AIMS Genetics

AIMS Genetics, 4 (3): 166-191
DOI: 10.3934/genet.2017.3.166
Received: 13 March 2017
Accepted: 30 June 2017
Published: 11 August 2017

http://www.aimspress.com/journal/Genetics

Review

The use of genotoxicity biomarkers in molecular epidemiology: applications in environmental, occupational and dietary studies

Carina Ladeira[1,2,3,*] **and Lenka Smajdova**[4]

[1] Environment and Health Research Group, Escola Superior de Tecnologia da Saúde de Lisboa-Instituto Politécnico de Lisboa (ESTeSL–IPL), Av. D. João II, Lote 4.69.01, 1990-096 Lisboa, Portugal
[2] Grupo de Investigação em Genética e Metabolismo, Escola Superior de Tecnologia da Saúde de Lisboa-Instituto Politécnico de Lisboa (ESTeSL–IPL), Av. D. João II, Lote 4.69.01, 1990-096 Lisboa, Portugal
[3] Centro de Investigação em Saúde Pública-Escola Nacional de Saúde Pública, (CISP-ENSP), Universidade Nova de Lisboa, Portugal
[4] Faculty of Social Sciences, London Metropolitan University, London, United Kingdom

*** Correspondence:** Email: carina.ladeira@estesl.ipl.pt; Tel: +35-121-898-0445;
Fax: +35-121-898-0460.

Abstract: Molecular epidemiology is an approach increasingly used in the establishment of associations between exposure to hazardous substances and development of disease, including the possible modulation by genetic susceptibility factors. Environmental chemicals and contaminants from anthropogenic pollution of air, water and soil, but also originating specifically in occupational contexts, are potential sources of risk of development of disease. Also, diet presents an important role in this process, with some well characterized associations existing between nutrition and some types of cancer. Genotoxicity biomarkers allow the detection of early effects that result from the interaction between the individual and the environment; they are therefore important tools in cancer epidemiology and are extensively used in human biomonitoring studies. This work intends to give an overview of the potential for genotoxic effects assessment, specifically with the cytokinesis blocked micronucleus assay and comet assay in environmental and occupational scenarios, including diet. The plasticity of these techniques allows their inclusion in human biomonitoring studies, adding important information with the ultimate aim of disease prevention, in particular cancer, and so it is important that they be included as genotoxicity assays in molecular epidemiology.

Keywords: molecular epidemiology; biomarkers; genotoxicity; micronuclei; comet assay; environment; occupation; diet

1. Introduction

Genetic factors are clearly important in terms of influencing individual susceptibility to carcinogens; however, external factors represent the greatest opportunity for primary prevention. By 'external factors' we mean those related with environment—a broad scope, including all non-genetic factors such as diet, lifestyle and infectious agents. In a more specific approach, environmental factors include natural or man-made agents encountered by humans in their daily life, upon which they have no or limited personal control. The most important 'environmental' exposures, defined in this strict sense, include outdoor and indoor air pollution and soil and drinking water contamination [1]. In a more specific environmental niche are the occupational settings. People who work in certain jobs may have a higher risk of cancer due to exposure to some chemicals, radiation, or other aspects of their work (ergonomics, complex networks of safety risks, and many and varied psychosocial factors). Activities such as agriculture, painting, and industry are examples where workers can handle certain chemicals or be exposed to hazardous agents that can increase the risk of developing cancer [2]. Diet is also included in environment, particularly in lifestyle, and recognition of its importance has increased in recent decades, since it is a factor linked to some types of cancer [3,4]. The molecular epidemiology approach, measuring molecular or cellular biomarkers as indicators of disease risk or exposure to causative or preventive factors, has applications in studies of environmental and occupational exposure, disease etiology, nutrition, lifestyle and others [5], particularly in biomonitoring of populations.

This review aims to demonstrate the importance of genotoxicity biomarkers, such as those provided by cytokinesis blocked micronucleus assay and comet assay, as molecular epidemiology tools in human biomonitoring studies. With this approach, it is possible to detect, and therefore, prevent disease, specifically cancer in a wide variety of exposures—environmental, occupational and from diet.

2. Molecular Epidemiology

Classical epidemiology has historically been the hallmark approach to demonstrate associations between exposure to hazardous substances and development of disease; however, inter-individual variation, i.e., genetic/individual susceptibility, did not have a place in this equation. The development of molecular biology and its use as a potential tool in epidemiological studies strengthened the identification of diseases associated with environmental exposures related to lifestyle, occupation, or ambient pollution. In molecular epidemiology, laboratory methods are employed to document the molecular basis and preclinical effects of environmental carcinogenesis [6-9].

Molecular epidemiology has the advantage of being directly relevant to human risk, unlike animal or other experimental models that require extrapolation to humans. Moreover, biomarker data on the distribution of procarcinogenic changes and susceptibility factors in the population can improve the estimation of cancer risk from a given exposure [10]. Increasingly, molecular epidemiology studies are incorporating panels of biomarkers relevant to exposure, preclinical effects and susceptibility, using blood and exfoliated cells, tissues and body fluids. These biomarkers are now being widely used in cross-sectional, retrospective, prospective and nested case-control epidemiologic studies, with the aim of improving our understanding of the causes of specific human cancers [5,11].

It is well established that maintaining the integrity of the genome is essential for normal cell function and any disruption in the process can lead to either cell death or cancer development [12], and so the majority of the available biomarkers used in molecular epidemiology studies are related to agents that cause DNA damage and are mutagenic [5,13]. Major gains in cancer prevention should stem from theoretically important strategies, namely regulations, public education programs, health surveillance, behavior modification, and chemoprevention programs and other interventions that adequately protect these groups from environmental carcinogens [10,14].

3. Biomarkers of Genotoxicity

Traditionally, biomarkers are defined as biomarkers of exposure, effect and individual susceptibility. For the purpose of this review, we will focus on biomarkers of effect. A biomarker can be any substance, structure or process that can be monitored in tissues or fluids and that predicts or influences health; or that assesses the incidence or biological behavior of a disease, but is not a measure of disease, disorder or health condition itself [15,16]. Ideally, biomarkers should be accessible (non-invasive), non-destructive, easy and cheap to measure [17,18].

One of the criteria for establishing associations between an exposure and disease is biological plausibility. In this context, biomarkers may contribute by illuminating some of the carcinogenic steps linked to a particular risk factor. This is possibly an undervalued area where biomarkers can make significant contributions to cancer epidemiology. If a particular chemical exposure from ambient air is associated with increased risk, the additional information that exposed individuals have higher levels of DNA damage would add support to the exposure-disease association [19].

Biomarkers of effect offer the opportunity to provide scientific confirmation of proposed exposure-disease pathways in human populations, since they can be elicited as a result of interaction of the biological system with the environment [20,21]. The increasing demand for information about health risks derived from exposure to complex mixtures calls for the identification of biomarkers to evaluate genotoxic effects associated with occupational and environmental exposure to chemicals, and other potential sources of damage. An important group of effect biomarkers are genotoxicity biomarkers, which have been developed in vitro (cells and cell lines), in vivo (animals) and ex vivo (cells from humans). Cytogenetic biomarkers are the most frequently used endpoints in human biomonitoring studies, and are extensively used to assess the impact of environmental, occupational and other factors in genetic (in)stability [20-22]. Among the wide range of cytogenetic biomarkers, micronuclei in lymphocytes provide a promising approach to assess health risks [23].

The most used biological matrices for studying genotoxic effects in human biomonitoring are blood lymphocytes and exfoliated cells, both being easy to sample. Lymphocytes circulate throughout the body, have a reasonably long life span, and can therefore be damaged in any specific target tissue by a toxic substance [24]. Exfoliated buccal cells have been effective in showing the genotoxic effects of lifestyle factors such as tobacco smoking, alcohol, medical treatments, such as radiotherapy as well occupational and environmental exposure, namely exposure to potentially mutagenic and/or carcinogenic chemicals, and in studies of chemoprevention of cancer (antioxidants) and evaluation of malignant transformation of preneoplastic lesions associated with oral squamous cell carcinoma [25-33].

3.1. Cytokinesis Blocked Micronucleus (CBMN) Assay

Living organisms may be exposed to mutagenic substances that cause cellular damage, which may be induced by chemical, physical or biological agents that affect DNA, chromosome replication and gene transcription, causing abnormalities that may lead to cancer and cell death [34].

