Abstract: This chapter is part of the book "Oxford Textbook of Oncology" that gives a whole view of cancer, approaching basic concepts of oncology, etiology, epidemiology, care and characteristics of cancer in specific places. It is focused on chemical carcinogens, reviewing the origin of these compounds, their classification and mode of action, their absorption and metabolism, and the methods available to test carcinogenicity. This title includes an extensive list of main chemical compounds and a schematic representation of the absorption and metabolism of direct and indirect chemical carcinogenesis, and the process of carcinogenesis. The title was written for qualified specialist doctors in the oncology research.

Keywords: Carcinogenesis, chemical compounds, genotoxicity, in vitro, in vivo, initiator, progressor, promoter

Chapter 17 Chemical carcinogens

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Introduction to chemical carcinogens

In a simplistic way, a carcinogen is any agent, the exposure to which increases the incidence of malignant neoplasia. Chemical carcinogens are able to induce tumor development in human or animals, to increase its incidence or malignancy, or shorten the time of tumor occurrence after getting into the body. This ability is attributed to many chemicals, giving a widespread perception of danger and threats (3).

Cancer results from the accumulation of irreversible DNA damage. These mutations may be inherited from parents or caused by exposure to outside agents. These factors include lifestyle (food, tobacco, alcohol consumption, sedentarism), natural exposures (ultraviolet light, radon gas, infectious agents), medical therapies (radiation, chemotherapy, hormones, drugs that suppress the immune system), pollution, workplace exposures and household exposures (5).

It is worth to note that carcinogens do not induce cancer development all the time. Carcinogens exposure increases the risk of development of one or more types of cancer, but any carcinogen is able to increase the risk of development of all types of cancer. The carcinogens have different potential for cancer development. Some of them may increase cancer risk after a short exposure, while others may cause

cancer only after prolonged and high-level exposure. The risk of cancer development in a particular person depends on the way, length and intensity of the exposure, and the person's genetic makeup (5).

Chemical carcinogens may be classified as natural chemicals, synthetic compounds, or mixtures of both that are synthesized or used for industrial, agricultural, or commercial purposes. Chemical carcinogens may be classed as either exogenous or endogenous. Exogenous carcinogens may cause cancer after penetrating the body, while endogenous carcinogens arise in the human or animal organism as a consequence of respiratory and/or food intake. Since the exogenous chemicals are more prone to cause cancer, only these will be considered in this chapter (6).

Exogenous chemical carcinogenesis is a very complex and multifactorial process, throughout which the gene-environment interactions. Polymorphisms of cancer susceptibility genes add further complexity. The exogenous carcinogens may directly damage DNA or indirectly, after activation into DNA-reactive intermediated or free-radical production. The exogenous carcinogens need to go into cells for carcinogenic activation and DNA damage, while many of the endogenous carcinogens are naturally occurring intracellular metabolic intermediates.

Hundreds of chemical compounds induce cancer and many thousands of additional compounds are suspected to be carcinogens. The number of naturally occurring chemicals present in the food supply or generated during the process of growing, harvesting, storage and preparation is enormous, probably exceeding one million different chemicals. The long period of latency in humans (the time between carcinogen exposure and tumor appearance may potentially be over 20 years) is the problem behind the identification of carcinogens in humans. This chapter addresses the main types of exogenous chemical carcinogens, their classification and their mode of action, absorption and metabolism, along with the principal tests available for evaluating chemical compounds' carcinogenicity.

A historical perspective on the identification of chemical carcinogens

The history of the identification of chemical carcinogens is based on epidemiologic studies and on animal experiments. Chemical carcinogenesis was first suggested more than 200 years ago. In 1771, the physician John Hill described a correlation between tobacco use (snuff) and the development of nasal tumours (7). Few years later, in 1775, the surgeon Percivall Pott verified that the chimney sweeps who crawled up chimneys to clean them with their bodies frequently suffered from skin cancer of the scrotum. He described the association between the contact of soot and cancer development, being the first to document the causal association between contact with chemical substances and cancer development (8). In 1895, Rehn reported a high incidence of urinary bladder cancer in workers of the European dye industry. More recently, observations have been made concerning the development of angiosarcomas in patients exposed to contrast agents for radiological imaging. The basic principles of chemical carcinogenesis exemplified by Hill, Pott, and Rehn's studies are as follows: human tumors typically appear as a consequence of long-term exposure and tumor incidence may be decreased by the implementation of measures to reduce carcinogen exposure, and tumors arising late in life may occur as a consequence of irreversible events taking place during early exposures (15). Based on these observations, several researchers conducted the first experimental studies on chemical carcinogenesis using laboratory animals in the early twentieth century. The first work was carried out in 1915 by Katsusaburo Yamagiwa and Koichi Ichikawa (16). They rubbed rabbits' ears with coal tar and later observed the development of malignant tumors at the site. These results confirmed the epidemiologic observations of scrotal and nasal tumors by Pott and Hill, respectively. In the meantime, other researchers evaluated the effects of several chemical carcinogens on the urinary bladder, liver, kidneys, pancreas, and lungs using laboratory animals.

