CHAPTER 22.

Analysis of key characteristics of human carcinogens

Daniel Krewski, Mustafa Al-Zoughool, Michael Bird, Nicholas Birkett, Mélissa Billard, Brittany Milton, Jerry M. Rice, Vincent J. Cogliano, Mark A. Hill, Julian Little, and Jan M. Zielinski (deceased) in collaboration with other participants (see the Contributors list) in the Workshop on Tumour Site Concordance and Mechanisms of Carcinogenesis, which was convened by IARC in April and November 2012 in Lyon

Introduction

Since its establishment in the early 1970s, the *IARC Monographs Programme* has evaluated more than 1000 agents with evidence of human exposure and for which some suspicion exists of an increased cancer risk to humans. The *IARC Monographs Programme* has developed detailed criteria against which to evaluate the available scientific evidence on the carcinogenic potential of such agents. These criteria, which are described in the Preamble to the *IARC Monographs* (Cogliano et al., 2004; IARC, 2006), are used to evaluate and integrate the evidence provided by human epidemiological studies, animal cancer bioassays, and information on possible biological mechanisms of action, to classify agents into one of the following categories: *carcinogenic to humans* (Group 1), *probably carcinogenic to humans* (Group 2A), *possibly carcinogenic to humans* (Group 2B), *not classifiable as to its carcinogenicity to humans* (Group 3), and *probably not carcinogenic to humans* (Group 4). These evaluations involve classifying the data from both the human and the animal studies as providing *sufficient evidence of carcinogenicity*, *limited evidence of carcinogenicity*, *inadequate evidence of carcinogenicity*, or *evidence suggesting lack of carcinogenicity*. The information on biological mechanisms of action may be evaluated as *strong*, *moderate*, or *weak*, and is taken into consideration in the overall evaluation.

The role of mechanistic information in evaluating carcinogenicity has increased substantially during the history of the *IARC Monographs Programme*. In 1991, IARC convened a Working Group on the Use of Data on Mechanisms of Carcinogenesis in Risk Identification, to explore how mechanistic data could be used to

identify agents with the potential to cause cancer in humans. The consensus report of the Working Group documented several mechanisms that were considered to be relevant to human carcinogenesis at that time, including genotoxicity, cell proliferation, receptor mechanisms in mitogenesis, alterations in DNA repair, intercellular communication, and immune defects and immunosuppression (Vainio et al., 1992). Toxicokinetic and other variables were also identified as factors affecting multistage carcinogenesis. Since 1991, IARC (2006) and other organizations – for example, the United States National Toxicology Program (National Toxicology Program, 2014) and the United States Environmental Protection Agency (EPA) (EPA, 2005) – have stressed the increasing importance of mechanistic information in cancer risk assessment. Related risk assessment practices concern mode of action (Meek et al., 2014) and pathways of toxicity (Krewski et al., 2014; Bourdon-Lacombe et al., 2015; Cote et al., 2016), as well as dosimetric considerations (Gurusankar et al., 2017).

This chapter examines the available data on mechanisms of action of the Group 1 agents identified up to and including Volume 106 of the *IARC Monographs* (Table 22.1). The present analysis is based on a review of human cancer mechanisms, conducted by the participants in the two-part Workshop on Tumour Site Concordance and Mechanisms of Carcinogenesis, which was convened by IARC in April and November 2012 in Lyon. This approach initially involved retrieval of information from the *IARC Monographs* on 24 toxicological end-points identified as likely indicators of biological processes at the cellular and molecular level and thought to be relevant to carcinogenesis. Information on these 24 end-points was derived from human in vivo, human in vitro, animal in vivo, and animal in vitro studies (see Al-Zoughool et al., 2019). During the November 2012 meeting, the Workshop participants identified 10 broader key characteristics of carcinogens (see Chapter 10, by Smith, and Smith et al., 2016). Information on these characteristics was extracted from the *IARC Monographs* and used to develop a database of key characteristics for Group 1 agents (see Al-Zoughool et al., 2019). This chapter focuses on the key characteristics of the Group 1 agents identified in the *IARC Monographs* up to and including Volume 106, and presents the results of an exploratory analysis of this database.

Methods

Key characteristics

As mentioned above, Chapter 10, by Smith, and Smith et al. (2016) describe 10 key characteristics of human carcinogens, as listed in Table 22.2. The toxicological end-points initially considered by the Workshop participants and used as indicators of these characteristics are also noted in Table 22.2. A brief summary of each of these characteristics and the associated toxicological end-points is provided below.

Characteristic 1: Is electrophilic or can be metabolically activated to electrophiles

The first characteristic refers to agents that act as electrophiles themselves or that can be metabolized to form electrophiles. An electrophile can react with cellular macromolecules such as DNA, RNA, and proteins to form adducts. Some chemical carcinogens are direct-acting electrophiles (e.g. formaldehyde; sulfur mustard, and ethylene oxide), whereas others require biotransformation by enzymes in a process termed metabolic activation (e.g. polycyclic aromatic hydrocarbons and benzene) (Miller, 1970).

Characteristic 2: Is genotoxic

Genotoxicity is the ability to induce DNA damage or other chromosomal alterations, as measured by three associated toxicological end-points: (i) DNA damage: an alteration in the chemical structure or integrity of DNA, including a break in a DNA strand, and/or chemical modifications such as covalent binding to the nucleotide bases (Hoeijmakers, 2009); (ii) gene mutations: changes in the normal nucleotide sequence of cellular DNA that may have a central role in human carcinogenesis (Ding et al., 2008); (iii) clastogenic effects reflect damage to chromosomes, including DNA breakage, or the rearrangement, gain, or loss of chromosome fragments (Snyder, 2010).

Characteristic 3: Alters DNA repair or causes genomic instability

Alterations in DNA repair result in defects in processes that monitor and correct DNA replication fidelity. Such defects can confer strong mutator phenotypes that result in genomic instability.

Characteristic 4: Induces epigenetic alterations

Induced epigenetic alterations are stable changes in gene expression and chromatin organization that are independent of mutation and that

Table 22.1. Number of Group 1 agents in Volumes 100–118 of the *IARC Monographs*, by type of agent[a]

Type of agent	Volume											Total
	100	105	106	107	109	110	111	113	114	117	118	
Pharmaceuticals	23	–	–	–	–	–	–	–	–	–	–	23
Biological agents	11	–	–	–	–	–	–	–	–	–	–	11
Arsenic, metals, fibres, and dusts	10	–	–	–	–	–	2[b]	–	–	–	–	12
Radiation	18	–	–	–	–	–	–	–	–	–	1[c]	19
Personal habits and indoor combustions	12	–	–	–	–	–	–	–	1[d]	–	–	13
Chemical agents and related occupations	33	1[e]	1[f]	2[g]	2[h]	1[i]	–	1[j]	–	1[k]	1[l]	43
Total	**107**	**1**	**1**	**2**	**2**	**1**	**2**	**1**	**1**	**1**	**2**	**121**

[a] At the time that the present analysis was conducted, mechanistic information was available only for the 109 Group 1 agents evaluated in the *IARC Monographs* up to and including Volume 106.
[b] Fluoro-edenite fibrous amphibole; occupational exposures associated with the Acheson process in the manufacture of silicon carbide fibres.
[c] Ultraviolet radiation from welding.
[d] Processed meat.
[e] Diesel engine exhaust.
[f] Trichloroethylene.
[g] Polychlorinated biphenyls (PCBs); dioxin-like PCBs.
[h] Outdoor air pollution; particulate matter in outdoor air pollution.
[i] 1,2-Dichloropropane.
[j] Lindane.
[k] Pentachlorophenol (PCP).
[l] Welding fumes.

can be inherited through cell division. Epigenetic phenomena include genomic imprinting, X-chromosome inactivation, global reconfiguration of the DNA methylome, changes in chromatin compaction states and histone modification patterns, and altered expression of microRNAs (miRNAs). These phenomena occur during organ development and contribute to the lineage-specific epigenome that is maintained over the lifetime of an organism.

Characteristic 5: Induces oxidative stress

Oxidative stress results from an imbalance between formation of reactive oxygen and detoxification of the radical species within cells and tissues. Reactive oxygen species induce a cascade of events that can include DNA mutation and oxidative DNA damage. Both are key events in carcinogenesis (Klaunig et al., 2011).

Characteristic 6: Induces chronic inflammation

Chronic inflammation can arise from persistent infection (e.g. with human papillomavirus or with *Helicobacter pylori*) as well as from exogenous irritants (e.g. silica or asbestos fibres). Persistent infection and chronic inflammation disrupt local tissue homeostasis and alter cell signalling, leading to the recruitment and activation of inflammatory cells. Strong links exist between inflammation and the induction of oxidative stress and genomic instability; this makes it difficult to separate out the relative importance of each of these mechanisms. These linkages might be the basis of the relationship between chronic inflammation and cancer (Multhoff and Radons, 2012).

Characteristic 7: Is immunosuppressive

Immunosuppression is an induced reduction in the capacity of the immune system to respond effectively to foreign antigens, including

Table 22.2. Key characteristics and toxicological end-points demonstrated by agents known to cause cancer in humans (adapted from Al-Zoughool et al., 2019)

Key characteristic	Corresponding toxicological end-points
Is electrophilic or can be metabolically activated to electrophiles	Metabolic activation Protein adducts ADME (differences in absorption, distribution, metabolism, and elimination)
Is genotoxic	DNA damage Cytogenetic/clastogenic effects Gene mutations
Alters DNA repair or causes genomic instability	DNA repair alteration, leading to genomic instability
Induces epigenetic alterations	Epigenetic alterations (DNA methylation, histone modification, and altered expression of microRNAs)
Induces oxidative stress	Oxidative stress
Induces chronic inflammation	Chronic inflammation Chronic irritation
Is immunosuppressive	Immune effects
Modulates receptor-mediated effects	Receptor-mediated effects Hormonal effects
Causes immortalization	Immortalization Alterations in telomere length
Alters cell proliferation, cell death, or nutrient supply	Cell-cycle effects Bystander effects Alterations in cell signalling pathways Angiogenic effects Cell death Inhibition of gap-junctional intercellular communication

antigens on tumour cells. In contrast to other key characteristics, immunosuppression does not play a direct part in transforming normal cells into tumour cells, but enables them to escape immune surveillance. Among other roles, the immune system also plays a major part in the inflammatory response to injury.

Characteristic 8: Modulates receptor-mediated effects

Modulation of receptor-mediated effects can occur when agents mimic the structure of endogenous ligands that bind to cells and activate cell surface receptors or intracellular receptors, thereby inducing or modifying a plethora of signal transduction pathways that, among other responses, stimulate cell proliferation. Receptor-mediated effects can induce hormonal effects whereby external agents can interfere with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body. These external factors can also demonstrate reactivity similar to endogenously produced hormones, which can lead to them mediating changes in homeostasis, reproduction, development, or behaviour.

Characteristic 9: Causes immortalization

Immortalization refers to a cell evading normal cellular senescence and proliferating indefinitely. In culture, normal cells have a fixed number of replication cycles before they enter cellular senescence and stop replicating. Evasion of senescence is frequently associated with activation of telomerase (Willeit et al., 2010) and plays a critical part in carcinogenesis (Reddel, 2000). Carcinogenesis may involve activation of a telomerase that prevents loss of telomere length, leading to immortalization of cells (Willeit et al., 2010).

Characteristic 10: Alters cell proliferation, cell death, or nutrient supply

Cell proliferation is affected by alterations in the rates of cell growth within a tissue. It may be a direct effect or a secondary regenerative effect after induction of cell death by cytotoxic agents. Two associated toxicological end-points are (i) cell-cycle effects, i.e. alterations in the functioning of the complex series of factors that control the cell cycle and cell division, which have been associated with carcinogenesis (Diaz-Moralli et al., 2013), and (ii) alterations in cell signalling pathways, which relate to the ability of the agent to interfere with cell signalling pathways, leading to expression of a carcinogenic trait or phenotype in the cell.

For cell death, necrosis triggers the invasion of cells such as macrophages into the affected area, and enhances the proliferation and spread of cancer cells. Defects in programmed cell death can cause cancer; evasion of apoptosis is a requirement for both neoplastic transformation and sustained growth of cancer cells.

Adequate cell nutrition is essential to proliferating cancer cells, and agents that promote or inhibit the growth of blood vessels (angiogenesis) will affect tumour growth.

Group 1 agents included in the analysis

Volume 100 of the *IARC Monographs* provided a review and update of the 107 Group 1 agents identified as of 2009. Since the publication of Volume 100, mechanistic information has become available on two additional Group 1 agents: diesel engine exhaust (reviewed in Volume 105; Benbrahim-Tallaa et al., 2012; IARC, 2013) and trichloroethylene (evaluated in Volume 106; Guha et al., 2012; IARC, 2014). Had these two agents been evaluated within Volume 100, they would have been included in Volume 100F; they have therefore been listed with other chemical agents and related occupations in Volume 100F*.

