Cell Biol Toxicol (2009) 25:5–32
DOI 10.1007/s10565-008-9072-z

Comet assay: a reliable tool for the assessment of DNA damage in different models

Alok Dhawan · Mahima Bajpayee · Devendra Parmar

Received: 13 December 2007 / Accepted: 17 March 2008 / Published online: 22 April 2008
© Springer Science + Business Media B.V. 2008

Abstract New chemicals are being added each year to the existing burden of toxic substances in the environment. This has led to increased pollution of ecosystems as well as deterioration of the air, water, and soil quality. Excessive agricultural and industrial activities adversely affect biodiversity, threatening the survival of species in a particular habitat as well as posing disease risks to humans. Some of the chemicals, e.g., pesticides and heavy metals, may be genotoxic to the sentinel species and/or to non-target species, causing deleterious effects in somatic or germ cells. Test systems which help in hazard prediction and risk assessment are important to assess the genotoxic potential of chemicals before their release into the environment or commercial use as well as DNA damage in flora and fauna affected by contaminated/polluted habitats. The Comet assay has been widely accepted as a simple, sensitive, and rapid tool for assessing DNA damage and repair in individual eukaryotic as well as some prokaryotic cells, and has increasingly found application in diverse fields ranging from genetic toxicology to human epidemiology. This review is an attempt to comprehensively encase the use of Comet assay in different models from bacteria to man, employing diverse cell types to assess the DNA-damaging potential of chemicals and/or environmental conditions. Sentinel species are the first to be affected by adverse changes in their environment. Determination of DNA damage using the Comet assay in these indicator organisms would thus provide information about the genotoxic potential of their habitat at an early stage. This would allow for intervention strategies to be implemented for prevention or reduction of deleterious health effects in the sentinel species as well as in humans.

Keywords Comet assay · In vivo · In vitro · Plants · Invertebrate and vertebrate animal models · Human monitoring

IITR Communication No. 2656

A. Dhawan (✉) · M. Bajpayee · D. Parmar
Developmental Toxicology Division,
Indian Institute of Toxicology Research
(formerly Industrial Toxicology Research Centre),
P.O. Box 80, M.G. Marg,
Lucknow 226 001, India
e-mail: dhawanalok@hotmail.com

Introduction

Ostling and Johanson (1984) were the first to quantify DNA damage in cells using a microgel electrophoresis technique known as "single cell gel electrophoresis or Comet assay". However, the neutral conditions they used, allowed the detection of only DNA double-strand breaks. Later, the assay was adapted under alkaline conditions by Singh et al. (1988), which led to a sensitive version of the assay that could assess both double- and single-strand DNA breaks as well as

the alkali labile sites expressed as frank strand breaks in the DNA. Since its inception, however, the assay has been modified at various steps (lysis, electrophoresis) to make it suitable for assessing various kinds of damage in different cells (Collins 2004; Speit and Hartmann 2005). The assay is now a well-established, simple, versatile, rapid, visual, and a sensitive, extensively used tool to assess DNA damage and repair quantitatively as well as qualitatively in individual cell populations (Olive and Banath 2006). Some other lesions of DNA damage such as DNA cross-links (e.g., thymidine dimers) and oxidative DNA damage may also be assessed using lesion-specific antibodies or specific DNA repair enzymes in the Comet assay. It has gained wide acceptance as a valuable tool in fundamental DNA damage and repair studies (Speit and Hartmann 2005), genotoxicity testing (Moller 2005), and human biomonitoring (Kassie et al. 2000; Moller 2006a).

Relative to other genotoxicity tests, such as chromosomal aberrations, sister chromatid exchanges, alkaline elution, and micronucleus assay, the advantages of the Comet assay include its demonstrated sensitivity for detecting low levels of DNA damage (one break per 10^{10} Da of DNA; Gedik et al. 1992), requirement for small number of cells (~10,000) per sample, flexibility to use proliferating as well as non-proliferating cells, low cost, ease of application, and the short time needed to complete a study. It can be conducted on cells that are the first site of contact with mutagenic/carcinogenic substances (e.g., oral and nasal mucosal cells). The data generated at single cell level allow for robust types of statistical analysis.

A limitation of the Comet assay is that aneugenic effects, which may be a possible mechanism for carcinogenicity (COM 2000), and epigenetic mechanisms (indirect) of DNA damage such as effects on cell-cycle checkpoints are not detected. The other drawbacks such as single cell data (which may be rate limiting), small cell sample (leading to sample bias), technical variability, and interpretation are some of its disadvantages. However, its advantages far outnumber the disadvantages, and hence, it has been widely used in fields ranging from molecular epidemiology to genetic toxicology.

The present review deals with various models ranging from bacteria to man used in the Comet assay for assessing DNA damage (Fig. 1).