The cytokinesis-blocked micronucleus (CBMN) assay is a comprehensive system for measuring DNA damage, cytostasis and cytotoxicity-DNA damage events scored specifically in once-divided binucleated cells. It is a method for assessing DNA damage caused by xenobiotics, allowing detection of effects caused by clastogenic agents (that provoke chromosome breakage) and aneugenic agents (abnormal chromosome segregation associated with loss) [34-38]. Other endpoints that can be measured are nucleoplasmic bridges (NPB), a biomarker of DNA misrepair and/or telomere end-fusions, and nuclear buds (NBUD), a biomarker of elimination of amplified DNA and/or DNA repair complexes [29,39].

The CBMN assay is regularly used as an in vitro test in genotoxicity testing (OECD 487) and it is the preferred method in human biomonitoring studies to detect cytogenetic effects after exposure to genotoxic agents. It is regarded as an indicator of mutagen sensitivity, a biological dosimeter of ionizing radiation exposure, a measure of DNA-repair capacity and genomic stability, and a predictor of cancer susceptibility/risk [40,41]. In summary, it is defined as a robust assay for genetic damage with applications in ecotoxicology, nutrition, radiation sensitivity testing both for cancer risk assessment and optimization of radiotherapy; as well as these applications in biomonitoring of human populations, it is important for testing new pharmaceuticals and other chemicals. There are expectations regarding the future development of an automated system that can reliably score the various endpoints which are possible with the CBMN assay [29].

3.2. Comet Assay

The comet assay (otherwise called single-cell gel electrophoresis—SCGE) is a simple, sensitive method for detecting DNA-strand breaks. DNA strand breaks can originate from the direct modification of DNA by chemical agents or their metabolites; from the processes of DNA excision repair, replication, and recombination; or from the process of apoptosis. Direct breakage of the DNA strands occurs when reactive oxidative species (ROS) interact with DNA. Alkali-labile sites generated by loss of bases in the DNA, are converted to strand breaks by alkaline treatment (pH above 13.1) and so are also detected with the comet assay [42].

This assay was adapted to measure oxidized purines and oxidized pyrimidines by incubation of the nucleoids (the DNA structures remaining after lysis of agarose-embedded cells) with bacterial DNA repair enzymes [43] including formamidopyrimidine DNA glycosylase (Fpg), which recognizes the oxidized purine 8-oxoguanine, one of the most studied molecules regarding oxidative damage [34,43].

Comet assay has become one of the standard methods for assessing DNA damage, with a wide range of applications, namely in genotoxicity testing, human biomonitoring and molecular epidemiology, ecogenotoxicology (monitoring environmental pollution by studying sentinel organisms), research on oxidative damage as a factor in disease, monitoring oxidative stress in animals or human subjects resulting from exercise, or diet, or exposure to environmental agents as well as fundamental research in DNA damage and repair [9,44-46].

The congruence of results between the comet assay and other endpoints such as micronuclei or sister chromatid exchanges (SCE), has been one of the principal reasons to increase the use of the comet assay as a biomarker for hazard assessment, particularly in monitoring the effects of occupational hazards [47-52].

4. Human Genome-Environment Interaction—Biomonitoring as a Tool

The relative contribution of genetics versus the environment to human illness has been debated for decades, as the so-called gene-environment interaction. The importance of environmental exposures has been supported by geographic differences in incidence of disease, by variation in incidence trends over time, and by studies of disease patterns in immigrant populations [53].

Understanding risks to human health in the light of human genome-environment interactions is one of the most compelling challenges in environmental public health. With the sequencing of the human genome, renewed interest in understanding the role of the environment as a cause of human disease has emerged. Genes are expressed in response to the environment [54] and there are two kinds of susceptibility genes: those that predispose to disease without exposure to environmental factors and those that increase risk only by interaction with environmental agents [53]. Information about environmental risk factors should point to genes that might modify the risk, and identification of susceptibility genes should help identify previously unrecognized environmental risk factors [53].

Human biomonitoring has tremendous utility providing an efficient and cost-effective means of measuring human exposure to hazardous substances establishing evidence that both exposure and uptake have been taking place [55,56]. This approach considers all routes of uptake and all sources which are relevant, making it an ideal instrument for risk assessment and risk management. It can identify new chemical exposures, trends and changes in exposure, establish distribution of exposure among the general population, identify vulnerable groups and populations with higher exposures, and identify environmental risks at specific contaminated sites with relatively low expenditure [56]. More attention should be given to monitoring populations which are known to be exposed to hazardous environmental contaminants and to providing reliable health risk evaluation, since that information is useful for supporting regulations on protection of the environment [57].

There are well-established national human biomonitoring survey programs worldwide, where a target population has been identified, questionnaires have been developed and sample collections have taken place. In Europe there are the German Environmental Survey (GerES, Germany), the Flemish Environment and Health Study (FLEHS, Belgium), the French National Survey on Nutrition and Health (ENNS, France), BIOAMBIENT.ES (Spain), Program for Biomonitoring the Italian Population Exposure (PROBE, Italy), Human Biomonitoring Project (CZ-HBM, Czech Republic). In America there are the Canada Health Measures Survey (CHMS) and the United States of America the National Health and Nutrition Examination Survey (NHANES), and in Asia, the Korea National Survey for Environmental Pollutans in the Human Body (KorSEP).

5. Environmental Exposure

Nowadays people have to suffer the mutagenic and carcinogenic effects of many genotoxic agents in daily life and working environments due to changing lifestyles and innovations, for instance, chemical substances such as drugs, food additives, pesticides, and nanomaterials [58].

Anthropogenic pollution has become inherent to the modern environment. The global and rapid increase in technogenic stress in the biosphere raises the question about possible consequences for biota, including man, acknowledging that all forms of life are inter-connected and that human health is strongly linked to the ecosystem's health [59]. Environmental chemicals and contaminants are ubiquitous, occurring in water, air, food and soil. While some chemicals are short-lived in the environment and may elicit no harmful effects in humans, other chemicals bioaccumulate or persist for a long time in the environment or the human body due to frequent exposure, potentially leading to adverse health effects [60].

A more integrated approach is needed to deal with the fact that adverse biological effects induced by exposure to complex pollutant mixtures are not easily interpreted from a set of chemical analyses. The toxic effect of different interacting pollutants can be either additive, synergistic or antagonistic [61]. Molecular epidemiology studies on populations environmentally or occupationally exposed to high levels of complex mixtures of urban air pollutants have revealed genotoxic effects in terms of increased incidence of DNA damage [5,62]. Atmospheric pollutants, such as carbon monoxide, ozone, nitrogen oxides, sulfur dioxide, polycyclic aromatic hydrocarbons, and particulate matter are examples of chemical agents that may lead to DNA damage [34] and pose a serious threat to the health and the well-being of humans. According to their physicochemical properties, for instance, polycyclic aromatic hydrocarbons (PAHs) are released into the environment from both natural and anthropogenic sources, and are highly mobile in the environment, allowing them to distribute across air, soil, and water, becoming effectively ubiquitous [63,64]. It is also of great importance to assess the risk of future health effects from accidental or occupational radiation exposure to humans in order to be able to take appropriate measures to protect exposed individuals [65]. Multidisciplinary approaches combining chemical, ecotoxicological and ecological data have been undertaken to develop effective methods for assessing the quality of the environment, identifying the extent of genetic changes that occur when organisms are exposed to chronic, low-level, anthropogenic pollutants in selected species, such as protozoa, dicotyledonous plants [61], *Scots pine* [59], invertebrate and vertebrate native marine species [66], and others.

It is important to note that the genotoxicity biomarkers are applied in ecotoxicological studies; moreover, the application of early warning (sublethal) biomarkers in water-river quality monitoring programs is highly recommended since some of the pollutants are also relevant from a human health perspective—causing endocrine disruption, immune responses, or genotoxicity [61]. However this paper will cover just the effects in humans and human cells. Table 1 summarizes some studies regarding to environmental exposure, namely air pollutants [67-69], heavy metals [70,71], herbicides [72], mobile radiation [73], pesticides [74,75], pollution mixture [76], PAHs [77,78], and pyrethroids [79].

Table 1. Studies of human populations related environmental exposures.