Although additional Group 1 agents have since been identified (Table 22.1), the present analysis is restricted to Group 1 agents identified in the *IARC Monographs* up to and including Volume 106, the most recent volume for which mechanistic information was available at the time of the present analysis. Group 1 agents not included in the present analysis are (i) polychlorinated biphenyls (PCBs) and dioxin-like PCBs (reviewed in Volume 107; Lauby-Secretan et al., 2013; IARC, 2016b), (ii) outdoor air pollution and (iii) particulate matter in outdoor air pollution (both evaluated in Volume 109; Loomis et al., 2013; IARC, 2016a), (iv) 1,2-dichloropropane (reviewed in Volume 110; Benbrahim-Tallaa et al., 2014; IARC, 2017a), (v) fluoro-edenite fibrous amphibole and (vi) occupational exposures associated with the Acheson process used in the manufacture of silicon carbide fibres (both evaluated in Volume 111; Grosse et al., 2014; IARC, 2017b); (vii) lindane (Volume 113; Loomis et al., 2015), and (viii) processed meat (Volume 114; Bouvard et al., 2015).

In some cases, the discussion of mechanisms of action in Section 4 ("Other relevant data") of the *IARC Monographs* is based on groups of agents that act via the same mechanism. For example, internalized radionuclides that emit α-particles are discussed in the *Monographs* as a group with the same mechanism of action. Birkett et al. (2019) reviewed the mechanistic information for 109 Group 1 agents identified in the *IARC Monographs* up to and including Volume 106. The 86 Group 1 agents for which separate mechanistic summaries are provided in the *IARC Monographs* up to and including Volume 106 are listed in Table 22.3, along with their relationship to the 111 distinct agents identified up to and including Volume 109 used by Krewski et al. (Chapter 21) in a parallel analysis of overlap between tumours and tumour sites in animals and humans.

Database of mechanistic characteristics

A database of toxicological end-points was assembled for the 86 Group 1 agents identified up to and including Volume 106 of the *IARC Monographs*. The database includes information from in vivo and in vitro studies in humans and animals. Information on the 24 toxicological end-points was retrieved from Section 4 ("Other relevant data") of the *IARC Monographs* (Al-Zoughool et al., 2019).

Recognizing that, among other limitations, new data may have become available since 2009, when the various parts of Volume 100 were compiled, PubMed searches were conducted to identify evidence on any of the 24 toxicological end-points linked to these agents that was not recorded in the *IARC Monographs* (Birkett et al., 2019). The mechanistic database distinguishes information derived from the *Monographs* from that found in the PubMed search, thereby permitting an assessment of the extent to which Section 4 ("Other relevant data") of the *IARC Monographs* captured all relevant information on these end-points. The analyses in the present chapter are restricted to information taken

Table 22.3. Relationship between 86 agents used in the analysis of key characteristics of human carcinogens and 111 agents used in the analysis of concordance between tumours and tumour sites in humans and animals

Volume[a]	Agent number	86 agents used in the analysis of key characteristics	111 agents used in the analysis of concordance between tumours and tumour sites in humans and animals
100A	1	Aristolochic acid	Aristolochic acid Aristolochic acid, plants containing
100A	2	Azathioprine	Azathioprine
100A	3	Busulfan	Busulfan
100A	4	Chlorambucil	Chlorambucil
100A	5	Chlornaphazine	Chlornaphazine
100A	6	Cyclophosphamide	Cyclophosphamide
100A	7	Ciclosporin	Ciclosporin
100A	8	Diethylstilbestrol	Diethylstilbestrol
100A	9	Estrogen-only menopausal therapy	Estrogen-only menopausal therapy
100A	10	Estrogen–progestogen menopausal therapy (combined)	Estrogen–progestogen menopausal therapy (combined)
100A	11	Estrogen–progestogen oral contraceptives (combined)	Estrogen–progestogen oral contraceptives (combined)
100A	12	Etoposide in combination with cisplatin (Group 2A) and bleomycin (Group 2B)	Etoposide Etoposide in combination with cisplatin and bleomycin
100A	13	Melphalan	Melphalan
100A	14	PUVA (psoralen–UVA photochemotherapy)	Methoxsalen in combination with UVA
100A	15	MOPP	MOPP
100A	16	Phenacetin	Phenacetin Phenacetin, analgesic mixtures containing
100A	17	1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (Methyl-CCNU)	1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (Methyl-CCNU)
100A	18	Tamoxifen	Tamoxifen
100A	19	Thiotepa	Thiotepa
100A	20	Treosulfan	Treosulfan
100B	21	*Opisthorchis viverrini* and *Clonorchis sinensis*	*Clonorchis sinensis* (infection with) *Opisthorchis viverrini* (infection with)
100B	22	Epstein–Barr virus	Epstein–Barr virus
100B	23	*Helicobacter pylori*	*Helicobacter pylori* (infection with)
100B	24	Hepatitis B virus	Hepatitis B virus
100B	25	Hepatitis C virus	Hepatitis C virus
100B	26	Human immunodeficiency virus type 1	Human immunodeficiency virus type 1
100B	27	Human papillomavirus	Human papillomavirus

Table 22.3. Relationship between 86 agents used in the analysis of key characteristics of human carcinogens and 111 agents used in the analysis of concordance between tumours and tumour sites in humans and animals (continued)

Volume[a]	Agent number	86 agents used in the analysis of key characteristics	111 agents used in the analysis of concordance between tumours and tumour sites in humans and animals
100B	28	Human T-cell lymphotropic virus type 1	Human T-cell lymphotropic virus type 1
100B	29	Kaposi sarcoma-associated herpesvirus	Kaposi sarcoma-associated herpesvirus
100B	30	*Schistosoma haematobium*	*Schistosoma haematobium* (infection with)
100C	31	Arsenic and inorganic arsenic compounds	Arsenic and inorganic arsenic compounds
100C	32	Asbestos (all forms, including actinolite, amosite, anthophyllite, chrysotile, crocidolite, and tremolite)	Asbestos (all forms, including actinolite, amosite, anthophyllite, chrysotile, crocidolite, and tremolite)
100C	33	Beryllium and beryllium compounds	Beryllium and beryllium compounds
100C	34	Cadmium and cadmium compounds	Cadmium and cadmium compounds
100C	35	Chromium(VI) compounds	Chromium(VI) compounds
100C	36	Erionite	Erionite
100C	37	Leather dust	Leather dust
100C	38	Nickel and nickel compounds	Nickel compounds
100C	39	Silica dust, crystalline, in the form of quartz or cristobalite	Silica dust, crystalline, in the form of quartz or cristobalite
100C	40	Wood dust	Wood dust
100D	41	Solar and UV radiation	UV radiation (bandwidth 100–400 nm, encompassing UVC, UVB, and UVA) UV-emitting tanning devices Solar radiation
100D	42	X- and γ-radiation	X- and γ-radiation Ionizing radiation (all types)
100D	43	Neutron radiation	Neutron radiation
100D	44	Internalized radionuclides that emit α-particles	Haematite mining with exposure to radon (underground) Plutonium-239 Internalized radionuclides that emit α-particles Thorium-232 (as Thorotrast) Radium-224 and its decay products Radium-226 and its decay products Radium-228 and its decay products Radon-222 and its decay products
100D	45	Internalized radionuclides that emit β-particles	Fission products including Sr-90 Radioiodines, including iodine-131 Phosphorus-32, as phosphate Internalized radionuclides that emit β-particles

Table 22.3. Relationship between 86 agents used in the analysis of key characteristics of human carcinogens and 111 agents used in the analysis of concordance between tumours and tumour sites in humans and animals (continued)

Volume[a]	Agent number	86 agents used in the analysis of key characteristics	111 agents used in the analysis of concordance between tumours and tumour sites in humans and animals
100E	46	Consumption of alcoholic beverages	Acetaldehyde associated with consumption of alcoholic beverages Alcoholic beverages Ethanol in alcoholic beverages
100E	47	Betel quid and areca nut	Areca nut Betel quid with tobacco Betel quid without tobacco
100E	48	Coal, indoor emissions from household combustion of	Coal, indoor emissions from household combustion of
100E	49	*N′*-Nitrosonornicotine (NNN) and 4-(Methyl-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK)	*N′*-Nitrosonornicotine (NNN) and 4-(Methyl-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK)
100E	50	Salted fish, Chinese-style	Salted fish, Chinese-style
100E	51	Second-hand tobacco smoke	Second-hand tobacco smoke
100E	52	Tobacco smoking	Tobacco smoking
100E	53	Tobacco, smokeless	Tobacco, smokeless
100F	54	Acid mists, strong inorganic	Acid mists, strong inorganic
100F	55	Aflatoxins	Aflatoxins
100F	56	Aluminium production	Aluminium production
100F	57	4-Aminobiphenyl	4-Aminobiphenyl
100F	58	Auramine production	Auramine production
100F	59	Benzene	Benzene
100F	60	Benzidine	Benzidine
100F	61	Benzidine, dyes metabolized to	Benzidine, dyes metabolized to
100F	62	Benzo[*a*]pyrene	Benzo[*a*]pyrene
100F	63	Bis(chloromethyl)ether; Chloromethyl methyl ether (technical grade)	Bis(chloromethyl)ether; Chloromethyl methyl ether (technical grade)
100F	64	1,3-Butadiene	1,3-Butadiene
100F	65	Coal gasification	Coal gasification
100F	66	Coal-tar distillation	Coal-tar distillation
100F	67	Coal-tar pitch	Coal-tar pitch
100F	68	Coke production	Coke production
100F	69	Ethylene oxide	Ethylene oxide
100F	70	Formaldehyde	Formaldehyde
100F	71	Iron and steel founding, occupational exposure during	Iron and steel founding, occupational exposure during

Table 22.3. Relationship between 86 agents used in the analysis of key characteristics of human carcinogens and 111 agents used in the analysis of concordance between tumours and tumour sites in humans and animals (continued)

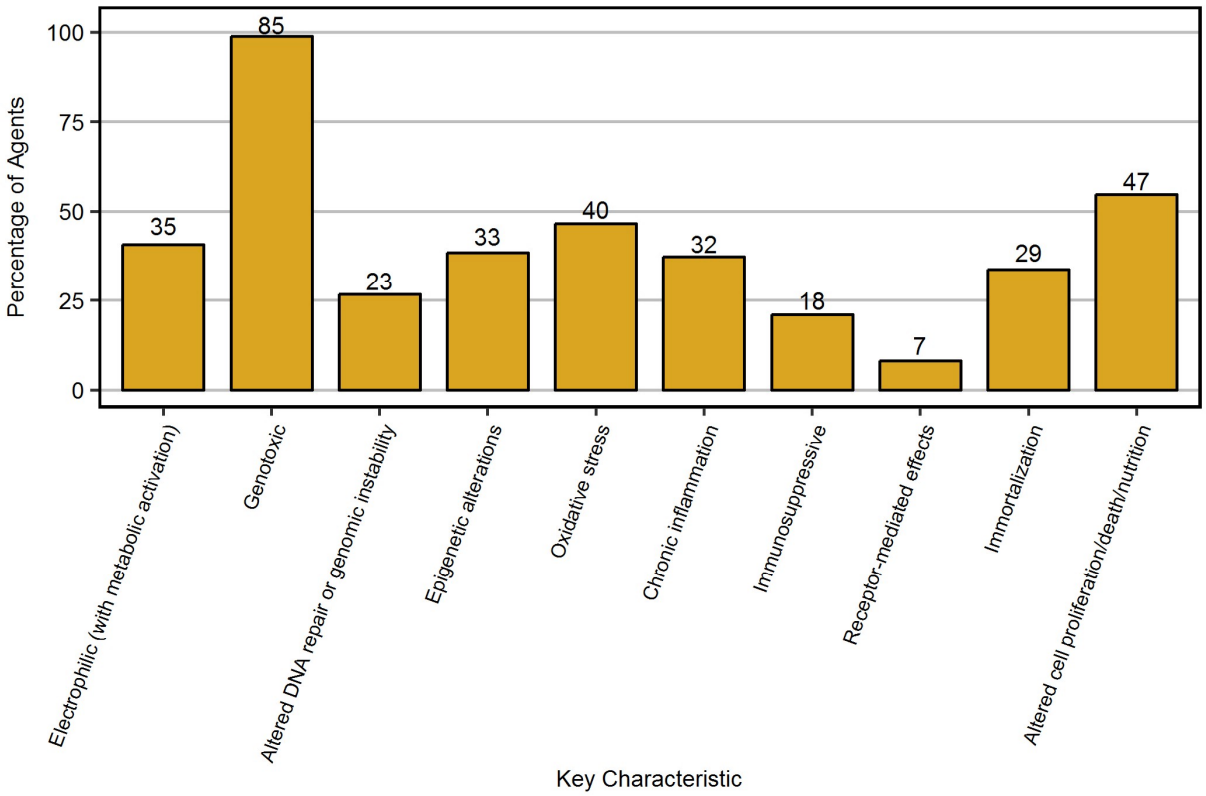
Volume[a]	Agent number	86 agents used in the analysis of key characteristics	111 agents used in the analysis of concordance between tumours and tumour sites in humans and animals
100F	72	Isopropyl alcohol manufacture using strong acids	Isopropyl alcohol manufacture using strong acids
100F	73	Magenta production	Magenta production
100F	74	4,4′-Methylenebis(2-chloroaniline) (MOCA)	4,4′-Methylenebis(2-chloroaniline) (MOCA)
100F	75	Mineral oils, untreated or mildly treated	Mineral oils, untreated or mildly treated
100F	76	2-Naphthylamine	2-Naphthylamine
100F	77	*ortho*-Toluidine	*ortho*-Toluidine
100F	78	Painter, occupational exposure as a	Painter, occupational exposure as a
100F	79	2,3,7,8-Tetrachlorodibenzo-*para*-dioxin, 2,3,4,7,8-Pentachlorodibenzofuran, 3,3′,4,4′,5-Pentachlorobiphenyl	2,3,4,7,8-Pentachlorodibenzofuran 2,3,7,8-Tetrachlorodibenzo-*para*-dioxin 3,3′,4,4′,5-Pentachlorobiphenyl
100F	80	Rubber manufacturing industry, occupational exposures in the	Rubber manufacturing industry, occupational exposures in the
100F	81	Shale oils	Shale oils
100F	82	Soot (as found in occupational exposure of chimney sweeps)	Soot (as found in occupational exposure of chimney sweeps)
100F	83	Sulfur mustard	Sulfur mustard
100F	84	Vinyl chloride	Vinyl chloride
105	85	Diesel and gasoline engine exhausts	Engine exhaust, diesel
106	86	Trichloroethylene	Trichloroethylene
107			Polychlorinated biphenyls[b]
109			Outdoor air pollution[b]
109			Particulate matter in outdoor air pollution[b]

UV, ultraviolet.

