Chapter 1
Current concepts in carcinogenesis

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1. Introduction

It is now well established that human diseases, particularly cancers, are the result of complex interactions between genetic and environmental factors, with the latter being definable in the broad sense of the term as general environmental and occupational exposure factors or even behavioural factors and social determinants.

The development of molecular biology and tumour genome research techniques and all the '-omics' technologies has led to a better understanding of the complexity of carcinogenesis, in particular by revealing numerous genetic and epigenetic changes and by highlighting the importance of new concepts in terms of the characteristics of dangers and exposures to carcinogens. Prompting us to revisit risk assessment methods, these concepts also concern the low doses and mixtures of carcinogens particularly present in work environments, the critical periods of exposure essential for understanding effects such as those of endocrine disruptors, and more generally the need for an adapted inclusive approach to exposures via the exposome concept[1].

2. Reminder of traditional concepts in carcinogenesis

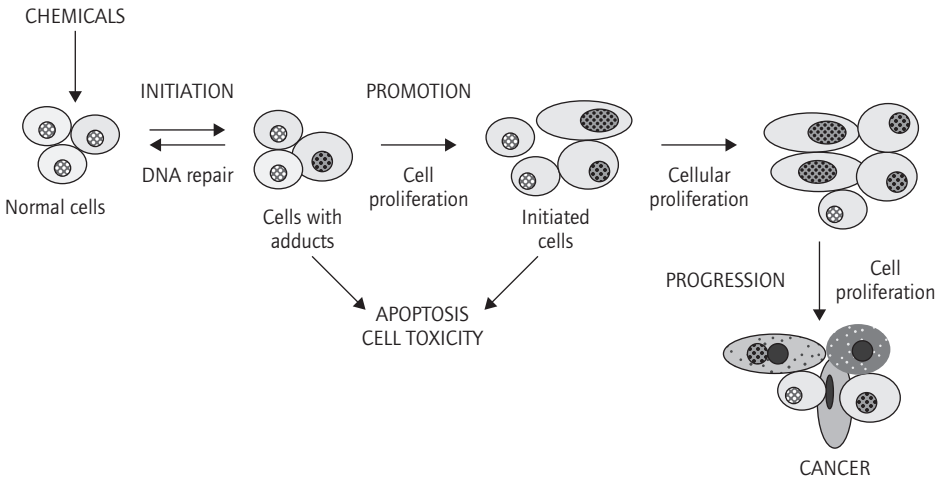
Carcinogenesis refers to the phenomena transforming a normal cell into a cancer cell, with the development of a cancer the culmination of a series of events stemming from the uncontrolled proliferation of malignant cells under the cumulative effect of multiple genetic changes. It is common to distinguish between several key stages in carcinogenesis (cf. Figure 1): initiation of the tumour, during which irreversible changes in the DNA (deoxyribonucleic acid) of the cell nucleus allow a normal cell to acquire properties that will gradually transform it into a tumour cell (initiated cell); tumour promotion phase, which involves the proliferation (abnormal multiplication) of the clone of the initiated cells; tumour progression phase, which marks the passage from precancerous lesions to malignant lesions, involving independent cell proliferation, invasive spread of the tumour and its capacity to metastasise.

1. The exposome encompasses the *totality* of human environmental (i.e. non-genetic) exposures from conception onwards, complementing the genome. It was first proposed in 2005 by a cancer epidemiologist Christopher Paul Wild, in an article entitled "Complementing the genome with an 'exposome': the outstanding challenge of environmental exposure measurement in molecular epidemiology" (Publisher's note).

In the end, the cancer cells gradually acquire a number of properties differentiating them from normal cells: capacity to proliferate; independence from environmental signals, particularly anti-proliferative signals; resistance to apoptosis (programmed cell death); and capacity for angiogenesis (formation of their own vascular system) and metastatic invasion and spread (dissemination through the blood or lymphatic system to distant organs).

