Use of DNA adducts to identify human health risk from exposure to hazardous environmental pollutants: the increasing role of mass spectrometry in assessing biologically effective doses of genotoxic carcinogens

Peter B. Farmer* and Rajinder Singh

Cancer Biomarkers and Prevention Group, Biocentre, University of Leicester, Leicester, LE1 7RH, United Kingdom

*Corresponding author:

Peter B. Farmer, Tel.: +44 [0]116 2231823, Fax: +44 [0]116 2231840

e-mail: pbf1@le.ac.uk

Abstract

The carcinogens to which humans are exposed are normally in the form of complex mixtures, and much effort has gone into determining the nature of the most significant carcinogenic components in these mixtures and their mechanisms of action. Essential to achieving this aim in exposed populations is the use of biomarkers, which can characterise the chemical nature of the carcinogens involved and identify key biological effects that result from the exposure. DNA adducts are particularly appropriate as biomarkers in the case of genotoxic carcinogens as they indicate the biologically effective dose of the genotoxin in the target tissue under study.

This review considers in particular the use of mass spectrometry (MS), which is having an increasing role in the determination of DNA adducts. Compared to other existing DNA damage detection methods, such as ^{32}P-postlabeling, HPLC-fluorescence or electrochemical detection, immunoassay based techniques and modified Comet assays, MS provides improved structural characterisation of adducts. Greater selectivity in the analyses is achieved by the use of tandem MS with selected reaction monitoring or constant neutral loss of ions. Use of capillary/nano liquid chromatography and micro/nano electrospray ionisation improves the analytical sensitivity and higher throughput may be obtained by the use of online-column switching. The application of microfluidics technology offers exciting new possibilities for interfacing sample preparation to the mass spectrometer. Despite these improvements in the use of MS for adduct detection, the main current requirement is to validate these methods both analytically and in molecular epidemiology studies. More knowledge of the stability of stored samples is required. Development of sensitive mass spectrometric DNA adductomic screening systems, and of long-term biomarkers (e.g. phosphotriester adducts that are not repaired

efficiently) seem important areas for the future assessment of the effects of human exposure to environmental genotoxins, together with studies of dose-response relationships at low doses.

1. INTRODUCTION

Biomarkers are playing an increasing role in the assessment of human exposure to hazardous environmental pollutants and in risk assessment to these compounds [1, 2]. As indicated in figure 1, biomarkers may be applied at any stage in the toxicological process, ranging from measurement of the external dose as an indicator of exposure to determining altered structure and function of cells as a biomarker of effect (Figure 1). All of these biomarkers have value in specific circumstances. Thus for example biomarkers towards the right of the scheme advise more on health effects, whereas those towards the left of the scheme inform us more on the nature of the environmental agent and its source and concentration.

In the ideal situation one would like to know of the linkages between biomarkers of exposure to biomarkers of effect and susceptibility and to adverse health effects, in order that one could derive risk assessment from the former. Although some examples exist where such relationships are known, e.g. blood levels of lead [3], and blood and hair levels of mercury [4], used as biomarkers for neurodevelopmental effects, this is generally not the case. More commonly, highly sensitive assays of exposure have been developed but the health effects associated with these is unknown, i.e. the nature of the dose-response relationship at low doses is unknown.

Endogenous formation of some biomarkers may additionally complicate the assessment of the external exposure, an example being ethylene oxide which is produced endogenously from metabolism of ethylene, possible sources of ethylene/ethylene oxide being methionine oxidation, lipid peroxidation, and the metabolising activity of intestinal bacteria [5]. Finally as environmental pollutants are normally present in complex mixtures there is also the possibility of synergistic or

antagonistic interactions which may affect the toxicity associated with a particular component.

In this review we will primarily focus on the determination of DNA adducts by mass spectrometry that indicates the biologically effective dose of environmental genotoxic carcinogens in humans. We will try to illustrate the progress in this field when compared to analysis of DNA adducts by other techniques and also highlight the limitations associated with the interpretation of DNA adduct determinations for genotoxic exposure and how these are being overcome.

2. BIOMARKERS OF BIOLOGICALLY EFFECTIVE DOSE OF GENOTOXIC CARCINOGENS: DNA ADDUCT DETERMINATION

Genotoxic carcinogens interact with nucleic acids to produce adducts, measurement of which is an indicator of the dose of active material which has reached the cells under study, termed the biologically active dose, in the individual being investigated. This thereby incorporates the effects of interindividual variation in absorption, metabolism and excretion of the compound which may affect risk assessment.

2.1. Methodology for human DNA adduct determination

The analytical methods for the determination of DNA adducts vary greatly in their sensitivity, selectivity, and practicality (e.g sample workup conditions, cost etc) for human studies [6,7]. All have their own advantages and disadvantages. Some of the most commonly used assays (e.g. ^{32}P-postlabelling and some immunoassays, see below) are capable of measuring mixtures of DNA adducts, and others (e.g. mass spectrometry (MS)) hold very high selectivity for determination of some individual

adducts. The sensitivities that can be achieved reach 1 adduct/10^{12} nucleotides, although the sensitivities of the more commonly used techniques are normally in the range of 0.1 to 1 adducts/10^{8} nucleotides. These are in a range which is suitable for most human studies, including those of DNA adducts derived from environmental and dietary sources and also for determination of endogenous levels of adducts. Thus for example adduct levels in white blood cell DNA resulting from environmental exposure to polycyclic aromatic hydrocarbons (PAHs) have been reported to be in the range 1-36 adducts/10^{8} nucleotides [8], and background adduct levels from many low molecular weight alkylating agents have been detected in the 1-10 adducts/10^{7} nucleotides range [9]. With regard to endogenous sources, normal metabolic processes generate both genotoxic agents and reactive oxygen species *in vivo* which can modify DNA bases. Oxidative DNA damage is the most abundant type of DNA base damage observed in human samples and accounts in total for at least 1 adduct/10^{5} nucleotides [9, 10].

We will first briefly mention the currently employed methodology for the determination of DNA adducts in human samples, with an indication of their particular advantages and disadvantages.

2.1.1. ^{32}P-Postlabelling

^{32}P-Postlabelling is a very versatile and widely applicable technique for DNA adduct determination, with a high sensitivity (0.1-1 adduct/10^{9} -10^{10} nucleotides) [11, 12]. However it is not a highly accurate quantitative technique. Following DNA digestion to nucleoside 3′-monophosphates, enrichment procedures to separate the adducted nucleotide from non-adducted nucleotides are normally used, such as n-butanol

extraction, nuclease P1 digestion (which removes the 3′-phosphate from normal (unmodified) nucleotides but not from some adducted nucleotides), immunoaffinity chromatography, or HPLC. The enriched product is 5′-phosphorylated with [γ-^{32}P]ATP by polynucleotide kinase, and chromatographed using 2-dimensional TLC or HPLC. The former is more sensitive but has lower resolution than HPLC. Structural information of adducts is gained from chromatographic comparison with standards. However the specificity of the postlabelling method for adducts from polyaromatic carcinogens has been put into some doubt by the work of Arif *et al.*, who questioned this chemical characterisation of adducts, detected by postlabelling and 2-dimensional TLC, from cigarette smoke-associated lung DNA [13].

The sensitivity of ^{32}P-postlabelling is limited by the specific activity of the isotope used, and by the yield of the labelling process, which should be determined if accurate quantitation is required.

2.1.2. Fluorescence-based techniques

The fluorescence characteristics of certain adducts allow their sensitive detection and quantitation. HPLC coupled to fluorescent detection has been widely used, for example for determination of DNA adducts of PAHs [14, 15] and of aflatoxin B_1 [16]. Sensitivities are as high as ca 1 adduct/10^8 nucleotides, although the use of this approach is limited as not all compounds hold suitable fluorescent properties. Extra selectivity may be achieved using synchronous fluorescence spectrophotometry (SFS) in which excitation and emission wavelengths are monitored synchronously [17, 18]. Of particular interest at present is the development of assays that combine capillary electrophoresis (CE) for separation of the adduct with laser induced fluorescence (LIF) for their detection [19, 20]. Schmitz *et al.* [21] have described the use of a

procedure that labels adducts with 4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacene-3-propionylethylenediamine (BODIPY FL EDA) as a fluorescence marker, followed by their detection by CE-LIF, a type of procedure which hopefully will have potential for ultrasensitive adduct detection. HPLC has also recently been used to separate the BODIPY FL labelled deoxynucleoside adducts of 4-aminobiphenyl (4-ABP) and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhiP) as well as the base adduct aflatoxin B_1-formamidopyrimidine, which were detected by LIF [22]. The detection limit of the labeled 4-ABP-C8-2′-deoxyguanosine adduct was estimated to be about 50 amol.

2.1.3. Immunoassay-based techniques

For reasons of ease of use, low cost, and ability to apply to high throughput studies, immunoassay holds great advantages for adduct detection assays [23, 24]. The sensitivity is high (up to ca. 1 adduct/10^8 -10^9 nucleotides) but clearly dependent on the antibody characteristics. One of the main limitations in the use of antibodies is the lack of availability of specific antibodies for all genotoxin adducts. Also some antibodies are not totally structure specific and show cross-reactivity with related compounds. Nevertheless in these cases they can be used as ‘class-specific’ antibodies. Competitive ELISA has been widely used, especially for benzo[a]pyrene (B[a]P) adducts, and sensitivity has been enhanced using a dissociation-enhanced lanthanide fluoroimmunoassay (DELFIA) [25]. Chemiluminescence provides another sensitive way to detect the end-product in adduct immunoassays, and this has been employed for example in human biomonitoring of B[a]P adducts [26] and in immunoslot-blot assays of the malondialdehyde adduct with guanine [27]. The latter immunoslot-blot method is particularly suitable for human studies as it only uses low

μg amounts of DNA [28]. Immunohistochemical detection of DNA adducts can be performed on either fixed cells or tissues sections providing qualitative or semiquantitative data. Immunohistochemical methods offer the advantage of the ability to detect DNA adducts in specific cell types within a tissue. Furthermore archived tissues stored for many years can also be examined for DNA adduct formation [23].

