

# CHEMICAL-INDUCED DNA DAMAGE AND HUMAN CANCER RISK

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Chemical carcinogenesis involves a complex series of events, the earliest of which typically include DNA damage and the fixation of DNA mutations. Sophisticated new techniques have been developed to quantify DNA damage and to correlate the amount of damage with cancer risk. Approaches such as these are underway in Linxian, China, where food contains high levels of DNA-damaging, carcinogenic polycyclic aromatic hydrocarbons (PAHs), and where there is high mortality from oesophageal cancer. Gaining better insight into the mechanisms by which PAH exposure might increase oesophageal cancer risk could lead to new strategies for cancer prevention.

POLYCYCLIC AROMATIC HYDROCARBONS (PAHs). Typically products of partial combustion of organic matter that consist of multiple fused benzene rings. These must be metabolized to react with cellular macromolecules, and many PAHs, such as benzo[*a*]pyrene, are known to be carcinogenic in humans.

Over two centuries ago clinicians and epidemiologists first reported the induction of human cancers as a result of exposure to chemical agents. In 1761, John Hill published reports of an increased incidence of nasal polyps in frequent users of snuff<sup>1</sup>, and in 1775 Percival Pott published a pioneering treatise on scrotal cancer in English chimney sweeps<sup>2</sup>. In the case of the chimney sweeps, scrotal tumours arose after years of intense exposure to POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) in chimney soot in individuals who bathed only rarely. Subsequently, the Danish Chimney Sweepers' Guild and other occupational authorities in many European nations recommended frequent bathing for chimney sweeps. The practice was so successful that about 100 years later Butlin<sup>3</sup> reported virtually no scrotal cancers among chimney sweeps in Europe, whereas the incidence in England, where bathing frequency had not changed significantly, remained high. The history of scrotal cancer in chimney sweeps serves to demonstrate two basic principles of CHEMICAL CARCINOGENESIS — that tumours typically arise in mid to late life as a result of long-term chronic exposure, and that measures designed to diminish exposure can decrease cancer incidence.

Since 1775 these basic principles have been observed repeatedly for each new chemical recognized to increase human cancer risk. Some notable examples include the association between lung cancer and cigarette smoking<sup>4</sup>; the induction of bladder cancer by benzidine and other AROMATIC AMINES<sup>5,6</sup>; the causation of mesothelioma by asbestos<sup>7</sup>; the association between

leukaemia and benzene exposure<sup>8,9</sup>; and induction of liver cancer by AFLATOXINS<sup>10,11</sup> and vinyl chloride<sup>12</sup>. In many instances, substantial levels of chemical exposures occurred before it became apparent that protection was necessary, whereas in other instances the demographics and economics of the affected populations made protection from exposure virtually impossible.

Although much progress has been made during the past ~200 years, localized areas exist in which specific cancers are prevalent and the aetiological agents remain obscure or unproven. In recent years, stories of regional pollution and increased cancer incidence in places such as Love Canal in upstate New York (see online links box), the increased incidence of childhood leukaemia among residents of Woburn, Massachusetts (see online links box), and the chemical-induced cancer cases investigated by the law firm of Masry and Vititoe — depicted in the film 'Erin Brockovich' — serve as cautionary tales for the dangers of chronic chemical exposure. These examples are just a few among many contaminated sites worldwide. But what do we know about the mechanisms by which chemicals cause cancer?

## Mechanisms of carcinogenesis

Many human cancers are associated with exposure to GENOTOXIC chemicals. There is typically a long period of time (years) between early events that include initial carcinogen exposure, the onset of DNA damage and the fixation of mutations, and the subsequent appearance of a tumour<sup>13–16</sup>. Evolution of the malignant phenotype

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## CHEMICAL CARCINOGENESIS

A long-term multistep process by which chemical carcinogens induce tumour formation. This process typically includes DNA damage and mutagenesis (initiation), followed by selective clonal expansion (promotion), a second mutagenic mechanism responsible for the transition to malignancy (conversion), and the ability of some malignant cells to acquire more aggressive characteristics (progression).

## AROMATIC AMINES

Hydrophobic compounds that consist of two or more benzene rings with one or more amino groups placed at varying positions on the ring structures. Aromatic amines are components of dye and rubber manufacturing processes, diesel-fuel combustion, ambient pollution, cigarette smoke and some heavily cooked meats. Several aromatic amines are implicated in the aetiology of human bladder and lung cancer.

## AFLATOXINS

Carcinogenic by-products of mould growth that are frequently found in high concentrations in corn, peanuts and grains produced and stored in very humid areas. Aflatoxins are implicated in the aetiology of human liver cancer.

