PART 1.

CONCORDANCE BETWEEN CANCER IN HUMANS AND IN EXPERIMENTAL ANIMALS

CHAPTER 1.

Electrophilic agents

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Introduction

In this chapter, electrophilic agents include direct-acting electrophilic chemicals and chemicals that are metabolized to reactive electrophiles. All of the chemicals discussed here are IARC Group 1 agents and as such can be characterized as *carcinogenic to humans*. Relevant carcinogens discussed in this chapter do not include pharmaceutical drugs classified in Group 1, which otherwise typically include alkylating cytotoxic agents.

Tumour sites identified in previous IARC evaluations of the carcinogenicity of several non-pharmaceutical organic compounds in humans and laboratory animals are shown in Table 1.1. For each of these agents, there was *sufficient evidence* of carcinogenicity from studies in rats and/or mice and, except for ethylene oxide, *sufficient evidence* of carcinogenicity from studies of exposed humans. For ethylene oxide, there was *limited evidence* of carcinogenicity in humans, but the classification of this chemical was raised to *carcinogenic to humans* (Group 1) based on strong mechanistic evidence of mutagenicity and clastogenicity, including the induction of sister chromatid exchange (SCE), chromosomal aberrations (CA), and micronuclei (MN) in workers exposed to ethylene oxide.

Among this group of chemicals, there is remarkable concordance in tumour sites with *sufficient evidence* or *limited evidence* of carcinogenicity in humans and *sufficient evidence* of carcinogenicity in rats and/or mice, for example for the liver (aflatoxins, trichloroethylene [TCE], and vinyl chloride), the lung (sulfur mustard), the lymphohaematopoietic system (benzene, 1,3-butadiene, and ethylene oxide), nasal tissue (formaldehyde), and the kidney (TCE). For bis(chloromethyl)ether (BCME), the lung and the nasal cavity were identified as target organs in humans and rats, respectively. In addition, angiosarcomas of the liver, which are rare tumours, were identified in humans, rats, and mice exposed to vinyl chloride.

In several instances, tumour sites identified in animals were not detected in epidemiological studies of exposed workers. These apparent discrepancies may be due to differences in susceptibility between humans and certain animal models, differences in exposure conditions between studies in animals and in humans, or limitations in

Table 1.1. Tumour sites in humans, rats, and mice for electrophilic agents

Agent	Humans		Rats	Mice
	Sufficient evidence	Limited evidence	Sufficient evidence	Sufficient evidence
Aflatoxins	Liver: hepatocellular carcinoma		Liver: hepatocellular carcinoma	
Benzene	Acute myeloid leukaemia	Acute lymphoblastic leukaemia Chronic lymphocytic leukaemia Multiple myeloma Non-Hodgkin lymphoma	Oral cavity: carcinoma Forestomach: squamous cell carcinoma Skin: squamous cell carcinoma Zymbal gland: carcinoma	Lymphoid tissue: lymphoma Haematopoietic tissue: granulocytic leukaemia Mammary gland: adenocarcinoma Lung: bronchiolo-alveolar carcinoma Zymbal gland: carcinoma Preputial gland: squamous cell carcinoma
Bis(chloromethyl)ether (BCME)	Lung		Nasal cavity: olfactory neuroblastoma	Soft tissue: sarcoma Skin: fibrosarcoma
1,3-Butadiene	Lymphohaematopoietic			Lymphoid tissue: lymphoma Soft tissue: haemangiosarcoma Liver: hepatocellular carcinoma Mammary gland: adenocarcinoma Lung: bronchiolo-alveolar carcinoma Forestomach: squamous cell carcinoma Harderian gland: carcinoma Preputial gland: squamous cell carcinoma
Ethylene oxide		Lymphohaematopoietic (non-Hodgkin lymphoma, multiple myeloma, chronic lymphocytic leukaemia) Breast	Brain: glioma Lymphoid tissue: lymphoma Peritoneum: mesothelioma	Lung
Formaldehyde	Nasopharynx Leukaemia	Paranasal sinuses	Nasal cavity: squamous cell carcinoma	
Sulfur mustard	Lung	Larynx		Lung
Trichloroethylene (TCE)	Kidney	Non-Hodgkin lymphoma Liver	Kidney	Liver Lung
Vinyl chloride	Liver: angiosarcoma Liver: hepatocellular carcinoma		Liver and extrahepatic: angiosarcoma Liver: hepatocellular carcinoma Mammary gland: adenocarcinoma Zymbal gland: carcinoma	Liver and extrahepatic: angiosarcoma Lung: bronchiolo-alveolar carcinoma Mammary gland: adenocarcinoma

epidemiological studies that reduce their power to detect excess cancer risk at particular sites. For example, the finding that mammary gland tumours were induced in female mice exposed to benzene, 1,3-butadiene, or vinyl chloride, whereas breast cancer risk was not elevated in exposed workers may be due to the fact that women were not included in many occupational cohort or case–control studies of these agents, sometimes because there were very few or no women in relevant workforces, or because they were exposed to much lower concentrations. In contrast, for hospital-based sterilization workers exposed to ethylene oxide, among whom there is a high proportion of women, increases in breast cancer incidence were observed. Exposure to ethylene oxide increased the incidence of mammary gland tumours in female mice but not in male mice. Thus, limitations in human cancer databases and sex-specific tumours in animal studies need to be considered in evaluations of site concordance for tumour induction between humans and animals.

The carcinogenicity of organic compounds and their organ specificity in animal models and in humans are influenced by numerous factors. This chapter focuses on factors that affect tissue dosimetry, factors that contribute to the multistep carcinogenic process for each agent, and factors that might account for interspecies and inter-individual differences in susceptibility.

Chemical disposition

The role of metabolism in the formation of the putative carcinogenic intermediates of the non-pharmaceutical organic compounds that are *carcinogenic to humans* is summarized in Table 1.2. Common among these 10 agents is the electrophilic nature of the parent chemical or a metabolite thereof. All of these agents either exist as direct-acting electrophilic species or can be metabolized to reactive electrophilic species. It is generally accepted that the ability of these electrophiles, whether alkylating agents, epoxides, or quinones, to react with nucleophiles, such as DNA, is key to the carcinogenicity of this group of agents (Table 1.1 and Table 1.2).

Because of the diversity in the biochemical and physical properties of organic electrophilic compounds, it is useful to first examine, in a general way, factors that are important for their absorption, distribution, metabolism, and elimination. These factors include, but are not limited to, (i) physiological, (ii) chemical, and (iii) metabolic determinants. Examples of physiological factors are alveolar ventilation rate, cardiac output, blood flow to organs, organ volumes, and body mass and composition (e.g. percentage body fat). Examples of chemical factors include the stability and reactivity of the parent compound and its metabolites, partition coefficients that control the distribution of organic compounds between blood and air or blood and tissues, rates of uptake from the gastrointestinal tract, and absorption through the dermis. For some organic chemicals, metabolic determinants such as the cellular capacity for metabolism and the affinity of metabolic enzymes for the compound are critical, both in activating the parent compound to the putative carcinogenic electrophilic metabolite and in transforming the parent compound or the electrophilic intermediate into a metabolite that can be readily eliminated. Molecular interactions of organic compounds, or their metabolites, with DNA and other critical macromolecules or receptors are also important, because these interactions can initiate the carcinogenic process or affect its progression. Determinants for metabolism or molecular interactions are dependent on the exposure history (including the intensity, duration, frequency, and route or routes of exposure, and the life stage of the exposed individual) and may differ according to the specific genotype of the individual (i.e. genetic polymorphisms in metabolic enzymes).

