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Key characteristics of 86 agents known to cause cancer in humans

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ABSTRACT

Since the inception of the International Agency for Research on Cancer (IARC) in the early 1970s, the *IARC Monographs Programme* has evaluated more than 1000 agents with respect to 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: (I) pharmaceuticals; (II) biological agents; (III) arsenic, metals, fibers, and dusts; (IV) radiation; (V) personal habits and indoor combustions; and (VI) chemical agents and related occupations. Data on biological mechanisms of action (MOA) were extracted from the *Monographs* to assemble a database on the basis of ten key characteristics attributed to 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 upon data 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 ten 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 agents 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 MOA of human cancer development.

KEYWORDS

carcinogenesis; mechanisms of action; key characteristics of carcinogens; toxicological endpoints; sources of information

Introduction



Since the establishment of the **International Agency for Research on Cancer (IARC)** 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 assess 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 determine and integrate this evidence provided by human epidemiological studies, animal cancer bioassays, and information on possible biological mechanisms of action (MOA), and subsequently 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),

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Not classifiable as to its carcinogenicity to humans
(Group 3),

Probably not carcinogenic to humans (Group 4).

These evaluations involve classifying the data from both the human and animal investigations as providing: (1) *sufficient evidence of carcinogenicity*, (2) *limited evidence of carcinogenicity*, (3) *inadequate evidence of carcinogenicity*, or (4) *evidence suggesting lack of carcinogenicity*. The information on biological MOA may be evaluated as *strong*, *moderate*, or *weak*, and taken into consideration in the overall evaluation.

The role of MOA findings 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 MOA data may be used to identify agents with the potential to induce cancer in humans. The consensus report of the Working Group documented several MOA that were considered to be relevant for 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 MOA observations in cancer risk assessment. Related risk assessment practices include mode of action (Meek et al. 2014) and pathways of toxicity (Bourdon-Lacombe et al. 2015; Cote et al. 2016; Krewski et al. 2014), as well as dosimetric considerations (Gurusankar et al. 2017).

The aim of this review is to examine available data on MOA of the Group 1 agents identified up to and including Volume 106 of the *IARC Monographs* (Table 1). The present analysis was based upon a review of human cancer mechanisms, conducted by 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 postulated to be relevant to carcinogenesis occurrence. Data on these 24 end-points was derived from human *in vivo*, human *in vitro*, animal *in vivo*, and animal *in vitro* studies (Al-Zoughool et al. 2019). During

Table 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.

the November 2012 meeting, the Workshop participants identified ten broader key characteristics of carcinogens (Smith et al. 2016). Information on these characteristics was extracted from the *IARC Monographs* and employed to develop a database of key characteristics for Group 1 agents (Al-Zoughool et al. 2019). The key characteristics of Group 1 agents identified in the *IARC Monographs* up to and including Volume 106 are the focus of this review, and results of an exploratory evaluation of findings of these key characteristics obtained from the *IARC Monographs* are presented.

Key characteristics of human carcinogens

Smith et al. (2016) introduced ten key characteristics of human carcinogens, as listed in Table 2. Toxicological end-points used as indicators of these characteristics are noted in Table 2. A brief

summary of each of these characteristics and associated toxicological end-points is provided below.

Characteristic 1: is electrophilic or may be metabolically activated to electrophiles

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

Characteristic 2: is genotoxic

Genotoxicity is the ability to induce DNA damage that leads to formation of DNA adducts, single- or double-strand breaks, or other chromosomal alterations, as measured by three associated toxicological end-points: (i) DNA damage: 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 display a critical role in human carcinogenesis development (Ding et al. 2008); (iii) clastogenic effects reflect damage to chromosomes, including DNA breakage, or the rearrangement, gain, or loss of chromosome fragments (Snyder et al. 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 may confer potent mutator phenotypes that subsequently yield genomic instability.

Characteristic 4: induces epigenetic alterations

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

Table 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

Table 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 tumors and tumor 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 tumors and tumor sites in humans and animals
100A	1	Aristolochic acid	Aristolochic acid
100A	2	Azathioprine	Aristolochic acid, plants containing 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
100A	13	Melphalan	Etoposide in combination with cisplatin and bleomycin Melphalan
100A	14	PUVA (psoralen–UVA photochemotherapy)	Methoxsalen in combination with UVA
100A	15	MOPP	MOPP
100A	16	Phenacetin	Phenacetin
100A	17	1-(2-Chloroethyl)-3-(4-methylcyclohexyl)- 1-nitrosoarea (Methyl-CCNU)	Phenacetin, analgesic mixtures containing 1-(2-Chloroethyl)-3-(4-methylcyclohexyl)- 1-nitrosoarea (Methyl-CCNU)
100A	18	Tamoxifen	Tamoxifen
100A	19	Thiotepa	Thiotepa
100A	20	Treosulfan	Treosulfan
100B	21	<i>Opisthorchis viverrini</i> and <i>Clonorchis sinensis</i>	<i>Clonorchis sinensis</i> (infection with) <i>Opisthorchis viverrini</i> (infection with)
100B	22	Epstein–Barr virus	Epstein–Barr virus
100B	23	<i>Helicobacter pylori</i>	<i>Helicobacter pylori</i> (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
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	<i>Schistosoma haematobium</i>	<i>Schistosoma haematobium</i> (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

(Continued)

Table 3. (Continued).