## Summary

- More than 200 years after the first reports of chemically induced cancer in humans, many carcinogenic chemicals have been identified in the environment and the workplace.
- Chemical carcinogenesis typically requires chronic exposure, followed by a period of years during which a complex series of events, involving DNA damage and alterations in gene expression, take place.
- The earliest carcinogen-induced events typically include DNA structural damage, which often occurs as the result of covalent binding of carcinogens to DNA (DNA-adduct formation). This results in DNA mutations, leading to alterations in protein structure and function that can result in tumorigenesis.
- In experimental models of chemical carcinogenesis, DNA adducts have been shown to be necessary, but not sufficient, for tumorigenesis. Proving such a relation in humans is difficult because it requires a correlation between DNA damage, which might have occurred 10–30 years earlier, and cancer incidence.
- In recent years, progress has been made in our ability to detect carcinogen-induced DNA damage in humans. Two studies have shown that increases in DNA-adduct levels are associated with cancer risk in humans.
- By analogy to experiments in animal models, administration of chemopreventive agents that reduce DNA-adduct formation might also reduce human cancer risk.
- In regions of the world such as Linxian, China, where dietary exposure to polycyclic aromatic hydrocarbons is suspected in the aetiology of high oesophageal cancer rates and where reducing the level of this exposure is considered economically unfeasible, chemoprevention might be appropriate.

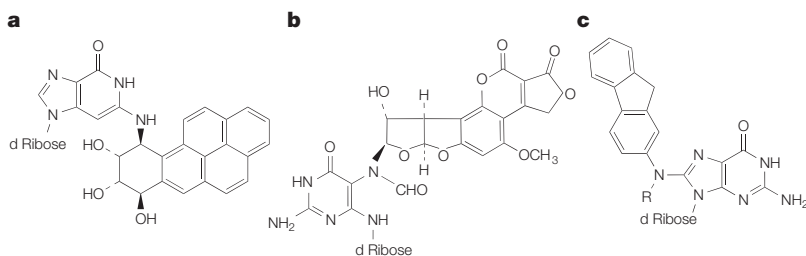
requires iterative alterations in gene expression that ultimately allow for the expansion of tumour subpopulations that have lost growth-control mechanisms and therefore have a proliferative advantage, compared with normal cells.

DNA damage is an important first step in this carcinogenic process. Chemical carcinogens can cause the formation of CARCINOGEN–DNA ADDUCTS (FIG. 1), or induce other modifications to DNA, such as oxidative damage and alterations to DNA ultrastructure (DNA-strand crosslinking, DNA-strand breakage, chromosomal rearrangements and deletions). Although cells possess mechanisms to repair many types of DNA damage, these are not always completely effective, and residual DNA damage can lead to the insertion of an incorrect base during DNA replication, followed by transcription

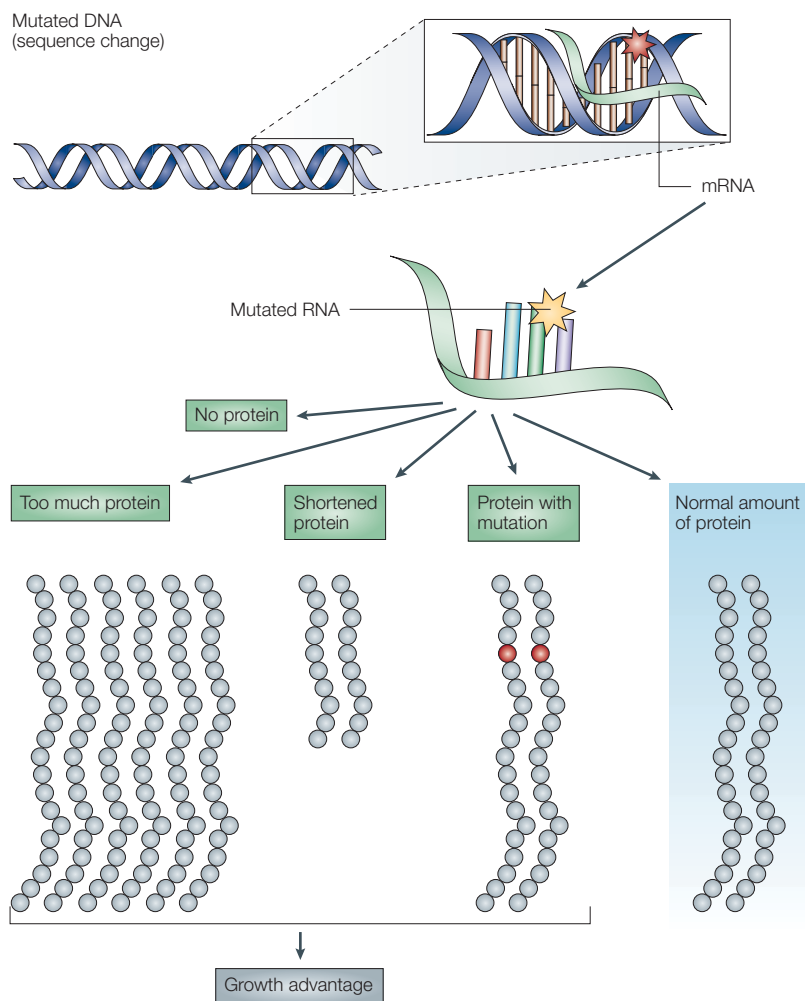
and translation of the mutated templates, ultimately leading to the synthesis of altered protein (FIG. 2). Mutations in an oncogene, tumour-suppressor gene or gene that controls the cell cycle can result in a clonal cell population with a proliferative or survival advantage. The development of a tumour requires many such events, occurring over a long period of time, and for this reason human cancer induction often takes place within the context of chronic exposure to chemical carcinogens. A well-known example of this is the long-term use of tobacco that is associated with increased lung cancer risk.

