evidence that a mechanism of tumor formation is not relevant to humans, such as the α2u-globulin-mediated mechanism that induces renal tumors in male rats, the substance is not classified as a carcinogen. If tumors are induced only in one sex of an animal species, it must carefully be assessed whether the development of these tumors is consistent with the postulated mechanism of tumor formation. Tumors observed in animal studies in tissues that lack a human equivalent are examined in light of the overall tumor response, i.e. the incidence of tumors in other organs. This requires a meticulous expert judgment on the assumed mechanism of tumor formation (ECHA, 2017).

2.3. IARC

The Preamble of the IARC (International Agency for Research on Cancer) highlights the importance of determining the relevance of the mechanism of tumor formation for humans. To clarify the mechanism of action, changes in the affected organs, tissues or cells caused by exposure are analyzed at three levels: changes in physiology, functional changes on the cellular level and changes at the molecular level. Physiological changes refer to exposure-related modifications to the physiology and/or response of cells, tissues and organs such as mitogenesis, inflammation, hyperplasia and metaplasia. Functional changes refer to exposure-related alterations in the signaling pathways used by cells to manage critical processes that are related to increased risk for cancer, for example alterations in cyclin-dependent kinases that govern cell cycle progression and changes in gap-junction-mediated intercellular communication. Molecular changes refer to exposure-related changes in key cellular structures at the molecular level, which are essentially related to genetic integrity, such as DNA strand breaks, mutations in genes or chromosomal aberrations. The studies of genotoxicity are described in view of the relevance of gene mutations and chromosomal aberrations/aneuploidy to carcinogenesis. If applicable, other relevant mechanistic data are included in the evaluation such as structure–activity relationships and data from gene expression

microarrays. An overview of the possible mechanisms of tumor formation is provided and discussed together with data on toxicokinetics and other data (IARC, 2006).

2.4. U.S. EPA

In the absence of sufficient, scientifically justifiable information on the mechanism of tumor formation, the U.S. EPA takes a protective default position regarding the interpretation of toxicological and epidemiological data. Therefore, in the absence of data on the mechanism of action, animal tumor findings are judged to be relevant to humans. The U.S. EPA prepared a framework as a tool for evaluating a mechanism of carcinogenic action hypothesized for a substance. Modified Bradford-Hill criteria can be useful for organizing thinking about aspects of causation. For the analysis of the mechanism of action, all relevant studies are evaluated based on the weight of evidence method, which points out strengths, weaknesses and uncertainties and discusses possible alternative conclusions. The mechanism of action is analyzed for all tumor types observed; this includes a description of the hypothesized mechanism of action as well as the sequence of key events. The relevance of a possible mechanism of action is evaluated within the context of a hazard assessment and not in regard to the level of risk. Human exposure data are therefore not included in the evaluation of the relevance of a hypothesized mechanism of action (U.S. EPA, 2005).

2.5. WHO – IPCS framework

The IPCS (International Programme on Chemical Safety) framework of the WHO (World Health Organization) is an approach used to evaluate the relevance of a mechanism of carcinogenic action in animals for humans. The individual steps of this framework are based on Bradford-Hill criteria of causality, which were originally developed for the interpretation of epidemiological studies (Hill, 1965). These include: 1. postulated mechanism of action, 2. key events; associated critical parameters, 3. dose–response relationships, 4. temporal association, 5. strength, consistency and specificity of association of key events and tumor response, 6. biological plausibility and coherence, 7. possible alternative mechanisms of action, 8. uncertainties, inconsistencies and data gaps, 9. assessment of mechanism of action (Boobis et al., 2006; Sonich-Mullin et al., 2001).

2.6. Summary

In the absence of sufficient evidence that the mechanism of tumor formation is not relevant to humans, IARC, ECHA, U.S. EPA and WHO consider tumor findings from animal studies to be applicable to humans. However, a critical examination is carried out to determine whether tumors are induced in only one or in several organs or in only one or in several species and whether the tumors themselves are relevant to humans. Benign tumors that are assumed to progress into malignant tumors are generally also included in the evaluation. In addition, the U.S. EPA includes a formalized framework for the assessment of hypothesized mechanisms of carcinogenesis in the method it uses to evaluate cancer formation.

A summary of the carcinogenicity classifications of the examples described in Section 7 by the above mentioned organizations is given in Table 3. The U.S. EPA is not included in the table because the substances under concern are not listed by the agency or were not evaluated regarding their carcinogenicity.

2.6.1. Advisory and legal concerns

The carcinogen classifications of the MAK Commission are advisory in nature comparable to those of the IARC. However, even though the MAK proposals are not directly legally binding they form the scientific basis for setting legally binding exposure limits by respective regulatory authorities in Germany and other countries.

US EPA is a regulatory agency authorized by Congress. The Toxic Substances Control Act (TSCA) of 1976 provides US EPA with authority to require reporting, record-keeping and testing protocols and to impose restrictions relating to chemical substances and/or mixtures.

The Committee for Risk Assessment (RAC) prepares the opinions of European Chemicals Agency (ECHA) related to the risks of substances to human health and the environment in the following REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) and CLP (Classification, Labelling and Packaging of substances and mixtures) processes. The final decisions are taken by the European Commission.

The Scientific Committee on Occupational Exposure Limits (SCOEL) assisted from 1995 to 2018 the European Commission to evaluate the potential health effects of occupational exposure to chemicals. The health-based scientific recommendations were used to underpin the regulatory initiatives on occupational exposure limit values for the protection of workers from chemical risks.

3. Evaluation of the quality of an experimental study

Before an experimental study is considered to be included into toxicological decision making by the MAK Commission, it is always necessary to assess the validity of its study design and of the conclusions drawn from the results of the study. Other organizations have already addressed this issue. Guidelines have been published on study design including the use of the proper study protocol and the selection of appropriate doses. Some examples are: The OECD Guideline for the testing of chemicals, here TG 451, “Carcinogenicity Studies” (OECD, 2018), the guidance document for the National Toxicology program (NTP) for the conduct of animal toxicity/carcinogenicity studies (NTP, 2011), the ICH Topic S1C(R2) “Dose Selection for Carcinogenicity Studies of Pharmaceuticals” (EMEA, 2008), “Guidance for Industry. Carcinogenicity Study Protocol Submissions” (FDA, 2002). There are also guidelines on validation and interpretation, such as the “Guidance for Industry. Statistical Aspects of the Design, Analysis, and Interpretation of Chronic Rodent Carcinogenicity Studies of Pharmaceuticals” (ECHA, 2017; FDA, 2001; Huff and Haseman, 1991; IARC, 2006; U.S. EPA, 2005; see also Section 2).

4. Mechanisms with no or only minor human relevance

The following chapter discusses mechanisms that lead to tumor formation in test animals, but have no or only minor human relevance. There are several different scenarios.

1. The carcinogenic effect of a particular test chemical is mechanistically linked to a very high dose used in the respective animal study. If this type of exposure cannot occur for humans under realistic conditions, even as a worst-case scenario, the findings obtained are regarded as not relevant for classification (discussed in Section 4.1 and 4.2).
2. The mechanism of tumor induction is based on parameters that differ strongly between humans and animals. This may be the case, for example, for differences in the metabolic competence of toxifying and detoxifying enzymes. In general, according to the guidelines of the MAK Commission, this would lead to the classification of the substance in the relevant Carcinogen Category (CC). However, the differences between humans and animals may be so large that, after careful consideration, the more plausible alternative may be not classifying the respective substance in one of the categories for carcinogens (discussed in Section 4.3).
3. Furthermore, a number of mechanisms of tumor formation have a very high species, strain or gender specificity and are thus not relevant to humans (discussed in Chapter 5).

4.1. MTD (maximally tolerated dose) and high dose effects

4.1.1. Definition of the MTD

The highest dose of a substance that may be tested in long-term carcinogenicity studies as stipulated by regulatory authorities is called the MTD. As the name itself indicates, this is a limit range. The most commonly used definition of the MTD can be found in the National Cancer Institute (NCI) publication by Sontag et al. (1976): "The MTD is defined as the highest dose of the test agent during the chronic study that can be predicted not to alter the animals' normal longevity from effects other than carcinogenicity." This was applied as the basis for selecting the doses used in carcinogenicity studies carried out by the NCI and the National Toxicology Program (NTP). In 1976, it was suggested that the dose chosen as the MTD should be one that, in a subchronic study, causes no more than a 10% weight decrement, as compared to the appropriate control groups, and does not produce mortality, clinical signs of toxicity or pathologic lesions (other than those that may be related to a neoplastic response) that would be predicted (in the long-term study) to shorten an animal's natural lifespan (Haseman, 1985). In carcinogenicity studies carried out according to the test guidelines of the OECD, the MTD is defined as the highest dose to produce toxic effects "without causing death and to decrease body weight gain by no more than 10% relative to controls". The MTD is used to decide whether the highest dose tested was adequate to give confidence in a negative result (OECD, 2012).

4.1.2. MTD tumor findings

The MTD as the highest dose tested in long-term carcinogenicity studies was deliberately chosen for toxicological assessments to ensure that all carcinogenic effects would be reported, even those that would generally not be observed due to statistical limitations in the small animal cohorts that are typically used in long-term carcinogenicity studies. However, MTD findings are increasingly coming under criticism because non-specific high-dose effects may yield "positive" responses that are not predictive of lower exposure levels (Ames and Gold, 1990; Carr and Kolbye, 1991; Greim and Albertini, 2012). This may cause carcinogenic effects and other associated effects that are not of importance in low dose ranges that are relevant to humans. In these cases, the decision not to classify a substance has to be based on the mechanism of action that is effective in the high dose range of the substance, but is not evident at lower levels of exposure, even in animal studies. For this reason, it must be carefully assessed in each case whether "MTD findings" are relevant for the classification of that substance as carcinogen.

Indeed, a large number of substances have been found to be carcinogenic at extremely high doses in animal studies that would not have been classified as carcinogens at lower doses. An evaluation of the results of the carcinogenicity studies in rats and mice, which was described in the 1989 Carcinogenic Potency Database (CPDB), concluded that 492 of 975 investigated substances, i.e. almost half of the substances, caused carcinogenic effects in at least one study (Gold et al., 1989). This can be explained by the fact that, for decades, the results of animal studies with very high doses (up to the MTD) have been used for the evaluation of carcinogenicity. The rationale was to increase the sensitivity of tests that were carried out with a limited number of animals. However, as early as in 1990, it was suspected that this approach leads to an overestimation of the "hazard" presented to humans by low, non-cytotoxic doses of carcinogens. The administration of substances at doses close to the MTD is postulated to increase mitogenesis (e.g. by stimulating regenerative growth), which in turn increases rates of mutagenicity and, thus, carcinogenicity (Ames and Gold, 1990). This may explain why 50% of all natural and synthetic pesticides tested in these studies showed a carcinogenic effect (Ames et al., 1990). The suitability of rodent carcinogenicity studies for predicting human cancer risk is also being critically debated in the pharmaceutical sector (Alden et al., 2011; Anisimov et al., 2005; Sistare et al., 2011).

In conclusion, the relevance of tumor findings from animal studies after exposure to high doses for predicting the tumor response at low doses, which are relevant to humans, needs to be critically examined (Greim and Albertini, 2012, 2015) with respect to high-dose effects such as "metabolic overloading" and "secondary carcinogenesis" (e.g. induced by toxic regenerative responses). However, in individual cases, this requires clear evidence that the respective mechanisms do not arise after exposure to concentrations that are relevant to humans, i.e. that human exposure occurs at levels far below the dose that may lead to a carcinogenic response (Haseman, 1985).

A question that is frequently asked concerns the human relevance of tumors that are observed in animal studies only close to the MTD. In these cases, the causative mechanism needs to be determined (Slikker et al., 2004a, b). The substance should in general not be classified as carcinogenic if it can unequivocally be ascertained that the mechanism that is effective in the high dose range close to the MTD qualitatively does not occur at lower doses and is therefore not relevant for exposure at the workplace even in worst case scenarios. In this case, the effect threshold has a mechanistic basis that can clearly be defined and used as a reason for non-classification.

Examples are biguanide derivatives. In two carcinogenicity studies (unpublished studies, ICI America Inc. and AstraZeneca, respectively), **polyhexamethylene biguanide** given to mice in the diet at the very high dose of 4000 mg/kg diet (males: 715 mg/kg body weight and day; females: 856 mg/kg body weight and day) induced vascular tumors (hemangiosarcomas). These were in the range of the historical controls at a middle dose of 1200 mg/kg diet. At the high dose, mortality was increased and body weights were reduced by 35%–42% in males and by 22%–33% in females, despite increased food consumption. As biguanides impair glucose utilization, they are used in anti-diabetic therapies. The effect is induced by reduced glucose absorption in the intestine and the suppression of gluconeogenesis and ATP production (ECHA RAC, 2011). Impaired mitochondrial oxidative phosphorylation leads to reduced ATP synthesis. Similar to cellular hypoxia, this leads to the activation of hypoxia-inducible factors (HIFs), which regulate a large number of cellular functions such as the transcription of the hormone erythropoietin (EPO). EPO stimulates the proliferation of red blood cells, promotes the vascular endothelial growth factor (VEGF), which regulates angiogenesis, and induces glycolytic enzymes (Tormos and Chandel, 2010). A consequence in mice is the induction of vascular tumors, which is, however, a typical "high dose effect". In humans, even a single dose at this high level would change glucose metabolism so dramatically that neither short-term nor long-term exposure at this level would be tolerated. Long-term exposure at the level that induces tumors in mice is therefore not possible in humans. In addition, substance-induced hemangioadenomas and hemangiosarcomas are extremely rare in humans. They have only been observed after high-level exposure of workers to vinyl chloride and in patients who received thorotrast (as a radiocontrast agent) for diagnostic purposes (Cohen et al., 2009). In summary, the tumors observed in mice after long-term exposure to biguanides are not relevant for classification because exposure of humans to a tumor-inducing dose is not possible; glucose metabolism will have been completely disrupted long before this level of exposure is reached. This is an example of a case in which the tumorigenic effect of a substance cannot be induced in humans under normal circumstances because of the limitation of the dose resulting from acute toxicity.

In the high dose range of the MTD, cytotoxic effects may also lead to "indirect genotoxic" effects that can initiate and accelerate the process of carcinogenesis. A key role plays the generation of reactive oxygen and nitrogen species (ROS and RNS, respectively) that may be initiated at very high doses by processes such as uncoupling of the mitochondrial respiratory chain (Filser et al., 2008; Wiseman and Halliwell, 1996). However, this does not mean that ROS and RNS are only induced in the high dose range. Increased ROS and RNS formation may play a role at low levels of exposure that are relevant to humans, for example as the

result of Fenton or Fenton-like reactions catalyzed by a transition metal or as the result of inflammatory reactions induced by xenobiotics. For this reason, the human relevance of these types of "indirect genotoxic" effects has to be assessed on a case-by-case basis.