2.1.4. Electrochemical detection

Electrochemical detection has been shown to be of sufficient sensitivity for adduct detection, in conjunction with HPLC separation, although its use is somewhat limited as not all compounds hold suitable electrochemical properties. Oxidative damage of DNA (notably 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG)) has been particularly well studied by this technique [29, 30] although great care needs to be taken to avoid artifactual formation of such oxidised products [31].

2.1.5. Comet assay (Single cell gel electrophoresis)

Single cell gel electrophoresis, or the Comet assay, is being increasingly used for the detection of single and double strand breaks resulting from genotoxin damage [32, 33]. Although the nature of the chemical that caused these cannot be identified from this approach, methods have been developed for making the procedure more specfic for certain lesions, e.g the use of formamidopyrimidine DNA glycosylase (Fpg) for detection of 8-oxodG. Unlike the other approaches described the Comet assay requires cells rather than isolated DNA for the analysis, which has to be taken into consideration in the design of molecular epidemiology studies.

2.2. Use of mass spectrometry (MS) in DNA adduct detection

The use of MS for the detection of DNA adducts has been the subject of several comprehensive reviews e.g. Singh and Farmer [34], with particular reference to electrospray ionisation (ESI)-liquid chromatography (LC)-MS, Esmans *et al.* [35] Andrews *et al.* [36], Doerge *et al.* [37], Koc and Swenberg [38] and Watson *et al.* [39]. The approaches most usually employ collision induced dissociation (CID) tandem MS (MS/MS) with selected reaction monitoring (SRM) for the determination of:

- adducted 2'-deoxynucleosides separated from enzymically digested DNA
- adducted purine bases derived from thermal depurination or repair of DNA

It is beyond the scope of this review to carry out a detailed survey of the applications of MS methods for DNA adduct measurements, but in order to illustrate the scope and diversity of these techniques, are the following examples, all published in the year prior to this publication (2006-2007).

2.2.1. Adducted 2'-deoxynucleosides separated from enzymatically digested DNA-experimental studies

A procedure was developed in our laboratory for determining the adduct of B[a]P with the exocyclic $-NH_2$ group of 2′-deoxyguanosine by LC-MS/MS SRM using a [$^{15}N_5$]-labelled internal standard [40]. The sensitivity was 3 adducts/10^8 nucleotides, and a good correlation was obtained with ^{32}P-postlabelling, although the levels of adducts from the latter were 3.7 fold lower. Mice treated with B[a]P showed a dose dependent increase in adducts in liver and lung DNA (see Figure 2).

Other recently published examples of LC-MS determination of adducted 2'-deoxynucleosides include: O^6 methyl- and O^6 ethyl-2′-deoxyguanosine in livers from

untreated mice, with a limit of sensitivity of < 0.2 adducts/10^8 nucleotides [41]; tamoxifen adducts in a study of hepatocarcinogenesis in rats [42]; pyridyloxobutyl adducts in rat tissue DNA derived from the tobacco specific nitrosamines NNN and NNK [43]; heptanone-etheno adducts in mouse tissue [44]; the oxidative lesions of guanine, oxazolone and 8-oxodG in rat liver [45]; 8-oxodG in chemopreventive trials in mice [46].

2.2.2. Adducted purine bases derived from thermal depurination of DNA- experimental studies

Recent studies on adducted purine bases derived from thermal depurination of DNA include our development of a sensitive assay for the determination of N-7 (2-hydroxyethyl)guanine. This approach (illustrated in Figure 3A) has a limit of detection of 0.1fmol on column equivalent to 6 adducts/10^9 nucleotides, sufficient to detect background (endogenously formed) levels of adducts in rats, 1.1-3.5 adducts/10^8 nucleotides [47]. Thermal depurination of DNA was also recently reported to have been used by Lao et al [43] to study pyridyloxobutyl adducted guanine, as a possible marker of tobacco specific nitrosamine exposure.

2.2.3. Human studies: determination of adducts in tissue DNA and of urinary modified bases or 2'deoxynucleosides derived from DNA repair or the nucleotide pool

We have recently completed a major study on the determination of 8-oxodG by LC-MS/MS in human lymphocyte DNA [28, 48]. The aim of this was to evaluate the relationship between exposure to environmental air pollution and endogenous oxidative DNA damage in human populations with varying exposure from three cities

(Prague (Czech Republic), Kosice (Slovak Republic) and Sofia (Bulgaria)). Populations exposed to urban pollution and controls were studied. The level of 8-oxodG was significantly increased in individuals in Kosice exposed to environmental air pollution compared to unexposed individuals. Of interest was the negative correlation between the level of 8-oxodG adducts and the level of total PAH (bulky) and B[a]P DNA adducts [48]. More recently we have studied the excretion of 8-oxodG in human urine [49], using an adaptation of the procedure we derived for 8-oxodG analysis in DNA. For urinary analysis the sample was analysed after purification with solid-phase extraction with a recovery of 81% and limit of detection of 5 fmol on column [49]. Levels of 0.41 $\pm$ 0.39 pmol/μmol creatinine were found in urine of human volunteers (Figure 3B).

Other recent examples of using MS for the determination of urinary modified bases and deoxynucleosides are: N-7 ethylguanine in urine from smokers and non-smokers [50]; etheno-DNA adducts in human urine after olive oil ingestion [51], aflatoxin B_1-guanine in human urine as a marker of intake of dietary aflatoxin B_1 [52]; Oltipraz is being examined as a possible chemopreventive agent for aflatoxin and its effect on DNA oxidation was studied by analysis of oxidized guanine derivatives in human urine by LC-MS/MS [53].

Other DNA adducts recently detected in human tissues include $1,N^2$-propanodeoxyguanosine adducts derived from acetaldehyde and crotonaldehyde in lung and liver tissues [54] and acetaldehyde-derived adducts (as a possible marker of ethanol genotoxicity) in human liver, measured after reduction as N^2 ethyl-2′-deoxyguanosine [55], 4-ABP adducts in DNA from human bladder [56]; endogenous cyclic DNA adducts derived from trans-4-hydroxy-2-nonenal with 2′-deoxyguanosine in human brain tissues [57] and N-7 ethylguanine in human liver [58].

2.2.4. Accelerator mass spectrometry (AMS)

For a limited number of LC-MS assays the sensitivity matches that of ^{32}P-postlabelling (the DNA adduct measuring technique which is most widely applicable and most commonly used), although this is not the case for many adducts. When greater sensitivity is required using mass spectral techniques, one possibility is to use AMS, which measures isotope ratios. This is the most sensitive analytical method so far available for detecting DNA adducts, with a limit of detection that may be as low as 1 adduct per 10^{12} nucleotides [59]. AMS has been used to detect the isotopic content of adducts after administration of labelled (^{14}C or ^{3}H) genotoxin [60]. Ethical permission is of course essential for such experiments where a labelled compound is administered to humans. Such approaches are clearly not applicable to human environmental studies but they give very valuable information in experimental studies (see below). Structural information on the adduct may be gained from chromatographic properties and comparison with standards. The sensitivity is limited by the background level of the isotope in 'normal' DNA. For interpretation of the results it is necessary to guard against the possibility of false positives because of biosynthetic incorporation, contamination or exchange of label to ensure that the results represent a true depiction of adduct levels.

Experimental animal and human applications of AMS are increasing in number in view of the extra sensitivity and consequently smaller sample size that is required for adduct analysis. Recent applications of the use of AMS include the detection of adducts from: [^{14}C]-acrylamide in mouse sperm DNA [61]; [^{14}C]-2,6-dimethyl, 3,5-dimethyl and 3-ethylaniline in mouse tissue DNA [62]; [^{14}C]-PhIP in human colon

DNA [63]; the novel anticancer agent '11-beta-dichloro' in tissue and xenografted tumours in mice [64]; methyl tert-butyl ether and tert-butyl alcohol in mouse liver, lung and kidney DNA [65].

An alternative approach to enable AMS to be used in human studies without the administration of a labelled compound is the development of postlabelling procedures to incorporate the isotope of interest into an unlabelled adduct, which is then determined by AMS. The approach of [^{14}C]-postlabelling, in which the adduct is labelled with a [^{14}C]-derivatising agent, seems promising [66, 67] although it is only at a very early stage of development.

2.3. Long lifetime DNA adducts

The lifetime of DNA adducts is very variable, dependent on their repair mechanism and their chemical stability, which must clearly be taken into consideration in designing biomarker studies. One measurement which would be of benefit to epidemiologists would be that of an adduct which was not repaired and which would give a long term assessment of genotoxin exposure. One possibility may be to determine phosphotriesters (PTEs) [68]. In addition to reacting with DNA bases, many genotoxic agents react with the oxygen of the internucleotide phosphodiester linkages to form PTEs. These compounds include N-alkylnitrosoureas, dialkylsulfates, alkyl methanesulfonates, cyclophosphamide and cyanoethylene oxide. S_N1 reacting compounds (e.g. nitrosoureas), which tend to have a higher proportion of O to N alkylation of nucleic acid bases, are also more reactive towards the phosphate group. In mammalian cells PTEs appear resistant to DNA repair although bacterial systems are capable of their removal [69]. As a consequence of their stability PTEs may accumulate with age and their measurement may be ideal to assess cumulative

genotoxic exposure. The biological consequences of PTE formation are so far unknown.