[a] *IARC Monographs* Volumes 100A (IARC, 2012e), 100B (IARC, 2012b), 100C (IARC, 2012a), 100D (IARC, 2012f), 100E (IARC, 2012d), 100F (IARC, 2012c), 105 (IARC, 2013), 106 (IARC, 2014), 107 (IARC, 2016b), and 109 (IARC, 2016a).

[b] Because the mechanistic sections for *Monographs* Volumes 107–109 were not available for review at the time that the present analysis was conducted, Group 1 agents in these volumes were not included in the present analysis.

Fig. 22.1. Key characteristics of 86 Group 1 agents. The number of agents is shown above each characteristic.



directly from the *IARC Monographs*: Birkett et al. (2019) present the results of a sensitivity analysis incorporating the additional information obtained through the PubMed search.

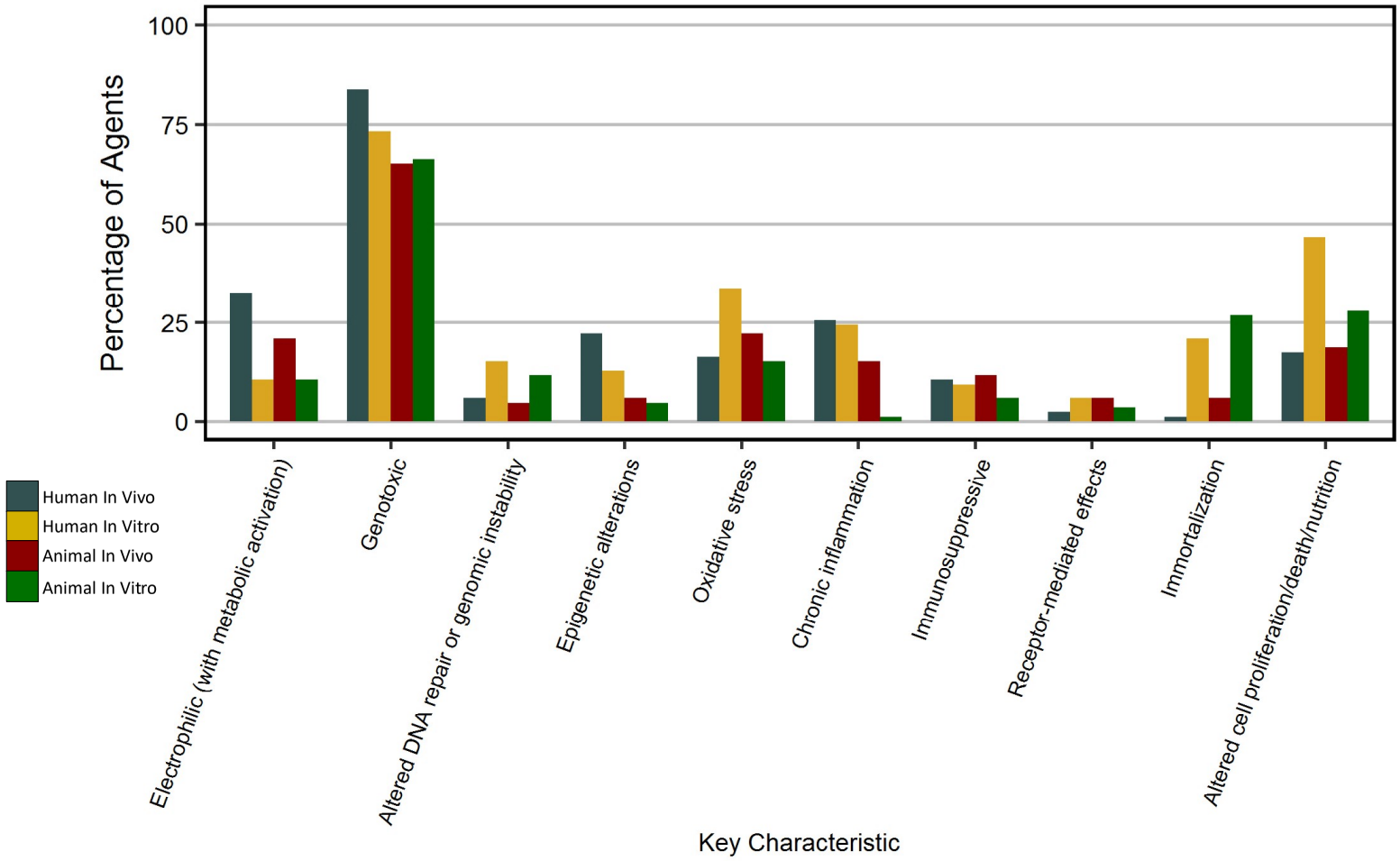
After the collection of information on the toxicological end-points identified by the Workshop participants during the April 2012 meeting, the database of key characteristics was then created by mapping the 24 toxicological end-points to the 10 characteristics as indicated in Table 22.2. As noted by Al-Zoughool et al. (2019), two of the toxicological end-points – susceptibility and changes in gene expression – did not link to any of the key characteristics, and thus were not included in the development of the database of key characteristics. Because the database includes information derived from human in vivo, human in vitro, animal in vivo and animal in vitro sources, it is possible to aggregate this information according to human and animal sources (by combining across in vivo and in vitro sources) or according to in vivo and in vitro sources (by combining across human and animal sources). Of primary interest here is aggregation across all four sources combined, to obtain an overall indicator of whether any of the key characteristics is associated with each of the 86 Group 1 agents of interest.

Statistical analysis

Descriptive statistical methods were used to explore the key characteristics associated with the 86 Group 1 agents, beginning with a tabulation of the number of agents demonstrating any of the 10 characteristics, both overall and stratified by the four sources of information noted above. To evaluate the extent to which the Group 1 agents demonstrated more than one key characteristic, the number of agents demonstrating multiple characteristics was also tabulated.

A heat map showing the number (0, 1, 2, 3, or 4) of sources of information (human in vivo, human in vitro, animal in vivo, and animal in vitro studies) supporting a given characteristic for a specified agent was prepared, to evaluate the consistency of information provided by different sources. A heat map showing the overlap between human and animal sources of information (after combining in vivo and in vitro sources in both cases) on the key characteristics was also prepared, to evaluate the extent to which there was overlap between these two sources.

Fig. 22.2. Sources of information on key characteristics of 86 Group 1 agents (sources are human in vivo, human in vitro, animal in vivo, and animal in vitro studies).



Overall mechanistic data were also tabulated by type of agent (pharmaceuticals; biological agents; arsenic, metals, fibres and dusts; radiation; personal habits and indoor combustions; and chemical agents and related occupations), to identify possible differences in mechanistic patterns by agent type.

Results

The key characteristics of the 86 Group 1 agents considered here are summarized in Fig. 22.1. The most prevalent mechanistic characteristic was "is genotoxic", followed by "alters cell proliferation, cell death, or nutrient supply", "induces oxidative stress", "is electrophilic or can be metabolically activated to electrophiles", and "induces chronic inflammation". Nearly all agents demonstrate genotoxicity as one of their mechanistic properties; a prominent exception is human immunodeficiency virus type 1 (HIV-1). Evidence of genotoxicity was provided by expression of the following toxicological end-points: DNA damage, gene mutations, and cytogenetic/clastogenic effects (including chromosomal aberrations, micronucleus formation, and aneuploidy).

Fig. 22.2 shows the key characteristics exhibited by the 86 agents classified according to the source of data (human in vivo, human in vitro, animal in vivo, and animal in vitro studies) on these characteristics. Information on all the mechanistic characteristics was available to different degrees from all four sources. Information on genotoxicity was available from each of the four sources for the majority of the agents. Human in vivo studies contribute the most evidence on four of the 10 key characteristics for these 86 agents, including "is genotoxic", "induces epigenetic alterations", and "induces chronic inflammation". Human in vitro studies provide the most information on an additional three key characteristics: "alters DNA repair or causes genomic instability", "induces oxidative stress", and "alters cell proliferation, cell death, or nutrient supply", and equivalent information to animal in vivo studies on "modulates receptor-mediated effects".

The prominence of human studies as sources of information on the key characteristics of human carcinogens may be attributed to the increasing use of molecular and genetic markers in human studies. Epidemiological studies conducted in the occupational or general environment often analyse biomarkers of DNA adduct formation, clastogenic effects, and gene mutations, all of which reflect DNA damage. Therefore, human in vivo studies are a major source of information on genotoxicity.

Fig. 22.3 shows the number of agents demonstrating multiple characteristics as evidenced from studies in animals and in humans. The 86 Group 1 agents considered here exhibit an average of approximately four key characteristics; the modal value is two characteristics, exhibited by 20 agents. All agents demonstrate at least one key characteristic, with two agents demonstrating nine characteristics and 14 agents showing six. No agent exhibited all 10 key characteristics.

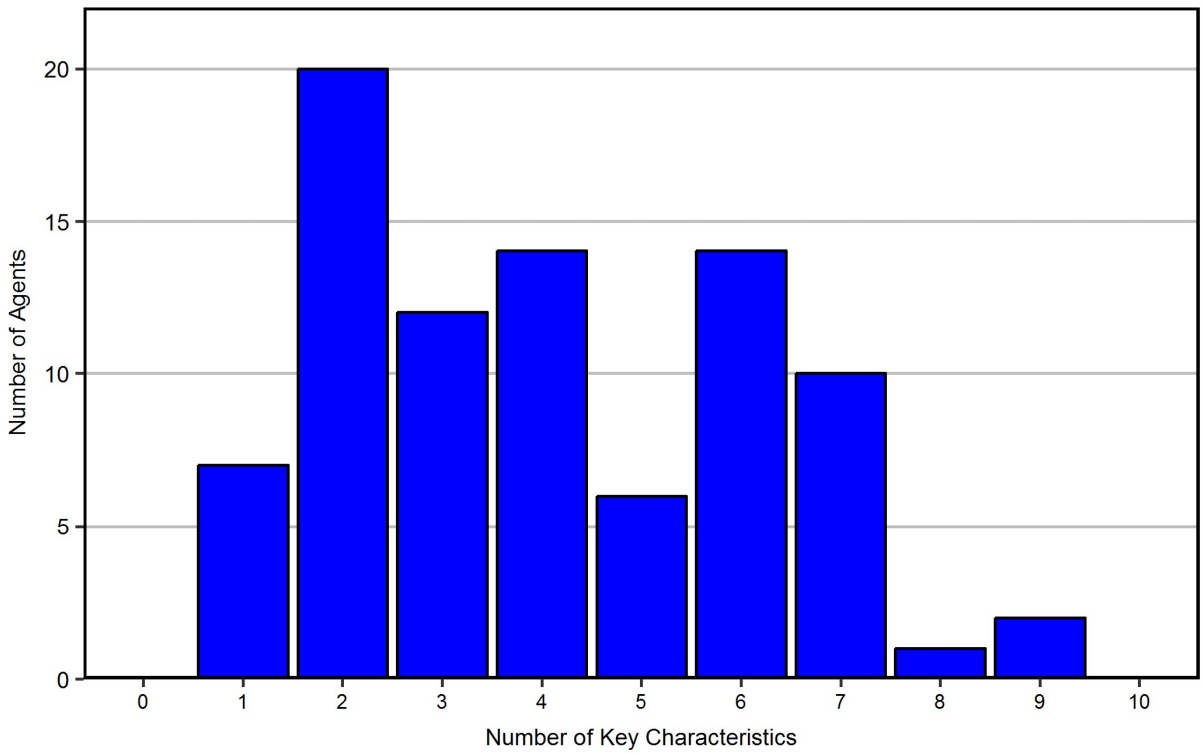
Fig. 22.4 presents a heat map indicating the strength of evidence of the different characteristics for the 86 individual Group 1 agents. The intensity of the colour reflects the number of sources of information (human in vivo, human in vitro, animal in vivo, and animal in vitro studies) on each key characteristic for each agent. As in Fig. 22.1, the single most prominent characteristic was genotoxicity: many agents (HIV-1 is a prominent exception) showed a positive response for genotoxicity in at least one of the four sources of information, and for many agents more than one source provided evidence of genotoxicity. For some agents (e.g. all radiation sources, some pharmaceutical agents, and some chemical agents), genotoxicity was demonstrated in all four test systems, confirming that genotoxicity is central to the carcinogenic pathways of these agents.