However, in many cases, the decision against classifying substances with positive tumor findings in animal studies in a Carcinogen Category cannot be based on mechanistic considerations. Under these circumstances, it is prudent to classify the substances in one of the categories for carcinogenic substances (generally in CC 4) and to indicate a MAK value, below which an exposure is regarded as being safe. Obviously, there is a gray area in which the decision for classification or non-classification has to be weighed carefully based on the established criteria.

4.2. Importance of species-specific differences in the activities of xenobiotic-metabolizing enzymes

The chronic toxicity and carcinogenicity of most carcinogens with human relevance is dependent upon the activity of toxifying and detoxifying enzyme systems, which can vary strongly between different species, both quantitatively and qualitatively. For this reason, it is important to take into consideration the species-specific enzyme systems that activate metabolic pathways, and their associated toxification and detoxification processes.

One example is an extremely efficient process in the liver of mice that involves the inactivation of the active metabolite of **aflatoxin B1**, the 8,9-epoxide, by an isoform of glutathione S-transferase. In consequence, mice are resistant to the hepatocarcinogenic effect of aflatoxin B1. Neither rats nor humans have a similarly efficient detoxifying enzyme (Degen and Neumann, 1981) and therefore are, unlike the mouse, highly susceptible to this hepatocarcinogen.

Another example that has been investigated extensively is **styrene** and the species-specific detoxification capacity towards its metabolite styrene-7,8-oxide, which has a direct alkylating effect, by microsomal epoxide hydrolase in the liver. Among the analyzed species, the activity of this enzyme is lowest in the mouse and then increases in the order mouse < rat < human (Mendrala et al., 1993; Seidegård et al., 1986). For this reason, styrene caused lung tumors only in mice, but not in rats (Greim, 2003a; IARC, 2002; NRC, 2014). Body burden levels determined by toxicokinetic investigations carried out in different species to assess the carcinogenic risk for humans have shown that changes in metabolic parameters in rats, mice and humans lead to similar changes in styrene and styrene-7,8-oxide concentrations in the blood. However, according to a physiologically based pharmacokinetic model, marked changes in the styrene-7,8-oxide concentrations in the blood of mice exposed by inhalation to styrene concentrations of 500 ml/m^3 were predicted because at this exposure concentration, glutathione-S-transferase (GST)-dependent detoxification of styrene-7,8-oxide has practically stopped functioning in the mouse due to the complete depletion of glutathione stores. Styrene-7,8-oxide concentrations in the blood thus reach a concentration that is high enough to saturate the elimination process for styrene-7,8-oxide by a second enzyme, epoxide hydrolase. For this reason, a slight increase in styrene-7,8-oxide will exceed the detoxification capacity of epoxide hydrolase, causing the alkylating agent to accumulate disproportionately. In contrast, a slight increase in the maximal activity of epoxide hydrolase would lead to a more efficient detoxification of styrene-7,8-oxide, thereby compensating for the loss of the glutathione-dependent pathway. Therefore, in mice, slight changes to the balance of styrene-7,8-oxide production and elimination through repeated administration drastically change the body burden levels of styrene-7,8-oxide. This phenomenon does not occur in rats and humans because of their higher epoxide hydrolase activity (Csanády et al., 1994), which means that mice are more sensitive to styrene exposure. In addition, there is a further plausible mechanistic explanation for the formation of lung tumors observed exclusively in styrene-exposed mice. In this species, lung metabolism of styrene is primarily mediated by the cytochrome P450 (CYP) 2F2 isoform, which is found in the Clara cells. The corresponding CYP2F4 in rats and CYP2F1 in humans and primates have a far lower metabolic capacity. In mice, CYP2F2 mediates not only styrene-7,8-oxide formation but also the formation of ring-hydroxylated epoxide derivatives, which cause cytotoxicity in Clara cells leading to regenerative hyperplasia and ultimately lung tumors (Cruzan et al., 2009, 2013). Based on the observations regarding species-specific differences in metabolism, it is likely that the assumed mechanism in the Clara cells in mice is not relevant to human lung cells to a biologically significant extent. However, the impact of circulating styrene-7,8-oxide and DNA adducts such as O6-deoxyguanosine-(O6-(2-hydroxy-1-phenylethyl)-2′-deoxyguanosine-3′-monophosphate) and N7-deoxyguanosine adducts observed in workers needs to be taken into account and may potentially play a role in organs other than the lung (IARC, 2002). Therefore, based on the data on the cancer risk for humans, the substance was classified in CC 5 (Greim, 2003a).

A general guideline cannot be established for the decision whether a substance should be classified in CC 4 or whether classification is not required. It will always be made on a case-by-case basis. In cases of uncertainty, a "conservative" approach should be followed with classification in CC 4 or in another carcinogen category if it is not possible to derive a MAK or BAT value.

4.3. Specific mechanisms of tumor formation that are not relevant to humans (see Table 1)

4.3.1. α2u-globulin-mediated kidney effects

Renal tubule tumors in male rats are one example that has undergone extensive investigation; these tumors are induced via the α2u-globulin-mediated mechanism. α2u-globulin is synthesized only in the liver of male rats, but not in the livers of female rats or other species (IARC, 1999b; Swenberg, 1993). The carcinogenic mechanism involves the binding of substances to this protein, leading to their accumulation in the kidney. This elicits cytotoxicity, which in turn induces a regenerative response in the form of cell proliferation. Clear evidence of this mechanism is provided by the following facts: lack of genotoxic activity of the substance and its metabolites; male rat specificity for nephropathy and renal tumorigenicity; verification of accumulation of α2u-globulin in renal cells; demonstration of the presence of a specific characteristic sequence of histopathological changes in short-term studies, of which protein droplet accumulation is obligatory; similarities in dose-response relationship of the tumor outcome with the histopathological end points (α2u-globulin accumulation, protein droplets and cell proliferation) and the reversible binding of the substance or metabolite to α2u-globulin (IARC, 1999a, b). In the adverse outcome pathway leading to the formation of kidney tumors in male rats, α2u-globulin plays a key role. The non-relevance of this mechanism for humans was ultimately concluded from the fact that this protein is not produced in humans, which means that this very well described tumor induction pathway is not applicable. Substances that induce these kinds of tumors in animals are, for example, **decahydronaphthalene** (Hartwig, MAK Commission, 2016a), **d-limonene** (Greim, 2006b, available only in German) and **2,2,4-trimethylpentane** (Hartwig, 2014a).

4.3.2. Tumors resulting from precipitate formation in the efferent urinary tract

Data available on **sodium saccharide, sodium ascorbate** (Note: no MAK documentations available) and **terephthalic acid** (Hartwig, 2009a) suggest that epithelial tumors of the bladder in rats are induced by the formation of a precipitate in the urine leading to cytotoxicity and increased regenerative cell proliferation. The most important indication for this assumption is that tumors can form only at urine concentrations at which the solubility product is exceeded. If critical concentration levels are not reached, the precipitate that can induce carcinogenicity

Table 1
Specific tumor formation in test animals, without or with only minor human relevance.

Mechanism	Tumor (Species)	Examples of substances
α2u-globulin-mediated	adenomas and carcinomas of the renal tubules (male rat)	decahydronaphthalene, D-limonene, 2,2,4-trimethylpentane
formation of precipitates in the urine	urothelial adenomas and carcinomas of the bladder (rat)	sodium saccharine, sodium ascorbate, terephthalic acid
higher sensitivity of the epithelium of the efferent urinary tract of male rats	carcinomas of the transitional epithelium of the bladder (male rat)	o-phenylphenol
proliferation of the mesothelium of the bladder of male mice	mesenchymal tumors including sarcomas of the bladder originating in the lamina propria (male mouse)	bifenthrin
hypoxia, uncoupling of oxidative phosphorylation	pheochromocytoma of the adrenal medulla (male rat)	furan
changes to thyroid hormone homeostasis through induction of glucuronosyltransferases or inhibition of peroxidases	follicular thyroid adenomas (rat)	spironolactone, sulfamethazine, thiabendazole
mediated by agonists of dopamine and GnRH	Leydig cell adenomas (male rats/mice)	nicotine
modulation of the dopaminergic tonus at dopamine D1 and D2 receptors or other membrane proteins	mesothelioma of the tunica vaginalis testis (male F344 rats)	acrylamide, methyl eugenol
PPARα-mediated	liver adenomas and liver carcinomas (rat, mouse)	clofibrate, trichloroacetic acid
severe irritation caused by non-DNA-reactive irritant substances after administration by gavage	forestomach, papillomas and squamous cell carcinomas (rat/mouse)	butylated hydroxyanisole (BHA)
lack of the respective organ in humans	Zymbal's gland, squamous cell carcinomas (rat)	chlorodifluoromethane
lack of the respective organ in humans	Harderian gland, adenomas/carcinomas (rat)	nitromethane

CYP: cytochrome P450; GnRH: gonadotropin-releasing hormone; PPARα: Peroxisome Proliferator Activated Receptor α.

does not form. In addition, there are important interspecies differences in the composition of the urine (protein concentration, total density and osmolarity) between rats and humans which affect the formation of precipitates or calculi (IARC, 1999b, d). Therefore, and because of the limitation of the carcinogenic effects to the high-dose range and the marked differences in the sensitivity of the epithelium of the efferent urinary tract between humans and rats, the corresponding tumors of the transitional epithelium are not considered relevant to humans as long as inflammatory or reactive alterations in the urothelium are not induced (Edler et al., 2014).

An example is **tributyl phosphate**, which was evaluated by the MAK Commission in 2000. Long-term studies reported local cell damage with reversible hyperplastic, proliferative and necrotic changes in the bladder of rats. Papillomas and, especially in the male animals, transitional cell carcinomas are found as a result of the damage to the bladder. These effects are not expected in humans at concentrations as long as inflammatory effects are not induced. Therefore, the MAK Commission has classified this substance as a carcinogen with a threshold in CC 4. (Greim, 2002b).

In the case of **o-phenylphenol (OPP)**, the MAK Commission concluded in 2016 that questions still remained as to the human relevance of the hyperplasia, papillomas and transitional cell carcinomas of the bladder observed in male F344 rats. The induction mechanism of cell proliferation has not completely been elucidated for OPP; the effects were, however, observed in the saturation range of metabolism. The pH of the urine or calculi alone was not considered the decisive factor in the formation of urothelial carcinomas (Hartwig, MAK Commission, 2016b, available only in German). For this reason, the substance has been classified in CC 4.

Mesenchymal tumors of the mouse bladder develop from the lamina propria, a layer of connective tissue that lies beneath the epithelium. **Bifenthrin**, a synthetic pyrethroid (Note: no MAK documentation available), is an example of a substance that induces mesenchymal tumors in the mouse bladder which is a non-invasive benign proliferation mainly in the submucosa (Butler et al., 1997). Up until this point, no tumor that originates in the submucosa (lamina propria) and is morphologically similar to the submucosal mesenchymal tumors of the mouse bladder has been described in humans. This type of tumor is not known to occur in other animal species. The following expert evaluation by Cohen (2011) is well accepted among scientists and government authorities: this type of bladder tumor seen in high-dose males that exceeded the MTD is mouse-specific, is not a carcinoma, is not invasive, does not metastasize, generally arises from inflammatory disorders and is without relevance to human.

4.3.3. *Pheochromocytomas resulting from hypoxia*

Pheochromocytomas originate in the chromaffin cells of the adrenal medulla. Induction of hypoxia is a common underlying mechanism, resulting, e.g., from impaired breathing, pulmonary toxicity or the uncoupling of oxidative phosphorylation (Greim et al., 2009; Ozaki et al., 2002). Inhalation studies with poorly soluble substances such as **talc** (NTP, 1993), **nickel (II) oxide** (NTP, 1996a), **nickel subsulfide** (NTP, 1996b), **cobalt sulfate heptahydrate** (NTP, 1998), **gallium arsenide** (NTP, 2000) and **indium phosphide** (NTP, 2001) suggest that there is a possible relationship between marked hypoxic conditions in the lungs (with the formation of fibrosis, inflammation and pulmonary tumors) and the formation of pheochromocytoma. Cobalt and nickel have also been shown to inhibit HIF1α (hypoxia-inducible factor 1α) degradation, thereby adapting cellular metabolism to oxygen deficient conditions (Greim et al., 2009).

The development of pheochromocytomas in animal studies under toxic conditions is assessed as a secondary effect that is of little relevance for conditions at the workplace. Pheochromocytomas rarely occur in humans, with about 30% caused by genetic factors. Up to now, there is no indication that substances that cause pheochromocytomas in animal studies also induce these tumors in humans. (Greim et al., 2009). **Furan** is an example of a substance that was not classified into a carcinogen category by the MAK Commission despite animal studies that yielded positive findings of pheochromocytoma (Greim, 2006c).

Although the pheochromocytomas observed in animal studies are not considered relevant to classification because of their mechanism of induction, this does not mean that substances that lead to the formation of these tumors in animal studies can generally be absolved of being carcinogenic risk factors. In fact, the above-mentioned metals are strong carcinogens (as shown by epidemiological data in the case of nickel (classified in CC 1) and by animal studies in the case of cobalt (classified in CC 2); in case of the latter, a recent NTP study also provides evidence of carcinogenicity in rats even after exposure to very low concentrations (NTP, 2016). Gallium arsenide was also classified in CC1 by the MAK Commission on the basis of its metabolites, which have been shown to be genotoxic and carcinogenic (Hartwig, 2014b). Generally, these metals induce - in addition to hypoxia - effects that are of human relevance and occur at concentrations that are much more realistic for humans. Of particular importance in this regard are indirect genotoxic mechanisms such as interference with DNA repair processes, tumor suppression functions, etc. (Beyersmann and Hartwig, 2008).

4.3.4. Tumors of the thyroid gland caused by induction of glucuronosyltransferases

Adenomas can be caused by increased TSH (thyroid stimulating hormone) levels in the thyroid gland of rats, which stimulate the function of the thyroid gland and thus lead to thyroid proliferative changes. Increased TSH levels can be caused by the induction of glucuronosyltransferases, which accelerate thyroxine (T4) clearance and reduce triiodothyronine (T3) levels. **Spironolactone** is an example of a substance mediating its carcinogenic effect in the thyroid gland of rats through induction of glucuronosyltransferases in liver. Another example is **thiabendazole**, which induces a slight, but statistically significant increase in thyroid adenomas in Sprague Dawley rats, but not in F344 rats. This is again related to the induction of glucuronosyltransferases. Accordingly, the substance has not been classified in one of the categories for carcinogens (Hartwig, 2009b, available only in German). An increase in TSH levels can also be caused more directly by the inhibition of thyroid peroxidase by substances such as **sulfamethazine**. Non-genotoxic substances such as spironolactone and sulfamethazine are not carcinogenic in humans as long as they do not disrupt thyroid hormone homeostasis (IARC, 2001).