Risk factor/exposure	Studied population/number of samples/sample	Genotoxicity biomarkers	Results			Refs.
Air pollutants (CO, NO_2, SO_2, benzene, O_3, PM10 and PM2.5)	Children (Northen Italy)/N = 181/exfoliated buccal cells	MN assay	MN mean ± SD: 0.29 ± 0.13. MN mean frequency of 0.29%: 2–3-fold higher than that considered as a “reference” value for children of this age.			[67]
Air pollutants: domestic heating (SO_2 and PM); traffic (NO_x VOCs)	Children (suburban, urban-traffic sites in Turkey)/N = 1.841 summer; N = 1.497 winter/buccal epithelial cells	MN assay		MN (‰) (mean ± SD)	BEC with MN (‰) (mean ± SD)	[68]
			Summer period	2.73 ± 1.98	2.28 ± 1.57	
			Winter period	1.87 ± 1.66	1.62 ± 1.33	
			p value	0.001	0.003	
			No statistical differences between summer and winter ($p > 0.05$) in suburban children.			
			Urban-traffic sites			
				MN (‰) (mean ± SD)	BEC with MN (‰) (mean ± SD)	
			Summer period	2.68 ± 1.99	2.68 ± 1.99	
			Winter period	1.64 ± 1.59	1.38 ± 1.15	
			p value	0.004	0.005	
			MN frequencies of urban-traffic children significantly higher in the summer than that of the winter ($p < 0.05$).			
Formaldehyde, nitrogen dioxide (NO2) in the air	Children 6–12 years old (living near chipboard-Viadana-Italy)/N = 413/oral mucosa cells	Comet assay MN assay	Children living near (<2 km) the chipboard industries — highest average exposure to formaldehyde.			[69]
			Comet assay	Mean		
			Tail intensity (%)	3.25		
			Tail lenght (μm)	11.69		
			Tail moment	0.20		

Continued on next page

<table>
<tr><td rowspan="5"></td><td rowspan="5"></td><td rowspan="5"></td><td colspan="4">Formaldehyde increase (0.20 μg/m^3) associated with a 0.13% (95% CI: 0.03, 0.22%) higher comet tail intensity, 0.007 (95% CI: 0.001, 0.012) higher tail moment.</td><td rowspan="5"></td></tr>
<tr><td colspan="4">Micronuclei assay (%)</td></tr>
<tr><td colspan="4">MN: 0.12</td></tr>
<tr><td colspan="4">NBUDs: 0.23</td></tr>
<tr><td colspan="4">NO$_2$ increase (2.13 μg/m^3) was associated with a 16% relative increase (RR = 1.16; 95% CI: 1.06, 1.26) in NBUDs.</td></tr>
<tr><td rowspan="4">Heavy Metals: arsenic, chromium, lead, manganese, molybdenum, zinc</td><td rowspan="4">Adults (working in the Panasqueira mine or living in the same region)/N = 122/blood samples</td><td rowspan="4">Comet assay (% DNAT)
MN assay</td><td></td><td>Controls</td><td>Environmentally exposed</td><td>p-value</td><td rowspan="4">[70]</td></tr>
<tr><td></td><td>Mean</td><td>Mean</td><td></td></tr>
<tr><td>% DNAT</td><td>12.40</td><td>24.58</td><td><0.001</td></tr>
<tr><td>MN (‰)</td><td>6.45</td><td>8.46</td><td>0.002</td></tr>
<tr><td rowspan="7">Heavy metals</td><td rowspan="7">Adults (average age: 35.41) in 5 Bosnian regions with extensive mining, industrial activities/N = 104/blood samples</td><td rowspan="7">CBMN assay.</td><td colspan="4">Frequencies—range and mean ± SD</td><td rowspan="7">[71]</td></tr>
<tr><td colspan="4">Total number of MN in BN cells: 1.00–27.00‰ and 8.35 ± 5.38.</td></tr>
<tr><td colspan="4">MN: 0.10–2.50% and 0.83 ± 0.54.</td></tr>
<tr><td colspan="4">NPB: 0.00–12.00‰ and 3.46 ± 2.89.</td></tr>
<tr><td colspan="4">NBUD: 0.00–10.00‰ and 2.40 ± 2.22.</td></tr>
<tr><td colspan="4">MN frequency (%) in BN cells no statistically significant differences between any of the studied group as compared to the control group ($p > 0.05$).</td></tr>
<tr><td colspan="4">NPBs differences were found to be statistically significant between 3 regions as compared to the controls ($p < 0.05$), and NBUDs in the local population of 1 region as compared to the control group ($p < 0.05$).</td></tr>
<tr><td rowspan="2">Herbicide (alachlor)</td><td rowspan="2">N = 1 male (age 43)/N = 1 female (age 30)/mononuclear isolated leukocytes</td><td rowspan="2">CBMN assay</td><td colspan="4">The induction of MN-BN in isolated lymphocytes was not statistically significant ($p = 0.18$) although one of the replicates at the highest concentration (20 μg mL^{-1}) was much higher than the other replicate, leading to a higher, but not statistically significant difference.</td><td rowspan="2">[72]</td></tr>
<tr><td colspan="4">Isolated blood lymphocytes</td></tr>
</table>

Continued on next page

			Alachlor [µg/mL]	MN (per 1000)	
			0.0	6.0 ± 0.0	
			2.5	6.0 ± 2.1	
			5.0	5.5 ± 0.7	
			10.0	6.8 ± 0.4	
			20.0	10.3 ± 4.6	
			Isolated human lymphocytes treated for last 51 h of a 72 h culture period.		
			Isolated human lymphocytes		
			Alachlor [µg/mL]	MN in BN cells (per 1000)	
			0.0	3.8 ± 0.4	
			2.5	4.8 ± 3.2	
			5.0	4.5 ± 0.7	
			10.0	4.8 ± 1.8	
			20.0	Too few dividing cells	
			40.0	Too few dividing cells	
			4 h treatment with alachlor		
			Alachlor [µg/mL]	MN in BN cells (per 1000)	
			0.0	6.5 ± 2.1	
			2.5	n.d.	
			5.0	n.d.	
			10.0	n.d.	
			20.0	4.5 ± 0.7	
			40.0	13.5 ± 3.5	
Mobile phone radiation	Male adults (age 20–30)/N = 300 (150 high mobile users and 150 low mobile users)/buccal epithelial cells	MN assay	Group I mean ± SD (0.77 ± 0.815).		[73]
			Group II mean ± SD (1.52 ± 1.176).		
			Significant increase in the mean MN count in group II in comparison to the group I (p-value < 0.0001).		

Continued on next page

			In group II, the MN count in the side of mobile phone use was found to be statistically significantly elevated (1.52 ± 1.176) in comparison to the opposite side (0.90 ± 0.3992).			
			MN mean count was found to be significantly increased in non-head phone users (2.08 ± 1.291) in comparison to headphone users (0.96 ± 0.699).			
Pesticides (complex mixtures): carbamates, organophosphates, pyrethroids	N = 239 agricultural workers/N = 231 unexposed controls/lymphocytes of peripheral blood (PBL) and exfoliated cells of the oral mucosa	CBMN assay in PBL MN assay			Mean ± SE	[74]
			BNMN	Control	12.25 ± 0.60	
				Exposed	11.40 ± 0.49	
			MNL	Control	13.82 ± 0.69	
				Exposed	12.55 ± 0.55	
			BCMN	Control	1.06 ± 0.10	
				Exposed	1.03 ± 0.09	
			MNBC	Control	1.18 ± 0.12	
				Exposed	1.12 ± 0.10	
Pesticides environmental exposure (through inhalation): glyphosate, liquid formulations of cypermethrin, chlorpyrifos	Children (age 4–14)/N = 50 pesticide spraying areas (Córdoba)/N = 25 children from the city of Río Cuarto (Córdoba), not exposed to pesticides/buccal mucosa cells	MN assay	MN mean per 1000 cells Marcos Juárez: 5.20 ± 0.58 Río Cuarto: 3.36 ± 0.63 Genotoxicity is present in a group of children in Marcos Juárez was higher compared from to the Río Cuarto.			[75]
Pollution containing: cadmium, lead, p,p'-DDE, hexachlorobenzene, PCBs, dioxin-like t,t'-muconic acid, 1-hydroxypyrene	Adult residents (age 50–65) from 9 areas with different types of pollution/N = 1583/peripheral blood cells	MN assay Comet assay (% DNA)		MN mean	% DNA mean	[76]
			Antwerp	7.30	1.69	
			Antwerp port	6.65	1.23	
			Fruit area	6.00	1.35	
			Olen	7.00	1.60	
			Ghent	7.25	2.03	
			Waste incinerators	8.60	2.24	