Most determinations of PTEs have been carried out by ^{32}P-postlabelling, commonly after gel separation [70] Separation of all the 16 possible species, from the 4 possible adjacent nucleotides, and identification of the adducted groups is challenging by this approach. Pioneering work using a mass spectrometric approach which may have more sensitivity and specificity was carried out by Haglund *et al.* on ethyl PTEs which were analysed by miniaturized LC ESI-MS/MS [71]. Recently Zhang *et al* have reported on a sensitive LC atmospheric pressure chemical ionisation (APCI) MS/MS method for determining the methyl PTE from thymidyl(3′,5′)thymidine with a limit of quantitation of 6.4 adducts/10^8 nucleotides [72] We have recently described the detection of phosphodiester adducts formed by the reaction of the diol epoxide metabolite of B[a]P with 2'-deoxynucleotides using negative ESI-MS/MS CID. These results lend support to the potential of phosphotriester adducts to be formed in DNA by B[a]P, which have been postulated to have adverse biological implications, such as the generation of DNA strand breaks [73]. Further development of such approaches to make them applicable for human monitoring seems desirable.

2.4. Assessment of mass spectral approaches for the detection of DNA adducts, recent advances and future prospects

The advantages of the use of MS for determination of DNA adducts are that it gives improved structural characterisation compared to other methods, and allows extremely accurate quantification. Also unlike most of the methods discussed above, the sensitivity and selectivity of mass spectrometric approaches continues to rise, as

new technological developments are continually taking place. Initially the advances in the use of MS were made by the introduction of new ionisation techniques such as ESI and matrix assisted laser desorption ionisation (MALDI), and the development of interfaces allowing introduction of samples directly from HPLC separations. ESI offers the advantage when compared to gas chromatography-MS that non-polar/volatile compounds can be directly analysed without the requirement for derivatization at high temperatures to make them more volatile prior to analysis. Tandem MS with SRM or constant neutral loss (CNL) of ions led to much greater selectivity, and the amount of DNA that was needed for an analysis decreased to a level that is acceptable for human studies. More recently improvements have continued by the introduction of capillary/nano LC and micro/nano-ESI, allowing even greater sensitivity [74, 75, 76]. Conventional MS techniques have not had a very high throughput in the past. However increased sample throughput can be obtained by using online-column switching valve technology which confers the the advantage that an enzymatically digested DNA sample can be analysed directly by LC-MS without the requirement for off-line pre-purification of the DNA adduct of interest from the vast excess of unmodified 2′-deoxynucleosides that are also generated. Thus online-column switching LC-MS is ideally suited for the large scale analysis of human DNA samples [77].

However MS does have some disadvantages. For example ^{32}P-postlabelling still has higher sensitivity than MS for many analyses, and is more widely applicable than MS particularly when the amount of DNA available for analysis is limited. The development of a validated mass spectral approach requires the availability of a standard, preferably labelled with stable isotopes, for accurate quantitation, which may be synthetically very challenging. However the use of stable isotope internal

standards does allow highly accurate and reproducible data to be obtained. MS is also resource intensive compared with many other adduct measuring techniques requiring expensive specialised equipment.

Finally, unlike ^{32}P-postlabelling, MS so far has not been routinely used to carry out a global screen of mixtures of adducts. However the potential of using mass spectrometry for the screening of DNA adducts has recently become apparent since it has been found that many adducted 2′-deoxynucleosides show a common fragmentation in positive ESI-MS/MS, this being the neutral loss of 116u following CID. This corresponds to the loss of the deoxyribose residue. Examples of this are shown in Figure 4 for adducts derived from B[a]P, dibenzo[a,l]pyrene (DB[a,l]P), malondialdehyde and 8-oxodG. Selective detection of these may be achieved by SRM of the MH^+ to $[MH\text{-}116]^+$ fragmentation. Multiple determinations of these adducts are possible, e.g. Figure 4E shows the DNA adducts from B[a]P and DB[a,l]P being monitored simultaneously following the dosing of HepG2 cells with an equimolar mixture of both PAHs, and many more adducts could be added to such an analysis. The concept of producing a screen of all DNA adducts in a sample by monitoring a large range of such MH^+ to $[MH\text{-}116]^+$ transitions was proposed by Kanaly *et al.* [78] and preliminary work on inter-tissue variation of adducts has been reported [79]. This technique holds great potential for gaining further structural information on adduct differences between populations exposed to environmental genotoxins and corresponding controls.

There are also exciting new possibilities for future improvements in interfacing sample separation to the mass spectrometer, with high sensitivity and minimal sample sizes, using LC chips. These use microfluidics-based technology for high-pressure nanoflow LC-MS systems, and incorporate the sample purification, separation and

electrospray interface on a single chip thus allowing the processing of lower volume samples with complete automation of the analysis procedure and the possibility of screening based applications [80, 81, 82].

2.5. Validation of DNA adduct determination and its use in risk assessment

In order for DNA adduct measurements to be used in risk assessment procedures, validation is necessary both for the analytical procedures and for the ability of adducts to predict disease in molecular epidemiological studies. Validation of many analytical methods for DNA adduct determination (with regard to the assay reliability, inter-laboratory variability, sampling strategy etc), and of their application to risk assessment is not yet complete. However examples where extensive analytical validation has occurred are ^{32}P-postlabelling [83, 84] and analysis of 8-oxodG [31, 85, 86, 87, 88]. Thorough inter-laboratory studies of assay reliability and variability have been performed for 4-ABP and also B[a]P DNA adducts in which radiolabeling was compared to ^{32}P-postlabelling, MS and immunoassay methods [26, 89, 90]. In all of these studies there were inter-laboratory comparison of DNA samples that were modified in vitro, extracted from exposed animals and, in the case of B[a]P extracted from human tissue. However further work still needs to be carried out using inter-laboratory trials for MS determination of DNA adducts.

With regard to the validation of the use of DNA adduct formation as a biological predictor of cancer, it is generally believed that the extent of DNA adduct formation is indicative of biological risk. The classic demonstration of a relationship of adducts with disease is the pioneering study by Ross *et al*. [91] which showed that the excretion of a urinary aflatoxin B_1-guanine adduct was statistically related to the subsequent incidence of hepatocellular carcinoma in a nested case control study of a

Chinese population who were ingesting aflatoxin B_1 in their diet [92]. A recent study by Gunter *et al.* have found that there was a 2.8-fold increase in the risk of colon adenoma in the quartile of individuals with the highest white blood cell PAH DNA adduct levels compared to the lowest quartile [93]. Many other associations have been drawn between adducts and biological effect in human studies, which have been well summarised in the review of Kyrtopoulos [94]. Overall there is mounting evidence that suggests that DNA adducts (the evidence being notably for bulky adducts) are associated with higher cancer risk on a population basis. However it should be noted that the nature of the dose-response relationship is uncertain at low doses and that there is evidence for saturation of some adduct levels (i.e. a non-linear dose response) at high exposure [95, 96]. Also to improve the interpretation of the results we need more information on intra-individual variation, and genetic factors affecting an individual's susceptibility.

3. CONCLUSION

Mass spectrometry has made enormous gains in its applicability for the detection of DNA adducts, and it is now possible to determine DNA adducts by MS with a sensitivity comparable to that of the other analytical approaches available for adduct detection, such as ^{32}P-postlabeling or immunoassays. Highly accurate and reproducible data can be obtained indicating that MS should provide more reliable results for biomonitoring of exposure to genotoxic carcinogens in human molecular epidemiological studies. Despite the potential availability of such data, one of the major questions remaining in the field of risk assessment to environmental carcinogens is whether or not there is a threshold in the dose response relationship. For human biomonitoring of such environmental chemicals, the current situation is

that the analytical methods for biomarkers of exposure have developed faster than our ability to understand the relationship between these biomarkers and biological outcome, i.e. we are not able to estimate risk directly from exposure biomarkers for many compounds. This is particularly the case for biomarkers of genotoxic carcinogens, where the DNA damage produced by genotoxic carcinogens (adducts) can often be detected at very low levels in human tissues, but biological effects are unknown, and may indeed be unmeasurable using existing assays. The result of this is that there is a paucity of data regarding the nature of the dose-response relationship at low doses, and also whether or not thresholds exist below which there is no tangible risk to humans.

Many highly sensitive mass spectrometric methods now exist for the determination of DNA adducts, and the main current requirement is to validate these both analytically and in molecular epidemiology studies. In view of the likely need to store samples over extended periods a greater understanding of stability of adducts is also required. Development of sensitive mass spectrometric DNA adduct screening systems, and of long-term biomarkers (e.g. adducts that are not repaired) seem important areas for the future assessment of the effects of human exposure to environmental genotoxins, together with studies of dose-response relationships at low doses.

Acknowledgements

The author acknowledges the Medical Research Council, grant number G0100873 for financial support. The authors are members of ECNIS (Environmental Cancer Risk, Nutrition and Individual Susceptibility) a network of excellence operating within the European Union 6th Framework Program, Priority 5: "Food Quality and Safety" (Contract 513943).

References

[1] Human Biomonitoring for Enviromental Chemicals, National Research Council of the National Academies, USA. The National Academies Press, USA., 2006.

[2] Guidance for the Interpretation of Biomonitoring Data, ECETOC Document No 44, 2005.

[3] R.L. Canfield, C.R Henderson, D.A. Cory-Slechta, C. Cox, T.A. Jusko, B.P.Lanphear, Intellectual impairment in children with blood lead concentrations below 10μg per deciliter, New Eng. J Med. 348 (2003) 1517-1526.

[4] D.C. Rice, R. Schoeny, K. Mahaffey, Methods and rationale for derivation of a reference dose for methylmercury by the U.S. EPA, Risk Anal. 23 (2003) 107-115.

[5] R. Thier, H.F. Bolt, Carcinogenicity and genotoxicity of ethylene oxide: New aspects and recent advances, Crit. Rev. Toxicol. 30 (2000) 595-608.

[6] P.B. Farmer, Exposure biomarkers for the study of toxicological impact on carcinogenic processes. In: P. Buffler, J. Rice, M. Bird, P. Boffetta (Eds.), Mechanisms of carcinogenesis. Contributions of molecular epidemiology, IARC Scientific Publication No. 157, IARC Press, Lyon, 2004. pp 71-90.

[7] D.H. Phillips, DNA adducts as markers of exposure and risk, Mutat. Res. 577 (2005) 284-292.