Studies involving chemical induction of tumours in animal models are typically performed using rats and mice, and hundreds of such studies attest to the essential role of DNA damage in the carcinogenic process<sup>14,15</sup>. In these models, dose-response curves for chemical exposure, DNA-adduct formation, mutagenesis and rates of tumour formation are closely correlated<sup>15–19</sup>. Furthermore, metabolic modulations that reduce the formation of DNA adducts in a given tissue, without reducing levels of chemical exposure, have been shown to reduce the number of tumours that develop<sup>20</sup>. DNA adducts also form in organs that do not develop tumours, indicating that tissue-specific risk factors, such as the proliferative capacity of a certain cell type, can also contribute to tumour induction. However, as tumours do not arise in the absence of DNA damage in these models, DNA-adduct formation is generally considered to be 'necessary but not sufficient' for tumour formation.

Although cancer induction in animal models has yielded valuable insights into the mechanisms of chemical carcinogenesis, the experiments that are necessary to define the relation between DNA damage and tumour induction in humans are still largely in progress. Tracing the pathway of events from human chemical exposure to DNA damage and then to the appearance of a tumour is a central challenge for MOLECULAR CANCER EPIDEMIOLOGY<sup>21</sup>. If chemically induced



**Figure 1 | Structures of some carcinogen-DNA adducts. a** | (7R)-N<sup>2</sup>-(10-(7β,8α,9α-trihydroxy-7,8,9,10-tetrahydro-benzo[a]pyrene)-yl)-deoxyguanosine (BPdG) is formed when 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydro-benzo[a]pyrene (BPDE) binds to deoxyguanosine. This is the major DNA adduct associated with mutagenicity and tumorigenicity. BPDE is derived from benzo[a]pyrene (BP), a polycyclic aromatic hydrocarbon that is ubiquitous in the environment and is formed from incomplete combustion of animal and vegetable matter. BP is carcinogenic in humans and animal models. **b** | 8,9-dihydro-8-(N5-formyl-2',5',6'-tri-amino-4'-oxo-N5-pyrimidyl)-9-hydroxy-aflatoxin B<sub>1</sub> (AFB<sub>1</sub>-N7-guanine) is formed when aflatoxin B<sub>1</sub> binds to deoxyguanosine. Aflatoxin B<sub>1</sub> is a natural product of *Aspergillus* mould growth. It binds to DNA and then forces opening of the deoxyguanosine imidazole ring. **c** | Shows an aromatic amine–DNA adduct. When R is a hydrogen atom, N-(deoxyguanosine-8-yl)-2-aminofluorene is formed. When R is an acetyl group, the molecule is called N-(deoxyguanosine-8-yl)-2-acetylaminofluorene. N-2-acetylaminofluorene is an aromatic amine and a recalled pesticide that has been studied extensively as an experimental carcinogen.



**Figure 2 | Generation of altered gene products.** DNA becomes structurally altered when a chemical carcinogen (red star) is bound to a DNA base — (this structurally altered DNA is known as a DNA adduct). The DNA adduct leads to the insertion of an incorrect base in the opposite DNA strand during replication. This mutation is then transcribed into the RNA, which is further translated into mutant protein. DNA adducts can therefore result in protein overexpression, underexpression or truncation, or a protein of normal size with a single amino-acid change(s) that alters protein function (folding, substrate binding or catalytic activity). Sometimes DNA adducts have no effects on protein levels or function (normal protein).

DNA damage is associated with increased cancer risk in humans, it might be possible to devise approaches for cancer prevention. For example, CHEMOPREVENTION could be used to inhibit DNA damage in instances when exposure to the carcinogen can not be altered. An ongoing study in Linxian, China is providing an opportunity to apply knowledge obtained from animal models to reduce cancer risk. In Linxian, inhabitants have an exceedingly high rate of oesophageal cancer, which might be caused in part by the high levels of PAHs in their diet.