Fig. 22.4 also shows that most agents exhibit multiple key characteristics, with evidence drawn from more than one source of mechanistic information. Radiation sources and tobacco smoke are associated with many of the key characteristics, suggesting that these agents act by multiple pathways.

Several Group 1 agents, including several occupational exposures, are complex mixtures of chemicals and other substances. Coal-tar pitch, occupational exposure to soot, and coke production have similar characteristics, probably due to the strong presence in relevant workplaces of polycyclic aromatic hydrocarbons, although other factors such as the nature of inorganic substances and their chemical composition could also have a role. Other occupationally relevant agents (e.g. exposures during iron and steel founding and aluminium production) demonstrate only a single key characteristic, although this may reflect the difficulty of testing for other characteristics in these occupational exposure situations.

The degree of overlap between human and animal sources of information on the 10 key characteristics

Fig. 22.3. Number of Group 1 agents demonstrating one or more key characteristics.



of human carcinogens is shown in the heat map in Fig. 22.5. This heat map, prepared by combining the in vivo and in vitro sources of information on the key characteristics for humans and for animals, indicates whether information on the key characteristics for a given agent is derived from both human and animal sources (reflecting concordance between humans and animals), from human sources alone, from animal sources alone, or from neither of these. These results indicate overlap between human and animal sources of information for several agents. The concordance is particularly strong for genotoxicity: information from both human and animal sources is available for 63 of the 85 agents demonstrating evidence of genotoxicity. Ten agents – diethylstilbestrol, Kaposi sarcoma-associated herpesvirus, arsenic and inorganic arsenic compounds, cadmium and cadmium compounds, asbestos, crystalline silica, solar and ultraviolet radiation, sulfur mustard, diesel and gasoline engine exhausts, and trichloroethylene – demonstrate overlap between human and animal sources of information for at least five of the key characteristics.

Comparisons between the results in Fig. 22.4 and Fig. 22.5 can provide additional insights into the key characteristics of the Group 1 agents considered here. For example, in the case of diethylstilbestrol, Fig. 22.4 indicates that there is information from 1, 2, or 3 sources on nine key characteristics (all except "induces oxidative stress"), but Fig. 22.5 clarifies that there are both human and animal data for only five of these. For chlornaphazine, Fig. 22.4 shows two sources of information, for "is electrophilic or can be metabolically activated to electrophiles" and "is genotoxic", whereas the corresponding data in Fig. 22.5 show overlap between human and animal sources only for "is electrophilic or can be metabolically activated to electrophiles", with human but not animal data on "is genotoxic".

Fig. 22.6 shows the key characteristics of the six categories of Group 1 agents considered in Volume 100: pharmaceuticals; biological agents; arsenic, metals, fibres, and dusts; radiation; personal habits and indoor combustions; and chemical

Fig. 22.4. Heat map showing the strength of evidence for key characteristics of 86 Group 1 agents according to the number of information sources (sources are human in vivo, human in vitro, animal in vivo, and animal in vitro studies).

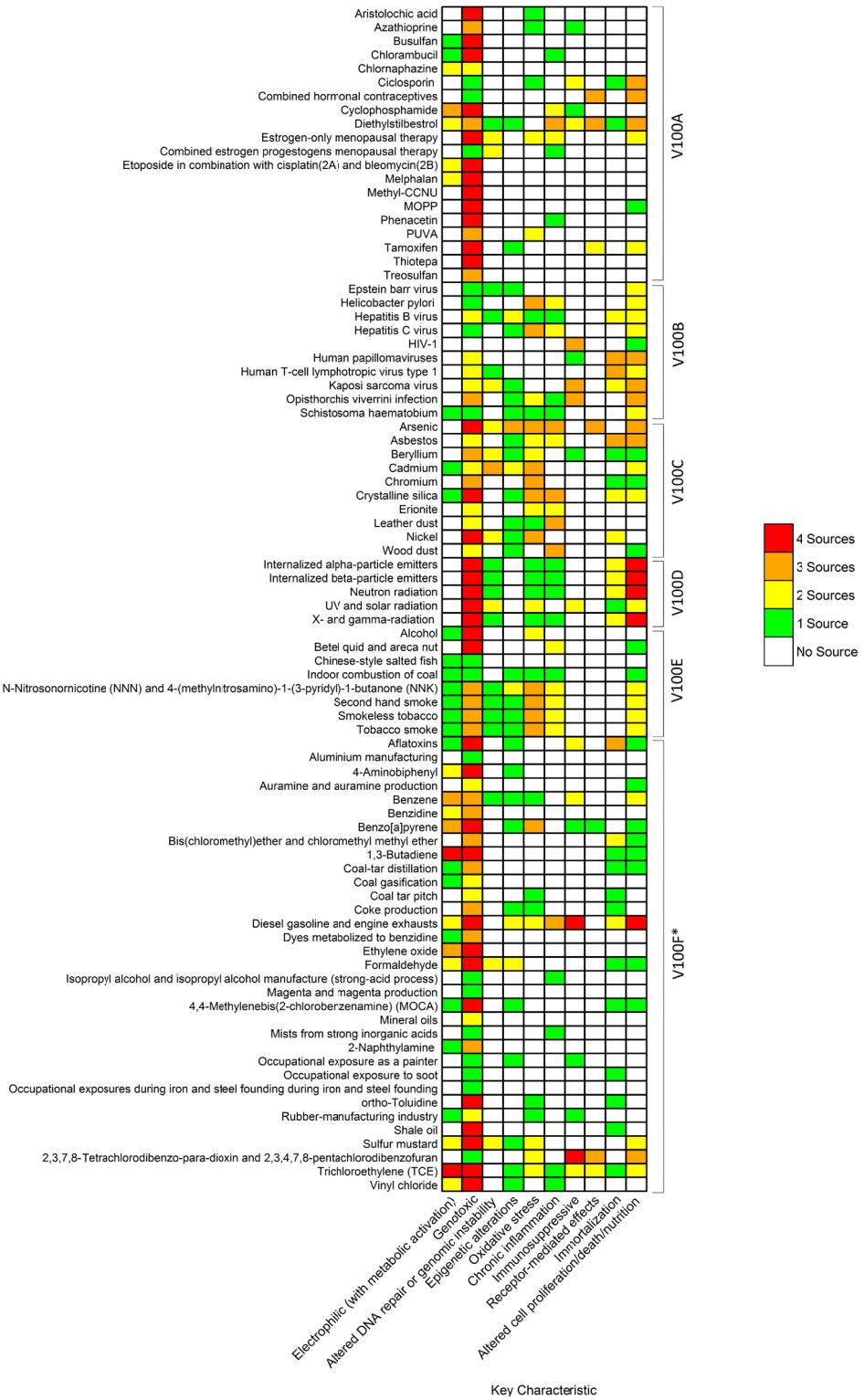


Fig. 22.5. Heat map showing the degree of concordance between human and animal sources of information on key characteristics of 86 Group 1 agents (after combining in vivo and in vitro sources of information for humans and for animals).

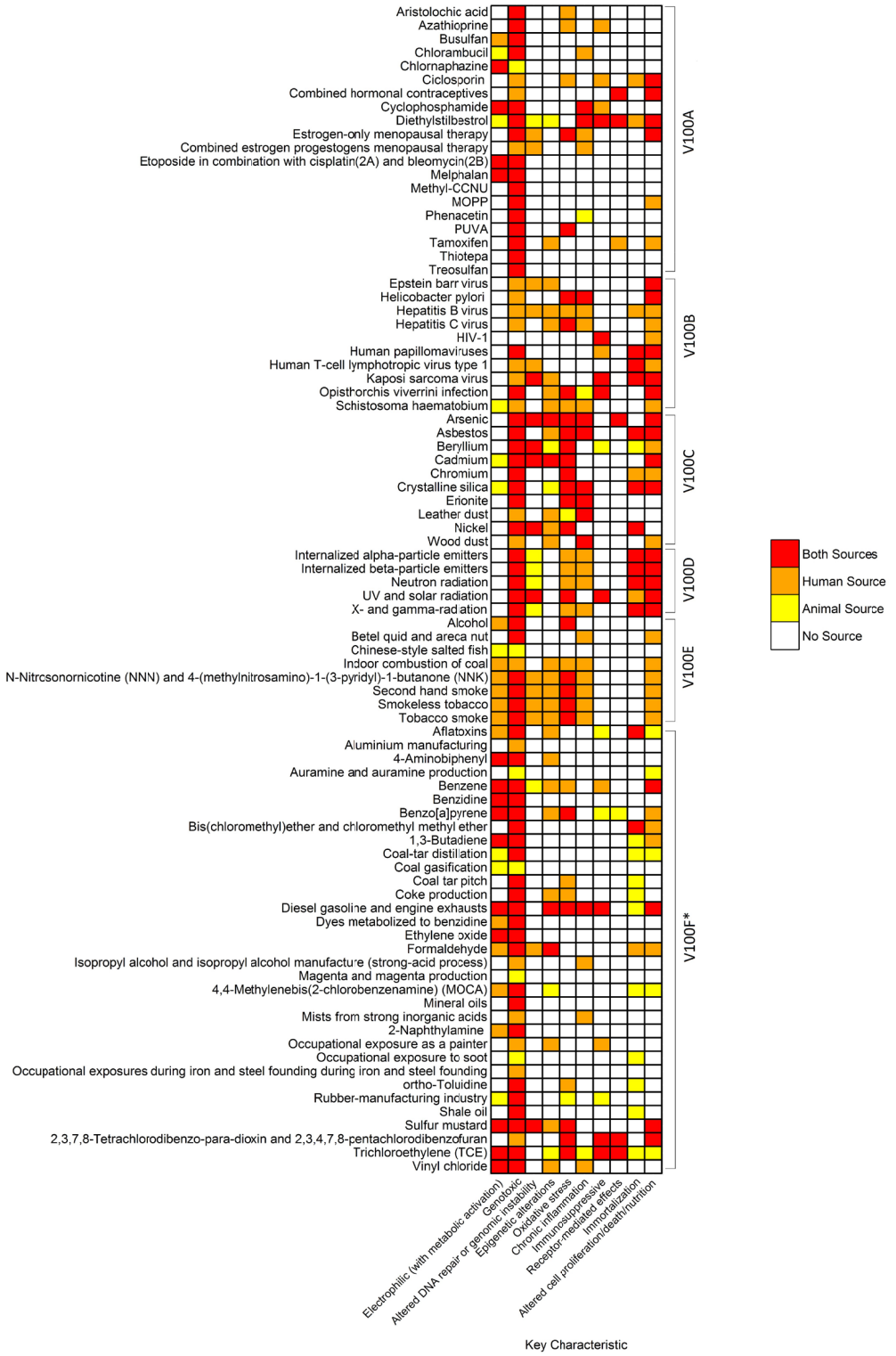


Fig. 22.6. Key characteristics of 86 Group 1 agents by type of agent (expressed as a percentage of the number of agents of each type demonstrating each of the 10 mechanistic characteristics): (a) pharmaceuticals; (b) biological agents; (c) arsenic, metals, fibres, and dusts; (d) radiation; (e) personal habits and indoor combustions; and (f) chemical agents and related occupations.