Continued on next page

			Rural area	7.00	1.97	
			Within an industrial area DNA strand break levels were almost three times higher close to industrial installations than 5 kilometres upwind of the main industrial installations ($p < 0.0001$).			
			Overall significant differences between areas were still observed for oxidative DNA damage ($p = 0.040$) and for DNA-strand breaks ($p < 0.001$) and for MN ($p = 0.11$).			
Polycyclic aromatic hydrocarbons (PAHs) in the air	Children (age: 6–15)/5 groups of Tabasco-Mexico 5 groups/peripheral blood lymphocytes	Comet assay		Exposed children	Control group	[77]
			Tail lenght	14.21–42.14	12.25	
			Tail/head	0.97–2.83	0.63	
PAHs and lead (Pb)	Children (age: 5–14), 2 most polluted cities-Katowice, Sosnowice/N = 74/peripheral blood lymphocytes	MN assay	MN mean: 4.44 Individual values reaching 17 MN cells per 1000 binucleated cells. Positive significant correlation was found between PbB and MN levels ($r = 0.347$, $p < 0.05$).			[78]
Pyrethroid insecticide	Males (age: 25–30)/N = 5/peripheral blood samples /human hepatoblastoma derived cell line HepG2	Alkaline comet assay with FPG	Dose dependent increase of DNA damage in both cell types, positive correlations between DNA damage in lymphocytes (tail DNA, $r = 0.982$, $p > 0.001$ and tail lenght, tail DNA, $r = 0.957$, $p > 0.001$. HepG2: tail DNA, $r = 0.848$, $p < 0.05$ and tail lenght, $r = 0.848$, $p < 0.05$.			[79]

6. Occupational Exposure

A wide range of chemicals that can act as environmental hazards, may also be exposure factors in specific occupational settings, and this is an extremely important consideration. For instance, besides the risks to the general public, atmospheric pollution can be considered an occupational health hazard to professional groups, such as traffic police or professional drivers working in urban areas [62], organic solvents [34, 80, 81], and others. Biomonitoring of exposure to toxic chemicals in the workplace is a fundamental tool to evaluate human health risks, supporting strategies to establish a safe work environment [82-85]. Table 2 summarizes some important occupational exposures, namely, antineoplastics [84], byproducts of petrol [85], formaldehyde [86], heavy metals [69,87,88], methyl bromide [89], organic solvents and smoke generated from biomass burning [34,80,81,90-92].

Occupational risk assessment may be defined as the qualitative and quantitative characterization of an occupational risk, i.e., the probability that an adverse health effect may result from human exposure to a toxic agent which is present in the occupational setting. It has three fundamental tools: environmental monitoring, health surveillance and biological monitoring. Risk assessment is meant to quantify the likelihood that a quantitatively defined occupational exposure of an individual (or group of individuals) to a chemical might result in adverse health effects [14,82].

National and international bodies set maximum allowable workplace concentrations for a wide range of substances. For instance, for airborne exposure to gases, vapors and particulates, recommended or mandatory occupational exposure limits (OELs) have been developed in many countries. The most widely used limits, called threshold limit values (TLVs), and are those issued in the United States of America by the American Conference of Governmental Industrial Hygienists (ACGIH). Specifically for airborne exposures, there are three other types of limit, namely the time-weighted average (TWA) exposure limit—the maximum average concentration of a chemical in air for a normal 8-hour working day and 40-hour week; the short-term exposure limit (STEL)—the maximum average concentration to which workers can be exposed for a short period (usually 15 minutes); and the ceiling value—the concentration that should not be exceeded at any time [83]. However, there is a need for revision of workplace limits to take also into account the levels of various agents that can cause allergies, for instance, in addition to occupational diseases. As new agents are identified they should be swiftly regulated.

Table 2. Studies of human populations related occupational exposures.

Risk factor/exposure	Studied population/number of samples/sample	Genotoxicity biomarkers	Results	Refs.
Antineoplastics	Occupationally exposed nurses N= 27/N = 111 non-exposed subjects/peripheral blood cells	CBMN assay	MN lymphocytes mean ± SE (range) Controls: 2.09 ± 0.312 (0–15) Exposed: 10.11 ± 2.053 (1–58) The occupationally exposed group showed significantly higher MN mean (p value < 0.001, Mann-Whitney test).	[84]
Benzene	Gasoline station attendants (GSA) N = 43/controls N = 28/whole blood, buccal exfoliated cells	Comet assay in whole blood MN assay in buccal exfoliated cells	DNA damage index, significant increase in the damage score in the GSA group compared to controls (Mann-Whitney test, $p < 0.001$). 3.8-fold higher in the GSA group compared to controls (Mann-Whitney test, $p < 0.001$).	[81]
Benzene and atmospheric pollutants	Gas station attendants (GSA N = 43) taxi drivers (TD N = 34)/persons without known occupational exposures (NE N = 22)/buccal cells, blood	MN assay buccal cells Comet assay blood lymphocytes	Micronucleus assay In the MN assay, no significant difference was observed among the groups ($p > 0.05$). Frequency of abnormal cells (MN/1000 cells): NE: 0.72 GSA: 2.70 TD: 1.30 Comet assay Significant increase in DNA damage index (DI) in GSA and TD groups comparing to NE group ($p < 0.001$).	[34]
Byproducts of petrol and lead	Workers of car and battery repair garages N = 60/control group N = 80 workers who were not	MN assay	MN mean (3000 cells per individual) Exposed: 8.22 Controls: 2.12 A significant difference ($p < 0.001$) was found between the exposed and the control.	[85]

Continued on next page

<table>
<tr><td></td><td>exposed to byproducts of petrol and lead/exfoliated cells of buccal mucosa</td><td></td><td></td><td></td></tr>
<tr><td>Formaldehyde</td><td>N = 46 workers occupationally exposed to formaldehyde (20–61 years old)/N = 85 unexposed individuals (20–53 years old)</td><td>CBMN assay in peripheral blood lymphocytes
MN assay in buccal cells</td><td>
<table>
<tr><td></td><td>MN in lymphocytes</td><td>NPB</td><td>NBUD</td><td>MN in buccal cells</td></tr>
<tr><td></td><td>Mean</td><td>Mean</td><td>Mean</td><td>Mean</td></tr>
<tr><td>Controls</td><td>0.81</td><td>0.18</td><td>0.07</td><td>0.16</td></tr>
<tr><td>Exposed</td><td>3.96</td><td>3.04</td><td>0.98</td><td>0.96</td></tr>
</table>
All genotoxicity biomarkers showed significant increases in exposed workers in comparison with controls (Mann-Whitney test, $p < 0.002$).</td><td>[86]</td></tr>
<tr><td>Heavy metals: arsenic, lead, chromium, manganese, molybdenum, zinc</td><td>Adults (workers in the Panasqueira/N = 122/blood samples</td><td>Comet assay (% DNA)
MN assay</td><td>
<table>
<tr><td></td><td>Controls</td><td>Occupationaly exposed</td><td>*p*-value</td></tr>
<tr><td></td><td>Mean</td><td>Mean</td><td></td></tr>
<tr><td>% DNA</td><td>12.40</td><td>18.73</td><td><0.001</td></tr>
<tr><td>MN (‰)</td><td>6.45</td><td>4.98</td><td>0.002</td></tr>
</table>
The occupationally exposed group showed significantly higher % DNA.</td><td>[69]</td></tr>
<tr><td>Heavy metals lead (Pb)</td><td>N = 90 male Pb recovery unit workers/N = 90 matched controls/peripheral blood lymphocytes, buccal exfoliated cells</td><td>Comet assay in PBL
MN assay in buccal exfoliated cells and PBL</td><td>
Comet assay
<table>
<tr><td></td><td>Comet tail lengh (μm)</td></tr>
<tr><td>Controls</td><td>8.15</td></tr>
<tr><td>Exposed</td><td>17.86</td></tr>
</table>
The results indicated that the exposed workers had a significantly higher mean comet tail length than that of controls ($p < 0.05$).
Micronucleus assay
<table>
<tr><td>MN frequency (‰)</td><td>Buccal cells</td><td>Lymphocytes</td></tr>
<tr><td>Controls</td><td>2.97</td><td>3.17</td></tr>
<tr><td>Exposed</td><td>4.66</td><td>6.46</td></tr>
</table>
Increased MN frequency in exposed subjects than in controls ($p < 0.05$).</td><td>[87]</td></tr>
<tr><td>Heavy metals: nickel</td><td>N = 204 male subjects (age: 18–50) in India/N = 102</td><td>Comet assay
MN assay</td><td>
<table>
<tr><td></td><td colspan="2">Basal DNA damage (μm)</td><td colspan="2">MN frequency (%)</td></tr>
<tr><td></td><td>Mean</td><td>Range</td><td>Mean</td><td>Range</td></tr>
</table></td><td>[88]</td></tr>
</table>