Chemical carcinogenesis was early recognized as a multistep process. So, Beremblum and Shubik (17) developed an experimental two-stage skin carcinogenesis model in mice. By applying polycyclic aromatic hydrocarbons and croton oil, they described two phases in carcinogenesis: initiation and promotion.

For the first time, chemical carcinogens were classified as initiators and promoters according to their involvement in each carcinogenesis phase. In 1954, Foulds (18) characterized a third stage, termed progression, to account for all post-initiation events that occur during carcinogenesis after promotion. The overview of DNA as genetic material by Avery, MacLeod, and McCarthy (19) and the description of the structure of DNA by Watson and Crick (20) showed that DNA was the cellular target for chemical carcinogens and its mutation was the key for understanding mechanisms of carcinogenesis. Generally, the evidence suggested that chemical carcinogens induce DNA damage, which may result in permanent mutations, and these events are necessary but not sufficient for a tumor appearance (15).

In the 1960s, the physician Lorenzo Tomatis working on primary prevention and environmental carcinogenesis perceived the growing need to objectively evaluate the carcinogenic potential/risks by an international groups of experts in chemical carcinogenesis. His vision and determination to provide a reliable source of knowledge and information on environmental and occupational causes of cancer led to the creation of the IARC Monographs Programme for evaluating cancer risks to humans from exposures to chemicals. As an expert in the field, Tomatis promoted the applicability and utility of experimental animal findings to identify carcinogens and prevent cancer in humans (21).

In a landmark paper in 1979, Ames (22) noted that damage to DNA appeared to be a major cause of most cancers and suggested that natural and man-made chemicals should be tested for their ability to damage DNA. Almost 200 years later, other researchers concluded that polycyclic aromatic hydrocarbons isolated from tar and soot caused skin tumors in laboratory animals similar to those described by Pott (23).

Carcinogenic classification and their mode of action

The list of substances known or suspected to cause cancer has been developed by two highly respected agencies: the IARC and the US National Toxicology Program. Other agencies, such as Food and Drug Administration and the National Cancer Institute, may comment on whether a substance may cause cancer and/or

what levels of exposure to a particular substance might be considered acceptable. The list of human carcinogens has increasing over years (26). The IARC has continuously updated the assessments of the chemical agents classified as carcinogenic to humans. This process is frequently complicated by the absence of a systematic method to do it.

IARC classification

The carcinogenicity of chemical compounds is classified by IARC as: Group 1 (carcinogenic to humans, when the evidence is sufficient), Group 2A (probably carcinogenic to humans, mainly for experimental carcinogens with limited data to humans), Group 2B (possibly carcinogenic to humans, mainly for experimental carcinogens with less than limited evidence from humans and less than sufficient evidence from animals) and Group 3 (not classifiable as to its carcinogenicity to humans, for agents that do not fall into any other category). The last publication of the IARC identified more than 100 agents as Group 1 (4) (Table 16.1).

Table 16.1 List of chemical compounds carcinogenic to human (Group 1) and probably carcinogenic to humans (Group 2A) according to IARC.

Group 1 (carcinogenic to human)		**Group 2A (probably carcinogenic to human)**	
Acetaldehyde	Melphalan	Acrylamide	Hydrazine
Aflatoxins	Methoxsalen (8-methoxypsoralen)	Adriamycin	Indium phosphide
4-Aminobiphenyl	Methyl-CCNU	Androgenic (anabolic) steroids	2-Amino-3-methylimidazo[4,5-f]quinoline
Areca nut	4,4'-Methylenebis(chloroaniline)	Azacitidine	Malathion
Aristolochic acid	2-Naphthylamine	Biomass fuel emissions	2-Mercaptobenzothiazole
Arsenic	Nickel compounds	Bitumens	Merkel cell polyomavirus (MCV)
Asbestos	N'-Nitrosonornicotine	Bischloroethyl nitrosourea	5-Methoxypsoralen
Azathioprine	4-(N-Nitrosomethylamino)-1-(3-pyridyl)-1-butanone	Captafol	Methyl methanesulfonate
Benzene	3,4,5,3',4'-Pentachlorobiphenyl	Carbon electrode manufacture	N-Methyl-N´-nitro-N-nitrosoguanidine
Benzidine and dyes metabolized to benzidine	2,3,4,7,8-Pentachlorodibenzofuran	Chloral	N-Methyl-N-nitrosourea
Benzo[a]pyrene	Pentachlorophenol	Chloramphenicol	Nitrate or nitrite
Beryllium and beryllium compounds	Phenacetin	alpha-Chlorinated toluenes (benzal chloride, benzotrichloride, benzyl chloride) and benzoyl chloride (combined exposures)	6-Nitrochrysene
Betel quid	Phosphorus-32, as phosphate	1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea	Nitrogen mustard
Bis(chloromethyl)ether and chloromethyl methyl ether (technical-grade)	Plutonium		1-Nitropyrene
	Polychlorinated biphenyls		
	Semustine		
	Shale oils		

