Guideline to the classification of carcinogenic compounds

guide for classifying compounds in terms of their carcinogenic properties, and for assessing their genotoxicity
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Subcommittee on the Classification of carcinogenic substances of the Dutch Expert Committee on Occupational Safety

to:

the Minister of Social Affairs and Employment

No. A10/07E, The Hague, August 26, 2010
The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is “to advise the government and Parliament on the current level of knowledge with respect to public health issues and health (services) research...” (Section 22, Health Act).

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Foreword

The Health Council of the Netherlands evaluates the carcinogenic and reproductive toxic properties of compounds to which people can be exposed in the course of their professional activities. To date, the Health Council has used a classification system based on the Dangerous Substances Directive (67/548/EEC). In 2009, the European Union replaced this Directive (67/548/EEC) with a new classification system based on the Globally Harmonised System (GHS).

As a consequence, the Subcommittee on the Classifying of Carcinogenic Substances has decided to update its own classification system as well. You have before you a new guideline for assessing the carcinogenic properties of compounds, containing a new classification system that the Subcommittee wishes to adopt.

The Hague, August 26, 2010
(signed)
Professor D. Kromhout
acting President of the Health Council of the Netherlands
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Guideline to the classification of carcinogenic compounds
Chapter 1

Introduction

1.1 Background

Carcinogenic compounds (or processes) can pose serious risks to the health of those who are exposed to them in the course of their work. Accordingly, the Working Conditions Decree includes rules on working safely with such compounds (or processes). Moreover, as a general rule all carcinogenic compounds should be replaced by substances with less hazardous properties. To clarify the issue of which compounds should be regarded as carcinogenic, the Ministry of Social Affairs and Employment (SZW) maintains a list of carcinogens.

At the request of the Minister of Social Affairs and Employment, the Health Council evaluates the carcinogenic properties of compounds in terms of the current level of knowledge. This evaluation is based on the European Union’s Dangerous Substances Directive (67/548/EEC). This directive, which was used until 2009, classifies carcinogens into three different categories. The Minister of Social Affairs and Employment then places compounds assessed by the Health Council as “The compound is known to be carcinogenic to man” or “The compound should be regarded as carcinogenic to man” on the above list of carcinogenic compounds. Compounds that have been classified by the European Union as Category 1 or 2 compounds are also included in this list.

In response to requests by the Minister of Social Affairs, the Health Council also assesses the mechanism or mechanisms that may account for the carcino-
genicity of a given compound. On the basis of this information, a decision can be made concerning the feasibility of deriving a safe (health-based) occupational exposure limit. If that is not possible, then the risks associated with the carcinogen in question are identified by calculating reference values (Health-based calculated occupational cancer risk values or HBC-OCRVs). The Minister of Social Affairs and Employment then uses these cancer risk values as the basis for setting a statutory limit value*.

In 2009, the European Union replaced the Dangerous Substances Directive (67/548/EEC) with a new classification system** based on the Globally Harmonised System. It would therefore seem logical for the Health Council to adopt this new system as the basis for assessing compounds’ carcinogenic properties as well. As the Minister of Social Affairs and Employment has also asked the Health Council for an opinion concerning the mechanism of action, the Council will have to refine or expand various elements of the new classification system.

1.2 Committee

This guideline was drawn up by the Subcommittee on the Classifying of Carcinogenic Substances, of the Dutch Expert Committee on Occupational Safety, hereafter referred to as “the Committee”. Details of the members of this Committee are given in Annex A of this advisory report.

1.3 Structure of the guideline

In chapter 2 of this guideline, the Committee summarises the classification system used by the Health Council until 2010. In chapter 3, the Committee briefly describes the European Union’s new classification system. Chapter 4 deals with developments in the area of carcinogenic mechanisms of action. In Chapter 5, the recommendations from the previous chapters are integrated into a modified classification system using standard formulations. Finally, chapter 6 gives details of the Committee’s approach with regard to the use of the new classification system.

* After the Economic and Social Council has been consulted concerning the technical and economic feasibility of such a step.

** The new EU regulation (EC) No 1272/2008 on classification, labelling and packaging of chemical substances and mixtures, the so called CLP Regulation entered into force on 20 January 2009.
Chapter 2

Dutch classification system until 2010

2.1 The classification system (based on Directive 93/21/EEC)

Throughout the world, compounds are classified into categories on the basis of their potentially carcinogenic properties. Take, for instance, the classification systems established by the European Union (EU, see Annex B), the International Agency for Research on Cancer (IARC, see Annex C) and the Committee for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK Kommission) of the German Research Foundation (Deutsche Forschungsgemeinschaft, see Annex D). These classification systems are often used for a range of different purposes. In the Netherlands, classifying compounds is carried out for the purpose of compiling a list of carcinogenic compounds*. The criteria used by the Health Council until 2010 were derived by a predecessor of the current Dutch Expert Committee on Occupational Safety from the guidelines drawn up by the European Union at that time**. Partly as a result of this, the Dutch classification system was comparable with that of the European Union (with the exception of one component, see section 2.2), aside from the fact that the Health Council converted the European categories into a classification system based on standard phrases. The advantage of using standard phrases rather

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* See Chapter 1.
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The subcommittee expresses its conclusions in the form of standard phrases:

<table>
<thead>
<tr>
<th>Judgment of the subcommittee</th>
<th>Comparable with EU class</th>
</tr>
</thead>
<tbody>
<tr>
<td>• <strong>This compound is known to be carcinogenic to humans.</strong></td>
<td></td>
</tr>
<tr>
<td>• It is stochastic or non-stochastic genotoxic.</td>
<td></td>
</tr>
<tr>
<td>• It is non-genotoxic.</td>
<td></td>
</tr>
<tr>
<td>• Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether it is genotoxic.</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>• <strong>This compound should be regarded as carcinogenic to humans.</strong></td>
<td></td>
</tr>
<tr>
<td>• It is stochastic or non-stochastic genotoxic.</td>
<td></td>
</tr>
<tr>
<td>• It is non-genotoxic.</td>
<td></td>
</tr>
<tr>
<td>• Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether it is genotoxic.</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
</tr>
<tr>
<td>• <strong>This compound is a suspected human carcinogen.</strong></td>
<td></td>
</tr>
<tr>
<td>• This compound has been extensively investigated. Although there is insufficient evidence for a carcinogenic effect to warrant a classification as ‘known to be carcinogenic to humans’ or as ‘should be regarded as carcinogenic to humans’, they indicate that there is cause for concern.</td>
<td></td>
</tr>
<tr>
<td>• This compound has been insufficiently investigated. While the available data do not warrant a classification as ‘known to be carcinogenic to humans’ or as ‘should be regarded as carcinogenic to humans’, they indicate that there is a cause for concern.</td>
<td></td>
</tr>
<tr>
<td>(A)</td>
<td></td>
</tr>
<tr>
<td>(B)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
</tr>
<tr>
<td>• <strong>This compound cannot be classified.</strong></td>
<td></td>
</tr>
<tr>
<td>4</td>
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</table>

than a numerical category was that these give a direct indication of a compound’s carcinogenic properties. The four categories used by the Health Council until 2010 were expressed by the following italicised standard phrases. In the case of compounds in the first two categories, an indication was also given of whether the substance in question was a genotoxic compound, which refers to the mechanism by which cancer can develop (see Section 2.3). For compounds in the third category, the adequacy of investigations into the compound in question was indicated by adding one of the standard phrases listed under this category.

