Review

NEW ASPECTS IN THE CLASSIFICATION OF CARCINOGENS*

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The existing systems of classification of carcinogens should include a distinction between genotoxic and non-genotoxic chemicals. For non-genotoxic chemicals, permissible exposure levels can be derived at which no relevant human cancer risks are anticipated. While genotoxic carcinogens can induce chromosomal effects without mutagenic action, non-DNA-reactive genotoxins affecting topoisomerase or the spindle, or those having an exclusively aneugenic effect can be carcinogenic only at high, toxic doses. Specific mechanisms of clastogenicity and processes of carcinogenesis based on reactive oxygen have practical thresholds. Since reactive oxygen species (ROS) are generally genotoxic, the question is whether chemicals that increase ROS production will add to endogenously produced background levels and lead to non-linear dose-effect relationships. Taking into account the presence of endogenous carcinogens, it is now becoming evident that carcinogenic risk extrapolation to low doses must be considered according to the mode of action.

KEY WORDS: *carcinogenesis, chromosomal genotoxins, dose-response, endogenous carcinogens, genotoxicity, reactive oxygen, threshold*

In the EU directives, carcinogens are classified according to a system which was introduced in the early eighties and which was based on the existing national systems. The decision making completely separates the sequential processes of "hazard assessment", which is directed towards classification, from labelling and "risk assessment" which is directed towards standard setting. Both the categorisation and the procedures for low-dose extrapolation of the risk of chemical carcinogens are now being discussed on the international level (1). The general idea is that the classification of carcinogens should be more based on mechanisms which trigger carcinogenic effects and should take more account of their potency. This proposal of a new classification of carcinogens includes additional considerations about germ-cell mutagens (2-4).

The German Senate Commission of the Deutsche Forschungsgemeinschaft (German Science Foundation) for the Investigation of Health Hazards in the Work Area (5, 6) has issued new recommendations to distinguish between five groups of proven and suspected carcinogens instead of three. Category 1 includes proven human carcinogens according to data on exposure, epidemiology and mechanisms of action. Category 2 includes suspected human carcinogens according to experimental animal data supported by data from epidemiological studies. Category 3 includes substances which raise concern because of their carcinogenic potency in experimental models, but which still lack relevant data. The proposed new categories are Category 4 "substances with carcinogenic potential for which genotoxicity plays no or at most a minor role. No significant

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contribution to human cancer risk is expected, provided that the Maximum Allowable Concentration (MAK) value is observed" and Category 5 of the new MAK system, which includes "substances with carcinogenic and genotoxic potential, the potency of which is considered so low that, provided that the MAK value is observed, no significant contribution to human cancer risk is to be expected."

CLASSIFICATION OF CARCINOGENS

For a number of chemical carcinogens (as well as for ionising radiation), the assumption of a linear dose-response relationship without threshold has been plausibly supported and the resulting linear non-threshold (LNT) extrapolation seems appropriate and scientifically well founded. This linear dose-response relationship seems adequate for a number of genotoxic carcinogens, such as aflatoxin B1, the tobacco-specific nitrosoketone N-methyl-nitrosopyridylbutanone NNK, and probably N,N-diethylnitrosoamine (7).

Other carcinogens may behave differently, but the precise nature of the dose-response at low doses has not sufficiently been established. However, extrapolation models connecting the high level risk to zero intercept have clearly resulted in the overestimation of risk, as was the case with vinyl acetate (7). It suggests that, in the low-dose range, a mechanism may be operative that prevents the formation of DNA damage, for instance DNA adducts, or translation of DNA adducts to mutations. Other relevant effects which promote tumour formation are the activation of oncogenes/inactivation of tumour suppressor genes as a consequence of DNA damage and the formation of preneoplastic lesions as a consequence of activated oncogenes (7). In principle, *in vitro* studies have already shown that that practical thresholds may apply for specific protective mechanisms such as metabolic inactivation. However, different tissues may differ in metabolic inactivation of the same compound (7-9).

Improvements in carcinogenicity testing in experimental animals and structure-activity correlations have provided new insights for the prediction of carcinogenic potential (10). There is a growing recognition that non-tumour data, such as information on metabolism, formation of DNA adducts, various other types of DNA damage, pharmacokinetic models, and the information on the mode of action can be important in elucidating the carcinogenic effect in the low concentration range. A review of non-tumour data for 1,3-butadiene, vinylchloride and benzene and general guidelines for their use are given in detail in *Albertini et al.* (10).

There is more uncertainty with other chemicals for which LNT extrapolation may be used as a default procedure. Precautionary considerations will in their case mostly lead to a conservative approach to linear low-dose extrapolation.

