CHAPTER 19.

Host susceptibility

Jane C. Caldwell, Ronald L. Melnick, and Lauren Zeise

Introduction

For several tumour sites (i.e. lung, lymphoid tissue, and digestive tract), concordance has often been observed among different species after exposure to a given IARC Group 1 human carcinogen (see Chapter 21, by Krewski et al.). When reported in epidemiological studies, these tumour sites are also noted in some or all of the animal species tested experimentally. There are several other tumour sites with fewer, or less common, tumour site concordances among the species studied.

Evaluation of concordance of cancer development in specific target organs between and within species is dependent on several factors. There are various limitations in epidemiological studies (e.g. statistical power, exposure assessment, follow-up, and misclassification), in experimental design of animal bioassays, in methods used to identify concordance, and in the degree to which the animal model captures the range of potential human response to the particular agent tested. An integral consideration for the development and use of these models is host susceptibility – the intrinsic and extrinsic factors that have an impact on variable response to carcinogens: genetic variation, health status, life stage, lifestyle, sex, and the impact of co-exposures. The microbiome can also play a critical part in host susceptibility.

This chapter focuses on examples in the *IARC Monographs* and in recent literature on how well animal models reflect the range of human susceptibility, how host susceptibility factors may modulate the impact of mechanistic events leading to tumour development between species and within species, and how host susceptibility factors may affect evaluations of tumour site concordance.

It can be difficult to parse out reasons for lack of tumour site concordance (i.e. lack of response or common responses between species). The factors alluded to above that are not strongly associated with host susceptibility include the following. Competing causes of mortality may prevent the development of late-developing tumours, or studies may lack statistical power to detect an increase in tumour incidence at sites with high background rates. Limitations in how the database on tumour site concordance was constructed may affect the types of responses observed (e.g. some studies may focus only on specific tumour outcomes or may not be designed to detect some types of tumours). Also, when the concordance database

was constructed, the identification of a site in animals required a significant response in multiple species or in both sexes even though one sex may be more susceptible at a particular site (i.e. the mammary gland in females). In addition, there may be mechanistic concordance between species in how an agent elicits effects (e.g. is able to induce genotoxicity or affect similar pathways), but host susceptibility factors may result in different site-specific neoplastic responses. Thus, host susceptibility may determine how and whether specific individual sites or target organ systems are influenced by mechanistic events associated with cancer induction.

Although host susceptibility factors have a modulating role in carcinogenesis and can affect underlying mechanistic events, they should not be confused with concepts such as modes of action and adverse outcome pathways. A mode of action is a well-defined and biologically plausible series of key events leading to an adverse effect (EPA, 2005a); an adverse outcome pathway is a construct that attempts to link an initiating event with an adverse outcome at a biological level of organization relevant to risk assessment (Ankley et al., 2010). Both the mode of action and the adverse outcome pathway concepts can have limitations for the determination of mechanistic concordance between species. For example, leukaemia induced by exposure to benzene appears to result from multiple mechanistic events, some of which are not well characterized and are difficult to quantitate or quantify, and they do not occur in an ordered sequence; these features limit the applicability of an approach based on mode of action to assess risk of leukaemia (McHale et al., 2012).

The mechanistic database assembled for IARC Group 1 carcinogens contains information on mechanistic characteristics, one or more of which are commonly exhibited, that can be used to identify and organize mechanistic information related to cancer induction. The database is organized in terms of whether an agent displays these key characteristics of carcinogens: (1) is electrophilic or can be metabolically activated to electrophiles, (2) is genotoxic, (3) alters DNA repair or causes genomic instability, (4) induces epigenetic alterations, (5) induces oxidative stress, (6) induces chronic inflammation, (7) is immunosuppressive, (8) modulates receptor-mediated effects, (9) causes immortalization, and/or (10) alters cell proliferation, cell death, or nutrient supply (see Chapter 10, by Smith; see also Smith et al., 2016).

These characteristics are not in themselves sufficient to explain all aspects of carcinogenesis (see Chapter 13, by Caldwell) but are indicative of multiple mechanisms and associated biological changes observed after exposure to carcinogenic agents. Similar to the limitations mentioned above for the database on tumour site concordance, the mechanistic database may present what was studied and reported, and reflect the depth to which an agent was treated in each review in the *IARC Monographs*. Identification and categorization of mechanistic data by use of these key characteristics cannot always predict tumour site concordance, because the information is at best collected in a way that provides only partial evidence on differences in host susceptibility.

Advances in the understanding of host susceptibility in tandem with the evolution of the knowledge on the mechanisms of carcinogenesis allow for greater understanding of both. The mechanistic data for all types of ionizing radiation (IARC, 2012f) are particularly informative with regard to mechanisms of genetic damage (mutation and epigenetic changes, and bystander effects; see Chapter 18, by Hill and Ullrich), as well as other host susceptibility factors from this large and rich database. The classic mutation theory of cancer no longer fully encompasses the mechanistic data for several carcinogens (e.g. benzene) that induce not only mutations but also a variety of epigenetic changes. With the present state of knowledge, the carcinogenic process cannot be confidently attributed to either a purely genetic or epigenetic process but probably involves both (see Chapter 12, by DeMarini).

Differences and similarities in apparent tumour types and targets between rodents and humans can result from a variety of factors that affect absorption, distribution, metabolism, and elimination (ADME) of the agent, as well as the wide range of inherent susceptibility elements associated with toxicodynamic factors. Differences in the expression of genes coding for enzymes that regulate these processes can contribute to differences in cancer susceptibility and tumour targets within a species, between species, and at various life stages when exposure occurs. In some instances, the apparent discordance between cancer outcomes in different species may be explained when these genetic factors are identified, when relevant animal models

are tested, and when more susceptible human populations are studied for carcinogenic effects.

Analogous transgenic, strain-specific, or species-specific animal models

Human and animal studies on cancer may be more effective in discerning tumour responses when those most at risk are studied, such as a susceptible subgroup within a human cohort and susceptible animal species and strains in a bioassay. Concordance of response between species may increase for a particular agent when mechanisms of carcinogenesis and subpopulations at risk are identified and more analogous transgenic or strain-specific animal models are examined. However, these are generally not known before an animal study is conducted. There may be gaps in the understanding of the impact of inherent variability on tumorigenesis, in the identification of susceptible human populations, and in the development of adequate animal models to detect a carcinogenic risk from an agent or exposure condition.

Inherent variability

Most animal studies used to identify a potential carcinogenic risk in humans are conducted in rodents under standard conditions (e.g. 2-year cancer bioassays) with one particular agent. However, humans are exposed throughout their lifetime to a mixture of agents (see below), and inherent biological variability among individuals is due to epigenetic and genetic variance (Zeise et al., 2013). The contribution of the inherited predisposition to diseases, such as cancer, has been an active area of research and has an impact on susceptibility analyses. Two proposed hypotheses for the inherited basis of complex genetic traits are that they result from "common disease–common variant" (i.e. many common alleles of small effect) or "common disease–multiple rare variants" (i.e. few rare alleles of large effect). Although genome-wide association study (GWAS) approaches have been based on the "common disease–common variant" hypothesis, they have not been successful in explaining genetic predisposition to disease (Zhang et al., 2011).

The understanding of the scope of human genetic variability now shows that although there is a great deal of similarity in the DNA sequences between individuals, rare gene mutations are abundant, are geographically localized across the world, are difficult to catalogue, and are possibly a consequence of the rapid spread and growth of the human population and weak purifying selection (Nelson et al., 2012). The breadth and scope of rare mutations have also been illustrated through studies of asthma that have attempted to discover the role of rare mutations in the "missing heritability" of the genetic contributions to disease. Although common variants at many loci have been associated with asthma, they do not account for overall genetic risk. A study of rare and low-frequency variants has reported ethnic specificity but was unable to account for the missing heritability of the disease (Igartua et al., 2015), which is relevant to that associated with cancer risk.

Whole-genome (i.e. exome) sequencing of common (i.e. minor allele frequency > 5%) and rare (i.e. minor allele frequency < 1%) alleles across 12 cytochrome P450 genes has identified many polymorphisms with pharmacogenetic effects, as well as 730 novel non-synonymous alleles with uncommon deleterious variations that, although individually rare, were present in 7.6–11.7% of the population studied (Gordon et al., 2014). Genetic variability in cell signalling and gene expression may be the result of the variants in regulatory regions of the genes, rather than being a consequence of variants in the genetic code that instructs how to build proteins, or in the regulatory code itself (see Chapter 13, by Caldwell).