### 2.2 Criteria for the Health Council’s classification system

The criteria for classification into the various categories established at that time was based on an assessment of the available evidence: to what extent could it be demonstrated (using reliable and accepted methods) that the compound in question was capable of causing tumours in exposed humans or animals. The criteria for the four categories are explained in more detail below. Another requirement of the evaluations was that the tumours involved should be malignant in nature.
Benign tumours were only included in the evaluations if they were likely to develop into malignant tumours. In addition, the evaluations also took account of information on the carcinogenic mechanisms of action, particularly genotoxicity*. This was because this information was used to determine whether it was possible to derive a health-based recommended exposure limit for the carcinogen in question (see Section 2.3).

Finally, the assessment was based on peer reviewed scientific publications or other publicly available information.

- **The compound is known to be carcinogenic to man**

The compound was classified as known to be carcinogenic to man if there was sufficient evidence for a causal relation between human exposure to the development of cancer. There was also likely to be a causal relation between the level of human exposure and the effect.

- **The compound should be regarded as carcinogenic to humans**

Compounds were presumed to be carcinogenic to man if there was sufficient evidence for a strong presumption that exposing humans to such substances may lead to the development of cancer. There was usually little epidemiological data, but long-term animal experiments and other relevant information suggested that the compound might cause cancer in humans.

The Committee classified a compound in this category if:

- positive results, *i.e.* a marked increase in the number of malignant tumours, were found in at least two animal species, or
- a positive result was found in a single animal species, supplemented by supporting evidence, such as:
  - a positive result in terms of genotoxicity
  - evidence of carcinogenicity or genotoxicity from metabolic or biochemical studies
  - induction of benign tumours in a second animal species
  - correspondence with chemically similar compounds that have proven to be carcinogenic (EU Category 1 or 2**).

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* Genotoxicity: the ability to irreversibly change information stored in DNA.
** In accordance with the EU classification system (based on Directive 93/21/EEC).
• The compound is a suspected human carcinogen

A compound was classified as a suspected human carcinogen if experimental animal data suggested that exposure might cause cancer. However, there was insufficient information/evidence for the compound to be placed in a higher category. This category had two subgroups:

• The compound has been well investigated. Additional experiments would not be expected to yield further relevant information with respect to classification.
• The compound has been insufficiently investigated. The available data are inadequate, the classification was provisional, further experiments were needed before a final decision could be taken.

This group included compounds, for example, which:

• in animals, increased tumour incidence in organs/tissues associated with a high “spontaneous tumour incidence”
• only increased tumour incidence in animals after exposure via routes that are less relevant for humans (e.g. intraperitoneal or intravenous), and not via exposure routes that are particularly relevant to humans (by inhalation, through the skin, or via the mouth)
• induced tumours in animals by means of a species-specific mechanism of action that was not relevant to humans
• caused tumours in one animal species, although there was no information on the genotoxicity of the compound in question.

• The compound cannot be classified

A compound was not classified by the Committee if a) insufficient information was available on its carcinogenicity, or b) sufficient data was available but it indicated that carcinogenicity in man was unlikely (the mechanism of action underpinning tumour development in some animal species was not relevant to man, for example, and no other data was available). Some more sensitive animal species naturally develop tumours more easily, i.e. they have a high rate of spontaneous tumour development. Nor, in general, were compounds classified that boost the incidence of such “spontaneous” tumours, but which caused no increase in other tumours in the same species (elsewhere in the body, for example).

Comparison of the classification criteria used by the Health Council and the EU

The classification criteria used by the Health Council were comparable to those used by the European Union, with the exception of one component. Where experimental animal studies found sufficient evidence of carcinogenicity, the Health Council classified the compound in question as “The compound should
be regarded as carcinogenic to humans”, which corresponded to Category 2 of the EU classification. However, if a compound had been shown to be non-genotoxic or a non-stochastic genotoxic carcinogen, then the European Union used Category 3 (similar to the Dutch classification “The compound is a suspected human carcinogen”). However, as far as the Health Council was concerned, the classification of this group of compounds remained unchanged. It did provide additional information (in the form of one of the standard phrases) on whether the compound in question was a non-genotoxic or non-stochastic genotoxic carcinogen.

2.3 Mechanism of action classified on the basis of genotoxicity data

In recent years, at the request of the Minister of Social Affairs and Employment, the Health Council has determined whether a genotoxic mechanism of action is involved in cancers caused by compounds classified as “known to be carcinogenic to man” or “should be regarded as carcinogenic to humans”. They expressed the genotoxicity of a compound using one of the following standard phrases:

- it is a genotoxic compound;
- it is a non-genotoxic compound;
- its genotoxicity has been insufficiently investigated. It is not known whether or not the compound is genotoxic.

The classification into genotoxic and non-genotoxic compounds was based on previously published advisory reports by the Health Council, including a 1996 report entitled *Beoordeling carcinogeniteit van stoffen*.12,13 The reasons for choosing this system of classification are set out below. The subsequent sections examine genotoxic and non-genotoxic carcinogens in detail.

The method of risk assessment used reflects the difference in mechanism of action. This is because the quantitative risk assessment for ‘stochastic’ genotoxic compounds involves linear extrapolation. This is used to derive Health-Based Calculated Occupational Cancer Risk Values (HBC-OCRVs). This is an exposure level (concentration in the air) that corresponds to a predefined accepted level of risk of death from cancer (set by the government). The HBC-OCRV implies that exposure (however low it may be) always carries a risk, and that there is no safe limit (threshold) below which there will be no cancer deaths whatsoever. In the case of non-stochastic genotoxic and non-genotoxic compounds, however, it is assumed that there is a safe limit (threshold). Here, the
method of risk assessment is based on deriving a threshold value of this kind, also referred to as the No-Observed-Adverse-Effect-Level. This effect level is also adjusted for various uncertainties (such as differences between experimental animal species and differences in responses between individual people), ultimately resulting in a health-based recommended occupational exposure limit (HBR-OEL). When establishing a limit value, the Minister of Social Affairs and Employment uses both the HBC-OCRV and the health HBR-OEL.

The classification by mechanism of action is based on factors such as the initiation-promotion model. This model describes cancer as a two-phase process. The first phase is initiation, in which in normal cells mutations are formed in their DNA at sites that are associated with carcinogenesis. This is followed by the promotion phase, in which the division of mutated cells is stimulated, resulting in a detectable malignant tumour. Often, a third phase – the progression phase – is also described. During this phase, for example, tumour cells develop the ability to spread throughout the surrounding tissue and the body (metastasis).

Only those compounds that are able to induce both the initiation phase and the promotion phase (complete carcinogens) are capable of independently giving rise to tumours. However, there are also compounds that are capable of initiation but not promotion, or vice versa. The former are known as initiators and the latter as promoters (co-carcinogens). Complete carcinogens and initiators are known as genotoxic carcinogens. Promoters or co-carcinogens, that are incapable of causing mutations in DNA, are referred to as non-genotoxic compounds. The term genotoxicity indicates an ability to induce potentially harmful changes to DNA. Genotoxic compounds are subdivided into carcinogens with stochastic and non-stochastic mechanisms of action (see next section).

It is also significant that many carcinogens do not acquire a genotoxic mechanism of action until they have been converted into DNA-reactive metabolites (bioactivation). In other words, the original compound is not carcinogenic, but its metabolites are. Being highly reactive, these DNA-reactive metabolites are usually quickly metabolised into innocuous breakdown products.