Humans are less sensitive to disturbances in thyroid hormone homeostasis than rats. Adult humans have an efficient store of T3 and T4 by means of iodinated thyreoglobulin, which is present in very large amounts in the colloid of the thyroid, and can maintain the supply of T3 and T4 for several months even if hormone synthesis is disrupted. In contrast, rats have only a limited storage capacity. In addition, humans have a transport protein for T3 and T4 available in the form of the thyroxine-binding globulin, which considerably prolongs the half-life of thyroid hormones in the blood as compared to rats, where the hormones are bound exclusively to albumin. A T3 deficiency induced by xenobiotics in rats thus very quickly stimulates TSH synthesis, thereby promoting the growth of the thyroid gland (Bartsch et al., 2018). As this mechanism has practically no relevance for humans, thyroid tumors induced in rats by the above-mentioned mechanisms are generally not considered relevant to humans and do not lead to classification by the MAK Commission.

4.3.5. Leydig cell tumors mediated by agonists of dopamine and GnRH

Leydig cell tumors induced in male rats and mice by effects mediated by agonists of dopamine and GnRH (gonadotropin-releasing hormone) are not considered relevant to humans. This is because GnRH and prolactin receptors are either not expressed or are expressed only at very low levels in the testes of humans. By contrast, other mechanisms that cause Leydig cell tumors are relevant to humans, such as the inhibition of testosterone biosynthesis, 5α-reductase and aromatase or estrogen agonism (Cook et al., 1999; RIVM, 2004).

4.3.6. Dopamine receptor mediated mesothelioma of the tunica vaginalis testis

A substance-induced increase in the incidence of mesothelioma of the tunica vaginalis testis was observed only in male F344 rats, but not in other rat strains (Sprague Dawley, Osborne Mendel and Wistar) or in B6C3F1 mice (Shipp et al., 2006). During the formation of spontaneous tunica vaginalis mesothelioma and several forms of substance-induced tunica vaginalis mesothelioma, autocrine growth factors induce mitosis in mesothelial cells. **Methyl eugenol** is one example (Maronpot et al., 2009). The assumed mechanism for the formation of tunica vaginalis mesothelioma, the modulation of the dopaminergic tonus at dopamine D1 and D2 receptors or other membrane proteins, which leads to an acceleration of age-dependent hormonal changes in male F344 rats, is not considered relevant to humans (Shipp et al., 2006).

4.3.7. PPARα-mediated liver tumors

Liver adenomas and carcinomas, which are only formed in rats and mice via processes mediated by the peroxisome proliferator activated receptor α (PPARα), are not considered relevant to humans. However, this type of assessment requires the exclusion of other mechanisms of carcinogenicity in addition to evidence of peroxisome proliferation and hepatocellular proliferation under the experimental conditions of carcinogenicity studies (IARC, 1994, 1996; RIVM, 2003). The response of the human liver to PPARα ligands is qualitatively and quantitatively different from the response seen in the livers of rats and mice. Evidence suggests that this is due to marked differences in PPARα-mediated gene regulation (different target genes, different receptor activities and different receptor levels) (Klaunig et al., 2003). Similar effects on peroxisomes were found neither in adequate studies in humans nor in human cells (IARC, 1994, 1996). Peroxisome proliferation was not observed in PPARα-humanized mice exposed to the prototype PPARα agonist Wy-14643 and, unlike wild-type mice, these mice did not develop hepatocellular carcinomas (Morimura et al., 2006).

The key events are the activation of PPARα, perturbation of cell proliferation and apoptosis, selective clonal expansion and a series of associative events involving peroxisome proliferation, hepatocyte oxidative stress and Kupffer-cell mediated events (Klaunig et al., 2003). Besides **clofibrate** (Note: no MAK documentation available), another example is **trichloroacetic acid** (Hartwig, MAK Commission, 2016c, available only in German). Trichloroacetic acid induces liver adenomas and carcinomas in B6C3F1 mice. The substance has been found to be a strong PPARα agonist and peroxisome proliferator. The level of peroxisome proliferation has been correlated with the total number of tumors per animal. Trichloroacetic acid was not classified in any of the categories for carcinogens because of the high spontaneous incidence of liver tumors in B6C3F1 mice and the mechanism of action that has no human relevance.

4.3.8. Effects after non-physiological routes of administration

Non-physiological administration of a test substance may completely change its toxicokinetics and thus the biological response in the body. For example, inorganic **zinc** compounds cause an increase in chromosomal aberrations in rats after intraperitoneal administration. Studies with intraperitoneal administration were not included in the evaluation of zinc and its inorganic compounds because the MAK Commission felt that intraperitoneal injection of high doses used as the route of administration bypasses the regulation of zinc homeostasis (Hartwig, 2010a). This does not mean, however, that results obtained by intraperitoneal administration are generally ignored by the Commission and are therefore never part of the evaluation process.

5. Species-, strain- or organ-specific tumors

A number of different tumor types observed in animal studies have been shown to lack human relevance because species-specific or strain-specific organ- or tissue-structures are involved in their development that have no human equivalent.

5.1. Tumors in species-specific organs

Tumors in organs and/or tissues that are only found in animals but do not exist in humans generally lack human relevance. Nevertheless, they can give evidence of a carcinogenic potential. The Zymbal's gland, Harderian gland or the forestomach in rats are examples of such organs. However, the induction of tumors in analogous cells and/or tissues in humans (e.g. squamous cell tumors) is possible (ECHA, 2017). Tumors in the forestomach of rats and mice are more frequently induced following exposure by oral routes of administration, in particular administration by gavage. After administration by gavage, very high local concentrations can be found in the forestomach, which may also lead to an increase in the exposure time of the respective epithelium. Humans do not have a forestomach; the esophageal epithelium and the epithelium of the oral cavity and pharynx are, however, possible targets. This has to be taken into consideration, for example in the case of DNA reactive substances. Non-DNA reactive, but irritant substances may

induce severe cytotoxicity, and thus cell proliferation and hyperplasia after oral administration because of the irritant effect and longer retention time in the forestomach (IARC, 1999c; RIVM, 2004). Forestomach tumors induced in rats and mice by the latter route of administration are, however, considered specific to rodents and without human relevance because substances are not retained for similar periods of time in any part of the human gastrointestinal tract (RIVM, 2004). Nevertheless, different considerations apply if tumors do not occur isolated in animal-specific organs; if additional organs are affected in experimental studies, tumors, even though specific to animals, will add evidence for a carcinogenic potential of the chemical under consideration.

5.2. Tumors in organs/tissues with a high spontaneous tumor incidence

The high spontaneous incidence of individual tumor entities in a given animal strain should also be mentioned in this context. Thus, the late onset of exclusively benign tumors at sites with a high spontaneous incidence (e.g. the liver) suggests a strain-specific genetically-fixed tumor susceptibility (U.S. EPA, 2005). A careful case-by-case examination based on the results on the historical range of tumors in the respective strains and other relevant information may lead the MAK Commission to conclude that it has no human relevance.

It should be pointed out that a high background tumor incidence observed in a certain test animal species or strain often correlates with a high sensitivity in respective carcinogenicity tests in these animals and therefore may also provide some advantage. However, the high dose effects that are discussed above and that are not relevant for classification are also particularly effective in the organs of these animals and induce the observed marked tumor responses. In addition, by its very nature, it is difficult to prove the statistical significance of tumor induction in cases in which there is a high spontaneous incidence.

Therefore, if the tumors are observed in an animal species with a high spontaneous incidence of tumors, it is necessary to examine each case individually. This is especially important if this type of tumor is a form frequently found in humans, such as lung tumors. In these cases, further evidence is required to decide on classification or non-classification.

5.2.1. Liver adenomas and carcinomas in B6C3F1 mice

A good example is provided by the high spontaneous incidence of liver tumors and the corresponding high sensitivity for chemical induction of liver adenomas and carcinomas in B6C3F1 mice, often used in carcinogenicity tests (Haseman et al., 1998; Maronpot, 2009). Untreated control animals from 21 inhalation studies carried out by the NTP (National Toxicology Program) from 1990 to 1997 yielded a percentage of liver carcinomas of 21.1% (221 of 1047 investigated animals, range: 9%–34%) in males and of 13.8% (150/1089, 0%–38%) in females and a percentage of liver adenomas of 24.6% (257/1047, 4%–48%) in males and of 14.1% (154/1089, 2%–40%) in females (Haseman et al., 1998). The main reason for the high spontaneous incidence of liver tumors and the high sensitivity for the induction of liver tumors by chemical substances is genetic predisposition. Thus, genetic susceptibility loci were identified in sensitive mouse strains such as C3H or B6C3F1 that are absent in non-sensitive strains such as C57BL (Drinkwater and Ginsler, 1986; Manenti et al., 1994). In addition, different responses to modifying exogenous factors may play a role in the varying degree of susceptibility to liver tumor formation of the different mouse strains (Maronpot, 2009). Assessing the relevance of chemically-induced liver tumors that occur very frequently and often exclusively in highly sensitive, but not in resistant, mouse strains leads to the question of how this information is to be applied to humans. Hepatocellular carcinoma (HCC) are among the most common tumors worldwide. However, the incidence of HCC is rather low in the absence of known risk factors that can induce chronic hepatitis such as a hepatitis B and C virus infection, exposure to aflatoxins and alcohol abuse. Therefore, with regard to HCC formation, humans appear to be one of the genetically less sensitive species. The tumor suppressor protein p53 may play a role here. Mutation often renders the *TP53* gene inactive in human HCCs, but not in those of mice. This indicates that its gene product plays a much more important role in the malignant transformation of hepatocytes in humans than it does in the sensitive mouse (Kress et al., 1992).

5.2.2. Lung tumors in rodents

The incidence of spontaneously occurring lung tumors in rodents also depends upon the species and the strain. The incidence of spontaneous and chemically-induced hyperplasia, adenomas and carcinomas in the lungs is higher in mice than in rats. The incidence of spontaneous lung tumors in mice is dependent upon the strain, as is the incidence of chemically-induced tumors. The incidence of spontaneous lung tumors in various mouse strains is, in descending order, 82% for A/J, 47% for SWR/J, 33% for BALB/c, 17% for CBA, 9% for C3H and 3% for C57BL6. The higher sensitivity of mice for lung tumors is caused by the pulmonary adenoma susceptibility 1 (Pas 1) locus. The varying sensitivity of the different mouse strains is attributed to a polymorphism of the Pas 1 locus. The strain-to-strain differences in the incidence of spontaneous lung tumors are not as striking in rats as they are in mice. In descending order, the incidences are 1.9% for F344, 1.8% for Lewis, 0.7% for Osborne Mendel, 0.6% for Brown Norway, 0.5% for both Sprague Dawley and Wistar, 0.4% for CD and 0% for ACI/N. The status of the Pas 1 locus in the rat strains has not yet been identified.

Genetic predisposition factors playing a critical role in certain mouse strains are probably of little relevance for humans, even though lung cancer is very common in humans but mainly caused by well-known risk factors such as tobacco smoke. However, positive lung tumor findings in sensitive rodent strains should always be understood as a warning sign and the MAK Commission has to decide on a case-by-case basis whether the positive tumor findings have human relevance. In cases of uncertainty, the substance should be classified in a carcinogen category (see also chapter 6.2.4).

5.2.3. Mononuclear cell leukemia in F344 rats

F344 rats have a high spontaneous incidence of mononuclear cell leukemia, which is also known as “large granular lymphocytic leukemia” (Maronpot et al., 2016). In NTP studies, the spontaneous incidence increased over the years of investigation: the prevalence of mononuclear cell leukemia in feeding studies in males was 51% in 1997 and 27% in 1990 (in each case with reference to the preceding seven years), and 28% and 18%, respectively, in females. In addition, spontaneous incidences are subject to great variability (Haseman et al., 1998). Mononuclear cell leukemia is extremely rare in other rat strains (Maronpot et al., 2016) and has not been observed in mice or hamsters (Caldwell, 1999). The only human correlate, a very rare and aggressive form of leukemia, has a different etiology: it is of viral origin (Maronpot et al., 2016; Thomas et al., 2007). However, the mechanism that leads to the development of mononuclear cell leukemia in F344 rats is not known. It is probably not genotoxic (Maronpot et al., 2016). Mononuclear cell leukemia therefore is considered to be a tumor that is specific to this rat strain and as such, without human relevance (Caldwell, 1999; Maronpot et al., 2016).

6. Assessment of benign tumors

When assessing the relevance of tumor findings in animal studies, one issue that needs to be addressed concerns how benign tumors and tumor precursors such as preneoplastic liver foci are to be judged. One of the decisive factors in this context is whether only adenomas were induced under experimental conditions, or if they were found in combination with carcinomas. Another factor that needs to be taken into consideration is the likelihood of the respective adenomas progressing

to malignancy in different tissues. The following paragraphs first discuss the approaches taken by other organizations.

6.1. Assessment by other organizations

According to the CLP classification criteria of the European Chemicals Agency (ECHA, 2017), the NTP (National Toxicology Program; Huff and Haseman, 1991), the Preamble of the IARC (International Agency for Research on Cancer; IARC, 2006) and the guidelines of the U.S. EPA (U.S. Environmental Protection Agency; U.S. EPA, 2005), adenomas play a key role in the classification of a substance as a carcinogen even in case a substance was only found to induce adenomas in animal experiments and no carcinomas it may be classified as a carcinogen. However, all organizations emphasize the importance of assessing each substance individually if only adenomas and no carcinomas are observed in animal studies.

6.1.1. ECHA

The CLP classification criteria developed by ECHA (2017) consider adenomas when assessing whether substances need to be assigned to a category of carcinogens. In general, any substance that induces adenomas or carcinomas fulfills the criteria for classification as a carcinogen. If a substance induces only adenomas in animal studies, it is generally classified in the category of “suspected carcinogens” (Category 2, CLP Annex I, 3.6.2.2.3). If a substance induces carcinomas in animal studies, it meets the criteria for Category 1B. However, even substances causing only benign tumors may be suspected of causing cancer if the observed tumors could potentially develop into carcinomas. Some benign tumors, such as brain tumors, present sufficient cause for concern by themselves even without knowing more about their malignant potential because they can cause mortality without developing into carcinomas.

6.1.2. NTP

In publications of the NTP, it was concluded that substance-induced neoplasia is an important toxicological indicator of a chemical's carcinogenic potential in rodents and should be made an integral part of the overall weight-of-the-evidence evaluation process (Huff et al., 1989). Another publication emphasized the relevance of adenomas in the assessment of carcinogenicity because, although 3.5% of the evaluated substances induced only adenomas, these were shown to have the potential to progress to malignant carcinomas. Of 143 substances investigated by the NTP in 524 long-term studies, 81 showed neoplastic responses in one or more of the studies and were therefore assessed to be carcinogenic. Of the 81 studies that were evaluated as “positive”, carcinomas were reported in 60 (74%) and the almost exclusive occurrence of benign neoplasms in 16 (20%). However, there was supporting evidence of a carcinogenic process because isolated carcinomas were observed in the same organ. Another five studies (6%) were found to be “positive” based on the occurrence of benign neoplasia alone. This is equivalent to 3.5% of the 143 substances tested. In contrast, of 200 substances that were evaluated by the NCI (National Cancer Institute), only 2 (1%) were evaluated as “positive” based on benign tumors (Huff and Haseman, 1991).