Absorption

Organic compounds can be absorbed after inhalation, dermal exposure, or ingestion. The sites and extent of absorption depend on the physicochemical characteristics of the compound. Highly reactive organic chemicals typically interact with tissues at the portal of entry, and toxicity and carcinogenicity are often most evident at these sites. Formaldehyde, for example, is a water-soluble, reactive volatile organic compound. After inhalation, formaldehyde interacts with the highly aqueous nasal mucosa, the first respiratory tract tissue that is encountered upon inspiration. In rats chronically exposed to formaldehyde vapours, the anterior portion of the nasal cavity was the site of tumour induction.

Sulfur mustard (also known as mustard gas) is another example of a reactive volatile organic compound that can be absorbed after inhalation or through dermal exposure. It is a lipophilic substance that easily penetrates into the skin and mucosal surfaces (Drasch et al., 1987; Somani and Babu, 1989), resulting in a high

Table 1.2. Mechanisms for formation of electrophilic species

Agent	Mechanism	Electrophilic species
Bis(chloromethyl)ether (BCME)	Direct-acting	Chloroalkyl ether
Chloromethyl methyl ether (CMME)	Direct-acting	Chloroalkyl ether
Ethylene oxide	Direct-acting	Epoxide
Formaldehyde	Direct-acting	Aldehyde
Sulfur mustard	Direct-acting	Sulfonium ion
Aflatoxins	Metabolic activation	Epoxide
Benzene	Metabolic activation	Quinone, epoxide, aldehyde
1,3-Butadiene	Metabolic activation	Epoxides
Trichloroethylene (TCE)	Metabolic activation	Epoxide, thioketene
Vinyl chloride	Metabolic activation	Epoxide

degree of bioavailability. Sulfur mustard is a bifunctional alkylating agent (IARC, 1987). Like for formaldehyde, there is evidence that DNA alkylation by sulfur mustard leads to formation of cross-links, inhibition of DNA synthesis and repair, and induction of point mutations and chromosome-type and chromatid-type aberrations (ATSDR, 2003). Some of these changes are observed in nasal tissue, consistent with the nose being a target organ for sulfur mustard.

BCME and chloromethyl methyl ether (CMME) are highly reactive organic compounds that belong to the group of chloroalkyl ethers, which are flammable, volatile, colourless liquids with highly irritating odours. In water and aqueous biological fluids, these substances are rapidly hydrolysed to form hydrochloric acid, methanol, and formaldehyde (Nichols and Merritt, 1973; National Toxicology Program, 2004). The carcinogenic effects of BCME are restricted to the epithelial tissue where exposure occurs, namely the lung for humans and respiratory tract tissues for laboratory animals exposed to BCME by inhalation. This portal-of-entry effect is consistent with the short half-life of BCME in aqueous media (ATSDR, 1989). BCME is among the most potent animal and human carcinogens known. The fact that BCME and CMME are powerful alkylating agents provides moderate to strong evidence that they operate by a genotoxic mechanism. This mechanism is likely to be similar to that of other strong alkylating agents, involving modification of DNA and resultant mutations. BCME can also be absorbed through the skin, and studies in which mice were exposed to BCME by dermal application demonstrated that this agent is a potent complete skin carcinogen, producing both papillomas and squamous cell carcinomas (Van Duuren et al., 1969).

Ethylene oxide is a water-soluble volatile organic compound that is relatively stable in aqueous solutions at neutral pH (half-life, approximately 72 hours). Because it is completely miscible with water, inhaled ethylene oxide will dissolve in the aqueous lining of the respiratory tract and diffuse into the blood. Thus, uptake of this organic compound will be substantial throughout the respiratory tract.

Volatile organic compounds that are not reactive or water-soluble are generally absorbed into the blood, primarily in the pulmonary region of the respiratory tract because of the large blood–tissue interface of the alveoli in that region. The blood–air partition coefficient is a key factor in determining the maximum levels of the organic compound that can be attained in the blood at any given concentration of the compound in air. For example, the blood–air partition coefficients for the carcinogens vinyl chloride, 1,3-butadiene, benzene, and TCE are 1.2, 1.5, 7.8, and 10 respectively. At equivalent air concentrations, higher levels of benzene and TCE will be present in the blood at steady state, compared with vinyl chloride and 1,3-butadiene.

The organic compounds discussed here can also be absorbed after oral exposure. Ingestion and subsequent oral absorption represent the greatest potential route of exposure to non-volatile organic compounds. Organic chemicals may enter the body via food or in liquids. Aflatoxin is a compound for which ingestion is considered the most important route of exposure. For example, human uptake of aflatoxins in quantities of nanograms to micrograms per day occurs mainly through consumption of contaminated maize and peanuts, which are dietary staples in some tropical countries. Uptake of aflatoxin M_1 in quantities

of nanograms per day occurs mainly via consumption of aflatoxin-contaminated milk, including breast milk (IARC, 2002). Once absorbed from the gastrointestinal tract, aflatoxins are transported via the hepatic portal blood to the liver, where they are metabolized. As discussed below, metabolism is key to understanding the carcinogenicity of aflatoxins.

Metabolic activation or detoxification and elimination

Metabolism plays a key role in both the activation and the detoxification of many organic compounds. The first step, generally called phase I metabolism, is oxidation to a metabolic intermediate. This intermediate becomes the substrate for the second step, phase II, in which it is enzymatically hydrolysed or conjugated with one of a variety of biological substrates, such as sulfate, glucuronic acid, or glutathione (GSH). Phase II reactions increase the water solubility of the chemical, which facilitates its excretion in urine, or increase the molecular weight, so that the agent is more readily eliminated in bile. Phase I metabolism of organic compounds can also result in formation of reactive intermediates that can spontaneously interact with critical macromolecules. For many organic chemicals, this is the first key step in the carcinogenic process.

Cytochrome P450 (CYP450) is the collective name of the family of enzymes responsible for the initial phase I metabolism of many organic compounds. Metabolic activation by various CYP450 isozymes is a key first step in the carcinogenic process for aflatoxin, benzene, 1,3-butadiene, TCE, and vinyl chloride. For example, in the mechanism of carcinogenicity of aflatoxins, the key steps involve: metabolism to a reactive *exo*-epoxide; binding of the *exo*-epoxide to DNA, resulting in the formation of DNA adducts; and miscoding during DNA replication, which leads to development of mutations, with eventual progression to tumours. Aflatoxin B_1, the most common and potent of the aflatoxins, is metabolized predominantly in the liver. In humans, CYP1A2, CYP2B6, CYP3A4, CYP3A5, and CYP3A7, as well as the phase II enzyme glutathione-*S*-transferase M1 (GSTM1; see below) are hepatic enzymes that mediate aflatoxin metabolism. In humans, the relative contribution of these enzymes in vivo depends not only on their affinity but also on their expression level in the liver, where CYP3A4 is predominant, mediating the formation of the aflatoxin B_1 *exo*-epoxide and aflatoxin Q_1. In humans, as in other species, the DNA binding and carcinogenicity of aflatoxin B_1 result from its conversion to the *exo*-8,9-epoxide by CYP3A4 (Essigmann et al., 1982). This epoxide is highly reactive and is the main mediator of cellular injury (McLean and Dutton, 1995). In contrast, CYP1A2 can generate some *exo*-epoxide but also a high proportion of *endo*-epoxide and aflatoxin M_1. Aflatoxins M_1 and Q_1, produced from aflatoxin B_1 by CYP1A2 and CYP3A4, respectively, are present in the urine of individuals exposed to aflatoxins (Ross et al., 1992; Qian et al., 1994).