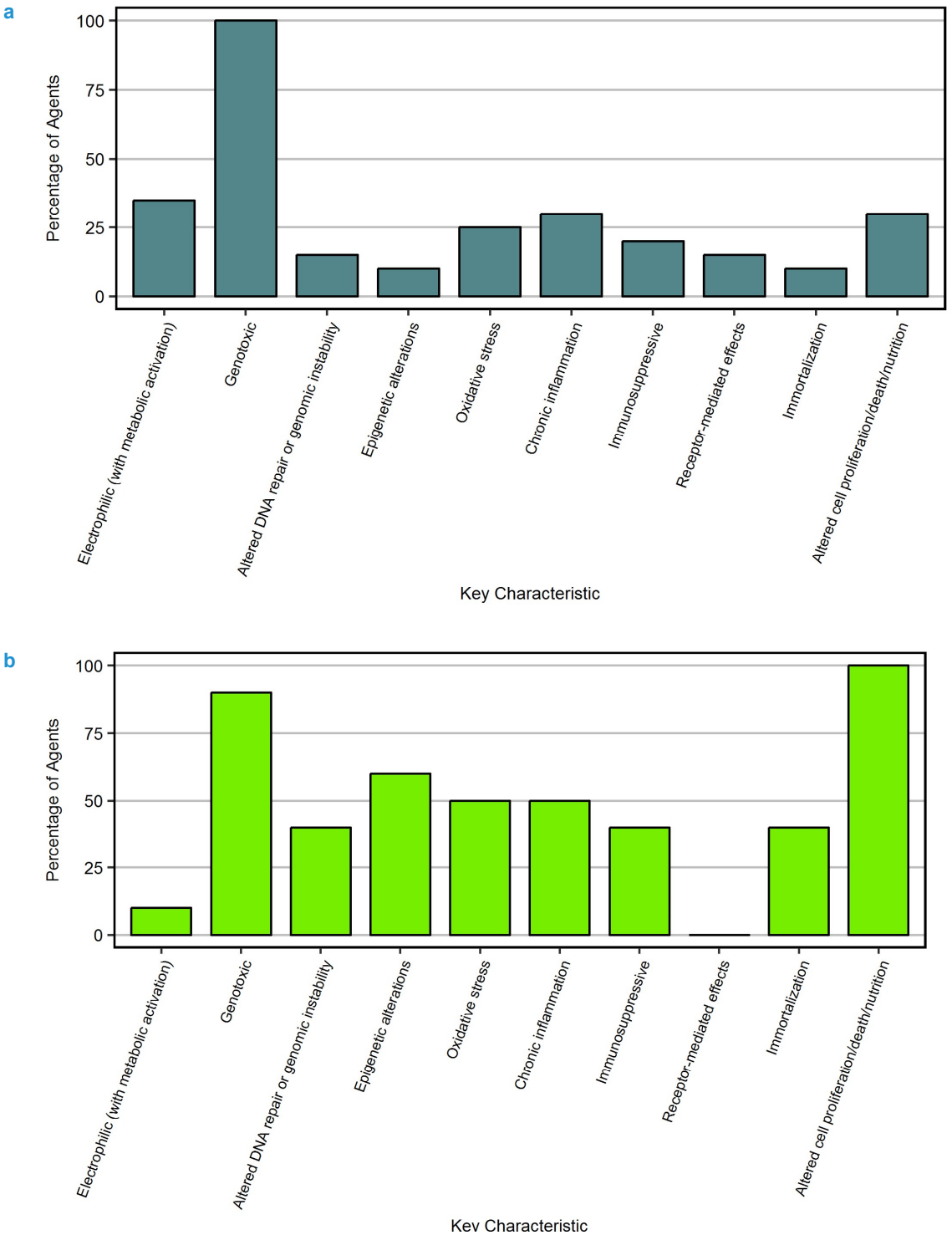
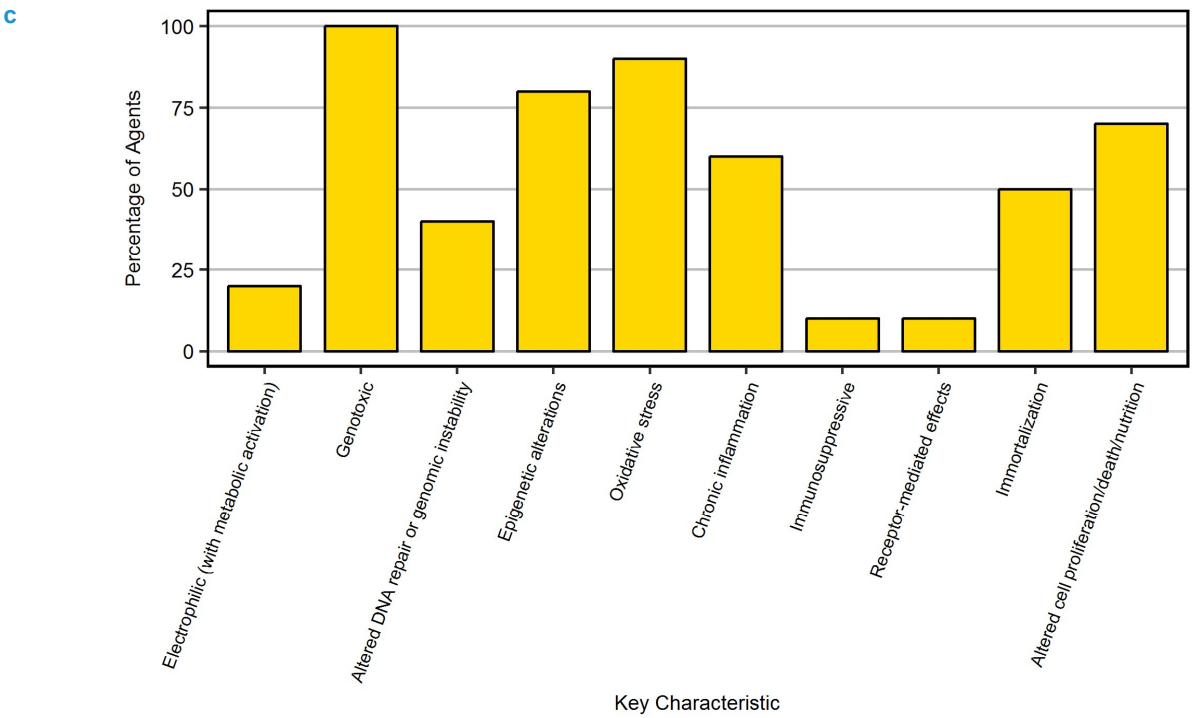


Fig. 22.6. Key characteristics of 86 Group 1 agents by type of agent (expressed as a percentage of the number of agents of each type demonstrating each of the 10 mechanistic characteristics): (a) pharmaceuticals; (b) biological agents; (c) arsenic, metals, fibres, and dusts; (d) radiation; (e) personal habits and indoor combustions; and (f) chemical agents and related occupations (continued).



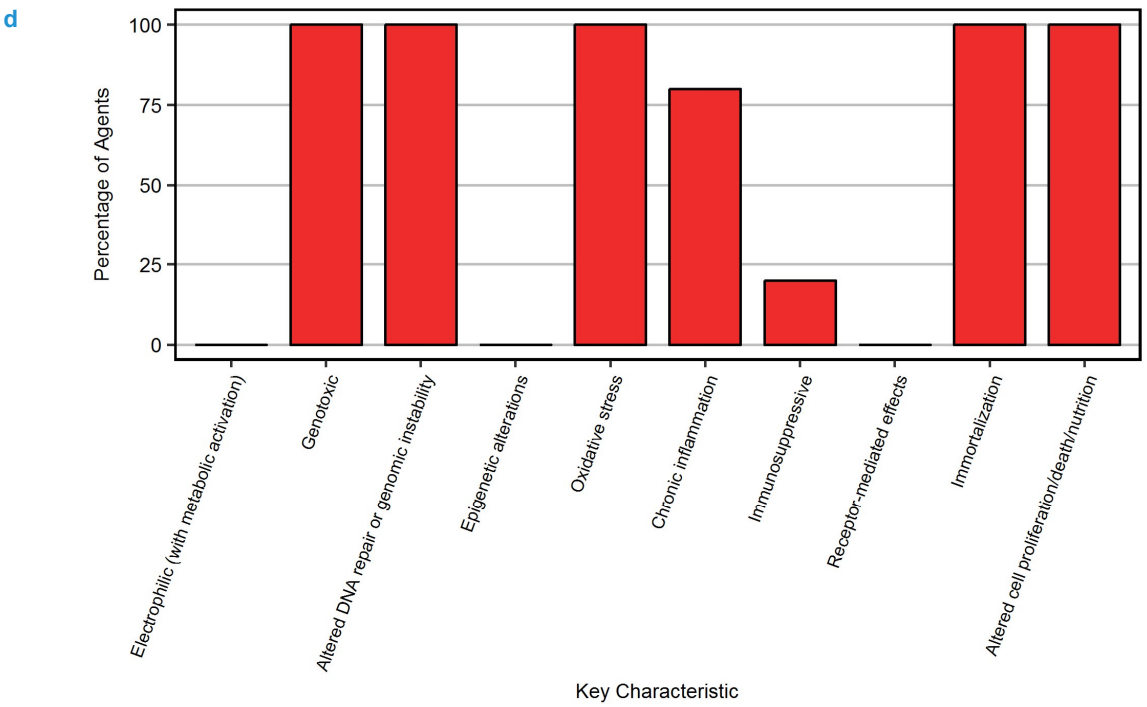
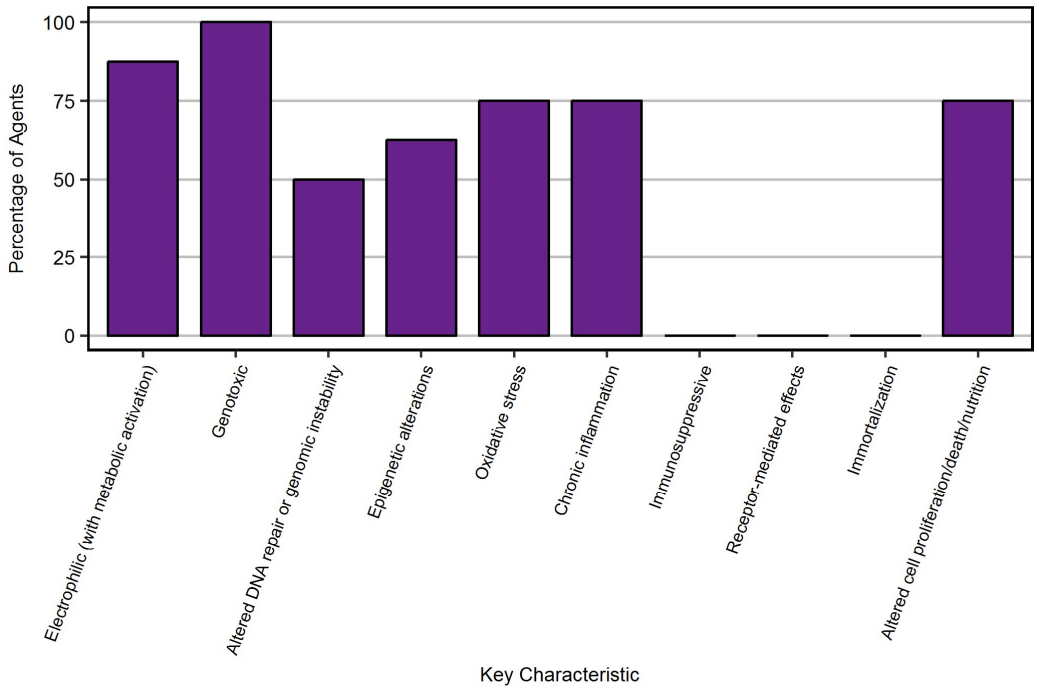
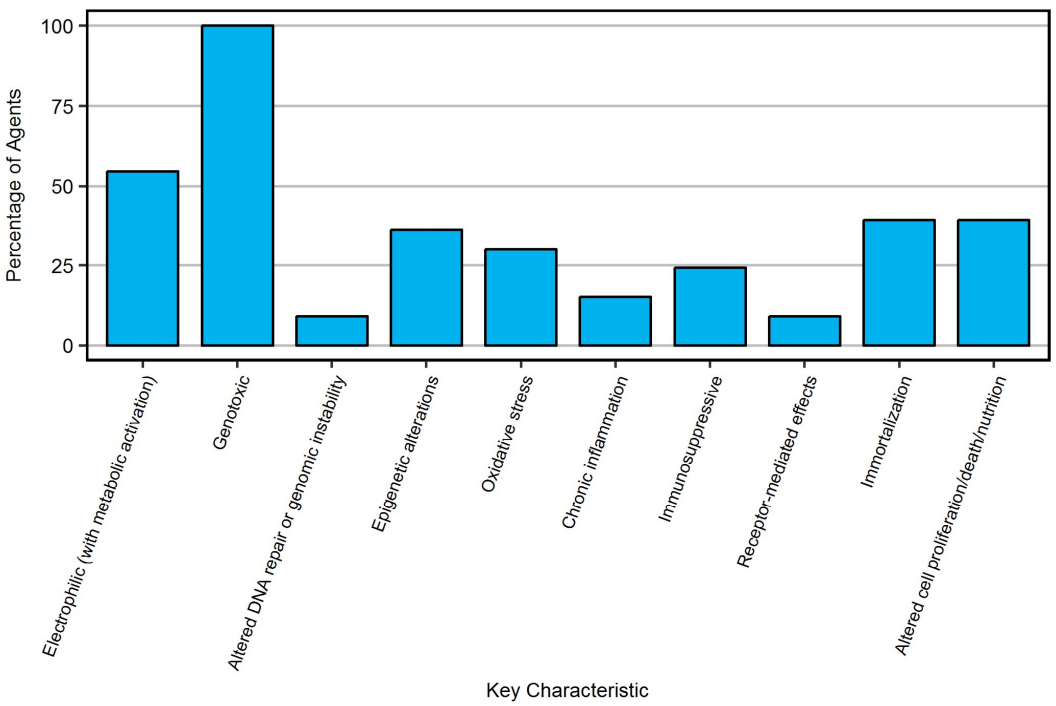


Fig. 22.6. Key characteristics of 86 Group 1 agents by type of agent (expressed as a percentage of the number of agents of each type demonstrating each of the 10 mechanistic characteristics): (a) pharmaceuticals; (b) biological agents; (c) arsenic, metals, fibres, and dusts; (d) radiation; (e) personal habits and indoor combustions; and (f) chemical agents and related occupations (continued).

e



f



agents and related occupations. Genotoxicity was the most prevalent key characteristic demonstrated by agents in the categories of pharmaceuticals; arsenic, metals, fibres, and dusts; personal habits and indoor combustions; and chemical agents and related occupations, and genotoxicity was exhibited by all agents in the category of radiation. Immortalization, genotoxicity, and altered cell proliferation, cell death, or nutrient supply are prominent characteristics of the biological agents. None of the biological agents demonstrated modulation of receptor-mediated effects, and none of the agents in the category of personal habits and indoor combustions appeared to act through modulation of receptor-mediated effects, through immunosuppression or through immortalisation. There are five agents in the category of radiation, all of which demonstrate the following key characteristics: is genotoxic; alters DNA repair or causes genomic instability; induces oxidative stress; causes immortalization; and alters cell proliferation, cell death, or nutrient supply. The profiles of key characteristics for pharmaceutical agents and for chemical agents and related occupations are remarkably similar, possibly reflecting the fact that despite their different exposure circumstances, some of these chemical entities may act via similar mechanisms.