2.3.1 **Genotoxic carcinogens**

Carcinogens that act by a stochastic genotoxic mechanism

This group includes compounds that (either in their unchanged form or as reactive metabolites) interact directly with DNA, causing damage (adducts, single- and double-strand breaks). If this damage is not repaired quickly or adequately,
gene mutations and chromosome abnormalities can occur at sites that are associated with carcinogenesis. Some examples are benzo[a]pyrene (DNA alkylation), methyl methane sulphonate (DNA alkylation) and reactive oxygen radicals (DNA breaks).

DNA changes brought about by interactions with genotoxic carcinogens are known as “hits”. Even at the lowest possible exposure (which, in theory, could involve just a single molecule), genotoxic carcinogens can still initiate the cancer process, although the risk is very small. This line of reasoning clearly indicates that when two molecules of carcinogen are present the risk involved is twice as great. In this way, a linear relationship could be created between exposure and the risk of a hit. This is also referred to as one-hit kinetics. It is based on the assumption that the probability of effective hits is directly proportional to the level of exposure.

The concept of linearity at low exposures also involves a number of assumptions. One of these is that the occurrence of DNA damage is a stochastic process, in which the state variable is “DNA damage” or “no DNA damage”, and in which the risk or probability of this resulting from exposure to a carcinogen is determined by chance and not by the degree of “DNA damage”. Another assumption is that there is no threshold value below which a compound that causes hits can be considered inactive. In other words, there is no dose at which the risk of a relevant effect is equivalent to zero. The one-hit kinetics at low exposure means that, however low the exposure involved, there is always an elevated risk of cancer. Accordingly, safety considerations dictate that it would be best to derive a HBC-OCRV.

Moreover, DNA changes are continuously being induced by genotoxic carcinogens that are either naturally present in food or in the environment, or that are generated by normal metabolic processes and inflammatory responses in the body, such as various reactive oxygen radicals (background hits). Much of the DNA damage is corrected by efficient DNA repair enzymes. With regard to risk evaluation, the Committee previously confined itself to assessing compounds’ ability to cause DNA damage, regardless of the body’s ability to repair such damage.

Carcinogens that act by a non-stochastic genotoxic mechanism

These include compounds that do not interact directly with DNA, but which can ultimately damage DNA indirectly. Various mechanisms are involved in this process, some of which are described below.
Inhibiting DNA repair. Cells possess DNA repair mechanisms that are capable of correcting many types of damage to their DNA. Accordingly, compounds capable of inhibiting that repair mechanism (e.g. by the inactivation of DNA repair enzymes) can cause permanent DNA damage – albeit indirectly. Salts of cadmium, arsenic and nickel are examples of compounds that act in this way.

Effects on the spindle apparatus. The spindle apparatus plays a major part in cell division, by controlling the segregation of chromosomes. The spindle apparatus, which is a complex of centrosomes and microtubules, is part of the cytoskeleton. Compounds such as vincristine and vinblastine sulphate, which interact with spindle apparatus structures, can cause chromosomal aberrations.

Topoisomerase inhibitors. Topoisomerases are enzymes that change the supercoiling of double-stranded DNA, by cutting one or both of its strands. They play an essential part in DNA transcription and replication, hence also in cell division. Topoisomerases are classified into a range of different types, depending on the exact nature of their function. These enzymes can be inhibited by cytostatic drugs, such as busulfan and taxol, resulting in breaks in DNA, chromosomal aberrations and cell death (apoptosis).

These are examples of non-stochastic genotoxic carcinogens that interact with proteins involved in DNA repair, DNA replication, and chromosome segregation. As they can ultimately damage the DNA or chromosomes, this group of compounds are classified as genotoxic compounds. However, they do not function in accordance with stochastic principles, as relevant DNA damage only occurs when the carcinogen inhibits the activity of the enzymes in question, or that of other proteins, to such an extent that their capacity for repair can no longer meet the requirement. Only then can sufficient damage accumulate for its effects to become significant and visible. Hence a threshold is involved.

2.3.2 Non-genotoxic carcinogens

These carcinogens are capable of promoting various phases of the cancer process without damaging DNA, either directly or indirectly. Such compounds are known as tumour promoters. Various mechanisms contribute to cancer processes that involve non-genotoxic routes, some examples of which are described below.

Regulation of gene expression. Some processes affect gene expression without changing the DNA sequence, nevertheless these changes in expression are transmissible to daughter cells. One example of such an effect is the hyper-methylation or hypo-methylation of gene promoter sequences, i.e. the C5 position of
cytosine in a CpG sequence. Changes in DNA methylation can cause genes to be activated or turned off, which can dramatically change a cell’s behaviour. Compounds suspected of causing cancer in this way include arsenite, dichloroacetic acid and trichloroacetic acid.

Disruption of hormonal balance. Some compounds can disrupt the hormonal balance and the functions of some hormones, either by accelerating the breakdown of hormones (e.g. thyroxine in rats) or because the compounds themselves exert a powerful hormonal effect (e.g. oestrogenic compounds). This increases the risk of tumour development, especially in hormone-sensitive organs. One example is the induction of thyroid cancer by polychlorinated biphenyls (PCBs).

Inhibition of gap junctional intercellular communication. The inhibition of intercellular communication adversely affects the differentiation, proliferation and migration of cells, as well as programmed cell death (apoptosis). Some compounds can affect the expression of the numerous genes involved in intercellular communication, as well as the activity and function of the proteins involved. Such compounds include phorbol esters (12-O-tetradecanoylphorbol-13-acetate (TPA / PMA)) and fluoranthene.

Regulation of growth factors and steroid hormones. Cell proliferation, cell differentiation, and programmed cell death are regulated and controlled by a range of factors that stimulate or inhibit growth, and by cytokines. The inhibition or stimulation of such factors can promote the cancer process. Some examples are the use of steroid hormones such as oestrogen and progesterone in hormone therapy, and possibly some phytoestrogens (at high intake levels). Another example is the protein hormone insulin, which can contribute to tumour growth in breast cancer, for instance.

Immunosuppression. Compounds such as cyclosporin and purine analogues can suppress the immune system. Any weakening of the immune system can lead to the uncontrolled growth of cancer cells.

Chronic tissue damage caused by irritation and cytotoxicity. Some carcinogens induce cancer by causing chronic tissue damage in organs. An example of this is the chronic renal toxicity caused by chloroform. The body responds either with massive regeneration or with an inflammatory reaction, involving inflammatory cells such as macrophages. This is basically a normal biological reaction that ends with the recovery of the affected tissue. However, chronic exposure and toxicity may well lead to the development of cancer (from cells initiated by endogenous mediators, for example).
Accordingly, as shown above, the mechanisms underlying non-genotoxic effects are many and varied. Non-genotoxic mechanisms can promote the growth of DNA-damaged cells by stimulating cell proliferation while inhibiting the immune response to initiated cells, for example. The combined effect is to promote continued growth to form detectable tumours and even metastases. However, this tumour-growth-promoting effect does not appear until the compound reaches exposure levels at which the mechanisms that promote such growth become manifest. This does not occur at lower levels of exposure, so the mechanism of action of non-genotoxic carcinogens clearly involves a threshold.
New European classification system: implementation of the Globally Harmonized System

A new harmonised classification system was recently drawn up: *the Globally Harmonized System of Classification and Labeling of Chemicals*, or the GHS system for short. The main goal of this initiative is to achieve worldwide standardisation and harmonisation in the classification and labelling of chemical compounds (including carcinogens). The GHS system was developed under a mandate from the United Nations Conference on Environment and Development (UNCED)*.

