Development of a database on key characteristics of human carcinogens

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ABSTRACT
A database on mechanistic characteristics of human carcinogenic agents was developed by collecting mechanistic information on agents identified as human carcinogens (Group 1) by the International Agency for Research on Cancer (IARC) in the IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. A two-phase process is described for the construction of the database according to 24 toxicological endpoints, derived from appropriate test systems that were acquired from data obtained from the mechanisms sections of the IARC Monographs (Section 4) and a supplementary PubMed search. These endpoints were then aligned with 10 key characteristics of human carcinogens that reflect the broader attributes of these agents relating to the development of cancer in humans. The considerations involved in linking of toxicological endpoints to key characteristics are described and specific examples of the determination of key characteristics for six specific agents (tamoxifen, hepatitis B virus, arsenic, ultraviolet and solar radiation, tobacco smoking, and dioxin) are provided. Data for humans and animals were tabulated separately, as were results for in-vivo and in-vitro sources of information. The database was constructed to support a separate analysis of the expression of these endpoints by 86 Group 1 carcinogens, in-vivo and in-vitro along with an analysis of the key characteristics of these agents.

KEYWORDS
human carcinogens; mechanisms of carcinogenesis; genotoxic; toxicological endpoint

Introduction
The majority of the early observations of chemical carcinogenesis linked personal exposures with certain types of cancer such as scrotal cancer in chimney sweeps or lung cancer in cigarette smokers. Subsequently, etiologic attention focused on the identification of the causative agents of cancer along multiple lines of investigation (Loeb and Harris 2008). Experimental research on polycyclic aromatic hydrocarbons (PAH) commenced a century ago (Yamagiwa and Ichikawa 1915), followed by studies on other chemical carcinogens, such as dimethyl-nitrosamine (Magee and Farber 1962), aflatoxins (Croy et al. 1978; Wogan 1966), and benzo[a]pyrene (Carrell et al. 1997; Sims and Grover 1974). The (1) identification of DNA as the genetic material regulating fundamental biological processes at the cellular and molecular level, (2) discovery of metabolic activation as a key process in chemical carcinogenesis (Miller and Miller 1966), and (3) growing insight into the role of DNA damage in aberrant cell function were key factors in achieving a more comprehensive understanding of mechanisms underlying human cancer development. Early descriptors of the mode of action of carcinogens were often based upon assays of ‘gross’ cytogenetic events including DNA strand-breakage and formation of micronuclei (MN) and chromosomal aberrations (CA). Most of the early mechanistic studies focused on the ability of agents to induce genotoxic effects. Recent advances in molecular and cell biology have elucidated the critical role of a number of cellular and molecular processes and pathways – including transcription factors, signaling molecules, and epigenetic events – involved in chemical-mediated carcinogenesis. In parallel with breakthroughs in molecular biology, advances in applying
high-throughput microarrays, toxicogenomics, and computational systems-biology have also increased our understanding of mechanisms underlying cancer occurrence (Krewski et al. 2011).

Molecular and genetic epidemiology provide knowledge regarding the biological mechanisms underlying human cancer development, including factors affecting individual susceptibility. This has led to a more comprehensive understanding of the role of polymorphic gene variants and gene-environment interactions in chemical-initiated carcinogenesis. Early conclusions regarding factors affecting inter-individual variation in response to carcinogen exposure were based upon investigations on the influence of the metabolism of carcinogens and on the production of DNA damage (Loeb and Harris 2008; Perera et al. 1982). More recently, the importance of the role of the cellular epigenome, cell signaling, apoptosis, inflammation, immune modulation, and receptor-mediated effects in cancer initiation and promotion has become understood (Sever and Brugge 2015). At present, the assessment of the mechanisms underlying cancer induction by different agents considers both functional and structural changes induced by carcinogens, in the context of multiple molecular mechanisms of action (Coleman and Tsongalis 2006). This, in turn, may shed light on the complex interactions among different agents that may increase human cancer risk (Guyton et al. 2009).

Although carcinogenesis is a complex process, common mechanistic characteristics and toxicological endpoints may be identified through examination of the biological processes that are modified by human carcinogens. A database on mechanistic characteristics of these agents was developed by collecting mechanistic information from the IARC Monographs. This database was subsequently used by Krewski et al. (2019) to analyze the key characteristics of human carcinogens developed by Smith (2019). The present study describes the construction of the database according to the key characteristics of human carcinogens, which was conducted under the guidance of participants in the IARC Workshops on ‘Tumour-site Concordance and Mechanisms of Carcinogenesis’, which convened in Lyon, in April and November 2012.

The development of the components of the database proceeded in two stages. At the initial meeting, the Workshop participants developed a list of 24 toxicological endpoints postulated to be related to the causation of human cancer. Data supporting these toxicological endpoints were retrieved from the IARC Monographs. At the second meeting, a list of 10 Key Characteristics of human carcinogens was adopted by the Workshop. A database of these characteristics was then developed by assigning each of the 24 toxicological endpoints to their appropriate key characteristic.

To populate the IARC cancer-mechanisms database, Section 4 on ‘Mechanistic and other relevant data’ of each Monograph that presented a Group-1 evaluation was examined to identify information pertaining to 24 toxicological endpoints. For each Group-1 agent, information on these endpoints was entered into the database, with separate entries for human in-vivo and in-vitro studies and for animal in-vivo and in-vitro investigations. Summary indicators for humans and animals were obtained by combining results across in-vivo and in-vitro sources of information for each agent; similarly, summary indicators for in-vitro and in-vivo sources were obtained by combining results across human and animal sources of information. Finally, an overall indicator of the expression of each endpoint combined across in-vivo/in-vitro sources was derived.

In addition to using the IARC Monographs as the main source of mechanistic information, a supplementary PubMed search was conducted to identify additional information available outside the Monographs. This search was not intended to be a comprehensive systematic review of the scientific literature on the mechanisms underlying human cancer occurrence but was conducted to verify whether important mechanistic results may have been missed in the Monographs or published after the time of their publication. Particular attention was paid to recent publications on epigenetics, since this area was still in development at the time when Monograph Volume 100 was compiled.

The main mechanistic analyses are reported by Krewski et al. (2019 this Volume), based upon information abstracted from the Monographs. It should be noted that the supplementary PubMed search did not comprise a complete systematic
review of the literature on toxicological endpoints expressed by these agents, as this would not have been feasible for all 86 Group-1 agents included in the mechanisms database.

**Toxicological endpoints in carcinogenesis**

**Development of the toxicological endpoints**

The participants identified 24 toxicological endpoints that may be related to cancer induction, including cellular and molecular changes associated with different stages of carcinogenesis. These endpoints included: DNA damage, oxidative stress, protein adducts, clastogenic effects, gene mutations, epigenetic alterations, changes in gene expression, alterations in cell-signaling pathways, metabolic activation, susceptibility, immune effects, chronic inflammation, cell death, chronic irritation, cell-cycle effects, DNA-repair alteration, receptor-mediated effects, hormonal effects, angiogenic effects, alterations in telomere length, inhibition of gap-junctional intercellular communication, bystander effects, immortalization and differences in absorption, distribution, metabolism and elimination (ADME). These endpoints are listed and defined in Table 1, along with prototypical assays that may be used to identify agents expressing these endpoints.

**Perspectives on the toxicological endpoints**

Evidence accumulated from molecular epidemiological studies demonstrated that the risk of chemically induced cancer may vary in different individuals as a function of inherited factors such as individual genotype as well as acquired factors such as environmental exposures (Rothman et al. 2001). On the other hand, epidemiological investigations amongst migrant populations showed greater importance of lifestyle as compared with genetics as a cause of cancer. This finding implies that many cancers might be prevented by lifestyle changes that reduce exposure to carcinogens. Risk is also affected by agent-related factors such as exposure conditions including dose, frequency, and duration as well as the health and nutritional status of the host. These variations in risk are most likely mediated by key mechanistic pathways involved in carcinogenesis.

Cancer risk may vary at different life stages. Exposures to exogenous agents might elicit different types and levels of adverse responses according to the age of the exposed individual. This may be related to different levels of exposure: the risk of ovarian cancer, for example, varies in response to reproductive characteristics such as hormone use and menopausal status (Moorman et al. 2008). In other cases, the risk of cancer occurrence may vary as a result of age-related changes in cellular structure or function. Das and Tyler (2013) noted the functioning of the chromatin apparatus appeared to change during aging, which might lead to alterations in genomic functions such as transcription and replication. DNA repair is another molecular process that decreases in effectiveness with age (Garm et al. 2013).