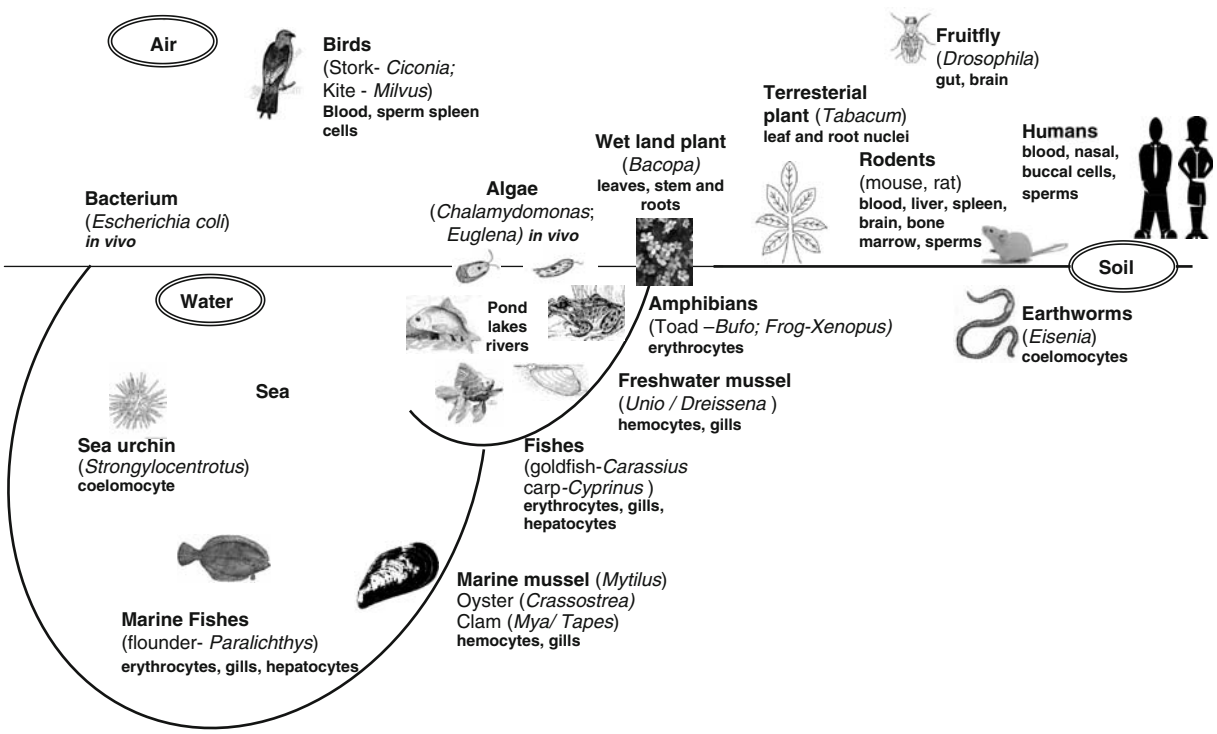

Fig. 1 Schematic diagram of the use of Comet assay in assessing DNA damage in different models from bacteria to humans

Bacteria

The first study to assess the genetic damage in bacteria treated with 12.5–100 rad of X-rays using Comet assay was conducted by Singh et al. (1999). In the study, neutral Comet assay was used for direct (visual) determination of DNA double-strand breaks in the single electrostretched DNA molecule of *Escherichia coli* JM101. Significant increase in the DNA breaks was induced by a dose as low as 25 rad, which was directly correlated to X-ray dosage. The study supported a hypothesis that strands of the electrostretched human DNA in the Comet assay represented individual chromosomes.

Plant models

Plant bioassays help detect genotoxic contamination in the environment (Maluszynska and Juchimiuk 2005). Plant systems can provide information about a wide range of genetic damage, including gene mutations and chromosome aberrations. The mitotic cells of plant roots have been used for the detection of clastogenicity of environmental pollutants, especially for in situ monitoring of water contaminants. Roots of *Vicia faba* and *Allium cepa* have long been used for assessment of chromosome aberrations (Grant 1999) and micronucleus (Ma et al. 1995). During the last decade, the Comet assay has been extensively applied to plants (leaves, shoot, and roots) to detect DNA damage arising due to chemicals and heavy metals in polluted soil (Table 1).

Comet assay in lower plants

Fungi

Schizosaccharomyces pombe has been used as a model organism to investigate DNA damage due to chlorinated disinfectant, alum, and polymeric coagulant mixture in drinking water samples (Banerjee et al. 2008). The authors observed a significantly higher ($P<$ 0.001) DNA damage in chlorinated water (i.e., tap water) when compared to untreated (negative control) or distilled water (laboratory control). Hahn and Hock (1999) used mycelia of *Sordaria macrospora* grown and treated with a variety of DNA-damaging agents directly on agarose minigels for assessment of genotoxicity using the Comet assay. DNA strand breaks were detected by an increase in the DNA migration from the nucleus. This model allowed for the rapid and sensitive detection of DNA damage by a number of chemicals simultaneously.

Algae

Aquatic unicellular plants like algae provide information of potential genotoxicity of the water in which they grow. Being single-celled, they can be used as a model for assessment of DNA damage and monitoring of environmental pollution utilizing Comet assay. Unicellular green alga *Chlamydomonas reinhardtii* was used for evaluation of DNA damage due to known genotoxic chemicals and also demonstrated that oxidative stress was better managed by the algal cells under light than dark conditions (Erbes et al. 1997). The Comet assay was found to be useful for evaluating chemically induced DNA damage and repair in *Euglena gracilis*, and responses were more sensitive than those of human lymphocytes under the same treatment conditions (Aoyama et al. 2003). The ease of culturing and handling *E. gracilis* as well as its sensitivity makes it a useful tool for testing the genotoxicity of chemicals and monitoring environmental pollution. A modified version of the Comet assay was used as an alternative technique to assess DNA damage due to UV radiation in *Rhodomonas* sp. (Cryptophyta), a marine unicellular flagellate (Sastre et al. 2001).

Comet assay in higher plants

V. faba has been widely used for the assessment of DNA damage using Comet assay. Strand breaks and abasic (AP) sites in meristematic nuclei of *V. faba* root tips were studied by the neutral and alkaline Comet assay (Angelis et al. 2000; Menke et al. 2000). The alkaline electrophoresis procedure was found to be most sensitive at low doses, while the neutral electrophoresis procedure yielded an optimal dose–response curve within a wider dose range. Angelis et al. (2000) also suggested that the Comet assay was able to detect a phenomenon resembling clastogenic adaptation at molecular level. Gichner and Plewa (1998) developed a sensitive method for isolation of nuclei from leaf tissue of *Nicotiana tabacum*. The method resulted in high resolution and constant, low