Benzene is also a substrate for CYP450 enzymes. In common with other compounds discussed in this section, benzene must be metabolized to become carcinogenic (Ross, 2000; Snyder, 2004). The initial metabolic step involves CYP450-dependent oxidation to benzene oxide, which exists in equilibrium with its tautomer oxepin. It has been reported that benzene is most likely to be metabolized via two CYP450 enzymes, rather than just one, and that the putative, high-affinity enzyme is active primarily at benzene concentrations below 1 ppm (Rappaport et al., 2009). Whereas CYP2E1 is the primary enzyme responsible for mammalian metabolism of benzene at higher levels of exposure (Valentine et al., 1996; Nedelcheva et al., 1999), CYP2F1 and CYP2A13 have been proposed as enzymes that metabolize benzene at environmental levels of exposure, i.e. below 1 ppm (Powley and Carlson, 2000; Sheets et al., 2004; Rappaport et al., 2009).

A large proportion of benzene oxide spontaneously rearranges to phenol, which is either eliminated via phase II conjugation or further metabolized to hydroquinone and 1,4-benzoquinone. The remaining benzene oxide is either hydrolysed to produce benzene-1,2-dihydrodiol (catechol), which is further oxidized to 1,2-benzoquinone, or conjugated with GSH to produce *S*-phenylmercapturic acid. Metabolism of the oxepin tautomer of benzene oxide is thought to open the aromatic ring; this yields the reactive muconaldehydes and muconic acid. Benzene oxide, the benzoquinones, the muconaldehydes, and the benzene dihydrodiol epoxides (formed from CYP450-mediated oxidation of benzene dihydrodiol) are electrophiles that react readily with peptides, proteins, and DNA (Bechtold et al., 1992; McDonald et al., 1993; Bodell et al., 1996; Gaskell et al., 2005; Henderson et al., 2005; Waidyanatha and Rappaport, 2005), thereby interfering with cellular function (Smith, 1996). The role of these different metabolites in the carcinogenicity of benzene remains unclear, but formation of benzoquinone from hydroquinone via myeloperoxidase in the bone marrow has been

suggested to be a key step (Smith, 1996). Indeed, there is considerable evidence for an important role of the metabolic pathway that leads to formation of benzoquinone, because the benzoquinone-detoxifying enzyme NAD(P)H:quinone oxidoreductase 1 (NQO1) protects mice against benzene-induced myelodysplasia (Long et al., 2002; Iskander and Jaiswal, 2005) and protects humans against the haematotoxicity of benzene (Rothman et al., 1997). However, this does not rule out adverse effects from other metabolites.

Similarly to the metabolism of benzene, the first step in 1,3-butadiene metabolism involves CYP450-mediated oxidation to epoxybutene (Himmelstein et al., 1997). At low concentrations of 1,3-butadiene, metabolism via the CYP2E1 isozyme predominates (IARC, 1999, 2008). Epoxybutene may be metabolized by conjugation with GSH mediated by glutathione *S*-transferase (GST) or by hydrolysis catalysed by epoxide hydrolase (EH) (Csanády et al., 1992; Himmelstein et al., 1997). It may also be oxidized to multiple diastereomers of diepoxybutane (Seaton et al., 1995; Krause and Elfarra, 1997), and dihydroxybutene formed by hydrolysis of epoxybutene may be oxidized to epoxybutanediol. Diepoxybutane and epoxybutanediol are also detoxified by GST or EH (Boogaard et al., 1996). Partial hydrolysis of diepoxybutane also produces epoxybutanediol. Bone marrow myeloperoxidase can also epoxidize 1,3-butadiene. Each of the epoxide intermediates may contribute to the mutagenicity and carcinogenicity of 1,3-butadiene. The formation of epoxybutanediol or diepoxybutane requires a second oxidation of butenediol or epoxybutene, respectively. At increasing exposure concentrations of 1,3-butadiene, competition between 1,3-butadiene and butenediol or epoxybutene for CYP450-mediated metabolism may limit the extent to which the second oxidation reaction may occur.

Vinyl chloride is another volatile organic compound that is primarily and rapidly metabolized in the liver (Reynolds et al., 1975; Ivanetich et al., 1977; Barbin and Bartsch, 1989; Lilly et al., 1998; Bolt, 2005) by CYP2E1 (WHO, 1999). The primary metabolites of vinyl chloride are the highly reactive chloroethylene oxide, which is formed in a dose-dependent process and has a half-life of 1.6 minutes in aqueous solution at neutral pH (Barbin et al., 1975; Dogliotti, 2006), and its rearrangement product chloroacetaldehyde (Bonse et al., 1975). Both can bind to proteins, RNA, and DNA and form etheno adducts in RNA and DNA. Chloroethylene oxide is more reactive with nucleotides than is chloroacetaldehyde (Guengerich and Watanabe, 1979).

Conjugation of chloroethylene oxide and chloroacetaldehyde with GSH eventually leads to the major urinary metabolites *N*-acetyl-*S*-(2-hydroxyethyl)cysteine and thiodiglycolic acid (Plugge and Safe, 1977). Chloroethylene oxide can also be detoxified to glycolaldehyde by microsomal EH, and chloroacetaldehyde can be converted to chloroacetic acid by aldehyde dehydrogenase 2 (ALDH2) (Guengerich and Watanabe, 1979; ATSDR, 2006; IARC, 2008).

Two metabolic pathways of TCE have been characterized in both humans and laboratory animals: oxidation and GSH conjugation (IARC, 2014). The major pathway is CYP450-mediated oxidation, resulting in the formation of a variety of short- and long-lived metabolites. Subsequent processing of oxidative metabolites involves reduction by alcohol dehydrogenase (ADH) and ALDH, and glucuronidation. The initial step in TCE oxidation in both humans and laboratory animals is catalysed by one of several CYP450 enzymes and results in the formation of an enzyme-bound intermediate (an oxygenated TCE-CYP450 transition state, TCE-O-CYP), which is chemically unstable. This intermediate can be converted to (i) the electrophile TCE epoxide, (ii) *N*-hydroxy-acetyl-aminoethanol, or (iii) chloral, which is in equilibrium with chloral hydrate. The majority of the conversion is directed towards chloral/chloral hydrate. TCE epoxide spontaneously generates dichloroacetyl chloride, another chemically unstable and reactive species, or oxalic acid, which is readily excreted in urine. Dichloroacetyl chloride undergoes spontaneous dechlorination to produce dichloroacetate. Chloral/chloral hydrate undergoes either reduction by ALDH or CYP450 to generate trichloroethanol, or oxidation by ALDH to form trichloroacetate. Trichloroacetate is typically the major urinary metabolite of TCE that is recovered, although trichloroethanol is also an important metabolite in urine. Trichloroethanol can be oxidized by CYP450 enzymes to yield trichloroacetate, or can undergo glucuronidation by uridine 5′-diphosphate (UDP)-glucuronosyltransferases to produce trichloroethanol glucuronide. In all species investigated, trichloroacetate and trichloroethanol/trichloroethanol glucuronide are formed in much larger quantities than other oxidative metabolites. There are quantitative differences in the extent of oxidative TCE metabolism among

species at higher exposures, but at lower exposures oxidation is limited by the hepatic blood flow.