[8] E. Kriek, M. Rojas, K. Alexandrov, H. Bartsch, Polycyclic aromatic hydrocarbon-DNA adducts in humans: relevance as biomarkers for exposure and cancer risk, Mutat. Res. 400 (1998) 215-231.

[9] R. De Bont, N. van Larebeke, Endogenous DNA damage in humans: a review of quantitative data, Mutagenesis 19 (2004) 169-185.

[10] B. Halliwell, Oxidative stress and cancer: have we moved forward? Biochem J. 401 (2007) 1-11.

[11] K. Randerath, E. Randerath. ^{32}P-Postlabelling methods for DNA adduct detection: overview and critical evaluation. Drug Metab. Rev. 26 (1994) 67-85.

[12] D.H. Phillips, Detection of DNA modifications by the ^{32}P-postlabelling assay. Mutat. Res. 378 1997 1-12.

[13] J.M. Arif, C. Dresler, M.L. Clapper, C.G. Gairola, C. Srinivasan, R.A. Lubet, R.C. Gupta, Lung DNA adducts detected in human smokers are unrelated to typical polyaromatic carcinogens, Chem. Res. Toxicol, 19 (2006) 295-299.

[14] M. Rojas, K. Alexandrov, F.J. van Schooten, M. Hillebrand, E. Kriek, H. Bartsch, Validation of a new fluorometric assay for benzo[a]pyrene diolepoxide-DNA adducts in human white blood cells: comparisons with ^{32}P-postlabeling and ELISA, Carcinogenesis 15 (1994) 557-560.

[15] R.W.L. Godschalk, I.T.M. Vermeer, E. Kriek, B. Floot, P.A.E.L. Schilderman, E.J.C. Moonen, J.C.S. Kleinjans, F.J. van Schooten, Comparison of ^{32}P-postlabelling and HPLC-FD analysis of DNA adducts in rats acutely exposed to benzo[*a*]pyrene, Chem. Biol. Inter. 104 (1997) 41-54.

[16] R.E. Sotomayor, M. Washington, L. Nguyen, R. Nyang'anyi, D..M. Hinton, M. Chou, Effects of intermittent exposure to aflatoxin B_1 on DNA and RNA adduct formation in rat liver: dose response and temporal patterns, Toxicol. Sci. 73 (2003) 329-338.

[17] C.C. Harris, G. LaVeck, J. Groopman, V.L. Wilson, D. Mann, Measurement of aflatoxin B_1, its metabolites, and DNA adducts by synchronous fluorescence spectrophotometry, Cancer Res. 46 (1986) 3249-3253.

[18] D.K. Manchester, A. Weston, J-S. Choi, G.E. Trivers, P.V. Fennessey, E. Quintana, P.B. Farmer, D.L. Mann, C.C. Harris, Detection of benzo[a]pyrene diol-

epoxide-DNA adducts in human placenta, Proc. Natl. Acad. Sci., USA 85, (1988) 9234-9247.

[19] E. Carrilho, A.P. Formenton-Catai, F.M. Lancas, D.M. Pereira, dGMP-BPDE DNA adduct investigation in environmentally exposed rural workers by capillary electrophoresis with laser-induced fluorescence detection, J. Brazilian Chem. Soc. 16 (2005) 220-226.

[20] X.C. Le, J. Xing, J. Lee, S.A. Leadon, M. Weinfeld, Inducible repair of thymine glycol detected by an ultrasensitive assay for DNA damage, Science 150 (1998) 1101-1102.

[21] O.J. Schmitz, C.C. Worth, D. Stach, M. Wiessler, Capillary electrophoresis analysis of DNA adducts as biomarkers for carcinogenesis, Angew. Chem. Int. Ed. Engl. 41 (2002) 445-448.

[22] H.G. Jang, M. Park, J.S. Wishnok, S.R. Tannenbaum, G.N. Wogan, Hydroxyl-specific fluorescence labeling of ABP-deoxyguanosine, PhIP-deoxyguanosine, and AFB_1-formamidopyrimidine with BODIPY-FL, Anal. Biochem. 359 (2006) 151-160.

[23] R.M. Santella, Immunological methods for detection of carcinogen-DNA damage in humans. Cancer Epidem. Biom. Prev. 8 (1999) 733-739.

[24] M.C. Poirier, Human exposure monitoring, dosimetry, and cancer risk assessment: the use of antisera specific for carcinogen-DNA adducts and carcinogen-modified DNA. Drug Metab. Rev. 26 (1994) 87-109.

[25] B. Schoket, W.A. Doty, I. Vincze, P.T. Strickland, G.M. Ferri, G. Assennato, M.C. Poirier, Increased sensitivity for determination of polycyclic aromatic hydrocarbon-DNA adducts in human DNA samples by dissociation-enhanced lanthanide fluoroimmunoassay (DELFIA), Cancer Epidem Biom Prev 2 (1993) 349-353.

[26] R.L. Divi, F.A. Beland, P.P. Fu, L.S. von Tungeln, B. Schoket, J. E. Camara, M. Ghei, N. Rothman, R. Sinha, M.C. Poirier, Highly sensitive chemiluminescence immunoassay for benzo[a]pyrene-DNA adducts: validation by comparison with other methods, and use in human biomonitoring. Carcinogenesis 23 (2002) 2043-2049.

[27] C. Leuratti, R. Singh, C. Lagneau, P.B. Farmer, J.P. Plastaras, L.J. Marnett, D.E.G. Shuker, Determination of malondialdehyde-induced DNA damage in human tissues using an immunoslot blot assay. Carcinogenesis 19 (1998) 1919-1924.

[28] R. Singh, B. Kaur, I. Kalina, T.A. Popov, T. Georgieva, S. Garte, B. Binkova, R.J. Sram, E. Taioli, P.B. Farmer, Effects of environmental pollution on endogenous oxidative DNA damage in humans, Mutat. Res. 620 (2007) 71-82.

[29] K. Hensley, K.S. Williamson, M.L. Maidt, S.P. Gabbita, P. Grammas, R.A. Floyd, Determination of biological oxidative stress using high performance liquid chromatography with electrochemical detection (HPLC-ECD), J. High Resolut. Chromatogr. 22 (1999) 429-437.

[30] H. Kasai, A new automated method to analyze urinary 8-hydroxydeoxyguanosine by a high-performance liquid chromatography-electrochemical detector system, J. Radiat, Res. 44 (2003) 185-189.

[31] European Standards Committee on Oxidative DNA Damage (ESCODD), Measurement of DNA oxidation in human cells by chromatographic and enzymic methods, Free Radical Biol. Med. 34 (2003) 1089-1099.

[32] A.R. Collins, The comet assay for DNA damage and repair: principles, applications, and limitations, Mol. Biotechnol. 26 (2004) 249–261.

[33] P. Moller, The alkaline Comet assay: towards validation in biomonitoring of DNA damaging exposures, Basic Clin. Pharmacol. Toxicol. 98 (2006) 336-345.

[34] R. Singh, P.B. Farmer, Liquid chromatography-electrospray ionization-mass spectrometry: the future of DNA adduct detection, Carcinogenesis 27 (2006) 178–96.

[35] E.L. Esmans, D. Broes, I. Hoes, F. Lemiere, K. Vanhoutte, Liquid chromatography-mass spectrometry in nucleoside, nucleotide and modified nucleotide characterization, J. Chromatogr. A 794 (1998) 109-27.

[36] C.L. Andrews, P. Vouros, A. Harsch, Analysis of DNA adducts using high-performance separation techniques coupled to electrospray ionization mass spectrometry J. Chromatogr. A 856 (1999) 515-26.

[37] D.R. Doerge, M.I. Churchwell, F.A. Beland, Analysis of DNA adducts from chemical carcinogens and lipid peroxidation using liquid chromatography and electrospray mass spectrometry, J. Environ. Sci. Health, Part C Environ. Carcin. Ecotox. Revs. 20 (2002) 1-20.

[38] H. Koc, J.A. Swenberg, Applications of mass spectrometry for quantitation of DNA adducts, J. Chromatogr. B 778 (2002) 323-43.

[39] D.G. Watson, C. Atsriku, E.J. Oliveira, Review: role of liquid chromatography-mass spectrometry in the analysis of oxidation products and antioxidants in biological systems, Anal. Chim. Acta 492 (2003) 17-47.

[40] R. Singh, M. Gaskell, R.C. Le Pla, B. Kaur, A. Azim-Araghi, J. Roach, G. Koukouves, V.L. Souliotis, S.A. Kyrtopoulos, P.B. Farmer, Detection and quantitation of benzo[a]pyrene derived DNA adducts in mouse liver by liquid chromatography-tandem mass spectrometry: comparison with ^{32}P-postlabeling, Chem. Res. Toxicol. 19 (2006) 868-878.

[41] M.I. Churchwell, F.A. Beland, D.R. Doerge, Quantification of O^6-methyl and O^6-ethyl deoxyguanosine adducts in C57BL/6N/$Tk^{+/-}$ mice using LC/MS/MS, J. Chromatogr. B 844 (2006) 60-66.

[42] V.P. Tryndak, L. Muskhelishvili, O. Kovalchuk, R. Rodriguez-Juarez, B. Montgomery, M.I. Churchwell, S.A. Ross, F.A. Beland, I.P. Pogribny, Effect of long-term tamoxifen exposure on genotoxic and epigenetic changes in rat liver: implications for tamoxifen-induced hepatocarcinogenesis, Carcinogenesis 27 (2006) 1713-1720.

[43] Y.B. Lao, P.W. Villalta, S.J. Sturla, M.Y. Wang, S.S. Hecht, Quantitation of pyridyloxobutyl DNA adducts of tobacco-specific nitrosamines in rat tissue DNA by high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry, Chem. Res. Toxicol. 19 (2006) 674-682.

[44] M.V. Williams, S.H. Lee, M. Pollack, I.A. Blair, Endogenous lipid hydroperoxide-mediated DNA adduct formation in min mice, J. Biol. Chem. 281 (2006) 10127-10133.

