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What is the meaning of 'A compound is carcinogenic'?

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ABSTRACT

Chemical Carcinogens are compounds which can cause cancer in humans and experimental animals. This property is attributed to many chemicals in the public discussion, resulting in a widespread perception of danger and threat. In contrast, a scientific analysis of the wide and non-critical use of the term ‚carcinogenic' is warranted. First, it has to be clarified if the compound acts in a genotoxic or non-genotoxic manner. In the latter case, an ineffective (safe) threshold dose without cancer risk can be assumed. In addition, it needs to be investigated if the mode-of-action causing tumors in laboratory animals is relevant at all for humans.

In case the compound is clearly directly genotoxic, an ineffective threshold dose cannot be assumed. However, also in this case it is evident that high doses of the compound are generally associated with a high cancer risk, low doses with a lower one. Based on dose-response data from animal experiments, quantification of the cancer risk is carried out by mathematical modeling. If the safety margin between the lowest carcinogenic dose in animals and the relevant level of exposure in humans exceeds 10,000, the degree of concern is classified as low. Cases, where the compound turns out to be genotoxic in one study or one test only but not in others or only *in vitro* but not *in vivo*, are particularly difficult to explain and cause controversial discussions. Also for indirectly genotoxic agents, an ineffective (threshold) dose must be assumed. The situation is aggravated by the use of doubtful epidemiological studies in humans such as in the case of glyphosate, where data from mixed exposure to various chemicals were used. If such considerations are mixed with pure hazard classifications such as 'probably carcinogenic in humans' ignoring dose-response behavior and mode-of-action, the misinformation and public confusion are complete. It appears more urgent but also more difficult than ever to return to a scientifically based perception of these issues.

1. Introduction

Toxicological risk assessment is a science-based approach aimed at describing the quantitative risk of adverse effects of chemicals, preferentially in humans. It is the final goal of toxicological risk assessment to provide a rational basis for eventual regulatory measures in order to avoid or exclude such adverse outcome. The methods and results are not only discussed among scientists and regulatory bodies but also in the public.

Likewise, there are frequently reports in the press about the occurrence of chemicals or pollutants in the environment, in food or in the human body. For the author, the editor *etc.* the question is at hand, if this news is worthwhile being published. A common denominator of such reports is the issue that a vulnerable target such as ‚the environment, nature, plants, animals or humans may be at risk of being harmed. If officials such as representatives of a government, an authority *etc.* comment such news, they often claim that a risk cannot be excluded completely. Such a notion is often misunderstood, *i.e.*, it seems to indicate that a realistic risk in fact exists. The novelty of the news increases dramatically if it can be made plausible that the occurrence of the, chemical may or will cause a real danger including damage to the vulnerable target.

If an agency, authority or another official body has made such a vague statement, this will be mentioned in the report. If the scientific analysis reveals a more or less equivocal picture, *i.e.*, some reports underpin a risk whereas others dismiss it, the report will in many instances tend to give more weight to the concerns than to the reliefs.

This way of communicating the facts follows an idea similar to the so-called ‚precautionary principle since it tends to be more ‚on the safe side and has the positive side effects that the news gains more attention. A common way to illustrate such concerns is the notion that the, chemical is 'suspected to cause cancer'. Such a comment reads much easier than the notion that, the institution X has expressed such concerns whereas institutions Y and Z have dismissed them.

Exactly the same situation occurred, *e.g.*, in the case of the herbicide glyphosate which was classified as ‚probably carcinogenic in humans 'by the International Agency for Research on Cancer, an institution of the World Health Organization (WHO) [1], whereas other institutions

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in the field such as the European Food Safety Authority [2], the Joint Meeting on Pesticide Residues (JMPR, also a WHO expert group) [3] and the German Federal Institute for Risk Assessment (BfR) [4] decided that it was, not relevant carcinogenic (or a similar wording). Nevertheless, several press releases added the attribute 'suspected to be carcinogenic' thus using the 'most negative' classification available. The latter isn't even wrong since there is one well-known institution having this point of view. However, it raises the general question how scientific institutions (two under the same umbrella of WHO) can come to such divergent conclusions in particular since they based them on the same publically available information. Thus, the question is what a classification as 'carcinogenic' is based upon and what the meaning of such a statement is. To get closer to an answer, it will be discussed first what our current understanding is on how a chemical can cause cancer.

