

## HEALTH BASED STANDARDS: ONCOLOGY

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### Contents

1. Introduction
  2. Cancer development as a multi step process
  3. Carcinogenicity tests
    - 3.1 Rationale
    - 3.2. Genotoxic tests
    - 3.3. Carcinogenicity tests
    - 3.4. Limited *in vivo* bioassay
    - 3.5. Case reports and epidemiology
  4. Classification of carcinogens
  5. Dose-response relationships for genotoxic carcinogens
  6. Risk figures for non-threshold carcinogens
  7. Standard establishment based upon risk figures
  8. Discussion – conclusions
- Acknowledgement  
Glossary  
Bibliography  
Biographical Sketch

### Summary

For genotoxic carcinogens there is no proof that a threshold exists. This means that human exposure to these substances is always associated with an increased risk. These substances therefore necessitate special attention in environmental policy and standard establishment shows specific features.

Carcinogenesis is a complex process involving multiple steps which are systematized in an initiation, promotion and progression phase.

Laboratory experiments are able to test different aspects of the genesis and the development of cancer. Tests for carcinogenicity in experimental animals, and short term genotoxicity tests including those measuring DNA damage, mutation induction and chromosomal changes, obtain essential information on the carcinogenicity of a substance. Widely used classification schemes for carcinogens are those developed by the International Agency for Research on Cancer (IARC) and the US Environmental Protection Agency (US EPA).

Although laboratory experiments also provide quantitative data on carcinogens, within the present state of knowledge it is difficult to define a predictable relationship between

the dose of a particular chemical required to produce cancer in test animals and the dose that would provide a similar incidence of cancer in humans. Thus quantitative estimations for humans, including those essential in standard establishment, are based upon epidemiological studies.

These estimations are based upon two particular concepts:

- a) the absence of a threshold dose and the notion that each increase of human exposure to a genotoxic carcinogen also increases the cancer risk,
- b) a linear dose effect relationship even at doses which are that low that no experimental or epidemiological data are available.

Consequently no safe exposure levels for most carcinogens can be provided. The WHO shows this by publishing standardized risk figures associated with the exposure to carcinogens.

Standards for carcinogenic substances can only be established after agreeing on an ethically loaded, societal acceptable risk. This value can be set at the level where environmental exposure to a carcinogen should not result in more than 1 cancer per one million exposed people on a lifetime basis.

In spite of the standards, prevention of exposure to carcinogens is the rule of the thumb in environmental policy.

## **1. Introduction**

Carcinogenic substances are dealt with in risk analysis and standard setting in a separate way mainly because cancer originates through an action mechanism which is fundamentally different from the mechanisms underlying acute and chronic toxicological effects. These differences entail aspects such as the dose-response relationship, which necessitate concepts different from those existing in toxicological standard setting. In particular, for genotoxic carcinogens, there is no proof that a threshold exists and therefore the safe dose concept, with its inherent use of a safety factor, does not apply. In this situation, quantitative risk assessment should be undertaken since human exposure to such substances offers a greater hazard compared with non-genotoxic carcinogens.

Carcinogens also have a specific place in environmental policy for a number of reasons:

- due to its dramatic impact on life and its quality, cancer has always been considered a health effect which needs to be avoided.
- various chemicals present in the environment are known to have carcinogenic properties, for example: benzene, soot, arsenic and its compounds, nickel compounds, 2,3,7,8-TCDD, radon, and its decay products.
- the number of cancers due to environmental exposure is difficult to estimate, but the impact of environmental exposure in particular on the incidence and mortality of cancer in urban conditions is well demonstrated.

- moreover there are important indications that especially in industrialized countries the relative impact of the environment as a cancer causing agent is increasing.
- environmentally induced cancers are a type of risk for which a particularly low degree of public acceptance exists.

All these arguments provide a context for environmental policy which pays particular attention to carcinogenic substances.

This article reviews the basic mechanisms of cancer and focuses on the aspects which necessitate particular risk analysis concepts. These concepts are provided and their impact on standard establishment for carcinogens is discussed. The list of environmentally relevant carcinogens is discussed.