This system was introduced into European Union regulations (see EU Directive 1272/2008) in early 2009. Annex E contains supplementary information about this system’s classification and criteria for carcinogenic compounds. It is briefly summarised below:

**Category 1: Known or presumed human carcinogens**

A substance is classified in Category 1 for carcinogenicity on the basis of epidemiological and/or animal data. A substance may be further distinguished in category 1A and 1B, based on the strength of evidence of human data (category 1A) or animal data (category 1B):

* See also www.unece.org
• Category 1A, known to have carcinogenic potential for humans, classification is largely based on human studies that establish a causal relationship between human exposure to a substance and the development of cancer evidence, or
• Category 1B, presumed to have carcinogenic potential for humans, classification is largely based on animal experiments for which there is sufficient evidence to demonstrate animal carcinogenicity.

In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans, together with limited evidence of carcinogenicity in experimental animals.

Category 2: suspected human carcinogens

The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations. Such evidence may be derived either from limited evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.
Chapter 4

Developments in the area of carcinogenic mechanisms of action

4.1 Introduction

Since the publication of the 1996 Health Council advisory report on the assessment of the carcinogenicity (and mechanisms of action) of chemicals, there have been enormous advances in our understanding of the relevant mechanisms in the areas of cell and molecular biology. However, this has yet to result in a fundamentally different approach to the risk assessment of carcinogenic compounds.

On the basis of these insights, the Committee envisions a future scenario in which it will be possible to differentiate more reliably between potential carcinogens in man on the basis of their anticipated exposure-response relationship. Moreover, it may even prove possible to define safe exposure levels for stochastic genotoxic carcinogens in man. Defence mechanisms and background effects might explain, for instance, why DNA damage and mutations caused by stochastic genotoxic compounds only become apparent at higher exposures.

4.1.1 Defence mechanisms

Various defence mechanisms have been identified at the molecular, cellular and organism level that are capable of preventing or halting the ‘genotoxic’ cancer process:

• harmful metabolites, such as oxygen radicals, are eliminated by antioxidants and radical scavengers;
• carcinogens are converted into harmless metabolites by biotransformation;
• DNA is repaired by certain enzymes;
• damage slows down cell division, allowing more time for DNA repair;
• the immune system recognises tumour cells and their precursors, and eliminates them.

Ultimately, it is the balance between exposure and defence mechanisms that determines the risk of DNA damage and the subsequent development of a tumour cell. If these defence mechanisms become overloaded, this will enhance the effects of carcinogens (or genotoxic carcinogens).

4.1.2 Background effects

Background effects can be caused by genotoxic carcinogens that are always present in people’s surroundings, for example in the environment and in food. They can also be caused by carcinogens that are formed by normal metabolic processes and by inflammatory responses in the body. One example is reactive oxygen radicals, which are known to be carcinogens with a stochastic genotoxic mechanism, another is the existence of certain “naturally occurring” DNA damage (DNA adducts) in cells.1,16 Accordingly, endogenous processes can also contribute to the risk of cancer.

Reactive oxygen species (ROS) are normally produced in large amounts by metabolic processes and by inflammatory reactions in the body. This may lead to oxidative DNA damage (oxidative stress). Ames and Gold (1991) estimated that – under steady state conditions – rat cells each contain about $1 \times 10^6$ (one million) oxidative DNA adducts, and that about $1 \times 10^5$ new oxidative DNA adducts are formed each day.1 At the cellular level, however, there is a powerful antioxidant defence system that eliminates ROS. There is also a DNA repair system, which ensures that ROS-induced damage to DNA is rapidly repaired. It is these highly efficient defence and repair systems that make it possible for man to live in an oxygen-rich environment.

Certain xenobiotic compounds that have been shown to be carcinogenic in animal studies – albeit at high exposure – are capable of producing ROS. It is suspected that this is the mechanism by which they cause cancer. The observation that there is no increase in tumours at low exposure can be explained by the fact that the amount of ROS produced by the carcinogen was negligible compared to the quantity of oxygen radicals generated by normal cellular processes. This means that there is no significant increase in the amount of ROS in the cell. At the lowest possible exposure (a single molecule) to xenobiotic carcinogens
that produce one or several ROS, one or several hits fade into insignificance compared to the number of hits caused by normal biological processes. Accordingly, there will be no increased risk of DNA damage at low exposure, which means that there is a threshold below which no significant effects are observed. Pyrocatechol and cadmium are examples of carcinogens that generate ROS.

In practice, however, it will not be easy to demonstrate the existence of such thresholds for compounds of this kind. This would involve carrying out a broad-based experimental animal study to obtain data on the status of oxidative stress at the cellular level in relation to tumour incidence as a function of exposure to the compound in question. This study is necessary to determine whether there is a causal link between that oxidative damage and carcinogenicity, and whether the existence of a threshold can be demonstrated experimentally. Where such data is unavailable, the Committee assesses genotoxic carcinogens that generate ROS as stochastic genotoxic carcinogens, given the genotoxicity of the ROS that is produced. This implies that linear extrapolation is the appropriate risk assessment methodology. However, if the exposure-response data unequivocally indicates the existence of a threshold and if it is also shown that no increase in oxidative stress occurs beneath that threshold then, according to the Committee, the cancer risk can be estimated using a threshold value.

In addition, with regard to the quantitative risk analysis of stochastic genotoxic carcinogens, it is important to determine the scope of any background effects involved. In the case of oxidative DNA damage, they would be high and might even be measurable using current technology. In other situations, that is not necessarily the case. Methyl methane sulphate, for example, induces characteristic DNA damage involving the creation of DNA adducts of the same type that occur naturally, albeit at a very low frequency. So low, in fact, that in everyday situations the natural background level is barely measurable with current technology. In situations like this, linear extrapolation is the only option.

4.2 Proposed classification by mechanism of action, based on genotoxicity

In the case of compounds that are assessed as “carcinogenic to man” or as “is regarded to be carcinogenic to man”, the Committee will continue, where possible, to indicate the exact carcinogenic mechanism (or mechanisms) of action involved. In this connection, when selecting the best risk calculation method, it is important to distinguish between a “stochastic genotoxic mechanism of action” and other mechanisms. This is because the Health Council uses the former to derive HBC-OCRVs, and the latter to derive a HBR-OELs. The Committee is
free to depart from this approach, provided that it has well-founded reasons for doing so.

Where there is doubt concerning the exact mechanism of action, the Committee takes a cautious approach and views the compound in question as a stochastic genotoxic carcinogen until such time as new data proves otherwise. In such cases, the Committee will proceed on the basis of a worst case scenario, which is that the compound in question is capable of initiating the cancer process. With this in mind, if a given compound has multiple carcinogenic mechanisms, at least one of which involves a stochastic genotoxic mechanism, then the Committee will recommend that the stochastic genotoxic mechanism be used as a basis for deriving an occupational limit value. In exceptional and specific cases, the Committee retains the option of deviating from this principle, for example, if data becomes available that indicates that other mechanisms play a dominant role.

In view of the above, the Committee will continue to use the current classification system, based on mechanism of action. This classification system is as follows:

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>In theory, base limit value on …</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotoxic</td>
<td></td>
</tr>
<tr>
<td>a Stochastic</td>
<td>HBR-OCR V</td>
</tr>
<tr>
<td>b Non-stochastic</td>
<td>HBR-OEL</td>
</tr>
<tr>
<td>Non-genotoxic</td>
<td>HBR-OEL</td>
</tr>
</tbody>
</table>

In addition, the Committee uses one of the following standard phrases:

- The compound acts by a stochastic genotoxic mechanism.
- The compound acts by a non-stochastic genotoxic mechanism.
- The compound acts by a non-genotoxic mechanism.
- Its genotoxicity has been insufficiently investigated. Therefore, the mechanism of action is not known.