Table 1 Comet assay for assessment of DNA damage—bacteria to humans

Model	Agent tested	Cell used	DNA damage	Reference
Bacteria				
Escherichia coli JM101	X-rays	Whole organism in vivo	↑	Singh et al. 1999
Plant models				
Euglena gracilis	1-methyl-3-nitro-1-nitrosoguanidine (MNNG), benzo[*a*]pyrene, mitomycin C and actinomycin D	Whole organism in vivo	↑	Aoyama et al. 2003
Chlamydomonas reinhardtii	4-nitroquinoline-1-oxide (4-NQO), *N*-nitrosodimethylamine, and hydrogen peroxide	Whole organism in vivo	↑	Erbes et al. 1997
Rhodomonas	UV (UVA + UVB) radiation	Whole organism in vivo	↑	Sastre et al. 2001
Vicia faba	*N*-methyl-*N*-nitrosourea (MNU) and methyl methanesulfonate (MMS)	Root tip meristematic cells	↑	Gichner and Plewa 1998
Tobacco *(Nicotiana tabacuml)*	Ethyl methanesulfonate	Nuclei from leaf tissue	↑	Gichner and Plewa 1998
	Age	Leaf nuclei		Koppen et al. 1999
	Kinetics of DNA repair	Leaf nuclei		Gichner et al. 2000
	Ethyl methanesulphonate (EMS) and *N*-ethyl-*N*-nitrosourea (ENU) maleic hydrazide (MH)	Whole roots in vivo	↑	Gichner 1999, 2003a
	O-phenylenediamine (*o*-PDA), hydrogen peroxide and ethyl methanesulphonate (EMS)	Isolated root nuclei	–	Gichner 2003b
	Heavy metal (Cd, Cu, Pb, and Zn)	Leaf nuclei	↑	Gichner et al. 2006
	Polychlorinated biphenyls		↑	Gichner et al. 2007
Potato plants (*Solanum tuberosum* var. *Korela*)	Heavy metal (Cd, Cu, Pb, and Zn)	Nuclei from leaf tissue	↑	Gichner et al. 2006
Phaeseolus vulgaris	Uranium	Root or shoot cells	–	Vandenhove et al. 2006
Impatiens balsamina	Cr^{+6} and airborne particulate	Stem, root and leaves.	↑	Poli et al. 1999
Bacopa monnieri L.	Ethyl methanesulfonate, methyl methanesulfonate cadmium	Nuclei isolated from roots and leaves	↑ Dose- and time-dependent roots > leaves	Vajpayee et al. 2006
Animal models				
Tetrahymena thermophila	Phenol, hydrogen peroxide, and formaldehyde, influent and effluent water samples	Whole animal in vivo	↑	Lah et al. 2004
Invertebrates				
Bivalves				
Freshwater bivalve zebra mussel (*Dreissena polymorpha*)	Polybrominated diphenyl ethers (pbdes)	Hemocytes	↑↑	Riva et al. 2007
	Sodium hypochlorite and chlorine dioxide) and peracetic acid		↑	Bolognesi et al. 2004
	Pentachlorophenol		↑	Pavlica et al. 2001
	Varying temperatures		↑	Buschini et al. 2003
	Polluted waters		↑	Klobucar et al. 2003
Mytilus edulis	Cadmium (Cd) and chromium (Cr)	Gills	–	Pruski and Dixon 2002
	Styrene	Haemolymph cells	↑	Mamaca et al. 2005
	Tritium	Haemocytes	↑	Jha et al. 2005
	Marine waters (Denmark), French Atlantic Coast	Gill and haemolymph	↑	Rank and Jensen 2003; Akcha et al. 2003
	Polycyclic aromatic hydrocarbons	Gill and haemocytes	↑	Large et al. 2002
	Seasonal variation	Haemocytes	↑	Shaw et al. 2000
Freshwater mussels (*Unio tumidus*)	Polyphenols	Digestive gland cells	↑	Labieniec and Gabryelak 2006

Golden mussel (*Limnoperna fortunei*)	Guaíba Basin water	Haemocytes	↑	Villela et al. 2007
Bivalve mollusc *Scapharca inaequivalvis*	Organotin compounds (MBTC, DBTC and TBTC)	Erythrocytes	↑	Gabbianelli et al. 2006
Mytilus galloprovincialis	Environmental stress	Haemocytes	↑	Frenzilli et al. 2001
	Heavy oil spill	Gills		Laffon et al. 2006a
	Cadmium	Digestive gland cells		Dailianis et al. 2005
Vent mussels (*Bathymodiolus azoricus*)	Hydrostatic pressure change	Haemocytes and gill tissues	↑	Dixon et al. 2004; Pruski and Dixon 2003
Green-lipped mussel (*Perna viridis*)	Benzo[a] pyrene	Haemocytes	↑	Siu et al. 2004
Freshwater mussel *Utterbackia imbecillis*	Chemicals used in lawn care (atrazine, glyphosate, carbaryl, and copper)	Glochidia	↑	Conners and Black 2004
Oyster (*Crassostrea gigas*)	Cryopreservation	Spermatozoa	↑	Gwo et al. 2003
Manila clam (*Tapes semidecussatus*)	Sediment-bound contaminants	Haemolymph, gill and digestive gland	↑	Coughlan et al. 2002; Hartl et al. 2004
Clams (*Mya arenaria*)	Petroleum hydrocarbons	Haemocytes and digestive gland cells	–	Hamouten et al. 2002
Earthworms				
Eisenia foetida	Chemical-treated soil	Coelomocytes	↑ Dose-dependant	Verschaeve et al. 1993
	Soil from coke ovens	Coelomocytes	↑	Salagovic et al. 1996
	Soil from industrialized contaminated areas	Coelomocytes	↑	Xiao et al. 2006
	Sediment from polluted river	Coelomocytes	↑	Rajaguru et al. 2003
	Wastewater irrigated soil	Coelomocytes	↑	Qiao et al. 2007
	Commercial parathion	Coelomocytes	↑	Bustos-Obregon and Goicochea 2002
	Imidacloprid and RH-5849	Sperm cells	↑	Zang et al. 2000
	PAH-contaminated soil and hydrogen peroxide, cadmium (in vitro)	Eleocytes	↑	Di Marzio et al. 2005
	Nickel chloride	Coelomocytes	↑	Reinecke and Reinecke 2004
Aporrectodea longa (Ude)	Soil samples spiked with benzo[*a*]pyrene (B[*a*]P) and/or lindane	Intestine and crop/gizzard cells	↑ Intestine > crop	Martin et al. 2005
Other invertebrates				
Fruitfly (*Drosophila melanogaster*)	Ethyl methanesulfonate (EMS), methyl methanesulfonate (MMS), *N*-ethyl-*N*-nitrosourea (ENU) and cyclophosphamide (CP)	Gut and brain cells of first instar larvae	↑	Siddique et al. 2005a, b
	Cypermethrin		↑	Mukhopadhyay et al. 2004
	Lechates of industrial waste		↑	Siddique et al. 2005b
	Cisplatin		↑	García Sar et al. 2008
Sea urchins (*Strongylocentrotus droebachiensis*)	Dispersed crude oil	Coelomocytes	↑ Concentration-dependent	Taban et al. 2004
Grass shrimp, (*Paleomonetes pugio*)	UV, benzo[*a*] pyrene, and cadmium	Embryos	↑ Damage and decreased repair	Hook and Lee 2004
	Estuarine sediments	Hepatopancreas	↑	Lee et al. 2004
	Coal combustion residues			Kuzmick et al. 2007
Sea anemone (*Anthopleura elegantissima*)	Hydrogen peroxide ethylmethanesulphonate (EMS) or benzo(a)pyrene (B[a]P)	Blood cells	↑ Dose response	Mitchelmore and Hyatt 2004
Vertebrates				
Fishes				
Chub (*Leuciscus cephalus*)	PAHs, PCBs, organochlorine pesticides (OCPs), as well as heavy metals	Hepatocytes	↑	Winter et al. 2004
	Exhaustive exercise	Erythrocytes	↑	Aniagu et al. 2006

Table 1 (continued)

