

# NATURE AND NURTURE – LESSONS FROM CHEMICAL CARCINOGENESIS

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Abstract | The roles of genetic constitution versus environmental factors in cancer development have been a matter of debate even long before the discovery of ‘oncogenes’. Evidence from epidemiological, occupational and migration studies has consistently pointed to environmental factors as the major contributing factors to cancer, so it seems reasonable to discuss the importance of chemical carcinogenesis in the present ‘age of cancer genetics’.

Epidemiological data on geographical and temporal variations in cancer incidence, as well as studies of migrant populations and their descendants that acquire the pattern of cancer risk of their new country, indicate that ‘environmental exposures’ make a substantial contribution to human cancers<sup>1,2</sup>. These studies, along with the investigation of lifestyle factors and habits, led to the conclusion that the great majority (over 80%) of cancer deaths in Western industrial countries can be attributed to factors such as tobacco, alcohol, diet, infections and occupational exposures, with diet (35%) and tobacco (30%) as the major contributors<sup>1</sup>. In a recent compilation of data, it was estimated that about 75% (smokers) or 50% (non-smokers) of all deaths due to cancer in the United States could be avoided by elimination of these risk factors<sup>3</sup>.

The view that environmental factors are a principal cause of sporadic cancers was reinforced by analyses of cancer cases in large cohorts of twins from Sweden, Denmark and Finland<sup>4</sup>, and by analyses of the Swedish family-cancer database, which encompasses about 10 million individuals<sup>5</sup>. Although heavily debated, the authors of both studies were able to demonstrate that the influence of exposures and factors not shared among siblings or relatives — termed as ‘non-shared environmental factors’ — predominates. For instance, in the family-cancer database these factors accounted for 79% of lung, 77% of urinary bladder, 69–75% of colorectal, and 60% of breast cancer cases. In principle, ‘non-shared environmental factors’ comprise causative factors, such as smoking, radiation, infections and occupational exposures, as well as sporadic mutations. *A priori*,

sporadic and random mutations have no predictable cause — they might be induced by environmental or genetic factors, or by the interaction of both. What are the roles of chemical carcinogens in the aetiology of cancer, a complex disease that is caused by the interplay of multiple genes and many environmental factors?

## Chemical carcinogens – from past to present

Based on his observations in Austrian mines and at several other places throughout Middle Ages Europe, Theophrastus Bombastus von Hohenheim, better known by the name Paracelsus, described the ‘wasting disease of miners’ in 1567 (REF. 6). He proposed that the exposure to natural ores such as realgar (arsenic sulphide) and others might have been causing this condition. A similar disease was noticed in the Erz Mountains of Eastern Europe that was much later known as the ‘lung cancer of the Schneeberg mines’ in Saxony<sup>7</sup>. Although this kind of cancer was eventually linked to radioactivity emitted from decay products of radon gas, rather than to arsenic<sup>8</sup>, Paracelsus was actually among the first to consider a chemical compound as an occupational carcinogen.

A more systematic account on the work-related ‘peculiar diseases’ in several different fields was published by Bernardino Ramazzini in 1700 (REF. 9), who could therefore be considered as the actual founder of occupational medicine. Later in the same century, the two English physicians John Hill and Percivall Pott described the occurrence of cancerous alterations in the nasal mucosa and at the skin of the scrotum in a few patients, and traced it to the local long-term exposure

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### Summary

- Associations between cancer and occupational exposures can be dated back into the sixteenth century.
- Today, about 200 different chemical compounds and mixtures are known or anticipated to be human carcinogens.
- The great majority of human chemical carcinogens require metabolic activation to elicit detrimental effects. The activity of 'xenobiotic metabolizing enzymes' such as cytochrome-P450-dependent monooxygenases, glutathione S-transferases, sulphotransferases and others are required for activation (toxication) of important carcinogens.
- Human carcinogens act through a variety of genotoxic and non-genotoxic mechanisms. DNA binding and induction of mutations in cancer-susceptibility genes, such as *TP53* and *KRAS*, are important mechanisms of tumour initiation. In addition, the accompanying ability of many compounds to promote the outgrowth of transformed cell clones has been acknowledged.
- The preferential formation of certain stereoisomers during metabolic activation of genotoxic carcinogens can determine the level of DNA damage, the efficiency of DNA repair, and the carcinogenic potency of a compound.
- Humans are exposed to mixtures of compounds with different degrees of biological activity. Analysis of compound-specific mutational patterns provides valuable clues on the contribution of individual chemicals (or single classes of chemicals) to the overall biological response to these mixtures observed in certain tissues.

to snuff<sup>10</sup> and to repetitive local contamination by soot<sup>11</sup>, respectively. In 1822, John Ayrton Paris noticed that arsenic fumes ("arsenical vapour") might contribute to the occurrence of scrotal skin cancer in the copper-smelting works of Cornwall and Wales<sup>12</sup>.

At the end of the nineteenth century it became evident that occupational exposures to certain chemicals or mixtures of chemicals had carcinogenic effects. For instance, Richard von Volkmann and Joseph Bell confirmed the early observation from

Pott by describing several scrotal skin tumour cases in the German and Scottish paraffin industry<sup>13,14</sup>. In the late 1880s, the English pathologist and dermatologist Sir Jonathan Hutchinson reported on the occurrence of skin cancer as a result of the medical use of arsenic<sup>15</sup>. In 1895, the surgeon Ludwig Wilhelm Carl Rehn reported the appearance of urinary bladder tumours among men employed in the German aniline dyestuff industry in the production of 'fuchsin' (magenta)<sup>16</sup>. This workplace was subsequently associated with frequent occurrence of cancer of the urinary tract, referred to as 'aniline cancer'.

By the year 1907 it was officially recognized in Great Britain that cancer of any cutaneous site could be caused by pitch, tar or tarry compounds<sup>17</sup>. Until then, physicians were only able to observe unexpected overall effects of occupational exposures, based on the rise of industrialization. The imperative next step was that of systematic inquiry and reproduction of the diseases in experimental systems. After many failures to induce cancer in laboratory animals, the Japanese pathologist Katsusaburo Yamagiwa and his assistant Koichi Ichikawa were the first to produce malignant epithelial tumours by application of coal tar to the ears of rabbits in 1915 (REF. 18). This pioneering experiment actually marked the transition into the modern era of experimental cancer research. During these years, the identification of the carcinogenic compound(s) in these mixtures was extremely difficult, because of their complexity, but success was achieved by Sir Ernest Kennaway and his co-workers at the Royal Cancer Hospital in London (FIG. 1). During this time, chemical synthesis routes for higher molecular weight polycyclic aromatic hydrocarbons (PAHs), such as dibenz[*a,h*]anthracene (DBA), were first described and these molecules were subsequently proven to induce skin cancer in mice<sup>19,20</sup>. The active component isolated from 2 tons of coal tar pitch turned out to be another PAH, the pentacyclic benzo[*a*]pyrene (BP)<sup>21</sup>.

Parallel studies with aromatic amines or related compounds supplemented experimental data on the carcinogenicity of industrial chemicals that had been



**Figure 1 | Sir Ernest Laurence Kennaway (1881–1958) and his co-workers.** In 1930, Kennaway and Hieger showed for the first time that single polycyclic aromatic hydrocarbons (PAHs), such as dibenz[*a,h*]anthracene, are tumorigenic in mouse skin<sup>19</sup>. In 1933, Cook, Hewett and Hieger isolated the carcinogen benzo[*a*]pyrene from coal tar pitch<sup>21</sup>. Boyland and Levi proposed in 1935 that toxic PAHs might either be intravitaly converted into more active pathogenic substances or detoxified<sup>75</sup>. Photo taken at The Cancer Hospital, London, May 9, 1934. From left to right, top row: Colin L. Hewett, Aaron Cohen, Geoffrey A. D. Haslewood, Alfred A. Levi. Middle row: Margaret E. Boyland, Eric Boyland, Frederick L. Warren, Izrael Hieger, Michael Knox, L. Dorothy Parsons. Front row: Harold Burrows, Ernest L. Kennaway, James W. Cook. Photo reproduced with permission from REF. 143 © Oxford University Press.

released in large amounts during this time. Although aniline and other aromatic amines failed to produce tumours in rabbits<sup>22</sup>, Hueper and colleagues showed that 2-naphthylamine (2-NA) induced bladder tumours in dogs when administered to the stomach or skin<sup>23</sup>. This experiment supported an earlier prediction from Otto Leichtenstern about the tumorigenicity of this compound in the human bladder<sup>24</sup>. In the meantime, others provided evidence for the hepatocarcinogenicity of aminoazo dyes such as *o*-aminoazotoluene<sup>25</sup> and *N,N*-dimethyl-4-aminoazobenzene (DAB, 'butter yellow') in

rats<sup>26</sup>. In 1941, the first report on tumorigenesis in bladder, liver, kidney, pancreas and lung of rats induced by 2-acetylaminofluorene (AAF), an arylamide intended to be used as a pesticide, had been published<sup>27</sup>.

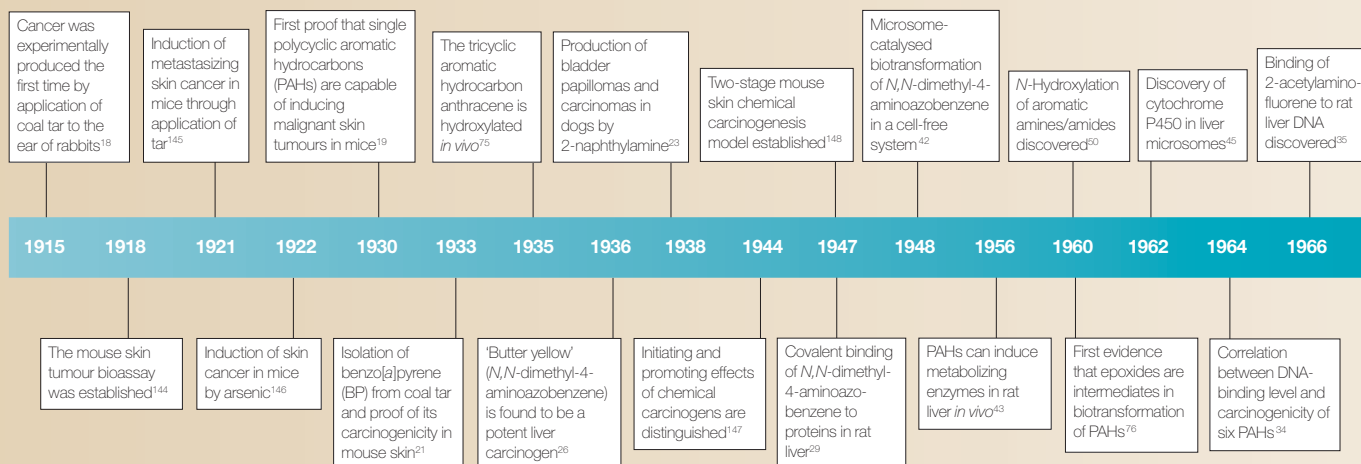
By 1940, evidence for chemical carcinogenesis in humans, initially fuelled from single case observations, had been complemented by experimental data on the bioactivity of pure and structurally defined compounds present in the industrial environment. Today, it is well established that a great diversity of compounds (as pure chemicals or present in mixtures) are tumorigenic in