Discussion

The present analysis of key characteristics of 86 agents classified as *carcinogenic to humans* (Group 1) by the *IARC Monographs Programme* was based on mechanistic information retrieved from the *IARC Monographs* (see Al-Zoughool et al., 2019 and Birkett et al., 2019). The profiles of key characteristics of these agents show several interesting patterns. First, all but seven agents exhibited multiple characteristics, an observation consistent with previous findings on the complexity and heterogeneity of carcinogenic pathways (Hanahan and Weinberg, 2011; Floor et al., 2012; Baker, 2014; Pickup et al., 2014; Roessler et al., 2014). Agents in the categories of biological agents; arsenic, metals, fibres, and dusts; personal habits and indoor combustions; and radiation demonstrated a wide spectrum of biological activity. Radiation has been linked to many hallmarks of cancer (Boss et al., 2014): this mechanistic profile, with multiple pathways involved for most radiation agents, is consistent with the broad spectrum of tumours associated with exposure to ionizing radiation (Chapter 21, by Krewski et al.). Viral oncogenesis is also multifaceted, and the multistep nature of viral oncogenesis is thought to be influenced by host genetic variability (Mesri et al., 2014).

Genotoxicity was the most prevalent mechanistic characteristic, demonstrated by 85 of the 86 agents considered, possibly reflecting the fact that the process of carcinogenesis necessarily involves genomic changes. This finding is consistent with an earlier evaluation of 180 Group 1, Group 2A, and Group 2B agents conducted by Bartsch and Malaveille (1989), who reported that 80–90% of the agents in these three categories demonstrated genotoxic characteristics. In the present analyses, genotoxicity was considered to include the following end-points: DNA damage, cytogenetic effects (including chromosomal aberrations, micronucleus formation, and aneuploidy), and gene mutations. Information drawn from the *IARC Monographs* showed that the overwhelming majority of the agents examined here induce one or more of these end-points. Even biological agents such as viruses that act primarily through non-genotoxic mechanisms induce cytogenetic effects and gene mutations as secondary events through chronic inflammation and oxidative stress.

Another important observation is that information on the key characteristics of the 86 Group 1 agents considered here is often derived from multiple sources (human in vivo, human in vitro, animal in vivo, and animal in vitro studies); for many agents, evidence is available from more than one of these sources. Concordance between animal and human sources of information was seen for several agents, particularly with respect to genotoxicity, an observation that lends additional support to the relevance of animal data for human cancer risk assessment.

Some caution must be used in interpreting the distribution of key characteristics across the Group 1 agents considered here. It is possible that the near universality of genotoxicity as a carcinogenic mechanism may be related to the way in which the *IARC Monographs* were compiled, with emphasis on the reporting of genotoxicity data. This would have been partially mitigated by the inclusion of mechanistic information from outside the *IARC Monographs* in the preparation of the mechanistic database evaluated separately by Birkett et al. (2019). It should also be noted that the *IARC Monographs* have been published over a long time span, extending from the early 1970s to the present (Saracci and Wild, 2015). Studies of agents in earlier *Monographs* would

have focused on changes such as DNA damage that could have been detected by the techniques available at that time. These agents may not have been evaluated exhaustively for pathways that have been identified more recently, such as those involving the multifactorial nature of carcinogenesis, or for the multiplicity of pathways operating during the process of agent-induced carcinogenesis.

A related limitation of the present analysis is that it did not distinguish direct genotoxicity of the agent or its metabolites from genotoxicity that occurs as a result of other responses, because this information was not generally provided in the *IARC Monographs* from which the mechanistic data on the Group 1 agents were abstracted. It is recommended that the distinction between primary and secondary genotoxic effects be noted in future *Monographs*, whenever possible.

Another limitation of the present results is that they are based on the information on mechanisms in Section 4 ("Other relevant data") of the *IARC Monographs*, which focused primarily on "established" and "likely" mechanisms. A full systematic review of the entire literature on biological mechanisms for all Group 1 agents was not undertaken, so the database may not reflect all mechanistic characteristics of the different agents. As a sensitivity analysis to examine the extent to which the *Monographs* captured most of the relevant information in this regard, Birkett et al. (2019) conducted a supplementary PubMed search to identify additional information on key characteristics not cited in the *Monographs*, or published since 2009. Although this sensitivity analysis was not based on an exhaustive search, it did identify additional information sources, of which the most notable was the identification of evidence for six additional agents that demonstrate modulation of receptor-mediated effects, beyond the seven agents noted in Fig. 22.1. Nonetheless, the overall findings are largely comparable with those presented without the additional data search (for further details, see Birkett et al., 2019).

As the *IARC Monographs Programme* has evolved from its inception in the early 1970s until the present time, the guidelines for carcinogen identification as set out in the Preamble to the *IARC Monographs* (IARC, 2006) have been updated from time to time, with increasing emphasis on the use of mechanistic information in the overall evaluation in the most recent updates. Nonetheless, the identification of Group 1 agents continues to rest heavily on the availability of *sufficient evidence* of carcinogenicity in epidemiological or clinical studies. Of the 111 distinct Group 1 agents identified up to and including Volume 109, no less than 102 demonstrated *sufficient evidence* of carcinogenicity in humans, and the remaining nine agents were placed in Group 1 after reference to mechanistic data or other considerations (as "mechanistic upgrades" according to the evaluation criteria outlined in the Preamble to the *IARC Monographs*; see Table 21.4, in Chapter 21, by Krewski et al.). Despite the inherent reliance on human epidemiological data in identifying agents that may increase human cancer risk, Section 4 ("Other relevant data") of the *IARC Monographs* increasingly provides detailed descriptions of the mechanisms by which agents under review may act, including agents not assigned to Group 1.

The epigenetic characteristics of the 74 Group 1 agents considered in Volumes 100A–E were assessed by Herceg et al. (2013). As in the present analysis, those authors used DNA methylation, histone modification, and altered expression of miRNAs as indicators of epigenetic alterations. They considered information from both the *IARC Monographs* and the general scientific literature, and identified 22 of the 74 Group 1 agents (30%) as demonstrating epigenetic effects. The present analysis, which examined Group 1 agents in Volumes 100A–F as well as Volumes 105 and 106, identified 33 of the 86 Group 1 agents (38%) as mediating epigenetic change.

In an earlier evaluation, Hernández et al. (2009) reported that 45 of the 371 agents (12%) in Groups 1, 2A, and 2B at the time of their analysis were not genotoxic. In their study, an agent was considered non-genotoxic if it showed negative results in the *Salmonella* mutagenicity assay (the Ames test) as well as in the mouse lymphoma assay, the in vitro chromosomal aberration test, the in vitro micronucleus test, the in vivo micronucleus test, and the in vivo chromosomal aberration test in bone marrow in rodents. These results support the role of non-genotoxic pathways in carcinogenesis, an observation that is reinforced by the prevalence of multiple characteristics of human carcinogens not associated with genotoxicity in the present analysis.

To ensure that all relevant evidence on the 10 key characteristics of human carcinogens is taken into account in future *Monographs* evaluations of agents that may cause cancer in humans, a carefully designed systematic review of the scientific literature would be required in conjunction with each evaluation.

However, to conduct a series of comprehensive systematic reviews of the key characteristics of all 86 agents considered in the present analysis would require a considerable effort, and was not attempted as part of the present project. The expert opinion of future IARC Working Groups charged with evaluating the mechanistic data on new agents selected for evaluation by the *IARC Monographs* would be of considerable value in this regard, but would ideally be supported by a concomitant systematic review of the relevant scientific literature on the key characteristics to ensure that the analysis would be as complete as possible.

Another issue that arises when discussing key characteristics of human carcinogens is whether indirect effects should be considered. Many agents have a direct carcinogenic effect, but in other cases the carcinogenic characteristic is the result of a secondary event along the mechanistic pathway. For example, cell proliferation can arise either as a result of a direct action of the agent on the cell or indirectly, as a result of cytotoxicity that stimulates cell proliferation to replace cells, through alterations in cell signalling without cytotoxicity, or via inhibition of cell proliferation that then results in selection of an altered clone of cells with a high proliferation rate. Although the downstream effect is the same (increased cell proliferation), the pathway leading to that result can be different. A similar issue arises with genotoxicity: many agents are not directly genotoxic but cause DNA damage by stimulating a chain of molecular changes (e.g. chronic inflammation). The current database does not contain the information needed to address these issues and cannot be used to draw conclusions about the detailed mechanism of action of an agent.

The 10 key characteristics are features of carcinogens rather than mechanisms. The analysis presented here does not address the sequence of events involved in carcinogenesis. For example, if the carcinogenic mechanism of action is being investigated for a genotoxic agent that requires metabolic activation, the mechanism needs to consider the entire metabolic pathway. If the agent is not metabolized to produce an electrophile, DNA damage will not occur. In such a case, biological effects that occur after induction of DNA damage also would not be observed. This sequential relationship is also apparent for characteristics such as chronic inflammation, which acts through the production of oxidative stress, release of cytokines, and stimulation of cell proliferation, which ultimately produces DNA damage.

The results of the present analysis can provide a basis for future efforts to categorize mechanistic data for carcinogens through a systematic review process. A full systematic review of all agents and all potential carcinogenic mechanisms is an intimidating prospect. However, such a review would provide a more comprehensive examination of mechanisms, because it would include studies that failed to find effects. It might also support a process that involves a sequence of mechanistic steps and mechanistic characteristics relevant to the development of cancer in humans.

The importance of systematic review in assembling all relevant evidence on a particular issue has been emphasized in the recent review of the EPA's Integrated Risk Information System (IRIS) (National Research Council, 2014) and is currently being implemented within the IRIS programme as a way of summarizing all relevant data in a comprehensive and reproducible manner. The EPA is also currently supporting the development of software tools specifically designed for systematic review of toxicological and epidemiological data (ICF, 2017).

The strong evidence linking genotoxicity to carcinogenesis is consistent with epidemiological data and experimental research. Genotoxic effects include the formation of DNA adducts or induction of single- and double-strand DNA breaks. Several lines of evidence from epidemiological studies and in experimental animals and model systems have shown that DNA adducts are strongly associated with cancer (Kriek et al., 1998; Phillips et al., 2015). Some genotoxic effects can lead to gene mutation, an important event in the pathway towards carcinogenesis, especially if it involves oncogenes or tumour suppressor genes. Chromosomal aberrations are another type of genetic alteration that occurs frequently in many tumours, especially solid tumours. Most tumour cells display aneuploidy, and for some tumours, characteristic chromosomal abnormalities have been identified (e.g. the Philadelphia chromosome in chronic myeloid leukaemia).

The complexity of the pathways involved in carcinogenesis and the fact that the cellular response to carcinogen exposure is modulated by host cell physiology, genetics, and other variables have prompted the development and application of sensitive assays that measure toxicity pathways and perturbations in the molecular functioning of the cell. The newly proposed toxicological testing paradigm (Krewski et al., 2014) focuses on high-throughput screening to detect changes in the molecular pathways of the cell in response to chemical exposure. This new

paradigm would be useful in comprehensive cancer risk assessment and would be able to detect distinct key mechanistic pathways operating after carcinogen exposure. In a similar initiative, the Kyoto Encyclopedia of Genes and Genomes (KEGG) website has compiled a comprehensive list of pathways associated with specific diseases (see the KEGG pathway database at http://www.genome.jp/kegg/pathway.html). KEGG also identified major in vitro assays that can be used to detect targets of these pathways. This attempt to understand the biological mechanisms of carcinogenesis is consistent with current practice of using in vitro assays to detect changes in critical signalling and other molecular pathways in cancer development, as proposed by Krewski et al. (2014).

Further analyses

The extensive database on key characteristics of human carcinogens developed here offers considerable potential for further analysis. More in-depth analyses are under way to explore the level of agreement between mechanistic data derived from human sources on the one hand and from animal sources on the other, as well as from in vivo and in vitro sources, issues that have received only limited attention here. An analysis of the key characteristics demonstrated by Group 1 agents on a site-specific basis is also planned; if agents that cause tumours at a specific site (e.g. the lung or the liver) are shown to demonstrate similar characteristics, this could provide new insights into site-specific carcinogenesis.