6.1.3. IARC

According to the Preamble of the IARC (2006), substances are classified as carcinogenic if adenomas found in animal studies can be defined as carcinoma precursors with certainty. This applies if a study were to find both adenomas and carcinomas in the same organ and cell type. In this case, the incidences of adenomas and carcinomas are to be assessed together/in combination. The IARC also emphasizes the need to assess the biological plausibility of preneoplastic findings. In the absence of malignancy risk data, the occurrence of only adenomas can also be evaluated as limited evidence of carcinogenicity (IARC, 2006).

6.1.4. U.S. EPA

The U.S. EPA (2005) clearly favors a case-by-case assessment if only adenomas are observed in animal studies. According to the U.S. EPA, incidences of benign and malignant tumors of the same cell type are to be considered separately but may be combined when scientifically defensible. In the assessment of adenomas, a wide range of possibilities needs to be considered: for example, benign tumors can also lead to serious health problems and critically impair organ function, such as in the case of a brain tumor. Adenomas are to be considered significant indicators of carcinogenicity and there is a need for further testing especially in the case when they are observed in a short-term test protocol and no conclusions can be made about a possible later progression to malignancy. Knowledge of the mechanism of action associated with an adenoma may aid in the interpretation of other tumor responses associated with the same substance. In other cases, observation of a benign tumor response alone may not be predictive for the occurrence of malignant tumors when other sources of evidence show no suggestion of carcinogenicity for this substance.

6.1.5. Summary

As long as the data do not prove otherwise, the IARC, ECHA and NTP assume the worst case and interpret adenomas as precursors of malignant tumors. Only the U.S. EPA considers the possibility that benign tumors may not represent a significant health risk.

6.2. Assessment of the risk of malignant transformation of preneoplastic and neoplastic lesions including adenomas

This chapter discusses how to evaluate the risk that different types of preneoplastic and benign neoplastic lesions including adenomas transform into malignant populations and whether there are adenomatous changes that do not need to be considered as precursors of carcinomas.

6.2.1. Hepatocellular preneoplasms and benign neoplasms

The liver is an important target organ in many carcinogenicity studies in rodents. However, the type of proliferative lesion and its human relevance needs to be considered. The assessment should take into account not only the late stages of the different types of hepatocellular lesions but also the early ones (Thoolen et al., 2012).

Focal nodular hyperplasia is a benign tumor of the liver that does not have malignant potential. With a prevalence of 3%–5% in the Western population, it is the second most common benign liver change after cysts and haemangiomas (Bastati-Huber et al., 2015). Differentiating focal nodular hyperplasia from hepatocellular adenoma has proven to be a diagnostic challenge (Bastati-Huber et al., 2015; Thoolen et al., 2012). Focal nodular hyperplasia is rarely observed in animal studies (Thoolen et al., 2012).

Hepatocellular adenoma is a benign epithelial tumor of the liver. However, unlike other benign liver tumors, it has a high potential for malignancy (Dietrich et al., 2005). The hepatocellular adenoma is the third most common benign neoplasm in humans; young women that take oral contraceptives account for 85% of the patients. The histological characteristics of hepatocellular adenoma are similar in rats and humans. Unlike focal nodular hyperplasia, hepatocellular adenomas are monoclonal and have a correspondingly high risk of malignancy (Thoolen et al., 2012). The risk of malignancy is about 10%; however, reference is also made to the difficulties inherent in making a differential diagnosis with respect to hepatocellular carcinomas (Dietrich et al., 2005; Dokmak et al., 2014). Hepatocellular adenomas with mutations in *CTNNB1* (coding for β-catenin) also have an increased risk of malignancy. Hepatocellular carcinomas in both rats and humans exhibit the malignancy patterns and histopathological characteristics typical of these tumors (Thoolen et al., 2012).

In rats, **preneoplastic foci** are considered to be precursors of hepatocellular adenomas and carcinomas; however, neoplasms do not

develop from all foci. The rat counterparts of the human classifications "large cell" and "small cell dysplasia", which are considered to be indicative of a malignant progression to hepatocellular carcinoma, are basophilic, eosinophilic and clear cell foci. The malignant potential of foci of different genotypes and phenotypes varies. As an example, the malignant potential of basophilic foci is greater than that of eosinophilic foci (Ito et al., 1995; Marsman and Popp, 1994). Liver cell foci occur spontaneously in older rats and other rodents. For example, the spontaneous incidence in two-year-old F344 rats was almost 100%. The foci can be induced by exposure to liver carcinogens, which shortens the latency period for tumor formation.

Liver cell dysplasia is often observed in cirrhotic livers and attributed with precarcinogenic properties. This means that its cytological properties are very similar to those of hepatocellular carcinomas. This is still a matter of controversy in the case of large-cell and small-cell dysplasia, but both have been considered to be precancerous lesions (Thoolen et al., 2012).

Hepatocarcinogenesis shares similarities between humans and animals, in particular in terms of early cellular and molecular markers. As an example, many similarities were found when the gene expression patterns of *CTNNB1*-mutated liver tumors were analyzed in mice and humans (see for example Stahl et al., 2005; Unterberger et al., 2014). Hepatocellular tumors display marked heterogeneity in humans, in particular during the late stages of tumor progression; this is not as evident in test animals. There is no universal molecular mechanism of hepatocarcinogenesis that is valid for humans, rats and mice (Maronpot et al., 2004). For example, tumors differ in their etiology in rodents and humans (Grisham, 1997; Maronpot et al., 2004). There are also marked differences in the mutation frequency of genes associated with tumor formation. *H-Ras* mutations, for instance, often lead to tumor formation in the livers of mice, but are much less frequently observed in hepatocellular carcinomas in rats and very rarely in humans (Grisham, 1997).

Mutagenic tumor initiating agents requiring metabolic activation may no longer be toxic in adenomas because of a lack of activating enzymes in these lesions (Buchmann et al., 1987). Therefore, the mechanism that accelerates malignant progression from adenomas to carcinomas can probably not be explained by the induction of mutations by a given carcinogen exposure. In the development of hepatocellular carcinomas, both genetic changes and the epigenetic activation of oncogenes or inactivation of tumor suppression genes are important mechanisms of tumor formation (Kanda et al., 2015; Tischoff and Tannapfel, 2008).

Even though the significance of preneoplastic lesions in the livers of rats and humans as carcinoma precursors and their human relevance are not completely understood (Thoolen et al., 2012), they can be considered biomarkers for carcinogenic activity. Kunz et al. were able to show that a quantitative evaluation of ATPase-deficient, preneoplastic foci in the livers of rats treated with *N*-nitrosomorpholine or *N*-nitrosodiethylamine can quantitatively predict the formation of adenomas and carcinomas in this organ (Kunz et al., 1983). Therefore, at the very least, substance-induced preneoplastic foci in the liver of rodents are a warning sign. However, if found as isolated lesions, they generally do not lead to classification into a carcinogen category.

6.2.2. Papillomas in the mouse skin initiation-promotion model

The MAK Commission has evaluated the significance of papilloma formation in the initiation-promotion model of the dorsal skin of mice for the classification of carcinogenic substances. They arrived at the conclusion that a tumor-promoting effect in mouse skin, which is induced by a nonspecific mechanism such as chronic irritation and only occurs at very high doses that lack human relevance, is not considered predictive of the development of skin tumors in humans (Schwarz et al., 2015). **Oleic acid** is one such example: In 2001, the MAK Commission classified the substance in CC 3A (Greim, 2002a) because of its tumor-promoting effect, but, following re-evaluation in 2016, withdrew it from this category (Hartwig, MAK Commission, 2016d).

The development of papillomas in experimental studies is largely restricted to the mouse. In other species such as rats or minipigs papillomas are only minimally or not induced at all. Likewise, human skin does not react to the application of chemical substances or to irritation by forming skin papillomas. As precancerous lesions (carcinoma *in situ*, actinic keratosis) and squamous cell carcinomas of the human skin do not develop via precursors of papillomas, there is no direct analogy between papillomas of the mouse skin and actinic keratosis. Papillomatous changes of the skin are quite common in humans and are often caused by viruses. However, there is no direct equivalence to chemically induced papillomas in the mouse skin because no progression into malignant lesions are known in humans after exposure to chemical noxa (Schwarz et al., 2015).

6.2.3. Fibroadenomas and adenocarcinomas in the mammary glands of rats

Tumors of the mammary glands in rats fall into different categories. As in humans, fibroadenomas in the mammary glands of rats are benign tumors that are made up of highly differentiated epithelial fractions and fibrous connective tissues. In most rat strains, the spontaneous incidence of fibroadenomas in females is between 20% and 40%. The period with the highest risk is between 31 and 36 months; in older rats, the incidence decreases again. In humans, fibroadenoma is the most common benign tumor in women of childbearing age. It most frequently forms during adolescence, pregnancy and menopause, i.e. during periods marked by relatively constant estrogen stimulation, while fibroadenomas form to a lesser degree in women whose cycles are characterized by frequent fluctuations in hormone levels (Russo, 2015).

Fibroadenomas are not considered to be precancerous lesions in rats and humans. However, adenocarcinomas that did arise in fibroadenomas were considered as separate (Rudmann et al., 2012).

Substance-induced tumors in the mammary glands of rats are generally hormone-dependent adenocarcinomas (Russo, 2015); these are considered relevant for classification. The frequency of adenocarcinomas induced by genotoxic carcinogens such as 7,12-dimethylbenz[a] anthracene, *N*-nitrosomethylurea and *N*-ethyl-*N*-nitrosourea can be modulated by various factors such as reproductive status, hormone treatments, feed, and the dose and time of carcinogen treatment (Rudmann et al., 2012). Initiation primarily takes place in the epithelium of the terminal end buds as they are developing into alveolar buds and terminal ducts. These structures are considered to be the counterparts of the terminal ductal lobular units of humans (Russo, 2015).

6.2.4. Lung tumors in rats and mice

In an assessment of substances evaluated by the IARC, the lungs were found to be the organ most frequently affected by tumors in both humans and rats (Krewski, 2014). Rodent lung tumors are predominantly bronchioloalveolar adenomas and carcinomas and follow a progressive continuum from hyperplasia to adenoma to carcinoma. Histologically, rodent lung tumors are very homogeneous. In contrast, human pulmonary carcinomas have a high degree of histologic heterogeneity and include squamous epithelial cells, neuroendocrine, mucinous and sarcomatoid cells, and multiple cell combinations. In addition, they exhibit a higher metastatic rate, higher stromal response, aggressive clinical behavior and lack of a clear continuum of proliferative lesions. Rodent lung tumors originate primarily in the peripheral lung and involve the distal bronchioles and alveolar acini, while bronchial or proximal bronchiolar tumors are very rare. In humans, the majority of lung tumors are caused by tobacco smoking. Most arise centrally or within the bronchi and are primarily squamous cell carcinomas or small cell carcinomas. Lung tumors in humans are categorized into two large groups: non-small cell lung cancer, which accounts for 80% of all cases of lung cancer, and small cell lung cancer. Non-small cell lung cancer comprises adenocarcinomas, squamous cell carcinomas, adenosquamous carcinomas, large cell carcinomas and sarcomatoid carcinomas. About 18% of lung tumors may be categorized as

malignant small cell lung tumors that have a neuroendocrine morphology and a very high metastatic potential. The remaining 2% are neuroendocrine tumors consisting of typical and atypical carcinoids (Pandiri, 2015). Pulmonary neuroendocrine tumors have not been found in rodents.

Atypical adenomatous hyperplasia in humans bears histologic similarities to alveolar hyperplasia in rodents and is therefore thought to be a precursor lesion for peripheral lung adenocarcinomas. In addition, an analysis of global gene expression changes in the lung tumors of humans and mice found a high degree of resemblance (Bonner et al., 2004; Pandiri et al., 2012; Pandiri, 2015; Stearman et al., 2005). These data suggest that lung tumors in mice are morphologically and molecularly similar to adenocarcinomas in humans and thus of relevance to humans. Therefore, adenocarcinomas in the lungs of rodents are in general relevant for classification.

7. Examples of substances

The following gives several examples of substances that were the subject of extensive discussion by the MAK Commission with regard to the human relevance of tumors observed in animal studies. All substances have a threshold for tumor induction; in some cases, carcinomas only occurred at very high doses. In the subsequent Sections 7.1 and 7.2, the substances are categorized into those that were ultimately classified in CC 4 and those that did not require classification, respectively. For an overview see Table 2. An overview of the carcinogenicity classifications by other organizations is given in Table 3.

7.1. Examples of substances classified in Carcinogen category 4

7.1.1. Nitrobenzene

Nitrobenzene induces adenomas and in some cases also carcinomas in the liver, kidneys and thyroid gland of rats and in the lungs and mammary glands of mice after exposure to high concentrations by inhalation (Hartwig, MAK Commission, 2017).

Arguments for classification:

- Tumors in two species and five organs.
- A mechanism of action that allows for the derivation of a threshold value (required for classification in CC 4), namely cytotoxicity and formation of ROS at higher doses.
- Structurally related to substances for which extensive data on carcinogenicity is available.
- Evidence of secondary genotoxicity in the high dose range.

Arguments against classification:

- Tumors observed in species/strains that show particular susceptibility in the target organs (thyroid gland/rat, kidneys/rat, lung/mouse; see also Section 4.4. and 5).
- Tumors observed in species/strains that have an increased spontaneous incidence for these tumors (mammary glands/mouse).
- Tumor formation in the range of “nonspecific toxicity” at very high doses that exceed the MTD.

Conclusion: Nitrobenzene is cytotoxic in different organs and in different species. Damage to the erythrocytes and the formation of superoxide radicals is observed. Nitrobenzene has the same metabolite (phenylhydroxylamine) as aniline (Hartwig, 2009b), which is responsible for the formation of ROS and tumor formation results from long-term damage. Based on the overall evaluation of the tumors, its non-linear dose–response relationship, and since genotoxicity only plays a subordinate role (Hartwig, 2009b), nitrobenzene has been classified in CC 4.

7.1.2. o-Phenylphenol (OPP) and sodium OPP

OPP induces liver tumors in male B6C3F1 mice and bladder tumors in male F344/DuCrj rats (Hartwig, MAK Commission, 2016b, available only in German).

Arguments for classification:

- Presence of carcinomas in two species: male mouse/liver, male rat/urinary bladder.
- A mechanism of action that allows for the derivation of a threshold value (required for classification in CC 4), namely bladder tumors in male rats in the saturation range of metabolism.
- Unanswered questions concerning the human relevance and extrapolation of findings from rat studies to humans because the induction mechanism of cell proliferation in the bladder is not fully understood.