Table 1 | **Selected human chemical carcinogens**

Compounds*	Main sources/uses	Affected organs/cancer type
<b>Aminoazo dyes</b>		
<i>o</i> -Aminoazotoluene	Pigments; colouring oils; immunosuppressant	Liver, lung, bladder
<i>N,N</i> -dimethyl-4-aminoazobenzene	Colour polishes; waxes (no longer in use)	Lung, liver
<b>Anticancer drugs</b>		
Melphalan	Chemotherapy	Leukaemia <sup>‡</sup>
Thiotepa	Chemotherapy (no longer in use)	Leukaemia <sup>‡</sup>
<b>Aromatic amines/amides</b>		
2-Naphthylamine	Dyes; antioxidant (no longer in use)	Bladder <sup>‡</sup>
4-Aminobiphenyl	Dyes; antioxidant (no longer in use); research tool	Bladder <sup>‡</sup>
2-Acetylaminofluorene	Model compound; tested as a pesticide	Liver, bladder
<b>Aromatic hydrocarbons</b>		
Benzo[ <i>a</i> ]pyrene	Coal tar; roofing; cigarette smoke	Skin, lung, stomach
2,3,7,8-Tetrachlorodibenzo- <i>p</i> -dioxin	No commercial use; tested as a pesticide	Lung <sup>‡</sup> , lymphoma <sup>‡</sup> , liver
Polychlorinated biphenyls	Flame retardants; hydraulic fluids	Liver, skin <sup>§</sup>
<b>Metals (and compounds)</b>		
Arsenic	Natural ores; alloys; pharmaceutical agent	Skin <sup>‡</sup> , lung <sup>‡</sup> , liver <sup>‡</sup>
Cadmium	Natural ores; pigments; batteries; ceramics	Lung <sup>‡</sup> , prostate <sup>‡</sup> , kidney <sup>‡</sup>
Nickel	Natural ores; alloys; electrodes; catalysts	Lung <sup>‡</sup> , nasal cavity <sup>‡</sup>
<b>Natural carcinogens</b>		
Aflatoxin B <sub>1</sub>	A mycotoxin (found in contaminated food)	Liver <sup>‡</sup>
Asbestos (fibrous silicates)	Thermal insulation; gaskets (declining usage)	Lung <sup>‡</sup> , mesothelioma <sup>‡</sup>
<b><i>N</i>-nitroso compounds</b>		
<i>N</i> -Nitrosodimethylamine	Polymers; batteries; nematocide (no longer in use)	Liver, lung, kidney
4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone	Research tool; cigarette smoke	Lung, liver
<b>Olefines</b>		
Ethylene oxide	Glycol and polyester production; sterilization	Leukaemia <sup>‡</sup> , lymphoma <sup>‡</sup>
Vinyl chloride (VC)	Plastics (PVC); co-polymers	Liver (angiosarcoma) <sup>‡</sup>
Trichloroethylene	Degreasing operations; adhesives; lubricants	Liver <sup>‡</sup> , kidney <sup>‡§</sup>
<b>Paraffines/ethers</b>		
1,2-Dichloroethane	VC production; solvent; degreaser (no longer in use)	Liver, lung, breast
Bis(chloromethyl)ether	Technical applications (rarely used)	Lung <sup>‡</sup>
Mustard gas (sulphur mustard)	Chemical warfare in First World War; research	Lung <sup>‡</sup>
Nitrogen mustard	Limited application as antineoplastic agent	Lung, skin, lymphoma

\*According to the National Toxicology Program 10<sup>th</sup> Report on Carcinogens, the compounds listed are known to be human carcinogens or reasonably anticipated to be human carcinogens<sup>28</sup>. This assessment is based on sufficient evidence of carcinogenicity in humans ('known') or animal models ('anticipated'). <sup>‡</sup>Tumour sites observed in humans. <sup>§</sup>Limited evidence in humans. PVC, poly VC.

Timeline | **Advances in 'chemical carcinogenesis' in the twentieth century**



humans<sup>28</sup>. A few examples from these lists of human chemical carcinogens are compiled in TABLE 1, and the history of carcinogen research is outlined in the TIMELINE.

**Metabolic activation of carcinogens**

**DNA binding.** In the pre-Watson and Crick era, before carcinogens were known to bind to DNA, the cancer-producing effects of DAB<sup>29</sup> or BP<sup>30</sup> were believed to be due to their interaction with proteins in specific tissues<sup>31</sup>. Later, when the sensitivity of detection was increased due to the availability of radioactively labelled chemicals, carcinogens such as N-methyl-bis(2-chloroethyl)amine ('nitrogen mustard')<sup>32</sup>, N-nitrosamines (for example, N-nitrosodimethylamine)<sup>33</sup>, PAHs (for example, BP)<sup>34</sup>, aromatic amines/amides (for example, AAF)<sup>35</sup>, and aminoazo dyes (for example, DAB)<sup>36</sup> were found to bind DNA *in vivo*. By the end of the 1960s, accumulating evidence pointed to a correlation between the DNA-binding capacity of a particular carcinogen and its biological potency. More than two decades after the discovery that mustard gas induced mutations in *Drosophila*<sup>37</sup>, all of these carcinogenic compounds were also regarded as mutagens<sup>38</sup>.

As chemical carcinogens were found to be covalently attached to cellular proteins and DNA, it became obvious that only sufficiently reactive, ELECTROPHILIC, compounds are capable of directly interacting with these macromolecules. Among known human carcinogens, however, only a few chemicals belong to the class of 'direct carcinogens' — for example, ethylene oxide, bis(chloromethyl)ether and some aziridine or nitrogen-mustard derivatives used in anticancer chemotherapy (TABLE 1). Conversely, NUCLEOPHILIC or chemically inert compounds such as aromatic and heterocyclic amines, aminoazo dyes, PAHs, N-nitrosamines, halogenated OLEFINS, and others represent the great majority of human carcinogens. As these chemicals do not react directly with cellular

constituents — they require enzymatic conversion into their ultimate carcinogenic forms — they are termed 'procarcinogens'.

**Metabolism.** Soon after first characterization of pure chemical carcinogens, the detection of hydroxylated derivatives (and their conjugation products) in the urine of animals treated with 2-NA<sup>39</sup>, DBA<sup>40</sup> or DAB<sup>41</sup> indicated that these compounds are metabolically converted *in vivo*. In 1948, James A. Miller and co-workers were the first to demonstrate the oxidative metabolism of a carcinogen, DAB, in a cell-free system containing rat liver MICROSOMES<sup>42</sup>. They also provided the first evidence that certain carcinogens, such as PAHs, are capable of promoting their own metabolism through induction of microsomal proteins<sup>43</sup>. At this time it was known that the enzyme(s) present in microsomes were MIXED-FUNCTION OXIDASES<sup>44</sup>, but it was not until the 1960s that CYTOCHROME P450 was discovered<sup>45</sup> and cytochrome-P450-dependent monooxygenases (CYPs) were shown to be associated with an NADPH-dependent reductase<sup>46</sup>.

The initial step during conversion of organic XENOBIOTICS into hydrophilic and excretable derivatives is mainly catalysed by CYP enzymes. At present, 57 genes encoding these enzymes have been identified in the human genome (see 'Cytochrome P450 Homepage' in the online links box). The forms of CYP that are most important for activation of carcinogens belong to families 1–3, and display broad and overlapping substrate specificities and tissue-specific expression patterns<sup>47,48</sup>. Whereas CYP1A1 is the most important form in human lung, CYP1A2, CYP2A6, CYP2E1 or CYP3A4 are mainly expressed in the liver, and CYP1B1 is expressed in almost all organs except liver and lung<sup>49</sup>. BIOTRANSFORMATION of carcinogens proceeds through intermediates that are capable of undergoing subsequent transferase-catalysed conjugation to polar molecules such as glutathione by glutathione S-transferases (GST), to glucuronic acid by glucuronosyltransferases,

**ELECTROPHILIC**  
Having an affinity for negative charge; molecules that behave as electron acceptors.

**NUCLEOPHILIC**  
Having an affinity for positive charge; molecules that behave as electron donors.

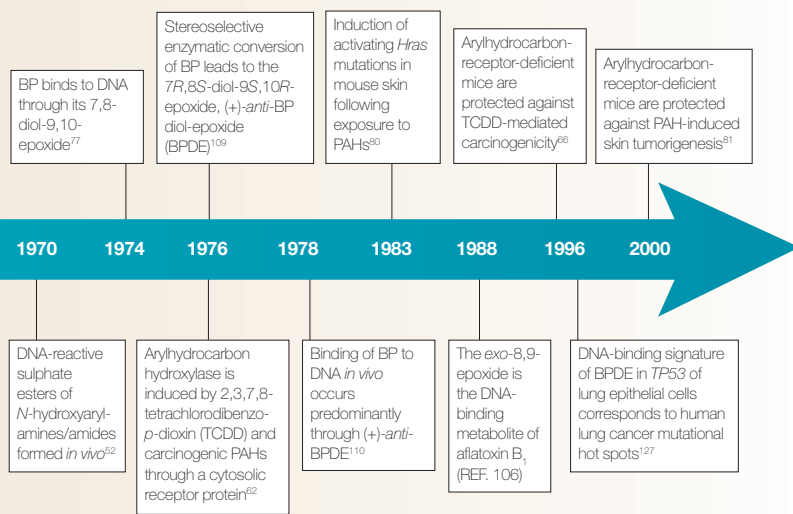
**OLEFINS**  
Hydrocarbons containing a carbon-carbon double bond; also known as alkenes.

**MICROSOMES**  
Vesicles formed from the endoplasmic reticulum when cells are disrupted; used in cell-free *in vitro* studies of biotransformation.

**MIXED-FUNCTION OXIDASES**  
Enzymes that catalyse oxidation-reduction reactions in which one atom of the oxygen molecule is incorporated into the organic substrate; the other oxygen atom is reduced and combined with hydrogen ions to form water. Also known as monooxygenases or hydroxylases.

**CYTOCHROME P450**  
Haem-containing protein involved in electron-transfer reactions.

**XENOBIOTICS**  
Chemical compounds that are foreign to the biological system.



or to small residues such as sulphate by sulphotransferases (SULT) and acetic acid by *N*-acetyltransferases (NAT). Unfortunately, activation into highly electrophilic intermediates also leads to covalent interaction with cellular macromolecules such as proteins or DNA (FIG. 2).

Although enzymes such as SULT, NAT and GST were originally thought to be involved only in detoxification steps of biotransformation, they can also contribute to the activation of certain procarcinogens *in vivo*. SULT and NAT enzymes catalyse sulphonation and acetylation of nucleophilic metabolites, whereas GST enzymes interact with electrophiles through the reactive cysteinyl residue in their cofactor glutathione (GSH). By the early 1960s, the importance of SULT and NAT in the conversion of carcinogens had been recognized. At this time, CYP-mediated *N*-hydroxylation was discovered to be the initial step in AAF-mediated liver carcinogenesis<sup>50</sup>. *N*-hydroxy-AAF was found to have greater carcinogenic activity than its mother compound<sup>51</sup>, even though it was unable to directly interact with DNA. Later, *N*-hydroxy-AAF was shown to undergo SULT-catalysed esterification into the ultimate, genotoxic form *N*-sulphoxy-AAF<sup>52</sup> (FIG. 2). It is now well known that *N*-hydroxy derivatives of procarcinogenic arylamines/amides, aminoazo dyes or heterocyclic amines are converted by NAT or SULT enzymes into highly reactive ester intermediates *in vivo* that bind to DNA through release of arylnitrenium ions<sup>53</sup>.

Although GST-mediated detoxification of reactive metabolites such as epoxides (from PAHs, olefines or aflatoxins) had been widely acknowledged since the early 1960s<sup>54</sup>, recent evidence points to the ability of GST enzymes to directly activate certain industrial chemicals. These include haloalkanes such as 1,2-dichloroethane and haloalkenes such as trichloroethylene (TCE; TABLE 1). These compounds are anticipated to be human carcinogens based on cancer studies in animals and the observation that biotransformation

pathways in animals are similar to those of humans<sup>28</sup>. In addition, limited epidemiological evidence for TCE-induced carcinogenicity in human organs such as the liver and kidney has been reported<sup>55</sup>. Mechanistically, there is considerable evidence for the formation of genotoxic GSH conjugates from VICINAL DIHALOALKANES (GSH episulphonium electrophiles)<sup>56</sup>, and for the kidney-specific cysteine conjugate  $\beta$ -lyase-dependent formation of genotoxic thioketenes, which contribute to the toxicity of haloalkenes in this tissue<sup>57</sup> (FIG. 2).

### Carcinogenic mechanisms

Despite the proof that most chemical carcinogens undergo metabolic conversion into DNA-reactive intermediates, some compounds do not bind to DNA and are not mutagenic, yet they are carcinogenic in animal models and possibly also in humans. An overview of the various modes of action of chemical carcinogens is presented in FIG. 3. What are some of the important non-genotoxic mechanisms?

**TCDD.** The non-genotoxic human carcinogen 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is a by-product of the manufacture of polychlorinated phenols that is inadvertently generated through waste incineration<sup>28</sup>. Both epidemiological evidence and mechanistic studies have indicated a relationship between exposure to this chemical and the occurrence of a range of cancers<sup>58</sup>. For example, in 1976, about 3,000 kg of chemicals, including up to 20 kg of TCDD, were released into the air in Seveso, Italy after a pipeline burst at a herbicide-producing plant. More than 30,000 people were exposed to these chemicals, and decades later cancer rates among those exposed were significantly increased compared with control populations. Numerous animal studies supported this view and confirmed TCDD-mediated carcinogenicity in various organs such as the liver, thyroid, lung, adrenal cortex, skin and lymph nodes<sup>59</sup>. In addition, this compound is a potent tumour promoter in liver and skin.