This multi-stage carcinogenesis process usually takes a long time, namely several years or even decades.

Figure 1 **Chemical carcinogenesis stages and the occurrences involved in each one**



Source: adapted from Oliveira *et al.* (2007)

Toxic substances act on the chromosomes and genetic makeup (DNA). Environmental carcinogens can be cancer initiators, directly genotoxic substances or promoters, which is particularly the case with numerous chemicals to which workers may be repeatedly exposed.

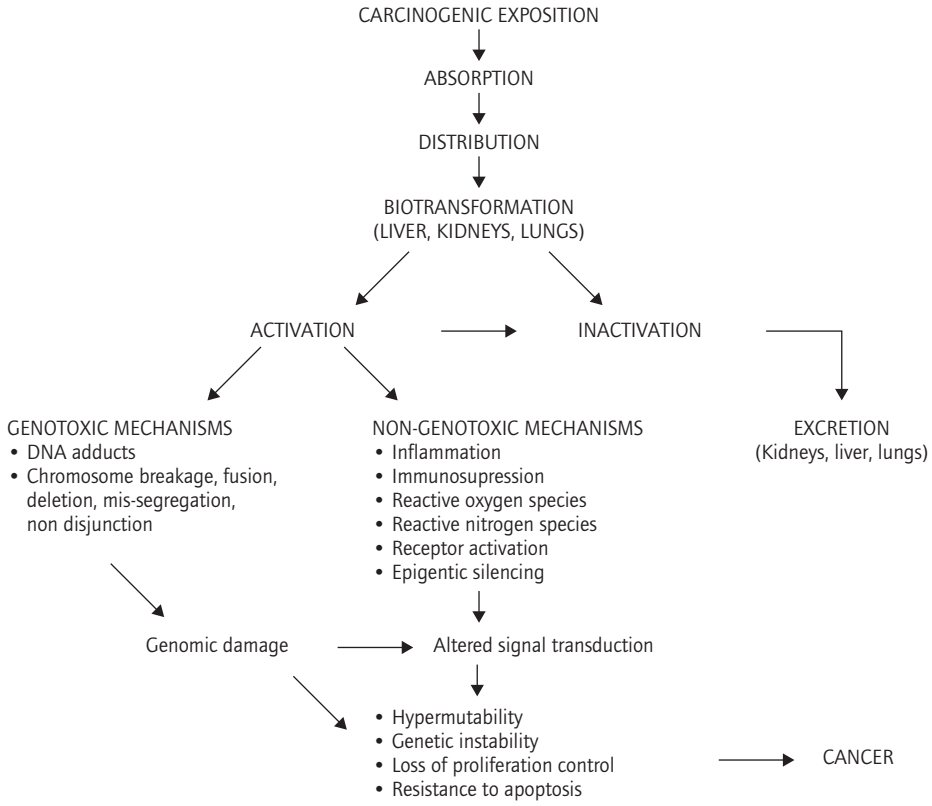
DNA is subject to constant attacks throughout the life of the cell, with DNA lesions usually being efficiently repaired by the repair mechanisms. However, the failure or suppression of the essential gene repair systems, particularly under the influence of environmental factors, can trigger or aggravate a cell transformation process and therefore a carcinogenesis mechanism. DNA replication allows the genetic material from a mother cell to be transmitted to daughter cells during cell division. If the DNA contains unrepaired lesions, this can cause gene mutations, i.e. sequence changes in the DNA molecules transmitted to the daughter cells or rearrangements in the DNA that manifest themselves as chromosome aberrations. Certain genes, such as oncogenes and tumour suppressor genes, are involved in the operation or control of crucial cell functions, such as growth, division, cell differentiation or apoptosis, which allows cell division to be balanced. Mutations occurring in these genes encourage cell transformation and the appearance of a clone of abnormal cells.