Conjugation with GSH is another important metabolic pathway for TCE, resulting in the formation of short-lived and reactive metabolites. The initial conjugation reaction occurs primarily in the liver to form (1,2-dichlorovinyl)glutathione (DCVG). Subsequent processing of DCVG occurs primarily in the kidney, which is a tumour target site in rats and humans. In the kidney, DCVG can be hydrolysed by γ-glutamyltransferase and cysteinylglycine dipeptidase to 1,2-dichlorovinyl-cysteine (DCVC), which may be *N*-acetylated to form a mercapturate or converted by β-lyase to generate a reactive thiolate that rearranges to form either chlorothioketene or chlorothioacetyl chloride (Dekant et al., 1988; Völkel and Dekant, 1998). Chlorothioketene and chlorothioacetyl chloride are highly reactive and chemically unstable, and are thought to be the molecular forms responsible for adduct formation with nucleic acids in the kidney (Müller et al., 1998a, b). For both humans and rodents, the information on urinary excretion of stable end-products is much more extensive for the oxidative pathway than for the GSH conjugation pathway. However, this is not an accurate indication of the overall flux through each pathway, because it does not account for the formation of reactive and chemically unstable metabolites via the GSH conjugation pathway.

As noted above, CYP450 enzymes are not the only enzymes involved in the metabolism of organic compounds. Formaldehyde, an important intermediate in one-carbon metabolism, is a substrate for several enzymes, including cytosolic ADH, mitochondrial ALDH, cytosolic GSH-dependent formaldehyde dehydrogenase, and catalase, as well as CYP2E1. One-carbon metabolism not mediated by CYP450 is central to many biological processes. In aqueous solution, formaldehyde is rapidly converted to its dihydroxy form, methanediol ($CH_2(OH)_2$, also known as formaldehyde hydrate or methylene glycol), which is in dynamic equilibrium with formaldehyde.

Direct-acting compounds

Some organic compounds discussed here are sufficiently reactive that they do not require metabolic activation as the first step in the carcinogenic process. Formaldehyde reacts readily and reversibly with amino groups to form Schiff bases, and with sulfhydryl groups resulting in the formation of *S*-hydroxymethylglutathione, which is oxidized by ADH3 to *S*-formylglutathione. *S*-formylglutathione is further metabolized by *S*-formylglutathione hydrolase to generate formate and GSH. Formate can also be formed non-enzymatically (Hedberg et al., 2002). Because formaldehyde reacts non-enzymatically with critical macromolecules (DNA and others), many of these enzymatic processes can be viewed as detoxification steps, especially those that lead to incorporation of the compound into the one-carbon pool.

Ethylene oxide is another direct-acting alkylating agent that reacts with nucleophiles without the need for metabolic transformation. The direct reaction of ethylene oxide with DNA is thought to initiate the cascade of genetic and related events that lead to cancer (Swenberg et al., 1990). The pathways of ethylene oxide metabolism can thus be considered detoxification pathways that increase the elimination of the parent chemical. Ethylene oxide is converted (i) by enzymatic and non-enzymatic hydrolysis to ethylene glycol, which is partly excreted as such and partly metabolized further via glycolaldehyde, glycolic acid, and glyoxylic acid to oxalic acid, formic acid, and carbon dioxide; and (ii) by conjugation with GSH.

Sulfur mustard, BCME, and CMME can also react spontaneously with biological molecules without the need for metabolic activation. For example, the reactivity of sulfur mustard with a wide variety of cellular macromolecules is well documented (IARC, 1975, 1987; ATSDR, 2003). The presence of two chlorine atoms makes it a strong bifunctional alkylating agent with a high chemical reactivity (Dacre and Goldman, 1996). The chlorine atom is typically released under formation of a carbonium ion, which then undergoes intramolecular cyclization to create a highly reactive compound. Formation of the carbonium ion is facilitated in aqueous solutions (Somani and Babu, 1989); this explains the sensitivity of mucosal tissues, such as the eye, to its effect (Solberg et al., 1997). Elevated concentrations of thiodiglycol, the major hydrolysis product of mustard gas, were detected in human urine after exposure to mustard gas vapour and aerosol (Jakubowski et al., 2000). BCME and CMME are rapidly hydrolysed in water and in aqueous biological fluids to form hydrochloric acid, methanol, and formaldehyde (Nichols and Merritt, 1973; National Toxicology Program, 2004).

Molecular interactions (DNA adducts, genetic alterations, etc.)

A common feature of the above-mentioned agents is that they either are direct-acting electrophiles or are

metabolized to electrophiles. The carcinogenicity of these chemicals is considered to be initiated by reaction of the electrophile with nucleophilic sites in DNA, leading to the induction of mutations, DNA strand breaks, and/or CA. However, additional processes may also be involved, for example free-radical-mediated oxidative stress, inhibition of DNA repair, inhibition of topoisomerase II, and immunosuppression. In addition to time-dependent variation in tissue concentrations of DNA-reactive metabolites of the chemicals described above, the likelihood that these compounds or their metabolites will bind to DNA and induce site-specific genetic alterations that lead to tumour development is a function of the physicochemical properties of the reactive agent (e.g. binding affinity for DNA or protein), several cellular features (including tissue concentrations of alternative binding biomolecules such as GSH, rates of cell division and cell death, and the activities and effectiveness of repair enzymes for DNA adducts), other physiological characteristics (e.g. age, sex, health status, and immunocompetence), and lifestyle factors (e.g. other exposures). Thus, multiple factors and mechanistic processes affect the tissue and species specificity for tumour development associated with exposures to each of the carcinogenic chemicals discussed in this chapter.

Table 1.3 presents 10 key characteristics of carcinogens (see Chapter 10, by Smith) that have been identified in in vivo and/or in vitro studies on the electrophilic agents reviewed in this chapter. What is most evident from Table 1.3 is that all these compounds produce DNA adducts in humans and animals, and cause mutations and cytogenetic alterations. Entries with weak evidence may reflect the availability of few or no published studies for certain characteristics of particular agents in animal or human tissues, rather than negative responses (Table 1.3).

BCME and CMME

The chloroalkyl ethers BCME and CMME are often referred to as powerful alkylating agents. However, because these compounds are short-lived in aqueous solution and undergo rapid hydrolysis, genotoxicity studies of BCME and CMME are sparse and have produced mixed results (IARC, 1987). Both compounds were mutagenic in bacteria (Mukai and Hawryluk, 1973; Anderson and Styles, 1978) and caused an increase in the frequency of CA in peripheral lymphocytes of exposed workers (Srám et al., 1983). BCME binds to guanine and adenine residues of calf thymus DNA in vitro (Goldschmidt et al., 1975). Both compounds induced unscheduled DNA synthesis in cultured human cells (Agrelo and Severn, 1981; Perocco et al., 1983) and cell transformation in Syrian hamster embryo cells (Casto, 1983) and cultured human fibroblasts (Kurian et al., 1990). The carcinogenicity of BCME is widely thought to involve mutagenesis resulting from alkylation of DNA bases (Bernucci et al., 1997). BCME and CMME may act synergistically with formaldehyde, one of their hydrolysis products. The likelihood of BCME–DNA adducts leading to mutations depends on the cellular content and activity of DNA repair enzymes such as methylguanine methyltransferase, and enzymes involved in mismatch repair and excision repair (Bernucci et al., 1997).