## 2. Cancer development as a multi step process

A cancer observed in the hospital is the result of a multistep process which happens over a period of many years. This means that the induction of cancer involves several consecutive, independent events. Cancer growth results from alterations in the genetic material of a cell which consequently obtains a selected growth advantage, and grows as a clonal expansion. The main steps involved in the origins and development of cancer are shown in Figure 1. Three phases are essential in this process:

- Initiation:** this is the first step in the process and involves an alteration of the cellular DNA by a reactive form of the carcinogen. Mutagenic chemicals, ionizing radiation, and viruses might cause such changes in the DNA. The initiated cell is regarded as a potentially malignant state, which might be converted to a cell with the capacity of unrestricted proliferation.
- However, before the carcinogen can react with the DNA it needs to be up-taken and transported by the body. During this process it can be metabolized and excreted. Moreover it needs to reach the DNA before the latter can be altered. The DNA-carcinogen reaction leads to translocation and amplification of specific genes, proto-oncogenes, which translate into a distinct expression of the properties of the altered cell. The altered or initiated cell, usually called a latent tumor cell or neoplastic cell, may stay dormant or, under specific circumstances (e.g. under the influence of growth promoting agents), may proliferate into preneoplastic clonal expansions.
- Initiation is thought to be dose related, which means that an increasing dose leads to greater numbers of initiated cells. It occurs only in a small proportion of the target cell population and with greater frequency if the cells in the tissue are rapidly dividing. This is because the DNA in the dividing cell is less protected and therefore more susceptible to chemical alterations.
- Promotion:** these preneoplastic clonal expansions, the cellular outgrowths of a dormant initiated cell, are called a promoted tumor; the underlying process, tumor promotion. A promoter is a substance which does not necessarily cause tumor development itself but which, by its action, permits a potentially carcinogenic mutation to be expressed. The promoting substance transforms the initiated cell into an abnormal, activated cell that may be the first cell of a tumor.

This transformation results in local cell proliferation usually leading to benign tumor formation.

- e) Dose and duration of exposure to the promoter are key factors. Under certain circumstances, a promoter can also be tumorigenic in itself. At this stage, the tumor is not yet malignant. The result is a group of tumor cells *in situ* which can only be microscopically observed.
- f) **progression:** involves the progress of a promoted population of cells into a clinically observable cancer. It is the stage where tumor cells become malignant and the unrestricted proliferation results in invasion of adjacent tissues and metastases. Metastases occur when cells from the tumor break off and are transported elsewhere in the body to give rise to new tumor masses. These may grow even more rapidly than the original tumor, which is called the primary tumor.