Volume ^a	Agent number	86 agents used in the analysis of key characteristics	111 agents used in the analysis of concordance between tumors and tumor sites in humans and animals
100E	46	Consumption of alcoholic beverages	Internalized radionuclides that emit β -particles 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	<i>N'</i> -Nitrosornicotine (NNN) and 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)	<i>N'</i> -Nitrosornicotine (NNN) and 4-(Methylnitrosamino)-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[<i>a</i>]pyrene	Benzo[<i>a</i>]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
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	<i>ortho</i> -Toluidine	<i>ortho</i> -Toluidine
100F	78	Painter, occupational exposure as a	Painter, occupational exposure as a
100F	79	2,3,7,8-Tetrachlorodibenzo- <i>para</i> -dioxin, 2,3,4,7,8-Pentachlorodibenzofuran, 3,3',4,4',5-Pentachlorobiphenyl	2,3,4,7,8-Pentachlorodibenzofuran 2,3,7,8-Tetrachlorodibenzo- <i>para</i> -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 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.

are independent of mutation and that might 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 microRNA expression. 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 occurs as an imbalance between formations of reactive oxygen species (ROS) and detoxification of the radical species within cells and tissues. Reactive oxygen species induce a cascade of events that include DNA mutation and oxidative DNA damage. Both are key events in carcinogenesis occurrence (Klaunig et al. 2011).

Characteristic 6: induces chronic inflammation

Chronic inflammation might arise from persistent infection as with human papillomavirus or with *Helicobacter pylori* as well as from exogenous irritants such as silica or asbestos fibers. Persistent infection and chronic inflammation disrupt local tissue homeostasis and alter cell signalling, leading to recruitment and activation of inflammatory cells. Strong links exist between inflammation and induction of oxidative stress and genomic instability which makes it difficult to separate out the relative importance of each of these mechanisms. These linkages to other pathways might be the basis for 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 antigens on tumor cells. In contrast to other key characteristics, immune suppression does not play a direct part in transforming normal cells into tumor 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 may occur when agents mimic the structure of endogenous ligands that bind to cells and activate cell surface receptors or intracellular receptors, thereby initiating or modifying a plethora of signal transduction pathways that, among other responses, stimulate cell proliferation. Receptor-mediated effects might produce hormonal effects whereby external agents interfere with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body. These external factors might also demonstrate reactivity similar to endogenously produced hormones, which lead to mediation of changes in homeostasis, reproduction, development, or behavior.

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 entering 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, resulting in 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 proliferation and spread of cancer cells. Defects in programmed cell death might induce carcinogenesis; 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) affect tumor growth.

Agents known to cause cancer in humans

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

Although additional Group 1 agents have since been identified (Table 1), the present assessment 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; IARC 2016b; Lauby-Secretan et al. 2013), (ii) outdoor air pollution and (iii) particulate matter in outdoor air pollution (both evaluated in Volume 109; IARC 2016a; Loomis et al. 2013), (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 fibers (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 MOA in Section 4 (“Other relevant data”) of the *IARC Monographs* is based upon groups of agents that act via a similar mechanism. For example, internalized radionuclides that emit α -particles are discussed in the *Monographs* as a group with the same MOA. Birkett et al. (2019) (this volume) 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 3, along with their relationship to the 111 distinct agents identified up to and including Volume 109 employed by Krewski et al. (2019a) in a parallel analysis of overlap between tumors and tumor sites in animals and humans.

Database of mechanistic characteristics

Al-Zoughool et al. (2019) assembled a database of toxicological end-points 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. Data on the 24 toxicological end-points was retrieved from Section 4 (“Other relevant data”) of the *IARC Monographs*. The database of key characteristics was then created by mapping the 24 toxicological end-points to the ten characteristics as indicated in Table 2 (see Al-Zoughool et al. 2019, for details).

Because the database includes observations 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.

A detailed analysis of the database developed by Al-Zoughool et al. (2019) was conducted in collaboration with participants in collaboration with other participants in the Workshop on Tumour

Site Concordance and Mechanisms of Carcinogenesis sponsored by the IARC in 2012 (Baan, Stewart, and Straif 2019). Highlights of this analysis are provided below.

Key characteristics of 86 human carcinogens

The key characteristics of the 86 Group 1 agents considered here are summarized in Figure 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 epigenetic alterations”. Nearly all agents demonstrated genotoxicity as one of their mechanistic properties; all but one of the 86 agents considered exhibited evidence of this characteristic (the one 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.

