G. Garban, et all. Journal of Agroalimentary Processes and Technologies, Volume XII, No. 2 (2006), 469-480
Review - Food Science Section

DEOXYRIBONUCLEIC ACID ADDUCTS AS BIOMARKERS OF EXPOSURE IN BIOMONITORING

Gabriela Gârban[1], Z. Gârban[2], G.D. Ghibu[3], Corina Auműller[3], Ariana-Bianca Velciov[2]

[1]Institute of Public Health Timişoara, Romania;
[2]Department of Biochemistry and Molecular Biology, Faculty of Food Processing Technology, Banat's University of Agricultural Sciences and Veterinary Medicine Timişoara, Romania
[3]Faculty of Chemistry and Environmental Engineering, University Politehnica Timişoara, Romania;

Abstract

Human exposures to chemicals found in certain foods, water and air due to the presence of environmental pollutants is linked to a higher risk for deoxyribonucleic acid (DNA) damage. It is believed that the initial step in chemical carcinogenesis is the covalent binding of a chemical to DNA macromolecule resulting DNA adducts. The covalent modification of DNA nucleobases by chemicals can alter the structure and in turn, the biological processing of the DNA by cellular proteins governing replication, transcription and repair. If not repaired or repaired incorrectly, these modifications may eventually lead to mutations and ultimately to cancer, especially if the adduct is located in an oncogene or tumor suppressor gene. Therefore the accumulation of DNA adducts can serve as a measure of the critical dose of carcinogen received at the target cells. Dosimetrical studies of the accumulation of critical DNA adducts, particularly under environmentally relevant conditions, can constitute important tools in the process of assessment of species susceptibility and risk assessment

***Keywords**: DNA-adducts, biomarkers of exposure, biomonitoring*

Introduction

Chemical substances which can participate to DNA adducts formation might be xenobiotics of food interest (e.g. ions of potentially toxic metals, mycotoxins, polycyclic aromatic hydrocarbons etc.) or xenobiotics of pharmaceutical interest (e.g. cyclophosphamide, cisplatin a.o.).

Any unprogrammed change in the structure of the DNA molecule, by DNA adducts formation, may be followed by serious biological consequences. As we previously showed, some environ-mental chemicals and/or physical agents, classified as carcinogens of genotoxicants, have the capacity to interact with DNA and damage the macromolecular structure, often with a concomitant adverse effect on biological integrity (Gârban, 1986; Shugart, 1995).

The measurement of adducts or other trace compounds in humans is referred to as *biological monitoring*, or *biomonitoring*. Biomonitoring is conducted by collecting samples of human fluids and/or tissues (such as urine, breast milk, blood or hair) in order to detect the presence of biomarkers of exposure.

1. Conceptual aspects on DNA adducts

1.1. Evolution of the adduct concept

The term "DNA adduct" is generally used to describe a covalent binding of a genotoxic substance to the macromolecule of deoxyribonucleic acid.

The compounds resulted from the interaction of DNA macromolecule with small molecules were investigated for the first time in the sixth decade of the 20^{th} century, and the results leaded to the idea of appearance of some bioincompatible structures that were named for the first time "molecular complexes". It was also used the term "molecular associations" (Bergman, 1968) but both denominations were inadequate with the characteristics of the studied chemical structures.

After years of physico-chemical investigations and mecanic-quantum calculations over the electronic density in the donor-acceptor relationship between DNA and small molecules, the term "adducts" was preferred (Grunberger, 1979; Gârban, 1985; Stich, 1988; Gârban, 1988), therefore this denomination will be used also in this paper to describe de chemical complex resulted from the covalent binding of a chemical substance to the DNA macromolecule.

1.2. Biogenesis of DNA adducts

Foreign chemical substances, also called xenobiotics, or their metabolites, may react with DNA macromolecules. The resulting

DNA-adducts can disrupt the biologic activity of DNA, leading to the perturbation of replication, transcription and even DNA-repair process.

Xenobiotics that can lead to DNA adduct, and therefore have carcinogenic potential, are divided in two categories: a) indirect-acting carcinogens and b) direct-acting carcinogens. Data concerning these aspects are presented below.

a) Indirect-acting carcinogens – xenobiotics that require metabolic activation prior to DNA adduct formation: benzo(a)pyrene (I); β-naphtylamine (II); 2-acetylaminoflorene (III); aflatoxin B_1 (IV); dimethylnitrosamine (V); benzidine (VI); vinyl chloride (VII); 2-amino-3-methylimidazo [4,5-*f*] quinoline (VIII); 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (IX). Their chemical structures are shown in figure 1.