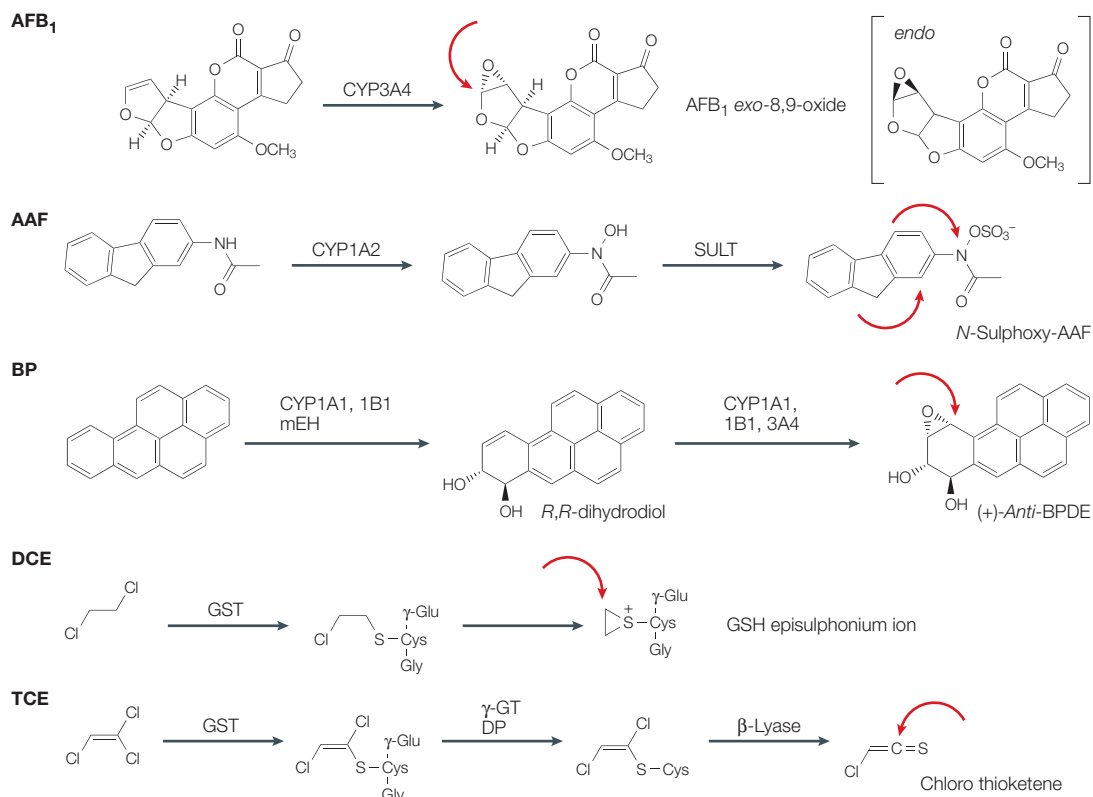
TCDD is the strongest agonist of the arylhydrocarbon receptor (AhR)<sup>60</sup>, a ubiquitous cytosolic protein originally discovered in connection with PAH-mediated induction of microsomal aryl hydrocarbon hydroxylase (AHH) activity<sup>61</sup>. This induction of AHH activity, which occurs when cells are exposed to TCDD, PLANAR PAHs such as BP, and polychlorinated biphenyls, is mediated by the AhR protein<sup>62</sup> (FIG. 4). The AhR belongs to the basic helix-loop-helix/PAS family of transcription factors<sup>63</sup>. Upon binding to one of its ligands, the complex translocates into the nucleus, heterodimerizes with AhR nuclear translocator, and then binds to specific xenobiotic-responsive elements (XREs; 5'-TNGCGTG-3'). Binding to XRE allows this complex to regulate the transcription of genes that encode a diverse set of proteins, including enzymes involved in xenobiotic metabolism, such as CYP1A1, CYP1B1, CYP1A2 and several GST isoforms<sup>64</sup>.

This process has been meticulously characterized, yet induction of xenobiotic metabolizing enzymes,

**BIOTRANSFORMATION**  
Enzymatically catalysed chemical alterations of a compound that occur within living organisms or cells.

**VICINAL DIHALOALKANES**  
Saturated hydrocarbons containing two halogen atoms (for example, chlorine) that are bonded to adjacent carbon atoms.

**PLANAR**  
Molecules that have two-dimensional structures.

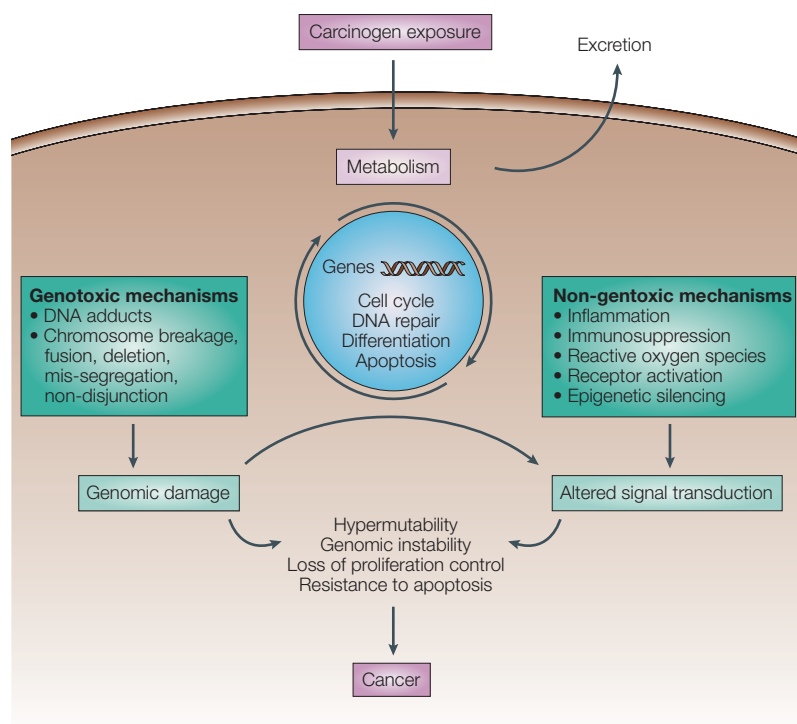


**Figure 2 | Enzymatic conversion of some selected human carcinogens towards their ultimate DNA-reactive metabolites.**

Activation of aflatoxin B<sub>1</sub> (AFB<sub>1</sub>), 2-acetylaminofluorene (AAF), and benzo[*a*]pyrene (BP) requires the activity of cytochrome-P450-dependent monooxygenases (CYPs). CYP3A4 activates AFB<sub>1</sub> at its 8,9-bond, resulting in the AFB<sub>1</sub> *exo*-8,9-oxide. The *endo*-diastereomer is not formed by CYP3A4, but might be formed in small amounts by CYP1A2. AAF is converted by CYP1A2 into *N*-hydroxy-AAF, which subsequently might undergo sulphotransferase (SULT)-catalysed esterification into the ultimate genotoxic form, the *N*-sulphoxy-AAF. BP is initially converted mainly by CYP1A1 or CYP1B1 into the 7,8-epoxide. This epoxide is a substrate of microsomal epoxide hydrolase (mEH), which produces the 7,8-dihydrodiol. Both reactions together stereoselectively form the *R,R*-dihydrodiol. Further epoxidation at the vicinal double bond catalysed by CYP1A1, CYP1B1 and CYP3A4 generates the ultimate genotoxic diol-epoxide of BP (BPDE). Of the four possible resulting diastereomers, the (+)-*anti*-BPDE is formed at the highest levels. 1,2-Dichloroethane (DCE) is activated by glutathione *S*-transferases (GSTs) into glutathione (GSH) half-mustard and GSH episulphonium electrophiles, which can bind directly to DNA. GST-catalysed conjugation of trichloroethylene (TCE) produces GSH adducts. Cleavage of the terminal amino acids by  $\gamma$ -glutamyltransferase ( $\gamma$ -GT) and cysteinylglycine dipeptidase (DP) activity give rise to cysteine (Cys) adducts that can be converted into genotoxic thioketenes by the kidney-specific cysteine conjugate  $\beta$ -lyase. The red arrows point to the position of the nucleophile (DNA, protein, GSH) attack. GSH conjugates of AFB<sub>1</sub> oxide or PAH diol-epoxides are detoxification products.

through TCDD-mediated AhR activity, is insufficient to explain its great variety of *in vivo* toxic effects, such as immunosuppression, teratogenicity and tumorigenicity. On the other hand, homozygous deletion of the *Ah* gene locus renders mice resistant to the entire spectrum of TCDD-mediated toxicities<sup>65</sup>, including the occurrence of early lesions in liver, lung and various other organs<sup>66</sup>. Conversely, a constitutively active form of the AhR can induce tumour formation in the glandular part of the stomach<sup>67</sup> and promote liver carcinogenesis in mice<sup>68</sup>. Recent studies have indicated that the biological effects induced by TCDD through AhR binding are mediated by the activation or inhibition of numerous proteins involved in cell and tissue homeostasis, such as factors that regulate cell growth, proliferation, differentiation and apoptosis<sup>69,70</sup> (FIG. 3). Microarray analysis of gene-expression patterns in human hepatoma HepG2 cells revealed that the levels of at least 112 (REF. 71) or 310 (REF. 70)

mRNAs were significantly up- or downregulated after the exposure to TCDD. For example, CYP1A1 expression was upregulated 12–16-fold. This primary response included changes in expression levels of proteins involved in cellular proliferation (serine/threonine kinases such as COT and NEK2, proto-oncoproteins such as KRAS), and guanine nucleotide exchange factors); cell-cycle regulation and apoptosis (cyclin B2, tumour-necrosis factor receptors, and heat-shock proteins); or extracellular-matrix turnover, signalling and cell adhesion (human enhancer of filamentation 1, metallothioneines and plasminogen activator inhibitors)<sup>70,71</sup>. AhR-ligand complexes also control signalling networks such as those triggered by hormones (oestrogen receptor- $\alpha$ ), hypoxia (hypoxia-inducible factor-1 $\alpha$ ; HIF1 $\alpha$ ), or protein kinases (SRC) — either through molecular crosstalk at DNA-binding sites or direct protein-protein interactions<sup>72,73</sup>.



**Figure 3 | Overview of genotoxic and non-genotoxic effects of carcinogens.** When chemical carcinogens are internalized by cells, they are often metabolized, and the resulting metabolic products are either excreted or retained by the cell. Inside the cell, carcinogens or their metabolic products can either directly or indirectly affect the regulation and expression of genes involved in cell-cycle control, DNA repair, cell differentiation or apoptosis. Some carcinogens act by genotoxic mechanisms, such as forming DNA adducts or inducing chromosome breakage, fusion, deletion, mis-segregation and non-disjunction. For example, carcinogenic ions or compounds of nickel, arsenic and cadmium can induce structural and numerical chromosome aberrations<sup>91,93,95</sup>. Others act by non-genotoxic mechanisms such as induction of inflammation, immunosuppression, formation of reactive oxygen species, activation of receptors such as arylhydrocarbon receptor (AhR) or oestrogen receptor (ER), and epigenetic silencing. Together, these genotoxic and non-genotoxic mechanisms can alter signal-transduction pathways that finally result in hypermutability, genomic instability, loss of proliferation control, and resistance to apoptosis — some of the characteristic features of cancer cells.

**PAHs.** In contrast to TCDD, carcinogenic PAHs require metabolic activation to elicit their detrimental effects. Although first isolated from coal tar, incomplete combustion of virtually any organic material can lead to the formation of PAHs. These compounds are therefore pervasive in the human environment<sup>74</sup>. The National Toxicology Program listed 15 individual PAHs as “reasonably anticipated to be human carcinogens” based on evidence from animal experiments<sup>28</sup>. PAH-containing mixtures such as coke-oven emissions, coal tar, pitch, soot or cigarette smoke are categorized as known human carcinogens based on epidemiological evidence for lung and skin cancer in workers exposed to these mixtures and lung cancer in smokers.