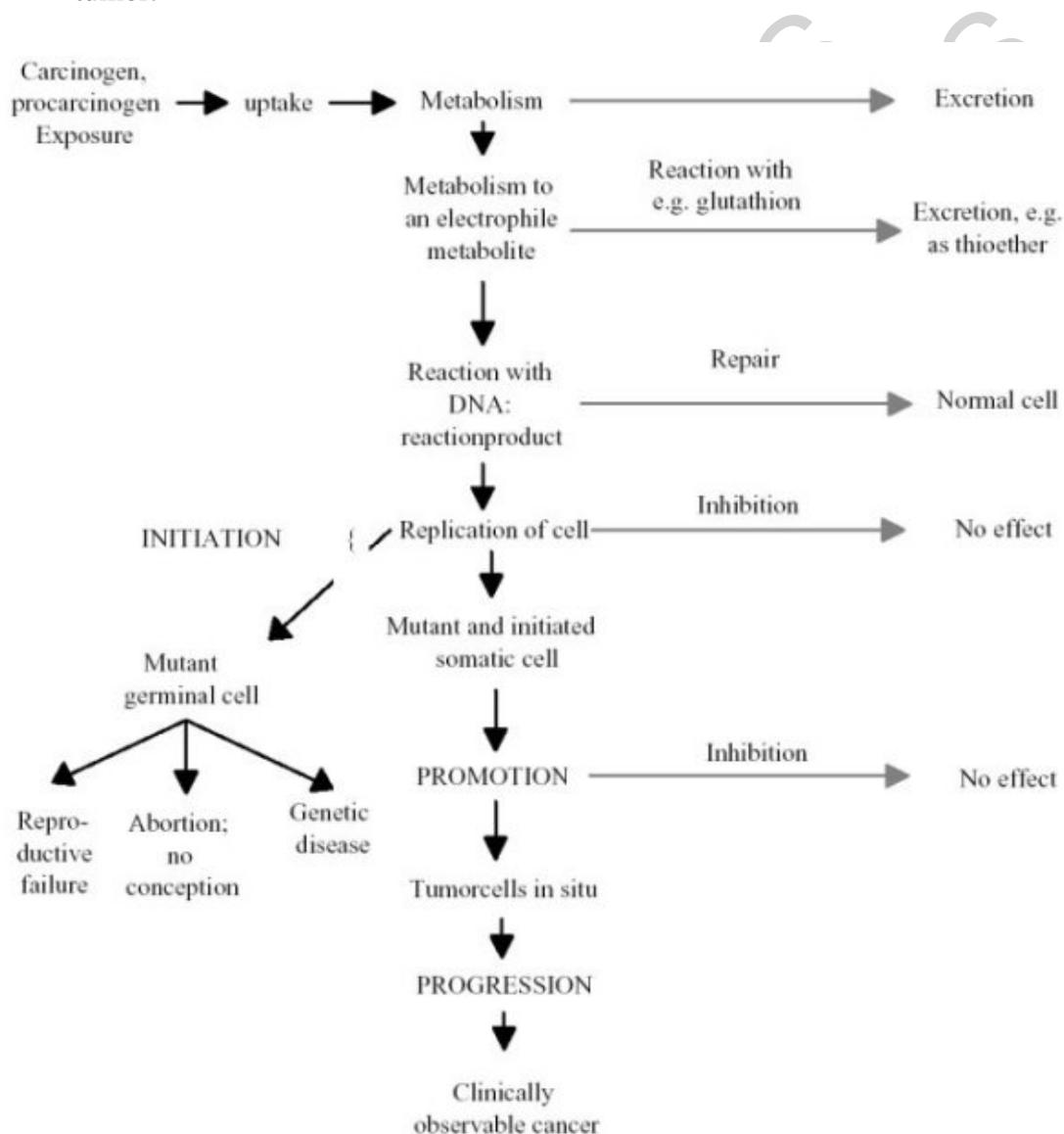


Figure 1. Neoplastic conversion, development and progression

The figure equally shows that the process can be stopped at well-defined different moments: damaged DNA can be repaired, an initiated cell can remain dormant; a group of promoted cells can be inhibited in their growth. In all these cases, no malignant, clinical observable cancer results.

Moreover initiation and promotion can be mediated by the same or by different substances.

Finally the figure shows how substances involved in the initiation of a cancer are linked to reproductive failure, spontaneous abortion or, more rarely, the birth of children with congenital malformations. A key issue in this respect is whether DNA in a somatic cell or a cell of the reproductive system is altered. DNA alteration in somatic cells can also contribute to aging or disfunctioning of cells in different organs.

The initiation-promotion-progression paradigm for cancer genesis does not apply under all conditions. For instance, this way of describing cancer process assumes that one needs to have (a) mutation(s) or genotoxic damage before cancer can arise. This causation theory is questioned. Moreover, it is unclear whether in situations where no mutations are at the base of the cancer process, threshold values apply.

### **3. Carcinogenicity tests**

#### **3.1 Rationale**

The first issue to be addressed in standard setting for carcinogens is to determine if an agent has the potential to elicit a carcinogenic response in humans.

The analysis of the cancer mechanism shows the complexity and the variety of biological targets as intermediate action points of the cancer process. To identify carcinogens and to detect their properties, laboratory assays aim at investigating different aspects of the development of cancer.

Since an alteration of the DNA is a prerequisite in the development of cancer, the demonstration of this property provides a basis to detect carcinogenic potential. These tests are usually *in vitro* tests, where the induction of mutations is detected in prokaryotic or eukaryotic cell systems or by unscheduled DNA repair in *in vitro* bioassays. Carcinogens acting via genetic alteration are called genotoxic carcinogens. For these genotoxic carcinogens no threshold has been demonstrated in most of the products. A chemical which is found to be genotoxic is unlikely to be acceptable for human exposure, unless the use or exposure is unavoidable.