Although the present analysis found that most Group 1 agents demonstrated multiple key characteristics, with an average of four characteristics per agent, no attempt was made to conduct a multivariate analysis of these characteristics to determine whether similar agents tended to express similar characteristics. Recalling that pharmaceuticals as a class demonstrated a mechanistic profile similar to that of chemical agents and related occupations, it is possible that the chemotherapeutic agents and some of the chemical agents act via the same carcinogenic mechanisms. Cyclophosphamide and benzene (once used as a chemotherapeutic agent) may have some commonality in this respect, as might treosulfan and butadiene through the formation of the same diepoxide. Further study of these two groups, in terms of both mechanism of action and tumour site concordance, may provide insight into tumours that result from long-term exposure to chemotherapeutic agents.

Searching for patterns within homogeneous classes of agents would also be of future research interest. For example, one could examine mechanistic patterns within subgroups of pharmaceuticals, including antineoplastic agents, hormonal products, immunosuppressants, and analgesic mixtures. In a similar vein, Shin et al. (2015) have recently used bioactivity profiles for 38 agents derived from high-throughput in vitro assays to investigate patterns of toxicity associated with different scenarios of use.

Exposure to a single agent may result in the development of more than one type of tumour, perhaps through different pathways that involve different mechanistic characteristics. It would be of interest to examine the key characteristics for agents associated with specific tumour types. This would extend the work of Krewski et al. (Chapter 21) that examined concordance between animals and humans for 39 tumour sites and 14 organ and tissue systems, based on the database on tumours and tumour sites in humans and experimental animals developed by Grosse et al. (Annex 1). The profiles of key characteristics of agents associated with specific tumour sites could be examined to obtain additional insights into the mechanisms by which specific tumours occur. It would be of particular interest to analyse whether certain tumour sites demonstrate signature profiles.

Extending the mechanistic database to include additional information such as structural alerts relevant to carcinogenesis could also be informative. Although the present version of the mechanisms database includes the International Union of Pure and Applied Chemistry (IUPAC) International Chemical Identifier (InChI) for key chemical coding (Stein et al., 2003; Heller et al., 2015), this information has not been taken into account in the analyses completed to date. One possible source of auxiliary information on toxicological end-points that may be related to the 10 key characteristics is EPA's Toxicity Forecaster (ToxCast) programme (Judson et al., 2014; Knudsen et al., 2015), which now includes in vitro, in vitro, and in silico data on diverse toxicological end-points for more than 10 000 chemical substances, some of which overlap with the set of Group 1 agents considered in this chapter. The ToxCast database also includes information on several hundred toxicological assays, which could enrich the database of key characteristics used in the present analysis.

With the elaboration of the 10 key characteristics articulated by

Smith (Chapter 10) and Smith et al. (2016), mechanistic evaluations of new agents undertaken within the *IARC Monographs Programme* are beginning to make use of these characteristics, including the use of formal methods of systematic review to identify relevant mechanistic information. This has been successfully attempted in recent evaluations of some organochlorine insecticides and some chlorophenoxy herbicides (Loomis et al., 2015; Volume 113) and of red meat and processed meat (Bouvard et al., 2015; Volume 114).

It is expected that the search strategies used in future mechanistic evaluations will be refined as experience with the key characteristics accumulates. In an earlier evaluation of evidence of epigenetic alterations for 28 Group 1 agents, Chappell et al. (2016) searched for evidence of DNA methylation, histone modification, and expression of non-coding miRNAs, as was done in the present analysis, but with the addition of several more detailed search terms, specifically long non-coding RNA (lncRNA), small RNA, chromatin, and promotor methylation. Chappell et al. (2016) noted that the great majority (89%) of the studies on lncRNAs included in their review reported alterations in miRNAs, leading to results largely consistent with the search terms used here: 43% (12 of 28) of the agents evaluated by these authors demonstrated evidence of epigenetic alterations, similar to the 38% (33 of 86) of agents included in the present analysis. Continued experience with the evaluation of the 10 key characteristics of human carcinogens can be expected to further refine the criteria used for their identification, including both the toxicological events associated with these key characteristics and the assays used as evidence of these events.

There could be value in revisiting the present retrospective analysis of the 86 Group 1 agents identified in the *IARC Monographs* up to and including Volume 106, with respect to the conduct of a series of comprehensive systematic reviews on the 10 key characteristics of these agents, followed by an in-depth evaluation of the findings of the systematic reviews by experts in relevant disciplines. The development of criteria for evaluating the weight of evidence for the key characteristics, similar to that included in the Preamble to the *IARC Monographs* for human and animal data (IARC, 2006), might be contemplated at that time. Group 1 agents identified beyond Volume 106 for which mechanistic information had become available could also be included in such an analysis.

Baker et al. (2016) have recently applied supervised machine learning techniques to classify PubMed literature according to the hallmarks of cancer (Hanahan and Weinberg, 2000, 2011). In a case study of basal cell carcinoma and melanoma, only 46,727 of 121,488 abstracts from their original systematic literature search were classified as relevant, reflecting the potential time savings that may be achieved through automatic classification. An approach to extracting information on the 10 key characteristics of human carcinogens would be to apply these machine learning techniques and biomedical text mining methods to identify, in an automated fashion, articles associating these key characteristics with specific Group 1 agents. Because of the sheer size of a full systematic review of mechanistic information on all Group 1 agents, the use of automated search algorithms of this type could offer considerable efficiency gains in identifying potentially relevant mechanistic information. Although this approach could expedite identification of relevant articles, expert opinion and application of weight-of-evidence criteria would still have value in reducing the errors in the assignment of key characteristics to specific agents.

Conclusions

In considering the results presented in this chapter, it is important to emphasize that these mechanistic analyses are a first step in understanding the biological mechanisms by which cancer may occur in humans. Although considerable effort was expended in developing the database of key characteristics and their analyses in this chapter, these results should be viewed as preliminary, to be refined through more exhaustive systematic reviews of the relevant scientific literature and/or through discussion with a broad panel of experts on the mechanisms of carcinogenesis. The 10 key characteristics were endorsed by the participants in the IARC Workshop on Tumour Site Concordance and Mechanisms of Carcinogenesis, which provided oversight for this project; additional experience with the exploration of these characteristics in cancer research will serve to define their utility more fully. Equally important is to consider the nature of the evidence needed to establish that specific mechanistic characteristics are associated with human carcinogens. The current database has relied on the demonstration of certain toxicological end-points as evidence of these mechanistic characteristics; further consideration of these and other possible markers of the key characteristics of human carcinogens is warranted.

Mechanistic considerations are becoming increasingly prominent in the *IARC Monographs*, thereby enriching the body of evidence on which future analyses of this type may be based. The authors are planning to update the mechanistic database to include *Monographs* published subsequent to Volume 106, a task that will be greatly facilitated by the documentation of key characteristics of agents evaluated in recent *Monographs*.

Summary

Since its inception in the early 1970s, the *IARC Monographs Programme* has evaluated more than 1000 agents with respect to their carcinogenic hazard; of these, up to and including Volume 119 of the *IARC Monographs*, 120 agents met the criteria for classification as *carcinogenic to humans* (Group 1). Volume 100 of the *IARC Monographs* provided a review and update of Group 1 carcinogens. These agents were divided into six broad categories: pharmaceuticals; biological agents; arsenic, metals, fibres, and dusts; radiation; personal habits and indoor combustions; and chemical agents and related occupations. Data on biological mechanisms of action were extracted from the *Monographs* to assemble a database on the basis of 10 key characteristics of human carcinogens. After some grouping of similar agents, the characteristic profiles were examined for 86 Group 1 agents for which mechanistic information was available in the *IARC Monographs* up to and including Volume 106, based on information derived from human in vivo, human in vitro, animal in vivo, and animal in vitro studies. The most prevalent key characteristic was "is genotoxic", followed by "alters cell proliferation, cell death, or nutrient supply" and "induces oxidative stress". Most agents exhibited several of the 10 key characteristics, with an average of four characteristics per agent, a finding consistent with the notion that cancer development in humans involves multiple pathways. Information on the key characteristics was often available from multiple sources, with many agents demonstrating concordance between human and animal sources, particularly with respect to genotoxicity. Although a detailed comparison of the characteristics of different types of agent was not attempted here, the overall characteristic profiles for pharmaceutical agents and for chemical agents and related occupations appeared similar. Further in-depth analyses of this rich database of characteristics of human carcinogens are expected to provide additional insights into the mechanisms of human carcinogenesis.

Acknowledgements

The authors are grateful to Kate Z. Guyton for many helpful comments, which resulted in both improvements in the presentation of this chapter and additional insights into the findings in this chapter, and to the editors for their thorough review of several preliminary drafts. Mélissa Billard was a Visiting Scientist at IARC during the summers of 2013 and 2014, working under the direction of Yann Grosse and Robert Baan on assembling the mechanistic database analysed in this chapter. Julian Little is the Canada Research Chair in Human Genome Epidemiology at the University of Ottawa. Daniel Krewski is the Natural Sciences and Engineering Research Council of Canada Chair in Risk Science at the University of Ottawa. This chapter is dedicated to the memory of Jan M. Zielinski, who succumbed to cancer in 2016; before becoming ill, Dr Zielinski led the analytical team that conducted the analyses reported here.

References

Al-Zoughool M, Bird M, Rice JM, Billard M, Birkett N, Krewski D, et al. (2019). Development of a database of toxicological end-points and key characteristics of agents known to cause cancer in humans. J Toxicol Environ Health B Crit Rev. (forthcoming)

Baker S, Silins I, Guo Y, Ali I, Högberg J, Stenius U, et al. (2016). Automatic semantic classification of scientific literature according to the hallmarks of cancer. Bioinformatics. 32(3):432–40. http://dx.doi.org/10.1093/bioinformatics/btv585 PMID:26454282

Baker SG (2014). A cancer theory kerfuffle can lead to new lines of research. J Natl Cancer Inst. 107(2):dju405 http://dx.doi.org/10.1093/jnci/dju405 PMID:25528755

Bartsch H, Malaveille C (1989). Prevalence of genotoxic chemicals among animal and human carcinogens evaluated in the IARC Monograph Series. Cell Biol Toxicol. 5(2):115–27. http://dx.doi.org/10.1007/BF00122647 PMID:2766027

Benbrahim-Tallaa L, Baan RA, Grosse Y, Lauby-Secretan B, El Ghissassi F, Bouvard V, et al.; International Agency for Research on Cancer Monograph Working Group (2012). Carcinogenicity of diesel-engine and gasoline-engine exhausts and some nitroarenes. Lancet Oncol. 13(7):663–4. http://dx.doi.org/10.1016/S1470-2045(12)70280-2 PMID:22946126

Benbrahim-Tallaa L, Lauby-Secretan B, Loomis D, Guyton KZ, Grosse Y, El Ghissassi F, et al.; International Agency for Research on Cancer Monograph Working Group (2014). Carcinogenicity of perfluorooctanoic acid, tetrafluoroethylene, dichloromethane, 1,2-dichloropropane, and 1,3-propane sultone. Lancet Oncol. 15(9):924–5. http://dx.doi.org/10.1016/S1470-2045(14)70316-X PMID:25225686

Birkett N, Al-Zoughool M, Bird M, Zielinski JM, Krewski D (2019). Carcinogenic mechanisms for 109 human carcinogens. J Toxicol Environ Health B Crit Rev. (forthcoming)

Boss MK, Bristow R, Dewhirst MW (2014). Linking the history of radiation biology to the hallmarks of cancer. Radiat Res. 181(6):561–77. http://dx.doi.org/10.1667/RR13675.1 PMID:24811865

Bourdon-Lacombe JA, Moffat ID, Deveau M, Husain M, Auerbach S, Krewski D, et al. (2015). Technical guide for applications of gene expression profiling in human health risk assessment of environmental chemicals. Regul Toxicol Pharmacol. 72(2):292–309. http://dx.doi.org/10.1016/j.yrtph.2015.04.010 PMID:25944780

Bouvard V, Loomis D, Guyton KZ, Grosse Y, Ghissassi FE, Benbrahim-Tallaa L, et al.; International Agency for Research on Cancer Monograph Working Group (2015). Carcinogenicity of consumption of red and processed meat. Lancet Oncol. 16(16):1599–600. http://dx.doi.org/10.1016/S1470-2045(15)00444-1 PMID:26514947

Chappell G, Pogribny IP, Guyton KZ, Rusyn I (2016). Epigenetic alterations induced by genotoxic occupational and environmental human chemical carcinogens: a systematic literature review. Mutat Res Rev Mutat Res. 768:27–45. http://dx.doi.org/10.1016/j.mrrev.2016.03.004 PMID:27234561

Cogliano VJ, Baan RA, Straif K, Grosse Y, Secretan MB, El Ghissassi F, et al. (2004). The science and practice of carcinogen identification and evaluation. Environ Health Perspect. 112(13):1269–74. http://dx.doi.org/10.1289/ehp.6950 PMID:15345338

Cote I, Andersen ME, Ankley GT, Barone S, Birnbaum LS, Boekelheide K, et al. (2016). The Next Generation of Risk Assessment multi-year study – highlights of findings, applications to risk assessment, and future directions. Environ Health Perspect. 124(11):1671–82. http://dx.doi.org/10.1289/EHP233 PMID:27091369

Diaz-Moralli S, Tarrado-Castellarnau M, Miranda A, Cascante M (2013). Targeting cell cycle regulation in cancer therapy. Pharmacol Ther. 138(2):255–71. http://dx.doi.org/10.1016/j.pharmthera.2013.01.011 PMID:23356980

Ding L, Getz G, Wheeler DA, Mardis ER, McLellan MD, Cibulskis K, et al. (2008). Somatic mutations affect key pathways in lung adenocarcinoma. Nature. 455(7216):1069–75. http://dx.doi.org/10.1038/nature07423 PMID:18948947

EPA (2005). Guidelines for carcinogen risk assessment (EPA/630/P-03/001F). Washington (DC), USA: U.S. Environmental Protection Agency. Available from: http://www.epa.gov/cancerguidelines/.