Apart from the obvious case of gender-specific cancers such as ovary or prostate, differences between females and males may be important in the response to agents that induce other types of cancer. Hochstenbach et al. (2012) reported gender differences in response to carcinogen exposure *in utero*: after dietary genotoxic and non-genotoxic treatments differences were observed in gene expression linked with the cell cycle, immune system, and more general cellular processes including post-translational modification. Levels of DNA methylation induced by prenatal exposure to cadmium were found to differ between male and female offspring (Kippler et al. 2013).

Genotype differs by race/ethnicity, and, consequently, the prevalence of variants in genes encoding critical metabolic enzymes or signaling molecules may vary in different groups. This may help explain variations in cancer incidence in different populations exposed to similar types and levels of carcinogenic agents (Derby et al. 2009; Park et al. 2015, 2014).

Gene–environment interactions are apparent in studies on biomarkers of biological effects such as DNA adducts (Iyer et al. 2014; Nock et al. 2007). Genetic variants might also interact differently with molecular mediators of pathways related to apoptosis, cell proliferation, and neoplastic processes, these being key processes in mutagenicity and carcinogenicity attributed to exposure to heavy metals (Koedrith et al. 2013; Kwon et al. 2013). Polymorphisms in key enzymes involved...
Table 1. Definitions of the 24 toxicological endpoints involved in carcinogenesis, and prototypical assays for each endpoint.

| Toxicological Endpoint | Definition | Description/Prototypical Assays |
|------------------------|-----------|---------------------------------|
| 1. DNA damage          | DNA damage is an alteration in the chemical structure of DNA, such as a DNA adduct, a break in a DNA-strand, a base missing from the DNA backbone, or a chemically changed nucleobase such as 8-oxodG. | A. Direct evidence of DNA damage—this category includes nuclear and mitochondrial DNA damage (in vitro or in vivo): 1. DNA adducts (NOTE: if detection is specifically focused on endogenously produced oxidative adducts such as 8-oxodG, this will be classified under oxidative damage, see below) Assays: a. Detection of radiolabel in isolated DNA, typically [3H] or [14C] followed by liquid scintillation counting b. UV-spectroscopy, fluorescence spectroscopy, NMR c. [32P]-Postlabelling followed by high-performance liquid chromatography (HPLC) or thin-layer chromatography (TLC) d. Accelerator mass spectrometry e. Immunoassays f. Mass spectrometry (typically GC-MS) g. 2D liquid chromatography/tandem mass spectrometry (LC/MS/MS) h. High-performance liquid chromatography (HPLC) interfaced with an electrochemical detector to detect ‘depurinating’ adducts as N7-alkylguanines 2. DNA strand-breaks (single- and/or double-strand breaks) Assays: a. Comet assay (single-cell gel electrophoresis) b. Alkaline elution assay c. Assays specific to DNA double-strand breaks (dsb), e.g. detection of factors specific for dsb (e.g. γH2AX) by immunofluorescence 3. DNA–protein cross-links; DNA–DNA cross-links a. Alkaline elution and comet assays b. Chromatin immunoprecipitation (ChIP) assays c. Selective K/Na dodecyl sulfate (SDS) precipitation of DNA associated with protein. |
|                        |           | B. Indirect indicators or biomarkers of DNA damage (in vitro or in vivo): 1. Sister chromatid exchange (SCE) 2. Unscheduled DNA synthesis (UDS) 3. Mitotic recombination and aneuploidy (mammalian and non-mammalian cells—e.g. Saccharomyces cerevisiae) 4. Chromosomal aberrations in plants (Tradescantia, Allium, Vicia) 5. Prokaryotic DNA damage and induction of DNA repair (e.g.umu test, prophage-induction test, rec differential survival test, SOS chromotest) 6. Formation of protein adducts (as indirect indicators of DNA adducts) |

(Continued)
| Toxicological Endpoint | Definition                                                                                                                                                                                                 | Description/Prototypical Assays |
|-----------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------|
| 2. Oxidative stress   | Oxidative stress is defined as a disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses within the cell. This compromises the cell’s ability to detoxify reactive intermediates or to repair the resulting damage. The effects of oxidative stress depend upon the extent of these changes, with a cell being able to overcome small perturbations and regain its original state. More severe oxidative stress can cause cell death and even moderate oxidation can trigger apoptosis, while more intense stresses may cause necrosis. Oxidative stress can be associated with a significant decrease in the effectiveness of antioxidant defenses, such as the glutathione response. | A. Cellular redox state          |
|                       | **Assays:**                                                                                                                                   | 1. Measurement of reactive oxygen species (ROS) with 2′,7′-dichloro-dihydrofluorescein diacetate (DCFH-DA) |
|                       | 2. Measurement of GSH/GSSG ratio                                                                                                               | B. DNA oxidation, oxidative DNA damage |
|                       | **Assays:**                                                                                                                                   | 1. Mass spectrometry             |
|                       | 1. Measurement of 8-oxodG by means of HPLC, combined with electrochemical detection                                                          | 2. Analysis of 8-oxodG by means of HPLC, combined with electrochemical detection |
|                       | 3. Comet assay combined with formamido-pyrimidine DNA-glycosylase (FPG) digestion                                                            | 3. Comet assay combined with formamido-pyrimidine DNA-glycosylase (FPG) digestion |
|                       | **Assays:**                                                                                                                                   | C. Lipid peroxidation             |
|                       | 1. Detection of modified lipids by matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS).                                     | 1. Detection of modified lipids by matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS). |
|                       | 2. TBARS (thiobarbituric acid-reactive substances) assay for detection of malondialdehyde (MDA)                                                | 2. TBARS (thiobarbituric acid-reactive substances) assay for detection of malondialdehyde (MDA) |
|                       | **Assays:**                                                                                                                                   | D. Oxidized proteins             |
|                       | 1. Protein carbonyl colorimetric assay                                                                                                         | 1. Protein carbonyl colorimetric assay |
| 3. Protein adducts    | Protein adducts are complexes formed when chemicals bind to protein molecules. They have been used in the assessment of carcinogen dose. Protein adducts are biomarkers of exposure to reactive xenobiotics which could also produce DNA adducts and lead to mutations. They are sometimes considered as indirect indicators/predictors of DNA damage. Alterations in protein function caused by adducts might also be important in the disruption of cellular control, which may lead to cancer. | Detection of protein adducts      |
|                       | **Detection of 3,4-Dihydroxy-L-phenylalanine as a biomarker of oxidative damage in proteins**                                                  | - 3,4-Dihydroxy-L-phenylalanine as a biomarker of oxidative damage in proteins |
|                       | **Immuno-complex of enzyme (ICE) assay for detection of DNA–protein covalent complexes (DPCCs)**                                               | - Immuno-complex of enzyme (ICE) assay for detection of DNA–protein covalent complexes (DPCCs) |
| 4. Clastogenic effects| The disruption or breakage of chromosomes, leading to sections of the chromosome being deleted, added, or rearranged                             | In vivo or in vitro              |
|                       | **In vivo or in vitro**                                                                                                                       | Chromosomal aberrations          |
|                       | **In vivo or in vitro**                                                                                                                       | Micronuclei                       |
|                       | **In vivo or in vitro**                                                                                                                       | Aneuploidy                       |
|                       | **In vivo or in vitro**                                                                                                                       | Abnormal karyotype               |
| Toxicological Endpoint | Definition | Description/Prototypical Assays |
|------------------------|------------|---------------------------------|
| 5. Gene mutation       | A change in the normal nucleotide sequence in cellular DNA. Mutations can be silent or produce alterations in mRNA leading to abnormal protein expression. They are usually caused by errors during DNA replication (often due to the presence of non-repaired adducts) or as result of DNA damage such as strand-breaks that could not be restored by DNA-repair mechanisms. These mechanisms often lead to base substitution, insertion, or deletion of one or more base pairs. They can produce major chromosomal restructuring (see clastogenic effects). Most carcinogens induce mutations via DNA adducts. Mutations relevant to cancer may occur in oncogenes (e.g. *K-RAS*), tumour-suppressor genes (*TP53*, *Tsc*, *VHL*) or genomic instability genes (e.g. genes encoding DNA-repair proteins). | Reversions and forward mutations in microorganisms. Mutations affecting oncogenes, tumour-suppressor genes, and other genes involved in cell-cycle control. In vitro 1. Ames assay (reversions) in *Salmonella typhimurium*, and reversions and forward mutations in *E. coli* 2. Other non-mammalian species—e.g., yeast or fungus (*Saccharomyces, Aspergillus*) 3. Mammalian mutation assays in endogenous genes used as markers for mutation—e.g. *Tk* (including mouse lymphoma assay), *Apt*, *Xprt*, *glycophorin A*, *Hprt/animal, HPRT/human* In vivo 4. Mammalian gene-mutation assays a. Transgenic rodent assays (*MutaMouse, BigBlue rat or mouse*) b. Rodent dominant lethal assay —embryonic death indicating mutation and chromosomal aberrations in male germ cells c. Mouse specific-locus mutation assay — germ-cell mutations in the male parent identified by phenotypic changes in the offspring d. Mouse spot test — somatic mutations in embryonic melanoblasts after transplacental exposure, identifiable in the offspring as spots of different colour in the coat 5. Mutation assays in non-mammalian species a. Plants (*Tradescantia, Allium, Vicia*) b. Insects (*Drosophila melanogaster*) i. Sex-linked recessive lethal assay — identifies heritable mutations in offspring ii. Somatic Mutation and Recombination Test [SMART] — identifies somatic mutations in wing (wing-spot test) and eye cells (eye-mosaic assay system) 6. Epigenetic effects Epigenetic events are cellular and physiological changes that are heritable, and not caused by alterations in the DNA sequence. Epigenetics describes the study of stable, long-term changes in the transcriptional potential of a cell. These effects can be caused by factors such as altered methylation of DNA, microRNA expression, and changes in chromatin and histone structure. DNA methylation, histone modification; alterations in microRNA expression in relevant genes 7. Changes in gene expression Alterations in the expression levels of genes that are active in the cell cycle and in related facets of cellular function. These changes frequently arise through the promotion of epigenetic effects. They can also be induced through a direct effect of the agent or via alterations in intracellular signalling, etc. Alterations in mRNA or microRNA expression in relevant genes and pathways. Epigenetic changes in genes associated with genomic instability (DNA replication and repair genes). 8. Alterations in cell-signalling pathways The ability of the agent to interfere with cell- signalling pathways leading to expression of carcinogenic trait/phenotype in the cell, e.g. facilitating cell invasion or induction of gene promotion for inflammatory mediators and oncogenes. Alterations in cell-signalling pathways such as the *Ras* pathway, the COX-2 pathway, the mitogen-activated protein kinase (MAPK) pathway, the ATM-p53 pathway |
Table 1. (continued).