Just as there is genetic variability in the human population that has an impact on host susceptibility to cancer, there is also variability within rodent strains and species. Genetic heterogeneity resulting from crossing different mouse strains has long been recognized as an issue of concern in the development of experimental mouse models, and has been used as an argument to create genetically inbred strains. Transgenic mice carrying exogenous DNA and gene-targeted knockout mice have both been used as models for studies on cancer and for identifying carcinogenic properties of chemicals (Lunardi et al., 2014).

Two examples illustrate the complexity and usefulness of studying cancer susceptibility with such models. Although they carried exactly the same mutation in K-*Ras*, mouse lung tumours that resulted from carcinogen-induced versus genetically engineered models appeared to develop along different mechanistic pathways (Westcott et al., 2015). Exposure of two strains of mice of different susceptibility (i.e. A/J and BALB/cBy) to the same treatment (3-methylcholanthrene, a polycyclic aromatic hydrocarbon found in tobacco smoke, and butylated hydroxytoluene) produced lung tumours

with different K-*Ras* mutations in codon 12, one resembling human tumours from smokers and the other resembling human tumours from non-smokers (Fritz et al., 2010). Thus, the same carcinogenic treatment given to different strains of mice produced tumours at the same site but with different K-*Ras* mutations. These examples highlight the utility of using different animal models to understand the mechanistic basis for tumour induction in diverse human populations, but they also demonstrate that studies in multiple animal models are needed.

Concerns have been raised about the sensitivity and design of accelerated cancer bioassays that use genetically modified mice. Design features (e.g. sample size, study duration, reproducibility, and genetic stability of the animals), pathway dependency of effects, and potentially different carcinogenic mechanisms render their utility for predicting human health risks uncertain, especially in terms of dose–response (Eastmond et al., 2013).

Although the use of isogenic mice to detect carcinogenicity of an agent should reduce the within-group variance and the number of animals required to detect a response, such mice fail to model the influence of genetic diversity. The genetically diverse inbred Collaborative Cross mouse strains and the heterogeneous Diversity Outbred mice derived from the same eight founder strains as the Collaborative Cross were developed to more accurately capture the impact of human variability on tumour responses (Threadgill et al., 2011; Churchill et al., 2012; French et al., 2015). Because they more accurately reflect human susceptibility, an order of magnitude greater sensitivity to chromosomal damage induced by benzene was observed in Diversity Outbred mice compared with the inbred B6C3F_1 mice (French et al., 2015). Other groups have used genetically diverse panels of inbred mice to better predict liver toxicity (Bradford et al., 2011) and kidney toxicity (Harrill et al., 2012).

Genetic polymorphisms

There are several examples of organic compounds where polymorphisms in metabolizing genes in the human population may cause an increased risk of cancer within certain subpopulations exposed to such agents (see Chapter 1, by Bond and Melnick). Inherited mutations in cancer-related genes (e.g. *TP53*, *BRCA1*, *APC*, and mismatch repair enzymes) have a low frequency in the population but can confer a high individual risk of cancer (Melnick, 2001). In such cases there may be more concordance of response between species when analogous transgenic or strain-specific animal models are also tested with that carcinogenic agent.

Transgenic mice that lack a functional epoxide hydrolase gene are more susceptible to the mutagenicity of butadiene, as are workers with low-activity epoxide hydrolase polymorphisms (see Chapter 1, by Bond and Melnick). Thus, a transgenic mouse model with a reduced ability to eliminate a mutagenic metabolite more closely simulates a susceptible human subpopulation. Polymorphisms in genes that encode enzymes involved in metabolism of aromatic amines (*N*-acetyltransferases: NAT1 and NAT2) have also been noted to have an impact on inter- and intraspecies differences in risk for cancer of the bladder; conflicting findings between studies are potentially a consequence of the interdependence of pathways to activate the parent compound and detoxify reactive metabolites at different rates in different tissues (IARC, 2012c).

Polymorphism in the human aldehyde dehydrogenase enzyme is related to risk of cancer from alcohol consumption in a complex way. Individuals who do not express the enzyme at all may have lower risk of cancer, because the acute effects they experience from alcohol intake (e.g. facial flushing and physical discomfort) cause them to abstain from alcohol consumption, whereas individuals with reduced expression of the enzyme would be able to drink alcohol and consequently would have a higher blood concentration of acetaldehyde (IARC, 2012d). The development of animal models to reflect this response is dependent on recognition of the role of these metabolic polymorphisms in forming or eliminating cancer-causing intermediates. Cancers related to alcohol consumption were first detected without consideration of enzyme polymorphisms. Later on, differential risks were associated with specific polymorphisms.

The main focus in pharmacogenetics has been on polymorphisms of genes encoding drug-metabolizing enzymes, based on the supposition that inter-individual differences in response were determined by such genetic differences and that the main genomic hazard was mutagenesis or physical damage to DNA (Szyf, 2007). However, human variability and susceptibility are influenced not only by genetic polymorphisms but also by differences in the epigenome and its regulatory features.

Time-dependent changes in global DNA methylation have been demonstrated in the same individuals in separate populations in widely

separated geographic locations, with familial clustering for both increased and decreased methylation (Bjornsson et al., 2008). The same study also showed considerable inter-individual variation with age, with differences in DNA methylation accruing over time among individuals who would be missed by studies that apply group averaging. Thus, a focus only on genetic polymorphisms does not consider the fact that epigenetic programming plays an equally important part in generating inter-individual differences in phenotype (Szyf, 2007), and that it should be taken into account in the analysis of such phenotypic diversity. Such inter-individual differences would also not be readily observed with conventional rodent models, for several reasons (see below).

Strain- and species-specific differences in ADME and susceptibility to biological agents

Most cancer bioassays have been conducted in rodents (see Chapter 21, by Krewski et al.). The genetic code has been described as conserved between humans and mice in terms of genome size, structure, and sequence composition, and although candidate regulatory sequences have been conserved and the chromatin landscape in cell lineages is relatively stable, there are interspecies differences in gene expression and regulation (see Chapter 13, by Caldwell), which may account for some apparent differences in susceptibility or specific tumour site concordance after exposure to a carcinogenic agent or condition. Other factors described for some agents in Volume 100 of the *IARC Monographs* focus primarily on ADME considerations and species-specific vulnerabilities to biological agents.

For example, with regard to induction of cancer of the bladder by aromatic amines, increased risks are consistently found in humans and in dogs exposed to, for example, 4-aminobiphenyl, benzidine, 4,4′-methylenebis(2-chloroaniline), and 2-naphthylamine. Several aromatic amines (e.g. *o*-toluidine and 2-naphthylamine) induce bladder tumours in rats (IARC, 2012c; see also Chapter 2, by Beland and Marques).

Multiple organ site carcinogenicity of aromatic amines in experimental animals is associated with metabolic activation of these agents to DNA-reactive intermediates via multiple pathways in target organs. For dogs, lack of *N*-acetylation of aromatic amines reduces elimination of the parent compound via a detoxification pathway (IARC, 2010), and their ability to store urine – as humans do – increases exposure to urinary metabolites that are hydrolysed in the bladder lumen epithelium to reactive electrophilic metabolites (IARC, 2012c). Indeed, infrequent voiding has been associated with increased DNA adduct formation in the bladder in dogs (Kadlubar et al., 1991). Thus, similarities between the metabolism of aromatic amines in dogs and metabolic polymorphisms in susceptible humans, and physiological similarities (i.e. the ability to store urine) between dogs and humans contribute to a stronger correspondence with respect to the target organ.

The mechanism of tumour induction by aromatic amines is similar between humans and rodents, but the target organ is not always the same; in rodents, there are multi-organ targets for exposure to these agents through similar effects on DNA from electrophilic metabolites. Cancer of the bladder is associated with exposure to 2-naphthylamine in humans, rats, dogs, hamsters, and monkeys, as well as with exposure to *o*-toluidine in humans and rats. In mice, however, tumours are seen in other tissues but not in the bladder. Exposure to benzidine is associated with cancer of the bladder in humans, but in rodents liver tumours, not bladder tumours, are observed. Conflicting findings between studies are potentially a consequence of the interdependence of pathways to activate the parent compound and detoxify reactive metabolites at different rates in different tissues (IARC, 2012c).