### 4.3 Endpoints of carcinogenic mechanisms of action

There is a wide range of test systems for identifying a given carcinogen's mechanism (or mechanisms) of action. Annex F contains a list of measurable endpoints and of the carcinogenic mechanisms of action that may be associated with them. Those requiring detailed assessments of these endpoints and of their value in determining potential genotoxicity should refer to the literature. In practice, the results of several types of tests are needed to arrive at a decision. In addition, it is
quite likely that a given carcinogen may have several different mechanisms of action.

The Committee uses the results of these tests to determine whether a compound has a genotoxic effect, and whether this effect is stochastic or non-stochastic in nature. In this connection, it works on the assumption that compounds that damage DNA indirectly by their interaction with proteins (e.g. by inhibiting DNA repair) theoretically involve a threshold, \textit{i.e.} they have a non-stochastic mechanism of action. Compounds that do damage DNA directly are assumed to have a stochastic mechanism of action. In addition, pragmatic decisions will be taken in some cases if the available experimental data so requires.
Guideline to the classification of carcinogenic compounds
Until 2010, the Health Council of the Netherlands used a classification system based on the Dangerous Substances Directive (EU Directive 67/548/EEC). In the past, the Health Council converted these European categories into a classification system based on standard phrases. The advantage was that these standard phrases give a direct indication of a compound's carcinogenic properties (see Chapter 2). Accordingly, the Health Council prefers to retain a system based on standard phrases.

In this chapter, the Committee proposes that the new GHS and the Dutch system referred to in Chapter 2 (based on standard phrases) be combined. In this connection, the Committee refers to the developments described in Chapter 4. The criteria for classification into a GHS category (see Annex E) are less detailed than the European Union’s previous system. They leave some scope for interpretation. In addition, a classification is always based on epidemiological studies and experimental animal studies. The data on mechanisms of action serves primarily as support.

In the following table, the Health Council summarises the proposed new classification system (based on EU Directive 1272/2008) for the Netherlands using the following italicised standard phrases. In addition, for compounds in Category 1A and Category 1B, it also uses one of the standard phrases mentioned to indicate whether a (stochastic) genotoxic mechanism is involved. That is not necessary for any of the other categories.
<table>
<thead>
<tr>
<th>Category</th>
<th>Judgement of the Committee (GRGHS)</th>
<th>Comparable with EU Category</th>
</tr>
</thead>
</table>
| 1A       | The compound is known to be carcinogenic to man.  
  - It acts by a stochastic genotoxic mechanism.  
  - It acts by a non-stochastic genotoxic mechanism.  
  - It acts by a non-genotoxic mechanism.  
  - Its potential genotoxicity has been insufficiently investigated.  
  - Therefore, the mechanism of action is not known.  | 1A |
| 1B       | The compound is presumed to be carcinogenic to man.  
  - It acts by a stochastic genotoxic mechanism.  
  - It acts by a non-stochastic genotoxic mechanism.  
  - It acts by a non-genotoxic mechanism.  
  - Its potential genotoxicity has been insufficiently investigated.  
  - Therefore, the mechanism of action is not known. | 2 |
| 2        | The compound is suspected to be carcinogenic to man. | 3 |
| 3        | The available data are insufficient to evaluate the carcinogenic properties of the compound. | Not applicable |
| 4        | The compound is probably not carcinogenic to man. | Not applicable |

The Committee uses the following criteria for the various categories.

**Category 1A: The compound is known to be carcinogenic to man**

A compound is classified as “carcinogenic to man” if there is sufficient evidence from epidemiological studies to support the existence of a causal relationship between human exposure and the development of cancer in those who were exposed to the compound in question. In addition, there is likely to be a causal relationship between exposure and effect. In some cases, a compound for which there is only limited evidence from epidemiological studies to support a relationship between exposure and the development of cancer can still be placed in this category if the studies in question are complemented by sufficient evidence from animal studies to establish the existence of such a relationship.

* Criteria have been developed to assess whether an epidemiological association also implies the existence of a causal link (see Hill published in 1965).
Category 1B: The compound is presumed to be carcinogenic to man

The compound is classified as “is presumed to be carcinogenic to man” if there is sufficient evidence to suggest that human exposure results in an increased risk of cancer developing in those exposed.

Positive epidemiological data are lacking, but based on chronic animal experiments and other relevant information, it is likely that the compound causes cancer in man. A compound is considered carcinogenic to man if positive results (a marked increase in the number of malignant tumours) have been obtained for at least two experimental animal species, or for a single species in two or more independent studies. If – in addition to two positive studies – negative studies are available, the Committee may decide, in exceptional cases, to place the compound in Category 2.

Category 2: The compound is suspected to be carcinogenic to man

The compound is classified as “suspected to be carcinogenic to man” if there is evidence from experimental animal studies showing that exposure to this compound can cause cancer. However, the information is insufficient to classify the compound as “is presumed to be carcinogenic to man”. There are a number of possible reasons for a compound to be classified into this category:

• Only one, good quality experimental animal study is available. This animal study demonstrated positive results.
• Several experimental animal studies are available, all of which produced positive results. These studies, however, are of less quality which precludes classification into Category 1B.
• Good-quality experimental animal studies have been conducted, but either the results do not give a clear picture or the data are open to interpretation. This is the case if, for example:
  • in animals, increased tumour incidence in organs/tissues is associated with a high “spontaneous tumour incidence”
  • in animals, tumour incidence is increased following exposure via routes that are less relevant to the human situation (e.g. intraperitoneal or intravenous).

In exceptional cases, a positive result in a single animal species can nevertheless lead to the compound being classified as “is presumed to be carcinogenic to man” (Category 1B). This is the case when there is a substantial amount of supportive evidence, such as (a) positive genotoxicity data, (b) evidence of carcino-
genicity or genotoxicity from metabolic or biochemical studies, (c) induction of benign tumours in a second animal species, (d) structural affinity with known carcinogens (Category 1A or Category 1B).

Category 3: The available data are insufficient to evaluate the carcinogenic properties of the compound

The compound is classified into this category if there is insufficient, good quality human or experimental animal data on a compound’s carcinogenicity.

Category 4: The compound is probably not carcinogenic to man

A compound is placed in this category when there is sufficient data from both epidemiological studies and experimental animal studies to suggest that carcinogenicity in man is unlikely. A number of good epidemiological studies and experimental animal studies have been published. These studies either found no exposure-induced tumours, or the tumours (including the mechanism of action) that did develop in some animal species were not relevant to man.
6.1 Assessing data quality

A classification process generally involves four distinct steps (see Annex G). Once all of the available data has been collected (step 1), the Health Council uses a set of quality criteria to systematically assess the quality of the epidemiological studies and experimental animal studies in question (step 2). These are based on guidelines issued by the Dutch Institute for Healthcare Improvement (CBO), which set out quality criteria for the human data (Annex H.1), and on a study by Klimisch et al. (1997) which provided quality criteria for experimental animal studies (Annex H.2). Studies of sufficient quality and of good quality can then be used for the classification (step 3).