Continued on next page

chromium	welders employed in welding plants, durations of exposure (1–24 years)/N = 102 subjects-control group/blood lymphocytes, buccal epithelial cells		Control	8.94	4.14–17.10	0.32	0.00–0.80	
			Welders	23.05	17.24–35.62	1.30	0.12–2.89	
			The results indicated that the welders had a larger mean comet tail length than that of the controls ($p < 0.001$).					
			Welders showed a significant increase in micronucleated cells compared with controls ($p < 0.001$).					
Methyl bromide	N = 31 Methyl bromide-exposed fumigation workers/n = 27 referents/blood lymphocytes and oropharyngeal cells	Oropharyngeal MN assay (buccal cells) lymphocyte MN assay (blood lymphocytes)	MN assay (MN/1000 buccal cells) mean:					[89]
			Workers: 2.00					
			Referents: 1.31					
			Two-sided *p*-value = 0.08.					
			Kinetochore-negative micronucleated cells/1000 lymphocytes mean:					
			Workers: 10.48					
			Referents: 10.41					
			Kinetochore-positive micronucleated cells/1000 lymphocytes mean:					
			Workers: 10.81					
			Referents: 10.44					
			No statistically significant differences were observed between workers and referents for mean kinetochore-negative lymphocyte MN.					
Organic solvent mixtures: acetone, 1-hexane, toluene, methylethylketone	N = 45 footwear industry workers: solvent based adhesive (SBA N = 29)/water solvent based adhesive (WSA N = 16)/N = 25 controls/blood, buccal cells	Comet assay CBMN assay		Control	WBA	SBA		[90]
			Comet assay (blood)					
			Damage index	3.44 ± 3.24	2.13 ± 2.45	8.35 ± 7.85		
			Damage frequency (%)	1.52 ± 1.31	0.78 ± 0.91	2.76 ± 1.99		
			Micronucleus test					
			MN (lymphocytes)	5.20 ± 2.33	3.88 ± 1.93	4.90 ± 2.34		
			NPB (lymphocytes)	3.00 ± 1.97	2.56 ± 2.53	3.69 ± 2.49		
			MN (exfoliated buccal cells)	0.62 ± 0.73	0.69 ± 0.87	1.15 ± 1.45		

Continued on next page

			The Comet assay results showed that there was a significant increase in the mean damage index for the SBA ($p < 0.001$) group in comparison to the WBA group and control ($p < 0.05$).			
			For the MN test in binucleated lymphocytes and exfoliated buccal cells, the 3 groups were not statistically different.			
Smoke generated by biomass burning	N = 23 sugar cane workers/N = 30 control group/blood lymphocytes, buccal exfoliated cells	MN assay	Micronucleus assay (MN/1000 cells)			[91]
				MN mean (lymphocytes)	MN mean (buccal cells)	
			Controls	1.27	9.70	
			Cutters	8.22	22.75	
			The MN frequencies in lymphocytes were higher ($p < 0.001$) in the sugar cane workers compared with the control group.			
			A higher MN frequency in exfoliated cells was obtained in the group of sugar cane cutters compared with the controls ($p < 0.001$).			
Toluene	N = 34 male industrial painters, occupationally exposed to toluene/N = 27 control group subjects with no history of occupational exposure/blood lymphocytes, buccal cells	Comet assay MN assay	Comet assay (DNA damage index):			[80]
			Controls: 39.4			
			Painters: 60.4			
			Significant increase in DNA damage index between painters and controls ($p < 0.001$).			
			Micronucleus assay (MN/1000 cells)			
			Controls: 2.24			
			Painters: 2.74			
			No significant difference between painters and controls ($p > 0.05$).			
	N = 34 women from shoemaking plants (n = 16 plant A + n = 18 plant B)/N = 19 controls/blood mononuclear lymphocytes	Comet assay		TM	% TDNA	[92]
			Controls	5.37 ± 2.48	18.18 ± 6.26	
			Workers plant A	5.85 ± 2.43	19.49 ± 5.80	
			Workers plant B	6.09 ± 1.91	20.26 ± 4.35	
Vehicle exhaust	N = 49 traffic police with outdoor activities N = 36 indoor workers from university/lymphocytes	CBMN assay		Mean ± S.D.	95% CI	[62]
			Controls	4.83 ± 1.84	4.20–5.46	
			Traffic police	7.06 ± 2.87	6.23–7.89	
			($p = 0.001$, Wilcoxon test).			

7. Diet

Dietary habits are recognized to be an important modifiable environmental factor influencing cancer risk and tumor development, and other diseases. Although some studies have estimated that about 30–40% of all cancers are related to dietary habits, the actual percentage is highly dependent on the foods consumed and the specific type of cancer [18,93,94]. Epidemiological studies on the role of environmental exposure to carcinogens in diet have identified specific cancers whose incidence is known to vary considerably among countries [89]; substantial increases in the risk of certain cancers are observed in populations migrating from low- to high-risk areas, and this suggests that international differences in cancer incidence can be attributed primarily to environmental or lifestyle rather than genetic factors [93,95]. Diet can influence cancer development in several ways, namely by direct action of carcinogens in food that can damage DNA, by dietary components that can change enzyme activity, or by inadequate intake of molecules involved in antioxidant protection, DNA synthesis, repair or methylation that can influence mutation rate or changes in gene expression [96], and others. It is important to note, however, that the role of dietary components with potential cancer chemopreventive activity is not the subject of this review [3].

Another perspective of diet related to cancer risk is unintended contamination, which can result from compounds used in agriculture (e.g., pesticides and herbicides in plant-based foods, and growth hormones or antibiotics used in animal farming), or food processing (e.g., preservatives, smoking) and food packaging (e.g., bisphenol A or phthalates). The latter are not known to directly cause cancer, but they may influence cancer risk in other ways—for example, by acting as hormone-like substances in the body [97]. Is important to note that heavy metals, such as cadmium or mercury, may enter the food chain, such as in fish, or they may enter through contamination or their natural presence in soil or water.

Many substances are added to foods to prolong shelf and storage life and to enhance color, flavor, and texture. The possible role of food additives in cancer risk is an area of great public interest [97]. Briefly, food additive is a substance not normally consumed as food by itself and not normally used as a typical ingredient of the food, whether or not it has nutritive value [98].

The presence of such chemical contaminants or other unwanted substances in food and feed is often unavoidable as some of these substances are ubiquitous in the environment. However, the collection of dietary intake data along with chemical analysis of biological samples allows human biomonitoring programs to identify chemical exposures that might be associated with diet [60].

The European Food Safety Authority (EFSA)—commissioned project to review the state of the art of human biomonitoring for chemical substances and its application to human exposure assessment for food safety, facilitated the identification of vulnerable populations (e.g., by age, sex, socioeconomic status, etc.) as well as chemical exposure associated with food intake [60]. An important and specific context where the studies in diet have been raising more attention and concerns are maternal diet during pregnancy, this being the main source of essential nutrients that are needed for optimal fetal and child development. This applies no just to diet itself but also to prenatal exposure to several environmental pollutants which enter the mother's body as food contaminants, such as dioxins, PAHs and polychlorinated biphenyls [99,100].

Table 3. Studies of human populations related dietary exposures.

Risk factor/exposure	Studied population/number of samples/sample	Genotoxicity biomarkers	Results			Refs.
Arsenic Cooked rice with > 200 µg/kg	Adults not significantly exposed to arsenic through drinking water (west Bengal-India)/N = 400/urothelial cells	MN assay		MN range	MN mean	[101]
			Whole cohort cooked rice arsenic (µg/kg)	0.50–4.98	2.12	
			Lowest cooked rise arsenic group ≤ 100		1.85	
			Highest cooked rice arsenic group > 300		3.23	
			Groups with mean cooked rice arsenic > 200 µg have significantly higher ($p < 0.05$) induction of genetic damage compared to each of the groups with mean cooked rice arsenic ≤ 200 µg/kg.			
Beauvericin and ochratoxin A	N = 1 female (age: 50)/human leukocytes PK15 cells	Comet assay	BEA (0.5 µM) and OTA (1 and 5 µM) as well as all toxin combinations produced a significant increase in tail moment compared to control cells ($p < 0.05$). BEA alone at either concentration had a significantly lower DNA damage than BEA and OTA combinations ($p < 0.05$).			[102]
Food additive benzoic acid	N = 2 adults (age: 24–25)/human peripheral blood lymphocytes	MN assay	Benzoic acid significantly increased micronucleus frequency (200 and 500 µg/mL). This increase was dose-dependent ($r = 0.79$).			[103]
Monosodium glutamate (MSG)	N = 3 adults (age: 23–26)/peripheral blood samples	CBMN assay Comet assay	MN assay:			[58]
			Increase dose dependent ($r = 0.96$).			
			Comet assay:			
			% Tail intensity: $r = 0.60$.			
			Mean tail lenght (mm): $r = 0.59$.			
			Tail moment: $r = 0.71$.			
			Increase dose dependent.			
Sodium sorbate (SS)	N = 2 adults (age: 24–25)/peripheral blood	MN assay Comet assay	SS increased SCEs/cell and MN frequency at 400 µg/mL and 800 µg/mL concentrations at both 24 h and 48 h compared to negative control.			[104]