| Toxicological Endpoint | Definition | Description/Prototypical Assays |
|------------------------|------------|--------------------------------|
| 9. Metabolites (reactive) | The agent under study is not itself reactive with DNA or other key cellular components. Instead, it requires enzymatic biotransformation (metabolic activation) in organs such as the liver, to produce active metabolites that are usually electrophilic. | Examples of metabolic activation include:  
1. Formation of an alkylating agent  
2. Oxidation to epoxide metabolites  
3. Formation of aryl nitrenium ion  
These can be identified through metabolism studies involving analysis of tissues and body fluids, and are often indicated by a positive result in mutagenicity assays observed only in the presence of a liver extract. |
| 10. Susceptibility | Susceptibility refers to individual variation in risk of developing cancer. This can arise from a range of factors including the presence of one or more inherited gene mutations (often marked by a family history that indicates an increased risk of disease) or exposures early in life (i.e. in utero, trans-placental, early postnatal, lactational) (Anderson et al. 2000). | Susceptibility may be gauged via variations in genotype (vulnerability or genetic predisposition), or by developmental stage (life stage).  
Genetic variation can be measured in vivo by analysis of single-nucleotide polymorphisms (SNPs), which may reveal different functions for critical gene variants. |
| 11. Immune effects | The immune system is a key factor in the response of the body to external foreign agents, particularly viruses, bacteria and parasites. Adverse effects on the functioning of the immune system can also result from exposure to chemical substances. The immune system has a major role in the inflammatory response to injury. Altered immune function may lead to an increased incidence or severity of infectious diseases or to cancer when the ability of the immune system to respond adequately to invading agents is suppressed. The inflammatory response to an agent can release cytokines and other factors that contribute to carcinogenesis. | Measures of altered function of the immune system that may lead to increased cancer risk (e.g. HIV-related effects)  
Mouse splenocyte assay |
| 12. Inflammation (chronic) | Many cancers arise from sites where chronic inflammation is observed. The tumour microenvironment, which is largely orchestrated by inflammatory cells, is an indispensable participant in the neoplastic process, fostering proliferation, survival and migration. In addition, tumour cells have co-opted some of the signalling molecules of the innate immune system, such as selectins, chemokines and their receptors for invasion, migration and metastasis. | Chronic inflammation leading to oxidative DNA damage and other lesions (etheno-dG, M$_2$G).  
Assays: light microscopy, cytokine assays, gene-expression profiles |

(Continued)
| Toxicological Endpoint | Definition | Description/Prototypical Assays |
|------------------------|------------|---------------------------------|
| 13. Cell death         | Programmed cell death (apoptosis) is one of mechanisms by which a cell protects itself from the consequences of DNA damage. In the presence of severe damage, the cell initiates a cascade that leads to its own destruction. Other signals can also trigger this effect. TP53 has a major role in the integrity of this process. Defects in programmed cell death can cause cancer (Tagawa et al., 2005). Evasion of apoptosis is a requirement for both neoplastic transformation and sustained growth of cancer cells (Hanahan & Weinberg, 2000, 2011). | Detection of cell death, including both inhibition and induction of apoptosis, autophagy, and necrosis. **Assays:** 1. Apoptosis-specific assays: a. TUNEL (TdT-mediated dUTP nick-end labelling) assay b. ISEL (in situ end labelling) c. DNA-ladder analysis for detection of DNA fragmentation d. Annexin-V analysis (membrane integrity) e. Detection of apoptosis-related proteins encoded by p53, Fas, Bcl-2/Bax (ratio), cytochrome c, caspases f. Light-microscopic evidence of apoptotic nuclei 2. Cytotoxicity and cell-viability assays a. Clonogenic cell survival b. Trypan-blue or propidium-iodide exclusion (membrane integrity) c. Cell-suspension counts by use of haemocytometer (manual) or Coulter counter (automated) d. Lactate-dehydrogenase (LDH) assay (membrane integrity) e. MTT (dye) and related tetrazolium salts such as MTS, XTT, or WSTs (water-soluble tetrazolium salts) — colorimetric assays f. ATP-based bioluminescence assays g. Sulfo-rhodamine B (SRB) assay h. Light-microscopic evidence of necrotic nuclei i. Light-microscopic evidence of missing cells in solid tissues j. Failure of appropriate background growth (Ames for cytostasis) or altered growth (e.g. decreased numbers of nuclei present for scoring in SCE assays). |
| 14. Irritation (chronic) | Chronic irritation can arise from a variety of external factors such as repetitive trauma and, exposure to acid (e.g. gastric acids). These factors create an environment of chronic inflammation which contributes to cancer (see endpoint 12, chronic inflammation). | Chronic irritation leading to chronic inflammation (see 12, above). |
| 15. Cell-cycle effects  | Cellular replication is controlled by a complex network of factors that regulate the cell-division cycle. These factors are responsible for preventing cell division in the presence of unrepaired DNA damage. Cell-cycle effects refer to an alteration of the functioning of this complex series of signalling pathways. | Detection of alterations in cell proliferation and cell-cycle effects (e.g. DNA replication changes, cell-cycle control, ploidy), mitogenesis. **Assays:** 1. Replicative DNA synthesis (RDS) 2. BrdU labelling 3. Proliferating cell nuclear antigen (PCNA) labelling. 4. Light-microscopic evidence of hyper-plasia (e.g. thickening of epithelium). 5. Light-microscopic evidence of bi-nucleate cells. 6. Flow cytometry |
| 16. DNA-repair alteration | To preserve genomic integrity, cells contain multiple mechanisms among which repair systems are very important. These can involve repairing DNA damaged by adducts, strand breaks, etc. Key repair mechanisms include: base-excision repair (BER) and nucleotide-excision repair (NER). Inherited abnormalities in DNA-repair function lead to enhanced cancer susceptibility (e.g. Xeroderma pigmentosum). | A. Inhibition of DNA-repair enzyme production or activity, loss of fidelity. B. Induction of DNA repair or transition from one repair pathway to another. C. mutations in DNA-repair enzymes. |
| Table 1. (Continued). |
|-----------------------|
| **Toxicological Endpoint** | **Definition** | **Assays** |
| 17. Receptor-mediated effects | Receptor-mediated effects are those that result from extracellular signalling by an agent that activates a specific receptor located on the cell surface. In turn, this signalling can lead to a response, depending on the receptor and downstream signal pathways. | Endocrine profiles, immunohistochemical density measurement, functional assays to assess the induction of biological effects of chemicals and analogues. |
| 18. Hormonal effects | Hormones are substances secreted by the body to control homeostasis, reproduction, development, and/or functioning of local or distant tissues (e.g., insulin and its control on glucose metabolism). External agents can interfere with the synthesis, secretion, transport, binding, functioning, or elimination of natural hormones in the body. These agents can also demonstrate a reactivity similar to that of endogenously produced hormones, thereby disturbing homeostasis. | Endocrine profiles, mammographic density measurement, ovariectomized animal model (to assess the role of endogenous ovarian function). |
| 19. Angiogenic effects | Angiogenesis refers to the process of inducing the growth of blood vessels. This physiological function is essential for growth and maintenance of organs and tissues. Tumour growth is characterized by the development of new blood vessels in the tumour tissue through secretion of various growth factors (e.g., vascular endothelial growth factor, VEGF). This is commonly considered to be a hallmark of a tumour rather than a characteristic of an exogenous agent. | Change in pro-angiogenesis factors, such as basic fibroblast growth factor (b-FGF). |
| 20. Alterations in telomere length | Telomeres are found at the ends of human chromosomes that form repetitive DNA sequences that act as the end of chromosomes. During each cycle of cell division, the telomere length is reduced, eventually leading to cellular senescence. Activation of a telomerase that prevents loss of telomere length leads to cell immortalization and tumorigenesis. | Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of extracted RNA to measure expression levels of the telomerase components. |
| 21. Inhibition of gap-junctional intercellular communication (GJIC) | Gap junctions are plaque-like structures on the cellular plasma membrane that form channels between cells, allowing the transmission of small molecules and ions between adjacent cells. Disruption of these communication pathways can cause loss of contact inhibition, which may lead to abnormal cell growth. | Inhibition of gap-junctional intercellular communication (GJIC) causes loss of contact inhibition between cells. Assays: 1. Transformation assay with Syrian hamster embryo cells. 2. Increased motility and invasiveness of cancer cell lines. |
| 22. Bystander effects | First identified in radiobiology, the bystander effect refers to the situation where non-irradiated cells exhibit effects caused by radiation as a result of chemical signals (messengers) received from nearby irradiated cells. These effects are often mediated through gap-junctional transfer of chemical agents. In cell culture, normal cells undergo a fixed number of replication cycles before they enter the senescence stage where the cell cycle is normal and senescence will prolong. Immortalization is frequently associated with activation of telomerase (see endpoint #20). | Assays: 1. Transformation assay with Syrian hamster embryo cells. 2. Cultured mouse fibroblasts. |
| 23. Immortalization | In cell culture, normal cells undergo a fixed number of replication cycles before they enter the senescence stage where the cell cycle is normal and senescence will prolong. Immortalization is frequently associated with activation of telomerase (see endpoint #20). | Oncogenic transformation, i.e., anchorage-independent growth, loss of contact inhibition. Assays: 1. Transformation assay with Syrian hamster embryo cells. 2. Increased motility and invasiveness of cancer cell lines. |
| 24. Absorption, distribution, metabolism, excretion (ADME) | Evidence for the absorption, distribution, metabolism, and elimination (ADME) of a chemical in vivo. | Pharmacokinetics/toxicokinetics (PK/TK), mass-balance studies, quantitative tissue distribution studies, metabolic profiling and identification. |
in the metabolism of carcinogenic agents such as cigarette smoke may play a role in susceptibility to cancer at several sites. This has been established for polymorphisms in several cytochromeP450 genes such as CYP1A1 in squamous cell carcinoma of the lung (Ji, Wang, and Suo 2012), CYP1A1 in cervical cancer (Roszak et al. 2014) and in arylamine N-acetyltransferase (NAT) in head-and-neck cancers (Khlifi et al. 2013).