There is an almost general agreement that genotoxic and non-genotoxic chemicals should be distinguished when assessing cancer risk to humans. Non-genotoxic carcinogens (hormones, tumour promoters, or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin [TCDD]) are characterised by a "conventional" dose-response, which makes it possible to derive the no-observed-adverse-effect-level (NOAEL). Including an uncertainty (or safety) factor makes it possible to derive permissible exposure levels at which no relevant human cancer risk is anticipated.

Risk assessment

Carcinogens are basically classified in a two-step hazard identification procedure, followed by risk characterization where hazard estimates are calculated to lifetime hazard after lifelong exposure to a virtual dose (Figure 1). The result describes a lifetime cancer risk related to daily intake by food, water or air, and is presented as cancer cases per 100.000 people. It is important to note that these data are related to a virtual scenario and that these cases are not to be expected in reality.

It is important to know how the tumourigenic dose is estimated to characterise the risk. The US Environmental Protection Agency (EPA) has proposed to use LED_{10} as the reference point. LED_{10} is the lower 95 % confidence limit for the dose which induces tumours in 10 % of exposed animals in experimental models (11-13).

As a descriptor of carcinogenic potency, *Dybing et al.* (12) have introduced the "T25 concept". As a recent example, they used this concept to assess the risk of acrylamide in food (13). Generally, the T25 dose is defined as the dose yielding a 25 % cancer incidence in an animal experiment (12). Again, the T25 method assesses the risk at low exposure, assuming proportionality through linear extrapolation (T25/linear). The T25/linear and other extrapolation methods based on metrics such as LED_{10} assume

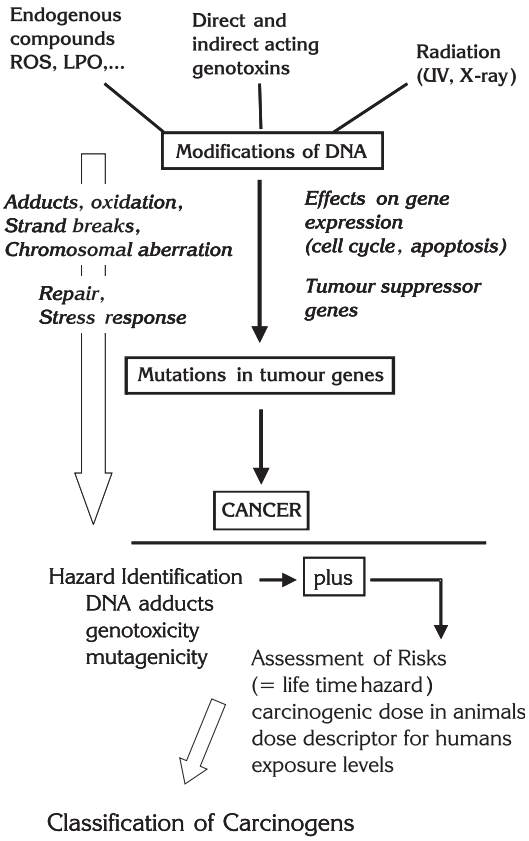

Figure 1 *The existing system of classification of genotoxic carcinogens through hazard identification and risk assessment*

linearity which may be invalid. Any risk calculated using the T25/linear method would provide a precise risk figure similar to the output obtained from the linearised multistage method (LMS) formerly used by the US EPA. However, uncertainties such as the false assumption of precision and non-linearity in the dose-response curve for tumour induction raised concerns against the use of T25/linear method for predicting human cancer risk (11).

NEW VIEWS ON THE MECHANISM OF GENOTOXIC CARCINOGENS

Insights into the mechanisms of a whole array of different genotoxic carcinogens have raised questions about the strict application of linear non-threshold (LNT) extrapolation models.

Compounds with an aneugenic but not mutagenic effect may be characterised as carcinogenic only at high, toxic doses (14). Effects such as aneuploidy, chromosome loss and non-disjunction have received particular attention (15-17). There is a wide consensus that dose-response thresholds should be defined for non-DNA reactive genotoxins such as aneugens (18, 19).

It is being discussed whether clastogenic mechanisms have a dose-effect threshold, as discussed for toposisomerase II poisons (20) or mechanisms based on reactive oxygen (21, 22). Yet, the general inclusion of clastogens is viewed critically by a number of authors (23).

Non-DNA-reactive genotoxins, such as topoisomerase inhibitors (20, 24) or the inhibitors of the spindle apparatus and associated motor proteins (16, 17, 25, 26), are considered to have "practical" thresholds and relevant arguments have been put forward in favour of this argument (15, 27). Colcemid and vinblastine induce aneuploidy by modifying the number of spindle fibres which regulate the segregation of chromosomes during mitosis and meiosis. It is expected that polyploidy or aneuploidy are induced only if most fibres are damaged, because of their redundancy in dividing cells. This effect helps to understand that a threshold was experimentally established.