2. Mechanisms of carcinogenicity of chemicals

In 1771, John Hill, a physician of London described a correlation between the use of tobacco (snuff) and nasal tumors [5], and 1775, the English physician Persival Pott observed that lean boys, so-called ‚chimney sweeps' crawling up the chimneys of to clean them with their bodies frequently suffered from skin cancer of the scrotum (described in [6]). About two hundred years later it was discovered that certain polycyclic aromatic hydrocarbons (PAHs) isolated from tar and soot caused similar types of skin cancer in laboratory animals. Probably, such PAHs had contributed to the tumors observed by Pott in the London chimney sweeps. Subsequently, the groundbreaking work by Elizabeth and James Miller [7], Jerina [8] and other researchers [9] showed that PAHs cannot cause cancer directly but need metabolic activation by cytochrome P450 (CYP) mono-oxygenases to do so. These enzymes are preferentially found in the endoplasmic reticulum of cells, *e.g.* in the liver cell, and are able to catalyze an enormous spectrum of chemical reactions most prominently the insertion of an oxygen atom in to organic substrates. Since the CYP enzymes are mainly located in the so-called microsomal fraction (mostly containing the endoplasmic reticulum) obtained by differential centrifugation of a tissue homogenate, they are also called microsomal mono-oxygenases. Their major function is the detoxification of a very broad spectrum of exogenous (and also endogenous) compounds by modifying their structure, *i.e.*, making them more hydrophilic and/or preparing them for further conjugation reactions (reviewed in [10]). Several chemical carcinogens such as carcinogenic polycyclic aromatic hydrocarbons, *e.g.* benzo(a)pyrene, are converted by CYP enzymes, however, into highly reactive unstable products. Due to their electrophilic properties, these are able to react with nucleophilic targets under formation of covalently bound adducts (Fig. 1). These targets are nucleophiles, *e.g.* proteins but also the genomic information, *i.e.* the nuclear DNA. If nuclear DNA is modified covalently a permanent change in the sequence of DNA bases called mutation or other changes in DNA structure may finally result [11,12].

Events of this type may lead to alterations in some cells not resulting in cell death but putting these cells on a 'track towards malignancy'. Such so-called 'initiated' cells bear genetic changes making them vulnerable to further steps or lesions. Furthermore, it is assumed that the process of malignization takes time and is subject to a variety of influencing factors. Since a single 'hit' is highly unlikely to result in a malignant cancer cell it is widely accepted that several genetic alterations have to occur before a malignancy develops, originating from the clonal expansion of a malignant cell [13]. On several (earlier) stages the process can be stopped or possibly even reversed. It is evident that all steps including the fate of the initial genetically altered cells are subject to a variety of responses of the host such as programmed death of the affected cell [14], repair of the DNA damage [15], attack by the immune system [16] *etc.*

Chemical carcinogenesis was recognized early as proceeding *via* distinct steps. Thus, an experimental two-stage skin carcinogenesis model was developed [17]. Chemicals which can facilitate or accelerate early steps in multi-stage carcinogenesis are called tumor promoters [18]. while compounds which enhance the growth and conversion of later stages such as precancerous hyperplasia are called tumor progressors [19]. It is not completely clear if such chemicals supporting the pathway towards malignancy do so only by modifying the survival and growth conditions of the cell or by helping to selects cells with a certain growth advantage within a mixed population of a tumorous lesion. Compounds acting as tumor promoters usually do not form reactive metabolites but act by modulating growth or cell death ('apoptosis') *via* receptor-mediated or other mechanisms [20,21]. It has to be kept in mind, however, that the available initiation-promotion regimens are simplified models which cannot reflect all facettes of chemical carcinogenicity [22].

Direct interaction of a chemical with the genome not necessarily requires metabolic activation. In fact some highly reactive chemicals used as chemical weapons but also certain drugs used in cancer chemotherapy are DNA-reactive in itself [23].

However, those chemicals which are relatively stable in the environment but require metabolic activation in the body are of much greater importance since they occur in food, environmental samples *etc.*

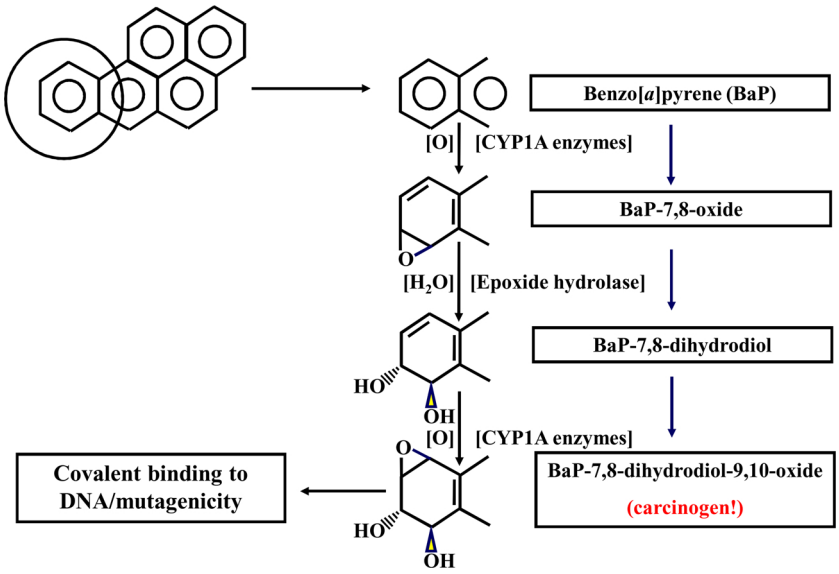

Fig. 1. Metabolic activation of benzo(a)pyrene into a directly genotoxic metabolite.