Sulfur mustard

The elimination of a chloride ion from sulfur mustard creates a highly reactive cyclic sulfonium ion that can alkylate cellular macromolecules including DNA, RNA, and proteins. Because of the presence of two chlorine atoms, sulfur mustard can act as a bifunctional alkylating agent, producing DNA interstrand or intrastrand cross-links, for example by binding to guanines on opposite strands or to neighbouring guanines on the same strand (Roberts et al., 1971; Walker, 1971; Shahin et al., 2001; Saladi et al., 2006). Such cross-links can inhibit DNA synthesis and cell division. Sulfur mustard-specific 2-hydroxyethylthioethyl–DNA adducts have been detected in in vitro systems and in multiple tissues of exposed animals (Somani and Babu, 1989; Fidder et al., 1994; van der Schans et al., 1994; Niu et al., 1996). Similar to the binding pattern for other alkylating agents, sulfur mustard-derived DNA adducts have been identified at N7 of guanine, N3 of adenine, and O6 of guanine (Fidder et al., 1994). O^6-alkylguanine DNA alkyltransferase is ineffective in repairing O^6-ethylthioethylguanine adducts (Ludlum et al., 1986). Thus, sulfur mustard can inhibit cell division by cross-linking of DNA strands and can produce mutations by inducing errors in DNA replication or repair.

Sulfur mustard induced mutations and CA in exposed animals and in a variety of in vitro systems (IARC, 1987). Further, *TP53* mutations (predominantly G → A transitions) were detected in DNA extracted from lung tumours of individuals exposed to sulfur mustard (Hosseini-khalili

Table 1.3. Levels of evidence[a] in humans and animals of key characteristics of carcinogens

Characteristic	Aflatoxins		Benzene		1,3-Butadiene		Ethylene oxide	
	Humans	Animals	Humans	Animals	Humans	Animals	Humans	Animals
Is electrophilic or can be metabolically activated to electrophiles	2	2	2	2	2	2	2	2
Is genotoxic	2	2	2	2	2	2	2	2
Alters DNA repair or causes genomic instability	1	1	1	1				
Induces epigenetic alterations	0	0	1	0	0	0	1	0
Induces oxidative stress	0	0	1	1	0	0	0	0
Induces chronic inflammation			0	0				
Is immunosuppressive	0	0	2	2	0	0	0	0
Modulates receptor-mediated effects			1	1				
Causes immortalization	0	0	0	0	0	0	0	0
Alters cell proliferation, cell death, or nutrient supply	0	1	1	1	1	0	0	0

Characteristic	Formaldehyde		Sulfur mustard		Trichloroethylene		Vinyl chloride	
	Humans	Animals	Humans	Animals	Humans	Animals	Humans	Animals
Is electrophilic or can be metabolically activated to electrophiles	2	2	2	2	2	2	2	2
Is genotoxic	2	2	2	2	2	2	2	2
Alters DNA repair or causes genomic instability	2	1	1	1	0	0		
Induces epigenetic alterations	1	1	0	0	0	1	0	0
Induces oxidative stress	0	0	1	1	0	0	0	0
Induces chronic inflammation					0	0		
Is immunosuppressive	0	0	0	0	1	1	0	0
Modulates receptor-mediated effects					0	1		
Causes immortalization	0	0	0	0	0	0	0	0
Alters cell proliferation, cell death, or nutrient supply	2	2	1	1	0	1	1	1

[a] 2 = strong evidence, 1 = moderate evidence, 0 = weak evidence.

et al., 2009). The base excision repair and nucleotide excision repair pathways were activated in human lymphoblastoid cell lines exposed to the sulfur mustard analogue 2-chloroethyl-ethylsulphide (Jowsey et al., 2009).

Ethylene oxide

Ethylene oxide is a direct-acting alkylating agent that reacts with nucleophiles, resulting in the formation of a variety of adducts in DNA, RNA, and protein. Numerous studies have demonstrated that ethylene oxide induces gene mutations and chromosomal changes in in vitro systems and in prokaryotic and eukaryotic organisms. 2-Hydroxyethyl–DNA adducts formed upon exposure to ethylene oxide have been observed in vivo at N7 of guanine, N3 of adenine, and O6 of guanine (Walker et al., 1992) and in vitro at N1 and N6 of adenine and at the N3 position of cytosine, uracil, and thymine (Tates et al., 1999). Genotoxicity resulting from ethylene oxide-induced DNA adducts may involve mispairing of altered bases, formation of abasic sites upon depurination of the adducts at N7 of guanine followed by insertion of a different base during DNA synthesis, or DNA strand breaks and subsequent chromosome breakage (Tates et al., 1999; Houle et al., 2006). In mice, ethylene oxide induced large deletion mutations, base-pair substitutions, and frameshift mutations (Walker and Skopek, 1993; Walker et al., 1997a, b). In tumours obtained from mice exposed to ethylene oxide, increases in K-*Ras* mutations with frequent G → T transversions at codon 12 and G → C transversions at codon 13 were reported (Houle et al., 2006; Hong et al., 2007). Evidence was also provided for the involvement of mutations in *p53*.

The carcinogenicity of ethylene oxide is thought to be due to the induction of gene mutations and/or chromosomal changes resulting from the formation of ethylene oxide-derived DNA adducts. Although evidence for the carcinogenicity of ethylene oxide was *sufficient* in experimental animals and *limited* in humans, the observed increases in the frequencies of CA, SCE, and MN in lymphocytes of exposed workers served as the basis for raising the classification of this alkylating agent to *carcinogenic to humans* (Group 1) (IARC, 1994, 2008).

Formaldehyde

Formaldehyde can react directly with cellular macromolecules including proteins and nucleic acids. The formaldehyde-specific DNA adduct N^6-hydroxymethyl-deoxyadenosine has been identified in lymphocytes of smokers (Wang et al., 2009). The genotoxicity of formaldehyde is well established: it induces mutations (point mutations, deletions, and insertions), CA, SCE, MN, DNA strand breaks, and DNA–protein cross-links in several in vitro and in vivo systems, including CA, SCE, and MN in nasal mucosal cells and/or lymphocytes of exposed humans (IARC, 2006). In animals exposed to formaldehyde, genotoxic effects were more consistently found in nasal tissues than in blood lymphocytes. In addition, formaldehyde produces irritation of the nose and pharynx in humans and laboratory animals. Genotoxicity and increased cell proliferation appear to be the major determinants of the nasal carcinogenicity of formaldehyde in humans and laboratory animals.

A mechanism for formaldehyde-induced myeloid leukaemogenesis might involve pancytopenia caused by genotoxicity leading to damage of primitive progenitor cells in the bone marrow; mutation of myeloid progenitor cells by formaldehyde and subsequent growth of a mutant phenotype may then lead to myeloid leukaemia. Evidence of a mild pancytopenic effect of formaldehyde or changes in ratios of lymphocyte subsets has been reported in exposed workers (Kuo et al., 1997; Ye et al., 2005; Tang et al., 2009; Zhang et al., 2010). In addition, colony formation by cultured progenitor cells that give rise to myeloid cells is inhibited by low concentrations of formaldehyde (Zhang et al., 2010). The observation of increased monosomy (loss) of chromosome 7 and trisomy (gain) of chromosome 8 in cultured myeloid progenitor cells obtained from the blood of workers exposed to formaldehyde may be relevant to the potential involvement of formaldehyde in leukaemogenesis, because these types of cytogenetic changes are frequently seen in myeloid leukaemia and myelodysplastic syndromes (Zhang et al., 2010).