Figure 2 illustrates 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. Genotoxicity data were available from each of the four sources for at least 65% of the agents. Human *in vivo* investigations contributed the most evidence on four of the ten key characteristics for these 86 agents, including “is electrophilic”, “is genotoxic”, “induces epigenetic alterations”, and “induces chronic inflammation”. Human *in vitro* studies provided 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 investigations as sources of information on the key characteristics of human carcinogens may be attributed to the increasing utilization of molecular and genetic markers in human studies. Epidemiological investigations conducted in the occupational or general environment often analyze 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 data on genotoxicity.

Figure 3 presents the number of agents demonstrating multiple characteristics as evidenced from

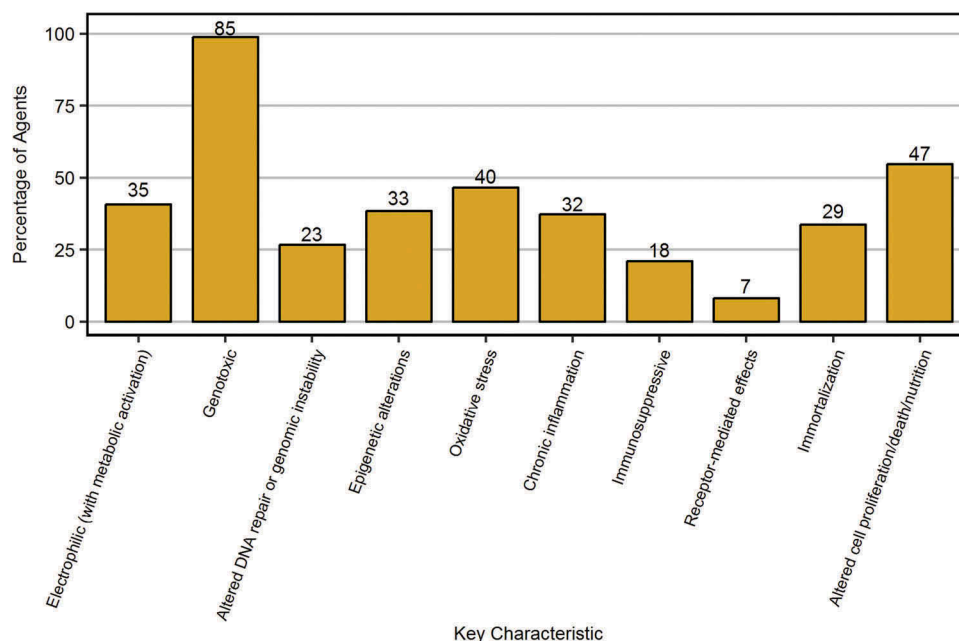


Figure 1. Key characteristics of 86 Group 1 agents (number of agents shown above each characteristic).

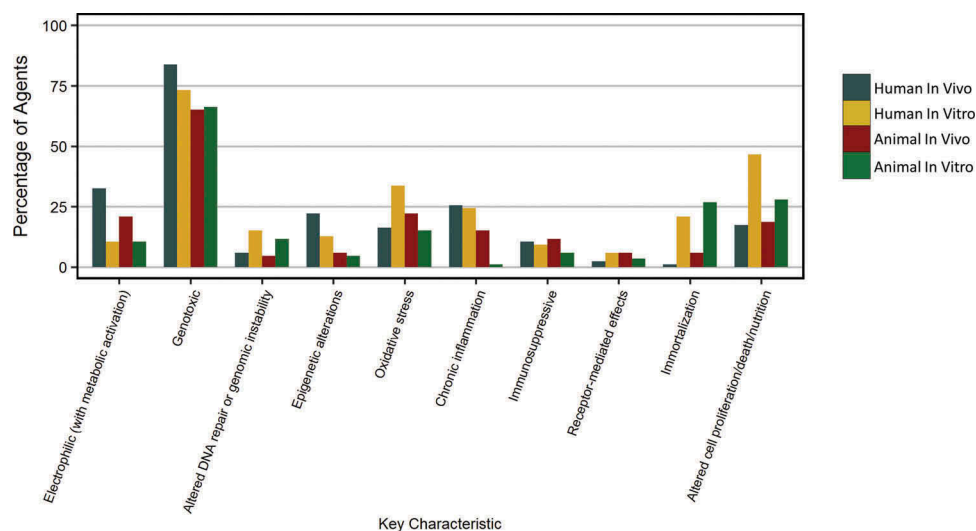


Figure 2. Sources of information on key characteristics of 86 Group 1 agents (sources are human in vivo, human in vitro, animal in vivo, and animal in vitro studies).

studies in animals and in humans. The 86 Group 1 agents considered here express 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 displaying six. No agent expressed all ten key characteristics.