Genotoxicity, especially when of local nature, may only be relevant under conditions of sustained local tissue damage and the associated increased cell proliferation. Cases in point are formaldehyde (28-30) and vinyl acetate (7, 31). Defining practical thresholds and health-based exposure limits for these two compounds may prove justified.

High doses of reactive oxygen species (ROS) or ROS promotors are clearly toxic. ROS are involved in many forms of tissue damage such as ischemia-reperfusion, atherosclerosis, radiation injury, aging and carcinogenesis (22, 32). Generally, "oxidative stress" is an important mechanism of indirect genotoxicity that is triggered by exposure to exogenous factors such as UV, ionising radiation, anoxia and hyperoxia. Other pathways are mediated by chemicals producing reactive oxygen species (33, 34). Paraquat and certain oxidants (potassium bromate, hydrogen peroxide) are the classical examples in this respect. Other exogenous sources of ROS are tobacco smoke, fatty acids, transition metals, ethanol, redox cycling compounds or physical irradiation by multiple sources. ROS interact with critical molecules within cells and with intracellular signalling, leading to cell death, mutagenesis and toxicities associated with lipid peroxidation. Increased oxidative stress and excessive ROS production cause damage to DNA modifying the

base and altering DNA strands, and can contribute to cancer. This is evident from many studies using sensitive methods for the detection for oxidative DNA damage (35).

Presently it is difficult to define the precise role of ROS-induced DNA damage in carcinogenesis and how genetic and epigenetic events induced by ROS affect cell transformation and progression of malignancies. Effects observed under oxidative stress (i.e. increased oxidative DNA damage) are inconclusive due to pronounced epigenetic effects of ROS on signal transduction and gene expression (36). Many aspects have been elucidated so far, indicating that at low levels of ROS adaptive responses are active on the side of repair and antioxidative defence, and this strengthens non-linear dose-response relationships between low and high levels of ROS (Figure 2).

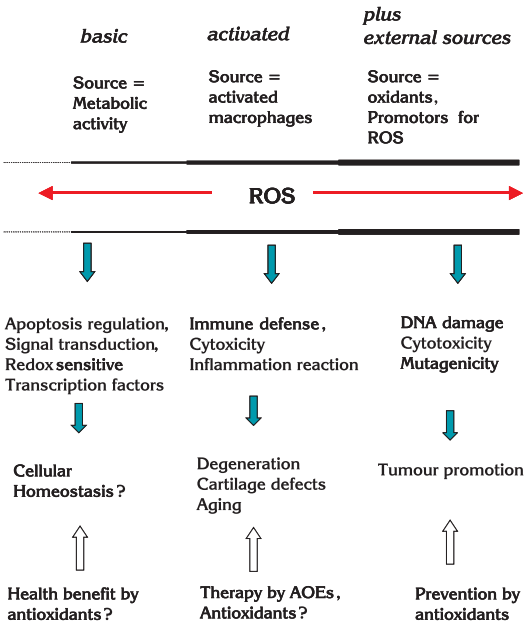

Figure 2 *Aspects of non-linear dose-response relationships between low and high levels of reactive oxygen species (ROS)*

In general, the idea that ROS-mediated processes of carcinogenesis should have at least practical thresholds is receiving more and more support from the scientific community (22).

ENDOGENOUS PROCESSES

Antioxidative defense against oxidative stress

High internal doses of ROS or high levels of ROS stimuli are clearly genotoxic, and in this context the effect of antioxidants is primarily beneficial. Regarding the role of antioxidants in cancer prevention strategies, numerous epidemiological studies support a protective role of dietary antioxidants in cancer prevention (35). On the other hand, epidemiological studies designed as intervention studies in high-risk subjects also indicate a tumour-promoting effect of beta-carotene supplements (37). This may be due to the fact that dietary antioxidants protect cancer cells from oxidative stress-induced suicide and thereby accelerate cancer progression.

It is now apparent that the balance between oxidants and antioxidants is very complex, and includes the role of ROS in a set of signalling pathways (33, 38, 39). For instance, ROS mediate apoptosis and affect NFkB, AP-1 and other transcription factors. Many other signal transduction pathways have shown sensitivity to ROS. *Allen and Tresini* (38) point out that the consequences of oxidant generation in cells are not limited to harmful effects, and *Martin and Barrett* (39) refer to ROS as "double-edged swords" in cellular processes, pointing out that low-dose effects can differ from high-dose effects. While the impact of ROS on physiologically relevant pathways in phagocytes is well known and crucial; low-dose effects of ROS on the cellular homeostasis are much less clear. It is therefore important to understand the impact of ROS on the cellular functions in order to elucidate potential harmful effects if the balance is counteracted by antioxidants.