Model	Agent tested	Cell used	DNA damage	Reference
Estuarine mullet (*Mugil* sp.) and sea catfish (*Netuma* sp.)	Organochlorine pesticides and heavy metals High temperature	Erythrocytes	↑	de Andrade et al. 2004a, b
Freshwater teleost fish *Mystus vittatus*	Endosulfan	Gill, kidney, and erythrocytes	↑ In all cells	Sharma et al. 2007
Eastern mudminnow (*Umbra pygmaea* L.)	Rhine water for 11 days	Blood erythrocytes	↑	Alink et al. 2007
Neotropical fish *Prochilodus lineatus*	Diesel water soluble fraction acute (6, 24, and 96 h) and subchronic (15 days) exposures	Erythrocytes	↑	Vanzella et al. 2007
Freshwater goldfish *Carassius auratus*	Technical herbicide Roundup containing Glyphosate salt ADDB and PBTA-6	Erythrocytes	↑↑ Dose-dependent	Cavas and Könen 2007 Masuda et al. 2004
Turbot (*Scophthalmus maximus* L.)	Sediment collected from polluted sites in Cork Harbour (Ireland)	Hepatocytes	↑	Hartl et al. 2007
Brazilian flounder *Paralichthys orbignyanus*	Contaminated estuary waters	Blood cells	↑↑	Amado et al. 2006
Bullheads (*Ameiurus nebulosus*)	Polycyclic aromatic hydrocarbon (PAH) and polychlorinated biphenyl (PCB) polluted waters	Erythrocyte	↑	Pandrangi et al. 1995
Carp (*Cyprinus carpio*)	Polycyclic aromatic hydrocarbon (PAH) and polychlorinated biphenyl (PCB)-polluted waters	Erythrocyte	↑	Pandrangi et al. 1995
Brown trout (*Salmo trutta fario*)	PCB77 (3,3′,4,4′-tetrachlorobiphenyl)	Erythrocytes	–	Belpaeme et al. 1996
Marine flatfish	Ethyl methanesulphate	Blood, gill, liver and kidney	↑ In all tissues	Belpaeme et al. 1998
Trout *Oncorhynchus mykiss*	Cryopreservation (Freeze-thawing)	Spermatozoa	Slight increase	Labbe et al. 2001
European eel (*Anguilla anguilla*)	Benzo[a]pyrene, Arochlor 1254, 2-3-7-8-tetrachlorodibenzo-p-dioxin and beta-naphthoflavone	Erythrocytes	↑	Nigro et al. 2002
Eelpout (*Zoarces viviparus*)	Oil spill (PAH)	Nucleated erythrocytes	↑	Frenzilli et al. 2004
Gilthead sea bream *Sparus aurata*	Copper	Erythrocytes	↑↑	Gabbianelli et al. 2003
Dab (*Limanda limanda*)	PAHs and PCBs polluted waters of English channel Gender and age	Blood cells	↑ In adults and males	Akcha et al. 2003
Hornyhead turbot (*Pleuronichthys verticalis*)	Sediments collected from a natural petroleum seep (pahs)	Liver cells	↑	Roy et al. 2003
In vitro				
Carp (*Cyprius carpio*)	Organic sediment extracts from the North Sea (Scotland)	Leukocytes	↑	Kammann et al. (2000)
Trout (*Oncorhynchus mykiss*)	Cadmium	Hepatocytes	↑	Risso-de Faverney et al. 2001
	Oxidative stress and its prevention by	Erythrocytes	↑	Villarini et al. 1998
	Indolinic and quinolinic nitroxide radicals		↓	
	Tannins		↓	Fedeli et al. 2004
	Diaryl tellurides and ebselen (organoselenium)		↓	Tiano et al. 2000
Zebrafish (*Danio rerio*)	Surface waters of German rivers, Rhine and Elbe	Hepatocytes and gill cells	↑	Schnurstein and Braunbeck 2001
Rainbow trout hepatoma cell line (RTH-149)	Water samples from the polluted Kishon river (Israel)	Liver	↑	Kamer and Rinkevich 2002
Rainbow trout gonad (RTG-2) cell line liver (RTL-W1) cell line	4-Nitroquinoline-*N*-oxide *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine, benzo [*a*] pyrene, nitrofurantoin, 2-acetylaminofluorene, and dimethylnitrosamine, and surface waters	Gonad epitheloid liver	↑ Dose-dependent response	Nehls and Segner 2005

Amphibians				
Amphibian larvae (*Xenopus laevis and Pleurodeles waltl*)	Cadmium ($CdCl_2$) Captan (*N*-trichloromethylthio-4-cyclohexene-1,2-dicarboximide)	Erythrocytes	↑ Concentration-and time-dependent	Mouchet et al. 2007; Mouchet et al. 2006
Amphibian larva (*Xenopus laevis*)	benzo(a)pyrene, ethyl methanesulfonate methyl methanesulfonate	Erythrocytes	–	Mouchet et al. 2005a
	aqueous extracts of five sediments from French channels		↑	Mouchet et al. 2005b
Toad (*Bufo raddei*)	Petrochemical (mainly oil and phenol) polluted area	Liver cells and erythrocytes	↑	Huang et al. 2007
Toad *Xenopus laevis* and *Xenopus tropicalis*	Bleomycin induced DNA damage and repair	Splenic lymphocytes	↑ DNA damage *X. tropicalis* > *X. laevis* DNA repair in *X. laevis* > *X. tropicalis*	Banner et al. 2007
Tadpoles of *Rana N. Hallowell*	Imidacloprid [1-(6-chloro-3-pyridylmethyl)-*N*-nitro-imidazolidin-2-ylideneamine] and RH-5849 [2′-benzoyl-l′-*tert*-butylbenzoylhydrazinel]	Erythrocytes	↑	Feng et al. 2004
Rana hexadactyla tadpoles	Sulfur dyes (Sandopel Basic Black BHLN, Negrosine, Dermapel Black FNI, and Turquoise Blue) used in the textile and tannery industries	Erythrocytes	↑↑	Rajaguru et al. 2001
Bullfrog (*Rana catesbeiana*) tadpoles	Herbicides AAtrex Nine-*O* (atrazine), Dual-960E (metalochlor), Roundup (glyphosate), Sencor-500F (metribuzin), and Amsol (2,4-d amine)	Erythrocytes	↑↑	Clements et al. 1997
Tadpole *Rana clamitans*, *Rana pipiens*	agricultural regions industrial regions	Erythrocytes	↑ Industrial regions > agricultural regions	Ralph and Petras 1997
In vitro				
Xenopus laevis	High peak-power pulsed electromagnetic field	Erythrocytes	↑ Due to rise in temperature	Chemeris et al. 2004
Birds				
Wild nestling white storks (*Ciconia ciconia*)	Heavy metals and arsenic	Blood cells	↑ Correlated with arsenic	Baos et al. 2006
	Toxic acid mining waste rich in heavy metals		↑↑	Pastor et al. 2001a, b; Pastor et al. 2004
Black kites (*Milvus migrans)*	Heavy metals and arsenic	Blood cells	↑ Correlated with copper and cadmium	Baos et al. 2006
	Toxic acid mining waste rich in heavy metals		↑ (2- to 10-fold)	Pastor et al. 2001a, b; Pastor et al. 2004
Turkey	Short term storage	Sperm	↑	Kotłowska et al. 2007
Chicken	T-2 toxin and deoxynivalenol (DON)	Spleen leukocytes	↑	Frankic et al. 2006
Chicken	Storage conditions (4°C)	Liver and breast muscle cells	↑ Liver cells > breast muscle cells	Faullimel et al. 2005
Rodents				
Aldh2 knockout mice	Ethanol	Hepatic cells	↑ Oxidative damage	Kim et al. 2007
P53(+/−) mice	Melphalan	Liver, bone marrow, peripheral blood and the distal intestine	DNA cross-links in all cells tested	Cordelli et al. 2004
SKH-1 mice	UV A + Fluoroquinolones (clinafloxacin, lomefloxacin, ciprofloxacin) UVA + 8-methoxypsoralene (8-MOP)	Epidermal cells	↑↑ For fluoroquino-lones ↓ for MOP	Wirnitzer et al. 2006
Dyslipidemic ApoE(−/−) mice	Ageing Diesel exhaust particles	Aorta, liver, and lung	↑ Oxidative damage in liver; in lung or aorta	Folkmann et al. 2007a; Folkmann et al. 2007b
Balb/c mice	Trypanosoma cruzi infection	Peripheral blood, liver, heart, and spleen cells	↑ In heart and spleen	Ribeiro et al. 2007
CD-1 mice	Lead acetate	Nasal epithelial cells, lung, whole blood, liver, kidney, bone marrow, brain and testes	↑ In all organs on prolonged exposure; in testes	Valverde et al. 2002
Swiss albino mice	Sanguinarine alkaloid, argemone oil	Blood, bone marrow cells and liver	↑ Dose-dependent in blood and bone marrow	Ansari et al. 2004, 2005
	Cypermethrin	Brain, liver, kidney, bone marrow, blood, spleen	↑	Patel et al. 2006