Arguments against classification:

- Carcinomas in a mouse strain (B6C3F1) that has been shown to have a high spontaneous incidence of liver tumors.
- Bladder tumors in rats which show increased susceptibility for this tumor type.
- Mechanism identified, but with no or questionable human relevance: bladder tumors in male rats in the saturation range of

Table 2
Overview of examples described in Section 7.

Substances classified in CC 4	Arguments for and against classification
Nitrobenzene	**against:** tumors in sensitive organs/species, only isolated cases of carcinomas at the level of the MTD **for:** cytotoxicity and damage to erythrocytes (phenylhydroxylamine), reactive oxygen species, tumor formation resulting from chronic damage, mechanism of action with human relevance
***o*-Phenylphenol**	**against:** bladder tumors in male rats in the saturation range of metabolism with overloading of detoxification via the sulfate conjugate; urothelium markedly less sensitive in humans than in male rats **for:** unanswered questions concerning the human relevance and extrapolation of the findings to humans because the mechanism of cell proliferation in the bladder is not fully understood
***N*,*N*-Dimethylformamide**	**against:** hepatocellular adenomas, carcinomas and hepatoblastomas only occur at doses at which necrosis is also induced **for:** liver carcinogenicity in rats and mice resulting from liver cell necrosis (causes rapid regenerative proliferation of hepatocytes) after long-term exposure to *N*,*N*-dimethylformamide or its metabolites
Substances not classified	
Imazalil	**against:** thyroid adenomas and carcinomas induced by a compensatory effect (induction of glucuronosyltransferases, followed by decreased T4 and increased TSH levels); liver adenomas in the range of the MTD only in the case of marked hepatotoxicity
2-Ethylhexanol	**against:** liver carcinomas in female mice only after MTD exceeded by toxic effects on the liver
Atrazine	**against:** no genotoxicity; early development of adenocarcinomas in the mammary gland attributed to premature aging of the reproductive system of female Sprague Dawley rats; not relevant to humans
Butoxyethanol	**against:** liver tumors in mice and pheochromocytomas in rats indirectly caused by hemolysis; humans are significantly less sensitive to this effect than rats or mice. This species difference is so great that the level of exposure required to induce tumors cannot be reached because of the limiting effect of irritation

Table 3
Carcinogenicity classification of examples described in Section 7.

Substance	MAK Commission	IARC	CLP EU, ECHA	SCOEL
Nitrobenzene	Category 4 (2016)	Group 2B (1996)	1.1.1.1.1.1.1.1 Carc Cat 2	Carcinogen with a non-genotoxic mechanism of action; an OEL of 0.2 ml/m^3 was set (2002)
***o*-Phenylphenol**	Category 4 (2015)	*o*-Phenylphenol: Group 3 (1999) *o*-Phenylphenol sodium salt: Group 2B (1999)	1.1.1.1.1.1.1.2 not classified as carcinogenic	not listed
***N,N*-Dimethylformamide**	Category 4 (2015)	Group 2A (2018)	1.1.1.1.1.1.1.3 not classified as carcinogenic	1.1.1.1.1.1.1.4 not carcinogenic (2006)
Imazalil	not classified as carcinogenic	1.1.1.1.1.1.1.5 not listed	1.1.1.1.1.1.1.6 Carc Cat 2	not listed
2-Ethylhexanol	not classified as carcinogenic	not listed	1.1.1.1.1.1.1.7 not classified as carcinogenic	not carcinogenic (2011)
Atrazine	not classified as carcinogenic	Group 3 (1999)	1.1.1.1.1.1.1.8 not classified as carcinogenic	not listed
Butoxyethanol	not classified as carcinogenic	Group 3 (2006)	1.1.1.1.1.1.1.9 not classified as carcinogenic	listed, but carcinogenicity not evaluated (2002)

IARC: Group 2A: Probably carcinogenic to humans; Group 2B: Possibly carcinogenic to humans; Group 3: not classifiable as to its carcinogenicity to humans.
CLP EU, ECHA: Carc Cat 1A: Known to have carcinogenic potential for humans; Carc Cat 1B: Presumed to have carcinogenic potential for humans; Carc Ca 2: Suspected human carcinogen.

metabolism with overloading of detoxification via the sulfate conjugate; moreover, the sensitivity of the human urothelium is markedly lower. Cell proliferation in the bladder of male rats only at doses that induced marked cytotoxicity.
- "Confounding through excessive toxicity": tumorigenic effects only at very high doses that exceed the MTD.
- Metabolism saturated (deactivation saturated, other metabolites).
- No genotoxicity; clastogenic only at high doses.

Conclusion: Cytotoxicity in combination with species-specific and sex-specific factors is considered to be the cause of the tumors observed only in the saturation range of metabolism after exposure to OPP and sodium OPP. A contributing factor is the high sensitivity of the urinary tract in male rats. These bladder tumors are not relevant to humans as long as inflammatory or reactive changes in the urothelium are not induced. However, in the case discussed here, some questions concerning the human relevance and extrapolation of findings in male rats to humans remain because the mechanisms underlying induction of cell proliferation is not fully understood (see also Section 4.4.2). Therefore the compound was classified in CC 4.

7.1.3. N,N-dimethylformamide

***N,N*-Dimethylformamide** induced hepatocellular carcinomas in male rats of the high exposure group and in mice of all exposure groups after long-term exposure by inhalation (Hartwig, MAK Commission, 2016e).

Arguments for classification:

- Hepatocellular carcinomas in male rats and in mice.
- Liver also a target organ of the toxic effect in humans.
- A mechanism of action that allows for the derivation of a threshold value (required for classification in CC 4): tumors are induced subsequent to induction of liver cell necrosis and regenerative proliferation of hepatocytes caused by *N,N*-dimethylformamide, or its metabolites *N*-methylformamide and methyl isocyanate.

Arguments against classification:

- Not genotoxic
- Tumors (hepatocellular adenomas and carcinomas, and hepatoblastomas) occur only at doses that induce necrosis.

Conclusion: *N,N*-dimethylformamide has no genotoxic potential. Long-term studies have shown that liver tumors are only observed following the induction of chronic, regenerative toxic effects. The tumors are considered to be a result of chronic damage and only occur after exposure at a level that leads to necrotic changes. Therefore the compound was classified in CC 4.

7.2. Examples of substances not classified in a carcinogen category

7.2.1. Imazalil

Imazalil causes hepatocellular adenomas and follicular adenomas and carcinomas of the thyroid gland in male rats. It also induces adenomas in the liver of male mice, but without a clear-cut dose-response relationship (Hartwig, 2014c).

Arguments for classification:

- Follicular adenomas and carcinomas of the thyroid gland in male rats and liver adenomas in male rats and mice.

Arguments against classification:

- Absence of liver carcinomas (only adenomas observed) in male rats and mice (see Section 6.2).
- Lack of a dose-response relationship with respect to liver adenomas

in mice; mouse strain used (Swiss) characterized by a high spontaneous incidence of liver tumors (see Section 5.2). No significant increase in thyroid tumors in rats; significant increase only when adenomas and carcinomas are combined for analysis (Bartsch et al., 2018; see Section 4.4.4).
- Liver adenomas only at marked hepatotoxicity and at the level of the MTD; imazalil induces liver enzymes in rats and mice and leads to liver damage and regenerative proliferation.
- Development of thyroid tumors in rats via a compensatory effect (induction of glucuronosyltransferases, followed by decreased T4 and increased TSH levels).
- Not genotoxic in vivo.

Conclusion: Tumors induced by imazalil are not due to a genotoxic effect. The substance induces liver enzymes and leads to degenerative liver damage. No liver adenomas are induced in the absence of hepatotoxicity. Liver carcinomas do not develop even at the level of the MTD. Only the combined incidence of adenomas and carcinomas in the thyroid gland is increased. The latter is caused by the induction of glucuronosyltransferases and thus decreased T4 and increased TSH concentrations. As long as the exposure remains at imazalil concentrations that are low enough not to affect T4 or TSH concentrations, no thyroid tumors are expected. In addition, humans are less sensitive to disturbances in thyroid hormone homeostasis than rats (see also Section 4.4.4) Therefore, imazalil was not classified in a carcinogen category.

7.2.2. 2-Ethylhexanol

2-Ethylhexanol induces liver carcinomas only in female B6C3F1-mice (Greim, 2003b).

Arguments for classification:

- Liver: focal hyperplasia and isolated carcinomas in female mice.

Arguments against classification:

- Tumor induction at levels far exceeding the MTD (15 of 50 animals died during the experiment or were in a moribund state).
- Postulated mechanism of action: peroxisome proliferator, metabolite of Bis(2-ethylhexyl) phthalate (DEHP), a well-documented activator of the peroxisome proliferator activated receptor α (PPARα). However, a (PPARα)-mediated effect is not plausible since tumors did not occur in both sexes. Therefore, “unspecific” hepatotoxicity likely to be the cause of tumor formation.
- Not genotoxic.

Conclusion: Isolated liver carcinomas only in female mice at levels that far exceed the MTD. Moreover, no genotoxicity observed. Therefore, 2-ethylhexanol was not classified in a carcinogen category.

7.2.3. Atrazine

In female Sprague Dawley rats, **atrazine** leads to a decreased latency period and to an increased incidence of adenocarcinomas in the mammary glands. No breast tumors were observed in F344 rats or mice at the same dose (Hartwig, 2013, available only in German).

Arguments for classification:

- Adenocarcinomas of the mammary gland in female Sprague Dawley rats.

Arguments against classification:

- Tumors only in Sprague Dawley rats, but not in F344 or Long Evans rats.
- Rat strain-specific differences known in the effects on the hypothalamus-pituitary-gonad axis.
- Mechanism of action: decline and loss of the LH (luteinizing hormone) surge, increase in estrogen and prolactin levels leading to premature aging of the reproductive system followed by formation of adenocarcinomas of the mammary gland.

Conclusion: Atrazine has no genotoxic effect. The early induction of adenocarcinomas in the mammary gland in female Sprague Dawley rats is caused by premature aging of the reproductive system. This mechanism of action is not relevant to humans because reproductive aging progresses differently in humans (exhaustion of the ovarian follicle, decrease in estrogen and prolactin levels). Therefore, atrazine was not classified in a carcinogen category.

7.2.4. Butoxyethanol

Following chronic inhalation, **2-butoxyethanol** induced benign and malignant pheochromocytomas in the adrenal medulla in female F344 rats, liver cell carcinomas and hemangiosarcomas in male B6C3F1 mice and squamous cell papillomas, associated with a concentration-dependent increase in tumors and epithelial hyperplasia in the forestomach of female B6C3F1 mice (Hartwig, MAK Commission, 2018).

Arguments for classification:

- Tumors in two species and three organs.
- Dose-dependent induction of tumors.

Arguments against classification:

- B6C3F1 mice exhibit a high spontaneous incidence of hepatocellular tumors (see also Section 5.2.1).
- Forestomach tumors in rats are not relevant to humans because the retention period in the human stomach is shorter, the gastric mucosa protects the stomach from irritants, and the localization of the enzymes that are necessary for acid production is not the same as in the forestomach of rodents.
- Mechanism of action: liver tumors in mice and pheochromocytomas in rats are very likely caused by hemolysis induced by butoxyethanol and butoxyacetic acid (the active metabolite). Humans are markedly less sensitive than rats and mice for hemolysis and the hemosiderosis in the liver that is caused by hemolysis. In human volunteers exposed to a saturated vapor atmosphere (reflecting inhalative plus skin exposure), butoxyacetic acid had only a minimal hemolytic effect.
- Not genotoxic.

Conclusion: Forestomach tumors in mice induced by the non-genotoxic substance 2-butoxyethanol are not relevant to humans. The liver tumors in mice and the pheochromocytomas in rats were very likely caused by the induction of hemolysis. Humans are markedly less sensitive than rats and mice for hemolysis and the hemosiderosis in the liver that is caused by hemolysis. The species differ so greatly in this respect that the level of exposure necessary for tumor formation in humans is not achieved because of the limiting effects of irritation, which limits the exposure at the workplace. For this reason, 2-butoxyethanol was withdrawn from CC.

8. Catalogue of criteria

The following criteria are used by the MAK Commission to decide whether a substance being assessed should or should not be classified in a carcinogen category:

Arguments FOR classification:

- Human data, in particular if dose-response relationships can be proven (CC 1).
- Genotoxicity (CC 4 only in the case that genotoxicity plays a minor role).

- Tumors in several organs, several species, via several routes of administration, irrespective of sex.
- Mechanism of tumor formation in the animal studies has human relevance and demonstrates a clear dose-response relationship. Special case: a mechanism of action that enables the derivation of a threshold value (e.g. hormonal effect, physiological regulatory mechanisms) for tumor induction allows classification in CC 4.
- Occurrence of rare tumors definitely induced by the substance.
- Structural relationship with substances for which carcinogenic effects have been demonstrated and for which extensive carcinogenicity data are available (Suspicion for carcinogenicity leading to classification in CC 3B)
- Induction of exclusively adenomas, but with a high risk of malignant progression to carcinomas (Suspicion for carcinogenicity leading to classification in CC 3B).

Arguments AGAINST classification:

- No genotoxicity (does not apply to CC 4).
- Species-/strain-specific tumors that lack human relevance (e.g. induction via pathways specific to that species); i.e. mechanism known and without human relevance (see Section 4.2, 4.3, 5.1).
- Carcinogenic responses in species/strains with high susceptibility towards development of the tumors in question and an increased spontaneous incidence of these tumors (see Section 5.2).
- Carcinogenic effects observed exclusively at very high doses that are in the range of the MTD (confounding effects mediated through excessive toxicity (see Section 4.1).
- Carcinogenic effects observed in animal studies at exposure levels that are much higher than would be tolerated by humans (requires examination of the exposure conditions in the animal studies, e.g. route of administration, duration and frequency, and their relevance to the conditions at the workplace).
- Tumor occurrence only at the application site in very sensitive test systems if target organs lack human relevance (see Section 5.1).
- Saturated metabolism (deactivation saturated, other metabolites) if this cannot occur in humans under realistic exposure conditions (see Section 4.2).
- Adenomas without a known potential for progression to malignancy (e.g. papillomas of the skin) (see Section 6.2.2).

9. Conclusions

When evaluating the safety of substances that are relevant for the workplace, the challenge is to draw the line between either their classification in one of the categories for carcinogens or, alternatively, no classification as carcinogen. As there are only data from animal studies, but no human data available for the majority of the substances, the interpretation of these data and the question of their extrapolation to humans play an essential role in the assessment process. As animal studies are often carried out under “extreme conditions” (of particular importance in this context being the exposure to very high doses close to or at the MTD), the relevance of these findings for humans under realistic exposure conditions needs to be critically examined in each individual case.