1,3-Butadiene

1,3-Butadiene can be metabolized to three different DNA-reactive epoxide intermediates, which are direct-acting mutagens (IARC, 2008). The major DNA adducts formed from these epoxide intermediates in rats and mice exposed to 1,3-butadiene are at the N7 position of guanine. These N7-guanine adducts can undergo spontaneous or glycosylase-mediated depurination, which leaves an apurinic site in the DNA. Epoxide

metabolites of 1,3-butadiene can also react at sites involved in base pairing and form adducts at the N3 position of cytosine, at N1 and N6 of adenine, and at N1 and N2 of guanine (Selzer and Elfarra, 1996a, b, 1997; Zhao et al., 1998; Zhang and Elfarra, 2004). An increase in the number of N1-trihydroxybutyladenine adducts was detected in lymphocytes of workers exposed to 1,3-butadiene (Zhao et al., 2000). Alkylation of N1-adenine by epoxybutene followed by hydrolytic deamination forms the highly mutagenic deoxyinosine (Rodriguez et al., 2001), which codes for incorporation of cytosine during DNA replication, leading to the generation of A → G mutations. Diepoxybutane is a bifunctional alkylating agent that can form monoadducts in DNA similar to those formed by epoxybutanediol, or DNA–DNA cross-links by binding at the N7 position of guanine of one DNA strand and at another site elsewhere in the DNA, such as the N7 of another guanine or the N1 of an adenine (Goggin et al., 2009). Depurination of these interstrand or intrastrand lesions can induce point mutations and large deletions. However, if diepoxybutane alkylates an adenine at N6 in DNA, an exocyclic adenine adduct is formed preferentially to DNA–DNA cross-linked products (Antsypovich et al., 2007).

1,3-Butadiene is genotoxic at multiple tissue sites in mice and rats, and its epoxide metabolites are mutagenic in a variety of in vitro systems. Deletion mutations and base substitution mutations induced by these alkylating agents are consistent with their DNA adduct profiles and include G → A transition mutations, G → C transversions, A → T transversions, and A → G transitions (Lee et al., 2002). Other genotoxic effects of 1,3-butadiene and its metabolites are induction of CA, SCE, and MN.

Genetic alterations in 1,3-butadiene-induced tumours in mice are of the same type as those frequently involved in the development of a variety of human cancers. The K-*Ras*, H-*Ras*, *p53*, *p16*/*p15*, and *β-catenin* mutations detected in tumours from exposed mice are probably the result of the DNA reactivity and the genotoxic effects of 1,3-butadiene-derived epoxides. Other DNA-alkylating metabolites of 1,3-butadiene (hydroxymethylvinylketone and crotonaldehyde) may also contribute to the mutagenicity and carcinogenicity of this compound. A consistent pattern of K-*Ras* mutations (G → C transversions at codon 13) was observed at multiple organ sites of 1,3-butadiene-induced cancers (Hong et al., 2000; Sills et al., 2001; Ton et al., 2007). Alterations in the *p53* gene in brain tumours in mice were mostly G → A transition mutations (Kim et al., 2005) that probably arose from miscoding at apurinic sites resulting from depurination of N7-guanine adducts. Inactivation of the tumour suppressor genes *p16* and *p15* may also be important in the development of 1,3-butadiene-induced lymphomas (Zhuang et al., 2000). Mammary gland adenocarcinomas induced by 1,3-butadiene in mice frequently had mutations in the *p53*, H-*Ras*, and *β-catenin* genes (Zhuang et al., 2002). Overall, these observations point to a genotoxic mechanism underlying the development of 1,3-butadiene-induced cancers.

Vinyl chloride

The carcinogenicity of vinyl chloride is probably caused by its highly reactive metabolite chloroethylene oxide and/or by the rearrangement product chloroacetaldehyde (Bonse et al., 1975). Both intermediates can bind to proteins, RNA, and DNA (Guengerich and Watanabe, 1979). Vinyl chloride is mutagenic in bacteria and mammalian cells. It is also clastogenic in vivo and in vitro, causing increases in the frequencies of CA, SCE, and MN (IARC, 2008). The major DNA adduct formed from chloroethylene oxide is at the N7 position of guanine. In addition, etheno DNA adducts (1,N^6-ethenoadenine, 3,N^4-ethenocytosine, N^2,3-ethenoguanine, and 1,N^2-ethenoguanine) have been identified after in vitro incubations with chloroethylene oxide, and levels of these adducts are increased in multiple organs of rats exposed to vinyl chloride by inhalation (Ciroussel et al., 1990; Guengerich, 1992; Swenberg et al., 2000). The etheno adducts, which may be involved in base-pair substitutions, are much more persistent than the N7-guanine adduct (Fedtke et al., 1990) and have demonstrated miscoding potential in vitro and in vivo, causing A → G transitions, A → T transversions, C → A transversions, C → T transitions, and G → A transitions (Singer et al., 1987; Cheng et al., 1991; Mroczkowska and Kuśmierek, 1991; Singer et al., 1991; Basu et al., 1993). The same types of mutation have been observed in the *TP53* and *RAS* genes in vinyl chloride-induced tumours. *TP53* mutations associated with exposure to vinyl chloride (frequently A → T transversions) were found in angiosarcomas in both humans and rats, and mutations in K-*RAS* were also associated with vinyl chloride-induced angiosarcomas in humans (IARC, 2008). Polymorphisms in *XRCC1*, a gene that encodes an enzyme that repairs etheno DNA adducts, may account

for inter-individual differences among exposed workers in susceptibility to genetic damage induced by vinyl chloride (Li et al., 2003).

Aflatoxins

Aflatoxin B_1 is genotoxic in prokaryotic and eukaryotic systems in vitro, including cultured human cells, and in vivo in humans and in a variety of animal species. Its metabolism to a reactive *exo*-8,9-epoxide results in DNA binding and formation of DNA adducts that lead to gene mutations, CA, SCE, MN, and mitotic recombination in a variety of in vivo and in vitro systems (IARC, 2002). Adduct formation in DNA at the N7 position of guanine represents more than 98% of the total adducts formed by the *exo*-8,9-epoxide (Guengerich et al., 1998). Depurination of this guanine adduct creates an apurinic site. Alternatively, the N7-guanine adduct may convert to the more stable ring-opened aflatoxin B_1–formamidopyrimidine adduct (Groopman et al., 1981). Differences in the mutational specificity of an apurinic site-containing genome (derived from depurination of aflatoxin B_1–N7-guanine) compared with that of a genome with the aflatoxin B_1–N7-guanine adduct itself, where mutations also occurred at the base 5′-adjacent to the site of the adducted guanine, suggest that intercalation of the aflatoxin moiety on the 5′ side of the modified guanine perturbs both the modified and the complementary DNA strands, causing interference with 5′ base pairing (Gopalakrishnan et al., 1990; Bailey et al., 1996). Thus, mutations resulting from aflatoxin B_1–N7-guanine adducts may not be due only to depurination.