However, associations between such polymorphisms and susceptibility to cancer are rather weak, and not consistent (Agundez 2004, 2008). Low-level environmental exposures may be relevant in genetically susceptible individuals. Nakachi et al. (1993) reported that polymorphisms of the CYP1A1 and GST (glutathione S-transferase) genes conferred an increased risk of lung cancer occurrence associated with a lower level of cigarette smoking. The slow-acetylator phenotype was correlated with a reduced clearance of the bladder carcinogen 4-aminobiphenyl after low-dose exposure to this agent (Vineis et al. 1994). This is important for risk assessment, where differences in levels and types of biomarkers and hence cancer risk development may be related to both environmental (dose) and host (genotype) factors.

Human exposure to environmental agents commonly involves complex mixtures of a wide range of different chemicals, such as occurs with cigarette smoking and several industrial manufacturing processes. Examples of carcinogenic mixtures include air pollution, volatile components in paint, diesel-engine exhaust, wood dust, and cigarette smoke. Cigarette smoke contains more than 7000 different chemicals. Occupational exposure as a painter involves exposure to over a thousand different substances. As the composition of the environmental mixtures varies appreciably in terms of chemical structure and physical characteristics such as particle-size distribution, gaseous and particulate components, their biological activity may also vary. Further, the composition of many of these mixtures is different across industrial sites and sectors. This composition varies with the changing nature of many industrial processes and may also exhibit seasonal variation. The presence of different chemicals in these mixtures indicates that the toxicological endpoints involved are more diverse than those associated with exposure to separate, single constituents of the mixture. Further, interactions between chemicals in complex exposures are possible, as outlined below. The evidence relating to mechanistic pathways that lead to the carcinogenicity of specific chemical compounds such as benzo[a]pyrene present in mixtures was rarely obtained from human exposure settings but rather from epidemiological evidence which tends to be available only for overall mixtures containing the specific compound of interest. This observation, along with data on structure–activity relationships and experiments with animals, is used to draw conclusions regarding the mechanism(s) of action of the agent itself.

Many examples of interaction among multiple exposures exist in the literature on co-exposures and resulting biological responses. Arsenic (As) was found to potentiate the genotoxicity of benzo[a]pyrene by inducing DNA adducts in Hep-1 mouse hepatoma cells (Maier et al. 2002), and by enhancing oxidative stress in human lung adenocarcinoma cells (Chen et al. 2013). Interaction can occur between ultraviolet radiation and heavy metals as measured by DNA damage in human keratinocytes and in SKH-1 hairless mice (Cooper et al. 2013). Another example is the synergistic effect of alcohol and tobacco smoke associated with an increased risk of upper digestive tract cancer (Pelucchi et al. 2006). More complex interactions involving biological agents include the interplay between exposure to aflatoxin and chronic infection with hepatitis B virus (HBV), which enhances the risk of hepatocellular carcinoma via several potential mechanisms including a higher frequency of TP53 mutations (Kew 2003) and elevated levels of DNA adducts (Kew et al. 2001). Similarly, environmental exposures to PAH appeared to increase the risk of liver cancer development among individuals with high aflatoxin exposure or chronic HBV infection (Wu et al. 2007). Evidence also indicates that consumption of Chinese-style salted fish might re-activate the Epstein-Barr virus, which is known to induce nasopharyngeal carcinoma.

The level of exposure to carcinogens plays an important part in carcinogenesis. Whereas experiments with high, toxic doses of inorganic As show little evidence of a mutagenic response (Klein et al. 2007) long-term, low-dose exposure to inorganic
As may increase mutagenesis, perhaps mediated by enhanced levels of reactive oxygen species (ROS) (Nesnow et al. 2002), as well as co-mutagenesis with other agents (Burns et al. 2004). Other underlying mechanisms observed at low concentrations of As include inhibition of DNA repair and changes in DNA-methylation patterns, and genomic instability (Sage et al. 2017). In contrast, clastogenic effects were not observed after low-dose exposure to beryllium (Be) (Strupp 2011). In another case, nickel-induced genotoxic effects including mitochondrial and chromosomal fractions were only observed at toxic levels (Doreswamy et al. 2004).

**Key characteristics of carcinogenic agents**

The 10 key characteristics displayed by carcinogens developed by the Workshop participants are: agent (1) is electrophilic or may be metabolically activated to an electrophile; (2) is genotoxic; (3) alters DNA repair or produces genomic instability; (4) induces epigenetic alterations; (5) produces oxidative stress; (6) induces chronic inflammation; (7) is immunosuppressive; (8) modulates receptor-mediated effects; (9) causes immortalization; or (10) alters cell proliferation, cell death or nutrient supply. These particular characteristics were not intended to be exhaustive but recognized by the participants as representing important attributes of human carcinogens. These characteristics are described in more detail by Smith et al. (2016, Smith (2019).

Some of the key characteristics of carcinogens listed in Table 2 may be considered themselves as primary events that trigger conversion to malignancy. Some agents induce the initial mutagenic changes in stem and progenitor cells that start the cancer process. There are other key characteristics that may pertain to later stages of carcinogenesis such as (1) enhancing the growth, malignancy, or metastasis of already developed tumors through suppression of immune surveillance; and (2) hormone-mediated growth stimulation in the case of tumors derived from cells with appropriate receptors as in the case of estrogens. Epigenetic silencing of tumor-suppressor genes may occur in different phases during the various steps in cancer development (Hattis et al. 2009). These distinctions have important implications for cancer risk assessment, since agents that induce early changes in the development of cancer may be more clearly associated with cancer as an endpoint than those with characteristics associated with later stages.