Busulfan	Silica dust	4-Chloro-ortho-toluidine	N-Nitrosodiethylamine
1,3-Butadiene	Soot	Chlorozotocin	N-Nitrosodimethylamine
Cadmium a	Sulfur mustard	Cisplatin	2-Nitrotoluene
Chlorambucil	Talc containing asbestiform fibres	Cobalt metal with tungsten carbide	Non-arsenical insecticides
Chlornaphazine	Tamoxifen	Creosotes	Petroleum
Chromium (VI) compounds	2,3,7,8-Tetrachlorodibenzo-para-dioxin	Cyclopenta[cd]pyrene	Pioglitazone
Coal	Thiotepa	DDT (4,4'-Dichlorodiphenyltrichloroethane)	Polybrominated biphenyls
Cyclophosphamide	Thorium-232	Diazinon	Procarbazine hydrochloride
Cyclosporine	Tobacco	Dibenz[a,j]acridine	1,3-Propane sultone
1,2-Dichloropropane	ortho-Toluidine	Dibenz[a,h]anthracene	Shiftwork that involves circadian disruption
Diethylstilbestrol	Treosulfan	Dibenzo[a,l]pyrene	Silicon carbide whiskers
Erionite	Trichloroethylene	Dichloromethane (methylene chloride)	Styrene
Estrogen	Vinyl chloride	Dieldrin, and aldrin metabolized to dieldrin	Styrene-7,8-oxide
Ethanol	Welding fumes	Diethyl sulfate	Teniposide
Ethylene oxide	Wood dust	Dimethylcarbamoyl chloride	Tetrabromobisphenol A
Etoposide		N,N-Dimethylformamide	3,3′,4,4′-Tetrachloroazobenzene
Strontium-90		1,2-Dimethylhydrazine	Tetrachloroethylene (perchloroethylene)
Fluoro-edenite fibrous amphibole		Dimethyl sulfate	Tetrafluoroethylene
Formaldehyde		Epichlorohydrin	1,2,3-Trichloropropane
Isopropyl alcohol Leather dust		Ethyl carbamate (urethane)	Tris(2,3-dibromopropyl) phosphate
Lindane		Ethylene dibromide	Vinyl bromide
Magenta production		N-Ethyl-N-nitrosourea	Vinyl fluoride
		Glycidol	
		Glyphosate	

The experts present at the first IARC meeting in 2012 originally identified 24 mechanistic end points for chemical carcinogens with several subcategories. This number was considered impractical, and the working group merged these categories into ten at the second meeting. They concluded that the human carcinogens frequently exhibit one or more of the following ten key characteristics/properties: *1*) electrophilic either directly or after metabolic activation; *2*) genotoxic; *3*) alter DNA repair or cause genomic instability; *4*) induce epigenetic alterations; *5*) induce oxidative stress; *6*) induce chronic inflammation; *7*) are immunosuppressive; *8*) modulate receptor-mediated effects; *9*) cause immortalization; *10*) alter cell proliferation, cell death, or nutrient supply (27). The knowledge of these characteristics is important to identify, organize and summarize information concerning the process of evaluation of carcinogens.

The electrophilic compounds are electron-seeking molecules that commonly form addition products (adducts) with cell macromolecules, namely DNA, RNA, lipids and proteins. Some of these agents act directly, while others require chemical conversion within the body or biotransformation by enzymes (metabolic activation). Sulfur mustards and ethylene oxide are examples of electrophilic agents (29).

The DNA damage generally does not change the linear sequence of DNA nucleotides, while a mutation is a change in the DNA sequence and usually arises when the cell tries to repair DNA damage (33). DNA damage includes DNA adducts, DNA strand breaks, DNA crosslinks and DNA alkylation. Based on their location or involvement of the genome, the mutations may be classified into three groups as gene or point mutations (in nucleotide sequence within a gene), chromosomal mutations (in nucleotide sequence that extend over multiple genes) or genomic mutations (duplication or deletion of nucleotide sequences of an entire chromosome). Aromatic amines are an example of genotoxic agents (21).

Some carcinogens act by changing the process that controls normal DNA replication or repairs DNA damage. Among these agents are cadmium, formaldehyde and arsenium. Epigenetic alterations are changes in gene expression and chromatin organization not caused by changes in the DNA sequence itself and may be inherited over cell divisions. Epigenetic changes, like changes in the DNA methylome and chromatin compaction states, and histone modification may affect gene expression and DNA repair dynamics, contributing to the carcinogenesis (39).

Some carcinogens act by influencing redox balance within target cells. An imbalance occurs in oxidative stress, favoring formation of reactive oxygen (ROS) and/or nitrogen species (RNS) instead of their detoxification. The oxidative damage is considered a major factor in the generation of DNA point mutations, deletions, insertions or chromosomal translocations, which may lead to oncogene activation and inactivation of tumor suppressor gene, initiating or promoting carcinogenesis (41). Asbestos is among the carcinogens able to induce cell injury by ROS (27). It is generally accepted that the chronic inflammation promoted by persistent infections by biological or chemical agents, like silica or asbestos fibers, leads to tumor development. The DNA repair, replication and maintenance of integrity decreases with ageing, and consequently DNA lesions may accumulate over years. In a young,

healthy organism, the majority or all DNA lesions are repaired or the affected cells are eliminated (apoptosis). Some authors suggest that the DNA repair plays a stronger role for the final outcome than the number of primary lesions (42).