Working on the urinary metabolites of anthracene, Eric Boyland and Alfred A. Levi (FIG. 1) proposed in 1935 that toxic PAHs might either be intravitaly converted into more active pathogenic substances or detoxified<sup>75</sup>. About 20–25 years later, epoxides were shown to be involved in this process<sup>76</sup>. Based on the observation that the enzymatically activated 7,8-dihydrodiol of BP binds to DNA with a higher affinity than its parent compound

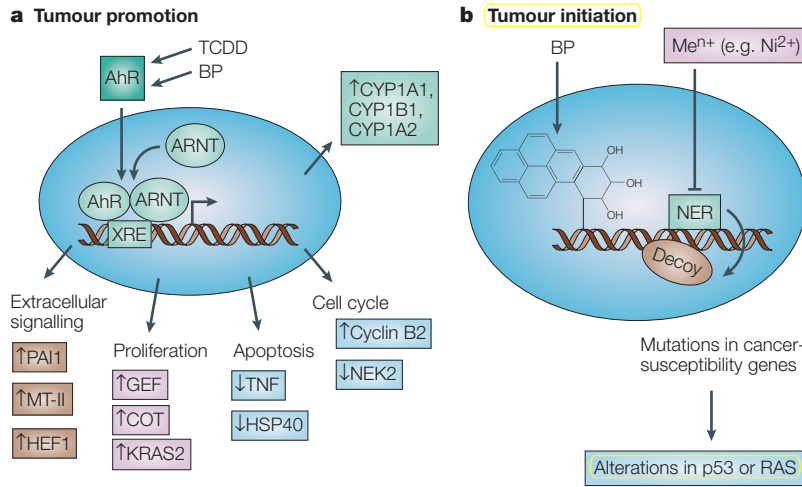
(BP), Sims *et al.* proposed that a secondary metabolite, the 7,8-dihydrodiol 9,10-epoxide (vicinal ‘diol-epoxide’), is actually the chemical species that covalently interacts with DNA<sup>77</sup> (FIG. 2). Subsequent work confirmed the central role of diol-epoxide metabolites in mediating the DNA-binding activity of BP and other carcinogenic PAHs. As they were also confirmed to be highly mutagenic and carcinogenic, diol-epoxides are regarded to be the ultimate metabolites in the initiation of PAH tumorigenesis<sup>78</sup>. It is well established that PAHs would not be carcinogenic if they were not metabolized by subsequent steps of EPOXIDATION and hydrolysis. Disruption of genes that encode enzymes and factors that are involved in this activation route, such as CYP1B1, microsomal epoxide hydrolase (mEH) or AhR, renders mice resistant to the biological effects of BP and others<sup>78,79</sup>.

Many potent PAHs act as ‘complete carcinogens’ in mice when repeatedly applied over a prolonged time period<sup>74</sup>. Such compounds can induce both somatic mutations in crucial genes through DNA binding (tumour ‘initiation’ phase) and subsequent outgrowth of irreversibly transformed cells (tumour ‘promotion’ phase). In the early 1980s, carcinogenic PAHs were shown to induce activating *Hras* mutations in the skin of mice<sup>80</sup>. Although oncogenic RAS constantly promotes cellular proliferation through multiple signalling pathways, repeated application of carcinogenic PAHs is required for maximal tumour induction. As the AhR protein is indispensable for PAH-mediated tumorigenesis<sup>81</sup>, it might be reasonable to assume that not only the initiating activity (through induction of metabolizing enzymes), but also the promotional activity of carcinogenic PAHs in skin depends on AhR-mediated gene expression (FIG. 4). This, of course, is not experimentally confirmable in the case of genotoxic PAHs, yet it would be in agreement with the tumour-promoting activity of TCDD in this organ. On the other hand, autoxidation of metabolically generated PAH phenols or PAH-induced tissue inflammation and concomitant recruitment of polymorphonuclear leukocytes are major sources of reactive oxygen species such as hydroxy or superoxide anion radicals<sup>78</sup>. These short-lived species induce a plethora of additional DNA modifications and modulate the expression of several genes involved in signal-transduction pathways that regulate proliferation at the tumour-promotion stage of carcinogenesis<sup>82</sup>.

**Arylamines/amides.** After the initial work with 2-NA, it was shown that other aromatic amines, such as 4-aminobiphenyl (ABP) or the arylamide AAF consistently induced formation of bladder tumours in dogs, but only occasionally in rodents<sup>83</sup>. Whereas the primary amines are known human bladder carcinogens, according to the evidence from cancer cases, AAF is only a suspected human carcinogen<sup>28</sup> (TABLE 1). In rodent tumour models, all of these compounds primarily induce tumours in the liver, lung or mammary gland, because of a high rate of *N*-hydroxylation in rodent liver cells. By contrast, humans and dogs primarily produce *N*-glucuronides in the liver, which are then

#### EPOXIDATION

A chemical reaction in which an oxygen is joined to an olefinic molecule to form a cyclic, three-membered ether. The products are known as oxiranes, epoxides or simply oxides.



**Figure 4 | Tumour promotion and tumour initiation.** Genotoxic carcinogens can induce damage in tumour suppressors or oncogenes in different ways, all of which contribute to the transformation of normal cells into tumour cells — this is known as the ‘tumour initiation’ stage in carcinogenesis. Some chemical carcinogens are also capable of promoting the outgrowth of those transformed cell clones and of contributing to the generation of visible tumour cell masses — this is known as the ‘tumour promotion’ stage in carcinogenesis. **a** | Chemical compounds such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) or benzo[*a*]pyrene (BP) result in tumour promotion through arylhydrocarbon receptor (AhR)-mediated signal transduction. Binding of TCDD or BP to AhR leads to activation and translocation of the complex into the nucleus. After heterodimerization with the AhR nuclear translocator (ARNT), the complex binds to xenobiotic-responsive elements (XREs) and induces the expression of a variety of different genes involved in carcinogen metabolism, including CYP forms 1A1, 1B1 and 1A2. It also changes the expression pattern of several factors involved in cellular growth and differentiation, such as plasminogen-activator inhibitor type 1 (PAI1), metallothionein II (MT-II), human enhancer of filamentation 1 (HEF1), guanine nucleotide exchange factor (GEF), COT and KRAS. Pro-apoptosis factors such as tumour-necrosis factor (TNF; superfamilies 3,6,8,9,10) and heat-shock protein 40 (HSP40) are downregulated, and cell-cycle genes can either be upregulated (such as cyclin B2) or downregulated (such as NEK2). The factors shown here are only a few examples of the great number of factors that have been shown by gene-expression analysis to be altered following carcinogen exposure. COT and NEK2 are serine/threonine kinases<sup>70,71</sup>. **b** | Tumour initiation occurs through DNA-adduct-derived mutations in cancer-susceptibility genes. DNA binding by genotoxic carcinogens such as activated BP leads to the induction of base pair or frameshift mutations in cancer-susceptibility genes such as *TP53* or *RAS*. The mutagenic potency of such polycyclic aromatic hydrocarbon diol-epoxide–DNA adducts can be increased due to inhibition of nucleotide excision repair (NER) by metal ions (Me<sup>n+</sup>; for example, Ni<sup>2+</sup>), or a result of NER factor immobilization at repair-resistant DNA-adduct sites, also known as decoy adducts.

**GENOTOXIC CARCINOGENS**  
Chemical carcinogens that are capable of causing damage to DNA. These can be mutagenic, clastogenic or aneugenic.

transported to the kidney. In the acidic environment of the urine, the amines are again released and subsequently converted into genotoxic derivatives in bladder epithelial cells<sup>83</sup>. *N*-hydroxylation and subsequent sulphate or acetoxy ester formation have been studied in detail and can sufficiently explain the genotoxicity of these compounds<sup>83,84</sup> (FIG. 2).

On the other hand, additional tumour-promoting activities were reported for AAF, indicating that it acts as a ‘complete carcinogen’ in rodent liver<sup>85</sup>. Chronic exposure of rats to AAF triggers adaptive responses in mitochondria permeability transition pores and BCL2 expression levels of hepatocytes that increase resistance to apoptosis<sup>86</sup>. There is evidence that this effect, which was fully reversible, is an early tissue response to the presence of reactive oxygen species that are generated through oxidation–reduction cycling of AAF metabolites such as 2-nitrosofluorene. As mitochondrial resistance is

established in the tissue before the clonal outgrowth of pre-neoplastic cells, this non-genotoxic effect contributes to the selection of resistant cells and hence to the tumour-promoting activity of AAF in liver.

**Epigenetics.** Based on their potency in inducing a p53-mediated DNA-damage response, GENOTOXIC CARCINOGENS can be distinguished from non-genotoxic carcinogens through expression profiling<sup>87</sup>. However, from the foregoing section it can be concluded that long-term exposure to low doses of genotoxic carcinogens also contributes to non-genotoxic alterations that promote the outgrowth of transformed cell clones *in vivo*. At early stages of tumorigenesis these non-genotoxic effects are reversible and require continuous presence of the compound. On the other hand, changes in gene-expression patterns caused by epigenetic alterations such as DNA methylation or histone acetylation have been observed in cells exposed to the metals nickel (Ni) and cadmium (Cd), or to the metalloid arsenic (As)<sup>88</sup>. Ionic Ni, Cd and As, along with their compounds, are established human carcinogens<sup>28</sup> (TABLE 1). All three of these inorganic carcinogens induce chromosome and DNA damage through a variety of mechanisms such as interference with DNA-repair processes and cell-cycle control<sup>89,91</sup>, or generation of reactive oxygen species<sup>92</sup>. In addition, metal-induced stimulation of cellular proliferation through signalling pathways and transcription factors such as extracellular-regulated kinases, NF-κB, HIF1α and MYC has been described<sup>93–95</sup>. There is now further evidence that Ni carcinogenicity also involves DNA hypermethylation<sup>96</sup> and histone deacetylation<sup>97</sup>, both of which contribute to heterochromatin condensation and the epigenetic silencing of tumour-suppressor genes such as *CDKN2A/INK4A* (REF. 98) or fragile histidine triad gene<sup>99</sup>. By contrast, the requirement of methyl groups in the metabolism of arsenic decreases intracellular methyl donor pools and causes aberrant over-expression of oncoproteins such as MYC, cyclin D1 and proliferating cell nuclear antigen<sup>100</sup> through global genome hypomethylation<sup>101</sup>.

**Molecular specificity**

The great majority of genotoxic carcinogens induce DNA damage and mutations in genes involved in the regulation of cellular growth, proliferation and death (FIG. 3). The principal DNA-damaging chemical species generated during biotransformation of several groups of carcinogens are shown in FIG. 2.

**DNA affinity.** Working on a small number of carcinogenic PAHs, Brookes and Lawley proposed in 1964 that “the DNA binding of these compounds shows a significant positive correlation with carcinogenic potency”<sup>34</sup>. So, the higher the level of DNA binding, the greater the potency of a chemical carcinogen? Today, numerous test systems enable a simple and fast characterization of the genotoxicity of carcinogens and the identification of their ultimate DNA-reactive metabolites. In mammalian cells, vicinal diol-epoxides from PAHs have been proven to exert extraordinarily strong mutagenic effects compared

ADDUCTS  
Covalent reaction products  
between chemicals and proteins  
or DNA.

with other metabolites from the same compounds<sup>102</sup>. Although PAH diol-epoxides vary considerably in their bioactivity, the level of mutations induced is quantitatively related to the level of diol-epoxide-DNA ADDUCTS at earlier time points. Furthermore, the tumorigenicity of different PAHs was found to correlate with DNA adduct levels induced in lung tissue<sup>103</sup>. Both the relationship between the DNA-binding level and mutagenicity, as well as the correlation between the overall DNA binding and carcinogenicity observed in mice, indicate that

DNA adduct formation is an important bioindicator of the cancer risk resulting from exposures to carcinogenic PAHs, and possibly to other genotoxic carcinogens<sup>104</sup>.