Table 1 (continued)

Model	Agent tested	Cell used	DNA damage	Reference
	Steviol	Stomach cells, hepatocytes, Kidney and testicle cells	↑ –	Sekihashi et al. 2002
	Apomorphine, 8-oxo-apomorphine-semiquinone	Brain cells	↑	Picada et al. 2003
	Ethanol, grape seed oligomer and polymer procyanidin fractions	Brain cells	↓ Ethanol-induced protection by grape seed	Guo et al. 2007
Male CBA mice	Pesticide formulations (Bravo and Gesaprim)	Hepatic cells, bone marrow cells spleen cells	↑↑	Zeljezic and Garaj-Vrhovac 2004
Isogenic mice	Sulfonamide, protozoan parasite Toxoplasma gondii	Peripheral blood cells, liver cells and brain cells	↑ In peripheral blood cells	Ribeiro et al. 2004
Cirrhotic rats	Rutin and quercetin	Bone marrow cells	↑↑	Da silva et al. 2002
In vitro				
FE1 Muta Mouse lung epithelial cell line.	Carbon black	Lung epithelial cell line	↑	Jacobsen et al. 2007
L5178Y mouse lymphoma cells	Ketoprofen, promazine, chlorpromazine, dacarbazine, acridine, lomefloxacin, 8-methoxypsoralen, chlorhexidine, titanium dioxide, octylmethoxycinnamate	Lymphoma cells	Positive with phototoxic compound	Struwe et al. 2007
Murine primary cultures of brain cells and a continuous cell line of astrocytes	Xanthine/xanthine oxidase, hydrogen peroxide Superoxide dismutase, catalase, or ascorbic acid.	Brain cells	↓ By antioxidants	Cemeli et al. 2003
Chinese hamster Ovary cell line (CHO)	Endosulfan	Ovary cells	↑	Bajpayee et al. 2006
	Cypermethrin, pendimethalin, dichlorovous			Patel et al. 2007
Humans				
Clinical				
Breast cancer patients and controls	Radiosensitivity	Peripheral blood mononuclear cells	↑	Djuzenova et al. 2006
Breast cancer patients and controls	Radiosensitivity	Peripheral blood mononuclear cells	↑ And reduced DNA repair	Popanda et al. 2003
Normal individuals	Chlorhexidine	Buccal epithelial cells and peripheral blood lymphocytes	↑	Eren et al. 2002
Transitional cell carcinoma patients and controls	DNA strand breaks	Exfoliated cells extracted from bladder washing	↑ In patients	Mckelvey-Martin et al. 1997
Aaxia telangiectasia heterozygote	X-irradiation	Peripheral leukocytes	↑ (three times high) in patients	Djuzenova et al. 1997
Nijmegen breakage syndrome (NBS) patients	X-irradiation	Peripheral blood mononuclear cells	↑ In patients	Bürger et al. 2006
Alzheimer disease patients	–	Peripheral blood mononuclear cells	↑ In patients	Migliore et al. 2005
Breast cancer patients	–	Peripheral blood mononuclear cells	↑ In patients	Sánches et al. 2004
Type 2 diabetes mellitus	Oxidative DNA damage	Peripheral blood cells	↑	Pitozzi et al. 2003
Cancer (testicular cancer, lymphoma and leukemia) patients	DNA integrity	Spermatozoa	Decreased DNA integrity	O'Donovan 2005
Dietary intervention				
Healthy subjects	Tomato drink	Blood	↓	Porrini et al. 2005
	Green vegetables	Lymphocytes		Kang et al. 2004