Classification

Carcinogenic classification is by no means consensual and is in most cases based on the carcinogens' mode of action. Other authors classify chemical carcinogens according to the function of their mechanisms of action, and according to their involvement with DNA as being genotoxic and non-genotoxic (mitogenic and cytogenic) (47).

Genotoxic/Non-genotoxic

Carcinogens may be grouped as genotoxic or non-genotoxic. Most of them are considered genotoxic agents. This classification is of paramount importance for chemical carcinogens' risk assessment (27).

Genotoxic carcinogens are those chemicals or their metabolites able to induce cancer by the direct alteration of the genetic material of a target cell. Genotoxic carcinogens exhibit a direct analogy between their structure and activity, are mutagenic in *in vitro* assays, are active in high doses, and may affect various animal species and injure diverse organs. DNA adducts are covalent bonds established with macromolecules and, if not removed prior to DNA replication, these adducts may result in mutations. If such mutations occur in critical oncogenes or in the tumor suppressor genes that control cell proliferation, cancer development may follow. Adduct repair is coordinated by numerous enzymes and is controlled by different genes. It may be done via the excision of bases or nucleotides, recombined repair or mismatched repair and direct-damage reversal. The detection of adducts suggests that chemical carcinogens were absorbed, metabolized, and distributed by tissues, thus fleeing from the body's detoxification and repair mechanisms. Adduct detection may be done by techniques such as immunohistochemistry, immunoassays with ^{32}P-post-labelling, mass spectrometry, enzyme-linked immunosorbent assay, mass spectrometry or

accelerator mass spectrometry (15). Each approach presents different advantages and limitations and the most appropriate method depends on the type of sample, level of damage and nature of the investigation, as well as practical considerations such as instruments and reagents' availability and costs. The genotoxic agents may act directly or after xenobiotic metabolism (metabolic activation). Some of the genotoxic compounds can cause DNA damage without direct interaction. In a general way, they act through the generation of ROS or other reactive metabolites (endogenous metabolites) that are DNA reactive, or through other mechanisms that damage DNA structure and integrity, like topoisomerase poisons. Once the chemical compounds that are relatively stable in the environment and require metabolic activation in the body are present in the food and environment, they are considered more important when compared with those that interact directly with the genome. In the case of genotoxic agents, an ineffective (safe) dose may not be assumed (3).

In 2004, Bolt et al. (47) suggested the separation of genotoxic compounds into two groups: those that react with DNA and those which are genotoxic at a chromosomal level. Compounds that react with DNA are subdivided into three groups: initiators (with unlimited doses), borderline, and weak genotoxic (which act via secondary mechanisms). In 2006, were classified chemical compounds that may act on chromosomal structure and induce aneuploidy and changes in chromosome number as clastogenic. Since then, DNA damage at the chromosome level is being studied as an essential part of chemical carcinogenesis (66).

Non-genotoxic carcinogens do not affect DNA directly, do not raise adducts, and are negative on mutagenicity tests carried out in *in vivo* and in *in vitro*, but they are capable of inducing cancer by secondary mechanism not related to direct DNA damage. Little is known about this group of carcinogens, but evidence from known non-genotoxic carcinogens suggests that multiple pathways need to be changed for cancer induction (68). Non-genotoxic carcinogens do not require metabolic activation, act as tumor promoters (commonly used in two-stage animal models), and have a huge diversity of mechanisms of cancer induction by acting as endocrine-modifiers, receptor mediators (enhance proliferation, suppress apoptosis), immunosuppressants, or inducers of tissue-specific toxicity and inflammatory responses leading to epigenetic alterations like changes in histone

acetylation, perturbation of DNA repair, and oxidative stress (69). The wide range of modes of action of these non-genotoxic agents, the tissue and species specificity and the absence of DNA damage, makes the prediction of their carcinogenic potential extremely hard. As opposed to genotoxic agents, an ineffective (safe) threshold dose without cancer risk may be assumed to these non-genotoxic agents (3).

Non-genotoxic compounds potentiate the effects of genotoxic compounds, do not demonstrate a direct association among structure and activity, and are conditioned by their concentration. They are tissue- and species-specific (72). Non-genotoxic carcinogens are classified as mitogenic and cytotoxic regarding to whether their activity is mediated by a receptor or not. Mitogenic compounds induce cell proliferation in target tissues through interaction with a precise cellular receptor (74). Cytotoxic carcinogens cause cell death in vulnerable tissues followed by compensatory hyperplasia (75). The more nearby cells augment the number of cell divisions through regenerative events, the more likely it is that they will end up being prematurely recruited for the cell cycle and that the time available for DNA repair will be inferior - this increases the probability of mutations occurring. On the other hand, necrotized cells are destroyed by the immune system and endogenous chemicals such as ROS, RNS, and proteolytic enzymes are produced. When production of these ROS and RNS exceeds the cellular anti-oxidant capacity, it may cause lipid peroxidation, oxidative DNA and RNA damage, oxidative damage to proteins, and DNA mutations. Mitogenic compounds should be present in adequate concentrations in order to promote their action. In contrast, the action of cytotoxic compounds is independent of their concentrations (76).