Figure 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 color 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 Figure 1, the single most prominent characteristic was genotoxicity: all agents except HIV-1 exhibited 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 such as all radiation sources, some pharmaceutical agents,

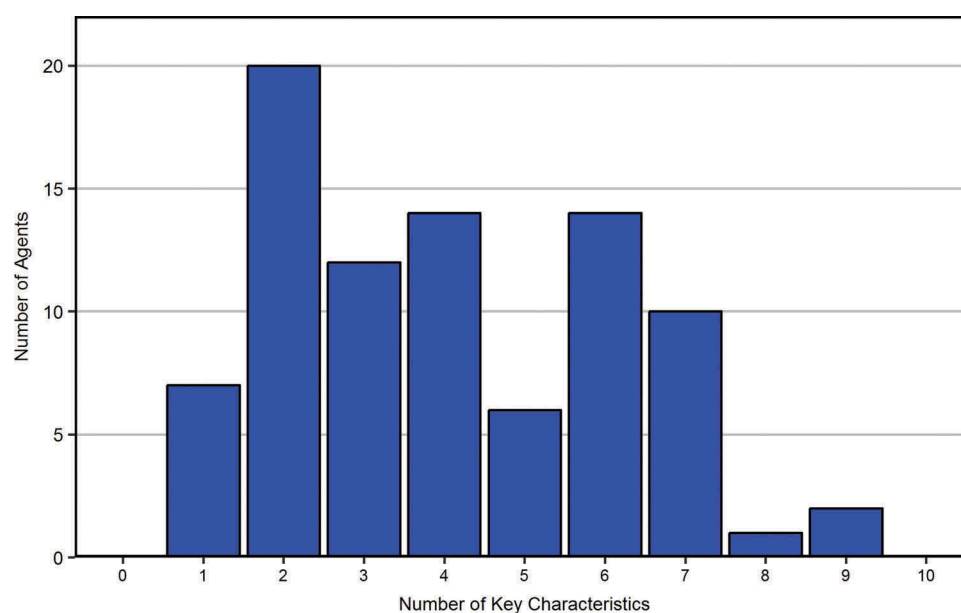


Figure 3. Number of Group 1 agents demonstrating one or more key characteristics.