Similarly, some of the mechanistic characteristics of carcinogenic agents may correlate better with cancer risk than others. Agents that react directly with DNA may form adducts or induce single- or double-strand breaks, such genotoxic effects are common to many Group-1 carcinogens. Several lines of evidence from epidemiological investigations, from studies with experimental animals, and from experimental in vitro systems demonstrated that DNA-adduct induction is strongly associated with cancer (Kriek et al. 1998; Phillips et al. 2015; Wiencke 2002). Genotoxic effects might lead to mutations. As noted above, gene mutation represents an important event in the pathway towards carcinogenesis, especially if it involves oncogenes or tumor-suppressor genes. RAS mutations that result from exposure to PAH are involved in the etiology of tumors (Ross and Nesnow 1999), and mutations in TP53 from other chemical exposures are linked to human cancers (Hussain and Harris 1999). Chromosomal changes are another type of genetic alteration frequently seen in many tumors. Consequently, agents that induce genomic instability such as benzene need to be regarded as potential carcinogens.

Other characteristics of carcinogenic agents including induction of changes in gene expression, altered cell proliferation, immunosuppression, inflammation, and oxidative stress may not reliably predict carcinogenicity (Hernández et al. 2009; Melnick, Kohn, and Portier 1996). One reason might be that those effects have also been implicated in other adverse health outcomes, and therefore lack specificity with respect to cancer. Alternatively, agents with these characteristics may be active during the process of neoplastic conversion and, therefore may be associated with a more advanced stage of the carcinogenic process, rather than acting as initiators (Amend and Pienta 2015; Brücher and Jamall 2014; Hanahan and Weinberg 2011). Several factors such as dose, frequency or duration of exposure may determine which toxicological endpoints would be elicited by specific agents and what their relative contribution might be to the overall process of carcinogenesis (Bolt and Huici-Montagud 2008).
Table 2. Linkage between the 24 toxicological endpoints and the 10 key characteristics of carcinogens.

| Key characteristic | Toxicological endpoints\(^a\) | Description |
|--------------------|-------------------------------|-------------|
| 1. Is electrophilic or can be metabolically activated to electrophiles | 3. Protein adducts | Formation of protein adducts indicates the presence of reactive metabolites, which are sometimes also considered as indirect indicators/predictors of DNA damage (see characteristic 2, below) |
| | 9. Metabolites (reactive) | Requires biotransformation (metabolic activation) to produce reactive metabolites, e.g. alkylating agents, epoxide metabolites, aryl-nitrenium ion |
| | 24. Absorption, distribution, metabolism, excretion (ADME) | Evidence for the absorption, distribution, metabolism and elimination (ADME) of the agent affecting its carcinogenicity |
| 2. Is genotoxic | 1. DNA damage | Direct evidence of DNA damage – this category includes nuclear and mitochondrial DNA damage (in vitro or in vivo): DNA adducts, DNA strand-breaks (single- and/or double-strand breaks), DNA–protein cross-links, DNA–DNA cross-links. Indirect indicators or biomarkers of DNA damage (in vitro or in vivo). |
| | 4. Clastogenic effects | Disruption or breakages of chromosomes leading to sections of the chromosome being deleted, added, or rearranged. |
| | 5. Gene mutation | Reversions and forward mutations in microorganisms. Mutations affecting oncogenes, tumour-suppressor genes, and other genes involved in cell-cycle control. |
| 3. Alters DNA repair or causes genomic instability | 16. DNA-repair alteration | Effects on key DNA-repair mechanisms such as base-excision repair (BER) and nucleotide-excision repair (NER). Inherited abnormalities in DNA-repair function lead to enhanced cancer susceptibility. |
| 4. Induces epigenetic alterations | 6. Epigenetics | Stable, long-term alterations in the transcriptional potential of a cell. These effects can be caused by factors such as altered methylation of DNA, micro-RNA expression, and changes in chromatin and histone structure. |
| 5. Induces oxidative stress | 2. Oxidative stress | Disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses within a cell. |
| 6. Induces chronic inflammation | 12. Inflammation (chronic) | Chronic inflammation and/or irritation leading to oxidative DNA damage. |
| | 14. Irritation (chronic), leading to chronic inflammation | Measures of altered function of the immune system that may lead to increased cancer risk (e.g. HIV-related effects). |
| 7. Is immunosuppressive | 11. Immune effects | Interference with cell-signaling pathways leading to expression of carcinogenic trait/phenotype in the cell, e.g. facilitating cell invasion or induction of genes for inflammatory mediators, oncogenes. |
| 8. Modulates receptor-mediated effects | 17. Receptor-mediated effects | Interference with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body. External agents can interfere with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body. |
| | 18. Hormonal effects | Interference with cell-signaling pathways leading to expression of carcinogenic trait/phenotype in the cell e.g. facilitating cell invasion or induction of gene promotion for inflammatory mediators, oncogenes. |
| 9. Causes immortalization | 23. Immortalization | A. Oncogenic transformation, i.e. anchorage-independent growth, loss of contact inhibition. B. Increased motility and invasiveness of cancer cell lines C. Cell transformation |
| | 20. Alterations in telomere length | Activation of a telomerase that prevents loss of telomere length, leading to immortalization of cells |
| 10. Alters cell proliferation, cell death, or nutrient supply | 8. Alterations in cell-signaling pathways | Interference with cell-signaling pathways leading to expression of carcinogenic trait/phenotype in the cell e.g. facilitating cell invasion or induction of gene promotion for inflammatory mediators, oncogenes. |
| | 13. Cell death | Induced defects in programmed cell death (apoptosis). Evasion of apoptosis is a requirement for both neoplastic transformation and sustained growth of cancer cells. |
| | 15. Cell-cycle effects | Detection of alterations in cell proliferation and cell-cycle effects (e.g. DNA-replication changes, cell-cycle control, ploidy), mitogenesis. Altered nutrient supply affects cell viability. |
| | 19. Angiogenic effects | Change in pro-angiogenesis factors |
| | 21. Inhibition of gap-junctional intercellular communication | Disruption of gap-junctional intercellular communication pathways that can cause a loss of ‘contact inhibition’ and abnormal cell growth. |
| | 22. Bystander effects | The bystander effect was first identified in radiobiology and refers to the situation where non-irradiated cells exhibit effects caused by radiation as a result of chemical signals (messengers) received from nearby irradiated cells. These effects are often mediated through gap-junction transfer of chemical agents. |

\(^a\)Toxicological endpoints 7 (changes in gene expression) and 10 (susceptibility) were not considered specific to any of the key characteristics, and are therefore not included in Table 2.
It is worth noting that there may be exceptions to the notions outlined above regarding the relevance of different key characteristics of carcinogens. Labib et al. (2012) reported that early changes in gene expression may provide a better indication of the likelihood of carcinogenic transformation than DNA adducts and mutant frequency in response to exposure to benzo[a]pyrene. Nonetheless, the 10 key characteristics were considered by the Workshop participants to provide useful descriptors of the properties of Group-1 agents that are related to biological mechanisms by which such agents induce cancer in humans.

**Linking the toxicological endpoints to the key characteristics**

In constructing the IARC database of mechanisms of human cancer, data on the 24 toxicological endpoints relevant to carcinogenesis were abstracted from the *IARC Monographs*, with supplementary information collected through a PubMed search. To construct a database of the 10 key characteristics, the 24 toxicological endpoints were linked to the characteristics as presented in Table 2. Apart from obvious assignments such as linking toxicological endpoint 6 – which includes epigenetic changes such as altered methylation of DNA, micro-RNA expression, and changes in chromatin and histone structure – with key characteristic 4, ‘causes epigenetic alterations’, several toxicological endpoints may all be associated with a single key characteristic, which reflects the broader scope of the latter in describing factors related to development of cancer in humans. Toxicological endpoints 3 (formation of protein adducts), 9 (formation of reactive metabolites), and 24 (the overall information on ADME) have been categorized under key characteristic 1 ‘is electrophilic or can be metabolically activated to electrophiles’, while DNA damage, clastogenic effects and gene mutation (endpoints 1, 4 and 5) are grouped under key characteristic 2, ‘is genotoxic’. The toxicological endpoints ‘changes in gene expression’ (endpoint 7) and ‘susceptibility’ (endpoint 10) were not considered to be specific to any of the 10 key characteristics, and thus not included. The database on the key characteristics of human carcinogens constructed in this manner was used by Krewski et al. (2019, this Volume) to explore the characteristics of Group-1 agents identified in the *IARC Monographs* through Volume 109, the last volume for which the final version was available at the time the mechanistic database was constructed.