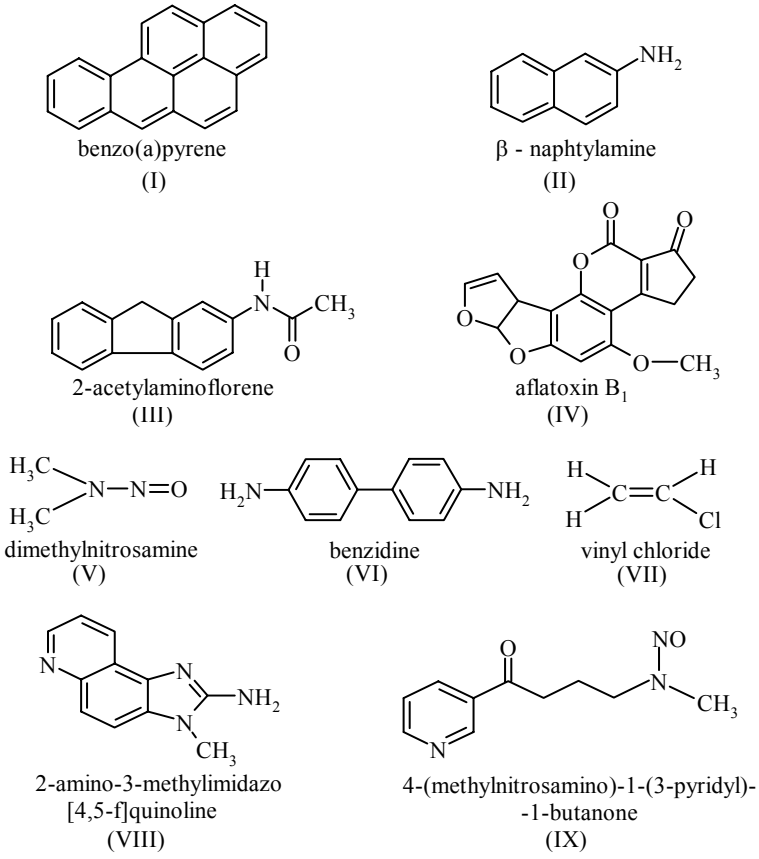
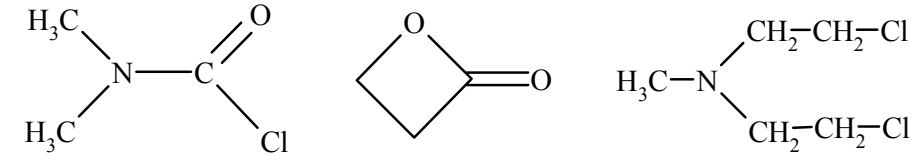


Fig. 1. Indirect-acting carcinogens – structural formula

b) Direct-acting carcinogens – xenobiotics that can damage DNA directly, without needing a metabolic activation in order to generate adducts. From this category we can enumerate: dimethylcarbamyl chloride (X); β-propiolactone (XI); nitrogen mustard (XII). The chemical structures of these substances are shown in figure 2.



dimethylcarbamyl chloride (X) | β-propiolactone (XI) | nitrogen mustard (XII)

Fig. 2. Direct-acting carcinogens – structural formula

During laboratory experiments it was found that xenobiotics which damage directly DNA yields adducts concentration that oscillates around similar values. As to xenobiotics that needs metabolic activation prior to DNA damage, the concentration of adducts is linked directly to the α-hidroxylation capacity of the target tissue. According to Lawley (1984), the α-hidroxylation capacity in human tissue, decreases in the order: hepatic tissue > renal tissue > pulmonary tissue.

When DNA-adducts occurs, the enzymatic process of DNA-repair begins. If the repair process fails, the anomaly of DNA macromolecular structure may lead to mutations and in the end to the apparition of cancer.