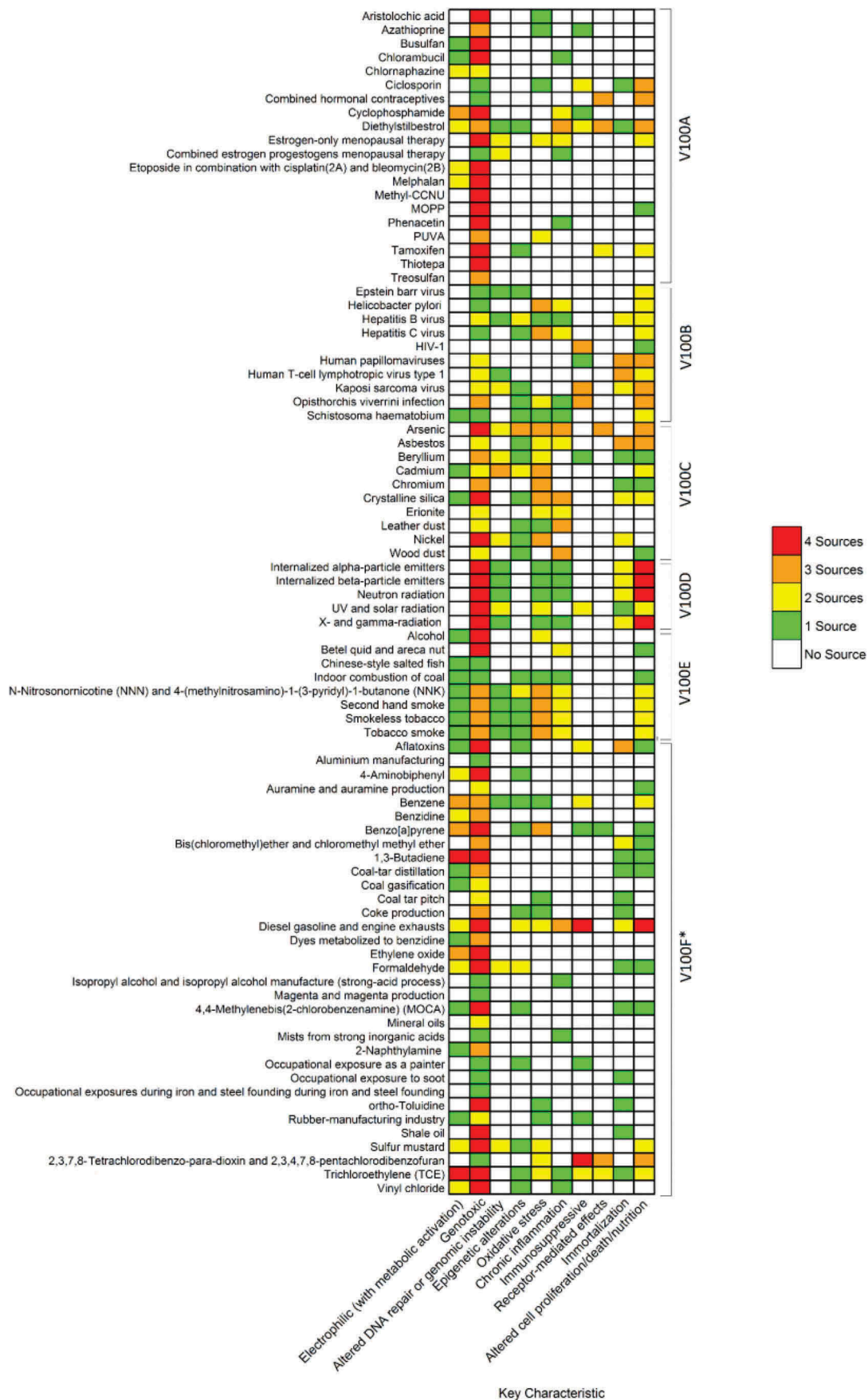


Figure 4. Heat map showing the strength of evidence for key characteristics of 86 Group 1 agents according to the number of information sources (sources are human in vivo, human in vitro, animal in vivo, and animal in vitro studies).

and some chemical agents, genotoxicity was demonstrated in all four test systems, confirming that genotoxicity is central to the carcinogenic pathways of these agents.

Figure 4 also shows that most agents exhibited 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 display similar characteristics, probably due to the strong presence in relevant workplaces of PAHs, although other factors such as the nature of inorganic substances and their chemical composition might also play a role. Other occupationally relevant agents, such as exposures during iron and steel founding and aluminium production, demonstrated 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 ten key characteristics of human carcinogens is shown in the heat map in Figure 5. This heat map, prepared by combining the *in vivo* and *in vitro* sources of data on key characteristics for humans and for animals, indicates whether findings on the key characteristics for a given agent are 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: observations from both human and animal sources are available for 63 of the 85 agents demonstrating evidence of genotoxicity. Ten agents – diethylstilbestrol (DES), 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 TCE –

demonstrate overlap between human and animal sources of information for at least five of the key characteristics.

Comparisons between the results in Figures 4 and 5 provide additional insights into the key characteristics of the Group 1 agents considered here. For example, in the case of DES, Figure 4 indicates that there are data from one, two, or three sources on nine key characteristics (all except “induces oxidative stress”), but Figure 5 clarifies that there are both human and animal data for only five of these. For chlornaphazine, Figure 4 shows two sources of information, for “is electrophilic or can be metabolically activated to electrophiles” and “is genotoxic”, whereas the corresponding data in Figure 5 demonstrate overlap between human and animal sources only for “is electrophilic or can be metabolically activated to electrophiles”, with animal but not human data on “is genotoxic”.

Figure 6 illustrates the key characteristics of the six categories of Group 1 agents considered in Volume 100: (A) pharmaceuticals; (B) biological agents; (C) arsenic, metals, fibers, and dusts; (D) radiation; (E) personal habits and indoor combustions (labelled ‘lifestyle agents’ in panel e); and (F) chemical agents and related occupations (labelled ‘chemical agents’ in panel f). Genotoxicity was the most prevalent key characteristic induced by substances in the categories A, B, C, E, F, and genotoxicity was exhibited by all agents in category D. Genotoxicity and altered cell proliferation, cell death, or nutrient supply are prominent characteristics of biological agents. None of the biological agents produced modulation of receptor-mediated effects, and none of the agents in the category V appeared to act through modulation of receptor-mediated effects, through immunosuppression, or through immortalization. There are five agents in the category D, all of which exhibited the following key characteristics: genotoxic; alters DNA repair or causes genomic instability; induces oxidative stress; causes immortalization; and altered cell proliferation, cell death, or nutrient supply. The profiles of key characteristics for A and F are remarkably similar, possibly reflecting the fact that despite their different exposure circumstances, some of these chemical entities may act via similar mechanisms.