**Illustrative examples of determination of key characteristics of Group-1 agents**

To illustrate the procedure that was followed to arrive at the assignment of key characteristics (KCs) for IARC Group-1 carcinogens based upon the corresponding toxicological endpoints given in Table 2, 6 Group-1 agents are considered in detail – one from each of the 6 Monograph Volumes 100A–F. These agents are: tamoxifen, hepatitis B virus (HBV), As and inorganic As compounds, solar and ultraviolet (UV) radiation, tobacco smoking, and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD).

Evidence of expression of the toxicological endpoints might originate from human or animal in-vivo or in-vitro sources. Following the identification of the toxicological endpoints listed in Table 1 associated with each of the six agents, the key characteristics of these agents were determined by use of the linkage between toxicological endpoints and key characteristics provided in Table 2.

It should be noted that *IARC Monograph* Volume 100 ‘A review of human carcinogens’ was developed to re-assess the carcinogenic hazards from exposure to the agents classified in Group 1. The Sections 4 on ‘Mechanistic and other relevant data’ of the respective Monographs were not intended to provide a comprehensive review of all mechanistic aspects of a given agent but rather sought to define ‘established’ and ‘likely’ mechanisms of carcinogenicity. Since the concept of ‘key characteristics’ was introduced by Smith et al. (2016), the assignment of these characteristics based only upon the contents of the Monographs would necessarily be incomplete. Although we are confident that the ‘established’ mechanisms have been properly identified in the Monograph for each agent, supplementary literature searches were conducted in order to possibly identify
additional ‘likely’ mechanisms that might be associated with additional key characteristics.

A detailed description of the results of the literature searches conducted to identify the toxico- logical endpoints (TEs) exhibited by the six selected agents is included in the appendix (see below). A summary of the corresponding key characteristics (KCs) of these agents, determined by mapping the TEs to the KCs using the linkages given in Table 2, is provided in the narrative form below and in tabular form in Table 3. This approach was employed to determine the key characteristics for all 86 Group-1 agents analyzed by Krewski et al. (2019, this Volume).

**Tamoxifen**

Based upon studies summarized in the appendix, the KCs of tamoxifen include genotoxicity (KC#2, based upon TEs #1, 4 and 5) in humans and animals in vivo and in human and animal cells in vitro, and induction of receptor-mediated effects (KC#8, based on TE#17) in humans in vivo and in human cells in vitro. Tamoxifen also induced cell proliferation alterations (KC#10, as evidenced by expression of TEs #13 and 15) in human cells in vitro, produced epigenetic changes (KC#4, as determined by TE#6) in human cells in vitro and in animals in vivo, and resulted in immortalization (KC#9, as determined by TE#20) in human cells in vitro. The updated PubMed search provided evidence that tamoxifen also produced oxidative damage (KC#5/TE#2) based upon in-vitro experiments in human and animal cells and in animal studies in vivo.

**Hepatitis B virus (HBV)**

The literature summarized in the appendix indicates that HBV is a pleiotropic virus that induces hepatocellular carcinoma (HCC) in accordance with a number of key characteristics. HBV infection induced oxidative stress (TE#2 mapped to KC#5) that led to DNA-adduct formation (TE#1 mapped to KC#2) in humans and animals, both in vivo and in vitro. Hepatitis B virus also interfered with DNA-repair mechanisms in human cells in vitro (TE#16 mapped to KC#3). HBV infection produced cytogenetic effects (TE#4, listed under KC#2), induced acute and chronic inflammation (TE#12 mapped to KC#6), and initiated oxidative stress (TE#2 mapped to KC#5). This agent also altered cell proliferation (KC#10, based upon TEs #13 and 15), in humans and animals in vivo and in vitro. HBV replication upregulated heat-shock proteins in human cells in vitro (TE#7). Integration of HBV-DNA into the host genome-changed expression of various genes in human HCC tissue and in human cells in vitro (TE#7) and produced mutations in key regulatory genes (KC#2 based upon TE#5) in human HCC tissue. The HBx protein induced alterations in cell signaling (KC#10 as determined by TE#8) in human HCC tissue and in vivo in animals. Epigenetic alterations (KC#4 based upon TE#6) are also important pathways for the carcinogenic effects mediated by HBV, where the virus interferes with DNA methylation, in humans in vivo and in vitro and in animal cells in vitro. HBV contributes to cell immortalization (KC#9 as determined by TEs #20 and 23) through interference with telomeres and integration in cellular oncogenes. The updated PubMed search provided additional recent data ‘epigenetic alterations’ (KC#4, TE#6), specifically histone deacetylation and differential microRNA expression found in humans in vivo and in human and animal cells in vitro. Although HBV is not DNA-reactive, it ‘is genotoxic’ (KC#2) under the definition of genotoxicity in Table 1, which includes not only DNA damage (TE#1) but also clastogenic effects (TE#4) and induction of gene mutation (TE#5), as noted above.

**Arsenic (As)**

Arsenic induces cancer by diverse mechanisms including genotoxicity (KC#2 as determined by TEs #1, 4 and 5) in humans in vivo and in human and animal cells in vitro. Some organic (methylated) arsenicals are mutagenic, whereas inorganic As compounds can act as co-mutagens. Arsenic also interferes with DNA-repair mechanisms (KC#3 based upon TE#16) as was reported in studies in human and animal cells in vitro. This metal also produced chronic inflammation (KC#6 based upon TE#12) based on investigations in humans in vivo and human cells in vitro. Oxidative stress (KC#5 as determined by TE#2)
Table 3. Key characteristics of six Group-1 agents based on toxicological endpoint(s) expresseda.

| Agent (Volume) | Key characteristics | Corresponding toxicological endpoint(s)b,c |
|---------------|---------------------|-------------------------------------------|
| Tamoxifen (100A) | Is genotoxic (KC2) | DNA damage (TE1); clastogenic effects (TE4); gene mutation (TE5). |
|               | Induces epigenetic alterations (KC4). | Epigenetics (TE6). |
|               | Induces oxidative stress (KC5). | Oxidative stress (TE2). |
|               | Modulates receptor-mediated effects (KC8). | Receptor mediated effects (TE17). |
|               | Causes immortalization (KC9). | Alterations in telomere length (TE20). |
|               | Alters cell proliferation, cell death or nutrient supply (KC10). | Cell death (TE13); cell-cycle effects (TE15). |
| Hepatitis B virus (100B) | Is genotoxic (KC2) | DNA-adduct formation (TE1); cytogenetic effects (TE4); gene mutations (TE5). |
|               | Alters DNA repair or causes genomic instability (KC3). | DNA-repair alteration (TE16). |
|               | Induces epigenetic alterations (KC4). | Epigenetics (TE6). |
|               | Induces oxidative stress (KC5). | Oxidative stress (TE2). |
|               | Induces chronic inflammation (KC6). | Chronic inflammation (TE12). |
|               | Causes immortalization (KC9). | Cell immortalization (TE23); interference with telomere length (TE20). |
|               | Alters cell proliferation, cell death or nutrient supply (KC10). | Alterations in cell signaling (TE8); cell death (TE13); cell-cycle effects (TE15); angiogenic effects (TE19). |
| Arsenic and inorganic arsenic compounds (100C) | Is genotoxic (KC2). | DNA damage (TE1); clastogenic effects (TE4); gene mutations (TE5). |
|               | Alters DNA repair or causes genomic instability (KC3). | Interference with DNA repair (TE16). |
|               | Induces epigenetic alterations (KC4). | Epigenetics (TE6). |
|               | Induces oxidative stress (KC5). | Oxidative stress (TE2). |
|               | Induces chronic inflammation (KC6). | Chronic inflammation (TE12). |
|               | Modulates receptor-mediated effects (KC8). | Receptor-mediated effects (TE17). |
|               | Causes immortalization (KC9). | Immortalization (TE23). |
|               | Alters cell proliferation, cell death or nutrient supply (KC10). | Alteration in cell-signaling pathway (TE8); cell death (TE13); cell-cycle effects (TE15); angiogenic effects (TE19). |
| Solar and ultraviolet radiation (100D) | Is genotoxic (KC2) | Induction of DNA damage (TE1); clastogenic effect (TE4); gene mutations (TE5). |
|               | Alters DNA repair or causes genomic instability (KC3). | Alteration in DNA repair (TE16). |
|               | Induces epigenetic alterations (KC4). | Epigenetics (TE6) from the updated PubMed search. |
|               | Induces oxidative stress (KC5). | Oxidative stress (TE2). |
|               | Is immunosuppressive (KC7). | Immunosuppression (TE11). |
|               | Causes immortalization (KC9). | Alterations in telomere length (TE20); Immortalization (TE23). |
|               | Alters cell proliferation, cell death or nutrient supply (KC10). | Alteration in cell signaling pathway (TE8); cell death (TE13); cell-cycle effects (TE15); angiogenic effects (TE19); bystander effects (TE22). |
| Tobacco smoking (100E) | Is electrophilic or can be metabolically activated to an electrophile (KC1) | Protein adducts (TE3); metabolites (TE9). |
|               | Is genotoxic (KC2). | DNA damage (TE1); clastogenic effects (TE4); gene mutation (TE5). |
|               | Alters DNA repair or causes genomic instability (KC3). | DNA repair alterations (TE16). |
|               | Induces epigenetic alterations (KC4). | Epigenetic effects (TE6). |
|               | Induces oxidative stress (KC5). | Oxidative stress (TE2). |
|               | Induces chronic inflammation (KC6). | Inflammation (TE12); chronic irritation (TE14). |
|               | Is immunosuppressive (KC7). | Immune effects (TE11). |
|               | Modulates receptor-mediated effects (KC8). | Receptor-mediated effects (TE17). |
|               | Causes immortalization (KC9). | Immortalization (TE23). |
|               | Alters cell proliferation, cell death or nutrient supply (KC10). | Alteration in cell signaling pathway (TE8); cell death (TE13); cell-cycle effects (TE15). |