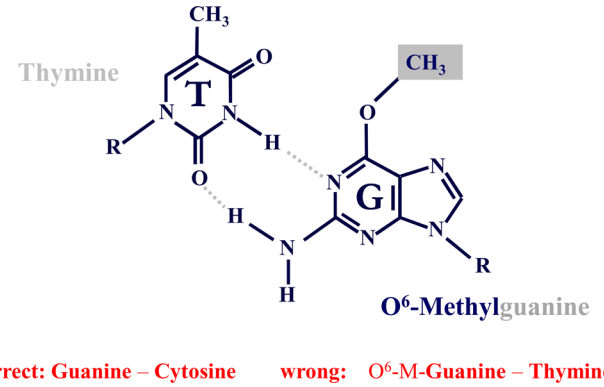

Fig. 2. Impact of guanine methylation at position O^6 on DNA base pairing.

Their transfer into the body and subsequent activation by the host's endogenous metabolic pathways has features of a 'Trojan horse'.

3. Genotoxic *versus* non-genotoxic

The issue of a direct genotoxic interaction *vs.* non-genotoxic mechanisms is of crucial interest for risk assessment of chemical carcinogens [24]. This fact is due to a dogma in toxicology saying that, in principle a single point mutation could be sufficient to finally cause a tumor. This hypothesis is based on early experiment with potent genotoxic (alkylating) carcinogens such as dimethyl-*N*-nitrosamine (DMN), which can act in a genotoxic manner *via* methylation of DNA bases after metabolic activation [25]. On a molecular level, *e.g.*, the guanine methylated at position ^{6}O undergoes wrong base pairing upon DNA replication resulting in point mutations, *i.e.*, a substitution of the regular pairing partner cytosine by thymine (Fig. 2). Thus, a permanent change in DNA sequence becomes manifest [26].

In several early studies by H. Druckrey with potent carcinogens such as N-nitrosamines [27], no deviation from linearity was observed in dose-response relationships between dose and number of cancer-bearing animals, even at very low dose levels. In a number in examples, however, deviations from linearity were observed, *e.g.*, with the genotoxic carcinogens 2-AAF [28], diethylnitrosamine [19] or dibenzo[a,l] pyrene) [29], in particular when the effects of very high or very low dose ranges were analyzed. Even in animal experiments with potent carcinogens, there is a dose level not causing a significant increase in tumors above background. Such analyses are often hampered by the fact that aging populations of higher animals exhibit increasing 'background' rates of tumors in the ‚untreated' control groups [30]. The age-dependent increase in cancer rates in humans may suggest a similar phenomenon to take place in humans [31,32]. However, the difficulties with this assumption were pointed out by Moolgavkar and Knudson [33] and Greenfield et al. [34].

Since, the exact reason for this observation is unknown it affects the interpretation of feeding studies with test compounds since the animals get a certain amount of tumors anyway, *i.e.*, even if the compound was not applied. It has been described that the overall fidelity of DNA repair, replication and maintenance of integrity decreases with ageing [35] while DNA lesions from 'background' factors may accumulate over a lifespan [36]. In a young healthy organism, it can be expected that a large portion if not all relevant DNA lesions are either repaired or the cells are eliminated. It is unclear why these defense mechanism are overruled at low dose levels of dimethyl-*N*-nitrosamine and if even lower dose levels are without effect. The perspectives to answer these questions are limited, of course, be the ‚spontaneous' background cancer incidence attributed to endogenous and 'unavoidable' exogenous factors [37]. The enormous numbers of animals required for a possible detection of low dose effects, besides the ethical problems with large animal experiments, are not feasible since minor bias factors such as infections, housing conditions, feed *etc.* may have a too strong impact. In consequence, these considerations indicate that we will not be able to finally answer the question of linearity of cancer risk at low doses. However, it may be that the DNA repair and maintenance capacity of a cell plays a stronger role for the final outcome than the amount of primary lesions [38].