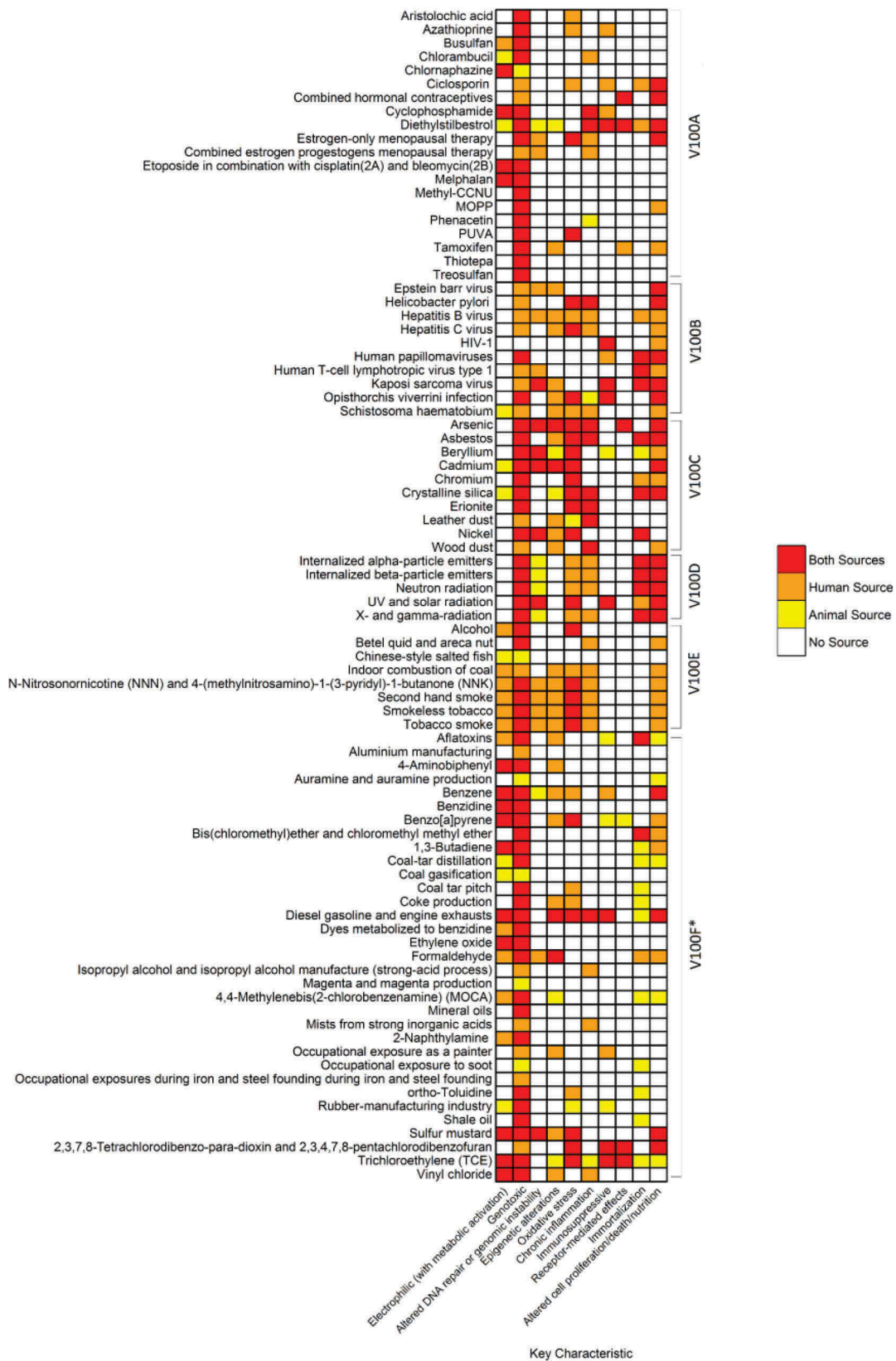


Figure 5. Heat map showing the degree of concordance between human and animal sources of information on key characteristics of 86 Group 1 agents (after combining in vivo and in vitro sources of information for humans and for animals).

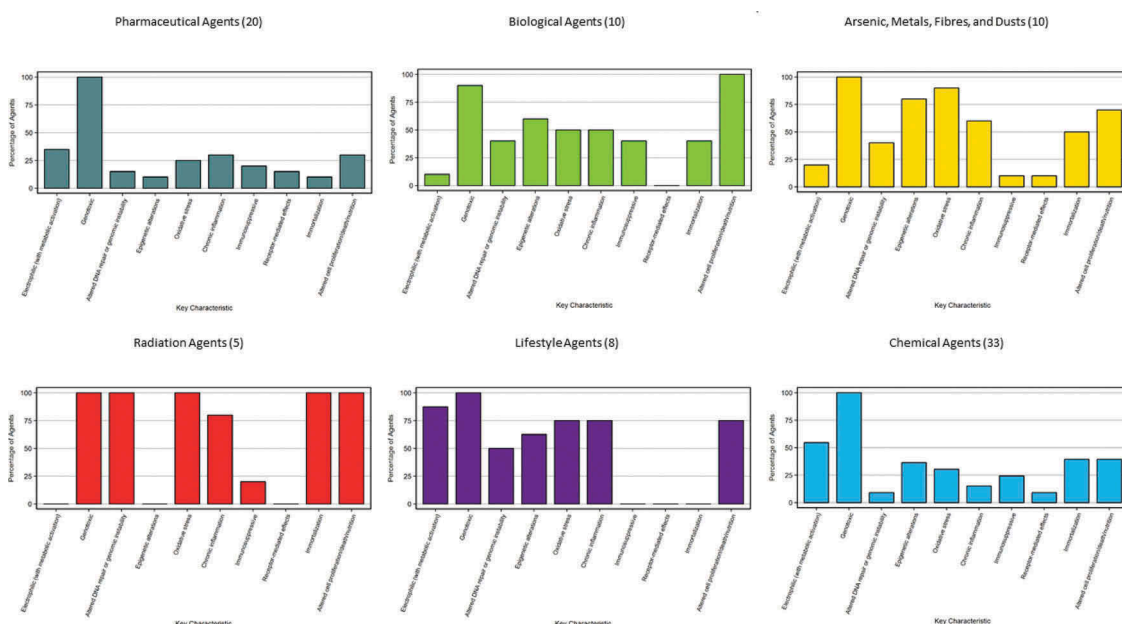


Figure 6. Key characteristics of 86 Group 1 agents by type of agent (expressed as a percentage of the number of agents of each type demonstrating each of the ten key characteristics).