Human exposure to asbestos has resulted in lung cancer, pleural and peritoneal mesothelioma, and cancer of the larynx and ovary (IARC, 2012a). The targets of this carcinogen are associated with its distribution. After inhalation, fibres may penetrate into the interstitium and translocate to the pleura or peritoneum or more distant sites. Asbestos has been shown to accumulate in the ovary in women (IARC, 2012a). Bronchial carcinomas and pleural mesotheliomas have been observed in rats after exposure to asbestos fibres, with no consistent increases reported for tumours at other sites. However, the Working Group for Volume 100C of the *IARC Monographs* (IARC, 2012a) noted that in many studies complete histopathology was not done, so it was not possible to observe a similar tumour pattern associated with carcinogen distribution.

The complexity of developing an appropriate animal model that takes into account similar distribution factors is further illustrated by the example of cancer induced by asbestos.

After inhalation of asbestos or synthetic fibres, Syrian golden hamsters are more susceptible than rats to induction of malignant pleural mesothelioma. More rapid translocation of synthetic vitreous fibres to the pleural space of hamsters compared with that in rats has been proposed as the reason for interspecies differences in susceptibility (Gelzleichter et al., 1999). Rats and hamsters are equally susceptible after direct intrapleural or intraperitoneal injection of the fibres, which circumvents differences in distribution.

Because rodents do not smoke, it is difficult to develop rodent models that mimic human smoking patterns and exposure to mainstream tobacco (see Chapter 5, by Hecht and DeMarini). However, rodents and other species have been used to study some of the carcinogenic components in cigarette smoke (e.g. polycyclic aromatic hydrocarbons, nitrosamines, aromatic amines, benzene, and butadiene). Although studies of the individual components of cigarette smoke have demonstrated genotoxicity, the development of murine models that reflect the induction of analogous forms of human lung cancer from smoking involves not only ADME considerations but also strain susceptibility (see above).

Standard animal cancer bioassays (i.e. 2-year testing in rats and mice) are not used to study biological agents that are specific to humans. Biological agents have evolved to preferentially target specific host species, specific organs or cell types within those species, and cell types with a specific differentiation status. There are data on the development and use of transgenic models to study biological agents with critical mechanistic evidence (see Chapter 9, by Lambert and Banks). With the exception of lymphoproliferative disease associated with Epstein–Barr virus (EBV), the use of surrogate hosts has not proven useful for assessing the carcinogenicity of human tumour viruses, and for several of them (e.g. EBV, Kaposi sarcoma-associated herpesvirus, and human papillomavirus), there is no understanding of cancer etiology in the context of natural viral infection (IARC, 2012b). Thus, determinations of interspecies concordance are hampered by the species specificity of most human tumour viruses.

In addition, human susceptibility and the identification of tumour targets of virally induced cancers involve many factors. The type of tumour induced is not only associated with the age of the subject but also related to stages of latency of the viral agent and the presence of susceptibility cofactors (e.g. variants or subtypes of the virus, gene polymorphisms and the immune status of the host, and environmental co-exposures that may lead to viral reactivation) (IARC, 2012b).

For EBV, specific latency transcription programmes that arise at specific stages in the viral life-cycle have been associated with specific tumours, i.e. latency I with EBV-related Burkitt lymphoma (BL), latency II with Hodgkin lymphoma and T-cell non-Hodgkin lymphoma, and latency III in immunocompromised individuals with lymphoproliferative disorders. In addition, three subtypes of BL are associated with EBV (endemic, sporadic, and immunodeficiency-associated), two of which primarily involve children (i.e. endemic and sporadic BL) (IARC, 2012b). Thus, the complexity of identifying target sites and susceptibility factors for these agents in humans also renders the analysis of tumour site and mechanistic concordance problematic.

Life stage

The timing of exposure to an agent during one's lifetime can affect the specific type of tumour that may arise, as well as the degree of cancer risk from such an exposure. Life stage as a susceptibility factor has been recognized and included in guidelines used by regulatory agencies in assessing cancer hazards and risks (EPA, 2005a, b). Although puberty and its associated biological changes could lead to changes in cancer susceptibility, exposures during that critical period and in that age group are seldom the subject of epidemiological studies; historically, the focus on cancer has been as a disease associated with ageing after extended exposure duration, with prolonged latency periods before the cancers appear (EPA, 2005b).

Cancer studies in rodents are generally designed to last somewhat less than a lifetime (2 years), beginning in early adulthood, and to mimic mostly occupational exposure circumstances (Melnick et al., 2008). With the exception of biological agents, radiation, or household exposures (IARC, 2012b, d, f), many data in cancer epidemiology come from exposures that occur in the workplace or upon the use of certain pharmaceuticals. Thus, these studies may not reveal the potential of exposures during the sensitive early-life period to induce childhood tumours, nor do they detect tumours with long latency periods.

Although similarities between childhood and adult cancers have been noted, childhood cancers generally are embryonic cell tumours (i.e. leukaemias, tumours of the brain

and the central nervous system, lymphomas, bone cancers, soft-tissue sarcomas, kidney cancers, eye cancers, and adrenal gland cancers), whereas adults generally develop more carcinomas (i.e. cancers of the skin, prostate, breast, lung, and colorectum). In addition, some tumours appear to be unique to the young, for example tumours of the kidney (Wilms tumour) or eye (retinoblastoma) (EPA, 2005b). Thus, another aspect of tumour site concordance between species is the difference in tumour types that may be observed in children versus adults.

A full assessment of cancer risks from childhood exposure to chemicals in the environment has been impeded by the relative rarity of childhood cancers, the lack of studies of the late effects of childhood exposure with sufficiently long follow-up, and the lack of relevant animal testing guidelines and assays focused on early-life or perinatal exposures (EPA, 2005b). However, some human carcinogens listed in Volume 100 of the *IARC Monographs* have been specifically identified as associated with increased risk of childhood cancer (i.e. radiation and certain pharmaceutical agents used in chemotherapy), as well as cancers occurring later in life after exposure during childhood.

In animals, several agents induce a higher incidence of tumours occurring later in life after perinatal exposure, for example diethylnitrosamine, benzidine, polybrominated biphenyls, and dichlorodiphenyltrichloroethane (DDT). For vinyl chloride, there appears to be greater susceptibility of weanling animals to the formation of DNA adducts (EPA, 2005b).

Along with the potential for more tumour types occurring after early-life exposure, the strength of the response (i.e. potency) may also be increased. There are examples of IARC Group 1 carcinogens for which potency is greatly increased in the young. For example, vinyl chloride is an agent for which young rodents are more susceptible for the target site and cell types (i.e. rare liver angiosarcomas and more common hepatocellular carcinomas) that are also observed in humans (see Chapter 1, by Bond and Melnick). The literature on cancer induced by exposure of animals to vinyl chloride is extensive and includes transplacental and perinatal exposures (IARC, 2012c). Barton et al. (2005) estimated the increase in potency of vinyl chloride for liver angiosarcomas to be 30-fold and for hepatomas to be about 50-fold in female rats after early-life exposure compared with exposure as adults.

As noted above, exposure to benzidine is associated with bladder cancer in humans and liver tumours in mice (IARC, 2012c). The ratio of potency after early-life versus adult exposure in studies with repeat exposures of juvenile and adult animals to benzidine is about 100 for liver cancer induction in male mice (Barton et al., 2005). This example illustrates an increased susceptibility in the young but an apparent lack of site concordance between humans and mice.

For most of the IARC Group 1 human carcinogens, there are data indicating genotoxicity as defined by the toxicological end-point of DNA damage (see Chapter 12, by DeMarini, and Chapter 22, by Krewski et al.). DNA damage has been noted to potentially exhibit a greater effect after early-life versus later-life exposure; this increased susceptibility has been attributed to more frequent cell divisions during development, which may enhance fixation of mutations, and the absence of key DNA repair enzymes in some embryonic cells, such as brain cells. In addition, increased risk may result from lack of fully functional components of the immune system during development, different functional operation of hormonal systems during different life stages, and induction of developmental abnormalities that can result in a predisposition to carcinogenic effects later in life (e.g. diethylstilbestrol) (EPA, 2005b). However, several other factors may also increase susceptibility in the young. The developmental origins of health and disease (DOHaD) hypothesis posits that environmental exposures during development increase susceptibility to cancer in adulthood through epigenomic reprogramming (Walker and Ho, 2012).