Genotoxic carcinogens are therefore capable of altering DNA and causing specific mutations in a gene or gene sequence (chromosome mutations). Some have a clastogenic effect (chromosome breakage) or aneugenic effect (anomalies in the distribution and number of chromosomes).

Tumour promoters do not interact directly with the DNA. They encourage genetic instability and carcinogenesis through various actions: stimulation of oxidative stress processes; proinflammatory effect; action on the immune defence system; involvement of epigenetic mechanisms or endocrine disruption effect, etc.

Numerous chemical substances are in fact pro-carcinogens needing metabolic activation in order to have a carcinogenic effect. The biotransformation that occurs via the body's metabolic pathways is intended to transform such substances into inert compounds so that they can be eliminated. During this process, however, these molecules can be metabolically activated, which means that they become capable of interacting with the DNA or triggering effects encouraging carcinogenesis (cf. Figure 2).

Figure 2 **Metabolic activation of chemical compounds and genotoxic and non-genotoxic actions**



Source: adapted from Oliveira *et al.* (2007)

Among these effects, oxidative stress plays a significant role, essentially by producing reactive oxygen species (ROS) such as free radicals, oxygenated ions and peroxides. These highly unstable oxygenated chemical species attack the cellular components such as the lipid membranes or DNA.

The production of ROS is normal in aerobic living organisms and cells possess an antioxidant system based on enzymes (catalase, glutathione peroxidase, superoxide dismutase, etc.) and small molecules (vitamins C and E). However, the imbalance between these two production and defence phenomena is a mechanism involved in carcinogenesis. It is also involved in the occurrence of chronic cardiovascular, inflammatory and neurodegenerative diseases and in ageing. Among the external agents that particularly cause oxidative stress, we can cite, for example, ionising radiation, ultraviolet radiation, air pollution and chemical agents such as certain pesticides or metals.

3. Concepts in epigenetics

There is now clear evidence that environmental exposures (chemical, physical, psychosocial, etc.) can influence the expression of genes involved in signalling pathways that are key to the cell by changing the genome environment. Epigenetics covers these changes in gene activity in the absence of a change in the DNA sequence. Epigenetic changes alter the chromatin structure[2] and its conformity, allowing gene expression to be altered. The best characterised epigenetic change is DNA methylation.

Epigenetic changes are transmissible during cell divisions, but differ from gene mutations affecting the DNA sequence, due to their potentially reversible character. The critical changes appearing in the cancer cells, such as the activation of oncogenes, the deactivation of tumour suppressor genes and failures to repair the DNA, can be caused not only by genotoxic mechanisms, but also by epigenetic mechanisms. The study of these mechanisms, which may be involved at all stages of carcinogenesis, is therefore essential not only to better diagnose and treat cancers, but also to prevent them. By extracting the DNA and chromatin from the cell nucleus, we can effectively characterise the epigenetic changes associated with environmental exposures and better understand how genes and environment interact to encourage the occurrence of diseases such as cancers.

The epigenome therefore seems to be a real biosensor for cumulative exposures to multiple ‘stressers’ of chemical and other origins. The ubiquity of these mechanisms, their potential involvement in all types of cancer in humans and their reversibility open the door to interesting prospects for identifying new and relevant biomarkers that could be used particularly in epidemiological research and for cancer prevention strategies.

2. Chromatin is the form that DNA takes in the cell nucleus. It is the basic substance of chromosomes and consists of DNA, RNA and proteins. There are two types of protein: histones and non-histone proteins.