The xenobiotics that have the potential to lead to the formation of adducts, are a variety of chemical compounds, which by their structure presents an increased chemical reactivity. These xenobiotics are generally electrophylic agents, converted especially by the enzymes system (cytochrome P-450), during biotransformation, into positively charged compounds. The resulted "electrophylic agents" interacts with negative charged biomolecules like proteins or the DNA macromolecule. Cytochrome P-450 is the most important enzyme system for activation of secondary genotoxic compounds. The highest concentration of P-450 enzymes involved in biotransformation of

xenobiotic compounds is found in liver, but P-450 enzymes are present in virtually all tissues (Ghibu, 2006).

2. Assessing exposure with DNA adducts

A biomarker has been defined as: a change in a biological response that can be related to an exposure to, or toxic effect of, an environmental chemical or chemicals. Deoxyribonucleic acid is found in the cells of all living organisms and is the carrier of inherited information.

It has been assumed that most structural alterations to DNA are intrinsically damaging events. This assumption might not be entirely correct since structural modifications may be repaired without further consequences, or may occur in regions of DNA of no known function, where they may persist without producing any adverse effects. Nevertheless, structural alternations to DNA still provide evidence of exposure to genotoxic xenobiotics that have passed all of the toxicokinetic barriers. Their detection in organisms provides information of a possible exposure to genotoxicants, thus they can be used as biomarkers of exposure.

Structural perturbations in DNA molecule that do persist and do not result in death of the cell in which they occur, may enhance other problems. In particular they may interfere with the fidelity of DNA replication. If this situation occurs, then the possibility exists for a new suite of biological markers of genotoxicity to be expressed ‘‘biomarkers of effect’’. These endpoints are linked to abnormal DNA occurrence in the somatic cells of the organism such as chromosomal aberrations etc.

3. Biomonitoring by DNA adducts determination

3.1. Conceptual basis of biomonitoring

Humans and other organisms are continually exposed to both naturally occurring substances (from soil or produced by plants, animals etc.) and synthetic ones (e.g. food additives, drugs, cosmeticals, by-products of combustion, etc.) in our environments.

Some chemicals are generated within our bodies; others are absorbed through eating, breathing, drinking, and through contact with

our surroundings. Understanding the effects of exposure to these substances and being able to measure the magnitude of the exposure and correlate the resulted values with the occurrence of a pathological process is crucial for the human health status.

Through biomonitoring it is relatively easy to establish for many types of substances that exposure have occurred at some point in time. But, taking into account the fact that many chemicals have a short half-life it is more difficult to determine the levels of exposure, the frequency of exposure and the time frame for the exposure.

Biomonitoring data alone do not constitute a complete exposure assessment; they complement other environmental monitoring data and modeling activities in estimating exposure. Studies of absorption, distribution, metabolism and excretion are needed to convert biomonitoring data into estimates of exposure. These exposure estimates would, in turn, need to be evaluated using toxicological data in order to be transformed into estimates of risk (http://www.accnewsmedia.com/docs/1300/1209.pdf - 2006).

3.2. Methods of DNA adducts determination – overview

Applications of DNA adducts in human biomonitoring is relatively recent and it has developed mainly during the past decade. DNA adduct biomonitoring has often been classified in the past as qualitative rather then quantitative, but today modern techniques are available for the quantitative determination of specific DNA adducts in humans. Furthermore, the increasing availability of quantitative human data makes DNA adducts very relevant to environmental and occupational risk assessment, and they may emerge as one of the main tools for risk assessment and environmental policy (Törnqvist, 1994).

To be useful for human biomonitoring a given method requires high sensitivity, because the levels of adducts are low. The sensitivity of the methods tends to depend much on the compound or chemical class studied. Additionally, any pre-purification of the adduct helps to boost sensitivity, but the amount of DNA may become limiting. As a reference point, 10 ml of human blood can yield up to 400 μg of DNA.

The most used methods for DNA-adducts detections, divided into those generally applicable methods for most types of DNA adducts and those applicable to certain classes only are presented in Table 1.