The finding that extremely low doses of a potent chemical carcinogen do not lead to significant increase in cancer incidence over the untreated control may indicate that the dose is not carcinogenic. It may also indicate, however, that the design of the study was not appropriate to identify a small increase. Nevertheless, the dogma of linearity of cancer response towards directly acting genotoxic carcinogens has received much criticism. It was derived not only from some observations with certain potent carcinogens but also from cancer studies with ionizing radiation, another DNA-damaging carcinogenic factor. The critical view on this dogma is based on its weak scientific justification [39] and biological plausibility since it appears unlikely that the natural defense and repair mechanism should be overruled even at infinitely low dose levels [40,41].

In addition, it is often ignored that the dose-response relationship for strong (alkylating) genotoxic carcinogens might be linear over a wide dose range but that this is not necessarily true for weak or borderline carcinogens [42]. Finally it is overlooked that even under the assumption of a complete lack of a 'threshold' of no effect (a dose which is not carcinogenic), over a wide dose-range higher doses cause more tumors, and lower dose less. Even if the compound was carcinogenic at very low doses, the number of tumors clearly has to be very low either. Likewise, smoking of one cigarette per year is not adding a relevant tumor risk. Labeling of chemicals as 'human carcinogens' does not tell us anything about the dose-response and the relevance of the risk [43].

The mode of action non-genotoxic carcinogens appears to be much more diverse. It includes not only the above-mentioned tumor promotion, a term which is tightly linked to certain two-stage animal models, but also a wide range of other mechanisms. These include receptor-mediated carcinogenesis (a typical feature of various tumor promoters causing enhanced proliferation, suppressing apoptosis *etc.*), epigenetic alterations such as changes in histone acetylation, perturbations of DNA repair, oxidative stress [44] and many others [45]. As a general rule, in case of enhanced tumor formation in an animal model without convincing evidence for genotoxicity, in particular *in vivo* genotoxicity, there is an urgent need for an in-depth investigation of the mode of action. A good example is the occurrence of pheochromocytoma in rats fed relatively high doses of vitamin D3 which is thought to be caused by a hyperproliferative response of chromaffin cells of the adrenal medulla, an effect not observed in humans [46,47]. Another well-described case is the induction of male rat kidney tumours by α-2-μ-globulin induction shown to be male rat specific and not relevant to humans [48]. Fundamental considerations on this problem and a framework of criteria (decision tree) for the evaluation of the relevance of carcinogenicity data from animals for the human situation were published by [49].

A special group of carcinogens is thought to be able to cause DNA damage without direct interaction. These indirect genotoxic compounds typically act *via* the generation of reactive oxygen species or other reactive (endogenous) metabolites which are DNA-reactive [50] or by other mechanisms which change DNA structure and integrity such as topoisomerase poisons [51].

For non-genotoxic [52] and also for indirectly genotoxic carcinogens, the existence of a threshold of effect or at least of a 'practical threshold' was suggested. A major argument for this suggestion is the notion, that such changes in DNA structure are likely to occur anyhow at low levels, *i.e.* under physiological conditions in the absence of any external 'chemical' [53]. One aspect related to this finding is the fact that endogenous metabolites, *e.g.* certain aldehydes [54], reactive oxygen species [55] *etc.* are able to react with nuclear DNA *in vivo*, probably contributing to what is called 'background cancer incidence'.

Taken together, the relevant mode of action of a chemical carcinogen in an animal study is a crucial piece of knowledge for a rationale risk assessment. Frequently, the situation is more complicated, however, with (apparently) contradictory findings on carcinogenicity in different studies, positive findings on genotoxicity in certain in vitro assays but not in others, lack of genotoxicity *in vivo etc.* For such cases, weight of evidence approaches have been applied [56,57] including considerations of the dose levels relevant for the human situation [58].

4. The quantitative aspect: risk *versus* hazard

Among toxicologists, it is well known that any chemical can cause adverse effects in living organisms as long as the dose is sufficiently high. Interestingly, this notion is widely unknown in the public discussion – probably because our ancestors already knew that there are 'poisonous' and 'edible' plants in our environment. This principle was helpful over thousands of years and even today it helps when collecting plants or mushrooms. Obviously the knowledge that 'a little bit of poison does not cause harm' or 'the dose makes the poison' (Paracelsus' law) [59] was not very practicable or safe. These old clichés still prevail in public making the discussion cumbersome, since they are incorrect from a scientific point of view. Even in the media, chemicals' are labeled as 'toxic' or 'carcinogenic' ignoring what the science of toxicology is about.