**Oesophageal cancer in Linxian**

The residents of Linxian, a mountainous county in north-east China (FIG. 3), are at high risk for developing cancer of the oesophagus and gastric cardia. The 20% mortality rate for these cancers in Linxian is among the

highest rate anywhere in the world<sup>22–24</sup>. Archaeological evidence of temples erected 2,000 years ago to the ‘Throat God’ indicates that these tumour types have been prevalent in Linxian for generations<sup>23</sup>. In addition, the lower cancer rates observed in the non-mountainous regions that surround Linxian indicate a geographically relevant aetiological component<sup>23</sup>. Oesophageal cancer in Linxian has been studied for approximately 40 years, but no single aetiological agent (for example, nutritional deficiencies, poor oral hygiene, nitrosamines, pickled foods or fungus-contaminated foods) has been strongly-associated with the observed oesophageal cancer incidence.

In Linxian, many houses have no chimneys, and a central fire serves both as a source of heat and the family cooking stove. The discovery of soot in lymph nodes of non-smoking individuals (anthracosis)<sup>25</sup>, and high levels of carcinogenic combustion products in both cooked and uncooked food<sup>26</sup>, has lead investigators to believe that ingested PAHs might contribute to the high oesophageal cancer rate in Linxian. Increased levels of 1-hydroxypyrene, a PAH metabolite, have been detected in the urine of many Linxian residents<sup>27</sup>, indicating that these individuals have been exposed to PAHs.

There are hundreds of types of PAHs, some of which, when metabolically activated, are carcinogenic in animal models and in humans<sup>28</sup>. Many PAHs are metabolized to products that bind covalently to DNA bases, forming stable DNA adducts<sup>14,28,29</sup>. A frequently studied member of the PAH class of compounds is BP — a human carcinogen that binds to deoxyguanosine in DNA (FIG. 1a), fitting into the minor groove of the DNA helix<sup>30</sup> (FIG. 4). To show that PAH-induced DNA damage might be the underlying cause of cancer in the Linxian population, it will be necessary to demonstrate that, in their early years, individuals who later developed oesophageal cancer sustained higher levels of PAH–DNA adducts than their matched, cancer-free contemporaries.

**Measurement of DNA adducts**

Methods to quantify DNA-adduct formation in human tissues have only become available in about the past 25 years. Some strengths and weaknesses of those in frequent current use are outlined in TABLE 1. The three most common approaches, in decreasing order of specificity, are mass spectrometry, immunoassays and immunohistochemistry, and <sup>32</sup>P-postlabelling. Mass-spectrometry (BOX 1a) is able to identify adducts based on molecular weight and is therefore considered the ‘gold standard’ for DNA-adduct identification, although expensive instrumentation is required. Immunoassays and immunohistochemistry use antisera that recognize specific carcinogen–DNA adducts or carcinogen-modified DNA; antibodies allow visualization of DNA adducts in individual nuclei (BOX 1b) of cells, but can be limited in specificity if there is cross-reactivity between structurally similar DNA adducts. DNA damage can, however, be visualized within the context of whole tissue in paraffin-embedded samples, if the adducts are chemically stable. These PAH–DNA adduct levels can then be semi-quantified using the automated cellular imaging system, a computerized

**GENOTOXIC**  
An agent or process that interacts with cellular DNA, either directly or after metabolic biotransformation, resulting in alteration of DNA structure. DNA-adduct formation comprises one type of genotoxicity.

**DNA ADDUCT**  
A covalent addition product formed by binding of a chemical to a DNA base.



Figure 3 | **Location of Linxian (Linzhou).** Residents of Linxian, a mountainous county in north-east China, are at high risk for developing cancer of the oesophagus and gastric cardia. This might be related to polycyclic aromatic hydrocarbon exposure.

bright-field microscopy technique.  $^{32}\text{P}$ -postlabelling involves digesting DNA and then adding a radiolabelled phosphate to 3' nucleosides. Normal nucleosides are then separated from those bound to adduct by thin-layer or other chromatography (BOX 1c). This approach is very sensitive, requiring only microgram quantities of DNA. However, it is virtually impossible to determine the structure of the labelled adduct based on the resulting chromatographic spots or high-performance liquid chromatography peaks.