Agents that change the DNA are called mutagens. Genotoxic carcinogens alter the DNA and therefore are mutagens. Therefore there is an important correspondence between carcinogens and mutagens. Over 90% of the mutagens are carcinogens. Only a minority of the carcinogens cause cancer through other pathways than the alteration of the DNA. These are the non-genotoxic carcinogens, which do not damage the DNA and usually become active in the development of cancer during the progression or promotion steps. The action mechanism of these non-genotoxic carcinogens is only partially known, but

the end result is usually an increased proliferation in specific tissues. This can be caused by excessive secretion of hormones, by injury, or can be receptor-mediated (e.g. peroxisome proliferation). Non-genotoxic carcinogens usually affect only one organ, and because of the nature of their indirect mechanism of action, there is a threshold for their action.

In a cancer bioassay, genotoxic as well as non-genotoxic carcinogens can be detected since the endpoint of this assay is the development of cancer. Carcinogenicity bioassays are performed when prolonged or continuous exposure of humans is likely. They are usually performed after testing a substance in a set of short-term genotoxicity tests.

### 3.2. Genotoxic tests

Genotoxicity testing is most useful in prescreening for potential carcinogenicity. The tests are designed to detect mutations in the genetic material. Consequently only potential genotoxic carcinogens can be detected and the final proof of their carcinogenic potential can only be provided by animal experiments.

*In vitro* genotoxicity testing usually involves at least two, but preferentially three, different points at several levels of biological complexity:

- an assay in a prokaryote,
- an assay in a eukaryote,
- an assay to detect DNA damage or an assay to detect adduct formation and an assay to detect chromosomal damage.

An assay in a prokaryote involves testing bacteria such as *Salmonella typhimurium* (Ames test) or *Escherichia coli*, where reverse mutations are used as an indication for genotoxicity. These tests detect mutations (e.g. the characteristic “not able to produce histidine” into the characteristic “being able to produce histidine”) of a strain of bacteria which are growth dependent and where the reverse mutation leads to independent growth which can be detected on a feeding layer devoid of growth factor. Over 200 *in vitro* genotoxicity assays have been described. A number of them are well standardized and validated.

In eukaryotic systems, yeast cells or preferably somatic cells are used. Systems also exist which use mammalian cells *in vitro*, in which unscheduled DNA synthesis is measured as an indication of genotoxicity.

Chromosome aberrations can be studied using *in vitro* mammalian cells both for numerical and structural aberrations, or for sister chromatid exchanges. In these tests loss of chromosomes and structural changes are studied by arresting the cells in mitosis. In the metaphases the chromosomes are counted, the abnormal chromosomes detected or the exchange of chromatids in a statistically sufficient number of cells evaluated.

When two out of three tests are positive, the genotoxicity of a substance is established. When all three tests are negative, there is good evidence that the substance has no genotoxic properties.

These basic tests can eventually be completed with a cell transformation test. These assays monitor the production of preneoplastic or neoplastic cells in culture. Therefore they provide an idea of essential steps in cellular carcinogens. However they are not grouped with the DNA damage, mutation and chromosomal tests, since the mechanisms by which chemicals induce cell transformation may not necessarily be the result of genetic change.

After *in vitro* testing and before long-term animal testing, *in vivo* genotoxicity testing is sometimes indicated. If the result of these tests is negative, the chances of the genotoxic substance being a carcinogen are smaller. Further testing with long-term bioassay may well result in non-carcinogenicity. As *in vivo* genotoxicity tests, the mouse bone marrow micronucleus test, the *in vivo* cytogenetic assay, the rodent liver genotoxicity test or the much less sensitive rodent dominant lethal test may be used. When these tests are negative, the likelihood of a substance being non-genotoxic in rodents is high and conversely a positive response may make it very likely that the substance in question will be a genotoxic rodent carcinogen.

In the mouse bone marrow micronucleus test, nuclear fragments derived from substance-treated mice are counted in a statistically sufficient number of bone marrow cells and compared with those from control animals.

In a rodent liver genotoxicity test, rats are treated with the test substance and liver cells in primary culture are exposed to tritiated thymidine to detect increased, unscheduled DNA repair (this is DNA synthesis occurring outside the normal DNA synthesis phase of the cell).

In the dominant lethal test, a serial mating technique is used. In this assay substance-treated males are mated with non-treated single virgin females for one oestrus cycle. By replacing the virgin female with another, the breeding study is continued for 70 days which is long enough to cover all stages of spermatogenesis. The detection of early embryonic deaths in the females is an indication of dominant lethality. In addition, this test also provides information about fertility.