Table 1. Methods for detection of DNA adducts in humans (after Hemminki, 1998 - modified)

Method	Advantage	Disadvantage
A. Methods used for most adducts		
^{32}P-postlabeling	High sensitivity; small amounts of DNA needed	Radiations; laborious and time consuming method
Immunoassays	Sensitive; easy to prepare columns; low cost	Raising antibodies ; low specificity
GC-MS	High specificity; suitable for small adducts	High costs; derivatization
Accelerator MS	High sensitivity; quantitative	High costs; radioactivity
B. Methods used for certain adducts		
Electrochemical	Low costs; easy technique; good sensitivity	Low specificity; contaminats
Fluorescence	Easy technique; good specificity	Requires large amount of DNA
Alkyltransferase	Low costs; easy technique; specific class of adducts	Unknown specificity for O_6-alkylguanines
Atomic absorption	High sensitivity; good specificity	Specific metals; platinum

3.2.1. Methods used for most adducts

In this category of methods we discuss: a) the isotopic method based on ^{32}P-postlabeling; b) immunoassays; c) mass spectrometry (MS) coupled to gas chromatography (GC) method; d) accelerator mass spectrometry (AMS)

a) Methods based on the ^{32}P-postlabeling assay. Were described in the early 1980s, is a sensitive technique and is now used extensively in human biomonitoring. The sensitivity depends on the use of high-specific-activity ^{32}P-ATP (adenosine 5′-triphosphate) in the kinase reaction, where a radioactive phosphate group is transferred to 3'-nucleotides.

The method has improved in specificity and cost since nuclease P1, butanol, high-performance liquid chromatography (HPLC), and immunoaffinity chromatography techniques have been used to pre-purify adducts before or after the kinase reaction (IARC, 1993).

b) Immunoassays. Over the last decade, highly specific polyclonal and monoclonal antibodies have been developed for the detection of DNA adducts formed by carcinogens such as by polycyclic aromatic

hydrocarbons, aromatic amines, mycotoxins, aldehydes, alkylating agents, chemotherapeutic agents, and ultraviolet light (Clingen, 1996). Antigens are DNA, nucleotides, or nucleosides that are reacted with the carcinogen. Both polyclonal antibodies and monoclonal antibodies are produced.

Immunoassays have been carried out as radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), and ultrasensitive enzyme radioimmunoassay (USERIA). Sensitivity depends on both the antibody used (i.e., its affinity constant) and the type of assay. The secondary antibody is conjugated to an enzyme that catalyzes a reaction forming a radioactive (USERIA), colored, or fluorescent (ELISA) product. The assays are generally sensitive enough to detect one adduct in 108 nucleotides in 50 μg of DNA (Santella, 1993).

c) Mass spectrometry (MS) coupled to gas chromatography (GC). In the last decades this method gained increasing ground in the analysis of DNA adducts (Allam, 1990). The technique is powerful, but the instrumentation is expensive. Derivatization of the samples has been one of the main obstacles so far. In its present form the technique appears most suitable to relatively small and abundant adducts that are analyzed as base derivatives.

d) Accelerated mass spectrometry (AMS). It is a technique for measuring isotope ratios with high selectivity, sensitivity, and precision. In general, AMS separates a rare stable or radioisotope from other isotopes and molecular ions of the same mass using a variety of nuclear physical techniques. The key physical steps allowing such quantitative and specific measurement are the production of negative ions from the sample to be analyzed; a molecular disassociation step to convert negatively charged molecular ions to positively charged nuclei, and the use of high energies which allow for the identification of ions with high selectivity (Turteltaub, 1990).

3.2.2. Methods used for certain adduct

These methods including: a) fluorescence spectroscopy; b) electro-chemical detection; c) O_6-alkyltransferase assay; d) atomic absorption spectroscopy, are applicable and valuable for a limited number of adducts. For example, synchronous fluorescence spectroscopy (SFS) can be applied in the identification of individual fluorescent species from a mixture of similar compounds. Fluorescence detection systems

are quite sensitive and have been successfully applied to the analysis of benzo(a)pyrene tetrols released hydrolytically from DNA.

3.3. Importance of DNA adducts determination

In biomonitoring, DNA adducts may be used generally to evaluate exposure to chemical xenobiotics of alimentary, pharmaceutical or toxicological nature. Therefore biomonitoring based on DNA adducts is important not only for medicine, but also for food science, pharmacology, and toxicology.

In *food science* biomonitoring based on DNA adducts can reveal important data regarding food safety. Thus food contamination with chemical xenobiotics with carcinogenic potential may be assessed (Gârban, 2003). An example that sustains our affirmations is the detection of DNA adducts with polycyclic aromatic hydrocarbons (PAH). Polycyclic aromatic hydrocarbons are a group of contaminants that are very often found in food. Processing of food (such as drying and smoking) and cooking of foods at high temperatures (grilling, roasting, frying) are major sources generating PAH. When exposed to PAH, adducts are found in most human tissues and once the samples are prelevated, DNA-PAH adducts can be investigated using one of the four available techniques: ^{32}P postlabeling, immunoassay, HPLC-fluorescence detection, and synchronous fluorescence spectroscopy (Rojas, 1994).