Discussion

The present analysis of key characteristics of 86 agents classified as *carcinogenic to humans* (Group 1) by the *IARC Monographs Programme* was based upon mechanistic data retrieved from the *IARC Monographs* (Al-Zoughool et al. 2019; 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 (Baker 2014; Floor et al. 2012; Hanahan and Weinberg 2011; Pickup, Mouw, and Weaver 2014; Roessler, Budhu, and Wang 2014). Agents in categories B, C, D, and F demonstrated a wide spectrum of biological activity. Radiation was 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 tumors associated with exposure to ionizing radiation (Krewski et al. 2019a). Viral oncogenesis is also multifaceted, and the multistep nature of viral oncogenesis is postulated to be influenced by host genetic variability (Mesri, Feitelson, and Munger 2014).

The observation that most Group 1 agents demonstrate multiple key characteristics is consistent with a recent analysis of additional agents

considered in Volumes 112–119 of the *Monographs Programme* by Guyton et al. (2018), who reported that 12 of 16 Group 1 and Group 2A agents identified subsequent to the evaluation of the 86 Group 1 agents reported here exhibited multiple key characteristics. Guyton et al. (2018) also noted that the vast majority (17/18) of Group 2B or Group 3 agents considered displayed at most one key characteristic. (This initial observation of fewer key characteristics identified for agents in lower classifications would be worth re-examining more broadly as additional data on key characteristics of agents evaluated within the *Monographs Programme* accumulate).

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 assessment 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 presented 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 agents examined here induce one or more of these end-points. Even biological agents such as viruses that act primarily through non-genotoxic mechanisms produce cytogenetic effects and gene mutations as secondary events through chronic inflammation and oxidative stress.

Another important observation is that findings 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 found 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. Notably, human evidence (*in vivo* or *in vitro*) has been more commonly used in past mechanistic evaluations than animal evidence, with human data being more often reported among the 86 agents considered here than animal data for the majority of key characteristics, possibly reducing the need for animal to human extrapolations in mechanistic evaluations. (The prominence of human sources of mechanistic information may reflect the fact that most Group 1 agents are identified largely on the basis of human evidence.) With respect to “induces chronic inflammation”, however, Guyton et al. (2018) recently reported that *in vivo* animal data have been most often used as evidence of this key characteristic in assessments of agents in Volume 112 and in subsequent *Monographs*.

Some caution needs to be employed in interpreting the distribution of key characteristics across Group 1 agents considered here. It is possible that the near universality of genotoxicity as a carcinogenic mechanism may be related to the manner in which the *IARC Monographs* were compiled, with emphasis on reporting of genotoxicity data. This would have been partially mitigated by inclusion of mechanistic information from outside the *IARC Monographs* in the preparation of the mechanistic database as 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). Investigations of substances in earlier *Monographs* focused on changes such as DNA damage that were detected using techniques available at that time. These compounds may not have been assessed exhaustively for pathways that were identified more recently, such as those involving the multifactorial nature of carcinogenesis, or 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 knowledge was not generally provided in the *IARC Monographs* from which the mechanistic data on the Group 1 agents were obtained. 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 upon 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, such that the database may not reflect all mechanistic characteristics of different agents. As a sensitivity analysis to examine the extent to which the *Monographs* captured most of the relevant knowledge in this regard, Birkett et al. (2019) conducted a supplementary PubMed search to identify additional data on key characteristics not cited in the *Monographs*, or published since 2009. Although this sensitivity analysis was not based upon an exhaustive search, it did identify additional information sources, of which the most notable was the identification of evidence for six additional agents that demonstrated modulation of receptor-mediated effects, beyond the seven agents noted in Figure 1. Nonetheless, the overall findings are largely comparable with those presented without additional data search (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 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 substances 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* (Krewski et al. 2019b). Despite the inherent reliance on human epidemiological data in identifying agents that may enhance human cancer risk occurrence, the *IARC Monographs* increasingly provides detailed descriptions of the MOA 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, Herceg et al. (2013) used DNA methylation, histone modification, and altered expression of microRNAs as indicators of epigenetic alterations. Information was considered from both *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 *Monographs* 100A–F as well as Volumes 105 and 106, identified 33 of the 86 Group 1 agents (38%) as mediating epigenetic alterations.