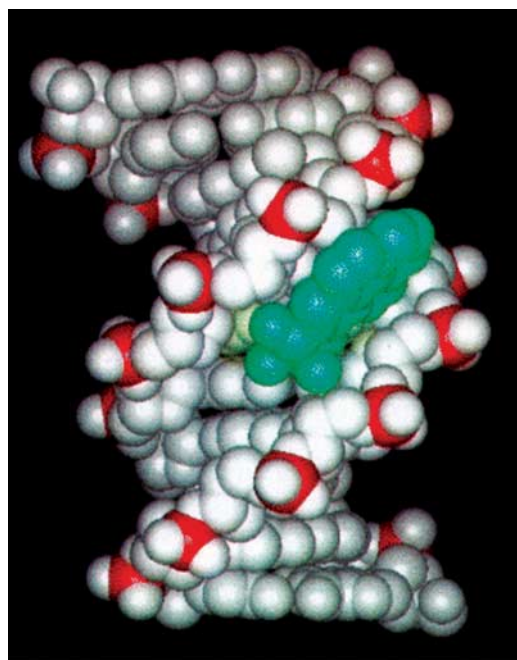


Figure 4 | **Structure of BPdG.** This space-filling model shows how enantiomeric 7 $\beta$ ,8 $\alpha$ -dihydroxy-9 $\alpha$ ,10 $\alpha$ -epoxy-7,8,9,10-tetrahydro-benzo[a]pyrene (BPdG; green) binds to deoxyguanine and fits into the minor groove of the DNA helix. Figure reproduced with permission from REF. 30 © (1997) American Chemical Society.

Most of these methods work well in experimental (animal and cell culture) models in which only one agent is under study. DNA from human tissues, however, is likely to contain many different types of adducts, so identifying a single adduct might not be possible without first separating the adduct of interest from the remaining species of DNA damage. Typically it is necessary to use a preparative step, such as chromatography, to isolate the adduct in question before identification by, for example, a mass-spectrometry-based approach.

Most methods to quantify DNA adducts are able to measure as little as one adduct per  $10^9$  nucleotides in 5–100 micrograms of DNA. The exception is accelerator mass spectrometry (AMS), which uses an approach similar to carbon dating and requires that the test compound contain a very low level of radioactivity; AMS requires very expensive instrumentation, but can detect as little as one adduct per  $10^{12}$  nucleotides. Using current methods, researchers have documented the presence of approximately 40 different types of DNA adducts in human tissues. Many human DNA adducts are derivatives of chemicals that are known to be carcinogenic in animal models but not yet proven to be carcinogenic to humans, whereas others are clearly formed from known human carcinogens. TABLE 2 shows examples of chemical carcinogens known to form DNA adducts and to cause cancers in humans<sup>10–12,31–51</sup>.

#### PAH exposure in Linxian

Recent developments in immunohistochemical methodology have made semi-quantification of PAH–DNA adducts possible in human tissue sections<sup>52,53</sup>. This particularly powerful approach allows for the use of paraffin-embedded archived material from studies that have insufficient frozen tissue to extract DNA, as was the case with the Linxian study. The samples available from Linxian included paraffin blocks of oesophageal biopsy and resection tissue collected over the past ~20 years. These samples were part of an endoscopic survey in a large prospective epidemiological study of cancer risk that began in 1985 and included careful patient follow-up and documentation of cancer development<sup>54–57</sup>. The oesophageal tissue samples were analysed by immunohistochemical analysis, and adducts were identified in the nuclei of whole tissue sections using antibodies specific for PAH–DNA adducts<sup>53</sup> (BOX 1b).

This study used an antiserum raised against a DNA modified by covalent binding of 7 $\beta$ ,8 $\alpha$ -dihydroxy-9 $\alpha$ ,10 $\alpha$ -epoxy-7,8,9,10-tetrahydro-benzo-[a]pyrene (BPdE) — a metabolite of BP — to deoxyguanosine<sup>58,59</sup>. This binding results in the formation of the (7R)-N<sup>2</sup>-(10-(7 $\beta$ ,8 $\alpha$ ,9 $\alpha$ -trihydroxy-7,8,9,10-tetrahydro-benzo[a]pyrene)-yl)-deoxyguanosine (BPdG) adduct (FIGS 1,4). This antiserum also recognizes several different types of PAH–DNA adduct<sup>60</sup>, but does not crossreact with unmodified (non-adducted) DNA or the unbound BP carcinogen.

When archived oesophageal endoscopic biopsy samples taken from Linxian residents in 1985 (REF. 53)

#### MOLECULAR CANCER EPIDEMIOLOGY

The application of biochemical and molecular markers in research designed to understand the continuum of events between exposure and disease, to design rational intervention strategies, and to identify groups of individuals who are most likely to be at risk of developing neoplastic disease.

#### CHEMOPREVENTION

Inhibition of carcinogenesis through the application of agents capable of blocking DNA-damage formation, enhancing DNA repair or accelerating cell death.