Hyperbaric oxygen and hyperoxia are the known factors of DNA damage *in vivo* and *in vitro* (34). On the other hand, hyperbaric oxygen therapy in humans seems to be mutagenic and clastogenic *in vitro*, but not *in vivo*. Repeated exposure to hyperbaric oxygen leads to an adaptive response, apparently in terms of increased DNA repair activity, compensating for the mutagenicity of oxidative DNA lesions. Moreover, it must be taken into account that genetic polymorphisms exist at the levels of xenobiotic metabolising enzymes, enzymes maintaining a redox balance in cells and enzymes involved in DNA repair (40, 41).

Endogenous oxidative DNA damage

Reactive oxygen species are formed in all aerobic organisms as unavoidable by-products of cellular oxygen metabolism. Endogenous sources of ROS produce significant DNA damage either through direct interaction with DNA or through reactive

intermediates from lipid peroxidation that couple to DNA bases. Endogenous ROS cause detectable background levels of DNA damage, namely in the form of oxidised bases (e.g. 8-oxoGua), apurinic (AP) sites, and strand breaks. Oxygen radicals also attack other cellular components such as lipids to generate reactive intermediates that couple to DNA and give rise to exocyclic etheno- and propane adducts (42, 43). Specifically, etheno-bridged exocyclic DNA adducts, namely 1,N^6-ethenodeoxyguanosine and 3,N^4-ethenodeoxycytidine, have been demonstrated at low and variable background levels in hepatic DNA of untreated rodents and in humans (44). Since reactive oxygen species are generally genotoxic, the question also arises whether chemicals that increase ROS production will add to an endogenously produced background level of DNA lesions or whether there are mechanisms which may produce non-linear dose-effect relationships.

Endogenous DNA lesions are genotoxic and mutagenic, and affect oncogenes and tumor suppressor genes. Thus, it is likely that oxidative DNA damage by endogenous sources is an important factor in the aetiology of human cancer.

DNA repair

DNA repair is a crucial factor in maintaining a low and steady level of DNA damage. The level of chromosome breaks in lymphocytes indicates an association between poor repair and cancer risk (45). DNA repair is implicated in processes that promote human cancer, and deeper insights into these processes are important for advancing cancer aetiology, prevention and therapy (41). A reduced DNA repair activity for the oxidative lesion of 8-oxoguanine DNA N-glycosylase was reported in lung cancer patients (46). This particular DNA repair activity was not different between smokers and non-smokers, but other factors from nutrients to life style and occupational exposure might well be involved.

A surprisingly low increase in oxidative DNA base modifications in repair-deficient knockout mice was associated with a substantial increase in mutation frequency in the liver. However, these effects were too small to increase spontaneous cancer incidence in knockout animals (47). This limited effect seems to be due to a back-up repair system for oxidative DNA damage. Although oxidative DNA lesions are an important event in the initiation of cancer cells, data also show that this alone may not be sufficient to cause cancer (36).

Other endogenous DNA adducts

The existence of "endogenous" DNA adducts has generally been accepted (48). The discussion first focused on so-called "I-compounds" ("indigenous compounds"), which are detected by the method of "32P-postlabelling" of DNA adducts (49). Physiological background levels differ between tissues and depend on sex, age and nutrition. Typically, one adduct per one million DNA bases is reported (48). These adducts will be associated with mutational consequences upon cell replication (50). In addition to their endogenous origin, these DNA adducts are typical products of the hepatic chemical carcinogens vinyl chloride and vinyl carbamate (44, 51, 52). Thus, a specific background of promutagenic DNA lesions is thought to arise from endogenous lipid peroxidation products (44). These DNA lesions are genotoxic, and they induce mutations that are common in mutated oncogenes and tumour suppressor genes. There is an argument that endogenous oxidative DNA damage could be an important factor in the aetiology of human cancers (36, 53).

Isoprene is a chemically defined endogenous carcinogen which, in physiologically activated form, serves to generate isoprenoids such as cholesterol, steroids, bile acids, and the side chain of K vitamins (54). Isoprene is metabolised into a di-epoxide (2-methyl-2,2'-bi-oxirane) with mutagenic and genotoxic properties. In long-term bioassays on mice, isoprene was clearly carcinogenic, with a potency of about 1/10 of 1,3-butadiene (55). The quantitative role of another chemically defined endogenous carcinogen, ethylene oxide, has been investigated more thoroughly (56, 57). Ethylene oxide is derived from endogenous ethylene and is therefore a natural metabolite (52, 58). Possible regulatory implications of the presence of this specific endogenous carcinogen for the regulation of its presence in consumer products have been discussed in literature (58, 59).