In an earlier assessment, Hernandez et al. (2009) reported that 45 of 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 the compound displayed negative results in the *Salmonella* mutagenicity assay (the Ames test) as well as in mouse lymphoma assay, *in vitro* chromosomal aberration, *in vitro* micronucleus, *in vivo* micronucleus, and *in vivo* chromosomal aberration test in bone marrow in rodents. These findings 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 ten key characteristics of human carcinogens is taken into account in future *Monographs* evaluations of agents that may induce cancer in humans, a carefully designed systematic review of the scientific literature would be required in conjunction with each assessment. However, to conduct a series of comprehensive systematic reviews of 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 mechanistic data on new agents selected for examination by *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 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 need to 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 might arise either as a result of a direct action of the agent on the cell or indirectly, (1) as a result of cytotoxicity that stimulates cell proliferation to replace cells, through alterations in cell signalling without cytotoxicity, or (2) via inhibition of cell proliferation that subsequently leads to selection of an altered clone of cells with a high proliferation rate. Although the downstream effect is similar as evidenced by enhanced cell proliferation, the pathway leading to that response may vary. A similar issue arises with genotoxicity where many agents are not directly genotoxic but produce DNA damage by stimulating a chain of molecular changes such as chronic inflammation. The current database does not contain the knowledge required to address these issues and cannot be used to draw conclusions regarding the detailed MOA of an agent.

The ten 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 MOA is being investigated for a genotoxic agent that requires metabolic activation, this mechanism needs to consider the entire metabolic pathway. If the agent is not metabolized to produce an electrophile, DNA damage does not occur. In such a case, biological effects that occur after induction of DNA damage also are not observed. This sequential relationship is also apparent for characteristics such as chronic inflammation, which acts through generation of oxidative stress, release of cytokines, and stimulation of cell proliferation, which ultimately produces DNA damage.

The results of the present analysis 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 was 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 investigations and experimental animals and model systems demonstrated that DNA adducts are strongly associated with cancer (Kriek et al. 1998; Phillips et al. 2015). Some genotoxic effects might lead to gene mutation

which is an important event in the pathway towards carcinogenesis, especially if it involves oncogenes or tumor suppressor genes. Chromosomal aberrations are another type of genetic alteration that occurs frequently in many tumors, especially solid tumors. Most tumor cells display aneuploidy, and for some tumors, characteristic chromosomal abnormalities were identified including the Philadelphia chromosome in chronic myeloid leukemia.

The complexity of the pathways involved in carcinogenesis and the fact that cellular response to carcinogen exposure is modulated by host cell physiology, genetics, and other variables prompted 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 (Andersen et al. 2010; Krewski et al. 2010, 2014) focused on high-throughput screening to detect changes in the molecular pathways of the cell in response to chemical exposure. This new paradigm might be useful in comprehensive cancer risk assessment and may 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 might be utilized to detect targets of these pathways. This attempt to understand the biological mechanisms underlying carcinogenesis development is consistent with current practice of using *in vitro* assays to detect changes in critical signalling and other molecular pathways in cancer development Krewski et al. (2010, 2014).

With the elaboration of the ten key characteristics articulated by Smith et al. (2016), mechanistic evaluations of new agents undertaken within the *IARC Monographs Programme* are beginning to make use of these characteristics, including utilization of formal methods of systematic review to identify relevant mechanistic information. It is expected that the search strategies employed in future mechanistic evaluations will be refined as experience with the key characteristics accumulates. In an earlier assessment of evidence of epigenetic alterations for 28 Group 1 agents, Chappell et al. (2016) searched for findings of DNA methylation,

histone modification and expression of non-coding micro RNAs (miRNAs), as was conducted in the present analysis, but with addition of several more detailed search terms, specifically long non-coding RNA (lncRNA), small RNA, chromatin, and promoter methylation. Chappell et al. (2016) noted that the vast majority (89%) of experiments on lncRNAs included in their review reported alterations in miRNAs, yielded findings consistent with search terms used here where 43% (12 of 28) of the agents demonstrated evidence of epigenetic alterations, similar to 38% (33 of 86) of compounds included in the present analysis. Continued experience with the evaluation of the ten key characteristics of human carcinogens may be expected to further refine the criteria used for their identification, including both toxicological events associated with these key characteristics and the assays employed as evidence of these events.

Conclusions

In considering the results presented in this review, it is important to emphasize that these mechanistic analyses are a first step in understanding the biological mechanisms underlying cancer occurrence in humans. These findings need to be viewed as preliminary and require refinement through more exhaustive systematic reviews of the relevant scientific literature and/or through discussion with a broad panel of experts on the mechanisms underlying carcinogenesis development. 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 recent update to the *Preamble to the IARC Monographs* (IARC, 2019) includes helpful guidance on how mechanistic information on potential human carcinogens needs to be

considered in future evaluations of agents that may potentially induce carcinogenesis in humans.

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