For this reason, the MAK Commission assesses tumors induced in animal studies as follows:

Mechanism of tumor formation:

1. In the case of a direct genotoxic effect (e.g., the substance itself or one of its metabolites causes damage to the genetic material), any tumor resulting from this effect in animal studies should always be evaluated as having human relevance. Accordingly, the substance is to be classified in one of the categories for carcinogens. The same applies in the case of an indirect genotoxic effect (e.g., the substance or one of its metabolites leads to the formation of reactive intermediates, such as reactive oxygen species, which then cause damage to the genetic material, or impair the repair of (endogenous) DNA damage or influence the accuracy of DNA polymerases). These are assessed as having human relevance if they are not limited to extremely high doses not relevant to human exposures (see below). In the case of substances that have a nongenotoxic mechanism of action, a case-by-case evaluation is required to determine whether the postulated mechanism of tumor induction is relevant to humans. If the matter of human relevance cannot be decided with certainty, the substance should be classified in one of the categories for carcinogens.
2. High dose effects: If tumors only occur in animal studies at extremely high doses, generally in the range of the MTD, then the next step is to examine whether the regenerative processes that are induced in the target organ by toxicity or indirect genotoxic effects are the cause of the carcinogenic effect and would not be expected to occur at lower doses. Likewise, mechanisms may become effective (e.g. precipitates in the target organ) in the high dose range that can reliably be ruled out at the lower doses that are relevant to humans. Furthermore, in isolated cases, acute toxic effects such as irritation limit the dose at a sufficient margin, ensuring that very high concentrations will definitely not be reached in the target organs in humans. In these cases, the substance is generally not classified in one of the categories for carcinogens. But if it is classified, Carcinogen Category 4 is the category of choice in most cases.
3. Species-specific mechanisms of tumor formation that are definitely not relevant to humans do not lead to classification, one such example being tumors that are induced in the renal tubules of male rats by an α2u-globulin-mediated mechanism.
4. In certain cases, it is necessary to establish whether the species (animal/human) differences in the activating/detoxifying metabolism of a substance which is carcinogenic in animal studies are quantitatively so pronounced that all human relevance can be ruled out. If these questions cannot be answered with certainty, the substance is classified in one of the categories for carcinogens.

Frequency and severity of the carcinogenic effect:

- Tumor findings in animal experiments gain weight if several organs are affected and/or tumors occur in several species. Their frequency is also a criterion for classification. Under certain circumstances, historical control incidences can be included in the evaluation. In each individual case, all arguments for and against have to be weighed against each other before making the decision to classify or refrain from classifying a substance. Therefore, it is not possible to develop a general guideline for all instances. The following points should be considered in the evaluation: Are the species/strains in which the tumors were observed known to have a high spontaneous incidence of these tumors in the target organ? Do the tumors only occur in highly sensitive strains? If so, classification may not be necessary in a case by case evaluation, given that the mechanism of tumor induction has been proven not to be relevant to humans.

Tumor precursors and benign tumors:

- If tumor precursors such as preneoplastic liver foci are the only response induced in animal studies, classification is not necessary. If they are observed together with adenomas, this can be considered further evidence of a potentially malignant response in the target organ. If both adenomas and carcinomas are observed in the target organ, these are in general assessed together. If there is possibility of human relevance, the substance is classified in one of the carcinogen categories.
- Tumors in organs that are not found in humans are considered to be indicators of potential carcinogenic activity, but do not generally lead to classification. Tumors that occur in humans but, unlike in

animals, are not caused by exposure to chemicals (e.g. skin papillomas), are in general not relevant for classification.

The MAK Commission uses this guidance document to make a well-founded and reasonable assessment of the possible carcinogenic risk for humans posed by the substance being evaluated under exposure scenarios that occur at the workplace. The assessments are to be made on a case-by-case basis, taking into consideration a weight-of-evidence evaluation process. If both human data and findings from animal studies are available, these should be analyzed together after examining the plausibility of causation according to Bradford-Hill criteria. As a rule, human data are weighted more heavily than findings from animal studies. The results of the assessments are documented in detail.

10. Conflicts of interest

The authors have no competing interests to declare. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

11. Funding body information

There is no funding body to be named.

Acknowledgements

The authors would like to thank all members and guests of the MAK Commission for critical reading of the manuscript and helpful suggestions. We also thank the Deutsche Forschungsgemeinschaft (DFG) for their continuous support. This work was financially supported by the DFG, HA2372/4-15.

Annex

Category 1 Substances that cause cancer in man and can be assumed to contribute to cancer risk. Epidemiological studies provide adequate evidence of a positive correlation between the exposure of humans and the occurrence of cancer. Limited epidemiological data can be substantiated by evidence that the substance causes cancer by a mode of action that is relevant to man.

Category 2 Substances that are considered to be carcinogenic for man because sufficient data from long-term animal studies or limited evidence from animals studies substantiated by evidence from epidemiological studies indicate that they can contribute to cancer risk. Limited data from animal studies can be supported by evidence that the substance causes cancer by a mode of action that is relevant to man and by results of in vitro tests and short-term animal studies.

Category 3 Substances that cause concern that they could be carcinogenic for man but cannot be assessed conclusively because of lack of data. The classification in Category 3 is provisional.

Category 3 A Substances that cause cancer in humans or animals or that are considered to be carcinogenic for humans for which the criteria for classification in Category 4 or 5 are in principle fulfilled. However, the database for these substances is insufficient for the establishment of a MAK or BAT value.

Category 3 B Substances for which in vitro or animal studies have yielded evidence of carcinogenic effects that is not sufficient for classification of the substance in one of the other categories. Further studies are required before a final decision can be made. A MAK or BAT value can be established provided no genotoxic effects have been detected.

Category 4 Substances that cause cancer in humans or animals or that are considered to be carcinogenic for humans and for which a MAK value can be derived. A non-genotoxic mode of action is of prime importance and genotoxic effects play no or at most a minor part provided the MAK and BAT values are observed. Under these conditions no contribution to human cancer risk is expected. The classification is supported especially by evidence that, for example, increases in cellular proliferation, inhibition of apoptosis or disturbances in cellular differentiation are important in the mode of action. The classification and the MAK and BAT values take into consideration the manifold mechanisms contributing to carcinogenesis and their characteristic dose-time-response relationships.

Category 5 Substances that cause cancer in humans or animals or that are considered to be carcinogenic for humans and for which a MAK value can be derived. A genotoxic mode of action is of prime importance but is considered to contribute only very slightly to human cancer risk, provided the MAK and BAT values are observed. The classification and the MAK and BAT values are supported by information on the mode of action, dose-dependence and toxicokinetic data.

References

Alden, C.L., Lynn, A., Bourdeau, A., Morton, D., Sistare, F.D., Kadambi, V.J., Silverman, L., 2011. A critical review of the effectiveness of rodent pharmaceutical carcinogenesis testing in predicting for human risk. Vet. Pathol. 48 (3), 772–784. https://doi.org/10.1177/0300985811400445.

Ames, B.N., Gold, L.S., 1990. Chemical carcinogenesis: too many rodent carcinogens. Proc. Natl. Acad. Sci. U.S.A. 87 (19), 7772–7776.

Ames, B.N., Profet, M., Gold, L.S., 1990. Nature's chemicals and synthetic chemicals: comparative toxicology. Proc. Natl. Acad. Sci. U.S.A. 87 (19), 7782–7786.

Anisimov, V.N., Ukraintseva, S.V., Yashin, A.I., 2005. Cancer in rodents: does it tell us about cancer in humans? Nat. Rev. Cancer 5 (10), 807–819.

Bartsch, R., Brinkmann, B., Jahnke, G., Laube, B., Lohmann, R., Michaelsen, S., Neumann, I., Greim, H., 2018. Human relevance of follicular thyroid tumors in rodents caused by non-genotoxic substances. Regul. Toxicol. Pharmacol. 98, 199–208. https://doi.org/10.1016/j.yrtph.2018.07.025.

Bastati-Huber, N., Pötter-Lang, S., Ba-Ssalamah, A., 2015. Focal nodular hyperplasia and hepatocellular adenoma (article in German). Der Radiologe 55 (1), 18–26. https://doi.org/10.1007/s00117-014-2704-9.

Beyersmann, D., Hartwig, A., 2008. Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms. Arch. Toxicol. 82 (8), 493–512. https://doi.org/10.1007/s00204-008-0313-y.

Bolt, H.M., Huici-Montagud, A., 2008. Strategy of the scientific committee on occupational exposure limits (SCOEL) in the derivation of occupational exposure limits for carcinogens and mutagens. Arch. Toxicol. 82, 61–64. https://doi.org/10.1007/s00204-007-0260-z.

Bonner, A.E., Lemon, W.J., Devereux, T.R., Lubet, R.A., You, M., 2004. Molecular profiling of mouse lung tumors: association with tumor progression, lung development, and human lung adenocarcinomas. Oncogene 23 (5), 1166–1176.

Boobis, A.R., Cohen, S.M., Dellarco, V., McGregor, D., Meek, M.E., Vickers, C., Willcocks, D., Farland, W., 2006. IPCS framework for analyzing the relevance of a cancer mode of action for humans. Crit. Rev. Toxicol. 36 (10), 781–792.

Buchmann, A., Schwarz, M., Schmitt, R., Wolf, C.R., Oesch, F., Kunz, W., 1987. Development of cytochrome P-450-altered preneoplastic and neoplastic lesions during nitrosamine-induced hepatocarcinogenesis in the rat. Cancer Res. 47, 2911–2918.

Butler, W.H., Cohen, S.H., Squire, R.A., 1997. Mesenchymal tumors of the mouse urinary bladder with vascular and smooth muscle differentiation. Toxicol. Pathol. 25 (3), 268–274.

Caldwell, D.J., 1999. Review of mononuclear cell leukemia in F-344 rat bioassays and its significance to human cancer risk: a case study using alkyl phthalates. Regul. Toxicol. Pharmacol. 30 (1), 45–53.

Carr, C.J., Kolbye Jr., A.C., 1991. A critique of the use of the maximum tolerated dose in bioassays to assess cancer risks from chemicals. Regul. Toxicol. Pharmacol. 14, 78–87.

Cohen, S., 2011. Expert Opinion Regarding the Two-Year Bioassays in Mice and Rats for Bifenthrin. Cohen, S. University of Nebraska Medical Center Department of Pathology and Microbiology, Nebraska Omaha, NE 68198-3135 March 28, 2011, Attachment B in: electronic submission of FMC Corporation to Californa Environmental Protection Agency, Office of Environmental Health Hazard Assessment, Sacramento, California. https://oehha.ca.gov/media/downloads/proposition-65/crnr/comments/fmc102416.pdf.

Cohen, S.M., Storer, R.D., Criswell, K.A., Doerrer, N.G., Dellarco, V.L., Pegg, D.G., Wojcinski, Z.W., Malarkey, D.E., Jacobs, A.C., Klaunig, J.E., Swenberg, J.A., Cook, J.C., 2009. Hemangiosarcoma in rodents: mode-of-action evaluation and human relevance. Toxicol. Sci. 111 (1), 4–18. https://doi.org/10.1093/toxsci/kfp131.

Cook, J.C., Klinefelter, G.R., Hardisty, J.F., Sharpe, R.M., Foster, P.M., 1999. Rodent Leydig cell tumorigenesis: a review of the physiology, pathology, mechanisms, and relevance to humans. Crit. Rev. Toxicol. 29 (2), 169–261.

Cruzan, G., Bus, J., Banton, M., Gingell, R., Carlson, G., 2009. Mouse specific lung tumors from CYP2F2-mediated cytotoxic metabolism: an endpoint/toxic response where data from multiple chemicals converge to support a mode of action. Regul. Toxicol. Pharmacol. 55 (2), 205–218. https://doi.org/10.1016/j.yrtph.2009.07.002.

Cruzan, G., Bus, J., Hotchkiss, J., Sura, R., Moore, C., Yost, G., Banton, M., Sarang, S.,

2013. Studies of styrene, styrene oxide and 4-hydroxystyrene toxicity in CYP2F2 knockout and CYP2F1 humanized mice support lack of human relevance for mouse lung tumors. Regul. Toxicol. Pharmacol. 66 (1), 24–29. https://doi.org/10.1016/j.yrtph.2013.02.008.

Csanády, G.A., Mendrala, A.L., Nolan, R.J., Filser, J.G., 1994. A physiologic pharmacokinetic model for styrene and styrene-7,8-oxide in mouse, rat and man. Arch. Toxicol. 68 (3), 143–157.

Degen, G.H., Neumann, H.G., 1981. Differences in aflatoxin B1-susceptibility of rat and mouse are correlated with the capability in vitro to inactivate aflatoxin B1-epoxide. Carcinogenesis 2 (4), 299–306.

Dietrich, C.F., Schuessler, G., Trojan, J., Fellbaum, C., Ignee, A., 2005. Differentiation of focal nodular hyperplasia and hepatocellular adenoma by contrast-enhanced ultrasound. Br. J. Radiol. 78 (932), 704–707.

Dokmak, S., Cauchy, F., Belghiti, J., 2014. Resection, transplantation and local regional therapies for liver adenomas. Expert Rev. Gastroenterol. Hepatol. 8 (7), 803–810. https://doi.org/10.1586/17474124.2014.917957.

Drinkwater, N.R., Ginsler, J.J., 1986. Genetic control of hepatocarcinogenesis in C57BL/6J and C3H/HeJ inbred mice. Carcinogenesis 7 (10), 1701–1707.

ECHA (European Chemicals Agency), July 2017. Guidance on the Application of the CLP Criteria - Guidance to Regulation (EC) No 1272/2008 on Classification, Labelling and Packaging (CLP) of Substances and Mixtures. Version 5.0. https://echa.europa.eu/documents/10162/23036412/clp_en.pdf/58b5dc6d-ac2a-4910-9702-e9e1f5051cc5, Accessed date: 20 December 2018.

ECHA RAC (Committee for Risk Assessment), 2011. Annex 1 Background Document to the Opinion Proposing Harmonised Classification and Labelling at Community Level of Polyhexamethylene Biguanide or Poly(hexamethylene) Biguanide Hydrochloride or PHMB. ECHA/RAC/CLH-O-0000001973-68-01/F. https://echa.europa.eu/documents/10162/b21b9828-ff70-ccd6-ae3d-96c4420b99c7, Accessed date: 20 December 2018.

ECHA/RAC-SCOEL, 2017. Joint Task Force ECHA Committee for Risk Assessment (RAC) and Scientific Committee on Occupational Exposure Limits (SCOEL) on Scientific Aspects and Methodologies Related to the Exposure of Chemicals at the Workplace. Final Report. . https://echa.europa.eu/documents/10162/13579/jtf_opinion_task_2_en.pdf/db8a9a3a-4aa7-601b-bb53-81a5eef93145, Accessed date: 20 December 2018 6 December 2017.