### 3.3. Carcinogenicity tests

Although carcinogenicity tests specifically designed to detect (only) cancers exist, the combined chronic toxicity/carcinogenicity bioassay is more commonly used. In this test the effects of a substance of a neoplastic or non-neoplastic nature can be determined.

Table 1 summarizes the main characteristics of these tests. They are almost exclusively performed using rats and mice. They begin with weanling or post-weanling males and females and cover the animals' lifespan of at least two years (rats) or 18 months (mice). Since information on the dose-response relationship is crucial, a sufficient number of dose groups should be used. At least three dose levels groups should be used with 50 animals per sex per group. The lowest dose should not interfere with growth and development and must not cause effects, whereas the group receiving the highest dose should show signs of toxicity. The highest dose should not exceed 5% of the diet unless macro-nutrients are examined. The intermediate dose would be in the mid range

between the high and low doses. As for traditional toxicity tests, caging, care, diet and water supply must be optimal and well controlled.

<b>Conditions</b>	Chemical identification of substance, its purity and chemical characteristics
<b>Route</b>	Oral, inhalatory
<b>Experimental animals</b>	Rat, mouse, (dog), (monkey)
<b>Number of animals</b>	50 per sex group (sometimes satellite groups); dogs and monkeys usually not more than 7 to 10 per group
<b>Dose level</b>	Control at least 3 dose groups, for proper quantitative risk assessment more dose groups
<b>Examinations</b>	<ul style="list-style-type: none"> <li>• Body weight, food consumption and water consumption at various intervals</li> <li>• Clinical examination at intervals of 10 to 20 animals per sex group</li> <li>• Gross examination: daily observation and extensive gross examination on termination</li> <li>• Histopathological examination in full of highest dose and controls where indicated, for other dose levels</li> </ul>
<b>Results</b>	Information on carcinogenic properties, tumor incidence in relation to dose, latency period, tumor multiplicity, potential for metastasis

Source: after Van Leeuwen and Hermens (1995)

Table 1. Carcinogenicity studies

The rate of exposure to the substance should be comparable to the anticipated exposure to humans. The frequency of exposure depends on the route of exposure. In oral studies, the substance is given daily, unless it is administered by gavage. In this latter event, exposure is usually restricted to 5 times a week, as usually occurs in inhalation studies, where exposure will generally be limited to 6 hours per day.

Careful daily clinical examination is required and action should be taken to minimize the loss of animals during the study due to autolysis or cannibalism. Body weight is measured weekly during the first 13 weeks and once every 4 weeks thereafter. Food and drinking water intake is recorded weekly during the first 13 weeks and thereafter every three months. Blood examinations are performed after 3, 6, 18 and 24 months on 20 animals per sex group. A differential blood count is performed on samples of animals from the highest dose group and the controls, and at lower levels when indicated. Urine analysis of 10 animals per sex group is performed at the same interval. At the end of an experiment a 50% survival rate is minimally expected for mice at 18 months and rats at 24 months. Complete gross examination is performed and histopathological examination is carried out on all tissues and organs from the highest dose group and the control group. Where indicated, the tissues and organs of lower dose groups should be examined and all tumors or lesions suspected of being tumors should be examined histopathologically.

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### **Biographical Sketch**

**Professor Luc Hens** obtained his Licentiate in Biology from the Free University of Brussels (VUB) in 1974, Aggregation of Higher Secondary School Teaching from the VUB in 1975, and PhD from the Faculty of Science of the VUB in 1981.

Professor Hens is a member of several professional societies and recipient of a number of honours and awards, including the prestigious award of the Belgian Royal Academy of Sciences and Arts which he was awarded in 1984. Currently he is the Head of the Department of Human Ecology at the VUB.

He has been responsible for organising and/or participating in several international research and postgraduate teaching programmes in many countries including Bolivia, Bulgaria, Brazil, Brussels, the Czech Republic, Ghana, Hungary, Turkey, the Ukraine and Vietnam.

To date the publications of Professor Hens number about 200 including twenty-six books. He is also the co-editor of the journals *Environment, Development and Sustainability* and *Environmental Pollution*. His teaching and research interests include environmental management, sustainable development, human ecology, and related issues.