In 2011, Cohen and Arnold (79) suggested a refinement of chemical carcinogen classification into two groups: chemicals that increase the risk of cancer that are non-DNA reactive and do so by increasing the number of DNA replications in the target cell population (increase cell proliferation) and those chemicals that are DNA reactive. This classification allows us to make the distinction between classes of chemicals based on their ability to generate DNA reactivity. To date, this is the basis for the classification of chemical carcinogens and forms the basis for the distinction of potential risks to humans in regulatory decision-making.

Initiator/Promoter/Progressor

The more recent description of the process of carcinogenesis is based not only on morphology or the impact of carcinogens, but on changes in gene expression and cell signaling. Cancer is a multistep process, involving different stages: initiation, promotion, progression, and metastasis. Considering its involvement in each step, several authors classify chemical carcinogens as initiators, promoters and progressors (76). It is worth to note that the initiation-promotion regimens are simplified models which may not reflect all features of chemical carcinogenicity. The dose-response relationship of the strong genotoxic agents may be linear over a wide range of doses, and this is not expected for weak or borderline carcinogen agents. It is also important to note that several human carcinogens act via multiple mechanisms, causing various biological changes in the carcinogenesis (27).

Tumor initiators are those compounds capable of inducing an initial driving DNA mutation, using numerous mechanisms in a dividing cell via direct or indirect mutagenesis, so that an initial clone of mutated cells may emerge. Initiators are chemicals that are DNA reactive, either directly or following metabolic activation. Genotoxicity is a required property of chemical compounds classified as initiators. They may induce DNA changes such as interruptions of the DNA chain, errors in DNA repair, or elimination of a base repair. Examples of carcinogens initiators include alkylating agents, polycyclic aromatic hydrocarbons, aromatic amines, metals (cadmium, chromium and nickel), aflatoxins, and nitrosamines (6).

Tumor promoters may promote, facilitate or accelerate early steps of carcinogenesis when applied repeatedly after initiators. These compounds usually do not form reactive metabolites, but act by modulating growth or cell death (apoptosis) via receptor-mediated or other mechanisms (86). Promoters may simultaneously act as initiators, though promoters are usually not initiators when used in isolation at the same dosage at which they promote. The promoter has to be present for weeks, months, and years to be effective and its effectiveness depends on its concentration in the target tissue. Some promoter agents are specific to a particular tissue, but others may act on several tissues at

the same time. Promoter compounds do not interact directly with DNA and lead to biological effects without being metabolically activated. They may induce some alterations in initiated cells, such as the alteration of cell-surface sensitivity to various growth factors, alteration of cell-surface glycoproteins and glycolipids, alteration of cell morphology, increased phospholipid and glucose metabolism, stimulation of DNA synthesis and cell proliferation, increased production of free oxygen radicals, induction of disproportionate DNA replication within one cell cycle via gene amplification, and preventing apoptosis (6,76). Initiators require the application of promoters to induce cancer development in experimental models. However, in studies of chemical carcinogenesis with prolonged exposure and using high doses, almost all promoters induced neoplasia without prior application of initiators. Examples of these are exposure to phenobarbital, benzene, and asbestos, which, even without the previous use of initiator agents, lead to neoplastic development (91). The following are examples of chemical promoters: diethylstilbesterol, cyclamates, phorbol 12-myristate 13-acetate, and saccharin (6).

Chemical carcinogens may also be classified as progressors. These agents move mutated cells on from the promotion to progression phase, i.e. they enable premalignant mutated cells irreversibly to attain the phenotype of fully malignant cells. Progressor agents include alkylating agents, arsenic salts, asbestos, and benzene (6). Complete chemical carcinogens are those that induce tumors, by themselves, usually with initiating, promoting, and progressing properties (15).

Chemical structure

According to their chemical structure, chemical carcinogens may be classified as polycyclic aromatic hydrocarbons, alkylating agents, aromatic amines/amides, aminoazo dyes, carbamates, halogenated compounds, natural carcinogens, metalloids and hormones. In Table 16.2 they are brought together under the

following headings: group, compound, mechanism of action, and affected organs/cancer type.

Table 16.2 Chemical carcinogenic agents.