Continued on next page

			Comet assay Negative control (c = 0 μg/mL) SS (c = 400 μg/mL) SS (c = 8000 μg/mL)	Average tail intensity (%) 2.73 10.91 5.97
			SS is genotoxic to the human peripheral blood lymphocytes in vitro at the highest concentrations.	
Synthetic food colorants Sunset yellow FCF and brilliant blue FCF	N = 10 adults/blood samples.	MN assay	MN frequency was increased with increasing concentrations of sunset yellow and brilliant blue. Sunset yellow, significant increases in the MN rates were detected 30 mg/mL and 40 mg/mL of the concentrations ($p < 0.05$). Brilliant blue, significant increases in the MN rates were detected 30 mg/mL and 40 mg/mL of the concentrations ($p < 0.05$).	[105]
Erythrosine (E127), tartrazine (E102), ponceau 4R (E124), sunset yellow (E110), brilliant blue FCF (E133), fast green (E143), carmoisine (E122), and indigo carmine (E132)	N = 1 adult/blood samples.	CBMN assay	Statistically significant increase in MN means induced by various food colors (multivariate analysis, $p = 0.001$ and pairwise comparisons, $p < 0.05$). Control = 10 100μg/mL = 12 ± 0.7 200μg/mL = 12.8 ± 0.8 300μg/mL = 13.7 ± 0.7	[98]

Table 3 summarizes some important studies in diet field, namely the exposure to arsenic [101], mycotoxins as contaminants in food items [102], food additives [103,104], flavor enhancers [58], and synthetic food colorants [98,105].

For many other compounds for which the effects on cancer risk are not clear, there may be other good reasons to limit exposure. But at the levels that these are found in the food supply, lowering cancer risk is unlikely to be a major reason to justify this. There are moves to redefine maximum permissible limits for food colorants, instead of setting arbitrary limits for food additives in general; for instance in the case of colorants, each dye should have an individual limit based on well controlled genetic studies [98].

8. Conclusions

Human biomonitoring is a scientifically-developed approach for assessing human exposures to natural and synthetic compounds from the environment, occupation, and lifestyle, including diet [56]. It is the only available tool to integrate exposures from all sources and provide data for epidemiological studies of strengths of associations, dose response relations, etc.; however, it does not differentiate the exposure by source. Furthermore, human biomonitoring alone cannot provide information on how long a chemical has been in the body. Additional data collected from questionnaires, interviews and exposure assessment, combined with background knowledge, may provide valuable information regarding sources [21,60].

Although there has been growing recognition for the need to incorporate complex interactions between environmental exposures together with genetic factors, in order to fully understand cancer and diseases causation, since genetic instability is the startup point of carcinogenesis, there is growing recognition that environmental challenges not only interact with genes but may also modulate genetic effects and influence phenotypes [106]. An optimistic message is the fact that cancer development is not an inevitable consequence of the aging process *per se*, although there is a partly avoidable increased likelihood of the requisite number of mutations occurred, and the human species is not inevitably destined to suffer a high incidence of cancer. This awareness has lent greater urgency to the search for more powerful tools for primary prevention, for early warning systems to identify causal environmental agents and flag risks well before a disease condition develops [5].

In conclusion, the potential benefits of biomarkers and molecular epidemiology in illness prevention justify a major commitment to the further development of human biomonitoring programs, the only available tool that combines exposure assessment from different sources and relates their effects, together with individual susceptibility, to the risk of disease.

Acknowledgements

The authors would like to acknowledge Professor Susana Viegas and Professor Carla Viegas for their contribution in conceiving the idea of this review and the CA15132 hCOMET COST Action–European Cooperation in Science and Technology.

Conflict of interest

The authors declare no conflict of interests.

References

1. Boffetta P, Nyberg F (2003) Contribution of environmental factors to cancer risk. *Br Med Bull* 68: 71-94.
2. Cancer Research UK, Cancer risk in the workplace. Cancer Research UK, 2016. Available from: http://www.cancerresearchuk.org/about-cancer/causes-of-cancer/cancer-risks-in-the-workplace.
3. Ladeira C, Gomes MC, Brito M (2014) Human nutrition, DNA damage and cancer: a review, In: *Mutagenesis*: *Exploring Novel Genes and Pathways*. Wageningen: Wageningen Academic Publishers, 73-104.
4. Key JT, Schatzkin A, Willett CW, et al. (2004) Diet, nutrition and the prevention of cancer. *Public Health Nutr* 7: 187-200.
5. Perera FP, Weinstein IB (2000) Molecular epidemiology: recent advances and future directions. *Carcinogenesis* 21: 517-524.
6. Portier CJ, Bell DA (1998) Genetic susceptibility: significance in risk assessment. *Toxicol Lett* 28: 185-189.
7. Vainio H (1998) Use of biomarkers—new frontiers in occupational toxicology and epidemiology. *Toxicol Lett* 102-103:581-589.
8. Bartsch H (2000) Studies on biomarkers in cancer etiology and prevention: a summary and challenge of 20 years of interdisciplinary research. *Mutat Res, Rev Mutat Res* 462: 255-279.
9. Dusinska M, Collins AR (2008) The comet assay in human biomonitoring: gene–environment interactions. *Mutagenesis* 23: 191-205.
10. Perera FP (1996) Molecular epidemiology: insights into cancer susceptibility, risk assessment, and prevention. *J Natl Cancer Inst* 88: 496-509.
11. Au WW (2007) Usefulness of biomarkers in population studies: From exposure to susceptibility and to prediction of cancer. *Int J Hyg Environ Health* 210: 239-246.
12. El-Zein R, Vral A, Etzel CJ, et al. (2011) Cytokinesis-blocked micronucleus assay and cancer risk assessment. *Mutagenesis* 26:101-106.
13. Husgafvel-Pursiainen K (2002) Molecular biomarkers in studies on environmental cancer. *J Epidemiol Community Health* 56(10):730-1.
14. Perera FP (2000) Molecular epidemiology: On the path to prevention? *J Natl Cancer Inst* 92: 602-612.
15. Goldstein B, Gibson J, Henderson R, et al. (1987) Biological markers in environmental health research. *Environ Health Perspect* 74: 3-9.
16. Fergurson L (2008) Biomarkers as endpoints in intervention studies. In: Wild, C., Vineis, P., Garte, S. Author, *Molecular Epidemiology of Chronic Diseases,* West Sussex: John Wiley & Sons Ltd, 255-266.
17. Schulte P, Mazzuckelli LF (1991) Validation of biological markers for quantitative risk assessment. *Environ Health Perspect* 90: 239-246.
18. Davis CD, Milner JA (2007) Biomarkers for diet and cancer prevention research: potentials and challenges. *Acta pharmacol Sin* 28: 1262-1273.
19. US Congress (1990) Genetic monitoring and screening in the workplace. Office of Technology Assessment.