There are, however, two exceptions from this scientific principle. One is a true exception, *i.e.*, an immunogenic (sensitizing) or allergenic reaction cannot generally be predicted based on the dose level [60]. Undoubtedly, extremely low doses of an allergen do not cause clinical symptoms. However, this 'threshold dose' seems to vary between individuals and for most of us any dose of the allergen is ineffective since we are not allergic towards the compound. The second exception is not a true one: it is the case of genotoxic carcinogens. There, the paradigm of a lack of an ineffective threshold dose is hampered by two facts. First, very low doses cause very low tumor incidences, even if the paradigm was true. The effect levels are likely to be 'virtually safe' if one tumor case in a million consumers is estimated. However, the calculation is usually made *via* a linear extrapolation over several orders of magnitude [61]. Furthermore, there is no tool to verify the estimate since the assumption is made of a probabilistic basis for a uniform dose level. Second, weak genotoxic carcinogens are also summarized under the same 'umbrella'. There, it is highly likely that indirect effects (which show an ineffective threshold dose) and biological defense mechanisms (which prevent damage) are active.

A typical example for a weak carcinogen is acrylamide. The process-generated carcinogen occurs as a contaminant in heated flour and potato products [62]. However, human exposure *via* the diet is in a range that would not allow a prediction of cancer incidences significantly exceeding background rates including background variability [63]. Thus, epidemiological studies on a relationship between acrylamide exposure and cancer risk have not indicated a robust effect so far. These (expected) findings have led to the recommendation to stop any further epidemiological studies on acrylamide exposure *via* food [64]. The fact that acrylamide exposure at the workplace did not convincingly show carcinogenicity in humans adds to these considerations and makes it likely that acrylamide acts as a weak carcinogen in humans. It appears problematic to apply the methodology designed for highly potent carcinogens for the risk assessment for such weak or borderline carcinogens as acrylamide.

The discussion becomes non-scientific if fundamental aspects of dose-response and mode of action are not at all taken into account. Statements such as 'carcinogenic dioxin was released' or 'the carcinogen glyphosate was found in beer' are of no value. As discussed above, such statements would be useful for risk assessment, if dioxin (or glyphosate) would be directly acting genotoxic carcinogens, which they are not. Furthermore, knowledge of the dose-response of the claimed carcinogenicity would be required. The latter aspect in particular is not considered by the International Agency for Research on Cancer classifying chemicals (and other agents) according to their carcinogenicity. These classifications such as 'carcinogenic in animals' are not necessarily wrong but according to the 'threshold paradigm' the carcinogenicity of dioxin in rodents is mediated *via* a non-genotoxic mode of action suggesting that there is a no-effect threshold of dose. This fact is of enormous relevance for the risk assessment process since it would allow the assumption that certain (small) dioxin levels do not represent any cancer risk. In the case of glyphosate the chemical is not genotoxic either in animal experiments [65]. The situation is complicated further by arguments on the use of data provided by the manufacturer and by the assessment of a commercial glyphosate-containing product which comprises several other chemicals. Furthermore, the classification was based on questionnaires of cancer cases among users (farmers), *e.g.* multiple myeloma, without knowledge of the actual exposure towards glyphosate, and of the exposure towards other herbicides *etc.* [66]. These examples demonstrate that an early agreement on the type and quality of data to be used is crucial to a successful risk assessment in particular when it comes to controversial issues.

5. Exposure as the dose metric: risk assessment and risk communication

According to Paracelsus' paradigm (see above) the dose makes the poison. This paradigm is of course true also for genotoxic carcinogens. Even under the assumption that very small doses still cause a very small (hypothetical) risk, the question of relevance has to be answered. In order to provide quantitative information on the risk estimate, various methods are in use, which will be presented as follows (Table 1).

In order to limit the cancer risk from chemicals, the so-called ALARA (‚As low as reasonably achievable') principle was introduced. It was applied, *e.g.*, to derive maximum values for certain carcinogenic food contaminants such as aflatoxins [67]. Although the ALARA principle represents an effective tool to protect the consumer [52], it is criticized since it is applied as a consensus between commercial producer, suppliers *etc.* and risk managers [68]. Thus it is sometimes considered as a 'good deal for industry' rather than a scientific derivation of a maximum exposure level.

Table 1
The most widely used methods in risk assessment of directly genotoxic carcinogens – pros and cons.

Method	Pros	Cons
ALARA/ALARP	easy to put into practice	no toxicological rational, based on practicability
extrapolation (virtually safe dose)	precise risk estimate based *e.g.* on experimental data	extrapolation across several orders of magnitude; difficult to communicate
MoE	**easy to communicate**	**strict 'cut-off' (< 10.000 danger?; > 10.000 no danger?), consequences for risk management are unclear**
TTC	generic approach lacking relevant toxicological data, based on structural analogy	should not be used if relevant toxicological data are available, analogy could be misleading

Abbreviations: ALARA, as low as reasonably achievable; ALARP, as low as reasonably practicable; MoE, margin of exposure; TTC, Threshold of Toxicological Concern.