Edler, L., Hart, A., Greaves, P., Carthew, P., Coulet, M., Boobis, A., Williams, G.M., Smith, B., 2014. Selection of appropriate tumour data sets for Benchmark Dose Modelling (BMD) and derivation of a Margin of Exposure (MoE) for substances that are genotoxic and carcinogenic: considerations of biological relevance of tumour type, data quality and uncertainty assessment. Food Chem. Toxicol. 70, 264–289. https://doi.org/10.1016/j.fct.2013.10.030.

EMEA (European Medicines Agency), 2008. ICH Topic S1C(R2) Dose Selection for Carcinogenicity Studies of Pharmaceuticals. EMEA, London, U.K. https://www.ema.europa.eu/documents/scientific-guideline/ich-s-1-c-r2-dose-selection-carcinogenicity-studies-pharmaceuticals-step-5_en.pdf, Accessed date: 20 December 2018.

FDA, 2002. Guidance for Industry: Carcinogenicity Study – Protocol Submissions. FDA CDER, Silver Spring, MD, USA. http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm078924.pdf, Accessed date: 20 December 2018.

FDA (Food and Drug Administration), 2001. Guidance for Industry: Statistical Aspects of the Design, Analysis, and Interpretation of Chronic Rodent Carcinogenicity Studies of Pharmaceuticals. Draft Guidance. FDA Center for Drug Evaluation and Research (CDER), Silver Spring, MD, USA. https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm079272.pdf, Accessed date: 20 December 2018.

Filser, J.G., Buters, J.T.M., Schwarz, L., Watkins, J.B., Stanley, L., Schlossmann, J., Hofmann, F., Feron, V.J., Jonker, D., Efferth, T., Kaina, B., Spielmann, H., Calow, P., Forbes, V.E., 2008. Chapter 2. Principles. In: Greim, H., Snyder, R. (Eds.), Toxicology and Risk Assessment: a Comprehensive Introduction. John Wiley & Sons Ltd, Chichester, United Kingdom, pp. 19–203.

Gold, L.S., Slone, T.H., Bernstein, L., 1989. Summary of carcinogenic potency and positivity for 492 rodent carcinogens in the carcinogenic potency database. Environ. Health Perspect. 79, 259–272.

Greim, H. (Ed.), 1999. Changes in the Classification of Carcinogenic Chemicals in the Work Area, Occupational Toxicants. vol. 12 Wiley-VCH, Weinheim.

Änderung der Einstufung krebserzeugender Arbeitsstoffe [available only in German]. In: Greim, H. (Ed.), Gesundheitsschädliche Arbeitsstoffe, Toxikologisch-arbeitsmedizinische Begründung von MAK-Werten, 30. Lieferung. Wiley-VCH, Weinheim.

Greim, H. (Ed.), 2006. Änderung der Einstufung krebserzeugender Arbeitsstoffe (available only in German), Gesundheitsschädliche Arbeitsstoffe, Toxikologisch-arbeitsmedizinische Begründung von MAK-Werten, 40. Lieferung. Wiley-VCH, Weinheim.

Greim, H. (Ed.), 2006. D-Limonen (MAK Value Documentation in German language, 2006), Gesundheitsschädliche Arbeitsstoffe, Toxikologisch-arbeitsmedizinische Begründung von MAK-Werten, 40. Lieferung. Wiley-VCH, Weinheim.

Greim, H. (Ed.), 2006. Furan (MAK Value Documentation in German language, 2006), Gesundheitsschädliche Arbeitsstoffe, Toxikologisch-arbeitsmedizinische Begründung von MAK-Werten, 40. Lieferung. Wiley-VCH, Weinheim.

Greim, H., Albertini, R., 2012. The rationale for thresholds for genotoxic carcinogens: synopsis and conclusions. In: Greim, H., Albertini, R.J. (Eds.), The Cellular Response to the Genotoxic Insult: the Question of Threshold for Genotoxic Carcinogens. Royal Society of Chemistry, pp. 1–20 Issues in Toxicology No. 13.

Greim, H., Albertini, R.J., 2015. Cellular response to the genotoxic impact: the question of threshold for genotoxic carcinogens. Toxicol. Res. 4, 36–45. https://doi.org/10.1039/C4TX00078A.

Greim, H., Hartwig, A., Reuter, U., Richter-Reichhelm, H.B., Thielmann, H.W., 2009. Chemically induced pheochromocytomas in rats: mechanisms and relevance for human risk assessment. Crit. Rev. Toxicol. 39 (8), 695–718. https://doi.org/10.1080/10408440903190861.

Grisham, J., 1997. Interspecies comparison of liver carcinogenesis: implications for cancer risk assessment. Carcinogenesis 18 (1), 59–81.

Hartwig, A. (Ed.), 2009. Thiabendazol (MAK Value Documentation in German language, 2009), Gesundheitsschädliche Arbeitsstoffe, Toxikologisch-arbeitsmedizinische Begründung von MAK-Werten, 46. Lieferung. Wiley-VCH, Weinheim. https://doi.org/10.1002/3527600418.mb14879kskd0046.

Hartwig, A. (Ed.), 2013. Atrazin (MAK Value Documentation in German language, 2013), Gesundheitsschädliche Arbeitsstoffe, Toxikologisch-arbeitsmedizinische Begründung von MAK-Werten, 54. Lieferung. Wiley-VCH, Weinheim. https://doi.org/10.1002/3527600418.mb191224d0054.

Hartwig, A. (Ed.), 2014. Trimethylpentane (All Isomers) (MAK Value Documentation, 2004), the MAK-Collection for Occupational Health and Safety Part I: MAK Value Documentations, 2014. Wiley-VCH, Weinheim. https://doi.org/10.1002/3527600418.mb2922248ise3814.

Hartwig, A. (Ed.), 2014. Arsenic and its Inorganic Compounds (With the Exception of Arsine) (MAK Value Documentation, 2014), the MAK-Collection for Occupational Health and Safety Part I: MAK Value Documentations, 2014. Wiley-VCH, Weinheim. https://doi.org/10.1002/3527600418.mb744038vere5716.

Hartwig, A. (Ed.), 2014. 1-(2-Allyloxy)-2-(2,4-dichlorophenyl)ethyl-1H-imidazole (Imazalil) [MAK Value Documentation, 2014), the MAK-Collection for Occupational Health and Safety Part I: MAK Value Documentations, 2014. Wiley-VCH, Weinheim. https://doi.org/10.1002/3527600418.mb3555444kske5715.

Hartwig, A., MAK Commission, 2016a. Decahydronaphthalene (MAK value documentation, 2015). MAK Collect Occup Health Saf 1, 1677–1703. https://doi.org/10.1002/3527600418.mb9117e5816.

Hartwig, A., MAK Commission, 2016b. ortho-Phenylphenol (OPP) und ortho-Phenylphenol-Natrium (OPP-Na) (MAK Value Documentation in German language, 2016). MAK Collect Occup Health Saf 1, 1067–1110. https://doi.org/10.1002/3527600418.mb9043verd0060.

Hartwig, A., MAK Commission, 2016c. Trichloressigsäure und Natriumtrichloracetat (MAK Value Documentation in German language, 2016). MAK Collect Occup Health Saf 1, 1955–1996. https://doi.org/10.1002/3527600418.mb7603d0061.

Hartwig, A., MAK Commission, 2016d. Oleic acid (MAK value documentation, 2016). MAK Collect Occup Health Saf 1, 455–460. https://doi.org/10.1002/3527600418.mb11280kske6017.

Hartwig, A., MAK Commission, 2016e. Dimethylformamide (MAK value documentation, 2016). MAK Collect Occup Health Saf 2, 8–20. https://doi.org/10.1002/3527600418.mb6812e6017.

Hartwig, A., MAK Commission, 2017. Nitrobenzene (MAK value documentation, 2017). MAK Collect Occup Health Saf 1, 1932–1982. https://doi.org/10.1002/3527600418.mb9895e6318.

Hartwig, A., MAK Commission, 2018. 2-Butoxyethanol (ethylene glycol monobutyl ether) (MAK value documentation, 2018). MAK Collect Occup Health Saf 1, 20–35. https://doi.org/10.1002/3527600418.mb11176e6419.

Haseman, J.K., 1985. Issues in carcinogenicity testing: dose selection. Fundam. Appl. Toxicol. 5 (1), 66–78.

Haseman, J.K., Hailey, J.R., Morris, R.W., 1998. Spontaneous neoplasm incidences in Fischer 344 rats and B6C3F1 mice in two-year carcinogenicity studies: a National Toxicology Program update. Toxicol. Pathol. 26 (3), 428–441.

Hill, A.B., 1965. The environment and disease: association or causation? Proc. R. Soc. Med. 58 (5), 295–300.

Huff, J.E., Eustis, S.L., Haseman, J.K., 1989. Occurrence and relevance of chemically induced benign neoplasms in long-term carcinogenicity studies. Cancer Metastasis Rev. 8 (3), 1–721.

Huff, J., Haseman, J., 1991. Long-term chemical carcinogenesis experiments for identifying potential human cancer hazards: collective database of the National Cancer Institute and National Toxicology Program (1976-1991). Environ. Health Perspect. 96, 23–31.

IARC, 1996. Some Pharmaceutical Drugs. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, vol. 66. IARC Lyon, France, pp. 391–426. https://monographs.iarc.fr/wp-content/uploads/2018/06/mono66.pdf, Accessed date: 20 December 2018.

IARC, 1999a. Some chemicals that cause tumours of the kidney or urinary bladder in rodents and some other substances. In: IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, vol. 73. IARC Lyon, France, pp. 307–327. https://monographs.iarc.fr/wp-content/uploads/2018/06/mono73.pdf, Accessed date: 20 December 2018.

IARC, 1999b. Species Differences in Thyroid, Kidney and Urinary Bladder Carcinogenesis. Scientific Publications 147. IARC, Lyon, France. http://publications.iarc.fr/Book-And-Report-Series/Iarc-Scientific-Publications/Species-Differences-In-Thyroid-Kidney-And-Urinary-Bladder-Carcinogenesis-1999, Accessed date: 20 December 2018.

IARC, 1999c. Predictive value of rodent forestomach and gastric neuroendocrine tumours in evaluating carcinogenic risks to humans. In: Views and Expert Opinions of an IARC Working Group, Lyon, France, 29 November–1 December 1999, IARC Technical Publication 39.

IARC, 1999d. Saccharin and its salts. In: IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, vol. 73. IARC, Lyon, France, pp. 517–624. https://monographs.iarc.fr/wp-content/uploads/2018/06/mono73.pdf, Accessed date: 20 December 2018.

IARC, 2001. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Some Thyrotropic Agents, vol. 79 IARC, Lyon, France. https://monographs.iarc.fr/wp-content/uploads/2018/06/mono79.pdf, Accessed date: 20 December 2018.

IARC, 2002. Some Traditional Herbal Medicines, Some Mycotoxins, Naphthalene and Styrene. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, vol. 82. IARC, Lyon, France, pp. 437–550. https://monographs.iarc.fr/ENG/Monographs/vol82/mono82.pdf, Accessed date: 20 December 2018.

IARC, 2006. Monographs on the Evaluation of Carcinogenic Risks to Humans - Preamble. IARC, Lyon, France. http://monographs.iarc.fr/ENG/Preamble/CurrentPreamble.pdf, Accessed date: 20 December 2018.

IARC (International Agency for Research on Cancer), 1994. Peroxisome Proliferation and its Role in Carcinogenesis. Views and Expert Opinions of an IARC Working Group, Lyon, France, 7–11 December 1994. IARC Lyon, France Technical Publication 24.

Ito, N., Hasegawa, R., Imaida, K., Hirose, M., Asamoto, M., Shirai, T., 1995. Concepts in multistage carcinogenesis. Crit. Rev. Oncol. Hematol. 21 (1–3), 105–133.

Kanda, M., Sugimoto, H., Kodera, Y., 2015. Genetic and epigenetic aspects of initiation and progression of hepatocellular carcinoma. World J. Gastroenterol. 21 (37), 10584–10597. https://doi.org/10.3748/wjg.v21.i37.10584.

Klaunig, J.E., Babich, M.A., Baetcke, K.P., Cook, J.C., Corton, J.C., David, R.M., DeLuca, J.G., Lai, D.Y., McKee, R.H., Peters, J.M., Roberts, R.A., Fenner-Crisp, P.A., 2003. PPARalpha agonist-induced rodent tumors: modes of action and human relevance. Crit. Rev. Toxicol. 33 (6), 655–780.

Kress, S., König, J., Schweizer, J., Löhrke, H., Bauer-Hofmann, R., Schwarz, M., 1992. p53 mutations are absent from carcinogen-induced mouse liver tumors but occur in cell lines established from these tumors. Mol. Carcinog. 6 (2), 148–158.

Krewski, D., 2014. Animal and Human Tumour Site Concordance. https://www.epa.gov/sites/production/files/2014-12/documents/2.6-krewski.pdf, Accessed date: 20 December 2018.

Kunz, H.W., Tennekes, H.A., Port, R.E., Schwartz, M., Lorke, D., Schaude, G., 1983. Quantitative aspects of chemical carcinogenesis and tumor promotion in liver. Environ. Health Perspect. 50, 113–122.

Manenti, G., Binelli, G., Gariboldi, M., Canzian, F., De Gregorio, L., Falvella, F.S., Dragani, T.A., Pierotti, M.A., 1994. Multiple loci affect genetic predisposition to hepatocarcinogenesis in mice. Genomics 23 (1), 118–124.

Maronpot, R.R., 2009. Biological basis of differential susceptibility to hepatocarcinogenesis among mouse strains. J. Toxicol. Pathol. 22 (1), 11–33. https://doi.org/10.1293/tox.22.11.

Maronpot, R.R., Flake, G., Huff, J., 2004. Relevance of animal carcinogenesis findings to human cancer predictions and prevention. Toxicol. Pathol. 32 (Suppl. 1), 40–48.

Maronpot, R.R., Nyska, A., Foreman, J.E., Ramot, Y., 2016. The legacy of the F344 rat as a cancer bioassay model (a retrospective summary of three common F344 rat neoplasms). Crit. Rev. Toxicol. 46 (8), 641–675. https://doi.org/10.1080/10408444.2016.1174669.

Maronpot, R.R., Zeiger, E., McConnell, E.E., Kolenda-Roberts, H., Wall, H., Friedman, M.A., 2009. Induction of tunica vaginalis mesotheliomas in rats by xenobiotics. Crit. Rev. Toxicol. 39 (6), 512–537. https://doi.org/10.1080/10408440902969430.

Marsman, D.S., Popp, J.A., 1994. Biological potential of basophilic hepatocellular foci and hepatic adenoma induced by the peroxisome proliferator, Wy-14,643. Carcinogenesis 15 (1), 111–711.

Mendrala, A.L., Langvardt, P.W., Nitschke, K.D., Quast, J.F., Nolan, R.J., 1993. In vitro kinetics of styrene and styrene oxide metabolism in rat, mouse, and human. Arch. Toxicol. 67 (1), 18–27.