TYPES OF THRESHOLDS

The proposition to differentiate between types of thresholds is based on the idea that a chemical can not produce genotoxic effect at very low or immeasurable target concentrations (60). Basically, non-genotoxic carcinogens have been connected

with a "real" (61) or "perfect" (7) threshold. So-called "apparent" thresholds have been connected with rapid degradation (toxicokinetics) of the chemical or with other or additional factors that limit target exposure (61). Definitions were later proposed for "absolute", "real or biological", "apparent" and "statistical" thresholds (61). A "statistical" threshold is attributed to mitotic spindle poisons. Similarly, *Hengstler et al.* (7) distinguish between "perfect" and "practical" thresholds, depending on the type of mechanism (Figure 3). *Streffer et al.* (62) distinguish four basic types/groups of chemical carcinogens:

- *Non-threshold genotoxic carcinogens*; the LNT model appears appropriate for the low-dose risk assessment. Regulations may be based on the ALARA principle ("as low as reasonably achievable"), technical feasibility, and other socio-political considerations.
- *Genotoxic carcinogens for which the existence of a threshold cannot be sufficiently supported*; in these cases the LNT model is used as a default assumption based on the precautionary principle.
- *Genotoxic carcinogens for which a "practical" threshold is supported by studies on mechanisms and/or toxicokinetics*; health-based exposure limits may be based on an established NOAEL.
- *Non-genotoxic carcinogens and non DNA-reactive carcinogens*; a "perfect" threshold for these compounds is associated with a NOAEL, and health-based exposure limits are to be derived.

Within this scheme, the distinctions between the groups B and C/D are of fundamental importance. These represent the most relevant areas of discussion and are also the major points of potential discrepancy in opinions. Key arguments referring to the chemicals acrylonitrile (group B), acrylamide (group B), formaldehyde (group C), vinyl acetate (group C) and trichloroethylene have been addressed in international discussions (7, 29). In the case of acrylamide, *Dybing and Sanner* (13) recently seconded the use of linear risk extrapolation as default, and it was noted that this procedure was "highly conservative".

CONCLUSION

In general, it is now becoming evident that a diversity of methods of carcinogenic risk extrapolation to low doses must be applied, depending on the mode of action. There is a wide array of current scientific opinions ranging from the restriction of threshold models for non-genotoxic carcinogens (1) to biological thresholds for both DNA- and non-DNA targeting chemicals (10, 63). The International Programme on Chemical Safety (IPCS) of the WHO has recently convened a Working Group on the "Harmonisation of Approaches to the Assessment of Risk from Exposure to Chemicals" to discuss a mode-of-action framework that addresses the relevance of animal carcinogenesis for humans.

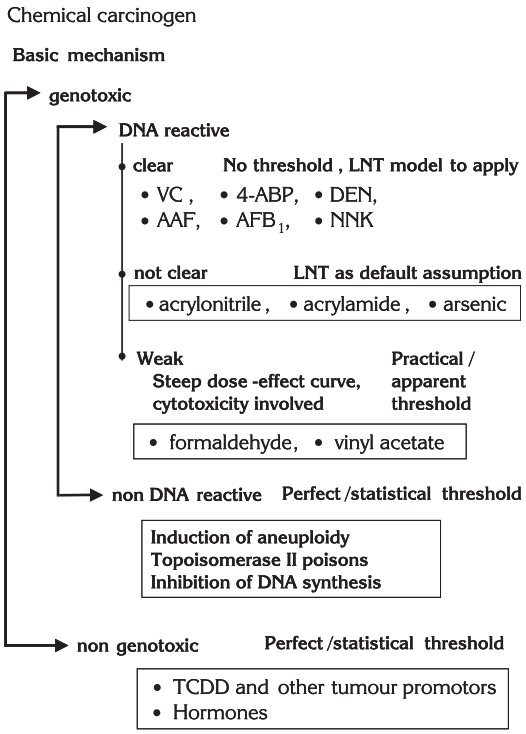

Figure 3 *Overview of non threshold and threshold models for risk assessment for genotoxic and non-genotoxic carcinogens*

REFERENCES

1. Seeley MR, Tonner-Navarro LE, Beck BD, Deskin R, Feron VJ, Johanson G, Bolt HM. Procedures of health risk assessment in Europe. Regul Toxicol Pharmacol 2001;34:153-69.
2. Adler D, Andrae U, Kreis P, Neumann HG, Thier R, Wild D. Recommendations for the categorization of germ cell mutagens. Int Arch Occup Environ Hlth 2000;73:428-32.
3. Bolt HM, Gelbke HP, Kimmerle G, Laib RJ, Neumann HG, Norpoth GH, Pott F, Steinhoff D, Wardenbach P.