In *pharmacology* biomonitoring based on DNA adducts is used especially in assessing the efficiency or the side effects of cytostatics. An example could be the anticancer agent tamoxifen which is a nonsteroidal antiestrogen used in adjuvant therapy of breast cancer (Hemminki, 1998). However tamoxifen has been shown to cause liver cancer and DNA adducts in experimental animals as well as in vitro. Endometrial cancer and other serious side effects of therapy have been reported in tamoxifen-treated patients. Therefore is important to investigate the levels of DNA adducts in humans and determine their utility in risk assessment.

Considering the expected low level of adducts and interference from background it is recommended to use for DNA-tamoxifen adducts detection a ^{32}P-postlabeling method coupled to HPLC and radioactivity detection (Hemminki, 1996).

Regarding *toxicology*, the use of DNA adducts based biomonitoring is important in studying the impact and end points of chemical toxics. In this case DNA adducts with inorganic compounds like toxic metals, e.g.: mercury, platinum etc. (using atomic absorption spectroscopy), or DNA adducts with organic compounds like nitrosamines or alkenes may be studied (Gârban, 2004).

DNA-alkenes adducts are a specific example. One of the most studied alkenes is styrene. The genotoxicity of styrene has been of interest worldwide because it is one of the few suspected mutagenic compounds to which there may be daily exposures in small quantities. Styrene is an example of the way in which chemical characterization led to standard compounds for postlabeling and to a quantification of O_6-guanine adducts in white blood cell's DNA of lamination workers.

The main technical consideration for small adducts, such as those produced by alkenes, is that purification from unmodified nucleotides has to be carried out prior to labeling to guarantee good labeling efficiency. Different chromatographic systems must be used because nuclease P1 and butanol extraction, applicable for PAH, may cause the loss of adducts. Among alkenes, DNA adducts of ethene have been measured in humans by the postlabeling assay and correlated to smoking (Kumar, 1996). The postlabeling assay would equally detect 7-alkylguanine derivatives of propene, butadiene and styrene.

Conclusions

Appearance of DNA adducts, as consequence of mutations and chromosomal aberrations are measurable in humans and are on the causal pathway, thus being highly relevant for cancer risk assessment. Even though this is the case, studies must meet the requirements of technical validity and unbiased selection and comparison of study populations.

Studies of DNA adduct in humans are becoming more quantitative and therefore provide data even for quantitative risk estimation.

Comparisons of biomarker levels to cancer risk in humans and in experimental animals would provide new data for risk assessment. It would be also important to encourage characterization of these biomarkers in human populations in terms of kinetics, tissue

distribution, and background variation. The relationship between target and surrogate tissues has to be established.

References

Allam, K., Saha, M., Giese, R.W. (1990). Preparation of electrophoretic derivatives of N7-(2-hydroxyethyl)guanine, an ethylene oxide DNA adduct. *Journal of Chromatography*, 499, 571-578.

Bergmann, E.D. (1968). *The interaction of aromatic hydrocarbons with nucleic acids and their constituents*, pp.207-216, in "Molecular Associations in Biology" (Pullman B., Ed.), Academic Press, New York-London,.

Clingen, P.H., Arlett, C.A., Roza, L. Mori, T. Nikaido, O., Green, M.H.L. (1995). Induction of cyclobutane pyrimidine dimers, pyrimidine(6-4)pyrimidone photoproducts, and Dewar valency isomers by natural sunlight in normal human mononuclear cells. *Cancer Research*, 55, 2245-2248.

Gârban Z., Holban Şt., Brazdă Maria, Ciubotariu D., Văcărescu G. (1985) Unele probleme de patologie moleculară în carcinogeneza chimică. I. Interacţiunea acidului deoxiribonucleic cu compuşi policiclici, *St. şi cerc. de biochim.,* 28 (1), 31- 40.