Morimura, K., Cheung, C., Ward, J.M., Reddy, J.K., Gonzalez, F.J., 2006. Differential susceptibility of mice humanized for peroxisome proliferator-activated receptor alpha to Wy-14,643-induced liver tumorigenesis. Carcinogenesis 27 (5), 1074–1080.

Neumann, H.G., Thielmann, H.W., Filser, J.G., Gelbke, H.P., Greim, H., Kappus, H., Norpoth, K.H., Reuter, U., Vamvakas, S., Wardenbach, P., Wichmann, H.E., 1998. Changes in the classification of carcinogenic chemicals in the work area. (Section III of the German List of MAK and BAT values). J. Cancer Res. Clin. Oncol. 124 (12), 661–669.

NRC (National Research Council), 2014. Review of the Styrene Profile in the National Toxicology Program 12th Report on Carcinogens. Excerpt from: Review of the Styrene Assessment in the National Toxicology Program 12th Report on Carcinogens: Workshop Summary. The National Academies of Sciences, Engineering, and Medicine, Washington D.C. https://www.ncbi.nlm.nih.gov/books/NBK241565/#sec_000037, Accessed date: 20 December 2018.

NTP, 1996a. Toxicology and Carcinogenesis Studies of Nickel Oxide (CAS No. 1313-99-1) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). NTP Technical Report Series No. 451. U.S. Department of Health and Human Services, National Institutes of Health, Bethesda, MD, USA. https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr451.pdf, Accessed date: 20 December 2018.

NTP, 1996b. Toxicology and Carcinogenesis Studies of Nickel Subsulfide (CAS No. 12035-72-2) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). NTP Technical Report Series No. 453. U.S. Department of Health and Human Services, National Institutes of Health, Bethesda, MD, USA. https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr453.pdf, Accessed date: 20 December 2018.

NTP, 1998. Toxicology and Carcinogenesis Studies of Cobalt Sulfate Heptahydrate (CAS No. 10026-24-1) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). NTP Technical Report Series No. 471. U.S. Department of Health and Human Services, National Institutes of Health, Bethesda, MD, USA. https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr471.pdf, Accessed date: 20 December 2018.

NTP, 2000. Toxicology and Carcinogenesis Studies of Gallium Arsenide (CAS No. 1303-00-0) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). NTP Technical Report Series No. 492. U.S. Department of Health and Human Services, National Institutes of Health, Bethesda, MD, USA. https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr492.pdf, Accessed date: 20 December 2018.

NTP, 2001. Toxicology and Carcinogenesis Studies of Indium Phosphide (CAS No. 22398-80-7) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). NTP Technical Report Series No. 499. U.S. Department of Health and Human Services, National Institutes of Health, Bethesda, MD, USA. https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr499.pdf, Accessed date: 20 December 2018.

NTP, 2011. Specifications for the Conduct of Studies to Evaluate the Toxic and Carcinogenic Potential of Chemical, Biological and Physical Agents in Laboratory Animals for the National Toxicology Program (NTP). https://ntp.niehs.nih.gov/ntp/test_info/finalntp_toxcarspecsjan2011.pdf, Accessed date: 20 December 2018.

NTP, 2016. Monograph on Cobalt and Cobalt Compounds that Release Cobalt Ions in Vivo. NTP Report on Carcinogens. U.S. Department of Health and Human Services, National Institutes of Health, Bethesda, MD, USA. https://ntp.niehs.nih.gov/ntp/roc/monographs/cobalt_final_508.pdf, Accessed date: 20 December 2018.

NTP (National Toxicology Program), 1993. Toxicology and Carcinogenesis Studies of Talc (CAS No. 14807-96-6) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). NTP Technical Report Series No. 421. U.S. Department of Health and Human Services, National Institutes of Health, Bethesda, MD, USA. https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr421.pdf, Accessed date: 20 December 2018.

OECD, 2018. Test No. 451: Carcinogenicity Studies, OECD Guidelines for the Testing of Chemicals, Section 4. OECD Publishing, Paris. https://doi.org/10.1787/9789264071186-en.

OECD (Organisation for Economic Cooperation and Development), 2012. Guidance Document 116, on the Conduct and Design of Chronic Toxicity and Carcinogenicity Studies, Supporting Test Guidelines 451, 452 and 453. series on testing and assessment, No. 116. second ed. OECD, Paris, France. https://www.oecd-ilibrary.org/docserver/9789264221475-en.pdf, Accessed date: 20 December 2018.

Ozaki, K., Haseman, J.K., Hailey, J.R., Maronpot, R.R., Nyska, A., 2002. Association of adrenal pheochromocytoma and lung pathology in inhalation studies with particulate compounds in the male F344 rat–the National Toxicology Program experience. Toxicol. Pathol. 30 (2), 263–270.

Pandiri, A., 2015. Comparative pathobiology of environmentally induced lung cancers in humans and rodents. Toxicol. Pathol. 43 (1), 107–114. https://doi.org/10.1177/0192623314556516.

Pandiri, A.R., Sills, R.C., Ziglioli, V., Ton, T.V., Hong, H.H., Lahousse, S.A., Gerrish, K.E., Auerbach, S.S., Shockley, K.R., Bushel, P.R., Peddada, S.D., Hoenerhoff, M.J., 2012. Differential transcriptomic analysis of spontaneous lung tumors in B6C3F1 mice: comparison to human non-small cell lung cancer. Toxicol. Pathol. 40 (8), 1141–1159. https://doi.org/10.1177/0192623312447543.

RIVM, 2004. Report 601516012/2004. Factsheets for the (Eco)toxicological Risk Assessment Strategy of the National Institute for Public Health and the Environment - Part IV. RIVM, Bilthoven, The Netherlands. http://rivm.openrepository.com/rivm/bitstream/10029/9008/1/601516012.pdf, Accessed date: 20 December 2018.

RIVM (National Institute for Public Health and the Environment), 2003. Report 601516010/2003. Factsheets for the (Eco)toxicological Risk Assessment Strategy of the National Institute for Public Health and the Environment - Part III. RIVM, Bilthoven, The Netherlands. http://www.rivm.nl/bibliotheek/rapporten/601516010.pdf, Accessed date: 20 December 2018.

Rudmann, D., Cardiff, R., Chouinard, L., Goodman, D., Küttler, K., Marxfeld, H., Molinolo, A., Treumann, S., Yoshizawa, K., INHAND Mammary, Zymbal's, Preputial, Clitoral Gland Organ Working Group, 2012. Proliferative and nonproliferative lesions of the rat and mouse mammary, Zymbal's, preputial, and clitoral glands. Toxicol. Pathol. 40 (6 Suppl. l) 7S-39S.

Russo, J., 2015. Significance of rat mammary tumors for human risk assessment. Toxicol. Pathol. 43 (2), 145–1570. https://doi.org/10.1177/0192623314532036.

Schwarz, M., Thielmann, H.W., Meischner, V., Fartasch, M., 2015. Relevance of the mouse skin initiation-promotion model for the classification of carcinogenic substances encountered at the workplace. Regul. Toxicol. Pharmacol. 72 (1), 150–157. https://doi.org/10.1016/j.yrtph.2015.03.014.

SCOEL, 2017. Methodology for Derivation of Occupational Exposure Limits of Chemical Agents. The General Decision-Making Framework of the Scientific Committee on Occupational Exposure Limits. European Commission, Directorate-General for Employment, Social Affairs and Inclusion. https://publications.europa.eu/en/publication-detail/-/publication/3c8ef3e0-48fc-11e8-be1d-01aa75ed71a1.

Seidegård, J., DePierre, J.W., Guenthner, T.M., Oesch, F., 1986. The effects of metyrapone, chalcone epoxide, benzil, clotrimazole and related compounds on the activity of microsomal epoxide hydrolase in situ, in purified form and in reconstituted systems towards different substrates. Eur. J. Biochem. 159 (2), 415–423.

Shipp, A., Lawrence, G., Gentry, R., McDonald, T., Bartow, H., Bounds, J., Macdonald, N., Clewell, H., Allen, B., Van Landingham, C., 2006. Acrylamide: review of toxicity data and dose-response analyses for cancer and noncancer effects. Crit. Rev. Toxicol. 36 (6–7), 481–608.

Sistare, F.D., Morton, D., Alden, C., Christensen, J., Keller, D., Jonghe, S.D., Storer, R.D., Reddy, M.V., Kraynak, A., Trela, B., Bienvenu, J.G., Bjurström, S., Bosmans, V., Brewster, D., Colman, K., Dominick, M., Evans, J., Hailey, J.R., Kinter, L., Liu, M., Mahrt, C., Marien, D., Myer, J., Perry, R., Potenta, D., Roth, A., Sherratt, P., Singer, T., Slim, R., Soper, K., Fransson-Steen, R., Stoltz, J., Turner, O., Turnquist, S., van Heerden, M., Woicke, J., DeGeorge, J.J., 2011. An analysis of pharmaceutical experience with decades of rat carcinogenicity testing: support for a proposal to modify current regulatory guidelines. Toxicol. Pathol. 39 (4), 716–744. https://doi.org/10.1177/0192623311406935.

Slikker Jr., W., Andersen, M.E., Bogdanffy, M.S., Bus, J.S., Cohen, S.D., Conolly, R.B., David, R.M., Doerrer, N.G., Dorman, D.C., Gaylor, D.W., Hattis, D., Rogers, J.M., Setzer, R.W., Swenberg, J.A., Wallace, K., 2004a. Dose-dependent transitions in mechanisms of toxicity. Toxicol. Appl. Pharmacol. 201 (3), 203–225.

Slikker Jr., W., Andersen, M.E., Bogdanffy, M.S., Bus, J.S., Cohen, S.D., Conolly, R.B., David, R.M., Doerrer, N.G., Dorman, D.C., Gaylor, D.W., Hattis, D., Rogers, J.M., Setzer, R.W., Swenberg, J.A., Wallace, K., 2004b. Dose-dependent transitions in mechanisms of toxicity: case studies. Toxicol. Appl. Pharmacol. 201 (3), 226–294.

Sonich-Mullin, C., Fielder, R., Wiltse, J., Baetcke, K., Dempsey, J., Fenner-Crisp, P., Grant,

D., Hartley, M., Knaap, A., Kroese, D., Mangelsdorf, I., Meek, E., Rice, J.M., Younes, M., 2001. IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. Regul. Toxicol. Pharmacol. 34 (2), 146–152.

Sontag, J.M., Page, N.P., Saffiotti, U., 1976. Guidelines for Carcinogen Bioassay in Small Rodents. DHHS Publication (NIH) 76-801. National Cancer Institute, Bethesda, MD, USA. https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr001.pdf, Accessed date: 20 December 2018.

Stahl, S., Ittrich, C., Marx-Stoelting, P., Köhle, C., Altug-Teber, O., Riess, O., Bonin, M., Jobst, J., Kaiser, S., Buchmann, A., Schwarz, M., 2005. Genotype-phenotype relationships in hepatocellular tumors from mice and man. Hepatology 42 (2), 353–361.

Stearman, R.S., Dwyer-Nield, L., Zerbe, L., Blaine, S.A., Chan, Z., Bunn Jr., P.A., Johnson, G.L., Hirsch, F.R., Merrick, D.T., Franklin, W.A., Baron, A.E., Keith, R.L., Nemenoff, R.A., Malkinson, A.M., Geraci, M.W., 2005. Analysis of orthologous gene expression between human pulmonary adenocarcinoma and a carcinogen-induced murine model. Am. J. Pathol. 167 (6), 1763–1775.

Swenberg, J.A., 1993. Alpha 2u-globulin nephropathy: review of the cellular and molecular mechanisms involved and their implications for human risk assessment. Environ. Health Perspect. 101 (Suppl. 6), 39–44.

Thomas, J., Haseman, J.K., Goodman, J.I., Ward, J.M., Loughran Jr., T.P., Spencer, P.J., 2007. A review of large granular lymphocytic leukemia in Fischer 344 rats as an initial step toward evaluating the implication of the endpoint to human cancer risk assessment. Toxicol. Sci. 99 (1), 3–19.

Thoolen, B., ten Kate, F.J.W., van Diest, P.J., Malarkey, D.E., Elmore, S.A., Maronpot, R.R., 2012. Comparative histomorphological review of rat and human hepatocellular proliferative lesions. J. Toxicol. Pathol. 25 (3), 189–199. https://doi.org/10.1293/tox.25.189.

Tischoff, I., Tannapfel, A., 2008. DNA methylation in hepatocellular carcinoma. World J. Gastroenterol. 14 (11), 1741–1748.

Tormos, K.V., Chandel, N.S., 2010. Interconnection between mitochondria and HIFs. J. Cell Mol. Med. 14 (4), 795–804. https://doi.org/10.1111/j.1582-4934.2010.01031.x.

Unterberger, E.B., Eichner, J., Wrzodek, C., Lempiäinen, H., Luisier, R., Terranova, R., Metzger, U., Plummer, S., Knorpp, T., Braeuning, A., Moggs, J., Templin, M.F., Honndorf, V., Piotto, M., Zell, A., Schwarz, M., 2014. Ha-ras and β-catenin oncoproteins orchestrate metabolic programs in mouse liver tumors. Int. J. Cancer 135 (7), 1574–1585. https://doi.org/10.1002/ijc.28798.

U.S. EPA (U.S. Environmental Protection Agency), 2005. Guidelines for Carcinogen Risk Assessment. March 2005. U.S. EPA, Washington DC, USA. https://www3.epa.gov/airtoxics/cancer_guidelines_final_3-25-05.pdf, Accessed date: 20 December 2018.

Wiseman, H., Halliwell, B., 1996. Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. Biochem. J. 313 (Pt 1), 17–29.

Greim, H. (Ed.), 2002. Oleic Acid (MAK Value Documentation, 2002), Occupational Toxicants, vol. 17 Wiley-VCH, Weinheim.

Greim, H. (Ed.), 2002. Tributyl Phosphate [MAK Value Documentation, 2002), Occupational Toxicants, vol. 17 Wiley-VCH, Weinheim.

Greim, H. (Ed.), 2003. Styrene (MAK Value Documentation), Occupational Toxicants, vol. 20 Wiley-VCH, Weinheim.

Greim, H. (Ed.), 2003. 2-Ethylhexanol [MAK Value Documentation, 2003), Occupational Toxicants, vol. 20 Wiley-VCH, Weinheim.

Hartwig, A. (Ed.), 2009. Phthalic Acid and its Isomers (Isophthalic Acid and Terephthalic Acid) (MAK Value Documentation), the MAK-Collection for Occupational Health and Safety Part I: MAK Value Documentations, vol. 25 Wiley-VCH, Weinheim.

Hartwig, A. (Ed.), 2010. Zinc and its Inorganic Compounds [MAK Value Documentation, 2010], the MAK-Collection for Occupational Health and Safety Part I: MAK Value Documentations Wiley-VCH, Weinheim. https://doi.org/10.1002/3527600418.mb744066e4914.