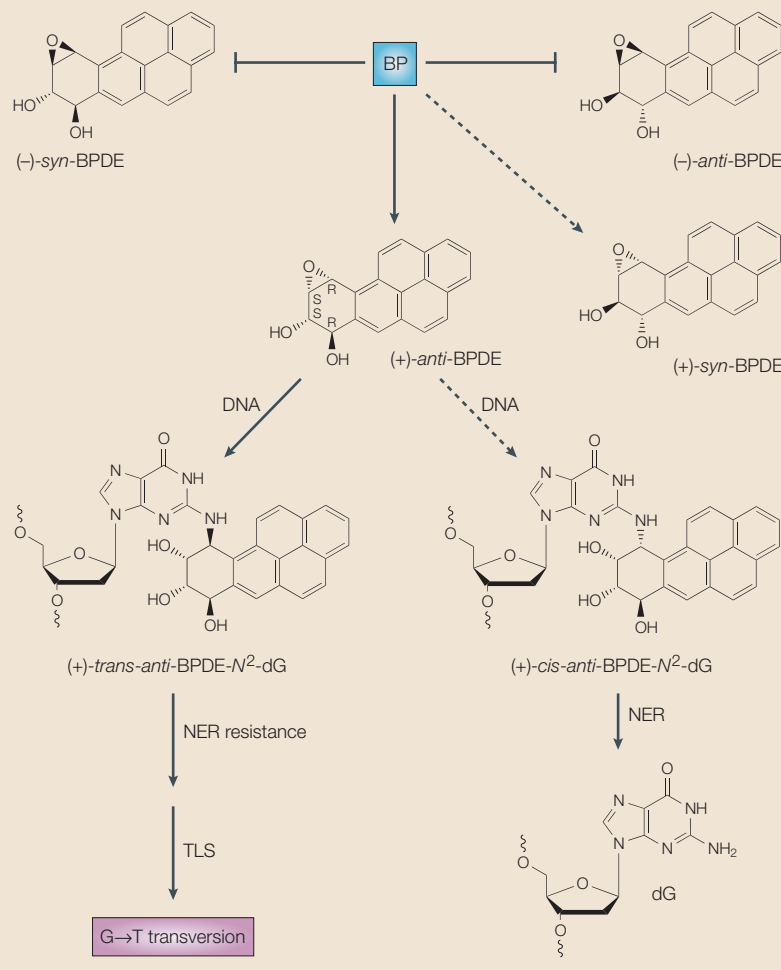
**Stereochemistry.** Enzymes involved in biotransformation of carcinogens operate with high regio- and stereoselectivity, and the metabolites formed can differ tremendously in their biological activity. An example of this is the CYP-mediated conversion of aflatoxin B<sub>1</sub> (AFB<sub>1</sub>), a potent natural carcinogen that has been linked to human liver cancer<sup>105</sup> (TABLE 1). Epoxidation of the olefinic bond at position 8,9 generates the ultimate carcinogenic intermediate<sup>106</sup>. Although the epoxide ring can be introduced from both sides of the furanocoumarine ring system, CYP3A4, the most important enzyme involved in AFB<sub>1</sub> activation in human liver, exclusively forms the *exo* isomer<sup>107</sup> (FIG. 2). As the spatial configuration of the epoxide moiety in the DNA–AFB<sub>1</sub> *exo*-8,9-oxide intercalation complex promotes the attack of the base, this isomer is about 1,000 times more genotoxic than its *endo* DIASTEREOMER<sup>108</sup>.

Similarly, metabolic activation of PAHs is highly selective. For BP, initial conversion at position 7,8 produces the *R,R*-dihydrodiol in high ENANTIOMERIC excess (FIG. 2). Subsequent epoxidation at position 9,10 then predominantly generates the diol-epoxide with '*R,S,S,R*'-configuration — the (+)-*anti*-BP-7,8-diol-9,10-epoxide [(+)-*anti*-BPDE]<sup>109</sup>. This isomer is the most potent mutagenic and carcinogenic species among all four diol-epoxide diastereomers that can be produced at this molecule region (BOX 1). Accordingly, BP-induced DNA damage predominantly results from covalent interaction of the (*R,S,S,R*) diol-epoxide with 2'-deoxyguanosine (dG) residues through *trans* opening of the epoxide moiety<sup>110,111</sup>. If not repaired, the resulting (+)-*trans*-*anti*-BPDE-*N*<sup>2</sup>-dG adduct can induce mutations, such as G→T transversions, during DNA replication.

'Bulky' DNA lesions such as those resulting from PAH diol-epoxides are subject to the nucleotide excision repair (NER) pathway<sup>112</sup>. It has been shown that (+)-*anti*-BPDE induces a different degree of NER activity depending on the way that the epoxide ring opens during adduct formation (*cis* versus *trans*)<sup>113</sup>. Whereas the (+)-*cis*-*anti*-BPDE-*N*<sup>2</sup>-dG adopts an intercalative conformation with concomitant displacement of the modified base, the (+)-*trans*-*anti*-BPDE-*N*<sup>2</sup>-dG displays an external conformation with the benzo[*a*]pyrenyl moiety accommodated in the minor DNA groove<sup>114</sup>. The local DNA distortion induced by the *cis* product is therefore much more severe and results in a 10 times faster removal through NER as compared with the *trans* product (BOX 1). In addition, poor enzymatic repair is preceded by an insufficiently activated DNA-damage checkpoint<sup>115</sup>. Analyses of the cell cycle and expression profiles revealed that the p53/p21-mediated G1 checkpoint was rarely activated, even at DNA-damage levels of about 180,000 *anti*-BPDE-DNA adducts/cell<sup>116</sup>. Both the lack of activation of cell-cycle arrest and insufficient adduct repair increase the likelihood of transforming mutations, because DNA replication continues from the damaged template<sup>117</sup> (BOX 1).

### Box 1 | Stereoselective activation of benzo[*a*]pyrene

Benzo[*a*]pyrene (BP) theoretically can be metabolized into four different 7,8-diol-9,10-epoxide stereoisomers (see figure). The (+)-*anti*-7*R*,8*S*-diol-9*S*,10*R*-epoxide of BP [(+)-*anti*-BPDE] is formed in great excess, both *in vitro* and *in vivo*, compared with the other three stereoisomers, the (+)-*syn*-, (-)-*syn*- and (-)-*anti*-BPDE. (+)-*Anti*-BPDE derives from further epoxidation of the *R,R*-dihydrodiol precursor, as shown in FIG. 2. Some small amounts of the (+)-*syn*-BPDE isomer are usually detectable as a result of further epoxidation of the *S,S*-dihydrodiol precursor (not shown). From all four possible stereoisomers, the (+)-*anti*-BPDE has the highest intrinsic genotoxic and carcinogenic potency. The major DNA adduct, the (+)-*trans*-*anti*-BPDE-*N*<sup>2</sup>-dG, derives from *trans* opening of the epoxide moiety. This adduct is more resistant to nucleotide excision repair than the *cis*-opened product, (+)-*cis*-*anti*-BPDE-*N*<sup>2</sup>-dG. Resistance to DNA repair and subsequent DNA replication across this 'bulky' DNA adduct — translesional synthesis (TLS) — can lead to the induction of G→T base pair transversions in cancer genes such as *TP53* or *KRAS*.



**Mutation profiles.** Mutations in RAS that impair its GTPase activity are commonly detected in human cancers and in animal models of chemical carcinogenesis<sup>118,119</sup>. In human bronchial epithelial cells it was shown that DNA-damaging metabolites of various carcinogens such as BP (*anti*-BPDE), AAF (*N*-acetoxy-AAF), and AFB<sub>1</sub> (AFB<sub>1</sub> 8,9-oxide) preferentially bind to dG residues within codon 12 of KRAS<sup>120</sup>. This site is most frequently mutated in human cancers including lung adenocarcinomas<sup>121</sup>. As compared to other sites, the ‘hot spot’ characteristic of codon 12 in lung cells is the combined result of the preferential binding of carcinogenic metabolites to this site and the inefficient repair of the resulting DNA adducts<sup>120</sup>. Although methylation at CpG sites in the vicinity of codon 12 of KRAS had no influence on the preferential binding of carcinogens at this position<sup>122</sup>, the presence of 5-methylcytosines greatly increased the binding of the ultimate genotoxic descendants of BP, ABP, AAF and AFB<sub>1</sub> at dG residues within the DNA-binding domain of the human TP53 gene<sup>123–125</sup>.

Analysis of the binding profiles of *N*-hydroxy-ABP and *anti*-BPDE at the TP53 locus in human bladder<sup>126</sup> and bronchial epithelial cells<sup>127</sup>, respectively, provided strong evidence for a role of these compounds in cancer initiation. In both cases, the codons (157, 248, 273) and the positions within the codons affected by *N*-hydroxy-ABP or *anti*-BPDE matched the TP53 mutations reported to be associated with human bladder or lung cancer. In congruence to the strand bias of G→T transversions associated with lung cancer of smokers, *anti*-BPDE-*N*<sup>2</sup>-dG adducts were almost exclusively formed at methylated CpG dinucleotides of the non-transcribed DNA strand, possibly as a result of the slow repair of this strand compared with its transcribed counterpart<sup>128</sup>.

### Assessing human cancer risk

The correlation between DNA-damage level and carcinogenic potency indicates that differences in the activity of enzymes that produce or detoxify DNA-reactive intermediates, or that repair the resulting DNA lesions, can influence individual cancer risk. However, the knowledge obtained from studies with single compounds in cancer models is limited — none

of the known xenobiotic metabolizing enzymes, including polymorphic variants, have strictly protumorigenic or anti-tumorigenic effects. Some enzymes that contribute to inactivation (detoxication) of one compound contribute to the toxicity of others. For example, GST enzymes have a detoxifying effect on PAH diol-epoxides, but they toxify haloalkanes/alkenes. One particular enzyme can also be involved in toxication and detoxication routes of the very same carcinogen, as mEH is involved in both the toxication and detoxication of PAHs.

Furthermore, humans are exposed to complex mixtures of compounds, rather than to single carcinogens. For instance, cigarette smoke contains about 60 known carcinogens from a variety of chemical classes including BP, ABP, 2-NA<sup>129</sup>, and traces of metal ions such as Ni, As and Cd<sup>130</sup>. As most laboratory studies have been conducted with single compounds, results cannot be directly extrapolated to the effects of mixtures. Multiple interactions between individual compounds can have additive, synergistic or antagonizing effects. At the level of DNA repair, for example, co-exposure of cells to Ni ions enhances BP-mediated mutagenesis through inhibition of NER of *anti*-BPDE-*N*<sup>2</sup>-dG adducts<sup>131</sup>. Also, the presence of structurally different lesions in the same DNA can inhibit repair, through sequestration of NER subunits by those modifications that are more repair resistant<sup>132</sup> (FIG. 4). Finally, the doses of carcinogens shown to induce cancer in animal models are very high, compared with typical levels of human exposure. Because of the unpredictability of the biological responses to carcinogens at incrementally low doses and because of species-specific effects, the way in which toxicologists extrapolate dose-response relationships from animals to humans is highly controversial<sup>133,134</sup>.

### Future directions

Based on the hypothetical assumption that single DNA lesions can induce tumour-promoting gene mutations, no safe threshold values can currently be defined for genotoxic carcinogens. However, zero-level exposures are unrealistic for most carcinogens. Background levels

#### Box 2 | Identification of a compound's mode of action and individuals at risk

The availability of genome-scale DNA sequence information, along with the development of cDNA and oligonucleotide microarray technology, has allowed researchers to study the effects of biohazards on cell- and tissue-specific gene-expression profiles in experimental animals and humans. Using microarray analysis, expression patterns of up to 10,000 genes can be determined from cells exposed to single compounds or mixtures. These studies have provided valuable information about the mechanisms of chemical agents in various biological systems. Using this ‘toxicogenomics’ approach, it is possible to obtain the gene-expression signatures for prototypic classes of toxicants that act through particular mechanisms and pathways, such as responses mediated through the arylhydrocarbon receptor. Signatures can also be compared, to determine mechanisms of unknown test compounds or mixtures. In cancer toxicology this technique might be used to quickly and efficiently assess exposures and to identify possible adverse effects of as yet unknown xenobiotics. This approach can also be used to identify precursors/pathways without the need for deciphering every detail of activation and detoxification routes. Moreover, it also allows for the identification of compound-mediated complex networks of additional gene–gene interactions that might be related in the broad-ranging interactive and tissue-specific biological outcomes observed. Most intriguing, however, are the possible applications in identifying individuals who are at risk of developing cancer in response to exposure to certain carcinogens. This study of interindividual variability in response to chemical exposures, based on genetic polymorphisms, is termed ‘toxicogenetics’<sup>138,139</sup>.

#### DIASTEREOMERS

Stereoisomers that are not related as mirror images to each other.

#### ENANTIOMER

One of the two non-superimposable mirror image forms of an optically active molecule.

of carcinogen–DNA adducts in human tissue samples from normal individuals were found to be in the range of 1 per  $10^7$  to 0.2 per  $10^8$  nucleotides<sup>135</sup>. Improving our understanding of the mechanisms of carcinogenic genotoxicity and the repair of such lesions (FIG. 3) will help us to determine the minimal levels of exposure that are required for adverse effects to occur, and to set reliable and acceptable concentration thresholds in the human environment<sup>136,137</sup>. At present, however, this goal only refers to single compounds. By far the greatest challenge will be to characterize the synergistic effects of complex mixtures. This is an important problem, as any risk assessment based on toxicological monitoring of only a few well-studied compounds is highly prone to misjudgement.