	Grape juice			Park et al. 2003
Smokers	Vitamin C supplementation	Blood lymphocyte	↓	Moller et al. 2004
Technical anesthesiology staff	Vitamin E and vitamin C	Blood lymphocyte	↓ In oxidative damage	Sardas et al. 2006
Occupational				
Airport personnel	Jet fuel vapors, jet fuel combustion products	Exfoliated buccal cells and lymphocytes	↑	Cavallo et al. 2006a
Agricultural workers	Pesticides	Lymphocytes	–	Piperakis et al. 2006
			↑	Zeljezic and Garaj-Vrhovac 2001; Paz-y-Mino et al. 2004
Rubber factory workers	Substances used in the rubber industry	Peripheral blood	↓ In exposed population	Laffon et al. 2006b
Outdoor workers in Mexico cities	Air pollutants	Blood lymphocytes	↑	Tovalin et al. 2006
Rickshawpullers	Exhaustive exercise	Lymphocytes	↑	Pandey et al. 2005
Nuclear medicine personnel	Ionizing radiation	Peripheral blood leukocytes	↑	Kopjar et al. 2005
Workers	Polycyclic aromatic hydrocarbon (PAH)	Human T and B lymphocytes and granulocytes	↑ B lymphocytes > T lymphocytes > granulocytes	Sul et al. 2002
	Benzene in printing	Human T and B lymphocytes and granulocytes	↑	Palus et al. 2003
	Lead (Pb) and cadmium (Cd)	Peripheral	↑	Chen et al. 2006
	Asbestos cement plant	Lymphocytes	↑	Dusinská et al. 2004
	Fenvalerate (FE) exposure	Lymphocytes	↑	Bian et al. 2004
	Organic solvents	Sperm	↑	Heuser et al. 2007
		Peripheral blood	↑	
	Coke oven emissions (coe)	Blood lymphocytes		Wang et al. 2007
	Welders (Cd, Co, Cr, Ni, and Pb)	Lymphocytes		Botta et al. 2006
	Pesticide formulators (organophosphorus pesticides)	Lymphocytes	↑	Shadnia et al. 2005
	Copper smelters (Inorganic arsenic)	Leukocytes	↑	Palus et al. 2005
	Chrome-plating workers (chromium -VI)	Lymphocytes	↑↑	Gambelunghe et al. 2003
	Workers in foundry and pottery (Silica)	Lymphocytes	↑	Basaran et al. 2003
Nurses	5-fluorouracil, cytarabine, gemcitabine, cyclophosphamide, and ifosfamide	Lymphocytes	Slight increase	Ursini et al. 2006
Lifestyle				
Normal individuals	Endurance exercise	Lymphocytes	↑	Mastaloudis et al. 2004
Active and passive smokers	Smoking	Lymphocytes	↑	Fracasso et al. 2006
Normal individuals	Smoking Diet (vegetarian or nonvegetarian)	Lymphocytes	↑	Hoffmann et al. 2005a, b; Dhawan et al. 2001; Speit et al. 2003
Rural Indian women	Biomass fuels	Lymphocytes	↑	Pandey et al. 2006
Normal individuals	Benzo(a)pyrene, beta-naphthoflavone (BNF)	Human umbilical vein endothelial cells (HUVEC)	↑	Annas et al. 2000
In vitro				
Episkin	UV, Lomefloxacin and UV or 4-nitroquinoline-N-oxide (4NQO) and protection by Mexoryl	Skin fibroblast cells	↑ Reduced by Mexoryl	Flamand et al. 2006
Sperms	Reproductive toxins	Male germ cells	↑	Shen and Ong 2000; Fraser 2004
Prostate tissues primary culture	2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine (PhIP), its *N*-hydroxy metabolite (*N*-OH-PhIP) and benzo[*a*]pyrene (B[a]P)	Prostrate cells	↑ Dose-related	Martin et al. 2002

Table 1 (continued)

Model	Agent tested	Cell used	DNA damage	Reference
Human keratinocytes	UVA or UVB	Skin cells	↑	Lehmann et al. 1998
MCF-7 cells	Estradiol	Breast cells	↑ Concentration-dependent	Bajpayee et al. 2005
JM1 cells	Estradiol	Lymphoblast cells	–	Bajpayee et al. 2005
HepG2 cells	Endosulfan	Liver cells		Lu et al. 2000
	Indirect acting genotoxins (cyclophosphamide)			Yusuf et al. 2000
Mini organ cultures of human inferior nasal turbinate epithelia	Sodium dichromate, N-nitrosodiethylamine (NDEA) and *N*-methyl-*N*-nitro-*N*-nitroso-guanidine (MNNG)	Nasal cells	↑ With sodium dichromate and MNNG —with NDEA	Buehrlen et al. 2007
	Mono(2-ethylhexyl) phthalate (MEHP), benzo[a]pyrene-7,8-diol-9, 10-epoxide (BPDE), or N-methyl-N′-nitro-N-nitrosoguanidine (MNNG)		↑ With BPDE and MNNG—with MEHP	Kleinsasser et al. 2004
Human lymphocytes	Heterocyclic amine and prevention by monomeric and dimeric flavanols and black tea polyphenols	Lymphocyte	↓ In oxidative damage	Dhawan et al. 2002
	C60 Fullerenes		↑	Dhawan et al. 2006
	Municipal sludge leachates		↑	Bakare et al. 2007

↑: significant increase in DNA damage, ↑↑: highly significant increase in DNA damage, ↓: decrease in DNA damage, –: no DNA damage reported

tail moment values for negative controls, and hence could be incorporated for in situ plant environmental monitoring.

The Comet assay has also been used to study the effect of age of plant on DNA integrity (Koppen et al. 1999) as well as kinetics of DNA repair (Gichner et al. 2000) in isolated nuclei from leaves of tobacco plants. A small but significant increase in DNA damage compared to controls was noted in heterezygous tobacco and potato plants grown on soil contaminated with heavy metal (Gichner et al. 2006). The tobacco and potato plants with increased DNA damage were also found to be severely injured (inhibited growth, distorted leaves), which may be associated with necrotic or apoptotic DNA fragmentation. No DNA damage was observed in the root or shoot cells of *Phaeseolus vulgaris* treated with different concentrations of uranium (Vandenhove et al. 2006). The ornamental plant *Impatiens balsamina* was used as a model to understand the genotoxic effect of Cr^{+6} and airborne particulate matter (Poli et al. 1999), which produced increased strand breaks in plant parts (stem, root, and leaves). Thus, this plant could be used for environmental biomonitoring studies involving air pollution and heavy metals.

The major drawback with plant models was the fact that exposure needs to be given in the soil, and it is difficult to say whether the result demonstrates synergies with other chemicals in the soil or non-availability of the toxicant due to its soil binding affinity. Therefore, Vajpayee et al. (2006) used *Bacopa monnieri* L., a wetland plant, as a model for the assessment of ecogenotoxicity using the Comet assay. In vivo exposure to cadmium (0.01–500 μM) for 2, 4, and 18 h resulted in dose- and time-dependent increases in DNA damage in the isolated roots and leaf nuclei, with roots showing greater DNA damage than leaves. In vitro (acellular) exposure of nuclei from leaves of *B. monneiri* to 0.001–200 μM cadmium resulted in significant ($P<0.05$) levels of DNA damage.

These studies revealed that DNA damage measured in plants using the Comet assay is a good model for assessment of genotoxicity of polluted environment, as in situ monitoring and screening can be accomplished. Higher plants can be used as an alternative first-tier assay system for the detection of possible genetic damage resulting from polluted waters/effluents due to industrial activity or agricultural runoffs.

Animal models

To assess safety/toxicity of chemicals/finished products, animal models have long been used. With the advancements in technology, knockouts and transgenic models have become common to mimic the effects in humans. Comet assay has globally been used for assessment of DNA damage in various animal models (Table 1).