Stoffe mit begründetem Verdacht auf krebserzeugendes Potential (Abschnitt III, Gruppe B der MAK-Werte-Liste): Probleme und Lösungsmöglichkeiten. Arbeitsmed Sozialmed Präventivmed 1988;23:139-44.
4. Neumann HG, Vamvakas S, Thielmann HW, Gelbke HP, Filser JG, Reuter U, Greim H, Kappus H, Norpoth KH, Wardenbach P, Wichmann H E. Changes in the classification of carcinogenic chemicals in the work area. Int Arch Occup Environ Hlth 1998;71:566-74.
5. Commission of the European Communities. Legislation on Dangerous Substances Classification and Labelling in the European Communities. Volume I. Luxembourg: Office for Official Publications of the European Communities; 2002.
6. Henschler D. Development of occupational limits in Europe. Ann Am Conf Ind Hyg 1985;12:37-40.
7. Hengstler JG, Bogdanffy MS, Bolt HM, Oesch F. Challenging dogma – Thresholds for genotoxic carcinogens? The case of vinyl acetate. Annu Rev Pharmacol Toxicol 2003;43:485-520.
8. Arand M, Herrero Plana ME, Hengstler JG, Lohmann M, Cronin A, Oesch F. Detoxification strategy of epoxide hydrolase the basis for a threshold in chemical carcinogenesis. EXCLI J 2003;2:22-30.
9. Oesch F, Herrero ME, Hengstler JG, Lohmann M, Arand M. Metabolic detoxification: Implications for thresholds. Toxicol Pathol 2000;28:382-7.
10. Albertini R, Clewell H, Himmelstein MW, Morinello E, Olin S, Preston J, Scarano L, Smith MT, Swenberg J, Tice R, Tavis C. The use of non-tumor data in cancer risk assessment: reflections on butadiene, vinyl chloride and benzene. Regul Toxicol Pharmacol 2003;37:105-32.
11. Roberts RA, Crump KS, Lutz WK, Wiegand HJ, Williams GM, Harrison PTC, Purchase IFH. Scientific analysis of the proposed uses of the T25 dose descriptor in chemical carcinogen regulation. Arch Toxicol 2001;75:507-512.
12. Dybing E, Sanner T, Roelfsema H, Kroese D, Tennant RW. T25 a simplified carcinogenic potency index: description of the system and study of correlations between carcinogenic potency and species/site specificity and mutagenicity. Pharmacol Toxicol 1997;80:272-9.
13. Dybing E, Sanner T. Risk assessment of acrylamide in foods. Toxicol Sci 2003;75:7-15.
14. Schoeny R. Use of genetic toxicology data in US EPA risk assessment: The mercury study report as an example. Environ Health Perspect 1996;104 Suppl 3:663-673.
15. Parry JM, Jenkins GJS, Haddad F, Bourner R, Parry EM. In vitro and in vivo extrapolations of genotoxin exposures: consideration of factors which influence dose-response thresholds. Mutat Res 2000;464:53-63.
16. Decordier I, Dillen L, Cundari E, Kirsch-Volders M. Elimination of micronucleated cells by apaptosis after treatment with inhibitors of microtubules. Mutagenesis 2002;17:337-44.
17. Kirsch-Volders M, Vanhauwaert A, Eichenlaub-Ritter U, Decordier I. Indirect mechanisms of genotoxicity. Toxicol Lett 2003;140/141:63-74.
18. Parry JM. Reflections on the implications of thresholds of mutagenic activity for the labelling of chemicals by the European Union. Mutat Res 2000;464:155-8.
19. Sarrif AM, Aardema MJ, Albertini S, Arni P, Henderson LM, Kirsch-Volders M, Vrijhof H. General Introduction. ECETOC-EEMS symposium on dose-response and threshold-mediated mechanisms in mutagenesis; 7 Sep 1998; Salzburg, Austria. Mutat Res 2000;464:1-2.
20. Lynch A, Harvey J, Aylott M, Nicholas E, Burman M, Siddiqui A, Walker S, Rees R. Investigations into the concept of a threshold for topoisomerase inhibitor-induced clastogenicity. Mutagenesis 2003;18:345-53.
21. Speit G, Autrup H, Crebelli R, Henderson L, Kirsch-Volders M, Madle S, Parry JM, Sarriff AM, Vrijhof H. Thresholds in genetic toxicology – concluding remarks. Mutat Res 2000;464:149-53.
22. Pratt IS, Barron T. Regulatory recognition of indirect genotoxicity mechanisms in the European Union. Toxicol Lett 2003;140/141:53-62.
23. Madle S, van der Hude W, Broschinski L, Jänig GR. Threshold effects in genetic toxicity: perspective of chemicals regulation in Germany. Mutat Res 2000;464:117-21.
24. Hengstler JG, Heimerdinger CK, Schiffer IB, Gebhard S, Sagemüller J, Tanner B, Bolt HM, Oesch F. Dietary topoisomerase-II poisons: contribution of soy products to infant leukemia? EXCLI J 2002;1:8-14.
25. Thier R, Bonacker D, Stoiber T, Böhm KJ, Wang M, Unger E, Bolt HM, Degen GH. Interaction of metal salts with cytoskeletal motor protein systems. 2003; Toxicol. Lett. 140/141:75-81.
26. Bonacker D, Stoiber T, Böhm KJ, Unger E, Degen GH, Thier R, Bolt HM. Chromosomal genotoxicity of nitrobenzene and benzonitrile. Arch Toxicol 2004; 78:49-57.
27. Crebelli R. Threshold-mediated mechanisms in mutagenesis: implications in the classification and regulation of chemical mutagens. Mutat Res 2000;464:129-35.
28. Bolt HM. Experimental toxicology of formaldehyde. J Cancer Res Clin Oncol 1987;113:305-9.
29. Bolt HM. Genotoxicity - threshold or not? Introduction of cases of industrial chemicals . Toxicol Lett 2003;140/141:43-51.
30. Morgan KT. A brief review of formaldehyde carcinogenesis in relation to rat nasal pathology and human risk assessment. Toxicol Pathol 1997;25:291-307.
31. Bogdanffy MS, Valentine R. Differentiating between local cytotoxicity, mitogenesis and genotoxicity in