[45] B. Matter, D. Malejka-Giganti, A.S. Csallany, N. Tretyakova, Quantitative analysis of the oxidative DNA lesion, 2,2-diamino-4-(2-deoxy-beta-D-erythro-pentofuranosyl)amino]-5(*2H*)-oxazolone (oxazolone), in vitro and in vivo by isotope dilution-capillary HPLC-ESI-MS/MS, Nucleic Acids Res. 34 (2006) 5449-5460.

[46] R.G. Tunstall, R. Sharma, S. Perkins, S. Sale, R. Singh, T.W. Gant, P.B. Farmer, W.P. Steward, A. Gescher, Cyclooxygenase-2 expression and oxidative DNA adducts in murine intestinal adenomas: modification by dietary curcumin and implications for clinical trials, Eur. J. Cancer 42 (2006) 415-421.

[47] D.A. Marsden, D.J.L. Jones, J.H. Lamb, E.M. Tompkins, P.B. Farmer, K. Brown, Determination of endogenous and exogenously derived N7-(2-hydroxyethyl)guanine adducts in ethylene oxide-treated rats, Chem. Res. Toxicol. 20 (2007) 290-299.

[48] R. Singh, R.J. Sram, B. Binkova, I. Kalina, T.A. Popov, T. Georgieva, S. Garte, E. Taioli, P.B. Farmer, The relationship between biomarkers of oxidative DNA

damage, polycyclic aromatic hydrocarbon DNA adducts, antioxidant status and genetic susceptibility following exposure to environmental air pollution in humans, Mutat. Res. 620 (2007) 83-92.

[49] M.S. Cooke, R. Singh, G.K. Hall, V. Mistry, T. Duarte, P.B. Farmer, M.D. Evans, Evaluation of enzyme-linked immunosorbent assay and liquid chromatography-tandem mass spectrometry methodology for the analysis of 8-oxo-7,8-dihydro-2′-deoxyguanosine in saliva and urine, Free Radical Biol. Med. 41 (2006) 1829-1836.

[50] M.R. Chao, C.J. Wang, L.W. Chang, C.W. Hu, Quantitative determination of urinary N7-ethylguanine in smokers and non-smokers using an isotope dilution liquid chromatography/tandem mass spectrometry with on-line analyte enrichment, Carcinogenesis 27 (2006) 146-151.

[51] P.R. Hillestrom, M.I. Covas, H.E. Poulsen, Effects of dietary virgin olive oil on urinary excretion of etheno-DNA adducts, Free Radical Biol. Med. 41 (2006) 1133-1138.

[52] P.A. Egner, J.D. Groopman, J.S. Wang, T.W. Kensler, M.D. Friesen, Quantification of aflatoxin-B_1-N^7-guanine in human urine by high-performance liquid chromatography and isotope dilution tandem mass spectrometry, Chem. Res. Toxicol. 19 (2006) 1191-1195.

[53] B. Glintborg, A. Weimann, T.W. Kensler, H.E. Poulsen, Oltipraz chemoprevention trial in Qidong, People's Republic of China: Unaltered oxidative biomarkers, Free Radical Biol. Med. 41 (2006) 1010-1014.

[54] S. Zhang, P.W. Villalta, M.Y. Wang, S.S. Hecht, Analysis of crotonaldehyde- and acetaldehyde-derived 1,N-2-propanodeoxyguanosine adducts in DNA from

human tissues using liquid chromatography electrospray ionization tandem mass spectrometry, Chem. Res. Toxicol. 19 (2006) 1386-1392.

[55] M.Y. Wang, N.X. Yu, L. Chen, P.W. Villalta, J.B. Hochalter, S.S. Hecht, Identification of an acetaldehyde adduct in human liver DNA and quantitation as N-2-ethyldeoxyguanosine, Chem. Res. Toxicol. 19 (2006) 319-324.

[56] B. Zayas, S.W. Stillwell, J.S. Wishnok, L.J. Trudel, P. Skipper, M.C. Yu, S.R. Tannenbaum, G.N. Wogan, Detection and quantification of 4-ABP adducts in DNA from bladder cancer patients, Carcinogenesis 28 (2007) 342-349.

[57] X. Liu, M.A. Lovell, B.C. Lynn, Detection and quantification of endogenous cyclic DNA adducts derived from trans-4-hydroxy-2-nonenal in human brain tissue by isotope dilution capillary liquid chromatography nanoelectrospray tandem mass spectrometry, Chem. Res. Toxicol. 19 (2006) 710-718.

[58] L. Chen, M. Wang, P.W. Villalta, S.S. Hecht, Liquid chromatography–electrospray ionization tandem mass spectrometry analysis of 7-ethylguanine in human liver DNA, Chem. Res. Toxicol. 20 (2007) 1498-1502.

[59] K.W. Turteltaub, K.H. Dingley, Application of accelerated mass spectrometry (AMS) in DNA adduct quantification and identification, Toxicol. Lett. 102-103 (1998) 435-439.

[60] K. Brown, E.M. Tompkins, I.N.H. White, Applications of accelerator mass spectrometry for pharmacological and toxicological research, Mass Spec. Rev., 25 (2006) 127-145.

[61] Q.Y. Xie, H.F. Sun, Y.F. Liu, X.F. Ding, D.P. Fu, K.X. Liu, Adduction of biomacromolecules with acrylamide (AA) in mice at environmental dose levels studied by accelerator mass spectrometry, Toxicol. Lett. 163 (2006) 101-108.

[62] P.L. Skipper, L.J. Trudel, T.W. Kensler, J.D. Groopman, P.A. Egner, R.G. Liberman, G.N. Wogan, S.R Tannenbaum, DNA adduct formation by 2,6-dimethyl-, 3,5-dimethyl- and 3-ethylaniline in vivo in mice, Chem. Res. Toxicol. 19 (2006) 1086-1090.

[63] M.A. Malfatti, K.H. Dingley, S. Nowell-Kadlubar, E.A. Ubick, N. Mulakken, D. Nelson, N.P. Lang, J.S. Felton, K.W. Turteltaub, The urinary metabolite profile of the dietary carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine is predictive of colon DNA adducts after a low-dose exposure in humans, Cancer Res., 66 (2006) 10541-10547.

[64] S.M. Hillier, J.C. Marquis, B. Zayas, J.S. Wishnok, R.G. Liberman, P.L. Skipper, S.R Tannenbaum, J.M. Essigman, R.G. Croy, DNA adducts formed by a novel antitumor agent 11-beta-dichloro in vitro and in vivo, Mol. Cancer Therap. 5 (2006) 977-984.

[65] Y. Yuan, H.F. Wang, H.F. Sun, H.F. Du, L.H. Xu, Y.F. Liu, X.F. Ding, D.P. Fu, K.X. Liu, Adduction of DNA with MTBE and TBA in mice studied by accelerator mass spectrometry, Environ. Toxicol. 22(2007) 630-635.

[66] E.M. Tompkins, P.B. Farmer J.H. Lamb, R. Jukes, K. Dingley, E. Ubick, K.W. Turteltaub, E.A. Martin, K. Brown, A novel ^{14}C-postlabeling assay using accelerator mass spectrometry for the detection of O^6-methyldeoxyguanosine adducts, Rapid Commun. Mass Spectrom. 20 (2006) 883-891.

[67] R. Goldman, B.W. Day, T.A. Carver, R.J. Mauthe, K.W. Turteltaub, P.G. Shields, Quantitation of benzo[a]pyrene-DNA adducts by postlabeling with C-14-acetic anhydride and accelerator mass spectrometry, Chem-Biol. Interact. 126 (2000) 171-183.

[68] K.V. Schooter, DNA phosphotriesters as indicators of cumulative carcinogen induced damage, Nature 274 (1978) 612-614.

[69] P. Bannon, W. Verly, Alkylation of phosphates and stability of phosphate triesters in DNA, Eur. J. Biochem. 31 (1972) 103-11.

[70] Y. Guichard, G.D.D. Jones, P.B. Farmer, Detection of DNA alkylphosphotriesters by ^{32}P-postlabeling: evidence for the non-random detection of phosphotriester lesions *in vivo*. Cancer Res. 60 (2000) 1276-1282.

[71] J. Haglund, W. Van Dongen, F. Lemiere, E.L. Esmans, Analysis of DNA-phosphate adducts in vitro using miniaturized LC-ESI-MS/MS and column switching: Phosphotriesters and alkyl cobalamins, J. Amer. Soc. Mass Spectrom. 15 (2004) 593-606.

[72] F. Zhang, M.J. Bartels, L.H. Pottenger, B.B. Gollapudi, M.R. Schisler, Quantitation of lower levels of the DNA adduct of thymidylyl(3'-5')thymidine methyl phosphotriester by liquid chromatography/negative atmospheric pressure chemical ionization tandem mass spectrometry, Rapid Commun. Mass Spectrom. 21 (2007) 1043-1048.

[73] M. Gaskell, B. Kaur, P.B. Farmer, R. Singh, Detection of phosphodiester adducts formed by the reaction of benzo[a]pyrene diol epoxide with 2'-deoxynucleotides using collision-induced dissociation electrospray ionization tandem mass spectrometry Nucleic Acids Res. 35 (2007) 5014-5027.

[74] J. Abian, A.J. Oosterkamp, E. Gelpi, Comparison of conventional, narrow-bore and capillary liquid chromatography/mass spectrometry for electrospray ionization mass spectrometry: practical considerations, J. Mass Spectrom. 34 (1999) 244-254.

[75] K. Vanhoutte, W. van Dongen, I. Hoes, F. Lemiere, E.L. Esmans, H. van Onckelen, E. van den Eeckhout, R.E.J. van Soest, A.J. Hudson, Development of a

nanoscale liquid chromatography/electrospray mass spectrometry methodology for the detection and identification of DNA adducts, Anal. Chem. 69 (1997) 3161-3168.