Lower animals

Tetrahymena thermophila is a unicellular protozoan widely used for genetic studies due to its well-characterized genome. Its uniqueness lies in the fact that it has a somatic and a germ nucleus in the same cell. Therefore, it has been validated as a model organism for assessing DNA damage using a modified Comet assay protocol standardized with known mutagens such as phenol, hydrogen peroxide, and formaldehyde (Lah et al. 2004). The method was then used for the assessment of genotoxic potential of influent and effluent water samples from a local municipal wastewater treatment plant (Lah et al. 2004). The method provided an excellent, low-level detection of genotoxicants and proved to be a cost-effective and reliable tool for genotoxicity screening of wastewater.

Invertebrates

Studies have been carried out on various aquatic (marine and freshwater) and terrestrial invertebrates (Table 1). The genotoxicity assessment in marine and freshwater invertebrates using the assay has been reviewed (Cotelle and Ferard 1999; Lee and Steinert 2003; Mitchelmore and Chipman 1998a). Cells from hemolymph, embryos, gills, digestive glands, and coelomocytes from mussels (*Mytilus edulis*; Rank et al. 2005), zebra mussel (*Dreissena polymorpha*), clams (*Mya arenaria*), and polychaetes (*Nereis virens*) have been used for ecogenotoxicity studies using the Comet assay. DNA damage has also been assessed in earthworms (Salagovic et al. 1996; Rajaguru et al. 2003) and fruit fly, *Drosophila*, (Bilbao et al. 2002; Mukhopadhyay et al. 2004). The Comet assay has been employed to assess the extent of DNA damage at polluted sites in comparison to reference sites in the environment, and in the laboratory, it has been widely used as a mechanistic tool to determine pollutant effects and mechanisms of DNA damage (Mitchelmore and Hyatt 2004).

Comet assay in Mussels

Freshwater and marine mussels have been used to study the adverse effect of contaminants in the aquatic environment, as they are important pollution indicator organisms. These sentinel species are adversely affected by pollution in the water bodies and thus provide the potential for environmental biomonitoring. The Comet assay in mussels can be used to detect a reduction in water quality caused by chemical pollution (Frenzilli et al. 2001; Jha et al. 2005; Rank et al. 2005; Steinert et al. 1998). *Mytilus edulis* has been widely used for Comet assay studies to evaluate DNA strand breaks in gill and digestive gland nuclei due to polycyclic aromatic hydrocarbons (PAHs) including benzo[*a*]pyrene (B[*a*]P; Large et al. 2002) and oil spills with petroleum hydrocarbons (Hamouten et al. 2002). However, the damage returned to normal levels after continued exposure to high dose (20 ppb-exposed diet) of B[*a*]P for 14 days. This was attributed to an adaptive response in mussels to prevent the adverse effects of DNA damage (Large et al. 2002). The green-lipped mussels (*Perna viridis*) also showed a similar result to B[*a*]P in water (Siu et al. 2004).

Significant levels of interindividual variability, including seasonal variations in DNA damage, have been reported from some studies in both laboratory and field (Wilson et al. 1998; Shaw et al. 2000, 2004; Frenzilli et al. 2001). Baseline monitoring has thus to be carried out over long time intervals. Temperature-dependent DNA damage was observed in hemocytes of freshwater mussel *Dreissena* polymorpha (Buschini et al. 2003), showing that the mussels are sensitive towards change in water temperatures. Thus, monitoring ecogenotoxicity with these species should take into account variations in temperatures. Findings have also suggested that antioxidant supplementation can improve the sensitivity of the Comet assay by lowering the baseline damage in untreated animals (Wilson et al. 1998).

Villela et al. (2006) used the golden mussel (*Limnoperna fortunei*) as a potential indicator organism for freshwater ecosystems due to its sensitivity to water contaminants. Comet assay in haemocytes of

freshwater Zebra mussel, *D. polymorpha* Pallas, was used as a tool in determining the potential genotoxicity of water pollutants (Bolognesi et al. 2004; Klobucar et al. 2003; Pavlica et al. 2001; Riva et al. 2007). Klobucar et al. (2003) suggested the use of Comet assay in haemocytes from caged, non-indigenous mussels as a sensitive tool for monitoring genotoxicity of freshwater. DNA damage and repair studies in vent mussels, *Bathymodiolus azoricus*, have been carried out to study the genotoxicity of naturally contaminated deep-sea environment (Dixon et al. 2004; Pruski and Dixon 2003). The vent mussels demonstrated similar sensitivity to environmental mutagens as that of coastal mussels and thus could be used for ecogenotoxicity studies of deep sea waters using the Comet assay.

In vitro Comet assay has also been used in cells of mussels. Dose–response increases in DNA strand breakages were recorded in digestive gland cells (Mitchelmore et al. 1998b), hemocytes (Rank and Jensen 2003), and gill cells (Wilson et al. 1998, Rank and Jensen 2003) of *M. edulis* exposed to both direct (hydrogen peroxide and 3-chloro-4-(dichloromethyl)-5-hydroxy-2[*5H*]-furanone) and indirect (B[*a*]P, 1-nitropyrene, nitrofurantoin and *N*-nitrosodimethylamine) acting genotoxicants. Digestive gland cells of *Unio tumidus* were also used for in vitro studies of DNA damage and repair due to pro-oxidative effect of polyphenolic compounds (Labieniec and Gabryelak 2004, 2006). Wilson et al. (1998) demonstrated potential application of the Comet assay to gill cells of *M. edulis* as a potential in vitro screen for agents destined for release or disposal into the marine environment.

Comet assay in other bivalves

Coughlan et al. (2002) showed that the Comet assay could be used as a tool for the detection of DNA damage in clams (*Tapes semidecussatus*) as biomonitor organisms for sediments. Significant DNA strand breaks were observed in cells isolated from haemolymph, gill, and digestive gland from clams exposed to polluted sediment (Coughlan et al. 2002; Hartl et al. 2004). Comet assay was used for the assessment of sperm DNA quality of cryopreserved semen in Pacific oyster (*Crassostrea gigas*), as it is widely used for artificial fertilization (Gwo et al. 2003). Gielazyn et al. (2003) demonstrated the use of lesion-specific DNA repair enzyme formamidopyrimidine glycosylase (Fpg) to enhance the usefulness and sensitivity of the Comet assay in studying oxidative DNA damage in isolated hemocytes from oyster (*Crassostrea virginica*) and clam (*Mercenaria mercenaria*).

The studies in mussels have shown the Comet assay to be a sensitive, but nonspecific, molecular biomarker of genotoxicity. One of the drawbacks when applying single-cell gel electrophoresis to field populations may be the adapability of the animals to high concentrations of contaminants (e.g., B[*a*]P), which may pose a major problem (Large et al. 2002). Also, seasonal variation and temperature altered both DNA damage baseline levels in untreated animals and cell sensitivity towards environmental pollutants under in vitro conditions (Buschini et al. 2003; Hartl et al. 2004). The Comet assay detecting DNA strand breaks has demonstrated that higher basal levels of DNA damage are observed in marine invertebrates; hence, the protocol followed in these animals should be considered for biomonitoring the ecogenotoxicity of a region (Machella et al. 2006).