In some cases, the newborn or young rodent may be a better model to assess human cancer risk for either children or adults. Components of diesel exhaust, an IARC Group 1 carcinogen (Benbrahim-Tallaa et al., 2012; IARC, 2013), appear to be metabolized in a similar fashion in rodents and humans at different stages of development. Nitroarenes (and, by extension, diesel exhaust) are activated to mutagens in humans and young rodents. Concordance of lung cancer risk is observed between young rodents and humans (see Chapter 5, by Hecht and DeMarini). Specifically, 1-nitropyrene (a component of diesel exhaust) is a compound that lacks evidence of carcinogenicity when exposure occurs in adult rodents, but it is carcinogenic in young adult or newborn rodents because of its more extensive metabolism to mutagens. Metabolism of 1-nitropyrene by adult humans resembles that of newborn

rodents. Accordingly, examination of bioassay data for exposures of adult rodents only would miss any similarity of cancer response between the two species.

In addition to the difficulty of developing adult rodent models that mimic human adult smoking patterns, the use of rodent models exposed in adulthood may not reflect susceptibility. Lung tumours can be induced in Swiss mice if exposure to mainstream cigarette smoke begins within 12 hours after birth, but not if exposures are delayed (Balansky et al., 2007; IARC, 2012d).

Diethylstilbestrol is an important example of a transplacental carcinogen where in utero exposure causes vaginal and uterine cancer in daughters but not in exposed mothers (IARC, 2012e; see Chapter 20, by Rice and Herceg). The effects of diethylstilbestrol on the developing reproductive tract of rodents are species- and strain-specific; neonatal exposure to diethylstilbestrol results in uterine adenocarcinomas in CD1 mice but not in C57BL/6 mice. Increased incidence of uterine tumours is seen in Eker rats (i.e. a strain that is tumour-prone because of a germline defect in the *Tsc2* tumour suppressor gene) but not in wild-type rats. CD1 mice exposed to diethylstilbestrol also exhibit permanent estrogen imprinting, morphological changes in the reproductive tract, and persistent expression of the *Ltf* (lactoferrin) and *c-Fos* genes (Cook et al., 2005).

In humans, exposure to inorganic arsenic compounds causes cancer of the lung, bladder, and skin, with limited evidence for cancer of the kidney, liver, and prostate (IARC, 2012a). Transplacental exposure to arsenic from oral intake by pregnant female mice induces lung bronchiolo-alveolar carcinomas and liver hepatocellular carcinomas in the offspring when they become adults; continuous exposure during adulthood was not required, and exposure only in adulthood did not induce these tumours (see Chapter 3, by Waalkes; IARC, 2004, 2012a). However, a recent study in male mice reported that low doses of arsenic in drinking-water given according to a scheme that more closely resembles human exposure (i.e. to parents before conception and throughout pregnancy and lactation, and to offspring after weaning and throughout adulthood) caused lung cancer, a response that has also been reported for in utero or early-life exposures in humans (Waalkes et al., 2014).

Bladder cancer has been induced in adult rats after chronic exposure to arsenic. The skin, kidney, and bladder have been reported as cancer targets in multiple rodent studies of inorganic arsenic upon co-exposure with other carcinogens, via drinking-water or transplacentally (IARC, 2004, 2012a). There is no identified rodent model for arsenic-induced cancer of the skin or lung after exposure by inhalation. Thus, arsenic is an example where carcinogenicity and tumour site concordance are dependent on experimental design, and especially on the impact of early-life exposures.

For some carcinogens, there may not be an appropriate animal model for human cancer risk from later-life exposures. The timing of exposure determines tumour patterns and is critical for tumour concordance relationships for estrogens. The Working Group for Volume 100A of the *IARC Monographs* (IARC, 2012e) cautioned that “estrogen products given with or without a progestogen have markedly different carcinogenic or anti-carcinogenic effects, and the same regimens may have markedly different effects in different organs and at different stages of women’s lives.”

To date, there are no mouse models for ovarian cancer that reflect the genetics and histology of human serous ovarian cancer, which is most often diagnosed in postmenopausal women (Smith et al., 2014). Tamoxifen has been given to these women to treat metastatic breast cancer, or to women who are at high risk of developing the disease. There is a concordant decrease in risk of breast cancer in such women and in female rodents treated chronically as adults. However, tamoxifen treatment also causes an increase in risk of endometrial cancer in postmenopausal women. In female mice and rats, perinatal exposure to tamoxifen is required to produce tumours of the reproductive tract (IARC, 2012e).

In children, several types of ionizing radiation show life stage-related differences in susceptibility that affect target sites and cancer risk later in life (see Chapter 18, by Hill and Ullrich; IARC, 2012f). Low-dose radiation at background levels has recently been reported to contribute to the risk of leukaemia and tumours of the central nervous system in children (Spycher et al., 2015). Children exposed to ionizing radiation from the atomic bombs in Japan and from the accident with the Chernobyl Nuclear Power Plant in Ukraine had an increased risk of thyroid cancer attributable to iodine-131 and its accumulation in the thyroid.

Ultraviolet radiation from tanning beds increases the risk of skin cancer, especially when exposure occurs at a younger age, i.e. an increased risk for malignant melanoma when first exposure occurs before

age 30 years, and for squamous cell carcinoma when first exposure occurs before age 20 years (IARC, 2012f). In mice exposed to this type of radiation, squamous cell carcinoma is regularly observed, but no malignant melanoma has been reported. However, transgenic mice that spontaneously develop malignant melanomas or that have melanocyte hyperplasia can develop early-onset malignant melanoma if exposure to ultraviolet radiation occurs neonatally, but not after the age of 6 weeks (IARC, 2012f). This example illustrates the complexity of developing an animal model that mimics human susceptibility. Target site susceptibility as well as age at which exposure occurs must be taken into account when evaluating tumour site concordance between species.

Influence of study design on determination of site concordance

Host susceptibility as well as the type of information collected in either human or animal studies influence the degree of tumour site concordance that can be identified and evaluated. Epidemiological research is often done in men, especially for occupational exposures. This can limit or preclude the detection of female-specific cancers in humans and thus site concordance between species.

The Working Group for Volume 100F of the *IARC Monographs* (IARC, 2012c) specifically noted that many plausible tumour sites identified in rodents have not been reported in humans, and gave the example of rats treated with aromatic amines that developed tumours in the mammary gland, an organ that has not been studied adequately as a potential target site in humans for cancer induced by aromatic amines. Epidemiological studies of aromatic amines have not considered breast cancer, because industrial cohorts were generally small and the relevant workforce did not include women. The lack of studies involving female subjects not only affects species concordance but also influences the weight of evidence of an effect when data on both sexes are required to identify a target site in experimental animals. For example, in the construction of the animal database to assess tumour site concordance (see Annex 1, by Grosse et al.), the same neoplastic effect is required in two animal species or in both sexes of one species. Breast cancer is rare in male rats as well as in men. Thus, limitations in epidemiological studies and sex differences in cancer response can also account for lack of tumour site concordance between humans and experimental animals.

Inaccurate diagnoses of disease or incorrect entries on death certificates can affect concordance determinations, especially for myeloproliferative and lymphoproliferative disorders, which can be described as extranodal or predominantly nodal, precursor or mature neoplasms, and which may have multiple cellular phenotypes. Changing codes in the International Classification of Diseases (ICD) can make it difficult to develop a conclusion from human studies. However, a multipotent haematopoietic stem cell is the precursor of myeloid or lymphoid progenitors that further give rise to several cell types (Greaves, 2004). Although the disease induced by an agent may be considered a “lymphoma or leukaemia”, the common progenitors overlap in haematopoietic cancers and complicate determinations of “target organ or target cell”.

For studies in humans, changes in classification schemes for haematopoietic cancers can present difficulties in target organ identification from different studies. Modern classifications of leukaemia and other lymphatic and haematopoietic malignancies are based on cytogenetic and molecular principles (Swerdlow et al., 2008) that do not always coincide with those of the ICD (IARC, 2012f). Although there may be concordance of haematopoietic cancers between or within species, the manifestation of disease may differ. Thus, the determination of tumour site concordance can be dependent on the definition and level of specificity of the target.