6.2 Assessment of study results

To assess the results of available studies, the Health Council defined four categories of evidence for both epidemiological studies and experimental animal studies (see below). Human and experimental animal studies are first evaluated separately. The assessment results are then combined. The Health Council has drawn up a decision chart for this purpose, with this guideline serving as the basis for the evaluation. The Committee is free to depart from the decision chart, provided that it gives reasons for doing so.
Assessment of results from epidemiological studies

++ Sufficient evidence of carcinogenicity. It is anticipated that a causal relationship exists between exposure to an agent and the development of cancer. That is to say, more than one study in human subjects has found a positive association between exposure and cancer, in which chance, bias and confounding can reasonably be excluded.

+ Limited evidence of carcinogenicity. Studies in human subjects have established that there is a positive association between exposure and cancer. However, the possibility that chance, bias and confounding may play an important part in this cannot be excluded with any certainty.

? There is little or no data to support statements concerning an association between exposure to an agent and cancer.

- Sufficient evidence for the absence of carcinogenicity in more than one human study.

Assessment of results from experimental animal studies

++ Sufficient evidence of carcinogenicity. A causal relationship has been established between exposure to an agent and malignant tumours in:
  a two or more animal species, or
  b in two or more independent studies using a single animal species.

+ Limited evidence of carcinogenicity. Experimental animal data suggests the presence of a carcinogenic effect, but no definitive conclusion can yet be drawn, as:
  a all of the available data comes from just a single animal study;
  b several positive animal studies have been published, but there are doubts about their quality in terms of design and interpretation;
  c while the available studies are of good quality, either the results do not provide a clear picture or the data are difficult to interpret (e.g. an increase in the number of benign tumours or tumours with a high background incidence).

? There is little or no data to support statements concerning an association between exposure to an agent and cancer.

- Sufficient evidence for the absence of carcinogenicity in experimental animal studies.
Classification based on assessment of human and experimental animal data

Based on an evaluation of the epidemiological data and experimental animal studies, the Health Council recommends classification into one of the four categories (step 4). With regard to compounds classified into Category 1A and Category 1B, the Committee also assesses whether a genotoxic mechanism is involved in the development of cancer.

<table>
<thead>
<tr>
<th>Epidemiology</th>
<th>++</th>
<th>+</th>
<th>?</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>++</td>
<td>1A</td>
<td>1A</td>
<td>1B</td>
<td>2</td>
</tr>
<tr>
<td>+</td>
<td>1A</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>?</td>
<td>1A</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>-</td>
<td>1A</td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

* This involves an overall assessment of the experimental animal data, taking into account whether or not the observed tumours are relevant to the human situation.
References

Guideline to the classification of carcinogenic compounds
Guideline to the classification of carcinogenic compounds
A  The Committee
B  European Union Directive 93/21/EEG
C  International Agency for Research on Cancer
D  Germany: MAK-Kommission
E  Globally Harmonized System
F  Endpoints of carcinogenic mechanisms of action
G  Steps in the classification process according to the European Agency for chemical substances
H  Judgement of the study quality for the purpose of classifying carcinogenic substances

Annexes
Guideline to the classification of carcinogenic compounds
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The Health Council and interests

Members of Health Council Committees are appointed in a personal capacity because of their special expertise in the matters to be addressed. Nonetheless, it is precisely because of this expertise that they may also have interests. This in itself does not necessarily present an obstacle for membership of a Health Council Committee. Transparency regarding possible conflicts of interest is nonetheless important, both for the President and members of a Committee and for the President of the Health Council. On being invited to join a Committee, members are asked to submit a form detailing the functions they hold and any other material and immaterial interests which could be relevant for the Committee’s work. It is the responsibility of the President of the Health Council to assess whether the interests indicated constitute grounds for non-appointment. An advisorship will then sometimes make it possible to exploit the expertise of the specialist involved. During the establishment meeting the declarations issued are discussed, so that all members of the Committee are aware of each other’s possible interests.
The categories used by the EU comprises:

• Category 1: substances known to be carcinogenic to man.
• Category 2: substances which should be regarded as if they are carcinogenic to man.
• Category 3: substances which cause concern for man owing to possible carcinogenic effects but in respect of which the available information is not adequate for making a satisfactory assessment.
  • Category 3A: substances are well investigated, but the data are insufficient for classification in category 2.
  • Category 3B: substances are insufficiently investigated, the data are inadequate but raise concern for man.
• No classification.

Category 1

Substances are classified in category 1 if there is sufficient evidence for a causal relationship between exposure to the substance and the development of cancer in humans. Classification in this category occurs mainly on the basis of epidemiological data.
Category 2

Substances are classified in this category if there is sufficient evidence to provide a strong presumption that human exposure to a substance may result in the development of cancer. Generally, the classification is based on a) appropriate long-term animal studies, and b) other relevant information.

For classification as a category 2 carcinogen either positive results in two animal species should be available or clear positive evidence in one species; together with supporting evidence such as genotoxicity data, metabolic or biochemical studies, induction of benign tumours, structural relationship with other known carcinogens, or data from epidemiological studies suggesting an association.

Category 3

In category 3 substances are classified which cause concern for man owing to possible carcinogenic effects but in respect of which the available information is not adequate for making a satisfactory assessment in category 2. Category 3 is divided in two subcategories:

3A substances which are well investigated but for which the evidence of a tumour-inducing effect is insufficient for classification in Category 2. Additional experiments would not be expected to yield further relevant information with respect to classification.

3B substances which are insufficiently investigated. The available data are inadequate, but they raise concern for man. This classification is provisional; further experiments are necessary before a final decision can be made.

For a distinction between Categories 2 and 3 the arguments listed below are relevant which reduce the significance of experimental tumour induction in view of possible human exposure. These arguments, especially in combination, would lead in most cases to classification in Category 3, even though tumours have been induced in animals:

- carcinogenic effects only at very high levels exceeding the ‘maximal tolerated dose’. The maximal tolerated dose is characterized by toxic effects which, although not yet reducing lifespan, go along with physical changes such as about 10% retardation in weight gain;
- appearance of tumours, especially at high dose levels, only in particular organs of certain species is known to be susceptible to a high spontaneous tumour formation;
• appearance of tumours, only at the site of application, in very sensitive test systems (e.g., intaperitoneal or subcutaneous application of certain locally active compounds);
• if the particular target is not relevant to man;
• lack of genotoxicity in short-term tests in vivo and in vitro;
• existence of a species – specific mechanism of tumour formation (e.g. by specific metabolic pathways) irrelevant for man.
Guideline to the classification of carcinogenic compounds
Annex C

International Agency for Research on Cancer

The International Agency for Research on Cancer (IARC) assesses the carcinogenic properties of substances, and uses the following classification system*.

Group 1

The agent is carcinogenic to humans (sufficient human data, or less than sufficient human data with sufficient evidence in experimental animals).

Group 2A

The agent is probably carcinogenic to human (limited (or exceptionally inadequate) evidence in humans and sufficient evidence of carcinogenicity in experimental animals).

Group 2B

The agent is possibly carcinogenic to humans (limited (or exceptionally inadequate) evidence in humans and less than sufficient evidence of carcinogenicity in experimental animals).

* Source: http://monographs.iarc.fr
Group 3

The agent is not classifiable as to its carcinogenicity to humans (inadequate evi-
dence in humans and limited or inadequate evidence of carcinogenicity in expe-
rimental animals).