[76] E.M. Ricicki, J.R. Soglia, C. Teitel, R. Kane, F. Kadlubar, P. Vouros, Detection and quantification of *N*-(deoxyguanosin-8-yl)-4-aminobiphenyl adducts in human pancreas tissue using capillary liquid chromatography-microelectrospray mass spectrometry, Chem. Res. Toxicol. 18 (2005) 692-699.

[77] D.R. Doerge, M.I. Churchwell, J-L. Fang, F.A. Beland, Quantification of etheno-DNA adducts using liquid chromatography, on-line sample processing and electrospray tandem mass spectrometry. Chem. Res. Toxicol., 13 (2000) 1259-1264.

[78] R.A. Kanaly, T. Hanaoka, H. Sugimura, H. Toda, S. Matsui, T. Matsuda, Development of the adductome approach to detect DNA damage in humans, Antiox. Redox Signal. 8 (2006) 993-1001.

[79] R.A. Kanaly, S. Matsui, T. Hanaoka, T. .Matsuda, Application of the adductome approach to assess intertissue DNA damage variations in human lung and esophagus, Mutat. Res. 625 (2007) 83-93.

[80] A.J. De Mello, Chip-MS: coupling the large with the small, *Lab on a Chip* 1 (2001) 7N-12N.

[81] P. Horvatovich, N.I. Govorukhina, T.H. Reijmers, A.G. Van der Zee, F. Suits R. Bischoff, Chip-LC-MS for label-free profiling of human serum. Electrophoresis 28 (2007) 4493-4505.

[82] J. Hardouin, M. Duchateau, , J. Joubert-Caron, M. Caron, Usefulness of an integrated microfluidics device (HPLC-Chip-MS) to enhance confidence in protein identification by proteomics, Rapid Commun. Mass Spectrom. 20 (2006) 3236-3244.

[83] D. H. Philllips, M. Castegnaro, Results of an interlaboratory trial of ^{32}P-postlabelling. In: D.H. Phillips, M. Castegnaro, H. Bartsch (Eds.), Postlabelling

methods for detection of DNA damage, IARC Scientific Publication No 124, IARC Press, Lyon, 1993, pp. 35-49.

[84] D.H. Phillips, M. Castegnaro, on behalf of trial participants, Standardization and validation of DNA adduct postlabelling methods: report of interlaboratory trials and production of recommended protocols, Mutagenesis 14 (1999) 301-315.

[85] ESCODD (European Standards Committee on Oxidative DNA Damage), Comparison of different methods of measuring 8-oxoguanine as a marker of oxidative DNA damage, Free Radical Res. 32 (2000) 333-341.

[86] ESCODD (European Standards Committee on Oxidative DNA Damage). Comparative analysis of baseline 8-oxo-7,8-dihydroguanine in mammalian cell DNA, by different methods in different laboratories: an approach to consensus, Carcinogenesis 23 (2002) 2129-2133.

[87] A.R. Collins, C. Gedik, S. Wood, A. White, J. Dubois, P. Duez, *et al.*, Inter-laboratory validation of procedures for measuring 8-oxo-7,8-dihydroguanine,8-oxo-7,8-dihydro-2′-deoxyguanosine in DNA, Free Radical Res. 36 (2002) 239-245.

[88] C.M. Gedik, A. Collins, Establishing the background level of base oxidation in human lymphocyte DNA: results of an interlaboratory validation study, FASEB J. 19 (2005) 82-84.

[89] F.A. Beland, D.R. Doerge, M.I. Churchwell, M.C. Poirier, B. Schoket, and M.M. Marques, Synthesis, Characterization, and quantitation of a 4-aminobiphenyl-DNA adduct standard, Chem Res. Toxicol. 12 (1999) 68-77.

[90] F.A. Beland, M.C. Poirier, E.D. Bowman, A. Weston, D.R. Doerge, M.I. Churchwell, L.S. Von Tungeln, S. Chen, P.P. Fu, S.J. Culp, B. Schoket, E. Gyorffy, J. Minárovits, High-performance liquid chromatography electrospray ionization tandem

mass spectrometry for the detection and quantitation of benzo[a]pyrene-DNA adducts, Chem. Res. Toxicol. 18 (2005) 1306-1315.

[91] R.K. Ross, J-M. Yuan, M.C. Yu, G.N. Wogan, G-S. Qian, J-T. Tu, J..D. Groopman, Y.T. Gao, B.E. Henderson, Urinary aflatoxin biomarkers and risk of hepatocellular carcinoma, Lancet 339 (1992) 943-946.

[92] G-S. Qian, R.K. Ross, M.C. Yu, J-M. Yuan, Y-T. Gao, B.E. Henderson, G.N. Wogan, J.D. Groopman, A follow-up study of urinary markers of aflatoxin exposure and liver cancer risk in Shanghai, People's Republic of China, Cancer Epidem Biom. Prev. 3 (1994) 3-10.

[93 M.J. Gunter, R.L. Divi, M. Kulldorff, R. Vermeulen, K.J. Haverkos, M.M. Kuo, P. Strickland, M.C. Poirier, N. Rothman, R. Sinha, Leukocyte polycyclic aromatic hydrocarbon–DNA adduct formation and colorectal adenoma, Carcinogenesis 28 (2007) 1426-1429.

[94] S.A. Kyrtopoulos, Biomarkers in environmental carcinogenesis: striving for a new momentum, Toxicol. Lett. 162 (2006) 3-15.

[95] J. Lewtas, D. Walsh, R. Williams, L. Dobias, Air pollution exposure-DNA adduct dosimetry in humans and rodents: evidence for non-linearity at high doses, Mutat. Res. 378 (1997) 51-63.

[96] F.J. Van Schooten, R.W.L. Godschalk, A. Breedijk, L.M. Maas, E. Kriek, H. Sakai, G. Wigbout, P. Baas, L. VantVeer, N. Van Zandwijk, ^{32}P-postlabelling of aromatic DNA adducts in white blood cells and alveolar macrophages of smokers: saturation at high exposures, Mutat. Res. 378 (1997) 65-75.

Figure legends:

Figure 1.
Biomarkers for exposure to an environmental toxin

Figure 2. Reprinted with permission from Chemical Research in Toxicology, 2006, volume 19, pp 868-878. Copyright 2006 American Chemical Society.

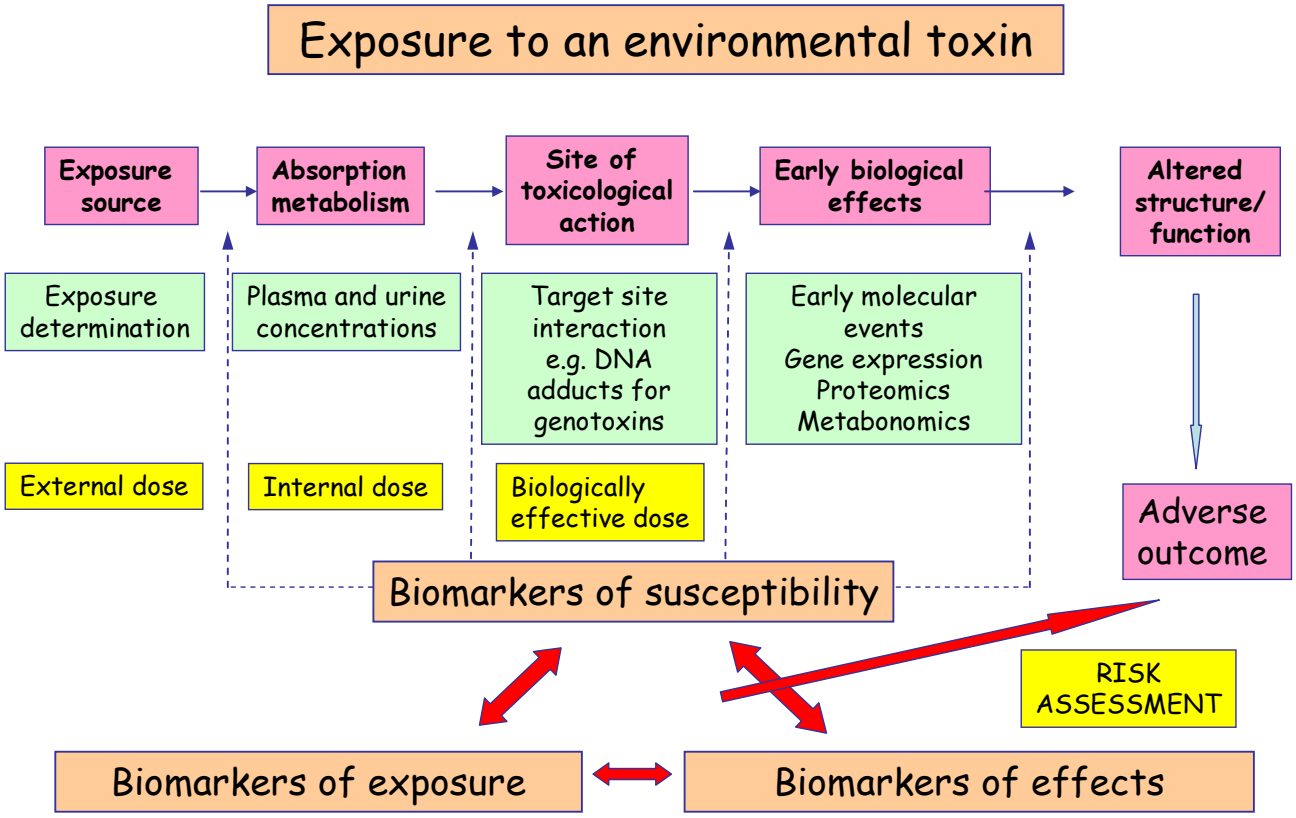
A. Determination of B[a]PDE-N^2dG adducts by LC-MS/MS SRM in mouse liver DNA (i.p., 200mg/kg B[a]P). Trace (a) is detection of the unlabelled adduct, and trace (b) of the [$^{15}N_5$]-labelled internal standard.
B. Formation of B[a]PDE-N^2dG adducts in mouse lung following dosing i.p. with 50, 100 and 200mg/kg B[a]P (nd = not determined, the DNA from 2 to 5 animals was analysed per time point, the error bars represent the standard deviation).