Similarly, any risk assessment that does not consider inter-individual variability in susceptibility to DNA damage following carcinogen exposure could also

underestimate the risk to those individuals who are most vulnerable. Therefore, it is important to identify individuals who are ‘at risk’ of carcinogenic effects, by means of toxicogenomics, toxicogenetics and molecular epidemiology (BOX 2)<sup>138,139</sup>. In this regard, we need to learn much more about the role and interplay of susceptibility and resistance genes targeted by human carcinogens or involved in modulating human responses to carcinogenic compounds. Beyond the range of known polymorphic enzymes that contribute to activation and excretion of carcinogens<sup>140,141</sup>, as well as to DNA repair, it seems reasonable to assume that a great variety of additional low-penetrance genetic variants determine sporadic cancer risk, through interaction with environmental factors that include chemical carcinogens<sup>142</sup>. These gene–environment interactions clearly deserve further attention as they could be sensitive and specific determinants of cancer incidence.

1. Doll, R. & Peto, R. The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. *J. Natl Cancer Inst.* **66**, 1191–1308 (1981).



**A landmark paper that compiled epidemiological evidence for a predominant role of environmental factors in human cancer.**

2. Kolonel, L. N., Altshuler, D. & Henderson, B. E. The multiethnic cohort study: exploring genes, lifestyle and cancer risk. *Nature Rev. Cancer* **4**, 519–527 (2004).
3. Peto, J. Cancer epidemiology in the last century and the next decade. *Nature* **411**, 390–395 (2001).
4. Lichtenstein, P. et al. Environmental and heritable factors in the causation of cancer. *N. Engl. J. Med.* **343**, 78–85 (2000).  
**Combined data on 44,788 pairs of twins indicate that the environment has the principal role in causing sporadic human cancer.**
5. Czene, K., Lichtenstein, P. & Hemminki, K. Environmental and heritable causes of cancer among 9.6 million individuals in the Swedish family-cancer database. *Int. J. Cancer* **99**, 260–266 (2002).
6. Theophrasti Paracelsi von Hohenheim. *Von der Bergsucht oder Bergkrankheiten drey Bücher* (Sebaldum Mayer, Dillingen, Germany, 1567).
7. Rostoski, Saupe & Schmorl. Die Bergkrankheit der Erzbergleute in Schneeberg in Sachsen (‘Schneeberger Lungenkrebs’). *Z. Krebsforsch.* **23**, 360–384 (1926).
8. Pirchan, A. & Siki, H. Cancer of the lung in the miners of Jáchymov (Joachimstal). Report of cases observed in 1929–1930. *Am. J. Cancer* **16**, 681–722 (1932).
9. Ramazzini, B. *De Morbis Artificum Diatriba* (Typis Antonii Capponi, Impressoris Episcopalis Supriorum Consensu, 1700).
10. Hill, J. *Cautions Against the Immoderate Use of Snuff. Founded on the Known Qualities of the Tobacco Plant; And the Effects it Must Produce when this Way Taken into the Body: And Enforced by Instances of Persons who have Perished Miserably of Diseases, Occasioned, or Rendered Incurable by its Use* (R. Baldwin and J. Jackso, London, 1761).
11. Pott, P. *The Chirurgical Works. Chirurgical Observations Relative to the Cataract, The Polypus of the Nose, The Cancer of the Scrotum, The Different Kinds of Ruptures, and The Mortification of the Toes and Feet* Ch. III 60–68 (Hawes, W. Clarke, and R. Collins, London, 1775).  
**Original work of Percivall Pott, who was a surgeon at St. Bartholomew’s Hospital London, that contains his seminal report on scrotal cancer in chimney sweeps. This observation raised the first possibility of cancer prevention.**
12. Paris, J. A. *Pharmacologica; or the History of Medicinal Substances, with a View to Establish the Art of Prescribing and of Composing Extemporaneous Formulae upon Fixed and Scientific Principles* 206–217 (F. & R. Lockwood, New York, 1822).
13. Volkmann, R. Ueber Theer- und Russkrebs. *Berl. Klin. Wochenschr.* **11**, 218 (1874).
14. Bell, J. Paraffin epithelioma of the scrotum. *Edinb. Med. J.* **22**, 135–137 (1876).

15. Hutchinson, J. On some examples of arsenic-keratosis of the skin and of arsenic-cancer. *Trans. Path. Soc. London* **39**, 352–393 (1888).
16. Rehn, L. Blasengeschwülste bei Fuchsin-Arbeitern. *Arch. Klin. Chir.* **50**, 588–600 (1895).  
**Report of three cases of urinary bladder tumours in the production of ‘fuchsin’ (magenta), a complex red dyestuff made from aniline and other aromatic amines.**
17. Henry, S. A. Occupational cutaneous cancer attributable to certain chemicals in industry. *Br. Med. Bull.* **4**, 389–401 (1947).  
**Compilation of about 4,000 cases of cutaneous cancer observed in certain factories of Great Britain. Provides interesting insights on the conditions and exposures at work during the first half of the twentieth century.**
18. Yamagiwa, K. & Ichikawa, K. Experimentelle Studie über die Pathogenese der Epithelialgeschwülste. *Mitt. Med. Fak. Kaiserl. Univ. Tokio* **15**, 295–344 (1915).
19. Kennaway, E. & Hieger, I. Carcinogenic substances and their fluorescence spectra. *Br. Med. J.* **1**, 1044–1046 (1930).
20. Cook, J. W. et al. Chemical compounds as carcinogenic agents. *Am. J. Cancer* **29**, 219–259 (1937).
21. Cook, J. W., Hewett, C. L. & Hieger, I. The isolation of a cancer-producing hydrocarbon from coal tar. *J. Chem. Soc.* 395–405 (1933).
22. Berenblum, I. & Bonser, G. M. Experimental investigation of ‘aniline cancer’. *J. Ind. Hyg. Toxicol.* **19**, 86–92 (1937).
23. Hueper, W. C. et al. Experimental production of bladder tumors in dogs by administration of beta-naphthylamine. *J. Ind. Hyg. Toxicol.* **20**, 46–84 (1938).
24. Leichtenstern, O. Ueber Harnblasenentzündung und Harnblasengeschwülste bei Arbeitern in Farbfabriken. *Dtsch. Med. Wochenschr.* **24**, 709–713 (1898).
25. Yoshida, T. Über die serienweise Verfolgung der Veränderungen der Leber der experimentellen Hepatomerzeugung durch o-Aminoazotoluol. *Trans. Jap. Path. Soc.* **23**, 636–638 (1933).
26. Kinosita, R. Researches on the carcinogenesis of the various chemical substances. (In Japanese) *Gann* **30**, 423–426 (1936).
27. Wilson, R. H., DeEds, F. & Cox, A. J. Jr. The toxicity and carcinogenic activity of 2-acetaminofluorene. *Cancer Res.* **1**, 595–608 (1941).
28. U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program. *10<sup>th</sup> Report on Carcinogens* [online], <<http://ehp.niehs.nih.gov/roc/toc10.html>> (Research Triangle Park, North Carolina, USA, 2004).
29. Miller, E. C. & Miller, J. A. The presence and significance of bound amino azodyes in the livers of rats fed p-dimethylaminoazobenzene. *Cancer Res.* **7**, 468–480 (1947).  
**First demonstration of the covalent binding of a chemical carcinogen to cellular macromolecules such as proteins.**
30. Miller, E. C. Studies on the formation of protein-bound derivatives of 3,4-benzpyrene in the epidermal fraction of mouse skin. *Cancer Res.* **11**, 100–108 (1951).

31. Miller, E. C. & Miller, J. A. *In vivo* combinations between carcinogens and tissue constituents and their possible role in carcinogenesis. *Cancer Res.* **12**, 547–556 (1952).
32. Wheeler, G. P. & Skipper, H. E. Studies with mustards. III. *In vivo* fixation of C<sup>14</sup> from nitrogen mustard-C<sup>14</sup>H<sub>5</sub> in nucleic acid fractions of animal tissues. *Arch. Biochem. Biophys.* **72**, 465–475 (1957).  
**Early paper describing the binding of a carcinogen to DNA in vivo.**
33. Magee, P. N. & Farber, E. Toxic liver injury and carcinogenesis. Methylation of rat-liver nucleic acids by dimethylnitrosamine *in vivo*. *Biochem. J.* **83**, 114–124 (1962).
34. Brookes, P. & Lawley, P. D. Evidence for the binding of polynuclear aromatic hydrocarbons to the nucleic acid of mouse skin: relation between carcinogenic power of hydrocarbons and their binding to deoxyribonucleic acid. *Nature* **202**, 781–784 (1964).  
**Seminal paper on the correlation between DNA-binding level and carcinogenicity of six selected polycyclic aromatic hydrocarbons.**
35. Sporn, M. B. & Dingman, C. W. 2-Acetamidofluorene and 3-methylcholanthrene: differences in binding to rat liver deoxyribonucleic acid *in vivo*. *Nature* **210**, 531–532 (1966).
36. Dingman, C. W. & Sporn, M. B. The binding of metabolites of aminoazo dyes to rat liver DNA *in vivo*. *Cancer Res.* **27**, 938–944 (1967).
37. Auerbach, C. & Robson, J. M. Chemical production of mutations. *Nature* **157**, 302 (1946).  
**The carcinogen ‘mustard gas’ induced mutations in *Drosophila*.**
38. Ames, B. N., Durston, W. E., Yamasaki, E. & Lee, F. D. Carcinogens are mutagens: a simple test system combining liver homogenates for activation and bacteria for detection. *Proc. Natl Acad. Sci. USA* **70**, 2281–2285 (1973).  
**Bruce Ames established the ‘Ames Assay’ for testing chemical-induced genotoxicity.**
39. Wiley, F. H. The metabolism of β-naphthylamine. *J. Biol. Chem.* **124**, 627–630 (1938).
40. Boyland, E., Levi, A. A., Mawson, E. H. & Roe, E. Metabolism of polycyclic compounds. 4. Production of a dihydroxy-1:2:5:6-dibenzanthracene from 1:2:5:6-dibenzanthracene. *Biochem. J.* **35**, 184–191 (1941).
41. Stevenson, E. S., Dobriner, K. & Rhoads, C. P. The metabolism of dimethylaminoazobenzene (Butter Yellow) in rats. *Cancer Res.* **2**, 160–167 (1942).
42. Mueller, G. C. & Miller, J. A. The metabolism of 4-dimethylaminoazobenzene by rat liver homogenates. *J. Biol. Chem.* **176**, 535–544 (1948).  
**First demonstration of microsome-catalysed biotransformation of a chemical carcinogen in vitro.**
43. Conney, A. H., Miller, E. C. & Miller, J. A. The metabolism of methylated aminoazo dyes. V. Evidence for induction of enzyme synthesis in the rat by 3-methylcholanthrene. *Cancer Res.* **16**, 450–459 (1956).
44. Brodie, B. B. et al. Detoxication of drugs and other foreign compounds by liver microsomes. *Science* **121**, 603–604 (1955).
45. Omura, T. & Sato, R. A new cytochrome in liver microsomes. *J. Biol. Chem.* **237**, 1375–1376 (1962).