carcinogen risk assessments: the case of vinyl acetate. Toxicol Lett 2003;140/141:83-98.
32. Kirkland D, Müller L. Interpretation of the biological relevance of genotoxicity results: the importance of thresholds. Mutat Res 2000;464:137-47.
33. Dalton TP, Shertzer HG, Puga A. Regulation of gene expression by reactive oxygen. Annu Rev Pharmacol Toxicol 1999;39:67-101.
34. Speit G, Dennog C, Radermacher P, Rothfuss A. Genotoxicity of hyperbaric oxygen. Mutat Res 2002;512:111-9.
35. Collins A. Oxidative DNA damage, antioxidants and cancer. Bioessays 1999;21:238-46.
36. Epe B. Role of endogenous oxidative DNA damage in carcinogenesis: what can we learn from repair-deficient mice? Biol Chem 2002;383:467-75.
37. Omenn G. Chemoprevention of lung cancer: the rise and demise of beta carotene. Annu Rev Public Health 1998;19:73-99.
38. Allen RG, Tresini M. Oxidative stress and gene regulation. Free Radic Biol Med 2000;28:463-99.
39. Martin KR, Barrett JC. Reactive oxygen species as double-edged swords in cellular processes: low-dose cell signaling versus high-dose toxicity. Hum Exp Toxicol 2002;21:71-5.
40. Forsberg L, de Faire U, Morgenstern R. Oxidative stress human genetic variation and disease. Arch Biochem Biophys 2001;389:84-93.
41. Caporaso N. The molecular epidemiology of oxidative damage to DNA and cancer. J Natl Cancer Inst 2003;95;1263-5.
42. Bartsch H, Nair J. Ultrasensitive and specific detection methods for exocyclic DNA adducts: markers for lipid peroxidaton and oxidative stress. Toxicology 2000;153:105-14.
43. Marnett LJ. Oxyradicals and DNA damage. Carcinogenesis 2000;21:361-70.
44. Nair J, Barbin A, Guichard Y, Bartsch H. 1-N6-Ethenodeoxyadenosine and 3-N4-ethenodeoxycytidine in liver DNA from humans and untreated rodents detected by immunoaffinity/32P-postlabelling. Carcinogenesis 1995;16:613-7.
45. Collins A, Harrington V. Repair of oxidative DNA damage: assessing its contribution to cancer prevention. Mutagenesis 2002;17:489-93.
46. Paz-Elizur T, Krypsky M, Blumenstein S, Elinger D, Schlechtman E, Livneh Z. DNA repair activity for oxidative DNA damage and risk of lung cancer. J Natl Cancer Inst 2003;95:1312-9.
47. Klungland A, Rosewell I, Hollenbach S, Larsen E, Daly G, Epe B, Seeberg E, Lindahl T, Barnes DE. Accumulation of premutagenic DNA lesions in mice defective in removal of oxidative base damage. Proc Natl Acad Sci 1999;96:13300-5.
48. Nath RG, Randerath K, Donghui L, Chung FL. Endogenous production of DNA adducts. Regul Toxicol Pharmacol 1996;23:22-8.
49. Randerath K, Putnam KL, Osterburg HH, Johnson SA, Morgan DG, Finch CE. Age-dependent increase of DNA adducts (I-compounds) in human and rat brain DNA. Mutat Res 1993;295:11-8.
50. Hang B, Medina M, Fraenkel-Conrat H, Singer B. A 55-kDa protein isolated from human cells shows DNA glycosylase activity toward 3 N4-ethenocytidine and the G/T mismatch. Proc Natl Acad Sci USA 1998;95:13561-6.
51. Laib RJ, Bolt HM. Trans-membrane-alkylation A new method for studying irreversible binding of reactive metabolites to nucleic acids. Biochem Pharmacol 1980;29:449-52.
52. Swenberg JA, Ham A, Koc H, Morinello E, Ranasinghe A, Tretyakova N, Upton PB, Wu KY. DNA adducts: effects of low exposure to ethylene oxide vinyl chloride and butadiene. Mutat Res 2000;464:77-86.
53. Marnett LJ, Riggins JN, West JD. Endogenous generation of reactive oxidants and electrophiles and their reactions with DNA and protein. J Clin Invest 2003;111:583-93.
54. Peter H, Wiegand HJ, Bolt HM, Greim H, Walter G, Berg M, Filser JG. Pharmacokinetics of isoprene in mice and rats. Toxicol Lett 1987;36:9-14.
55. Placke ME, Griffis L, Bird M, Bus M, Persing RL, Cox LA. Chronic inhalation oncogenicity study of isoprene in B6C3F1 mice. Toxicology 1996;110:253-62.
56. Bolt HM. Quantification of endogenous carcinogens. The ethylene oxide paradox Biochem Pharmacol 1996;52:1-5.
57. Thier R, Bolt HM. Carcinogenicity and genotoxicity of ethylene oxide: new aspects and recent advances. Crit Rev Toxicol 2000;30:595-608.
58. Filser JG, Kreuzer PE, Greim H, Bolt HM. New scientific arguments for regulation of ethylene oxide residues in skin-care products. Arch Toxicol 1994;68:401-5.
59. Bolt HM. The carcinogenic risk of ethene (ethylene). Toxicol Pathol 1998;26:454-6.
60. Seiler JP. Apparent and real threshold: a study on two mutagens In: Scott D, Bridges BA, Sobels FH, editors. Progress in genetic toxicology. Amsterdam, The Netherlands: Elsevier / North Holland Biomedical Press; 1977. p. 233-8.
61. Kirsch-Volders M, Aardema M, Elhajouji A. Concept of threshold in mutagenesis and carcinogenesis. Mutat Res 2000;464:3-11
62. Streffer C, Bolt HM, Fillesdal D, Hall P, Hengstler JG, Jacob P, Oughton D, Priess K, Rehbinder E, Swaton E. Environmental standards - dose-effect relations in the low dose range and risk evaluation. Berlin - Heidelberg - New York: Springer Verlag; 2004.
63. Sofuni T, Hayashi M, Nohmi T, Matsuoka A, Yamada M, Kamata E. Semi-quantitative evaluation of genotoxic activity of chemical substances and evidence for a biological threshold of genotoxic activity. Mutat Res 2000;464:97-104.