Table 1 | **Methods for DNA-adduct measurement**

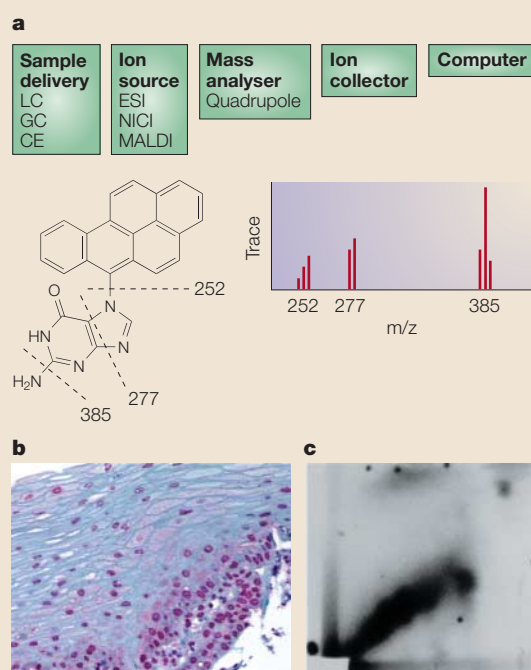
Method	Brief synopsis	Strengths	Weaknesses
<sup>32</sup> P-postlabelling	DNA is digested into nucleotides that are labelled on the 5' end with <sup>32</sup> P; adducts are separated by high-performance liquid chromatography or thin-layer chromatography	Requires about 10 µg DNA; highly sensitive; no requirement for costly instruments	DNA-adduct structure not identifiable; requires radioactive labelling; relatively labour intensive
Immunoassays	Antisera specific for a DNA adduct or a modified DNA is used in competitive immunoassays, with various end points (radioactivity, colour, fluorescence or chemiluminescence)	Antiserum defines specificity; highly sensitive; quantification by standard curve; high throughput and inexpensive	Antiserum may crossreact with similar adducts; requires 50–100 µg of DNA
Immunohistochemistry	Uses same antisera as immunoassays to stain tissue sections; antiserum binding revealed by fluorescence or bright-field microscopy; adducts stain nuclei	Allows for adduct localization; useful for archived human tissue; adaptable to large studies; high throughput	Less sensitive than other methods; requires many controls; can be semi-quantitative using costly instruments
Mass spectrometry	Involves derivitization of samples/standards; structural identification of adducts; preparation can use gas chromatography, ionization, electrospray and other approaches	Specific adduct structural identification; quantification possible	Requires 100 µg DNA; expensive equipment

and tissue from oesophagectomies performed in Linxian in the spring/summer of 1995 (REF. 52) were examined, PAH–DNA adducts were detected in most samples (BOX 1b). Controls for this study included oesophageal tissue from unexposed laboratory-housed monkeys, as well as from autopsies of smokers and non-smokers in the United States.

Interestingly, there were **no PAH–DNA adducts detected in oesophageal tissue samples taken from smokers in the United States.** Rough calculations were therefore performed to compare lifetime PAH exposures, through diet and smoking, in Linxian and in the United States. The BP content of cooked and uncooked food taken from homes in Linxian (3.1–13.8 ng/g food)<sup>26</sup> is

Box 1 | **Methods used to measure carcinogen–DNA adducts**

Several different approaches are used to measure carcinogen–DNA adducts. The figure panels demonstrate some of these approaches. Mass spectroscopy (MS) is the most specific approach. In panel a, MS has different possibilities for sample delivery — liquid chromatography (LC), gas chromatography (GC) or capillary electrophoresis (CE). Ion sources include electrospray ionization (ESI), negative-ion chemical ionization (NICI) and matrix-assisted laser desorption ionization (MALDI). Once the ions are collected, the signals are analysed by computer programs. Panel a also shows what the MS data for detection of the *N*<sup>2</sup>-(benzo[*a*]pyren-6-yl)-guanine adduct would look like. *m/z* denotes mass/charge ratio, where charge is essentially 1; *m/z* therefore indicates the molecular weight of the fragment being studied. The numbers show *m/z* ratios for different fragments of the molecule. DNA adducts can also be detected in fixed tissue samples by immunohistochemistry. Panel b shows immunohistochemical staining of human oesophageal tissue samples from a person in Linxian. Pink colour indicates nuclei of cells that contain polycyclic aromatic hydrocarbon (PAH)–DNA adducts, as detected with antisera. Finally, <sup>32</sup>P-postlabelling can be used to detect DNA adducts in microgram amounts of DNA. In this approach, nucleotides are labelled with <sup>32</sup>P and separated by thin-layer chromatography. Adducts can then be separated away from free nucleotides and quantified. Panel c shows a thin-layer chromatogram of an aromatic DNA adduct (diagonal black line) detected in DNA purified from lung cells of a smoker. Panel c reproduced with permission from REF. 67 © (1990) Wiley-Liss.