Floor SL, Dumont JE, Maenhaut C, Raspe E (2012). Hallmarks of cancer: of all cancer cells, all the time? Trends Mol Med. 18(9):509–15. http://dx.doi.org/10.1016/j.molmed.2012.06.005 PMID:22795735

Grosse Y, Loomis D, Guyton KZ, Lauby-Secretan B, El Ghissassi F, Bouvard V, et al.; International Agency for Research on Cancer Monograph Working Group (2014). Carcinogenicity of fluoro-edenite, silicon carbide fibres and whiskers, and carbon nanotubes. Lancet Oncol. 15(13):1427–8. http://dx.doi.org/10.1016/S1470-2045(14)71109-X PMID:25499275

Guha N, Loomis D, Grosse Y, Lauby-Secretan B, El Ghissassi F, Bouvard V, et al.; International Agency for Research on Cancer Monograph Working Group (2012). Carcinogenicity of trichloroethylene, tetrachloroethylene, some other chlorinated solvents, and their metabolites. Lancet Oncol. 13(12):1192–3. http://dx.doi.org/10.1016/S1470-2045(12)70485-0 PMID:23323277

Gurusankar R, Yenugadhati N, Krishnan K, Hays S, Haines D, Zidek A, et al. (2017). The role of human biological monitoring in health risk assessment. Int J Risk Assess Manag. 20(1–3):136–97. http://dx.doi.org/10.1504/IJRAM.2017.082561

Hanahan D, Weinberg RA (2000). The hallmarks of cancer. Cell. 100(1):57–70. http://dx.doi.org/10.1016/S0092-8674(00)81683-9 PMID:10647931

Hanahan D, Weinberg RA (2011). Hallmarks of cancer: the next generation. Cell. 144(5):646–74. http://dx.doi.org/10.1016/j.cell.2011.02.013 PMID:21376230

Heller SR, McNaught A, Pletnev I, Stein S, Tchekhovskoi D (2015). InChI, the IUPAC International Chemical Identifier. J Cheminform. 7:23. http://dx.doi.org/10.1186/s13321-015-0068-4 PMID:26136848

Herceg Z, Lambert MP, van Veldhoven K, Demetriou C, Vineis P, Smith MT, et al. (2013). Towards incorporating epigenetic mechanisms into carcinogen identification and evaluation. Carcinogenesis. 34(9):1955–67. http://dx.doi.org/10.1093/carcin/bgt212 PMID:23749751

Hernández LG, van Steeg H, Luijten M, van Benthem J (2009). Mechanisms of non-genotoxic carcinogens and importance of a weight of evidence approach. Mutat Res. 682(2–3):94–109. http://dx.doi.org/10.1016/j.mrrev.2009.07.002 PMID:19631282

Hoeijmakers JH (2009). DNA damage, aging, and cancer. N Engl J Med. 361(15):1475–85. http://dx.doi.org/10.1056/NEJMra0804615 PMID:19812404

IARC (2006). Preamble to the *IARC Monographs*, amended January 2006. Available from: https://monographs.iarc.fr/previous-preamble/.

IARC (2012a). Arsenic, metals, fibres, and dusts. IARC Monogr Eval Carcinog Risks Hum. 100C:1–499. Available from: http://publications.iarc.fr/120 PMID:23189751

IARC (2012b). Biological agents. IARC Monogr Eval Carcinog Risks Hum. 100B:1–441. Available from: http://publications.iarc.fr/119 PMID:23189750

IARC (2012c). Chemical agents and related occupations. IARC Monogr Eval Carcinog Risks Hum. 100F:1–599. Available from: http://publications.iarc.fr/123 PMID:23189753

IARC (2012d). Personal habits and indoor combustions. IARC Monogr Eval Carcinog Risks Hum. 100E:1–575. Available from: http://publications.iarc.fr/122 PMID:23193840

IARC (2012e). Pharmaceuticals. IARC Monogr Eval Carcinog Risks Hum. 100A:1–437. Available from: http://publications.iarc.fr/118 PMID:23189749

IARC (2012f). Radiation. IARC Monogr Eval Carcinog Risks Hum. 100D:1–437. Available from: http://publications.iarc.fr/121 PMID:23189752

IARC (2013). Diesel and gasoline engine exhausts and some nitroarenes. IARC Monogr Eval Carcinog Risks Hum. 105:1–704. Available from: http://publications.iarc.fr/129. PMID:26442290

IARC (2014). Trichloroethylene, tetrachloroethylene, and some other chlorinated agents. IARC Monogr Eval Carcinog Risks Hum. 106:1–514. Available from: http://publications.iarc.fr/130. PMID:26214861

IARC (2016a). Outdoor air pollution. IARC Monogr Eval Carcinog Risks Hum. 109:1–448. Available from: http://publications.iarc.fr/538.

IARC (2016b). Polychlorinated biphenyls and polybrominated biphenyls. IARC Monogr Eval Carcinog Risks Hum. 107:1–502. Available from: http://publications.iarc.fr/131.

IARC (2017a). Some chemicals used as solvents and in polymer manufacture. IARC Monogr Eval Carcinog Risks Hum. 110:1–276. Available from: http://publications.iarc.fr/547.

IARC (2017b). Some nanomaterials and some fibres. IARC Monogr Eval Carcinog Risks Hum. 111:1–316. Available from: http://publications.iarc.fr/552.

ICF (2017). DRAGON: an online tool for systematic review. Fairfax (VA), USA: ICF. Available from: https://www.icf.com/resources/solutions-and-apps/dragon-online-tool-systematic-review.

Judson R, Houck K, Martin M, Knudsen T, Thomas RS, Sipes N, et al. (2014). In vitro and modelling approaches to risk assessment from the U.S. Environmental Protection Agency ToxCast programme. Basic Clin Pharmacol Toxicol. 115(1):69–76. http://dx.doi.org/10.1111/bcpt.12239 PMID:24684691

Klaunig JE, Wang Z, Pu X, Zhou S (2011). Oxidative stress and oxidative damage in chemical carcinogenesis. Toxicol. Appl Pharmacol. 254(2):86–99. http://dx.doi.org/10.1016/j.taap.2009.11.028 PMID:21296097

Knudsen TB, Keller DA, Sander M, Carney EW, Doerrer NG, Eaton DL, et al. (2015). FutureTox II: *in vitro* data and *in silico* models for predictive toxicology. Toxicol Sci. 143(2):256–67. http://dx.doi.org/10.1093/toxsci/kfu234 PMID:25628403

Krewski D, Westphal M, Andersen ME, Paoli GM, Chiu WA, Al-Zoughool M, et al. (2014). A framework for the next generation of risk science. Environ Health Perspect. 122(8):796–805. http://dx.doi.org/10.1289/ehp.1307260 PMID:24727499

Kriek E, Rojas M, Alexandrov K, Bartsch H (1998). Polycyclic aromatic hydrocarbon-DNA adducts in humans: relevance as biomarkers for exposure and cancer risk. Mutat Res. 400(1–2):215–31. http://dx.doi.org/10.1016/S0027-5107(98)00065-7 PMID:9685648

Lauby-Secretan B, Loomis D, Grosse Y, El Ghissassi F, Bouvard V, Benbrahim-Tallaa L, et al.; International Agency for Research on Cancer Monograph Working Group (2013). Carcinogenicity of polychlorinated biphenyls and polybrominated biphenyls. Lancet Oncol. 14(4):287–8. http://dx.doi.org/10.1016/S1470-2045(13)70104-9 PMID:23499544

Loomis D, Grosse Y, Lauby-Secretan B, El Ghissassi F, Bouvard V, Benbrahim-Tallaa L, et al.; International Agency for Research on Cancer Monograph Working Group (2013). The carcinogenicity of outdoor air pollution. Lancet Oncol. 14(13):1262–3. http://dx.doi.org/10.1016/S1470-2045(13)70487-X PMID:25035875

Loomis D, Guyton K, Grosse Y, El Ghissasi F, Bouvard V, Benbrahim-Tallaa L, et al.; International Agency for Research on Cancer Monograph Working Group (2015). Carcinogenicity of lindane, DDT, and 2,4-dichlorophenoxyacetic acid. Lancet Oncol. 16(8):891–2. http://dx.doi.org/10.1016/S1470-2045(15)00081-9 PMID:26111929

Meek ME, Boobis A, Cote I, Dellarco V, Fotakis G, Munn S, et al. (2014). New developments in the evolution and application of the WHO/IPCS framework on mode of action/species concordance analysis. J Appl Toxicol. 34(1):1–18. http://dx.doi.org/10.1002/jat.2949 PMID:24166207

Mesri EA, Feitelson MA, Munger K (2014). Human viral oncogenesis: a cancer hallmarks analysis. Cell Host Microbe. 15(3):266–82. http://dx.doi.org/10.1016/j.chom.2014.02.011 PMID:24629334

Miller JA (1970). Carcinogenesis by chemicals: an overview – G. H. A. Clowes Memorial Lecture. Cancer Res. 30(3):559–76. PMID:4915745

Multhoff G, Radons J (2012). Radiation, inflammation, and immune responses in cancer. Front Oncol. 2:58. http://dx.doi.org/10.3389/fonc.2012.00058 PMID:22675673

National Research Council (2014). Review of EPA's Integrated Risk Information System (IRIS) Process. Washington (DC), USA: National Academies Press. http://dx.doi.org/10.17226/18764

National Toxicology Program (2014). Report on carcinogens. 13th ed. Research Triangle Park (NC), USA: Department of Health and Human Services, Public Health Service.

Phillips TD, Richardson M, Cheng YS, He L, McDonald TJ, Cizmas LH, et al. (2015). Mechanistic relationships between hepatic genotoxicity and carcinogenicity in male B6C3F1 mice treated with polycyclic aromatic hydrocarbon mixtures. Arch Toxicol. 89(6):967–77. http://dx.doi.org/10.1007/s00204-014-1285-8 PMID:24888377

Pickup MW, Mouw JK, Weaver VM (2014). The extracellular matrix modulates the hallmarks of cancer. EMBO Rep. 15(12):1243–53. http://dx.doi.org/10.15252/embr.201439246 PMID:25381661

Reddel RR (2000). The role of senescence and immortalization in carcinogenesis. Carcinogenesis. 21(3):477–84. http://dx.doi.org/10.1093/carcin/21.3.477 PMID:10688868

Roessler S, Budhu A, Wang XW (2014). Deciphering cancer heterogeneity: the biological space. Front Cell Dev Biol. 2:12. http://dx.doi.org/10.3389/fcell.2014.00012 PMID:25364720

Saracci R, Wild CP (2015). International Agency for Research on Cancer: the first 50 years, 1965–2015. Lyon, France: International Agency for Research on Cancer. Available from: http://publications.iarc.fr/449.

Shin H-M, Ernstoff A, Arnot JA, Wetmore BA, Csiszar SA, Fantke P, et al. (2015). Risk-based high-throughput chemical screening and prioritization using exposure models and in vitro bioactivity assays. Environ Sci Technol. 49(11):6760–71. http://dx.doi.org/10.1021/acs.est.5b00498 PMID:25932772

Smith MT, Guyton KZ, Gibbons CF, Fritz JM, Portier CJ, Rusyn I, et al. (2016). Key characteristics of carcinogens as a basis for organizing data on mechanisms of carcinogenesis. Environ Health Perspect. 124(6):713–21. http://dx.doi.org/10.1289/ehp.1509912 PMID:26600562

Snyder RD (2010). Possible structural and functional determinants contributing to the clastogenicity of pharmaceuticals. Environ Mol Mutagen. 51(8–9):800–14. http://dx.doi.org/10.1002/em.20626 PMID:20872827

Stein SE, Heller SR, Tchekhovskoi DV (2003). An open standard for chemical structure representation: the IUPAC Chemical Identifier. In: Proceedings of the International Chemical Information Conference, Nimes, France, 19–22 October 2003. Tetbury, United Kingdom: Infonortics; pp. 131–43.

Vainio H, Magee PN, McGregor DB, McMichael AJ, editors (1992). Mechanisms of carcinogenesis in risk identification. Lyon, France: International Agency for Research on Cancer (IARC Scientific Publication No. 116).

Willeit P, Willeit J, Mayr A, Weger S, Oberhollenzer F, Brandstätter A, et al. (2010). Telomere length and risk of incident cancer and cancer mortality. JAMA. 304(1):69–75. http://dx.doi.org/10.1001/jama.2010.897 PMID:20606151