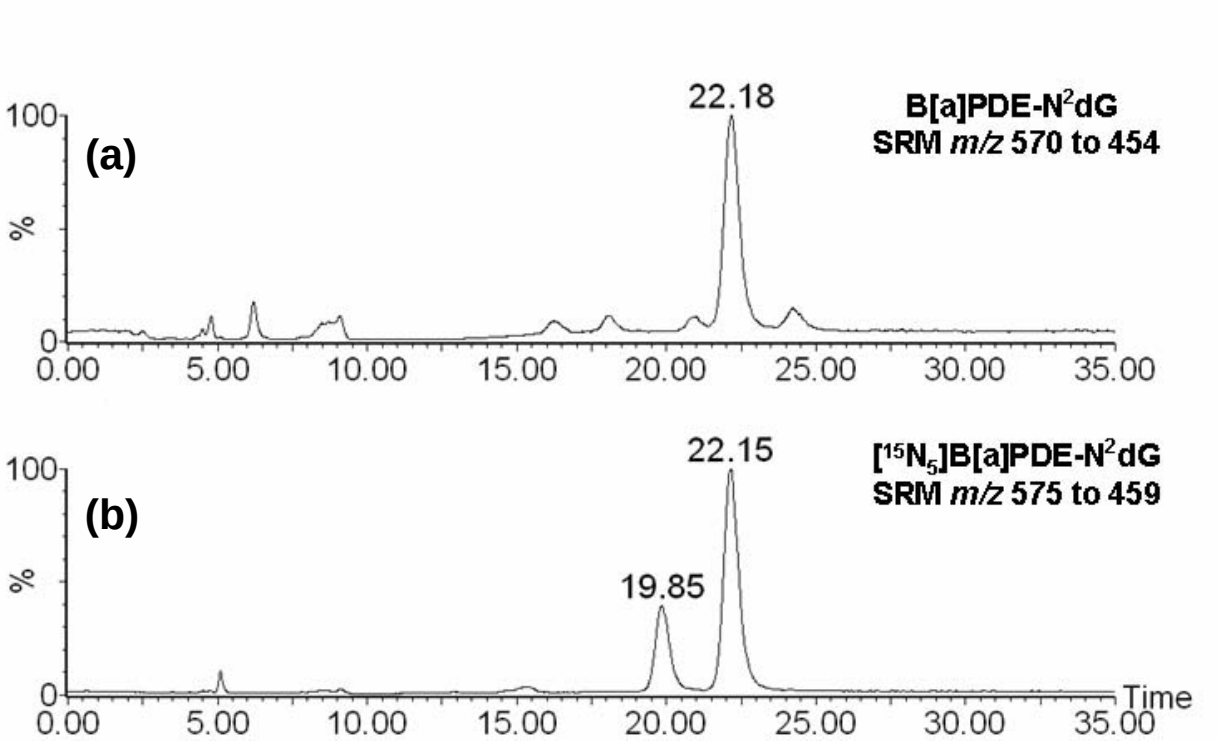
Figure 3.
A. Reprinted with permission from Chemical Research in Toxicology, 2007, volume 20, pp 290-299. Copyright 2007 American Chemical Society.

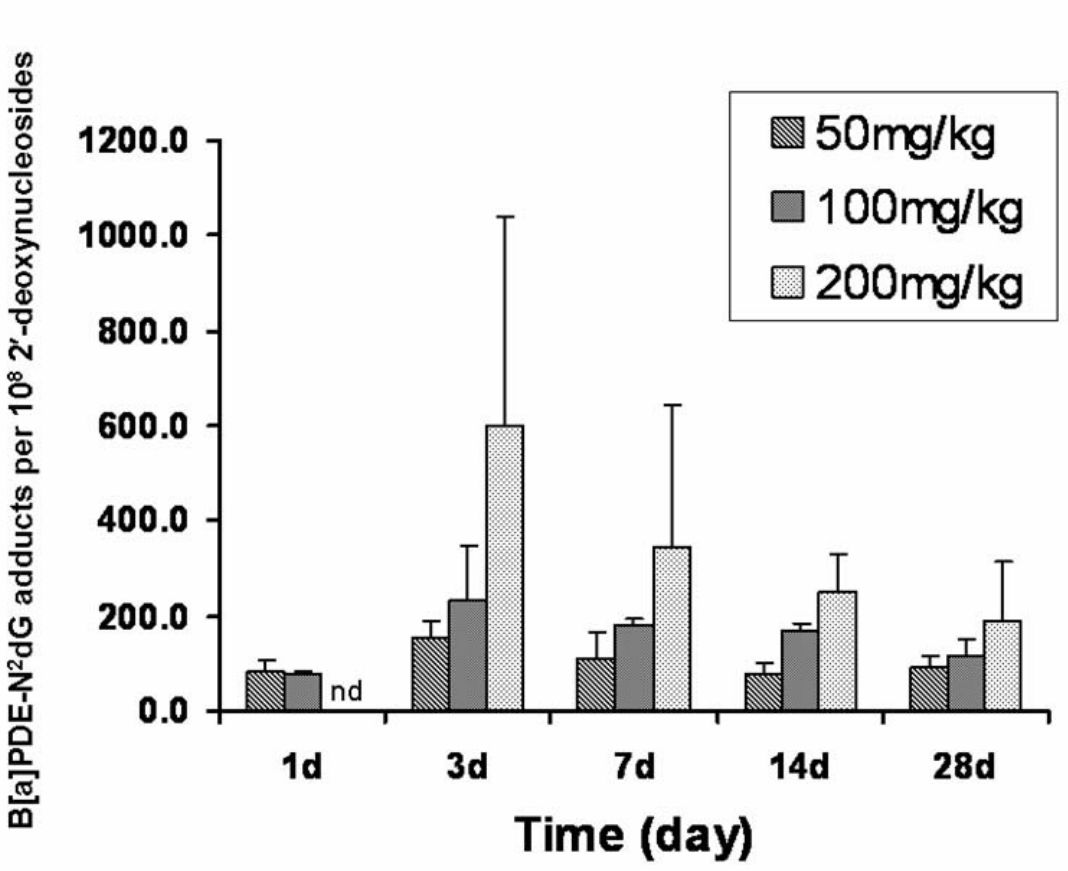
LC-MS/MS SRM ion chromatogram for the analysis of N-7 (2-hydroxyethyl)guanine in liver DNA from a control rat. Trace (a) is detection of the [$^{15}N_5$]-labelled internal standard, and trace (b) of the unlabelled adduct.
B. LC-MS/MS SRM ion chromatogram for the determination of 8-oxodG in human urine following SPE purification. Trace (a) is detection of the unlabelled adduct, and trace (b) of the [$^{15}N_5$]-labelled internal standard[1].

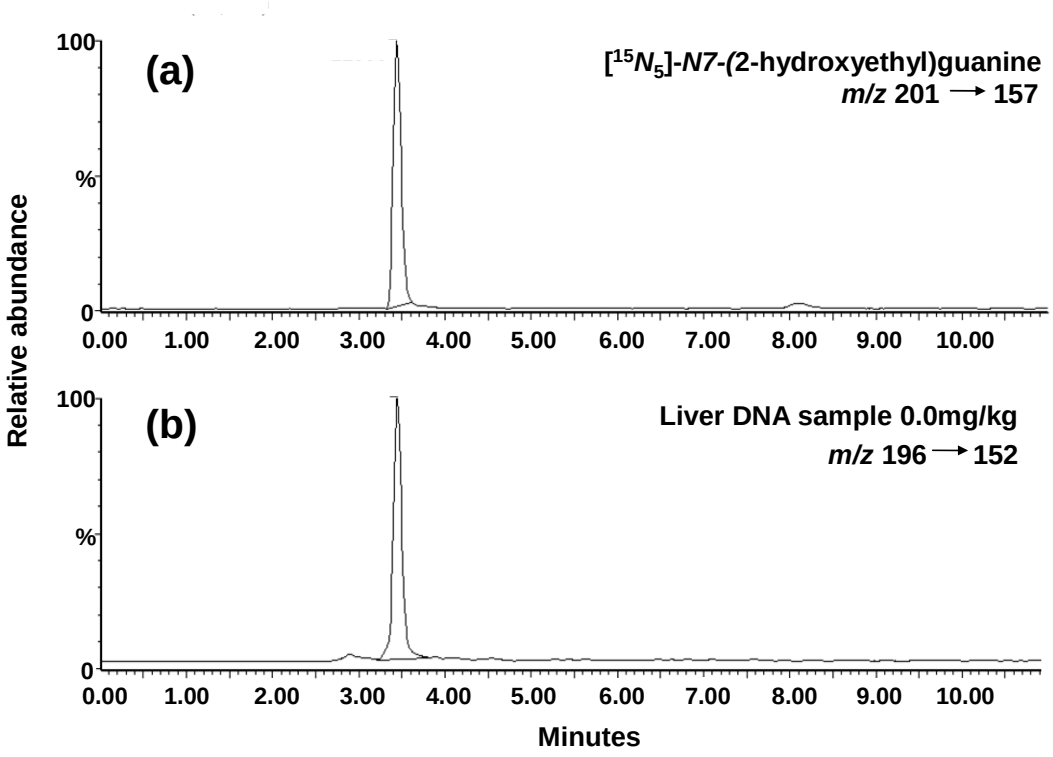
Figure 4.
ESI MS/MS CID product ion spectra of **(A)** 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), and adducts with 2′-deoxyguanosine of **(B)** malondialdehyde (M_1dG), **(C)** benzo[a]pyrene diol epoxide (B[a]PDEdG) and **(D)** dibenzo[a,l]pyrene diol epoxide (DB[a,l]PDEdG), showing the common neutral loss of 116u from MH^+. **(E)** shows the simultaneous SRM traces obtained for the detection of mass transitions (MH^+ to $[MH-116]^+$) corresponding to adducts from DB[a,l]PDEdG and B[a]PDEdG. These traces are derived from enzymatically digested and SPE purified DNA obtained from HepG2 cells dosed with a 1μM equimolar mixture of the two PAHs.