Group 4

The agent is probably not carcinogenic to humans.
Besides the European classification system, in Germany also another system of classification is used. This system is set up by the German MAK-Kommission of the German Forschungsgemeinschaft. In the system five categories are distinguished.*

Category 1

Substances that cause cancer in man and can be assumed to make a significant contribution 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 animal studies substantiated by evidence from epidemiological studies indicate that they can make a significant contribution to cancer risk. Limited data from animal studies can be

* Source: http://www.dfg.de
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 3A**

Substances for which the criteria for classification in Category 4 or 5 are fulfilled but for which the database is insufficient for the establishment of a MAK value.

**Category 3B**

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 with carcinogenic potential for which 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 significant 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. To characterize the cancer risk, the manifold mechanisms contributing to carcinogenesis and their characteristic dose-time-response relationships are taken into consideration.

Examples of substances classified in this category are: aniline, chloroform, DEHP, formaldehyde, glutaraldehyde, lindane, tetrahydofurane, and 2,3,7,8-TCDD.
Category 5

Substances with carcinogenic and genotoxic effects, the potency of which is considered to be so low that, provided the MAK and BAT values are observed, no significant contribution to human cancer risk is to be expected. The classification is supported by information on the mode of action, dose-dependence and toxicokinetic data pertinent to species comparison.

Examples of substances in this category are: acetaldehyde, ethanol, and styrene.
Guideline to the classification of carcinogenic compounds
Definitions

The term *carcinogen* denotes a chemical substance or a mixture of chemical substances which induce cancer or increase its incidence. Substances which have induced benign and malignant tumours in well performed experimental studies on animals are considered also to be presumed or suspected human carcinogens unless there is strong evidence that the mechanism of tumour formation is not relevant for humans.

Classification of a chemical as posing a carcinogenic hazard is based on the inherent properties of the substance and does not provide information on the level of the human cancer risk which the use of the chemical may represent.

Classification criteria for substances

For the purpose of classification for carcinogenicity, chemical substances are allocated to one of two categories based on strength of evidence and additional considerations (weight of evidence). In certain instances, route specific classification may be warranted.

* Source: http://www.unece.org
Classification as a carcinogen is made on the basis of evidence from reliable and acceptable methods, and is intended to be used for chemicals which have an intrinsic property to produce such toxic effects. The evaluations should be based on all existing data, peer-reviewed published studies and additional data accepted by regulatory agencies.

Carcinogen classification is a one-step, criterion-based process that involves two interrelated determinations: evaluations of strength of evidence and consideration of all other relevant information to place chemicals with human cancer potential into hazard categories.

Strength of evidence involves the enumeration of tumours in human and animal studies and determination of their level of statistical significance. Sufficient human evidence demonstrates causality between human exposure and the development of cancer, whereas sufficient evidence in animals shows a causal relationship between the agent and an increased incidence of tumours. Limited evidence in humans is demonstrated by a positive association between exposure and cancer, but a causal relationship cannot be stated. Limited evidence in animals is provided when data suggest a carcinogenic effect, but are less than suffi-
cient. The terms “sufficient” and “limited” are used here as they have been defined by the International Agency for Research on Cancer (IARC).

**Additional considerations (weight of evidence):** Beyond the determination of the strength of evidence for carcinogenicity, a number of other factors should be considered that influence the overall likelihood that an agent may pose a carcinogenic hazard in humans. The full list of factors that influence this determination is very lengthy, but some of the important ones are considered here.

The factors can be viewed as either increasing or decreasing the level of concern for human carcinogenicity. The relative emphasis accorded to each factor depends upon the amount and coherence of evidence bearing on each. Generally there is a requirement for more complete information to decrease than to increase the level of concern. Additional considerations should be used in evaluating the tumour findings and the other factors in a case-by-case manner.

Some important factors which may be taken into consideration, when assessing the overall level of concern are:
- tumour type and background incidence
- multisite responses
- progression of lesions to malignancy
- reduced tumour latency.

Additional factors which may increase or decrease the level of concern include:
- whether responses are in single or both sexes
- whether responses are in a single species or several species
- structural similarity or not to a chemical(s) for which there is good evidence of carcinogenicity
- routes of exposure
- comparison of absorption, distribution, metabolism and excretion between test animals and humans
- the possibility of a confounding effect of excessive toxicity at test doses
- mode of action and its relevance for humans, such as mutagenicity, cytotoxicity with growth stimulation, mitogenesis, immunosuppression.

**Mutagenicity:** It is recognized that genetic events are central in the overall process of cancer development. Therefore evidence of mutagenic activity *in vivo* may indicate that a chemical has a potential for carcinogenic effects.
The following additional considerations apply to classification of chemicals into either Category 1 or Category 2. A chemical that has not been tested for carcinogenicity may in certain instances be classified in Category 1 or Category 2 based on tumour data from a structural analogue together with substantial support from consideration of other important factors such as formation of common significant metabolites, e.g. for benzidine congener dyes.

The classification should also take into consideration whether or not the chemical is absorbed by a given route(s); or whether there are only local tumours at the site of administration for the tested route(s), and adequate testing by other major route(s) show lack of carcinogenicity.

It is important that whatever is known of the physico-chemical, toxicokinetic and toxicodynamic properties of the substances, as well as any available relevant information on chemical analogues, i.e. structure activity relationship, is taken into consideration when undertaking classification.

It is realized that some regulatory authorities may need flexibility beyond that developed in the hazard classification scheme. For inclusion into Safety Data Sheets, positive results in any carcinogenicity study performed according to good scientific principles with statistically significant results may be considered.

The relative hazard potential of a chemical is a function of its intrinsic potency. There is great variability in potency among chemicals, and it may be important to account for these potency differences. The work that remains to be done is to examine methods for potency estimation Carcinogenic potency as used here does not preclude risk assessment. The proceedings of a WHO/IPCS workshop on the Harmonization of Risk Assessment for Carcinogenicity and Mutagenicity (Germ cells)-A Scoping Meeting (1995, Carshalton, UK), points to a number of scientific questions arising for classification of chemicals, e.g. mouse liver tumours, peroxisome proliferation, receptor-mediated reactions, chemicals which are carcinogenic only at toxic doses and which do not demonstrate mutagenicity. Accordingly, there is a need to articulate the principles necessary to resolve these scientific issues which have led to diverging classifications in the past. Once these issues are resolved, there would be a firm foundation for classification of a number of chemical carcinogens.

Background guidance

Excerpts from monographs of the International Agency for Research on Cancer (IARC) Monographs Programme on the Evaluation of the Strength of Evidence of Carcinogenic Risks to Humans follow [as in 2.6.1 and 2.6.2].
Carcinogenicity in humans

The evidence relevant to carcinogenicity from studies in humans is classified into one of the following categories:

a sufficient evidence of carcinogenicity: the Working Group considers that a causal relationship has been established between exposure to the agent, mixture or exposure circumstance and human cancer. That is, a positive relationship has been observed between the exposure and cancer in studies in which chance, bias and confounding could be ruled out with reasonable confidence;

b limited evidence of carcinogenicity: A positive association has been observed between exposure to the agent, mixture or exposure circumstance and cancer for which a causal interpretation is considered by the Working Group to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence.

In some instances the above categories may be used to classify the degree of evidence related to carcinogenicity in specific organs or tissues.