Another traditional method is the derivation of a ‚Virtually safe dose' (VSD)' which is obtained by linear extrapolation of animal-derived dose-response data. An apparent (additional) risk of $1:10^6$ is considered as acceptable, the related dose level is called 'virtually safe' [61]. An obvious drawback of this method is the option to calculate a precise number of (additional) cancer victims based on a very rough extrapolation over several orders of magnitude. The pretended (but not real) precision stands in sharp contrast to the fact that those hypothetical victims can never be identified because an average exposure is assumed which causes cancer in a few individuals on a stochastic basis. The hypothesis can thus neither be confirmed nor discarded by data analysis.

A more modern concept describes the distance between the current dose level in a human population (*e.g.* the average exposure or its 95% percentile *etc.*) and a reference dose found in experimental animals (rarely in human studies). This so-called Margin of Exposure (MoE) method usually uses a so-called benchmark dose (BMD) causing a certain metric effect (*e.g.* a 10% increase in tumor incidence, *i.e.* BMD_{10}). Since the variability of the data has to be taken into account, the lower 95% confidence limit of the BMD, called BMDL (*e.g.* $BMDL_{10}$) is calculated (Fig. 3). The distance between this value, also called 'Point of Departure' (POD), and the actual exposure level of interest is then called MoE. If a MoE of higher than 10.000 is determined, EFSA guidance [69] recommend to classify the exposure as 'of low concern'. A MoE of $>$ 1.000.000 is classified as 'of no concern' whereas a MoE of $<$ 10.000 is considered to indicate 'concern'. This approach takes into account the low precision of extrapolations and the limitations of data modeling. However, it cannot overrule the dogma of 'lack of threshold', *i.e.* 'low concern' still means that there is some concern. Attempts to calculate 'safety limits' by dividing the POD dose by 10.000, therefore, give a wrong signal. In general the MoE approach can be communicated quite easily since it illustrates quite well the idea of a 'distance of safety'. The rather arbitrary selection of the value of 10.000, however, leads to misunderstandings in the sense that MoE levels slightly below 10.000 would indicate an urgent need for action. In addition, the 'true' dose response curve particularly in the low dose range is usually unknown (for reasons described above). Thus carcinogens with steep and flat dose-response curves of tumor response are dealt with in the same manner. Clearly, this approach needs refinement in the future to make it more adequate for the individual chemical.

Finally, the 'Theshold of Toxiclogical Concern' (TTC) concept was developed [70] which makes use of the notion that certain structural elements in chemicals are 'notorious' for carcinogenicity (and genotoxicity). It is well known that, *e.g.*, many aromatic amines, acetoxy compounds, hydrazines *etc.* are genotoxic carcinogens in animal studies. If a ‚new 'compound with a lack of toxicological data has to be assessed, the TTC concept assumes that the compound acts in similar way as its structural analogs. By applying a distribution analysis of the carcinogenic dose levels, a general intake estimate for humans, and a linear extrapolation the additional risk is thought to be limited to 1 in a million, if the daily intake does not exceed 0.15 µg per person. It was already limited in the original publication by taking out certain structural elements with a possible high carcinogenic potency (some of them not being directly genotoxic at all) such as steroids, azo compounds or dioxins. The concept would provide a low cost option for risk assessment without animal experiments, and has been further refined [71].

6. Risk assessment: stepwise approaching the 'true risk'

Any toxicological ('risk assessment') usually comprises a prediction of the hypothetical cancer risk of compound, in particular if the chemical is positive in genotoxicity testing or even in long-term carcinogenicity studies in animals. In order to start this effort, it is mandatory to find an agreement on the type and quality of data to be used. Papers by Sonich-Mullin et al. [72] or McGregor et al. [73] made attempts defined a number of such criteria for experimental studies to be suitable for risk assessment of possible chemical carcinogens. In most cases such data are used instead of cancer data from human epidemiology. This is due to the fact that such case-control studies often are insufficient to characterize complex effects of environmental factors on disease development [74]. Rare exceptions are tobacco smoking and long-term consistent exposure at the workplace [75]. In most other cases, many factors influence the development of human cancer, many of them being unknown and/or not monitored. Since the usually observed very low dose levels are thought to result in a very low cancer incidence, huge cohorts would be needed. Such attempts were successful in the past when certain interventions with drugs or food supplements were studied [76,77].