Sažetak

NOVI ASPEKTI U KLASIFIKACIJI KANCEROGENA

U postojećem sistemu klasifikacije kancerogenih tvari utvrđena je razlika između genotoksičnih i negenotoksičnih kemikalija. Za negenotoksične kemikallije mogu se izvesti pretpostavljeni stupnjevi izlaganja kod kojih ne postoji značajan rizik od pojave raka kod ljudi. Za genotoksične kancerogene mogući su na primjer inducirani kromosomski efekti bez početka procesa mutageneze, dok genotoksični toksini koji se ne vežu za DNA-molekulu, a djeluju na topoizomere ili diobeno vreteno ili su aneugeni, izazivaju kancerogene efekte jedino u visokim, toksičnim dozama. Za specifične mehanizme klastrogenog djelovanja i procesa kancerogeneze koji se baziraju na reaktivnom kisiku postoji prag početka procesa. Kako su vrste kemikalija reaktivne na kisik (ROS) u načelu genotoksične, pojavljuju se pitanja da li kemikalije koje povećavaju produkciju ROS-vrsta treba pridodati endogenim kancerogenima pozadinskog stupnja koji uzrokuju nelinearni odnos doze i učinka. Uzimajući u obzir rasprave o prisutnosti endogenih kancerogena, sada postaje jasno da se kancerogeni rizik od niskih doza mora uzeti u obzir sukladno načinu njihova djelovanja.

KLJUČNE RIJEČI: *endogeni kancerogeni, genotoksičnost, granične vrijednosti, kancerogeneza, kromosomski genotoksini, odnos doze i učinka, reaktivni kisik*

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