Gârban, Z., Daranyi, G., Tămaş M., Miklos, J, Borza I., Nemeş R., Precob, V. (1986). Investigations on the action of cis-platinum on the deoxyribonucleic acid in vitro and in vivo*, "14th Intemational Cancer Congress",* Abstracts of Lectures, Symposia and Free Communications, Vol.3, p.1187, Budapest-Hungary, August 21-27, Publ.by S.Karger AG-Basel/Akadémiai Kiadó Budapest, 1986.

Gârban, Z., Văcărescu, G., Daranyi, G., Popeţi, D., Eremia, I., Maurer, A. (1988). Chemical structure-biological activity relationship in the interaction of DNA with cis-platinum, *"l4th Intemational Congress of Biochemistry",* Abstracts, Vol.V, p.80, Prague, July 10-15.

Gârban, Z., Gârban, G. (2003). *Nutriţia umană. Vol.I*, ediţia 3-a, Editura Orizonturi Universitare Timişoara.

Gârban, Z. (2004). *Xenobiotice chimice de interes alimentar,* Editura Eurobit Timişoara.

Ghibu, G.D., Gârban, G., Gârban, Z. (2006). Adducts of DNA with nitrosamines, *The Journal of Agroalimentary Processes and Technologies* - In press.

Grunberger, D., Weinstein, I.B. (1979). Biochemical Effects of the Modification of Nucleic Acids by Certain Polycyclic Aromatic Carcinogens, pp.105-149, in *Progress in nucleic acids research and molecular biology* (Ed. Cohn W.E.), Academic Press, New York.

Hemminki, K., Rajaniemi, H. Lindahl, B., Moberger, B. (1996). Tamoxifen-induced DNA adducts in endometrial samples from breast cancer patients, *Cancer Research*, 56, 4374-4377.

Hemminki, K., Bykov, V., Yang, K., Rajaniemi H. (1998). Use of DNA adducts in Biomonitoring, In: *Biomarkers: Medical and Workplace Applications*, Mendelsohn L., Mohr L.C., and. Peeters J.P, (Eds), pp. 133-135, Joseph Henry Press, Washintong D.C.

Kumar, R., Hemminki. K. (1996). Separation of 7-methyl and 7-(2-hydroxyethyl)-guanine adducts in human DNA samples using a combination of TLC and HPLC, *Carcinogenesis*, 17, 485-492.

Lawley, P.D. (1984). Carcinogenesis by alkylating agents, In: *Chemical Carcinogens*, Searle, C.E. (Ed), ACS Monograph 182, pp. 325-484. American Chemical Society, Washington DC.

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

Santella, R., Hemminki, K. Tang, D. Paik, M. Ottman, R. Young, T.L., Savela, K. Vodickova, L. Dickey, C. Whyatt, R., Perera, F.P. (1993). PAH-DNA adducts in white blood cells and urinary 1-hydroxypyrene in foundry workers. *Cancer Epidemiology Biomarkers and Prevention*, 2, 59-62.

Shugart, L.R. (1995). Biomarkers of DNA damage. In *Ecotoxicity and Human Health*, F.J. de Serres and A.D. Bloom (Eds), pp. 123-141, Lewis Publishers Inc., Boca Raton.

Stich, H.F., Dunn, B.P. (1988). *Methods for Detecting DNA Damaging Agents in Humans: Applications in Cancer Epidemiology and Prevention*., pp. 137-147 (Bartsch H., Hemminki K., O'Neill I.K., Editors), IARC Sci. Publ. No. 89, International Agency for Research on Cancer, Lyon.

Törnqvist, M., Ehrenberg, L. (1994). On cancer risk estimation of urban air pollution. *Environmental Health Perspectives*, 102 (4), 173-181.

Turteltaub, K.W., Felton, J.S., Gledhill, B.L., Vogel, J.S., Southon, J.R. Caffee, M.W., Finkel, R.C., Nelson, D.E., Proctor, I.D., Davis, J.C. (1990). Accelerator mass spectrometry in biomedical dosimetry: Relationship between low-level exposure and covalent binding of heterocyclic amine-carcinogens to DNA. *Proceedings of the National Academy of Sciences*, USA, 87, 5288-5292.

*** International Agency for Research on Cancer (IARC) (1993). *Postlabeling Methods for Detection of DNA Adducts*, D. H. Phillips, M. Castegnaro, and H. Bartsch, (Eds), Scientific Publication of IARC no. 124. IARC, Lyon.

*** http://www.accnewsmedia.com/docs/1300/1209.pdf, 2006