The highest likelihood of identifying a human cancer risk may come from the study of sensitive subgroups with increased susceptibility to an agent or groups of agents. If multiple disease categories are lumped together and sensitive subpopulations are not distinguished, it may be difficult to detect a subtle but real cancer response in epidemiological studies. For example, taking into account the influence of genetic polymorphisms or the heterogeneity of tumour phenotypes will improve the ability to determine the risk to specific subpopulations for colon cancer after excessive alcohol consumption (Schernhammer et al., 2010).

Similarly, designing animal studies in such a way that rare tumours can be detected may increase the ability to determine a response and establish site concordance or mechanistic concordance between species. In epidemiology, rare tumours are considered a special type of finding, and they constitute a data set

that is different from the tumours that occur more commonly. For example, asbestos-induced mesothelioma is a rare tumour associated with exposure to a specific agent. In addition to the role of organ distribution of asbestos fibres in the determination of tumour site concordance, asbestos carcinogenicity also provides an example of the importance of tumour rarity for the determination of a response after exposure, either in humans or in animal bioassays: untreated controls from lifetime studies of asbestos exposure in five strains of rats and Syrian hamsters showed zero incidence of mesothelioma in 1175 rats and 253 hamsters (IARC, 2012a).

Many reports of animal bioassay data only highlight statistical significance to identify a positive tumour finding. Because of the relatively small number of animals involved in rodent bioassays, these studies may lack statistical power to identify rare tumours induced by a specific agent. As noted above, the use of a genetically heterogeneous strain of mice increased the ability to determine a genotoxic response to benzene (French et al., 2015).

Use of multiple strains of rats and mice in chronic studies of trichloroethylene enhanced the likelihood of observing increases in the incidence of rare kidney tumours and improved the probability of showing concordance with the finding of increased risk of kidney cancer through epidemiology. The epidemiological database for the current Scientific Publication also includes a study showing lower risk of kidney cancer among individuals with genetic polymorphisms that reduced their ability to produce mutagenic metabolites from trichloroethylene. Thus, tumour site concordance was more easily observed when rare tumours were detected in multiple strains of rats and mice, and when genetic polymorphisms were taken into account in human studies (Guha et al., 2012; Chiu et al., 2013; IARC, 2014).

In some cases, tumour site concordance between humans and experimental animals may be more evident when studies use rodent strains in which there is a lower background rate of more common tumours as well (e.g. the use of mice with lower body weight and decreased background tumour rates; see the discussion below), but with enough sensitivity to detect a response.

As illustrated by the example of exposure to ultraviolet radiation, a specific cell type (i.e. the melanocyte) at the origin of skin cancer in humans may not lead to skin cancer in wild-type mice (IARC, 2012f). Different cell types within a target organ may have different mechanisms of tumour development, susceptibilities, and cancer phenotypes that depend on the life stage at which exposure occurs. The determination of tumour site concordance between species can depend on the degree of specificity of the target description (i.e. cellular vs organ) in addition to cancer phenotype.

The risk of liver cancer from cigarette smoking illustrates how timing of exposure and interspecies differences in susceptibility are related to specific phenotypes of hepatocellular tumours. There is an increased risk of hepatocellular carcinoma in adult humans who smoke cigarettes. However, the strongest risk of hepatoblastoma (an embryonal hepatocellular tumour) is associated with paternal smoking before conception and a median age at diagnosis of 12 months; the timing of exposure for the cancer response is consistent with the identification of cigarette smoking as a germ cell mutagen in humans (IARC, 2012d).

Like in humans, hepatocellular adenomas and carcinomas occur in aged rodents, and background occurrence rates depend on species, strain, and sex. However, hepatoblastoma is extremely rare in rodents and, unlike in humans, this tumour usually occurs in aged rather than in young animals (Turusov et al., 2002). Therefore, at the organ level the liver is a similar target for cancer induction from exposure to cigarette smoke for adults and children. However, interspecies tumour site concordance is more difficult to demonstrate if cellular phenotype, life stage susceptibility, and age and timing of exposure are not taken into account.

Lifestyle, disease status, and co-exposures

Cancer susceptibility involves not only genetic predisposition but also the myriad of exposures experienced over a lifetime, at home and at work, and the various other microenvironments in which voluntary and involuntary choices affect cancer risk. Genetic and environmental interactions involving complex pathways, multiple genes, and multiple exposures have been suggested to provide an explanation for the inability of GWAS approaches to account for the missing heritability of most complex diseases, and for the failure of analyses of rare mutations to account for asthma (Schadt and Björkegren, 2012).

The “exposome” concept encompasses the totality of exposures from conception onwards, complementing the genome, instead of focusing on single exposure–health effect relationships (Vrijheid et al.,

2014). The exposome includes three broad domains of non-genetic exposures: the internal environment (e.g. endogenous hormones, the gut microflora, and ageing), specific external exposures (e.g. chemical contaminants, lifestyle factors such as tobacco use, and occupation), and the general external domain (which includes influences such as stress, the urban–rural environment, and climate) (Wild, 2012).

Tumour site concordance can be affected by the inherent nature of the conditions under which each species is studied to assess cancer risk. Human study subjects have a wide and varied range of co-exposures, whereas studies in experimental animals involve relatively uniform exposures in highly controlled environments. As noted above, changes in expression levels of metabolizing enzymes through genetic polymorphisms have been a focus of research, but metabolism is also affected by environmental co-exposures, which are less well studied. Also, many solvents have similar exposure targets, and in humans these exposures often occur together with co-exposures that have the potential to increase the effects of solvents (Caldwell et al., 2008). However, studies of solvents in general may mask effects of specific agents, for example trichloroethylene (Vermeulen et al., 2012). Lifestyle and co-exposures (e.g. obesity, alcoholism, nutritional status, a compromised immune system, and viral infections) can affect environmental cancer risk, but they are often not considered in animal models of carcinogenicity, nor are they typically addressed in human studies.

In humans, lifestyle choices and previous exposure during development that may change set points in genetic control and cell signalling can affect cancer susceptibility. In addition, exposures to preceding generations have been identified as affecting susceptibility to cancer. In experimental studies, transgenerational endocrine effects have been identified in the third generation of mice after the exposure to diethylstilbestrol (Ziv-Gal et al., 2015).

Obesogens have not been evaluated for carcinogenicity by the *IARC Monographs*. However, prenatal exposure to obesogens that activate the constitutive androstane receptor in neonates may affect susceptibility by causing permanent changes in enzyme expression and metabolism of environmental agents encountered as adults (Caldwell, 2012). Consequently, obesity associated with prenatal environmental exposures may render the subject more susceptible to cancer later in life. Increased background levels of all cancers have been observed in conjunction with increased body weight and obesity in rodent bioassays (Rao et al., 1987; Leakey et al., 2003).

Such changes in background tumour incidence and altered susceptibility will affect the detection of site concordance, especially across data sets that span many decades of research. Site concordance may be more difficult to detect, because exposure-induced and background tumours are harder to distinguish in small groups of animals. Site concordance may be detected more frequently between animal models and humans when obesity status is taken into account. Such is the case with liver cancer (Caldwell, 2012).

The proportion of the population that is overweight or obese has increased substantially over the past few decades. Type 2 diabetes, non-alcoholic fatty liver disease, cardiovascular disease, and increased body mass index are risk factors for liver cancer, and diabetes induces synergistic actions with other variables, such as viral hepatitis and alcohol consumption (Fan et al., 2009).

Immune system status can affect human responses to carcinogen exposures, and thus influence the ability to determine site concordance between species (e.g. lack of concordance in responses because animal models are used that do not also take immunosuppression into account). With the increasing survival of patients with the acquired immune deficiency syndrome (AIDS), associated cancers in West Africa have been reported to be Kaposi sarcoma, non-Hodgkin lymphoma, cervical cancer, anogenital cancer, and liver cancer (Tanon et al., 2012). Infection with human immunodeficiency virus (HIV) or immunosuppression causes a higher risk for lymphomas, i.e. a 400-fold increase in risk of non-Hodgkin lymphoma in the presence of HIV infection (Bassig et al., 2012). However, the increases in the incidence of non-Hodgkin lymphoma can only be partially explained by the HIV epidemic (Bassig et al., 2012).