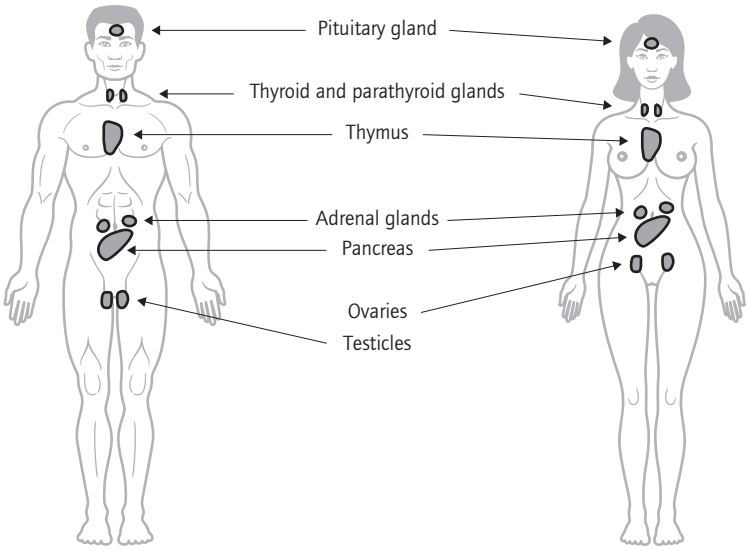
4. Endocrine disruptors and cancers

The reported increase in the frequency of certain cancers such as breast, prostate and testicular cancers, as also in fertility and metabolic problems and urogenital defects in children, is all that is needed to point to the contribution of endocrine disruptor exposures to the increased risks of these pathologies that we are now seeing.

Exposure to endocrine disruptors is in fact suspected of having many adverse health effects in humans: male fertility problems, with a trend towards a reduction in the concentration and quality of sperm; defects in the male reproductive system, such as cryptorchidism (testicular malposition) and hypospadias (urethral malposition); female reproductive problems, such as abnormalities in sexual differentiation, ovarian function, fertility, embryo implantation and gestation; sexual maturation problems (for example: early puberty); disruptions in thyroid function; metabolic disorders, diabetes and obesity; immune system alterations; increased frequency of hormone-dependent cancers such as testicular, prostate, breast and other cancers.

Endocrine disruptors are exogenic substances or mixtures (i.e. foreign to the living organism) that can alter the normal function of the body's hormonal system. This system consists of numerous endocrine glands such as the pituitary gland, thyroid gland, adrenal glands, pancreas, ovaries in women and testicles in men (cf. Figure 3). These organs secrete hormones that are carried by the blood and that are essential to the efficient functioning of the human body, controlling essential functions such as growth and development, body temperature regulation, metabolisms and the reproductive system.

Figure 3 **Endocrine system**



Endocrine disruptors can interfere with a natural hormone at all stages, from hormone synthesis and production, through transport, to binding to a receptor, action or elimination. At cell level, there are multiple potential action mechanisms. An endocrine disruptor can therefore bind to a natural hormone cell receptor (such as oestrogen receptors) and have an agonist effect (imitating the hormone) or, on the other hand, an antagonist effect (blocking the action of the hormone). It can bind to other types of receptor that are not hormone-specific, disrupt cell signals, interfere with the genome or epigenome pathways, and so on. The most common disruption effects involve the disruption of oestrogen, androgen, thyroid hormone and cortisol activity and the disruption of the metabolic functions of carbohydrates and lipids.

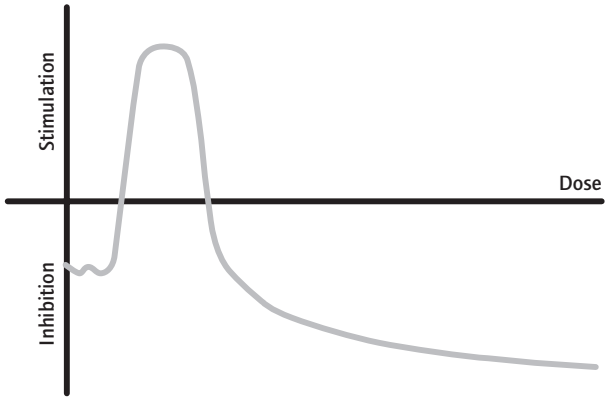
Numerous substances with an endocrine disruption effect are used in or produced by industry: plasticisers such as bisphenol A (BPA) are used in the manufacture of rigid and transparent polycarbonates (baby bottles, recyclable bottles, etc.) or are found in the epoxy resins of beverage can coatings; phthalates, which make plastics more flexible and facilitate their shaping, are present in many PVC articles; polybrominated flame retardants are used in the composition of furniture foams, carpets and electronic equipment; perfluorinated compounds are used in many industrial applications (non-stick coatings of kitchen utensils, treatment of textiles, packaging, etc.); reprotoxic glycol ethers have commonly been used for their solvent properties, particularly in paints, inks or adhesives; parabens are particularly used in the cosmetics industry; persistent organic pollutants such as polychlorobiphenyls (PCBs) were previously used in electric transformers or certain pesticides (DDT, chlordecone, etc.).