A specific AGG → AGT transversion mutation at codon 249 of the *TP53* tumour suppressor gene in human hepatocellular carcinomas is associated with exposure to aflatoxin B_1 (Gomaa et al., 2008). G → T transversion mutations are predominant in cell culture systems and animal models and are consistent with the formation of the major aflatoxin B_1-derived N7-guanine adduct. This is because adenine is most commonly inserted opposite the apurinic site. However, other types of mutation have also been observed with aflatoxin B_1, including G → C transversions and G → A transitions in DNA repair-deficient xeroderma pigmentosum cells (Levy et al., 1992); this suggests that DNA repair deficiency may influence the frequency and distribution of mutations within a gene. Aflatoxin B_1 may contribute to genomic instability in hepatocellular carcinomas (Kaplanski et al., 1997) by inducing mitotic recombination and loss of heterozygosity. The concurrent presence of hepatitis B virus, which causes chronic active hepatitis and cirrhosis, increases the incidence of hepatocellular carcinomas caused by aflatoxins in humans (IARC, 2002).

Trichloroethylene

Data from human studies suggest that exposure to TCE increases the frequency of CA in peripheral lymphocytes (Tabrez and Ahmad, 2009) and leads to mutations in the von Hippel–Lindau tumour suppressor gene *VHL* in renal cell carcinoma (Brüning et al., 1997; Brauch et al., 1999), but these findings have been reported in only a limited number of studies. TCE exposure induced MN both in vitro (Wang et al., 2001; Robbiano et al., 2004; Hu et al., 2008) and in vivo (Hrelia et al., 1994; Kligerman et al., 1994; Robbiano et al., 2004). Although TCE itself appears to be incapable of inducing gene mutations, it has shown potential to affect DNA and chromosomal structure. The formation of DNA adducts (Mazzullo et al., 1992; Cai and Guengerich, 2001) and the mutagenicity of TCE in vitro are dependent on the presence of metabolic activation systems (IARC, 2014). There is strong evidence that the GSH-conjugated metabolites of TCE, particularly DCVC, are genotoxic, and some of the oxidative metabolites (TCE epoxide, dichloroacetate, and chloral/chloral hydrate) may also be genotoxic. Thus, biotransformation of TCE can produce genotoxic metabolites, particularly in the kidney, where in situ metabolism occurs (IARC, 2014).

Both genotoxic and non-genotoxic mechanisms may contribute to the carcinogenicity and toxicity of TCE at other sites, including the liver, the lung, and the haematopoietic system. In addition to genotoxicity, epigenetic alterations, oxidative stress, cytotoxicity, and altered rates of cell division or apoptosis may be involved in tumour induction in the liver or lung. The immunotoxicity of TCE may be involved in the development of haematopoietic cancers. However, the data are inadequate for reliable conclusions to be drawn about causal relationships between non-genotoxic mechanisms and TCE-induced tumours in humans or laboratory animals (IARC, 2014). From toxicity and carcinogenicity studies in humans and laboratory animals, there is strong evidence for the kidney as a target tissue for TCE-induced tumour formation. The database supporting the non-genotoxic mechanism of kidney carcinogenesis is *moderate*. However, the *strong evidence* of genotoxicity of DCVC, the kidney metabolite of TCE, supports the overall conclusion that the evidence for

a genotoxic mechanism of kidney carcinogenesis is *strong*. The evidence for the liver as a target tissue for TCE, from cancer assays and toxicity findings in laboratory animals, is *strong*. The evidence for non-genotoxic and/or genotoxic mechanisms of liver carcinogenesis is *moderate*. The available data suggest multiple non-genotoxic mechanisms and the potential for genotoxic mechanisms from the TCE metabolites dichloroacetate and chloral hydrate.

Benzene

Benzene induced CA, SCE, and MN in bone marrow cells of exposed mice, CA in bone marrow cells of exposed rats, and CA and mutations in human cells in vitro. CA in human peripheral lymphocytes have long been associated with occupational exposure to benzene (Forni, 1979; IARC, 1982; Eastmond, 1993; Zhang et al., 2002; Holecková et al., 2004). As noted above, metabolism of benzene produces several electrophilic agents (benzene oxide, in equilibrium with its tautomer oxepin, muconaldehyde, benzoquinone, and benzene dihydrodiol epoxide) that can react with DNA or proteins. DNA binding and adduct formation may not be the major steps in the development of benzene-induced leukaemias (Whysner et al., 2004). Although the mechanisms of benzene-induced carcinogenesis and the potential relative roles of each of these metabolites are not fully known, there is strong support for the involvement of clastogenic and aneugenic effects, such as formation of CA, MN, and DNA strand breaks.

Exposure to benzene has been associated with chromosomal changes that are commonly observed in acute myeloid leukaemia, including those comprising loss of various parts of the long arm of chromosome 5 or 7, or complete loss of these chromosomes, gain of the entire chromosome 8, and an increased frequency of translocations between chromosomes 8 and 21 in peripheral lymphocytes of exposed workers (Smith et al., 1998; Zhang et al., 1999, 2002). Benzene and its quinone metabolites are inhibitors of topoisomerase II, leading to increased frequencies of DNA cleavage complexes and DNA double-strand breaks; this effect can result in the formation of chromosome translocations and inversions (Hutt and Kalf, 1996; Lindsey et al., 2004, 2005; Deweese and Osheroff, 2009). Other potential pathways involved in benzene-induced acute myeloid leukaemia include mutagenesis (possibly through generation of reactive oxygen species), epigenetic changes due to altered methylation status, decreased immunosurveillance (Cho, 2008; Li et al., 2009), haematotoxicity and alterations in stem cell pool size (Rothman et al., 1997), and inhibition of gap-junction intercellular communication (Rivedal and Witz, 2005). Thus, multiple mechanisms are likely to be involved in benzene-induced leukaemogenesis. Benzene produces multiple cytogenetic abnormalities in human lymphocytes (Tough and Brown, 1965; Picciano, 1979; Smith and Zhang, 1998; Zhang et al., 2002) and induces specific chromosomal changes associated with non-Hodgkin lymphoma in human lymphocytes (Zhang et al., 2007). Induction of DNA double-strand breaks and chromosomal rearrangements in lymphoid cells in combination with immunosuppression by benzene might be the cause of lymphoma.

The carcinogenicity of the group of electrophilic chemicals discussed above is likely to be due to interaction between the parent electrophile or one or more electrophilic metabolites and nucleophilic DNA, leading to point mutations and induction of CA. These effects have been observed in humans, in animals, and in in vitro systems. In addition, production of reactive oxygen species, inhibition of DNA synthesis or repair, and cytotoxicity/cell proliferation could complement DNA modification to enhance DNA damage. Tumour outcome can result from certain DNA adducts leading to mutations and dysregulation initially described with reference to proto-oncogenes and tumour suppressor genes. For benzene, chromosomal translocations, in combination with haematotoxicity or immunosuppression, are associated with increased risk of haematopoietic cancer in humans. The extent to which other processes (inflammation, oxidative stress, immunosuppression, epigenetic alterations, and immortalization) might contribute to the carcinogenicity of this class of chemicals in general is limited by the availability of few or no published studies that address these effects.

Polymorphisms and susceptibility

Susceptibility to the carcinogenic effects of organic compounds may derive from acquired characteristics, such as altered expression of certain enzymes, or from genetic factors, such as enzyme polymorphisms. Polymorphisms of enzymes involved in the metabolism of organic compounds are likely to be responsible for individual differences in activation and detoxification reactions that control tissue levels of electrophilic intermediates. The enzymes that catalyse epoxide formation and elimination are polymorphic in human

populations, and some isozymes may be induced by a variety of environmental and pharmaceutical agents. For example, factors that explain differences in the response to aflatoxin between human individuals and between animal species and strains include the proportion of aflatoxin metabolized to the *exo*-8,9-epoxide (mainly by CYP450 enzymes) relative to other, much less toxic metabolites, and the prevalence of pathways that lead to the formation of non-toxic conjugates with reduced mutagenicity and cytotoxicity (Guengerich et al., 1998).