An example of a common co-exposure that affects cancer risk is that of aflatoxin B_1 contamination of food supplies, which tends to be highest in areas with high prevalence rates of infection with hepatitis B and C viruses. While aflatoxin B_1 and particularly its epoxide metabolite are potent mutagens by themselves, infection with hepatitis B virus greatly amplifies the risk of liver cancer from aflatoxin exposure (IARC, 2012b), i.e. from 4-fold with aflatoxin alone to 60-fold in the

presence of infection with hepatitis B virus (Wu-Williams et al., 1992; Yu and Yuan, 2004). As discussed previously, aflatoxin metabolism and the attendant risk are also affected by polymorphisms of detoxification or activation pathways (IARC, 2012c).

Aflatoxin is one of several agents for which carcinogenicity in experimental animals was established or highly suspected before epidemiological studies confirmed its carcinogenicity in humans (IARC, 2012d). Aflatoxin B_1 is a liver carcinogen in humans, rats, tree shrews, trout, and several types of transgenic mice, but not in wild-type mice (IARC, 2012c). The resistance of adult mice to aflatoxin carcinogenesis has been suggested to result from constitutive hepatic expression of an α-class glutathione-*S*-transferase, mGSTA3-3, a detoxifying enzyme with a high affinity for aflatoxin B_1 8,9-epoxide (IARC, 2012c). However, aflatoxin is a liver carcinogen in newborn mice (Vesselinovitch et al., 1972). Therefore, risk of aflatoxin-induced liver cancer serves as an example not only of the effects of co-exposure but also of the influence of genetic polymorphism and age as susceptibility factors.

Finally, using the framework of the hallmarks of cancer (Hanahan and Weinberg, 2011), a task force of 174 scientists from 28 countries who participated in the Halifax Project ("Getting to know cancer") published a series of reviews that evaluated exposures to mixtures in the environment that may have the potential to contribute to cancer risk (Harris, 2015). Cumulative effects of individual chemicals that had not been identified as carcinogens were reviewed for actions on key pathways and mechanisms related to carcinogenesis and were reported to plausibly produce carcinogenic synergies (Goodson et al., 2015). The modification of human responses to carcinogens from co-exposures would not be reflected in current animal cancer bioassays of individual agents, and would thus affect the demonstration of tumour site concordance.

Microbiome effects

Included in the exposome concept is a more recently described component of gene–environment interactions that influence cancer susceptibility in humans and experimental animals: the contribution to cancer risk of the microbiota living on and in humans. These microbiota include 100 trillion (10^{14}) microbial cells, outnumbering human somatic and germ cells combined by 10-fold (Bultman, 2014), and a quadrillion (10^{15}) viruses that interact with one another and with the host immune system in ways that influence disease outcome. As humans age and develop, so do their microbiota. These microbiota and the genes they encode are collectively known as the microbiome (Clemente et al., 2012). The microbiome differs across species and individuals, and its effects on tumour site concordance have yet to be determined. However, its potential effect on human susceptibility to many chronic diseases, as well as cancer, is an emerging subject of research.

The composition of the microbiome varies across anatomical sites; the gut microbiome is highly enriched in genes involved in carbohydrate metabolism, in contrast to the relatively few genes in the human genome that encode carbohydrate-metabolizing enzymes (Bultman, 2014). The microbiome not only alters metabolic pathways in the human gut but is also linked to host susceptibility to metabolic diseases (Suez et al., 2014) and other multifactorial diseases. Microbial imbalance (dysbiosis) usually involves shifts in the relative abundance of commensal microbes. Inter-individual differences in arsenic-induced disease are associated with differences in arsenic metabolism; disturbances of the gut microbiome phenotype have also been reported to affect the biotransformation of arsenic (Lu et al., 2014).

Shifts in the microbiome have also been associated with several types of cancer, and two dominant phyla normally associated with healthy individuals (i.e. the gram-negative Bacteroidetes and the gram-positive Firmicutes) were underrepresented in colorectal tumour tissue compared with adjacent normal colonic tissue from the same individuals (Bultman, 2014).

Modulation of microbiota in mouse models of cancer has demonstrated that cancer susceptibility and progression are affected by concurrent changes in inflammation, the genomic stability of the host cell, and the production of metabolites that function as histone deacetylase inhibitors to epigenetically regulate host gene expression. Specific diets associated with changes in cancer susceptibility (e.g. increased consumption of red meat and higher intake of dietary fibres) have also been associated with corresponding changes in the microbiome (Bultman, 2014).

Altering the composition of the microbiota in transgenic mice prone to colorectal cancer led to a lower cancer incidence as a result of reduced provision of carbohydrate-derived metabolites that fuel hyperproliferation of colon epithelial cells, without changes in inflammation or DNA damage induction (Belcheva et al., 2014). Microbiota have also been implicated in the activation of the innate

immune response against tumours (Iida et al., 2013; Viaud et al., 2013). The interplay between human carcinogenic pathogens and the microbiome as well as the linkage between dysbiosis and carcinogenesis have recently been reviewed (Dzutsev et al., 2015).

Conclusions

This chapter discusses the importance of considering host susceptibility factors and their modulation of tumour response in interpreting findings of tumour site concordance between species, or lack thereof. Examples are given of how discordance can result from lack of studies covering sensitive sexes, subgroups, or life stages. Examples are also provided in which polymorphisms in metabolizing genes were associated with sensitive subpopulations, and where experimentally sensitive rodent strains were studied that also had sensitivity because of similar capacity for increased activation or reduced detoxification (e.g. in the case of butadiene, aromatic amines, and alcohol consumption). For aromatic amines, anatomical and physiological similarity (infrequent voiding of the bladder) between humans and dogs increases DNA adduct formation and ultimately tumour development at the same site, i.e. the bladder. More challenging in study design is to account for lifestyle, with its attendant co-exposures to exogenous chemicals and its influences on the microbiome.

The analyses of tumour site concordance are dependent on the types of information and databases available at present. Such analyses are limited by the underlying available studies, which may not provide adequate coverage of host susceptibilities. Animal models that cannot reflect the intrinsic and extrinsic factors that have an impact on biological variability in humans may not have adequate sensitivity to detect all targets of carcinogenicity occurring in humans. Similarly, limitations in epidemiological studies affect their ability to detect many tumour responses observed in animals.

Transgenic animal models as well as highly diverse outbred mouse strains and panels of diverse inbred strains have been developed as an approach to model the genetically highly diverse human species. Diversity Outbred mouse models may be used for future bioassays to obtain a better direct estimate of genetic contribution to variance, and these assays may detect potential human tumour sites missed by studies in genetically homogeneous strains. These models may also provide a platform to study other susceptibility factors, such as co-exposures or obesity. However, the use of such mouse models will involve greater expense; heritability estimates suggest that sample sizes should be increased by a factor of 3 to obtain the same precision as with isogenic mice (French et al., 2015). Other issues to be considered for Diversity Outbred models would be the percentage survival, the tumour rates in the controls, and the limited historical database that is used in the interpretation of data from current animal models. When these models are applied to address these issues, they may prove to be an invaluable resource for determining the impact of host susceptibility and of the intrinsic and extrinsic factors on variable responses to carcinogens.

Acknowledgements

The authors wish to thank D.M. DeMarini, M. Marty, S. Vulimiri, V.J. Cogliano, and N. Keshava for their helpful comments on this manuscript.

Disclaimer

This article was reviewed and approved for publication by the National Center for Environmental Assessment, United States Environmental Protection Agency. Approval does not signify that the contents reflect the views of the agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use. The views expressed are those of the authors and do not necessarily reflect those of the California Office of Environmental Health Hazard Assessment.