Furthermore, sporadic exposure towards traces of genotoxic carcinogens occurs from many chemicals including air contaminants (polycyclic aromatic hydrocarbons [78]), food process contaminants (benzo [a]pyrene, acrylamide [79]), natural food contaminants (aflatoxins [80]), natural food constituents (alkenylbenzenes [81]) *etc.* and it appears to be a great challenge to separate the individual effects from each other [82]. Furthermore, it appears unacceptable to ignore a

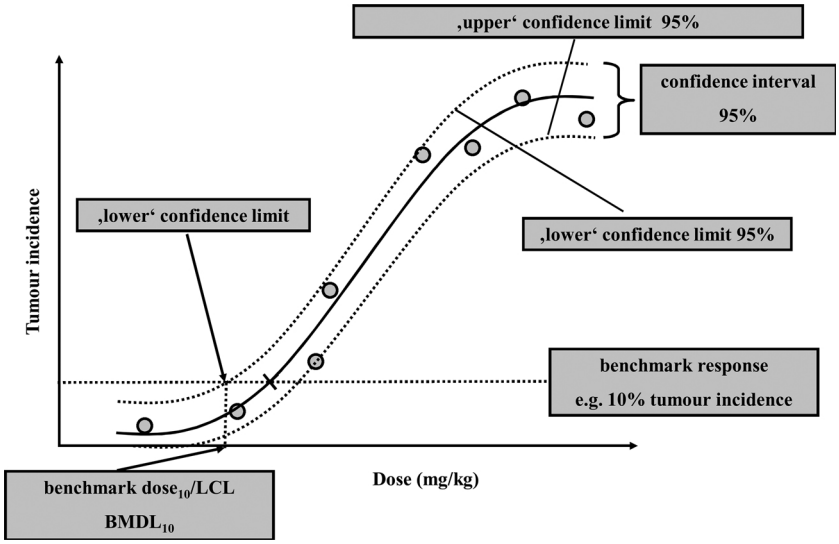

Fig. 3. Concept of benchmark dose modeling and calculation of a lower confidence limit for a 10% increase in tumor incidence ($BMDL_{10}$ value). LCL, lower confidence limit.

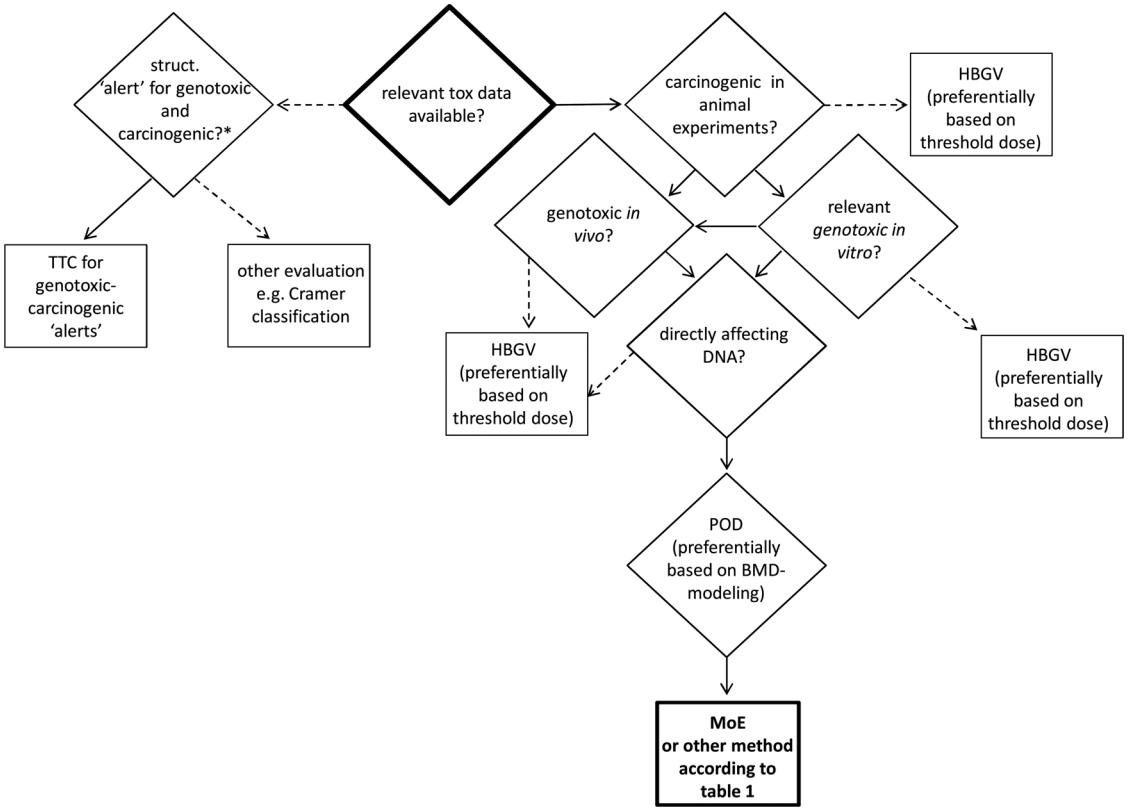

Fig. 4. Decision tree for the risk assessment of chemical carcinogens. A structural ‚alert' is a chemical moiety (group) which is notorious for its genotoxic carcinogenicity (*e.g.* a *N*-nitroso function). Abbreviations: BMD, benchmark dose; HBGV, health-based guidance value; MoE, margin of exposure; POD, point of departure; TTC, Threshold of Toxicological Concern. Straight arrows: yes; dotted arrows: no.

possible risk just because no ‚cancer cases 'can be identified which unequivocally are due to the chemical of interest.