**NESTED CASE–CONTROL STUDY**

In this study approach, samples are collected from an apparently healthy group of individuals who might be at high risk for a particular type of disease. At a later time, archived samples from the group are examined for an association between specific biomarkers and disease incidence. A 'nested' case–control approach is used to determine the cancer risk of individuals who were positive for this biomarker.

comparable to that of overcooked charbroiled meat in the United States (2.5 ng/g food)<sup>61</sup>. However, in the United States charbroiled meat is not typically consumed daily. Assuming an adult consumes 500 grams of staple food per day in Linxian<sup>62</sup>, the cumulative BP intake between the ages of 15 and 55 (the average age of oesophageal cancer diagnosis in Linxian<sup>57</sup>) would probably be 23–100 mg. Choosing the same time frame and projecting a consumption of 300 grams (about 10 ounces) of heavily charbroiled beef once a week by people in the United States, the total amount of BP intake for the same 40-year time period would be about 1.6 mg. Tobacco smoke also contains high levels of BP, and an individual who smokes 20 cigarettes a day for the same 40-year time period is potentially exposed to a maximal dose of 500 ng/day<sup>63</sup>, or about 7.3 mg in 40 years. So, using conservative estimates, over 40 years of exposure, the amount of BP ingested by the average individual from Linxian (23–100 mg) is considerably higher than that of a one-pack-a-day cigarette smoker (about 7.3 mg) or the average charbroiled-meat eater in the United States. The excessively high levels of PAH exposure among Linxian residents, coupled with the high levels of PAH–DNA adducts detected in oesophageal samples, could contribute to the increased rate of oesophageal cancer in this population.

**Human DNA adducts and cancer risk**

The presence of measurable PAH–DNA adducts in oesophageal samples collected in Linxian in 1985 indicated that PAH exposure extended back almost 20 years<sup>53</sup>. How did PAH–DNA adduct levels correlate with cancer incidence? The type of epidemiological study used to answer this question — the prospective **NESTED CASE–CONTROL STUDY** — is arguably one of the most powerful approaches available to establish an association between a biomarker and cancer risk. However, this type of study design requires a large bank of samples archived many years before the cancers appear, as well as extensive and careful follow-up to determine which

individuals did and did not develop cancer. Data collected from the Linxian population offers the unique opportunity to determine whether or not individuals with high PAH–DNA adduct levels in 1985 were more likely to develop oesophageal cancer, compared with matched individuals with low levels of PAH–DNA adducts. These experiments are underway.

Two studies (described below) have already correlated increased levels of carcinogen–DNA adduct formation with increased relative risk of cancer in individuals who have been exposed to chemical carcinogens. Prospective studies have examined associations between aflatoxin B<sub>1</sub> (AFB<sub>1</sub>)–DNA adducts and liver cancer risk in individuals with chronic dietary AFB<sub>1</sub> exposure<sup>50,51</sup>, and PAH–DNA adducts and lung cancer risk in tobacco smokers<sup>64</sup>. Both of these pioneering studies have correlated increased levels of carcinogen–DNA adduct formation with increased relative risk of cancer in individuals who have been chronically exposed to carcinogens. Clearly, there is a need to extend this type of study to many different cancers and chemical exposures, but these findings indicate that chemoprevention approaches to reduce DNA-adduct formation are needed, as they could lower cancer risk.

**Liver cancer** In some areas of China, liver cancer has long been associated with two key factors — hepatitis B virus (HBV) infection and dietary exposure to aflatoxins<sup>65</sup>. In a study designed to estimate the relative contributions of HPV and aflatoxin exposure to the aetiology of liver cancer in high-risk populations, blood and urine samples were collected from almost 20,000 Chinese men in Shanghai between 1986 and 1989 (REFS 50,51). During a 7-year follow-up period, 50 men developed liver cancer, and investigators chose 267 age- and residence-matched controls from the cohort as a comparison group. Samples of blood were assayed for HBV infection and samples of urine were assayed for the AFB<sub>1</sub>–N7-guanine (FIG. 1b), which is the excised aflatoxin–DNA adduct. Men with measurable urinary AFB<sub>1</sub>–N7-guanine adducts, and no serum anti-HBV antibodies, were over nine times more likely to develop liver cancer than men with no measurable aflatoxin exposure and no HBV infection. So, exposure to aflatoxin conferred a ninefold increased liver cancer risk.

In this population, the demographics and economics of exposure preclude removal of the carcinogen from the food supply, so investigators attempted to reduce AFB<sub>1</sub> genotoxicity by administering an agent that would prevent formation of AFB<sub>1</sub>–N7-guanine adducts. In a rodent model, dietary chemoprevention with ethoxyquin decreased the quantity of excreted AFB<sub>1</sub>–N7-guanine adduct in liver and substantially reduced the liver tumour incidence after oral aflatoxin administration<sup>20</sup>. Among volunteers in China, dietary chlorophyllin reduced levels of urinary AFB<sub>1</sub>–N7-guanine adduct<sup>66</sup>, and it remains to be seen whether or not this will become a promising protective agent against liver, and possibly other, cancers.