Group	Compounds	Major origins	Mechanism of action	Affected organs/Cancer type
Polycyclic aromatic hydrocarbons	Benzo[a]pyrene	Charcoal broiled foods Cigarette smoke	DNA adducts	Skin, lungs, stomach
	Dimethylbenz[a] anthracene	Diesel exhaust Residential heating	DNA adducts	Liver, skin
Alkylating agents	Nitrosamides (N-ethyl-N-nitrosurea; N-methyl-N-nitrosurea; N-methyl-N-nitro-N-nitrosoguanidine)	Chemical solvents	DNA adducts, methylation and ethylation reactions	Liver, lungs, kidneys, brain
	Nitrogen mustards (chlorambucil, cyclophosphamide)	Cancer chemotherapy	DNA adducts, DNA strand breaks, DNA alkylation	Leukaemia Nose
	Ethylene oxide; propylene oxide vinyl chloride		DNA adducts	Liver, lung, tumours from hematopoetic system
Aromatic amines/amides	Aniline dyes, 2-naphthylamine, benzidine, 2-acetylaminofluorene	Oil refining, synthetic polymers, dyes, adhesives and rubbers, pharmaceuticals, pesticides, explosives, cigarette smoke, hair dyes, diesel exhaust, burning/pyrolysis of protein-rich	DNA adducts	Liver, urinary bladder

Group	Compounds	Major origins	Mechanism of action	Affected organs/Cancer type
		vegetable matter		
	4-Aminobiphenyl	Industrial exposition, cigarette smoke	DNA adducts	Urinary bladder
Aminoazo dyes	o-Aminoazotoluene; *N,N*-dimethyl-4-aminoazobenzene	Dyes and pigments	Adducts with DNA and haemoglobin	Liver, lungs, urinary bladder
Carbamates	N-methylcarbamate esters: propoxur	Insecticides	Chromosome aberration, gene mutation, cell transformation	Liver, kidneys and testes degeneration
Halogenated compounds	Trichloroethylene, methylene chloride, chloroform, chloroisoprene, trichlorobenzene	Industry involved in the production of polymers, pesticides, and fire retardants	Somatic mutations, modification of cell cycle pathways	Kidney, liver and lung
Natural carcinogens	Aflatoxin B1	Food contamination (grains, nuts, peanut butter) by *Aspergillus flavus*	Forms adducts with guanine, react with RNA and proteins	Liver
	Asbestos	Environmental media (air, water and soil); human activities (product manufacture, construction activities and transport)	Mutagenecity	Mesothelioma, lung
	Ptaquiloside	*Pteridium aquilinum*	DNA adducts	Urinary bladder
Metals	Arsenic	Natural and anthropogenic sources (drinking water, gold mining activities, etc.)	Cell cycle checkpoint dysregulation, DNA damage response, abnormal chromosomal segregation,	Skin, lungs, liver, lungs, prostate, kidneys, urinary bladder

Group	Compounds	Major origins	Mechanism of action	Affected organs/Cancer type
			defects in cell cycle checkpoints, disabled apoptosis, telomere dysfunction, altered chromatin structure	
	Cadmium	Burning of coal and tobacco	Interferes with antioxidant defence mechanisms, inhibit apoptosis	Lungs, nasal cavity, breast
	Nickel	Industrial processes	Oxidative stress, recombination and repair of DNA	Respiratory cancer
	Chromium	Industrial processes	DNA adducts, oxidative DNA damage	Lungs and nasal cavity
Hormones	Ethinyl estradiol	Medicinal exposure	Cell cycle	Uterus and prostate
	Estradiol	Medicinal exposure	Cell cycle	Breast
	Tamoxifen	Medicinal exposure	Cell cycle arrest	Breast
	Estrogen	Medicinal exposure	Cell cycle	Breast cancer, endometrial cancer, ovarian cancer

Absorption and metabolism of chemical carcinogens

Following exposure, chemical carcinogens may be absorbed in a number of ways, such as ingestion, inhalation, skin absorption, injection, or other possible contamination routes, and distributed across several tissues (Figure 16.1). Absorption depends on the physicochemical properties of the substance. Substances absorbed orally pass through the liver and only then are distributed in the body. Those absorbed in the lungs are distributed by the blood prior to reaching the liver at a later stage. Those chemical carcinogens classified as direct act directly on DNA, causing mutations and forming DNA adducts without being metabolized. These chemicals are also defined as activation-independent carcinogens and ultimate carcinogens. Examples of direct-acting carcinogens include alkyl or aryl epoxides, nitrosoureas, nitrosamides, and certain sulfonate and sulfate esters. Approximately 25% of all carcinogens are direct carcinogens (6). The relative carcinogenic strength of direct-acting carcinogens depends in part on the relative rates of interaction between the chemical and genomic DNA, as well as competing reactions with the chemical and other cellular nucleophiles. The relative carcinogenic activity of direct-acting carcinogens is dependent upon such competing reactions and also on detoxification reactions. Chemical stability, transport, and membrane permeability determine the chemicals' carcinogenic activity. Direct carcinogens are typically carcinogenic at multiple sites and in all species examined (111).