Finally, the risk assessment of cumulative exposure to multiple chemicals with *e.g.* genotoxic potential, requires novel methods [83]. An integrated approach taking into account low dose exposure [84] and mechanistic data was suggested [85].

From these considerations, it appears urgent to establish both a list of criteria for the classification of chemical carcinogens based on their mode of action and to provide directions for a subsequent quantitative risk assessment methodology based on this classification. There are several systematic case studies published in the scientific literature aiming exactly at this goal. They include a threshold-driven approach for a carcinogen considered to be non-genotoxic [86].

From these considerations, it is even more urgent to establish a list of criteria (decision tree) to make a modern risk assessment transparent and scientifically sound. An attempt to approach this aim is presented in Fig. 4. It is finally based on the precautionary paradigm that no ineffective threshold dose can be assumed for a genotoxic and carcinogenic compound.

In particular it is not feasible, however, to assume a lack of threshold for genotoxic compounds acting in an indirect way, *i.e.*, not directly damaging the DNA. Many carcinogens act in this way, *i.e.*, they lead to generation of reactive oxygen species [87], inhibit DNA repair or DNA-processing enzymes [88] mostly as a result of tissue damage, inflammation *etc.* Furthermore, it cannot be assumed for so-called non-genotoxic carcinogens (tumor-promoters, epigenetic carcinogens) *etc.* that their dose-response curve lacks a threshold. The latter group comprises compounds suppressing apoptosis of pre-neoplastic cells or triggering the proliferation of such lesions. Many of these biochemical outcomes are triggered *via* receptor activation being widely assumed to require a certain threshold level of the receptor agonist. This assumption has been criticized, however, by some authors [89].

Finally, *in vitro* findings indicating genotoxicity are sometimes taken into account in a non-critical manner. Many assays are not representative of direct genotoxicity, are poorly reproducible, and have numerous technical difficulties identified over the years, such as the impact of pH, osmolality, cytotoxicity, *etc.* For these reasons, guidelines for the implementation of genotoxicity testing have been developed (reviewed in [90]).

It is well known from several studies that many substances are positive in genotoxicity tests in vitro but not *in vivo*. Examples are the amino acid cysteine [91], the endogenous peptide glutathione or the natural food constituent quercetin [92]. These compounds are not genotoxic or carcinogenic *in vivo* [93]. The *in vitro* findings are due to artificial chemical effects probably involving activation of/*via* atmospheric oxygen.

7. Summary

Chemical carcinogens are compounds that can induce cancer in humans or animals. This property is attributed to many chemicals and composite mixtures in the public communication and is considered as particularly alarming. However, this perception can lead to wrong conclusions and needs to be replaced by a scientific approach to the problem. As a first step the question must be answered if the compound acts as a genotoxic or a non-genotoxic carcinogen. In the latter case, an ineffective threshold dose can be assumed. Doses below this threshold should be classified as non-carcinogenic. Furthermore, it is mandatory to evaluate if the Mode of Action leading to cancer in experimental animals is relevant in humans. If the compound is clearly genotoxic, it has to be evaluated if this effect is indirect or direct (or both), *i.e.* if the reactive form of the compound directly binds to/interacts with DNA or not. In the latter case, the existence of an ineffective threshold dose cannot be assumed *a priori*. Furthermore, this approach is feasible only

if this type of DNA damage has been shown to occur *in vivo*, since a number of *in vitro* assays for genotoxicity are susceptible to artifacts and the outcome may not to be relevant *in vivo*. Even in case of a direct genotoxic effect occurring *in vivo*, it is noteworthy that 'high' doses correlate with higher risk than 'low' doses. Estimating the risk in quantitative terms is usually based on animal data by mathematical modeling (curve fitting). If the outcome of this approach results in a estimated Margin of Exposure of more than 10.000 between a low but clearly carcinogenic dose in animals and human exposure, the concern is considered as low. The discussion becomes more difficult in case of weakly genotoxic substances or compounds which are genotoxic in vitro only, *e.g.* in one assay but in others. The use of epidemiological data in such cases is highly problematic since they may show correlations but no clear scientific evidence. Furthermore, the publication of hazard-based classifications such as 'likely to cause cancer in humans' without taking into account the mode-of action and dose-response adds to the wide-spread confusion. It is the challenge for modern toxicology to replace this type of classification by evidence based, mechanistically sound estimates of the real risk.

Transparency document

The Transparency document associated with this article can be found in the online version.

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