In terms of work environments, several major industries are affected with regard to both production and use: pharmaceutical and chemical industries, agriculture, etc. Dermal exposures may predominate, such as for BPA and the exposure of cashiers or printers when handling thermal tickets, glycol ethers for painters, or pesticides among appliers. Inhalation is sometimes the main route, involving exposures to certain metals like lead. Dietary intake, a predominant route for certain endocrine disruptors such as BPA, can be added to the other routes of entry into the body.

Currently, the risks of occupational exposure to endocrine disruptors are still largely invisible. The health effects vary significantly depending on the substance – with these effects potentially appearing in the offspring of men and women who have been exposed – and on the specific conditions of exposure. Three important concepts need to be taken into account in this respect when assessing and preventing risks: the possibility of low-dose effects with specific dose-effect relationships; not uncommon co-exposures to several endocrine disruptors; and the critical periods of exposure, particularly the perinatal period when the mother becomes pregnant.

For certain endocrine disruptors such as BPA, experimental studies report specific non-monotonic dose-response or dose-effect relationships (cf. Figure 4).

Figure 4 **Inverted U curve (non-monotonic effect)**



The low-dose effects observed therefore seem to be greater, even in comparison to those observed at an average or high dose.

The diethylstilbestrol (Distilbene®) case, in which it was observed that cancers could originate in the foetus, has promoted the concept of an 'exposure window'. Much work currently indicates that, at certain critical periods (prenatal and perinatal periods and puberty), the body is particularly sensitive to endocrine disruptors, with the effect becoming apparent at a much later stage. Research, particularly experimental research conducted on several animal or human lines, also shows that effects can be transmitted to offspring or subsequent generations, particularly carcinogenic effects. Mother-child cohorts are currently being monitored to confirm these effects caused by a range of endocrine disruptors.

Lastly, the issue of low-dose cocktail effects is also the focus of current thinking on endocrine disruptors, given exposure to complex mixtures in food or the environment. Substances can interact, resulting in additive, synergistic or sometimes antagonistic effects.

One substance can also sometimes have multiple effects, for example both carcinogenic or mutagenic and endocrine-disrupting. This is the case with Distilbene®, a medication which has caused vaginal, breast and uterine cancers in the daughters of treated women, the insecticide Chlordecone (Kepone) for prostate cancer in the Antilles, or dioxins such as 2,3,7,8-TCDD, which is classified in Category 1 (known carcinogen) by the International Agency for Research on Cancer (IARC). Associations between exposure to other endocrine disruptors such as pesticides and plasticisers (BPA, phthalates) and the occurrence of various hormone-dependent cancers (breast, thyroid, uterine, prostate, ovarian, testicular) have been observed in certain experimental or epidemiological studies.

Given the very large number of substances on the European market for which the level of toxicological information is insufficient, we must improve our knowledge of these

mechanisms so that the toxicity pathways activated by endocrine disruptors can be better understood and described. This approach involving the toxicity pathways, which may allow action to be taken without waiting for sufficient information on the thousands of substances, is the subject of major research programmes initiated at international level (see the exposome concept further on).