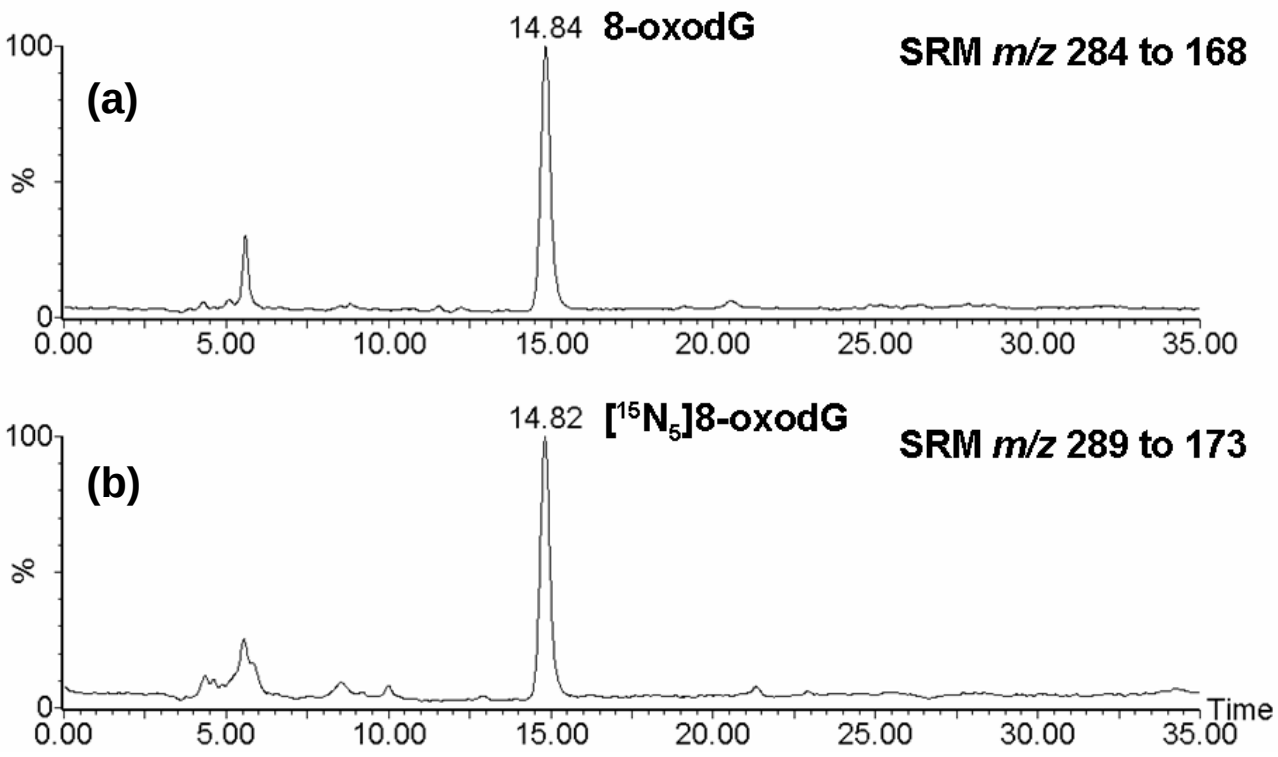
Footnote 1: This figure was published in Free Radical Biology and Medicine, 41, M.S. Cooke, R. Singh, G.K. Hall, V. Mistry, T.L. Diago, P.B. Farmer, M.D. Evans, Evaluation of enzyme-linked immunosorbent assay and liquid chromatography-tandem mass spectrometry methodology for the analysis of 8-oxo-7,8-dihydro-2′-deoxyguanosine in saliva and urine, pp. 1829-1836, Copyright Elsevier, 2006.

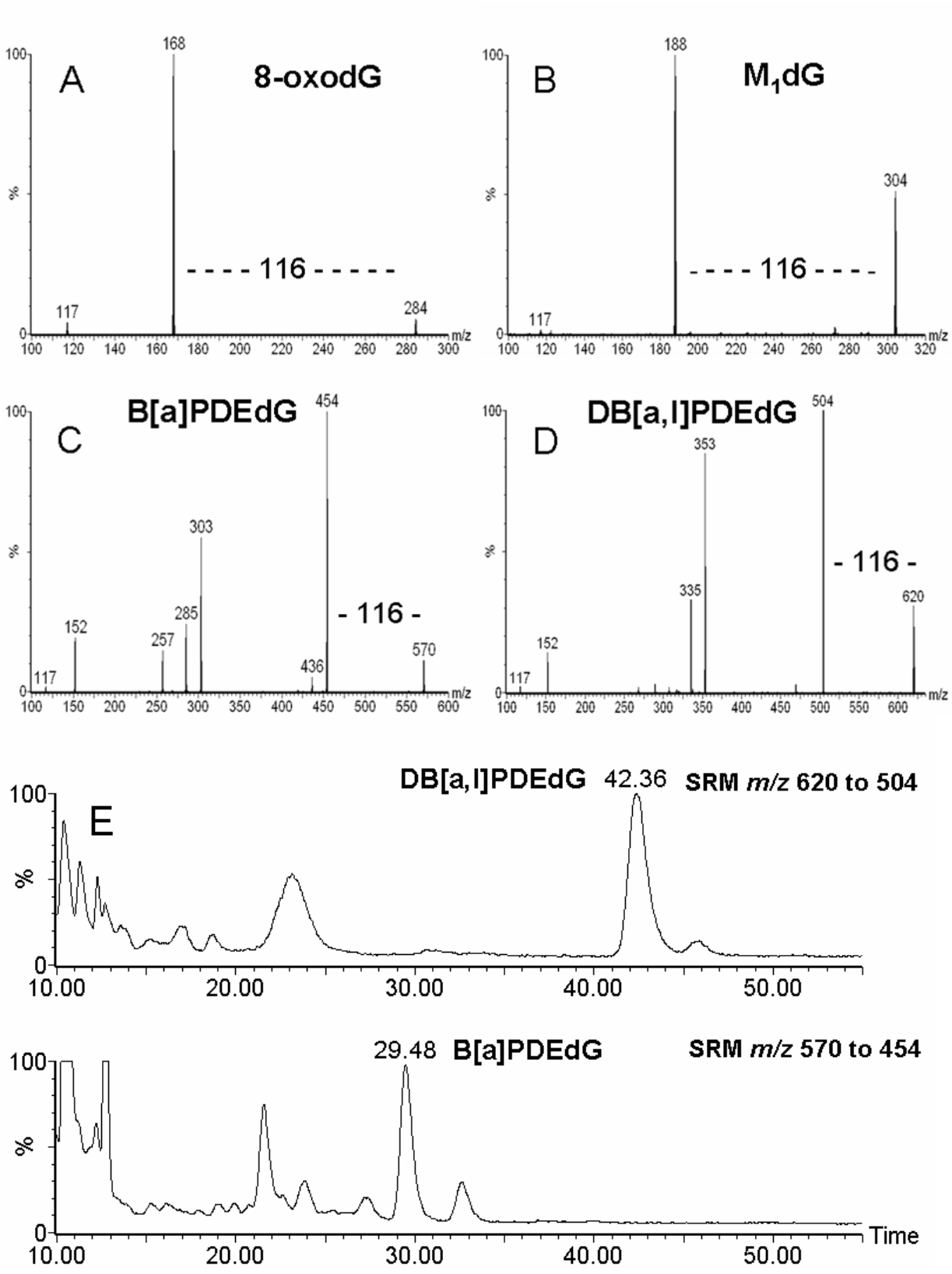

Exposure to an environmental toxin
Exposure source
Absorption metabolism
Site of toxicological action
Early biological effects
Altered structure/ function
Exposure determination
Plasma and urine concentrations
Target site interaction e.g. DNA adducts for genotoxins
Early molecular events Gene expression Proteomics Metabonomics
External dose
Internal dose
Biologically effective dose
Adverse outcome
Biomarkers of susceptibility
RISK ASSESSMENT
Biomarkers of exposure
Biomarkers of effects


(a)
22.18
B[a]PDE-N²dG
SRM m/z 570 to 454
100
%
0
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
(b)
22.15
[¹⁵N₅]B[a]PDE-N²dG
SRM m/z 575 to 459
19.85
100
%
0
Time
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00


B[a]PDE-N²dG adducts per 10⁸ 2'-deoxynucleosides
1200.0
1000.0
800.0
600.0
400.0
200.0
0.0
nd
1d
3d
7d
14d
28d
Time (day)
50mg/kg
100mg/kg
200mg/kg


(a)
[15N5]-N7-(2-hydroxyethyl)guanine
m/z 201 → 157
100
%
0
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00 10.00
(b)
Liver DNA sample 0.0mg/kg
m/z 196 → 152
100
%
0
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00 10.00
Relative abundance
Minutes


14.84 8-oxodG
SRM m/z 284 to 168
(a)
100
%
0
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
14.82 [15N5]8-oxodG
SRM m/z 289 to 173
(b)
100
%
0
Time
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00


A
8-oxodG
168
117
284
- - - - 116 - - - - -
B
M_1dG
188
304
117
- - - - 116 - - - -
C
B[a]PDEdG
454
303
285
257
152
117
436
570
- 116 -
D
DB[a,l]PDEdG
504
353
335
620
152
117
- 116 -
E
DB[a,l]PDEdG 42.36 SRM m/z 620 to 504
29.48 B[a]PDEdG SRM m/z 570 to 454
%
m/z
Time
10.00
20.00
30.00
40.00
50.00