20. Barrett JC, Vainio H, Peakall D, et al. (1997) 12th meeting of the scientific group on methodologies for the safety evaluation of chemicals: susceptibility to environmental hazards. *Environ Health Perspect* 105: 699-737.
21. Ladeira C, Viegas S (2016) Human biomonitoring—An overview on biomarkers and their application in occupational and environmental health. *Biomonitoring* 3: 15-24.
22. Battershill JM, Burnett K, Bull S (2008) Factors affecting the incidence of genotoxicity biomarkers in peripheral blood lymphocytes: impact on design of biomonitoring studies. *Mutagenesis* 23: 423-437.
23. Knudsen LE, Hansen AM (2007) Biomarkers of intermediate endpoints in environmental and occupational health. *Int J Hygiene Environ Health* 210: 461-470.
24. Cavallo D, Ursini CL, Rondinone B et al. (2009) Evaluation of a suitable DNA damage biomarker for human biomonitoring of exposed workers. *Environmental and Molecular Mutagenesis* 50 (9):781–790.
25. Fenech M, Crott J, Turner J, et al. (1999) Necrosis, apoptosis, cytostasis and DNA damage in human lymphocytes measured simultaneously within the cytokinesis-block micronucleus assay: description of the method and results for hydrogen peroxide. *Mutagenesis* 14:605-612.
26. Majer BJ, Laky B, Knasmüller S, et al. (2001) Use of the micronucleus assay with exfoliated epithelial cells as a biomarker for monitoring individuals at elevated risk of genetic damage and in chemoprevention trials. *Mutat Res* 489: 147-172.
27. Burgaz S, Erdem O, Cakmak G, et al. (2002) Cytogenetic analysis of buccal cells from shoe-workers and pathology and anatomy laboratory workers exposed to n-hexane, toluene, methyl ethyl ketone and formaldehyde. *Biomarkers* 7: 151-161.
28. Proia NK (2006) Smoking and smokeless tobacco-associated human buccal cell mutations and their association with oral cancer—A Review. *Cancer Epidemiol Biomarkers Prev* 15: 1061-1077.
29. Fenech M (2007) Cytokinesis-block micronucleus cytome assay. *Nat Protoc* 2: 1084-1104.
30. Holland N, Bolognesi C, Kirschvolders M, et al. (2008) The micronucleus assay in human buccal cells as a tool for biomonitoring DNA damage: The HUMN project perspective on current status and knowledge gaps. *Mutat Res* 659: 93-108.
31. Thomas P, Fenech M (2011) Buccal micronucleus cytome assay. *Methods Mol Biol* 682: 235-248.
32. Cerqueira EMM, Meireles JRC (2012) The use of the micronucleus test to monitoring individuals at risk for oral cancer. In: *The Research and Biology of Cancer*, Hong Kong: Icon Press Ltd, 1-26.
33. Kashyap B, Reddy PS (2012) Micronuclei assay of exfoliated oral buccal cells: means to assess the nuclear abnormalities in different diseases. *J Cancer Res Ther* 8: 184-191.
34. Göethel G, Brucker N, Moro AM, et al. (2014) Evaluation of genotoxicity in workers exposed to benzene and atmospheric pollutants. *Mutat Res Genet Toxicol Environ Mutagen* 770: 61-65.
35. Fenech M (1997) The advantages and disadvantages of the cytokinesis-block micronucleus method. *Mutat Res* 392: 11-18.
36. Fenech M (2000) The in vitro micronucleus technique. *Mutat Res* 455: 81-95.
37. Fenech M, Crott JW (2002) Micronuclei, nucleoplasmic bridges and nuclear buds induced in folic acid deficient human lymphocytes-evidence for breakage-fusion-bridge cycles in the cytokinesis-block micronucleus assay. *Mutat Res* 504: 131-136.

38. Mateuca R, Lombaert N, Aka PV, et al. (2006) Chromosomal changes: induction, detection methods and applicability in human biomonitoring. *Biochimie* 88: 1515-1531.
39. Fenech M (2006) Cytokinesis-block micronucleus assay evolves into a ‘cytome’ assay of chromosomal instability, mitotic dysfunction and cell death. *Mutat Res* 600: 58-66.
40. Fenech M, Kirsch-Volders M, Natarajan AT, et al. (2011) Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells. *Mutagenesis* 26: 125-132.
41. Speit G (2013) Does the recommended lymphocyte cytokinesis-block micronucleus assay forhuman biomonitoring actually detect DNA damage induced by occupational and environmental exposure to genotoxic chemicals? *Mutagenesis* 28: 375-380.
42. Moller P, Knudsen LE, Loft S, et al. (2000) The comet assay as a rapid test in biomonitoring occupational exposure to DNA-damaging agents and effect of confounding factors. *Cancer Epidemiol Biomarkers Prev* 9: 1005-1015.
43. Collins A, Dusinska M (2009) Applications of the comet assay in human biomonitoring. In: Dhawan, A., Anderson, D., Author, *The Comet Assay in Toxicology*, Cambridge: Royal Society of Chemistry, 201-202.
44. Collins AR (2004) The comet assay for DNA damage and repair: principles, applications, and limitations. *Molecular Biotechnol* 26: 249-261.
45. Collins AR (2009) Investigating oxidative DNA damage and its repair using the comet assay. *Mutat Res* 681: 24-32.
46. Azqueta A (2009) Detection of oxidised DNA using DNA repair enzymes. In: Dhawan, A., Anderson, D., Author, *The Comet Assay in Toxicology*, Cambridge: Royal Society of Chemistry, 58-63.
47. Valverde M, Rojas E (2009) Environmental and occupational biomonitoring using the comet assay. *Mutat Res* 681: 93-109.
48. Valverde M, Rojas E (2009) The comet assay in human biomonitoring. In: Dhawan, A., Anderson, D., Author, *The Comet Assay in Toxicology*. Cambridge: Royal Society of Chemistry, 227-251.
49. Digue L, Orsière T, De Méo M, et al. (1999) Evaluation of the genotoxic activity of paclitaxel by the in vitro micronucleus test in combination with fluorescent in situ hybridization of a DNA centromeric probe and the alkaline single cell gel electrophoresis technique (comet assay) in Human T-Lymphocytes. *Environ Mol Mutagenesis* 34: 269-278.
50. Hoffmann H, Speit G (2005) Assessment of DNA damage in peripheral blood of heavy smokers with the comet assay and the micronucleus test *Mutat Res* 581: 105-114.
51. Vasquez MZ (2010) Combining the in vivo comet and micronucleus assays: a practical approach to genotoxicity testing and data interpretation. *Mutagenesis* 25: 187-199.
52. Minozzo R, Deimling LI, Santos-Mello R (2010) Cytokinesis-blocked micronucleus cytome and comet assays in peripheral blood lymphocytes of workers exposed to lead considering folate and vitamin B12 status. *Mutat Res/Genet Toxicol Environ Mutagen* 697: 24-32.
53. Olden K, Guthrie J (2001) Genomics: implications for toxicology. *Mutat Res* 473: 3-10.
54. Toscano WA, Oehlke KP (2005) Systems biology: new approaches to old environmental health problems. *Int J Environ Res Public Health* 2: 4-9.
55. Sexton K, Needham L, Pirkle J (2004) Human biomonitoring of environmental chemicals. *Am Sci* 92: 38.

56. Angerer J, Ewers U, Wilhelm M (2007) Human biomonitoring: state of the art. *Int J Hygiene Environ Health* 210: 201-228.
57. Au WW, Cajas-Salazar N, Salama S (1998) Factors contributing to discrepancies in population monitoring studies. *Mutat Res, Fundam Mol Mech Mutagen* 400: 467-478.
58. Ataseven N, Yüzbaşıoğlu D, Keskin AÇ, et al. (2016) Genotoxicity of monosodium glutamate. *Food Chem Toxicol* 91: 8-18.
59. Geras'kin SA, Kimb JK, Oudalova AA (2005) Bio-monitoring the genotoxicity of populations of Scots pine in the vicinity of a radioactive waste storage facility. *Mutat Res* 583: 55-66.
60. Choi J, Morck TA, Joas A, et al. (2015) Major national human biomonitoring programs in chemical exposure assessment. *Environ Sci* 2: 782-802.
61. Dagnino A, Bo T, Copetta A, et al. (2013) Development and application of an innovative expert decision support system to manage sediments and to assess environmental risk in freshwater ecosystems. *Environ Int* 60: 171-182.
62. Maffei F, Hrelia P, Angelini S, et al. (2005) Effects of environmental benzene: Micronucleus frequencies and haematological values in traffic police working in an urban area. *Mutat Res* 583: 1-11.
63. Kim K-H, Jahan SA, Kabir E (2013) A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health effects. *Environ Inter* 60: 71-80.
64. Song XF, Chen ZY, Zang ZJ (2013) Investigation of polycyclic aromatic hydrocarbon level in blood and semen quality for residents in Pearl River Delta Region in China. *Environ Int* 60: 97-105.
65. Grawe ́ J, Biko J, Lorenz R, et al. (2005) Evaluation of the reticulocyte micronucleus assay in patients treated with radioiodine for thyroid cancer. *Mutat Res* 583: 12-25.
66. Harvey JS, Lyons BP, Page TS, et al. (1999) An assessment of the genotoxic impact of the Sea Empress oil spill by the measurement of DNA adduct levels in selected invertebrate and vertebrate species. *Mutat Res* 441: 103-114.
67. Ceretti E, Feretti D, Viola GC, et al. (2014) DNA damage in buccal mucosa cells of pre-school children exposed to high levels of urban air pollutants. *PLoS One* 2: 1-9.
68. Demircigil GÇ, Erdem O, Gaga EO, et al. (2014) Cytogenetic biomonitoring of primary school children exposed to air pollutants: micronuclei analysis of buccal epithelial cells. *Environ Sci Pollut Res Int* 21: 1197-1207.
69. Marcon A, Fracasso ME, Marchetti P, et al. (2014) Outdoor formaldehyde and NO_2 exposures and markers of genotoxicity in children living near chipboard industries. *Environ Health Perspect* 122: 639-645.
70. Coelho P, García-Lestón J, Costa S, et al. (2013) Genotoxic effect of exposure to metal(loid)s. A molecular epidemiology survey of populations living and working in Panasqueira mine area, Portugal. *Environ Int* 60: 163-170.
71. Mesic A, Nefic H (2015) Assessment of the genotoxicity and cytotoxicity in environmentally exposed human populations to heavy metals using the cytokinesis-block micronucleus cytome assay. *Environ Toxicol* 30: 1331-1342.
72. Kligerman AD, Erexson GL (1999) An evaluation of the feasibility of using cytogenetic damage as a biomarker for alachlor exposure. *Mutat Res* 441: 95-101.