(Continued)
is also a key characteristic of As-induced carcinogenesis as evidenced from studies in human cells in vitro and animals in vivo. Altered cell proliferation (KC#10 based upon TEs #8, 13, 15, and 19) by As was evident in all of the four test systems. Epigenetics was a key characteristic for As (KC#4 based upon TE#6) in studies in the four test systems. Arsenic exhibited some receptor-mediated effects (KC#8 as determined by TE#17). Inorganic As compounds induced neoplastic transformation in human and animal cells in vitro, which contributes to immortalization (KC#9 based on TE#23). The updated PubMed search provided supporting data for KCs noted above on the basis of data on TEs found in the Monographs but did not identify new KCs.

**Ultraviolet (UV) and solar radiation**

UV radiation is genotoxic (KC#2 based upon TEs #1, 4 and 5) as evidenced from studies in humans in vivo, and animals in vitro. The updated PubMed search found investigations that reported genotoxicity in humans in vivo and in vitro. Altered DNA-repair mechanisms (KC#3 as determined by TE#16) were observed in human and animal cells in vitro. Oxidative stress (KC#5 based on TE#2) was also noted in human and animal cells in vitro. Immunosuppression (KC#7 based upon TE#11) is a key characteristic of UV that was found in human and animal experiments in vivo. UV radiation also induced immortalization (KC#9 based upon TEs #20 and 23) in human cells in vitro. Altering cell proliferation, cell death or nutrient supply (KC#10 as determined by TEs # 8, 13, 19, and 22) was a key characteristic of UV from evidence in human cells in vitro.

The updated PubMed search provided further data in support of the genotoxicity (KC#2) of UV and solar radiation noted above in addition to that reported in the Monographs, including recent findings of sister chromatid exchange in animal cells in vitro (TE#1) and of MN detected in animals in vivo (TE#4). The search also identified new studies that demonstrated epigenetic effects (KC#4) as a key characteristic for UV radiation, based upon induced DNA hypermethylation and histone hypo-acetylation in human and animal cells in vitro (TE#6, mapped to KC#4).

**Tobacco smoking**

Components of tobacco smoke are electrophiles or may be metabolized to electrophiles (KC#1, based upon TE#3). Tobacco smoke is genotoxic (KC#2, based upon TEs #1, 3, 4 and 5) as reported in studies in humans and animals in vivo and human and animal cells in vitro. This agent mixture also initiates genomic instability (KC#3, based upon TE#16) as noted in all four test systems. Other mechanisms underlying tobacco-associated carcinogenesis include epigenetic effects (KC#4, based upon TE#6) in humans in vivo and human cells in vitro, oxidative stress (KC#5, determined by TE#2) in humans in vivo and human and animal cells in vitro, chronic inflammation (KC#6, determined by TE#12 and 14), and immunosuppression (KC#7, based upon TE#11).
Receptor-mediated effects attributed to various tobacco products were found in different test systems (KC#8, based upon TE#17). Tobacco smoke also produces immortalization through the malignant transformation of human cells in vitro (KC#9, determined by TE#23). Further, tobacco smoke components alter cell proliferation and cell death modify cell signaling in humans in vivo, and produce cell-cycle effects in humans in vivo and human cells in vitro (KC#10, determined by TEs #8, 13, and 15). The updated PubMed search provided further data for KCs noted above from TEs found in the Monographs, but in addition, identified modulation of receptor-mediated responses as illustrated in studies in all four test systems.

2,3,7,8-tetrachlorodibenzo-para-dioxin (TCDD)

TCDD induces DNA strand breaks (KC#2, determined by TE#1) in human cells in vitro associated with oxidative stress (KC#5, determined by TE#2). TCDD is mutagenic in bacteria, yeast, and mammalian cells and clastogenic in humans and animals in vivo and in human and animal cells in vitro (KC#2, based upon TEs #4 and 5). This agent produces genomic instability (KC#3, based upon TE#16) in animal cells in vitro, and induces oxidative stress (KC#5, determined by TE#2) in human cells in vitro and animals in vivo. TCDD is immunosuppressive and immunotoxic (KC#7, based on TE#11), based upon evidence from humans and animals in vivo, and from human and animal cells in vitro. This chemical also induces numerous receptor-mediated effects (KC#8, determined by TEs #17 and 18) as reported in a large number of studies in all four test systems. TCDD also alters cell proliferation and cell death (KC#10 determined by TEs #8, 13 and 15) in all test systems. TCDD induced immortalization of human and animal cells in vitro (KC#9, based upon TE#23). The updated PubMed search provided further data for KCs noted above from TEs found in the Monographs. The search also identified more recent experiments that showed epigenetic responses (KC#4, determined by TE#6) as a key characteristic for TCDD based upon changes in methylation of regulatory genes, acetylation, and methylation of histones, and transgenerational effects. Although TCDD is not directly DNA-reactive, this agent-induced DNA strand breaks (TE#1) attributed to oxidative stress. TCDD was also clastogenic (TE#4) in various assay systems (see the appendix for further details).

Key characteristics of 86 Group-1 agents

The approach described in the preceding section to identify the key characteristics of the six selected agents from Volume 100 was carried out for all 86 Group-1 agents considered by Birkett et al. (2019). A summary of the key characteristics of these agents based upon mechanistic information cited in the IARC Monographs is illustrated in Figure 1. An in-depth analysis of these characteristics, including prevalence of each of the 10 key characteristics overall and by type agent including pharmaceuticals; biological agents; As, metals, fibers, and dusts; radiation; personal habits and indoor combustions; and chemical agents and related occupations; the multiplicity of key characteristics demonstrated by these agents; and the sources of information on the key characteristics (human in-vivo and in-vitro as well as animal in-vivo and in-vitro) are provided by Krewski et al. (2019). Birkett et al. (2019) presented a brief sensitivity analysis of the prevalence of the key characteristics incorporating mechanistic information from the updated PubMed search not cited in the IARC Monographs. (This latter analysis shows that most of the relevant mechanistic information on the 10 key characteristics of human carcinogens was captured in Sections 4 of the Monographs.)

Discussion

The mechanistic database described in this paper may be used to understand the key characteristics of the 86 Group-1 agents included in the database. In reviewing different toxicological endpoints expressed by agents identified as human carcinogens in the IARC Monographs, along with their corresponding key characteristics as depicted in Figure 1, varying patterns are apparent for different groups of agents, with some exhibiting multiple and varied toxicological pathways, and others demonstrating a narrower range of pathways. The database may be employed to explore such patterns, including differences in DNA-reactive vs non-DNA reactive pathways that
Figure 1. Key characteristics of 86 Group-1 agents (cells shaded in grey reflect evidence of key characteristics from one or more animal or human in vivo or in vitro sources).
contribute to human cancer development, although a clear separation between these two broad pathways is not apparent. DNA-reactive agents such as busulfan are in some cases chemically reactive by themselves, others such as N′-Nitrosornicotinone might be metabolized to electrophiles that react with the nucleophilic centers in the cell including nucleotides thereby generating DNA adducts (Adam et al. 2016). In contrast, some agents produce DNA damage through indirect means as evidenced by altered cellular redox process, particularly affecting the nuclear redox state – independent of its cytoplasmic counterpart (Go and Jones 2010) – by generating ROS that subsequently react with DNA. Oxidative damage to DNA leads to several types of lesions including mutations, single-strand breaks, DNA–protein crosslinks, chromosomal abnormalities, and translocations. Mutations that arise from oxidative stress were found to activate oncogenes and inactivate tumor-suppressor genes (Klaunig et al. 2011).