46. Lu, A. Y. H. & Coon, M. J. Role of hemoprotein P-450 in fatty acid  $\omega$ -hydroxylation in a soluble enzyme system from liver microsomes. *J. Biol. Chem.* **243**, 1331–1332 (1968).
47. Guengerich, F. P. & Shimada, T. Oxidation of toxic and carcinogenic chemicals by human cytochrome P-450 enzymes. *Chem. Res. Toxicol.* **4**, 391–407 (1991).
48. Shimada, T., Oda, Y., Gillam, E. M. J., Guengerich, F. P. & Inoue, K. Metabolic activation of polycyclic aromatic hydrocarbons and other procarcinogens by cytochromes P450 1A1 and P450 1B1 allelic variants and other human cytochromes P450 in *Salmonella typhimurium* NM2009. *Drug Metab. Disp.* **29**, 1176–1182 (2001).
49. Nishimura, M., Yaguti, H., Yoshitsugu, H., Naito, S. & Satoh, T. Tissue distribution of mRNA expression of human cytochrome P450 isoforms assessed by high-sensitivity real-time reverse transcription PCR. *Yakugaku Zasshi* **123**, 369–375 (2003).
50. Cramer, J. W., Miller, J. A. & Miller, J. C. *N*-Hydroxylation: a new metabolic reaction observed in the rat with the carcinogen 2-acetylaminofluorene. *J. Biol. Chem.* **235**, 885–888 (1960).
51. Miller, E. C., Miller, J. A. & Hartmann, H. A. *N*-Hydroxy-2-acetylaminofluorene: a metabolite of 2-acetylaminofluorene with increased carcinogenic activity in the rat. *Cancer Res.* **21**, 815–824 (1961).
52. DeBraun, J. R., Smith, J. Y. R., Miller, E. C. & Miller, J. A. Reactivity *in vivo* of the carcinogen *N*-hydroxy-2-acetylaminofluorene: increase by sulfate ion. *Science* **167**, 184–186 (1970).
- First evidence that sulphate esters of *N*-hydroxyarylamines or -amides are the ultimate carcinogenic metabolites of the corresponding parent compounds formed *in vivo*.**
53. Dipple, A. DNA adducts of chemical carcinogens. *Carcinogenesis* **16**, 437–441 (1995).
54. Booth, J., Boyland, E. & Sims, P. An enzyme from rat liver catalysing conjugations with glutathione. *Biochem. J.* **79**, 516–524 (1961).
55. Wartenberg, D., Freyner, D. & Scott, C. S. Trichloroethylene and cancer: epidemiological evidence. *Environ. Health Perspect.* **108** (Suppl. 2), 161–176 (2000).
56. Guengerich, F. P. Activations of dihaloalkanes by thiol-dependent mechanisms. *J. Biochem. Mol. Biol.* **36**, 20–27 (2003).
57. Anders, M. W. & Dekant, W. Glutathione-dependent bioactivation of haloalkenes. *Annu. Rev. Pharmacol. Toxicol.* **38**, 501–537 (1998).
58. McGregor, D. B., Partensky, C., Wilbourn, J. & Rice, J. M. An IARC evaluation of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans as risk factors in human carcinogenesis. *Environ. Health Perspect.* **106** (Suppl. 2), 755–760 (1998).
59. Huff, J., Lucier, G. & Tritscher, A. Carcinogenicity of TCDD: experimental, mechanistic, and epidemiologic evidence. *Annu. Rev. Pharmacol. Toxicol.* **34**, 343–372 (1994).
60. Piskorska-Pliszczynska, J., Keys, B., Safe, S. & Newman, M. S. The cytosolic receptor binding affinities and AHH induction potencies of 29 polynuclear aromatic hydrocarbons. *Toxicol. Lett.* **34**, 67–74 (1986).
61. Conney, A. H., Miller, E. C. & Miller, J. A. Substrate-induced synthesis and other properties of benzpyrene hydroxylase in rat liver. *J. Biol. Chem.* **228**, 753–766 (1957).
- Describes BP-mediated induction of arylhydrocarbon hydroxylase in rat liver.**
62. Poland, A., Glover, E. & Kende, A. S. Stereospecific, high affinity binding of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin by hepatic cytosol. Evidence that the binding species is receptor for induction of aryl hydrocarbon hydroxylase. *J. Biol. Chem.* **251**, 4936–4946 (1976).
63. Gu, Y. Z., Hogenesch, J. B. & Bradfield, C. A. The PAS superfamily: sensors of environmental and developmental signals. *Annu. Rev. Pharmacol. Toxicol.* **40**, 519–561 (2000).
64. Nebert, D. W., Puga, A. & Vasilou, V. Role of the Ah receptor and the dioxin-inducible [Ah] gene battery in toxicity, cancer, and signal transduction. *Ann. NY Acad. Sci.* **685**, 624–640 (1993).
65. Gonzalez, F. J. & Fernandez-Salguero, P. The arylhydrocarbon receptor. Studies using the AhR-null mice. *Drug Metab. Dispos.* **26**, 1194–1198 (1998).
66. Fernandez-Salguero, P. M., Hilbert, D. M., Rudikoff, S., Ward, J. M. & Gonzalez, F. J. Arylhydrocarbon receptor-deficient mice are resistant to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced toxicity. *Toxicol. Appl. Pharmacol.* **140**, 173–179 (1996).
- Reports that mice deficient for the AhR are fully protected against the toxic effects of TCDD in the liver, thymus, heart, kidney, pancreas, spleen, lymph nodes and uterus.**
67. Andersson, P. et al. A constitutively active dioxin/aryl hydrocarbon receptor induces stomach tumors. *Proc. Natl Acad. Sci. USA* **99**, 9990–9995 (2002).
68. Moennikes, O. et al. A constitutively active dioxin/aryl hydrocarbon receptor promotes hepatocarcinogenesis in mice. *Cancer Res.* **64**, 4707–4710 (2004).
- Unequivocal proof that the AhR is crucial in mediating the tumour-promoting activities of receptor agonists such as TCDD, polycyclic aromatic hydrocarbons and polychlorinated biphenyls.**
69. Mimura, J. & Fujii-Kuriyama, Y. Functional role of AhR in the expression of toxic effects by TCDD. *Biochim. Biophys. Acta* **1619**, 263–268 (2003).
70. Frueh, F. W., Hayashibara, K. C., Brown, P. O. & Whitlock, J. P. Jr. Use of cDNA microarrays to analyze dioxin-induced changes in human liver gene expression. *Toxicol. Lett.* **122**, 189–203 (2001).
71. Puga, A., Maier, A. & Medvedovic, M. The transcriptional signature of dioxin in human hepatoma HepG2 cells. *Biochem. Pharmacol.* **60**, 1129–1142 (2000).
72. Carlson, D. B. & Perdew, G. H. A dynamic role for the Ah receptor in cell signaling? Insights from a diverse group of Ah receptor interacting proteins. *J. Biochem. Mol. Toxicol.* **16**, 317–325 (2002).
73. Enan, E. & Matsumura, F. Identification of c-Src as the integral component of the cytosolic Ah receptor complex, transducing the signal of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) through the protein phosphorylation pathway. *Biochem. Pharmacol.* **52**, 1599–1612 (1996).
- The protein kinase SRC is activated in cytosolic liver preparations through attachment to the AhR and upon binding to TCDD.**
74. Luch, A. in *The Carcinogenic Effects of Polycyclic Aromatic Hydrocarbons* (ed. Luch, A.) 1–18 (Imperial College, London, in the press).
75. Boyland, E. & Levi, A. A. Metabolism of polycyclic compounds. I. Production of dihydroxydihydroanthracene from anthracene. *Biochem. J.* **29**, 2679–2683 (1935).
76. Boyland, E. & Sims, P. Metabolism of polycyclic compounds. 16. The metabolism of 1:2-dihydrodiphthalene and 1:2-epoxy-1:2:3:4-tetrahydrodiphthalene. *Biochem. J.* **77**, 175–181 (1960).
77. Sims, P., Grover, P. L., Swaisland, A., Pal, K. & Hewer, A. Metabolic activation of benzo[a]pyrene proceeds by a diol-epoxide. *Nature* **252**, 326–328 (1974).
- First experimental evidence that the vicinal 'bay-region' 7,8-dihydrodiol 9,10-epoxide is the ultimate DNA-binding metabolite of the aromatic hydrocarbon BP.**
78. Luch, A. & Baird W. M. in *The Carcinogenic Effects of Polycyclic Aromatic Hydrocarbons* (ed. Luch, A.) 19–96 (Imperial College, London, in the press).
79. Gonzalez, F. J. The use of gene knockout mice to unravel the mechanisms of toxicity and chemical carcinogenesis. *Toxicol. Lett.* **120**, 199–208 (2001).
80. Balmain, A. & Pragnell, I. B. Mouse skin carcinomas induced *in vivo* by chemical carcinogens have a transforming *Harvey-ras* oncogene. *Nature* **303**, 72–74 (1983).
- Genomic DNA from skin carcinomas of mice sequentially treated with a chemical initiator (7,12-dimethylbenz[*a*]anthracene) and a promotor (12-*O*-tetradecanoylphorbol-13-acetate) of carcinogenesis contained an activated RAS oncogene and morphologically transformed fibroblasts.**
81. Shimizu, Y. et al. Benzo[a]pyrene carcinogenicity is lost in mice lacking the aryl hydrocarbon receptor. *Proc. Natl Acad. Sci. USA* **97**, 779–782 (2000).
- Demonstrates that BP-mediated tumorigenesis in subcutaneous or epidermal mouse tissue requires the presence of a functional AhR.**
82. Klauing, J. E. & Kamendulis, L. M. The role of oxidative stress in carcinogenesis. *Annu. Rev. Pharmacol. Toxicol.* **44**, 239–267 (2004).
83. Freudenthal, R. I., Stephens, E. & Anderson, D. P. Determining the potential of aromatic amines to induce cancer in the urinary bladder. *Int. J. Toxicol.* **18**, 353–359 (1999).
84. Beland, F. A. & Kadlubar, F. F. Formation and persistence of arylamine DNA adducts *in vivo*. *Environ. Health Perspect.* **62**, 19–33 (1985).
85. Neumann, H. G., Ambis, S. & Bitsch, A. The role of nongenotoxic mechanisms in arylamine carcinogenesis. *Environ. Health Perspect.* **102** (Suppl. 6), 173–176 (1994).
86. Klöhn, P. C. et al. Early resistance to cell death and to onset of the mitochondrial permeability transition during hepatocarcinogenesis with 2-acetylaminofluorene. *Proc. Natl Acad. Sci. USA* **100**, 10014–10019 (2003).
87. Van Delft, J. H. M. et al. Discrimination of genotoxic from non-genotoxic carcinogens by gene expression profiling. *Carcinogenesis* **25**, 1265–1276 (2004).
88. Bombail, V., Moggs, J. G. & Orphanides, G. Perturbation of epigenetic status by toxicants. *Toxicol. Lett.* **149**, 51–58 (2004).
89. Hartwig, A. et al. Interference by toxic metal ions with DNA repair processes and cell cycle control: molecular mechanisms. *Environ. Health Perspect.* **110** (Suppl. 5), 797–799 (2002).
90. McMurray, C. T. & Tainer, J. A. Cancer, cadmium and genome integrity. *Nature Genet.* **34**, 239–241 (2003).
91. Hughes, M. F. Arsenic toxicity and potential mechanisms of action. *Toxicol. Lett.* **133**, 1–16 (2002).
92. Kawanishi, S., Hiraku, Y., Murata, M. & Oikawa, S. Oxidative damage and repair: the role of metals in site-specific DNA damage with reference to carcinogenesis. *Free Radic. Biol. Med.* **32**, 822–832 (2002).
93. Beyersmann, D. & Hechtenberg, S. Cadmium, gene regulation, and cellular signalling in mammalian cells. *Toxicol. Appl. Pharmacol.* **144**, 247–261 (1997).
94. Qian, Y., Castranova, V. & Shi, X. New perspectives in arsenic-induced cell signal transduction. *J. Inorg. Biochem.* **96**, 271–278 (2003).
95. Kasprzak, K. S., Sunderman, F. W. Jr. & Salnikow, K. Nickel carcinogenesis. *Mutat. Res.* **533**, 67–97 (2003).
96. Lee, Y. W. et al. Carcinogenic nickel silences gene expression by chromatin condensation and DNA methylation: a new model for epigenetic carcinogens. *Mol. Cell. Biol.* **15**, 2547–2557 (1995).
97. Zhang, Q. et al. Inhibition and reversal of nickel-induced transformation by the histone deacetylase inhibitor trichostatin A. *Toxicol. Appl. Pharmacol.* **192**, 201–211 (2003).
98. Govindarajan, B. et al. Reactive oxygen-induced carcinogenesis causes hypermethylation of *p16<sup>INK4a</sup>* and activation of MAP kinase. *Mol. Med.* **8**, 1–8 (2002).
99. Kowara, R. et al. Reduced Fhit protein expression in nickel-transformed mouse cells and in nickel-induced murine sarcomas. *Mol. Cell. Biochem.* **255**, 195–202 (2004).
100. Chen, H. et al. Association of *c-myc* oncogene overexpression and hyperproliferation with arsenite-induced malignant transformation. *Toxicol. Appl. Pharmacol.* **175**, 260–268 (2001).
101. Zhao, C. Q., Young, M. R., Diwan, B. A., Coogan, T. P. & Waalkes, M. P. Association of arsenic-induced malignant transformation with DNA hypomethylation and aberrant gene expression. *Proc. Natl Acad. Sci. USA* **94**, 10907–10912 (1997).
102. Glatt, H. R. in *The Carcinogenic Effects of Polycyclic Aromatic Hydrocarbons* (ed. Luch, A.) 283–314 (Imperial College, London, in the press).
103. Ross, J. A. et al. Adenomas induced by polycyclic aromatic hydrocarbons in strain A/J mouse lung correlate with time-integrated DNA adduct levels. *Cancer Res.* **55**, 1039–1044 (1995).
104. Poirier, M. C. Chemical-induced DNA damage and human cancer risk. *Nature Rev. Cancer* **4**, 630–637 (2004).
105. Kensler, T. W., Qian, G. S., Chen, J. G. & Groopman, J. D. Translational strategies for cancer prevention in liver. *Nature Rev. Cancer* **3**, 321–329 (2003).
106. Baertschi, S. W., Raney, K. D., Stone, M. P. & Harris, T. M. Preparation of the 8,9-epoxide of the mycotoxin aflatoxin B<sub>1</sub>; the ultimate carcinogenic species. *J. Am. Chem. Soc.* **110**, 7929–7931 (1988).
- Key experiment with chemically synthesized AFB<sub>1</sub> exo-8,9-oxide that provided an unequivocal proof that this epoxide is the DNA-binding metabolite of the mycotoxin.**
107. Guengerich, F. P. et al. Activation and detoxification of aflatoxin B<sub>1</sub>. *Mutat. Res.* **402**, 121–128 (1998).
108. Iyer, R. S. et al. DNA adduction by the potent carcinogen aflatoxin B<sub>1</sub>; mechanistic studies. *J. Am. Chem. Soc.* **116**, 1603–1609 (1994).
109. Yang, S. K., McCourt, D. W., Roller, P. P. & Gelboin, H. V. Enzymatic conversion of benzo[a]pyrene leading predominantly to the diol-epoxide *r*-7,*t*-8-dihydroxy-*t*-9,10-oxo-7,8,9,10-tetrahydrobenzo[a]pyrene through a single enantiomer of *r*-7,*t*-8-dihydroxy-7,8-dihydrobenzo[a]pyrene. *Proc. Natl Acad. Sci. USA* **73**, 2594–2598 (1976).
110. Koreeda, M. et al. Binding of benzo[a]pyrene 7,8-diol-9,10-epoxides to DNA, RNA, and protein of mouse skin occurs with high stereoselectivity. *Science* **199**, 778–780 (1978).
111. Cheng, S. C., Hilton, B. D., Roman, J. M. & Dipple, A. DNA adducts from carcinogenic and noncarcinogenic enantiomers of benzo[a]pyrene dihydrodiol epoxides. *Chem. Res. Toxicol.* **2**, 334–340 (1989).
112. Friedberg, E. C. How nucleotide excision repair protects against cancer. *Nature Rev. Cancer* **1**, 22–33 (2001).
113. Hess, M. T., Gunz, D., Luneva, N., Geacintov, N. E. & Naegeli, H. Base pair conformation-dependent excision of benzo[a]pyrene diol epoxide-guanine adducts by human nucleotide excision repair enzymes. *Mol. Cell. Biol.* **17**, 7069–7076 (1997).
- Indicates that the efficiency of NER of bulky aromatic hydrocarbon-DNA adducts greatly depends on the stereochemistry and the conformation of the lesion induced.**