Carcinogenicity in experimental animals

The evidence relevant to carcinogenicity in experimental animals is classified into one of the following categories:

1 sufficient evidence of carcinogenicity: The Working Group considers that a causal relationship has been established between the agent or mixture and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (i) two or more species of animals or (ii) in two or more independent studies in one species carried out at different times or in different laboratories or under different protocols;

2 exceptionally, a single study in one species might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset;

3 limited evidence of carcinogenicity: the data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (i) the evidence of carcinogenicity is restricted to a single experiment; or (ii) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the study; or (iii) the agent or mixture increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential, or of certain neoplasms which may occur spontaneously in high incidences in certain strains.
Guideline to the classification of carcinogenic compounds
## Annex F

### Endpoints of carcinogenic mechanisms of action

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Mechanism(s)</th>
<th>Genotoxic</th>
<th>Non genotoxic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Stochastic</td>
<td>non Stochastic</td>
</tr>
<tr>
<td>DNA-adducts</td>
<td>• direct interaction with DNA</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>• inhibition of DNA-repair enzymes</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>DNA-breaks</td>
<td>• direct interaction with DNA</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>• replication of damaged DNA</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>• inhibition of DNA-repair enzymes</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Gene mutations (mutations, deletions, amplifications, breaks, translocations)</td>
<td>• replication of damaged (alkylation)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>• inhibition of DNA-repair enzymes</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Structural chromosome aberrations (translocations, deletions, sister chromatid exchanges)</td>
<td>• replication of damaged DNA (alkylation, intercalation, cross-links)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>• inhibition of DNA-repair enzymes</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>• inhibition of topoisomerases</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Numerical chromosome aberrations (aneuploidy, polyploidy; micronuclei)</td>
<td>• replication of damaged DNA (alkylation, intercalation, cross-links)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>• inhibition of topoisomerases</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>• disturbance of spindle apparatus</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Changed gene expression</td>
<td>• all possibilities mentioned above</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>• epigenetic: DNA-hypo- of hyper-methylation to cytosine</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>• epigenetic: disturbance of (de)acetylation of histones</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>• disturbance of receptor-directed regulation of gene transcription</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
Changes in normal cell proliferation and differentiation, induction and cell transformation

- all possibilities mentioned above
- disturbance of hormone equilibrium - - +
- immune suppression - - +
- cytotoxicity and chronic irritation - - +
- disturbed activity of growth factors and signaling factors - - +
- disturbed receptor mediated regulation of cell division - - +
- disturbed intracellular communication (via gap junctions) - - +

* Gene mutations, structural and numerical chromosome aberrations can result ultimately in changed gene expression and cell proliferation.
Steps in the classification process

according to the European Agency for chemical substances

Step 1:
Gather all available information
↓
Step 2:
Examine information to ensure it is adequate and reliable
↓
Step 3:
Evaluate available information against classification criteria
↓
Step 4:
Decide on appropriate classification

Source: http://echa.europa.eu/
Guideline to the classification of carcinogenic compounds
Judgement of the study quality for the purpose of classifying carcinogenic substances

H.1 Epidemiological research

In assessing the weight of contribution of epidemiological studies in classifying carcinogenic substances, it is important to estimate the quality of those studies. This can be done systematically by using a certain set of criteria (see also the guidelines by the Dutch Institute for Healthcare Improvement). These criteria need to cover the essential characteristics of the study. For carcinogenic effects caused by exposure of carcinogens, in practice two study designs are of importance: 1) patient and control study, and 2) cohort study. In exposure-related cancer research most types of studies have a cohort design. Since the design of the case-control and cohort studies significantly differ, the quality criteria differ as well, as shown below.

Quality criteria for cohort studies
a Is there a clear hypothesis formulated prior to starting the study?

b Is the composition of the exposed group done in such a way that at the beginning of the follow-up the disease risks are comparable between the exposed and the ‘non-exposed’ reference group? In other words, do both groups have the same cancer incidence pattern if the compound under investigation would not be a carcinogen (the healthy worker effect is no reason to decline the study)?
Guideline to the classification of carcinogenic compounds

c Is the status of the disease assessed in a comparable way regarding the exposed and reference group?
d Is the follow-up done in a reliable way, also regarding completeness?
e Is the statistical analyses performed adequately, and did it include corrections for differences in ages, duration and period of follow-up?
f Is the influence of confounding factors that could add to the observed adverse health effects, adequately controlled?

The criteria \textit{a, b, c, d and e} must always be met. Regarding criterion \textit{f}, in case of co-exposure to a known carcinogen, it should be plausible that co-exposure did not influence the observed effects. To fulfill criterion \textit{f}, it is not necessary to control on potentially strong confounders, such as smoking and alcohol consumption.

\textbf{Quality criteria for case-control studies}

\textit{g} Is there a clear hypothesis formulated prior to starting the study?
\textit{h} Are the patient and control groups composed in such a way that the prevalence of exposure is comparable between them when there is no relationship between disease and exposure?
\textit{i} Is the exposure assessed in a valid way, independent from and without knowing the disease state?
\textit{j} Is the statistical analyses performed adequately?
\textit{k} Is the state of disease assessed in a valid way, independent from the state of exposure?
\textit{l} Is the influence of confounding factors controlled adequately, either by statistical analysis or by making up the patient groups?

The criteria \textit{g, h, i, and j} must always be met. Beside that it is allowed that one of the criteria \textit{k or l} is not met. At least, in case of co-exposure to a known carcinogen, it should be plausible that co-exposure did not influence the observed effects. This additional criterion only holds for co-exposure which is strongly correlated to the exposure under investigation, and not for instance to smoking in case of lung and bladder cancer.

\textbf{H.2 Animal experiments}

Klimisch \textit{et al.} (1997) have set a number of criteria that can be used to systematically evaluate the quality of animal experiments. These criteria have been
adopted or processed by various authorities. Studies that are classified in reliability category 1 and 2 appear to be suitable for basing a classification.

<table>
<thead>
<tr>
<th>Code of reliability</th>
<th>Category of reliability</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Reliable without restriction</td>
</tr>
<tr>
<td>1a</td>
<td>‘Good laboratory practice’ guideline study (OECD, EC, EPA, FDA, etc.)</td>
</tr>
<tr>
<td>1b</td>
<td>Comparable to guideline study</td>
</tr>
<tr>
<td>1c</td>
<td>Test procedure in accordance with national standard methods (AFNOR, DIN, etc.)</td>
</tr>
<tr>
<td>1d</td>
<td>Test procedure in accordance with generally accepted scientific standards and described in sufficient detail</td>
</tr>
<tr>
<td>2</td>
<td>Reliable with restrictions</td>
</tr>
<tr>
<td>2a</td>
<td>Guideline study without detailed documentation</td>
</tr>
<tr>
<td>2b</td>
<td>Guideline study with acceptable restriction</td>
</tr>
<tr>
<td>2c</td>
<td>Comparable to guideline study with acceptable restrictions</td>
</tr>
<tr>
<td>2d</td>
<td>Test procedure in accordance with national standard methods with acceptable restriction</td>
</tr>
<tr>
<td>2e</td>
<td>Study well documented, meets generally accepted scientific principles, acceptable for assessment</td>
</tr>
<tr>
<td>2f</td>
<td>Accepted calculation method</td>
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<tr>
<td>2g</td>
<td>Data from handbook or collection of data</td>
</tr>
<tr>
<td>3</td>
<td>Not reliable</td>
</tr>
<tr>
<td>3a</td>
<td>Documentation insufficient for assessment</td>
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<tr>
<td>3b</td>
<td>Significant methodological deficiencies</td>
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<tr>
<td>3c</td>
<td>Unsuitable test system</td>
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<td>Abstract</td>
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<tr>
<td>4d</td>
<td>Original reference not translated</td>
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<tr>
<td>4e</td>
<td>Documentation insufficient for assessment</td>
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</tbody>
</table>

Source: Klimisch e.a. 1997.
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