Examples of direct-acting genotoxic carcinogens include chemotherapeutic drugs such as busulfan, chlorambucil, methyl-CCNU, melphalan, and the industrial chemicals ethylene oxide and sulfur mustard. All of these are electrophilic, direct-acting alkylating agents. Substances whose electrophilic metabolites react with DNA to form DNA adducts include benzo[a]pyrene found in tobacco smoke and in many industrial processes, several therapeutic agents such as tamoxifen, thiopeta, and treosulfan, and some industrial agents and processes (4-aminobiphenyl, benzidine, dyes meta-lized to benzidine, 2-naphthylamine, o-toluidine, auramine production, magenta production, coal gasification, coal-tar distillation, coal production). Heavy metals and fibers act predominantly by inducing the formation of ROS, thus initiating genomic instability, chromosomal aberrations, DNA strand breaks, and DNA–protein crosslinks.

Chemical agents that are not DNA-reactive predominantly lipophilic compounds and biological agents (HPV, human T-cell lymphotropic virus, Opisthorchis viverrini, Schistosoma haematobium, and Helicobacter pylori) act through a variety of mechanisms, generally involving interference with cell-signaling, resulting in diverse molecular changes and endpoints such as altered cell proliferation and migration, disruption of apoptosis, modification in gene expression, cellular immortalization and cell transformation. Other non-genotoxic mechanisms involve interference with the epigenetic mechanisms by inducing alterations in DNA methylation or histone modifications. Hormonal therapies and agents mimicking hormonal action such as TCDD induce receptor-mediated tissue-specific and agent-specific cell proliferation, mitogenesis, and other events.

The majority of the Group-1 human carcinogens demonstrate genotoxicity as a key characteristic. The proportion of non-genotoxic carcinogens among known (Group 1), probable (Group 2) and possible (Group 3) human carcinogens as classified by the IARC was evaluated by Hernández et al. (2009) who noted among 371 agents in Groups 1, 2A and 2B that were tested for genotoxicity, only 45 (12%) exhibited a non-genotoxic mode of action.

Some agents interfere with multiple pathways that involve the induction of DNA damage as well as other types of effects not involving DNA reactivity. Examples of the latter include changes in gene expression, activation of cell-signaling pathways, immunosuppression, and inflammation. Further, DNA damage induced by chemical exposures may occur as a secondary or tertiary effect of the cascade of events mediated through the metabolism of the agent or its reaction with cellular constituents such as receptor binding. It was found that TCDD metabolism results in enhanced production of reactive intermediates that increase oxidative stress and subsequently induce oxidative DNA damage (Knerr and Schrenk 2006). Chronic inflammation from exposure to fibers results in genotoxic damage (Catalán et al. 2017). The chemotherapeutic agent etoposide produces genetic damage without chemically interacting with DNA. The postulated pathway, in this case, involves binding to the topoisomerase IIα enzyme (Alessandra, Zanetta, and Biamonti 2015). The etoposide-topoisomerase IIα complex interferes with DNA re-ligation, thereby enhancing the production of DNA double-strand breaks. In other cases, the prevalent non-genotoxic pathway may eventually lead to a genotoxic event, as is the case in HBV-related hepatocellular carcinoma (HCC) where enhanced cellular proliferation from inflammatory responses to chronic viral infection produces double-strand DNA breaks and facilitate viral integration (Hollingworth and Grand 2015). Similarly, genomic instability associated with chromosomal
aberrations and MN formation in response to exposure to heavy metals, including As, cadmium, Be and nickel, may result from interference with DNA repair (Morales et al. 2016).

For some agents, both a primary mechanistic pathway and a secondary pathway contributing to carcinogenesis might be identified, based upon chemical structure and in-vitro experiments. However, the relative importance of each of these pathways for the carcinogenic process may be difficult to estimate in agent-induced human cancers. One reason is that multiple biomarkers and events are invoked during the conversion to malignancy. An example of this is tamoxifen, for which the evidence of a genotoxic pathway in induction of human endometrial tumors is less compelling than that of receptor-binding through an estrogen-receptor-dependent pathway. Similarly, for estrogen-induced cancer, it is difficult to conclude whether the receptor-mediated responses to the hormone or the genotoxic effects of the estrogens or their associated by-products are the underlying cause.

After review of the mechanisms of Group-1 agents included in Volume 100 and subsequent Volumes of the IARC Monographs, it becomes evident that multiple mechanisms operate for many carcinogenic agents. It is challenging to determine which mechanism predominates in the development of human cancers. Since mechanistic data display important implications for cancer risk assessment, including information on dose–response relationships, it is critical to consider the interrelationship of the key characteristics of human carcinogens, which may in turn also be informative with respect to the complex interactions among different carcinogens (Guyton et al. 2009).

Comprehensive knowledge of the various mechanistic pathways and elicited toxicological endpoints during carcinogenesis is of vital importance in cancer risk assessment. The focus in the next decade may likely focus on the pathway(s) and molecular targets – commonly modulated by exposure to carcinogenic agents – that are relevant to the carcinogenic process. This approach might become more feasible with the application of molecular “-omics” techniques to detect virtually all global changes in cellular constituents and processes after exposure to potentially carcinogenic agents. Further, recent developments in molecular biology, especially in the area of high-throughput molecular technology and advanced genome-wide scanning hold the promise of fostering understanding of the major etiological pathways and landmark events involved in cancer development. Recent genome-wide association studies and analyses revealed the driver genes that are most commonly mutated in cancers (Chen et al. 2017). Vogelstein et al. (2013) noted that most of the signaling pathways that these genes control and regulate were identified.

Understanding how pathways are perturbed, and determining which pathways are activated in different settings of tumorigenesis may exert an important impact on understanding the heterogeneity in the response to carcinogenic agents. This may also lead to the development of more effective individualized therapies and prevention strategies. Further, an interdisciplinary application of this research to molecular cancer epidemiology might aid in identification and subsequent validation of biomarkers along the pathway of chemical carcinogenesis, and potentially identify genetic variants that determine inter-individual differences in cancer risk occurrence. Early detection of mutations in genes controlling critical pathways in exposed individuals may provide evidence of a ‘molecular abnormality’ that leads to carcinogenic conversion even before mutated cells undergo clonal expansion.

Properly validated biomarkers corroborated in multiple studies might be utilized for cancer prognosis, and in defining strategies to reduce cancer-related morbidities and mortality (Ulrich and Ambrosone 2008). Genome-wide association investigations have been used to find genetic biomarkers associated with poor prognosis as in colorectal cancer (Bacolod and Barany 2011). Variation in cancer susceptibility is one of the toxicological endpoints considered in the current review, although it was not linked directly to any of the key characteristics of human carcinogens. Susceptibility is an important biomarker of a range of host factors mainly controlled by variations in genotype that affect the host response to carcinogenic agents. This endpoint potentially modulates each of the 10 key characteristics. It is an essential component of molecular epidemiological studies to understand inter-individual differences in susceptibility to chemically induced cancer. Low-penetrant but highly frequent
gene-allele variations controlling toxicokinetics (cytochrome p450; Gold et al. 2009), and to a lesser extent toxicodynamics (estrogen receptor; Zheng et al. 2003), play an important role in population-attributable risk in chemical carcinogenesis. Variants in other genes contributing to cellular dysfunction such as RAS, TP53 or BRCA1 make even stronger contributions to individual susceptibility to cancer. Knowledge of individual genetic variations provides opportunities for more effective and individualized cancer therapy (Safgren et al. 2015).

A major challenge in development and validation of reliable molecular biomarkers is to assess the background signals of genetic damage, especially lesions that are due to endogenously produced oxidative stress attributed to reactive intermediates in normal cellular metabolism (Swenberg et al. 2011). The relative contribution of environmental and endogenous DNA damage to overall carcinogenesis is difficult to determine but is important in attempts to reliably correlate exposure to exogenous carcinogenic agents with critical endpoints of carcinogenesis. However, major technological advances in high-throughput molecular assays may be used to develop biobanks of integrated biological responses (the concept of the ‘exosome’) of individuals exposed to well-defined environmental agents, with the promise of earlier detection and eventual prevention of cancer (Wild, Scalbert, and Herceg 2013). Marsit (2015) applied these techniques to understand the influence of environmental exposures on the epigenome.

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