References

Ankley GT, Bennett RS, Erickson RJ, Hoff DJ, Hornung MW, Johnson RD, et al. (2010). Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environ Toxicol Chem. 29(3):730–41. http://dx.doi.org/10.1002/etc.34 PMID:20821501

Balansky R, Ganchev G, Iltcheva M, Steele VE, D'Agostini F, De Flora S (2007). Potent carcinogenicity of cigarette smoke in mice exposed early in life. Carcinogenesis. 28(10):2236–43. http://dx.doi.org/10.1093/carcin/bgm122 PMID:17522065

Barton HA, Cogliano VJ, Flowers L, Valcovic L, Setzer RW, Woodruff TJ (2005). Assessing susceptibility from early-life exposure to carcinogens. Environ Health Perspect. 113(9):1125–33. http://dx.doi.org/10.1289/ehp.7667 PMID:16140616

Bassig BA, Lan Q, Rothman N, Zhang Y, Zheng T (2012). Current understanding of lifestyle and environmental factors and risk of non-Hodgkin lymphoma: an epidemiological update. J Cancer Epidemiol. 2012:978930 http://dx.doi.org/10.1155/2012/978930 PMID:23008714

Belcheva A, Irrazabal T, Robertson SJ, Streutker C, Maughan H, Rubino S, et al. (2014). Gut microbial metabolism drives transformation of MSH2-deficient colon epithelial cells. Cell. 158(2):288–99. http://dx.doi.org/10.1016/j.cell.2014.04.051 PMID:25036629

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

Bjornsson HT, Sigurdsson MI, Fallin MD, Irizarry RA, Aspelund T, Cui H, et al. (2008). Intra-individual change over time in DNA methylation with familial clustering. JAMA. 299(24):2877–83. http://dx.doi.org/10.1001/jama.299.24.2877 PMID:18577732

Bradford BU, Lock EF, Kosyk O, Kim S, Uehara T, Harbourt D, et al. (2011). Interstrain differences in the liver effects of trichloroethylene in a multistrain panel of inbred mice. Toxicol Sci. 120(1):206–17. http://dx.doi.org/10.1093/toxsci/kfq362 PMID:21135412

Bultman SJ (2014). Emerging roles of the microbiome in cancer. Carcinogenesis. 35(2):249–55. http://dx.doi.org/10.1093/carcin/bgt392 PMID:24302613

Caldwell JC (2012). DEHP: genotoxicity and potential carcinogenic mechanisms – a review. Mutat Res. 751(2):82–157. http://dx.doi.org/10.1016/j.mrrev.2012.03.001 PMID:22484601

Caldwell JC, Keshava N, Evans MV (2008). Difficulty of mode of action determination for trichloroethylene: an example of complex interactions of metabolites and other chemical exposures. Environ Mol Mutagen. 49(2):142–54. http://dx.doi.org/10.1002/em.20350 PMID:17973308

Chiu WA, Jinot J, Scott CS, Makris SL, Cooper GS, Dzubow RC, et al. (2013). Human health effects of trichloroethylene: key findings and scientific issues. Environ Health Perspect. 121(3):303–11. http://dx.doi.org/10.1289/ehp.1205879 PMID:23249866

Churchill GA, Gatti DM, Munger SC, Svenson KL (2012). The Diversity Outbred mouse population. Mamm Genome. 23(9–10):713–8. http://dx.doi.org/10.1007/s00335-012-9414-2 PMID:22892839

Clemente JC, Ursell LK, Parfrey LW, Knight R (2012). The impact of the gut microbiota on human health: an integrative view. Cell. 148(6):1258–70. http://dx.doi.org/10.1016/j.cell.2012.01.035 PMID:22424233

Cook JD, Davis BJ, Cai SL, Barrett JC, Conti CJ, Walker CL (2005). Interaction between genetic susceptibility and early-life environmental exposure determines tumor-suppressor-gene penetrance. Proc Natl Acad Sci U S A. 102(24):8644–9. http://dx.doi.org/10.1073/pnas.0503218102 PMID:15937110

Dzutsev A, Goldszmid RS, Viaud S, Zitvogel L, Trinchieri G (2015). The role of the microbiota in inflammation, carcinogenesis, and cancer therapy. Eur J Immunol. 45(1):17–31. http://dx.doi.org/10.1002/eji.201444972 PMID:25328099

Eastmond DA, Vulimiri SV, French JE, Sonawane B (2013). The use of genetically modified mice in cancer risk assessment: challenges and limitations. Crit Rev Toxicol. 43(8):611–31. http://dx.doi.org/10.3109/10408444.2013.822844 PMID:23985072

EPA (2005a). 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/.

EPA (2005b). Supplemental guidance for assessing susceptibility from early-life exposure to carcinogens (EPA/630/R-03/003F). Washington (DC), USA: U.S. Environmental Protection Agency. Available from: http://www.epa.gov/ttnatw01/childrens_supplement_final.pdf.

Fan JG, Farrell GC; Asia-Pacific Working Party for Prevention of Hepatocellular Carcinoma (2009). Prevention of hepatocellular carcinoma in nonviral-related liver diseases. J Gastroenterol Hepatol. 24(5):712–9. http://dx.doi.org/10.1111/j.1440-1746.2009.05776.x PMID:19646014

French JE, Gatti DM, Morgan DL, Kissling GE, Shockley KR, Knudsen GA, et al. (2015). Diversity Outbred mice identify population-based exposure thresholds and genetic factors that influence benzene-induced genotoxicity. Environ Health Perspect. 123(3):237–45. PMID:25376053

Fritz JM, Dwyer-Nield LD, Russell BM, Malkinson AM (2010). The *Kras* mutational spectra of chemically induced lung tumors in different inbred mice mimics the spectra of *KRAS* mutations in adenocarcinomas in smokers versus nonsmokers. J Thorac Oncol. 5(2):254–7. http://dx.doi.org/10.1097/JTO.0b013e3181c8ce04 PMID:20101149

Gelzleichter TR, Bermudez E, Mangum JB, Wong BA, Janszen DB, Moss OR, et al. (1999). Comparison of pulmonary and pleural responses of rats and hamsters to inhaled refractory ceramic fibers. Toxico Sci. 49(1):93–101. http://dx.doi.org/10.1093/toxsci/49.1.93 PMID:10367346

Goodson WH 3rd, Lowe L, Carpenter DO, Gilbertson M, Manaf Ali A, Lopez de Cerain Salsamendi A, et al. (2015). Assessing the carcinogenic potential of low-dose exposures to chemical mixtures in the environment: the challenge ahead. Carcinogenesis. 36(Suppl 1):S254–96. http://dx.doi.org/10.1093/carcin/bgv039 PMID:26106142

Gordon AS, Tabor HK, Johnson AD, Snively BM, Assimes TL, Auer PL, et al.; NHLBI GO Exome Sequencing Project (2014). Quantifying rare, deleterious variation in 12 human cytochrome P450 drug-metabolism genes in a large-scale exome dataset. Hum Mol Genet. 23(8):1957–63. http://dx.doi.org/10.1093/hmg/ddt588 PMID:24282029

Greaves MF (2004). Biological models for leukaemia and lymphoma. In: Buffler P, Rice JM, Baan R, Bird M, Boffetta P, editors. Mechanisms of carcinogenesis: contributions of molecular epidemiology. Lyon, France: International Agency for Research on Cancer (IARC Scientific Publication No. 157); pp. 351–372.

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

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

Harrill AH, Desmet KD, Wolf KK, Bridges AS, Eaddy JS, Kurtz CL, et al. (2012). A mouse diversity panel approach reveals the potential for clinical kidney injury due to DB289 not predicted by classical rodent models. Toxicol Sci. 130(2):416–26. http://dx.doi.org/10.1093/toxsci/kfs238 PMID:22940726

Harris CC (2015). Cause and prevention of human cancer. Carcinogenesis. 36(Suppl 1):S1. http://dx.doi.org/10.1093/carcin/bgv047 PMID:26106134

IARC (2004). Some drinking-water disinfectants and contaminants, including arsenic. IARC Monogr Eval Carcinog Risks Hum. 84:1–477. Available from: http://publications.iarc.fr/102 PMID:15645577

IARC (2010). Some aromatic amines, organic dyes, and related exposures. IARC Monogr Eval Carcinog Risks Hum. 99:1–658. Available from: http://publications.iarc.fr/117 PMID:21528837

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 (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

Igartua C, Myers RA, Mathias RA, Pino-Yanes M, Eng C, Graves PE, et al. (2015). Ethnic-specific associations of rare and low-frequency DNA sequence variants with asthma. Nat Commun. 6:5965. http://dx.doi.org/10.1038/ncomms6965 PMID:25591454

Iida N, Dzutsev A, Stewart CA, Smith L, Bouladoux N, Weingarten RA, et al. (2013). Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. Science. 342(6161):967–70. http://dx.doi.org/10.1126/science.1240527 PMID:24264989

Kadlubar FF, Dooley KL, Teitel CH, Roberts DW, Benson RW, Butler MA, et al. (1991). Frequency of urination and its effects on metabolism, pharmacokinetics, blood hemoglobin adduct formation, and liver and urinary bladder DNA adduct levels in beagle dogs given the carcinogen 4-aminobiphenyl. Cancer Res. 51(16):4371–7. PMID:1868460