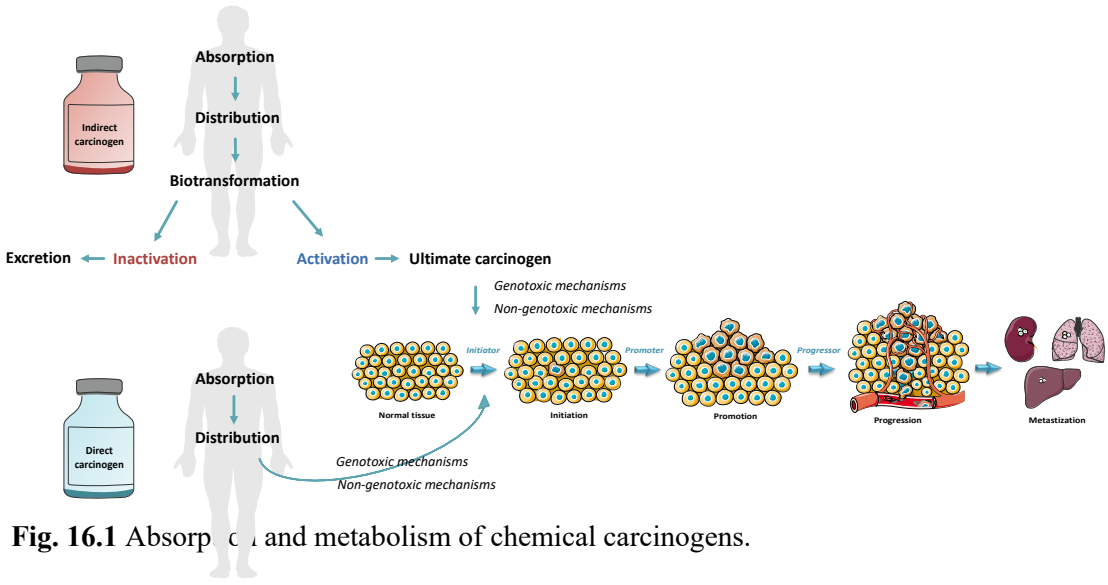

Fig. 16.1 Absorp[illegible] and metabolism of chemical carcinogens.

On the other hand, approximately 75% of chemical carcinogens, require metabolic activation to be carcinogenic and are labelled as indirect, procarcinogens or indirect-acting genotoxic carcinogens. Examples of indirect-acting carcinogens include polycyclic aromatic hydrocarbons, aromatic amines, alkyl nitrosamines, or aflatoxin B1. The terms procarcinogen, proximate carcinogen, and ultimate carcinogen have been coined to classify the parent compound (procarcinogen) and its metabolite form as well as the intermediate (proximate carcinogen) or final form (ultimate carcinogen) that reacts with DNA. The final form of the carcinogen is most likely to be the chemical species that results in mutation and neoplastic transformation. Indirect-acting genotoxic carcinogens usually produce their neoplastic effects, not at the site of exposure (as seen with direct-acting genotoxic carcinogens) but at the target tissue where their metabolic activation occurs. Metabolic activation occurs mainly in the liver at the plain endoplasmic reticulum, where the cytochrome P450 is abundant, and/or in other enzymes located in urothelium, skin, gastrointestinal system, oesophagus, kidneys, and lungs. The final product is an electrophilic compound that directly interacts with proteins, RNA, and DNA to form adducts (113). The P450 system not only activates chemical carcinogens but also other drugs. Although some of these metabolic processes lead to activation in reactive electrophiles, many actually lead to inactivation of the chemicals by increasing aqueous solubility and leading to their increased excretion either in urine or feces (79). Thus, exposure to any chemical initiates competing metabolic pathways for activation *versus* inactivation (79). The specificity of the activation systems of diverse tissues depends on genetic polymorphisms, which control the expression and distribution of the P450 enzyme and the resulting susceptibility to cancer development. Most tested chemical carcinogens were reported to be positive in the following organs in different mammal species: liver, lung, mammary gland, stomach, vascular system, kidney, hematopoietic system and urinary bladder. Metabolic pathways are equally important for both humans and animals, although qualitative and quantitative differences among them do exist. These differences led to incorrect interpretations when animal models are used in the research and analysis of carcinogenic properties of chemical compounds (115).

Testing for carcinogenicity

Before the classification as a carcinogen, the carcinogenicity of a chemical compound should be previously assessed by a scientific approach (21). A substance is defined as carcinogenic after an extensive study by researchers. One or more agencies evaluate the evidence and determine it to be a cause of cancer (5). Testing to see if something may induce cancer is often difficult. Experimental assays with animal models and *in vitro* assays as well as epidemiological studies allow the recognition of carcinogenic chemical compounds and the analysis of many aspects of chemical carcinogenesis. However, these tests do not always give clear answers. In some cases, the compounds seem to be genotoxic in one study or one test only (e.g. only *in vitro* or *in vivo*). This is difficult to explain and cause controversial discussions (3). In some cases, the compounds are classified as "likely to cause cancer in humans" without considering the mode of action and dose response, leading to confusion. Labeling chemical compounds as "human carcinogens" does not indicate nothing about the dose-response and the relevance of the risk.

Animal models

Studies in animals are important to study the mode of action of the chemical carcinogens and for risk assessment. From an experimental point of view, a chemical compound is considered carcinogenic when its administration to laboratory animals induces a statistically significant increase in the incidence of one or more histological types of neoplasia, compared with the animals in the control group which were not exposed to the compound (3).