73. Banerjee S, Singh NN, Sreedhar G, et al. (2016) Analysis of the genotoxic effects of mobile phone radiation using buccal micronucleus assay: A comparative evaluation. *J Clin Diagn Res* 10: 82-85.
74. Pastor S, Creus A, Parrón T, et al. (2003) Biomonitoring of four European populations occupationally exposed to pesticides: use of micronuclei as biomarkers. *Mutagenesis* 18: 249-258.
75. Bernardi N, Gentile N, Mañas F, et al. (2015) Assessment of the level of damage to the genetic material of children exposed to pesticides in the province of Córdoba. *Arch Argent Pediatr* 113: 126-131
76. De Coster S, Koppen G, Bracke M, et al. (2008) Pollutant effects on genotoxic parameters and tumor-associated protein levels in adults: A cross sectional study. *Environ Health* 7: 26.
77. Rodríguez TG, Aldeco RG, Alvarez HB, et al. (2008) Genotoxicity in child populations exposed to polycyclic aromatic hydrocarbons (PAHs) in the air from Tabasco, Mexico. *Int J Environ Res Public Health* 5: 349-355.
78. Mielzyńska D, Siwińska E, Kapka L, et al. (2006) The influence of environmental exposure to complex mixtures including PAHs and lead on genotoxic effects in children living in Upper Silesia, Poland. *Mutagenesis* 21: 295-304.
79. Nagya K, Rácz G, Matsumotoa T, et al. (2014) Evaluation of the genotoxicity of the pyrethroid insecticide Phenothrin. *Mutat Res, Genet Toxicol Environ Mutagen* 770: 1-5.
80. Moro AM, Brucker N, Charão M, et al. (2012) Evaluation of genotoxicity and oxidative damage in painters exposed to low levels of toluene. *Mutat Res, Genet Toxicol Environ Mutagen* 746: 42-48.
81. Moro AM, Charãoa MF, Brucker N, et al. (2013) Genotoxicity and oxidative stress in gasoline station attendants. *Mutat Res, Genet Toxicol Environ Mutagen* 754: 63-70.
82. Marco P, Priestly B, Buckett K (1998) Carcinogen risk assessment. Can we harmonise? *Toxicol Lett* 102-103: 241-246.
83. International Labour Organization (ILO). Chemical Exposure Limits. ILO 2011. Available from: http://www.ilo.org/safework/info/publications/WCMS_151534/lang--en/index.htm .
84. Ladeira C, Viegas S, Pádua M, et al. (2014) Assessment of genotoxic effects in nurses handling cytostatic drugs. *J Toxicol Environ Health* 77: 879-887.
85. Martino-Roth MG, Viégas J, Amaral M, et al. (2002) Evaluation of genotoxicity through micronuclei test in workers of car and battery repair garages. *Genet Mol Biol* 25: 495-500.
86. Ladeira C, Viegas S, Carolino E, et al. (2011) Genotoxicity biomarkers in occupational exposure to formaldehyde—The case of histopathology laboratories. *Mutatn Res, Genet Toxicol Environ Mutagen* 721: 115-120.
87. Grover P, Rekhadevi PV, Danadevi K, et al. (2010) Genotoxicity evaluation in workers occupationally exposed to lead. *Int J Hygiene Environ Health* 213: 99-106.
88. Danadevi K, Rozati R, Banu BS, et al. (2004) Genotoxic evaluation of welders occupationally exposed to chromium and nickel using the comet and micronucleus assays. *Mutagenesis* 19: 35-41.
89. Calvert GM, Talaska G, Mueller CA, et al. (1998) Genotoxicity in workers exposed to methyl bromide. *Mutat Res, Genet Toxicol Environ Mutagen* 417: 115-128.
90. Heuser VD, Andrade MV, Silva J, et al. (2005) Comparison of genetic damage in Brazilian footwear-workers exposed to solvent-based or water-based adhesive. *Mutat Res* 583: 85-94.

91. Silveira HC, Schmidt-Carrijo M, Seidel EH, et al. (2013) Emissions generated by sugarcane burning promote genotoxicity in rural workers: A case study in Barretos, Brazil. *Environ Health* 12: 87.
92. Pitarque M, Vaglenov A, Nosko M, et al. (1999) Evaluation of DNA damage by the comet assay in shoe workers exposed to toluene and other organic solvents. *Mutat Res* 44: 115-127.
93. Strickland PT, Groopman JD (1995) Biomarkers for assessing environmental exposure to carcinogens in the diet. *Am J Clin Nutr* 61: 710-720.
94. Sutandyo N (2010) Nutritional carcinogenesis. *Acta Med Indones* 42: 36-42.
95. Anand P, Kunnumakkara AB, Kunnumakara AB, et al. (2008) Cancer is a preventable disease that requires major lifestyle changes. *Pharm Res* 25: 2097-2116.
96. Willett W, Giovannucci E, et al. (2006) Epidemiology of diet and cancer risk. In: Shils, M.E., Shike, M. Author, *Modern Nutrition in Health and Disease*, Philadelphia: Lippincot Williams and Wilkins, 1627.
97. The American Cancer Society, Food additives, safety, and organic foods. The American Cancer Society medical and editorial content team, 2012. Available from: https://www.cancer.org/healthy/eat-healthy-get-active/acs-guidelines-nutrition-physical-activity-cancer-prevention/food-additives.html .
98. Swaroop VR, Dinesh RD, Vijayakumar T (2011) Genotoxicity of synthetic food colorants. *J Food Sci Eng* 1: 128-134.
99. Duarte-Salles T, Mendez MA, Meltzer HM, et al. (2013) Dietary benzo(a)pyrene intake during pregnancy and birth weight: Associations modified by vitamin C intakes in the Norwegian mother and child cohort study (MoBa). *Environ Int* 60: 217-223.
100. Papadopoulou E, Caspersen IH, Kvalem HE (2013) Maternal dietary intake of dioxins and polychlorinated biphenyls and birth size in the Norwegian mother and child cohort study (MoBa). *Environ Int* 60: 209-216.
101. Banerjee M, Banerjee N, Bhattacharjee P, et al. (2013) High arsenic in rice is associated with elevated genotoxic effects in humans. *Sci Rep* 3: 1-8.
102. Klarić MS, Darabos D, Rozgaj R, et al. (2010) Beauvericin and ochratoxin A genotoxicity evaluated using the alkaline comet assay: Single and combined genotoxic action. *Arch Toxicol* 84: 641-650.
103. Yılmaz S, Ünal F, Yüzbaşıoğlu D (2009) The in vitro genotoxicity of benzoic acid in human peripheral blood lymphocytes. *Cytotechnology* 60: 55-61.
104. Mamur S, Yüzbaşıoğlu D, Unal F, et al. (2012) Genotoxicity of food preservative sodium sorbate in human lymphocytes in vitro. *Cytotechnology* 64: 553-562.
105. Kus E, Eroglu HE (2015) Genotoxic and cytotoxic effects of sunset yellow and brilliant blue, colorant food additives, on human blood lymphocytes, *Pak J Pharm Sci* 28: 227-230.
106. Spitz MR, Bondy ML (2010) The evolving discipline of molecular epidemiology of cancer. *Carcinogenesis* 31: 127-134.