Similarly, the expression of enzymes involved in aflatoxin metabolism can be modulated with chemopreventive agents, resulting in inhibition of DNA adduct formation and hepatocarcinogenesis, as has been demonstrated in rats. Oltipraz is a chemopreventive agent that increases GSH conjugation and inhibits the activity of some CYP450 enzymes (e.g. CYP1A2). Results from clinical trials with oltipraz in China are consistent with experimental data in showing that after dietary exposure to aflatoxins, modulation of the metabolism of aflatoxins with oltipraz can lead to reduced levels of DNA adducts (IARC, 2002; Kensler et al., 2005).

Increased susceptibility to the toxic effects of benzene has been linked to genetic polymorphisms that increase the rate of metabolism of benzene to active intermediates or decrease the rate of detoxification of these active intermediates (Rothman et al., 1997; Xu et al., 1998; Kim et al., 2004).

Enzyme polymorphisms also affect the metabolism of 1,3-butadiene. Genetic polymorphisms in GST and microsomal EH affect the in vitro mutagenicity of 1,3-butadiene-derived epoxides or the in vivo mutagenicity of 1,3-butadiene in occupationally exposed workers (Wiencke et al., 1995; Abdel-Rahman et al., 2003). The extent to which these enzyme polymorphisms influence the carcinogenicity of 1,3-butadiene is not known. The genotoxic effects of 1,3-butadiene can be modulated by alterations in key determinants of its metabolism; this suggests that markers of individual susceptibility can be identified. For example, mice that lack a functional microsomal EH (*mEH*) gene were more susceptible than wild-type mice to the mutagenic effects of 1,3-butadiene or diepoxybutane (Wickliffe et al., 2003). EH activity varies considerably among humans. 1,3-Butadiene-exposed workers with the genotype for low-activity EH were reported to be more susceptible to 1,3-butadiene-induced genotoxicity (assessed by *HPRT* mutant frequency in lymphocytes) than individuals with the more common *EH* genotype (Abdel-Rahman et al., 2001, 2003). No significant effects were observed for induction of *HPRT* mutations or SCE in individuals with *GSTM1* or *GSTT1* polymorphisms (Abdel-Rahman et al., 2001). MN frequencies were higher among 1,3-butadiene-exposed workers in China with polymorphisms in *GSTM1* and/or *GSTT1* compared with workers with the wild-type genes (Cheng et al., 2013). These differences in response are consistent with the known important roles of EH and GST in the detoxification of 1,3-butadiene epoxides in tissues in which these intermediates are produced.

Ethylene oxide is a substrate of the GST isozyme T1 (Hayes et al., 2005). This detoxifying enzyme is polymorphic, and a relatively large proportion of the population (about 20% of Caucasians and almost 50% of Asians) has a homozygous deletion (*GSTT1*-null genotype) (Bolt and Thier, 2006). As expected, these individuals show a significantly elevated level of hydroxyethyl valine in their haemoglobin, due to the presence of endogenous ethylene oxide (Thier et al., 2001). Nevertheless, the influence of this genetic trait on the formation of this type of adduct as a result of exposure to exogenous ethylene oxide in the workplace is less clear.

In the cytoplasm of erythrocytes obtained from 36 individuals, ethylene oxide was eliminated 3–6 times as fast in samples from so-called conjugators (defined by a standardized conjugation reaction of methyl bromide and GSH; 75% of the population) as in samples from individuals who lack this GST-specific activity (the remaining 25%). In whole-blood samples incubated with ethylene oxide, an increase in the frequency of SCE was observed in lymphocytes from the non-conjugators but not in lymphocytes from the conjugators (Hallier et al., 1993).

The carcinogenicity and toxicity of TCE, particularly in the liver and kidney, are associated with its metabolism. There are inter-individual differences, both in humans and in rodents, in the formation of TCE metabolites that are thought to be responsible for the toxic and carcinogenic effects of TCE in the kidney and liver. The susceptibility to adverse health effects of TCE may be influenced by genetics, sex, life stage, and other conditions that influence the extent and nature of the metabolism of this chemical. Polymorphisms in metabolism genes in both oxidative (e.g. *CYP2E1*, *ADH*, and *ALDH*) and GSH conjugation (e.g. GSTs) pathways have been studied

in connection with susceptibility to TCE toxicity and carcinogenicity. Polymorphisms in genes for organic anion transporters (OAT1 and OAT3) in the kidney may also influence the rates of uptake and the extent of cellular accumulation of DCVG or DCVC. With respect to life-stage susceptibility, data are available to support the influence of differences in exposure (e.g. transplacental transfer or exposure through breast milk in early life stages) or life stage-specific differences in toxicokinetics. Lifestyle factors (e.g. consumption of alcoholic beverages) may also affect TCE metabolism, and nutrition or obesity may affect internal concentrations of TCE and its metabolites.

Genes involved in DNA repair act to maintain the integrity of the genome by removing lesions (i.e. adducts) that – if left unrepaired – could result in mutations or chromosomal damage. Individuals with defects in genes that encode DNA repair enzymes are at elevated risk for certain cancers (Poulsen et al., 1993). Heterozygous carriers may also have increased susceptibility, because of suboptimal levels of repair.

The reactive aflatoxin B_1 metabolite *exo*-8,9-epoxide induced a higher mutation frequency in a shuttle vector plasmid transfected into DNA repair-deficient xeroderma pigmentosum cells (human fibroblasts) compared with repair-proficient cells; the location of mutations was affected by repair proficiency (Levy et al., 1992).

Polymorphisms in genes involved in repair of DNA double-strand breaks (*WRN* [Werner syndrome], *TP53*, *BLM* [Bloom syndrome], *RAD51*, and *BRCA1*) can modify susceptibility to benzene-induced haematotoxicity in exposed workers (Shen et al., 2006; Lan et al., 2009; Ren et al., 2009).

Mice deficient in nucleotide excision repair were more susceptible than wild-type mice to the mutagenic effects of 1,3-butadiene or its reactive metabolites epoxybutene and diepoxybutane (Wickliffe et al., 2007).

Chicken cells deficient in the Fanconi anaemia complementation groups/breast cancer A (FANC/BRCA) pathway are hypersensitive (with reduced survival) to formaldehyde at levels measured in human plasma (Ridpath et al., 2007). This observation is consistent with an essential role for this pathway in the repair of DNA–protein cross-links caused by formaldehyde, and suggests that patients with Fanconi anaemia (a genetic disorder that is characterized by progressive pancytopenia) may have increased susceptibility to leukaemia from formaldehyde.

A common polymorphism in the DNA repair gene *XRCC1* is a biomarker of susceptibility to *TP53*-induced mutations in workers exposed to vinyl chloride (Li et al., 2003). In workers exposed to 1,3-butadiene, MN frequencies were higher in peripheral lymphocytes of individuals with polymorphisms in *XRCC1* compared with individuals carrying the wild-type repair gene (Wang et al., 2010).

In summary, genetic polymorphisms and variability in expression of enzymes due to induction or inhibition of constitutive enzyme levels can have considerable impact on the carcinogenic process. Determining the existence and functional role of genetic polymorphisms in cancer etiology is an active area of research in molecular epidemiology.

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