Leakey JE, Seng JE, Allaben WT (2003). Body weight considerations in the B6C3F$_1$ mouse and the use of dietary control to standardize background tumor incidence in chronic bioassays. Toxicol Appl Pharmacol. 193(2):237–65. http://dx.doi.org/10.1016/j.taap.2003.07.006 PMID:14644626

Lu K, Mahbub R, Cable PH, Ru H, Parry NMA, Bodnar WM, et al. (2014). Gut microbiome phenotypes driven by host genetics affect arsenic metabolism. Chem Res Toxicol. 27(2):172–4. http://dx.doi.org/10.1021/tx400454z PMID:24490651

Lunardi A, Nardella C, Clohessy JG, Pandolfi PP (2014). Of model pets and cancer models: an introduction to mouse models of cancer. Cold Spring Harb Protoc. 2014(1):17–31. http://dx.doi.org/10.1101/pdb.top069757 PMID:24371312

McHale CM, Zhang L, Smith MT (2012). Current understanding of the mechanism of benzene-induced leukemia in humans: implications for risk assessment. Carcinogenesis. 33(2):240–52. http://dx.doi.org/10.1093/carcin/bgr297 PMID:22166497

Melnick RL (2001). Occupational chemical carcinogenesis. In: Bingham E, Cohrssen B, Powell CH, editors. Patty's industrial hygiene and toxicology. 5th ed. Volume 1: New York, USA: Wiley; pp. 117–67.

Melnick RL, Thayer KA, Bucher JR (2008). Conflicting views on chemical carcinogenesis arising from the design and evaluation of rodent carcinogenicity studies. Environ Health Perspect. 116(1):130–5. http://dx.doi.org/10.1289/ehp.9989 PMID:18197312

Nelson MR, Wegmann D, Ehm MG, Kessner D, St Jean P, Verzilli C, et al. (2012). An abundance of rare functional variants in 202 drug target genes sequenced in 14,002 people. Science. 337(6090):100–4. http://dx.doi.org/10.1126/science.1217876 PMID:22604722

Rao GN, Piegorsch WW, Haseman JK (1987). Influence of body weight on the incidence of spontaneous tumors in rats and mice of long-term studies. Am J Clin Nutr. 45(1 Suppl):252–60. PMID:3799516

Schadt EE, Björkegren JL (2012). NEW: network-enabled wisdom in biology, medicine, and health care. Sci Transl Med. 4(115):115rv1. http://dx.doi.org/10.1126/scitranslmed.3002132 PMID:22218693

Schernhammer ES, Giovannucci E, Kawasaki T, Rosner B, Fuchs CS, Ogino S (2010). Dietary folate, alcohol and B vitamins in relation to LINE-1 hypomethylation in colon cancer. Gut. 59(6):794–9. http://dx.doi.org/10.1136/gut.2009.183707 PMID:19828464

Smith ER, Wang Y, Xu XX (2014). Development of a mouse model of menopausal ovarian cancer. Front Oncol. 4:36. http://dx.doi.org/10.3389/fonc.2014.00036 PMID:24616881

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

Spycher BD, Lupatsch JE, Zwahlen M, Röösli M, Niggli F, Grotzer MA, et al.; Swiss Pediatric Oncology Group; Swiss National Cohort Study Group (2015). Background ionizing radiation and the risk of childhood cancer: a census-based nationwide cohort study. Environ Health Perspect. 123(6):622–8. PMID:25707026

Suez J, Korem T, Zeevi D, Zilberman-Schapira G, Thaiss CA, Maza O, et al. (2014). Artificial sweeteners induce glucose intolerance by altering the gut microbiota. Nature. 514(7521):181–6. http://dx.doi.org/10.1038/nature13793 PMID:25231862

Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al. (2008). WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon, France: International Agency for Research on Cancer.

Szyf M (2007). The dynamic epigenome and its implications in toxicology. Toxicol Sci. 100(1):7–23. http://dx.doi.org/10.1093/toxsci/kfm177 PMID:17675334

Tanon A, Jaquet A, Ekouevi DK, Akakpo J, Adoubi I, Diomande I, et al.; IeDEA West Africa Collaboration (2012). The spectrum of cancers in West Africa: associations with human immunodeficiency virus. PLoS One. 7(10):e48108. http://dx.doi.org/10.1371/journal.pone.0048108 PMID:23144732

Threadgill DW, Miller DR, Churchill GA, de Villena FP (2011). The Collaborative Cross: a recombinant inbred mouse population for the systems genetic era. ILAR J. 52(1):24–31. http://dx.doi.org/10.1093/ilar.52.1.24 PMID:21411855

Turusov VS, Torii M, Sills RC, Willson GA, Herbert RA, Hailey JR, et al. (2002). Hepatoblastomas in mice in the US National Toxicology Program (NTP) studies. Toxicol Pathol. 30(5):580–91. http://dx.doi.org/10.1080/01926230290105802 PMID:12371667

Vermeulen R, Zhang L, Spierenburg A, Tang X, Bonventre JV, Reiss B, et al. (2012). Elevated urinary levels of kidney injury molecule-1 among Chinese factory workers exposed to trichloroethylene. Carcinogenesis. 33(8):1538–41. http://dx.doi.org/10.1093/carcin/bgs191 PMID:22665366

Vesselinovitch SD, Mihailovich N, Wogan GN, Lombard LS, Rao KVN (1972). Aflatoxin B_1, a hepatocarcinogen in the infant mouse. Cancer Res. 32(11):2289–91. PMID:4343225

Viaud S, Saccheri F, Mignot G, Yamazaki T, Daillère R, Hannani D, et al. (2013). The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. Science. 342(6161):971–6. http://dx.doi.org/10.1126/science.1240537 PMID:24264990

Vrijheid M, Slama R, Robinson O, Chatzi L, Coen M, van den Hazel P, et al. (2014). The Human Early-Life Exposome (HELIX): project rationale and design. Environ Health Perspect. 122(6):535–44. PMID:24610234

Waalkes MP, Qu W, Tokar EJ, Kissling GE, Dixon D (2014). Lung tumors in mice induced by "whole-life" inorganic arsenic exposure at human-relevant doses. Arch Toxicol. 88(8):1619–29. http://dx.doi.org/10.1007/s00204-014-1305-8 PMID:25005685

Walker CL, Ho SM (2012). Developmental reprogramming of cancer susceptibility. Nat Rev Cancer. 12(7):479–86. http://dx.doi.org/10.1038/nrc3220 PMID:22695395

Westcott PM, Halliwill KD, To MD, Rashid M, Rust AG, Keane TM, et al. (2015). The mutational landscapes of genetic and chemical models of *Kras*-driven lung cancer. Nature. 517(7535):489–92. http://dx.doi.org/10.1038/nature13898 PMID:25363767

Wild CP (2012). The exposome: from concept to utility. Int J Epidemiol. 41(1):24–32. http://dx.doi.org/10.1093/ije/dyr236 PMID:22296988

Wu-Williams AH, Zeise L, Thomas D (1992). Risk assessment for aflatoxin B_1: a modeling approach. Risk Anal. 12(4):559–67. http://dx.doi.org/10.1111/j.1539-6924.1992.tb00712.x PMID:1336206

Yu MC, Yuan JM (2004). Environmental factors and risk for hepatocellular carcinoma. Gastroenterology. 127(5 Suppl 1):S72–8. http://dx.doi.org/10.1016/j.gastro.2004.09.018 PMID:15508106

Zeise L, Bois FY, Chiu WA, Hattis D, Rusyn I, Guyton KZ (2013). Addressing human variability in next-generation human health risk assessments of environmental chemicals. Environ Health Perspect. 121(1):23–31. PMID:23086705

Zhang J, Chiodini R, Badr A, Zhang G (2011). The impact of next-generation sequencing on genomics. J Genet Genomics. 38(3):95–109. http://dx.doi.org/10.1016/j.jgg.2011.02.003 PMID:21477781

Ziv-Gal A, Wang W, Zhou C, Flaws JA (2015). The effects of *in utero* bisphenol A exposure on reproductive capacity in several generations of mice. Toxicol Appl Pharmacol. 284(3):354–62. http://dx.doi.org/10.1016/j.taap.2015.03.003 PMID:25771130