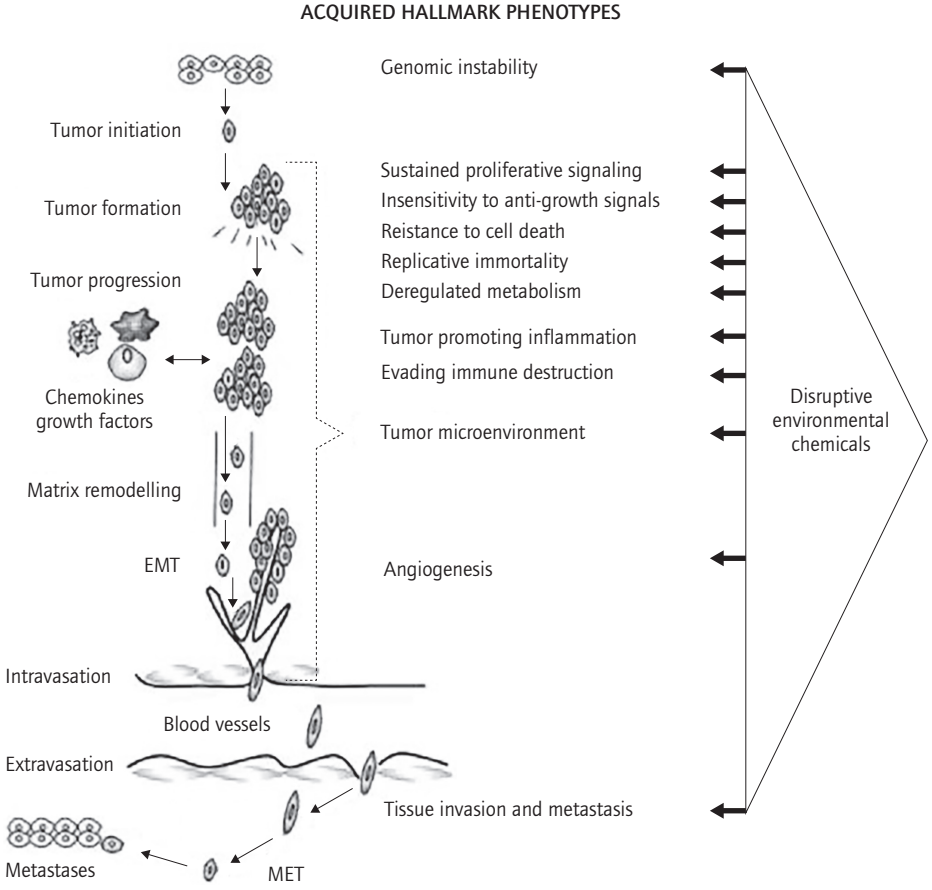
5. The issue of low doses and mixtures of chemical agents

Advances made in understanding the complexity of carcinogenesis, and more generally the mechanisms of interaction between toxic substances and the genome and epigenome, justify the extent of the research currently being carried out to better assess the potential effects of mixtures of chemical agents at low doses in combination with lifelong exposures, from the perinatal period to the end of the working life and beyond. Significant gaps still exist in the understanding of toxicity data for numerous substances that are, however, widely used. For example, only 50 % of the chemical agents classified by the EPA (the US Environmental Protection Agency) as high production volume chemicals have undergone minimum carcinogenicity testing, while the possibility of low-dose effects, often not anticipated and studied, further complicates the issue.