Studies should be adequately designed to detect the effects of small doses of the carcinogen agents, and the relevance of the mode of action of the carcinogens in laboratory animals for humans should be carefully investigated (3). It is worth it to note that a high number of animals is required (some ethical problem due to the high number of animal may arise) and the variation of conditions, like housing conditions, feed, infections, among others, may have a great impact on the results.

Animal models should reflect the exposure to carcinogens or the genetic predisposition that is present in at-risk humans. In addition, pathological lesions should reflect the molecular changes and histological characteristics seen in human cancers (117). The standard approach to carcinogenicity testing is to

conduct two-year bioassays in small laboratory rodents (rats and/or mice). However, this kind of assay uses large numbers of animals, is time-consuming and expensive and is also fraught with sources of controversy regarding the relevance of the mode of action to humans or the dose used in the study compared with human exposure levels (79). The uncertainty in the extrapolation of results is particularly high for non-genotoxic carcinogens. This is because non-genotoxic carcinogens are likely to have a dose-response curve that is not linear and that includes a threshold. Furthermore, they could induce cancer in animals via a mechanism that is not applicable to humans. Also, laboratory animals and man do not always metabolize chemical carcinogens in the same way. Achieving a positive result in a conventional assay only indicates that there is a potential hazard. Its significance for human health will depend on other factors, several of which need additional studies. These models do not mimic human exposure conditions and humans are exposed to an enormous complex of chemicals. Despite their limitations, rodent models are useful tools for identifying dietary carcinogens and anticarcinogens.

In vitro assays

In vitro models may be used to identify and to study chemical carcinogens. *In vitro* assays use prokaryotic, human, and animal cells, mimic some key stages of *in vivo* multistep carcinogenesis, measure induction of phenotypical alterations, have differing levels of complexity, and may overcome the ethical aspects related to animal experiments, as well as being faster, more cost-efficient and less reliance on animals. *In vitro* studies are more prone to artefacts and their conclusions may be not relevant for *in vivo* assays. In the case of weakly genotoxic agents, the chemical compounds are genotoxic in *in vitro* assays only (3). Despite this, the *in vitro* models have been shown to have a good concordance with rodent bioassay results, detecting both genotoxic and non-genotoxic carcinogens. However, we do not have appropriate cell lines available which appropriately mimic the *in vivo* response, all the metabolic activation and inactivation processes are not maintained *in vitro*, and current *in vitro* approaches are unable to address the frequent occurrence of organ interactions that are implicated in many toxic end points. The first test described to evaluate the carcinogenic properties of chemical compounds *in vitro* was the

malignant transformation of Syrian hamster embryo cells (120). In 1970 the Ames test emerged. This test semi-quantitatively analyses a chemical's capacity to induce mutations in *Salmonella typhimurium* in a culture medium improved by using microsomatic enzymes. Between 70% to 90% of identified chemical carcinogens show positive results on the Ames test. Due to the high correlation that exists between mutagenicity and carcinogenicity, the Ames test is still used to assess the carcinogenic potential of chemicals. A plethora of *in vitro* genotoxicity assays have been developed during the past four decades, which were designed to identify mechanisms by which a chemical causes cancer but also to identify chemical carcinogens. We highlight the micronucleus assay, the chromosomal aberration assay, the comet assay and the microRNA changes (123).

Epidemiological studies

Another important method to identify chemical carcinogens is through epidemiologic studies, in which human populations are examined to determine which chemical compounds might be associated with cancer development. Global epidemiological studies have identified environmental and occupational chemicals as potential carcinogens. Epidemiological studies are retrospective and unless a big number of individuals are studied their levels of sensitivity is low (125). Epidemiological advances in the identification of chemical carcinogens are limited for several reasons. Only relatively high risks may be detected, epidemiological surveys are based on observations of the effects resulting as a consequence of exposure that took place many years before, humans do not live in a controlled environment, and there are usually many years (often decades) between exposure to a chemical carcinogen and cancer development.

Some epidemiological studies performed in humans are doubtful, such as in the case of glyphosate, where the data from mixed exposure to various compounds were used. The epidemiological data may be problematic. In a general way, these studies may show correlations but without clear scientific evidence (3).

Other methods

Computational approaches for genotoxicity prediction have emerged over two decades. The carcinogenic capacity of a chemical substance may be observed

using software that thoroughly reproduces human's physiological and metabolic processes and relates them to the molecular configuration of the evaluated substance. These chemical compounds' characteristics are correlated to the molecular structure of chemical, physical, and toxicological properties (129).

Statistical learning methods have been explored as a new advance in genotoxicity prediction without any restrictions on the features of structures or types of molecules. As an alternative to focusing on specific structural characters or a particular group of related molecules, these methods classify molecules into genotoxic positive or non-genotoxic agents, based on their general structural and physicochemical properties, regardless of their structural and chemical types (131).

By combining data from both type studies, scientists do their best to make an educated assessment of a substance's cancer-causing ability.

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