Table 2 | **Carcinogenic chemicals that form DNA adducts in humans**

Chemical	Source	Cancer type	References
Aflatoxin B <sub>1</sub>	Mouldy food	Liver cancer	10,11,50,51
Aristolochic acid	Chinese herbs	Kidney cancer	31,32
4-Aminobiphenyl	Dye/rubber manufacture; tobacco smoke	Bladder cancer	33–37
Benzidine	Dye manufacture	Bladder cancer	37,38
Benzo[a]pyrene (and other PAHs)	Tobacco smoke; ambient pollution; industrial waste	Lung cancer	39–41
Butadiene	Manufacture of resins, plastics, synthetic rubber	Leukaemia	42,43
MOCA	Chemical/dye manufacture	Lung and bladder cancer	37,44
NNK and NNN	Tobacco smoke; smokeless tobacco	Lung and head and neck cancer	39,45,46
Procarbazine and dacarbazine	Chemotherapeutic drugs	Leukaemia	47–49
Vinyl chloride	Polyvinylchloride manufacture	Liver cancer and angiosarcoma	12,37

MOCA, 4,4'-methylene-bis-(2-chloroaniline); NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, N'-nitrosomnicotine; PAH, polycyclic aromatic hydrocarbon.

**Lung cancer.** Tobacco smokers are exposed to a complex mixture of many different carcinogenic PAHs as well as aromatic amines (FIG. 1c), and tobacco-related nitrosamines that are known to damage DNA. The chronic nature of this exposure over a period of years makes tobacco smoke an effective lung carcinogen and also complicates the search for cancer-specific biomarkers. A nested case-control study<sup>64</sup>, within the prospective **Physicians Health Study** (see online links box), was used to investigate the relationship between PAH-DNA adduct levels in white blood cells and lung cancer risk. The Physicians Health Study involved male physicians in the United States, 15,700 of whom gave blood samples between 1982 and 1984, and 89 of whom had developed lung cancer by 1998. Among the current smokers, the 36 with lung cancer had a two times higher mean leukocyte PAH-DNA adduct level (11.4 adducts per 10<sup>8</sup> nucleotides) compared with the 64 smokers who did not have lung cancer (5.6 adducts per 10<sup>8</sup> nucleotides). Among healthy smokers, those with the highest PAH-DNA adduct levels were found to be about three times more likely to be diagnosed with lung cancer, compared with those with 50% fewer PAH-DNA adducts. Potential chemopreventives for smokers have been proposed to include dietary fruits and vegetables, although no chemoprevention studies, within the context of a nested case-control study design, have been reported at present.

#### Future directions

Since 1775 when Sir Percival Pott published his treatise on scrotal cancer in chimney sweeps<sup>2</sup> we have learned much about the underlying molecular mechanisms of cancer development, including the importance of DNA damage in **initiating and sustaining the carcinogenic process**. One way of reducing the incidence of cancer

worldwide is to identify and reduce the effects of carcinogenic exposure in regions such as Linxian. Monitoring human DNA damage will not necessarily lend clues as to the nature of an unknown aetiological agent, but identification and chemical characterization of DNA adducts in human tissues will allow investigators to confirm that a suspected exposure has occurred, and possibly to design appropriate prevention and/or chemoprevention strategies. Biomarker research in general, and DNA adduct measurements in particular, can be **used to uncover aspects of cancer aetiology that remain hidden** in the face of **classic** epidemiological approaches. Biomarker research should allow investigators to tease out multifactorial aetiological elements, one or two at a time; however, as can be seen from the examples presented here, such studies are **slow and exceedingly laborious**, requiring interdisciplinary cooperation to amass large human sample banks, document incident cancers and develop/validate different types of biomarkers.

The high levels of PAH-DNA adducts detected in oesophageal samples taken from Linxian residents over the past decades indicate that PAHs might contribute to oesophageal cancer aetiology. Our next step is to examine the association between the extent of oesophageal PAH-DNA adduct formation and the risk of oesophageal cancer in a large number of cases and controls. If PAH-DNA levels do not prove to be correlated with oesophageal cancer risk, there might be other factors involved in this complex story, and the task of generating hypotheses and validating biomarkers will continue. If DNA adduct studies are able to identify individuals who are most likely to develop neoplastic disease, there remains the potential to apply primary preventive measures, such as changing heating and cooking practices, or chemopreventive strategies, to reduce PAH-DNA adduct formation.

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#### Competing interests statement

The author declares no competing financial interests.

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