114. Geacintov, N. E. *et al.* NMR solution structures of stereoisomeric covalent polycyclic aromatic carcinogen-DNA adducts: principles, patterns, and diversity. *Chem. Res. Toxicol.* **10**, 111–146 (1997).
115. Khan, Q. A. & Dipple, A. Diverse chemical carcinogens fail to induce G<sub>1</sub> arrest in MCF-7 cells. *Carcinogenesis* **21**, 1611–1618 (2000).
116. Wang, A. *et al.* Response of human mammary epithelial cells to DNA damage induced by BPDE: involvement of novel regulatory pathways. *Carcinogenesis* **24**, 225–234 (2003).
117. Lehmann, A. R. Replication of damaged DNA in mammalian cells: new solutions to an old problem. *Mutat. Res.* **509**, 23–34 (2002).
118. Ross, J. A. & Nesnow, S. Polycyclic aromatic hydrocarbons: correlation between DNA adducts and *ras* oncogene mutations. *Mutat. Res.* **424**, 155–166 (1999).
119. Malumbres, M. & Barbacid, M. *RAS* oncogenes: the first 30 years. *Nature Rev. Cancer* **3**, 459–465 (2003).
120. Feng, Z. *et al.* Preferential DNA damage and poor repair determine *ras* gene mutational hotspot in human cancer. *J. Natl Cancer Inst.* **94**, 1527–1536 (2002).  
**Codon 12 of KRAS in bronchial epithelial cells is a DNA-binding 'hot spot' of the cigarette smoke carcinogen BP, due to both a preferential binding of anti-BPDE at the first dG in this codon and the inefficient DNA repair that follows.**
121. Bos, J. L. *Ras* oncogenes in human cancer: a review. *Cancer Res.* **49**, 4682–4689 (1989).
122. Hu, W., Feng, Z. & Tang, M.-S. Preferential carcinogen-DNA adduct formation at codons 12 and 14 in human *K-ras* gene and their possible mechanisms. *Biochemistry* **42**, 10012–10023 (2003).
123. Chen, J. X., Zheng, Y., West, M. & Tang, M. S. Carcinogens preferentially bind at methylated CpG in the p53 mutational hot spots. *Cancer Res.* **58**, 2070–2075 (1998).
124. Feng, Z., Hu, W., Rom, W. N., Beland, F. A. & Tang, M. S. *N*-hydroxy-4-aminobiphenyl-DNA binding in human p53 gene: sequence preference and the effect of C5 cytosine methylation. *Biochemistry* **41**, 6414–6421 (2002).
125. Denissenko, M. F., Chen, J. X., Tang, M. S. & Pfeifer, G. P. Cytosine methylation determines hot spots of DNA damage in the human p53 gene. *Proc. Natl Acad. Sci. USA* **94**, 3893–3898 (1997).
126. Feng, Z., Hu, W., Rom, W. N., Beland, F. A. & Tang, M. S. 4-Aminobiphenyl is a major etiological agent of human bladder cancer: evidence from its DNA binding spectrum in human p53 gene. *Carcinogenesis* **23**, 1721–1727 (2002).
127. Denissenko, M. F., Pao, A., Tang, M. S. & Pfeifer, G. P. Preferential formation of benzo[*a*]pyrene adducts at lung cancer mutational hotspots in p53. *Science* **274**, 430–432 (1996).  
**Landmark paper in molecular epidemiology that provides an aetiological link between BP exposure and human lung cancer based on mutations at codons 157, 248 and 273 of cellular TP53.**
128. Denissenko, M. F., Pao, A., Pfeifer, G. P. & Tang, M. S. Slow repair of bulky DNA adducts along the nontranscribed strand of the human p53 gene may explain the strand bias of transversion mutations in cancers. *Oncogene* **16**, 1241–1247 (1998).
129. Hecht, S. S. Tobacco carcinogens, their biomarkers and tobacco-induced cancer. *Nature Rev. Cancer* **3**, 733–744 (2003).
130. International Agency for Research on Cancer. IARC *Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Volume 83, Tobacco Smoke and Involuntary Smoking*. (IARC Press, Lyon, 2004).
131. Hu, W., Feng, Z. & Tang, M. S. Nickel (II) enhances benzo[*a*]pyrene diol epoxide-induced mutagenesis through inhibition of nucleotide excision repair in human cells: a possible mechanism for nickel (II)-induced carcinogenesis. *Carcinogenesis* **25**, 455–462 (2004).
132. Buterin, T. *et al.* Trapping of DNA nucleotide excision repair factors by nonrepairable carcinogen adducts. *Cancer Res.* **62**, 4229–4235 (2002).  
**Demonstrates that the NER-catalysed excision of (+)-trans-anti-BPDE-dG fails when its deoxycytosine base pair is deleted. This is known as the 'prototypic decoy adduct'. Bulky lesions opposite deoxycytosine deletion are also normally present in cells as an intermediate after replicative bypass of AAF or BP adducts.**
133. Lewtas, J. *et al.* in *Methods for Genetic Risk Assessment* (ed. Brusick, D. J.) 125–169 (Lewis Publishers, Boca Raton, Florida, 1994).
134. Calabrese, E. J. & Baldwin, L. A. Toxicology rethinks its central belief. *Nature* **421**, 691–692 (2003).
135. Farmer, P. B. & Shuker, D. E. G. What is the significance of increases in background levels of carcinogen-derived protein and DNA adducts? Some considerations for incremental risk assessment. *Mutat. Res.* **424**, 275–286 (1999).
136. Wiltse, J. A. & Dellarco, V. L. U. S. Environmental Protection Agency's revised guidelines for carcinogen risk assessment: evaluating a postulated mode of carcinogen action in guiding dose-response extrapolation. *Mutat. Res.* **464**, 105–115 (2000).
137. Henderson, L., Albertini, S. & Aardema, M. Thresholds in genotoxicity responses. *Mutat. Res.* **464**, 123–128 (2000).
138. Nulwaisry, E. F., Bittner, M., Trent, J., Barrett, J. C. & Afshari, C. A. Microarrays and toxicology: the advent of toxicogenomics. *Mol. Carcinog.* **24**, 153–159 (1999).  
**Discussion of the advantages of cDNA microarray-based expression profiling as a highly sensitive marker for detection, monitoring and characterization of biohazardous compounds in the human environment ('ToxChip' technology).**
139. Hamadeh, H. K. *et al.* Prediction of compound signature using high density gene expression profiling. *Toxicol. Sci.* **67**, 232–240 (2002).
140. Gonzalez, F. J. The role of carcinogen-metabolizing enzyme polymorphism in cancer susceptibility. *Reprod. Toxicol.* **11**, 397–412 (1997).
141. Vineis, P. Individual susceptibility to carcinogens. *Oncogene* **23**, 6477–6483 (2004).
142. Balmain, A., Gray, J. & Ponder, B. The genetics and genomics of cancer. *Nature Genet.* **33** (Suppl.), 238–244 (2000).
143. Boyland, E. History and future of chemical carcinogenesis. *Br. Med. Bull.* **36**, 5–10 (1980).
144. Tsutsui, H. Über das künstlich erzeugte Carcinoid bei der Maus. *Gann* **12**, 17–21 (1918).
145. Bloch, B. & Dreifuss, W. Ueber die experimentelle Erzeugung von Carcinomen mit Lymphdrüsen- und Lungenmetastasen durch Teerbestandteile. *Schweiz. Med. Wochenschr.* **51**, 1033–1037 (1921).
146. Leitch, A. & Kennaway, E. L. Experimental production of cancer by arsenic. *Br. Med. J.* **II**, 1107–1108 (1922).
147. Friedewald, W. F. & Rous, P. The initiating and promoting elements in tumor production. An analysis of the effects of tar, benzopyrene, and methylcholanthrene on rabbit skin. *J. Exp. Med.* **80**, 101–126 (1944).
148. Berenblum, I. & Shubik, P. A new, quantitative, approach to the study of the stages of chemical carcinogenesis in the mouse's skin. *Br. J. Cancer* **1**, 383–391 (1947).

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The author declares no competing financial interests.

#### Online links

##### DATABASES

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##### FURTHER INFORMATION

Agency for Toxic Substances and Disease Registry:  
<http://www.atsdr.cdc.gov>

Cytochrome P450 Homepage:

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General National Institutes of Health web site on Toxicology and Environmental Health:

<http://sis.nlm.nih.gov/Tox/ToxMain.html>

Genetic polymorphisms of cytochrome P450 enzymes:

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International Agency for Research on Cancer (IARC)

Monographs Programme on the Evaluation of Carcinogenic

Risks to Humans: <http://monographs.iarc.fr>

IARC web site on polychlorinated dibenzo-*p*-dioxins:

<http://www.inchem.org/documents/iarc/vol69/dioxin.html>

National Toxicology Program: <http://ntp-server.niehs.nih.gov>

Toxicology Data Network of National Institutes of Health:

<http://toxnet.nlm.nih.gov>

United States Environmental Protection Agency Integrated

Risk Information System: <http://www.epa.gov/iris>

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