In a recent scientific review, a group of researchers identified several examples of non-monotonic dose-effect relationships for substances for which the low-dose effects could not be predicted in advance through those observed at a high dose. These environmental disruptors may, depending on the situation, affect the various stages of carcinogenesis by influencing the acquisition of the phenotypic characteristics of cancer cells (cf. Figure 5), in particular: genomic instability and mutations, which allow DNA changes to be transmitted from one cell to daughter cells via not only mutations but also epigenetic changes; inflammation which, in addition to disrupting immune defence adaptation phenomena, encourages the growth of tumour cells and contributes to their survival, to angiogenesis and to the metastatic process; and deregulation of the cell metabolism. Said scientific review cites several dozen examples of disrupting substances capable of acting on the key mechanisms of carcinogenesis (numerous metals, pesticides, various organic compounds, endocrine disruptors, nanomaterials, etc.), with over half potentially having these low-dose effects, some with a non-monotonic dose-effect relationship profile.

Growing knowledge of the biology of cancer therefore suggests that the cumulative effects of these chemical substances involve various pathways that are relevant for assessing the carcinogenic risk, with the possibility of synergistic mechanistic effects not necessarily taken into account in the current methods of regulatory assessment of the risks posed by chemical substances, based on common toxicity mechanisms or modes of action.

Figure 5 **Disruptive potential of environmental exposures to mixtures of chemicals**



Source: adapted from Goodson III W.H. *et al.* (2015)

6. The exposome concept

All the current concepts described here concerning epigenetics, endocrine disruptors, low doses, cumulative and integrated exposures, and the possibility of cocktail effects ultimately lead to the more general exposome concept. This term was proposed by C. Wild to describe all the environmental exposures of an individual throughout his/her life, i.e. from the period of conception. The "environmental exposures" concept is very broad as it not only covers all the chemical, physical and biological exposures, but also the behavioural, psychological, social and other factors. In particular, the relationship between the external environment and the internal environment of the organs and cells, themselves subject to multiple influences from the external exposures combined with the influences of various internal regulation systems (hormone metabolism, intestinal microflora, inflammation, oxidative stress, ageing, etc.), is a crucial element of this concept, ultimately leading to exposomes for these various targets being taken into account.

Lastly, as seen above with endocrine disruptors, the timing of the exposure, encompassing critical periods in life such as the perinatal period, is an important dimension. C. Wild in fact highlights that, 'unlike the genome, the exposome is a highly variable and dynamic entity that evolves throughout the individual's life': hence the difficulty in terms of measurement, needing to incorporate both the qualitative and the quantitative dimension. Given the rise in chronic diseases, he rightly insists on the need for public health to be capable of developing exposure measurement methods that can be as precise as those developed for describing the human genome. The contribution of the '-omics' technologies, used to better understand the mechanisms of pathologies such as cancer, could therefore help in assessing exposures by establishing the signature or fingerprint of specific external environmental exposures combined with internal factors. This involves examining, for example, whether these exposures result in measurable changes to the epigenome, transcriptome (the set of all RNA messengers), metabolome (the set of all metabolites and small molecules) or proteome (the set of all proteins). The challenge is also to validate, from that point, relevant effect and exposure biomarkers that can be used to monitor the population, in particular through large cohorts formed from the general or working population.

The importance of these methodological developments is entirely clear as they have the potential to allow the effects of combined exposures, cocktails of chemical agents or combinations of chemical, physical and other exposures to be predicted. Being able to study the potential toxic or carcinogenic effects of the mixtures of chemical agents most commonly found, for example, in food or water is clearly relevant, but it is impossible to do this for the infinite number of combinations of the thousands of chemical compounds to which humans are potentially exposed. Thanks to current methods, it is, however, now possible to quickly identify, for a contaminant or for categories of contaminants, the activated receptors or signalling pathways at cell level, as well as the main toxicity pathways. This in turn allows us to study the interactions between toxicity pathways and establish predictive toxicity modelling tools for numerous families of substances (databases, mapping, etc.), as is currently happening in some major international programmes.

Ultimately, all these current concepts clearly illustrate the huge complexity of today's environmental exposures and risks. Being able to tackle the challenge of understanding this complexity within risk assessment, by integrating new concepts and appropriate methods (biology, modelling, etc.), is a major concern for occupational, environmental and public health and prevention.

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