Comet assay in earthworm

The Comet assay applied to earthworms is a valuable tool for monitoring and detection of genotoxic compounds in terrestrial ecosystems (Salagovic et al. 1996; Zang et al. 2000; Table 1). As the worms feed on the soil they live in, they are a good indicator of the genotoxic potential of the contaminants present in the soil and thus used as a sentinel species. Verschaeve et al. (1993) demonstrated a dose–response with the extent of DNA damage in coelomic leucocytes (coelomocytes) of earthworms (*Eisenia foetida*) from soil treated with different chemicals as an indication of soil pollution.

Coelomocytes from *E. foetida* demonstrated increased DNA damage when worms were exposed to soil samples from polluted coke oven sites (Salagovic et al. 1996) or industrialized contaminated areas (Xiao et al. 2006) and even sediment samples from polluted river system (Rajaguru et al. 2003). An insecticide, parathion, produced DNA strand breaks at all time points and doses in the sperm cells of *E. foetida* (Bustos-Obregon and Goicochea 2002), while dose–effect relationships were displayed by two pesticides, Imidacloprid and RH-5849, in the same species (Zang et al. 2000), showing that pesticides could also have adverse effects on non-target species. In vitro exposure

of coelomocytes primary cultures to nickel chloride as well as whole animals either in spiked artificial soil water or in spiked cattle manure substrates exhibited increased DNA strand breaks due to the heavy metal (Reinecke and Reinecke 2004). The eleocytes, a subset of coelomocytes, exhibited increased DNA strand breaks under both in vitro and in vivo conditions and could be used as a sensitive biomarker for genotoxicity in earthworms (Di Marzio et al. 2005). Another earthworm, *Aporrectodea longa* (*Ude*), when exposed to soil samples spiked with B[*a*]P and/or lindane, demonstrated intestinal cells to be more sensitive to the effect of the genotoxicants than the crop/gizzard cells (Martin et al. 2005).

Fourie et al. (2007) used five earthworm species (*Amynthas diffringens*, *Aporrectodea caliginosa*, *Dendrodrilus rubidus*, *Eisenia foetida*, and *Microchaetus benhami*) to study genotoxicity of sublethal concentrations of cadmium sulphate, with significant DNA damage being detected in *E. foetida* followed by *D. rubidus* and *A. caliginosa*. The study showed a difference in sensitivity of species present in an environment and its influence on the genotoxicity risk assessment. Hence, for environmental biomonitoring, specific species have to be kept in mind to reduce false negative results.

Comet assay in Drosophila

The simple genetics and developmental biology of *Drosophila melanogaster* has made it the most widely used insect model and has been recommended as an alternate animal model by the European Centre for the Validation of Alternative Methods (ECVAM; Benford et al. 2000). Recently, *Drosophila* has evolved as a model organism in toxicological studies (Mukhopadhyay et al. 2003; Nazir et al. 2003). *D. melanogaster* has also been used as an in vivo model for assessment of genotoxicity using Comet assay (Bilbao et al. 2002; Mukhopadhyay et al. 2004; Siddique et al. 2005a, b; Table 1). Neuroblast cells of third instar larvae, DNA repair deficient in nucleotide excision repair (mus201), and a mechanism of damage bypass (mus308) have been used for mechanistic studies (Bilbao et al. 2002).

Third instar larvae of *D. melanogaster* (Oregon R +) were validated for genotoxicity assessment using a modified Comet assay (Siddique et al. 2005a, b). As the cells of Drosophila are smaller than mammalian cells, modifications in the Comet assay were done, e.g., higher concentration of agarose (for the smaller size of Drosophila cells), removal of dimethyl sulfoxide (DMSO) from lysing solution (DMSO is toxic to the cells), and lower electrophoresis time (for improved performance of the assay). This modified protocol was validated in gut and brain cells using well-known alkylating agents, i.e., ethyl methanesulfonate (EMS), methyl methanesulfonate (MMS), *N*-ethyl-*N*-nitrosourea (ENU), and cyclophosphamide (CP), which were mixed in standard Drosophila diet and produced a significant dose-dependent response (Siddique et al. 2005a, b). Cypermethrin, a synthetic pyrethroid, even at low concentrations (at 0.002 ppm), and leachates of industrial waste produced significant dose-dependent increase in DNA damage in the brain ganglia and anterior midgut of *D. melanogaster* (Mukhopadhyay et al. 2004; Siddique et al. 2005b). Results from Comet assay have also shown a direct correlation between the concentrations of cisplatin adducts and DNA damage in somatic cells of *D. melanogaster* (García Sar et al. 2008).

In vitro studies using Drosophila S2 cells demonstrated that the ectopically expressed DNA glycosylases (dOgg1 and RpS3) reduced the oxidized guanosine (8-OxoG) but contributed to increased DNA degradation due to one of the constituents of the DNA repair system (Radyuk et al. 2006).

The studies in Drosophila have shown it to be a good alternate to animal model for the assessment of in vivo genotoxicity of chemicals using the Comet assay.

Comet assay in other invertebrates

Nereis virensa, a polychaete, plays an important role in the distribution of pollutants in sediments due to their unique property of bioturbation. These worms are similar to earthworms in soil and can be used for genotoxicity assessment of sediments. Intracoelomic injection of B[*a*]P was given to the worms, and Comet assay was conducted on coelomocytes (De Boeck and Kirsch-Volders 1997). Nereis species was, however, not found to be suitable for assessing PAH genotoxicity probably due to its lack of metabolic capability to convert B[*a*]P to its toxic metabolite (De Boeck and Kirsch-Volders 1997).

DNA damage was assessed in neuroblast cells of brains of first instars of grasshoppers (*Chorthippus*

brunneus) exposed to various doses of zinc from a polluted site to understand the mechanism of toxicity in insects due to industrial pollutants (Augustyniak et al. 2006).

The estuarine grass shrimp, *Palaemonetes pugio*, exposed to coal combustion residues from coal-fired electrical generation, were studied for DNA damage using Comet assay. Chronic exposure caused DNA damage in hepatopancreas cells of adult shrimps as compared to reference shrimp (Kuzmick et al. 2007). Comet assay in planarians is an important test for environmental monitoring studies, as these are simple organism with high sensitivity, low cost, and high proliferative rate (Prá et al. 2005). The genotoxic potential of water from Diluvio's Basin was evaluated in planarians where increase in pollutants towards the basin led to an increase in the DNA damage in these species (Prá et al. 2005). Significant increase of primary DNA damage was observed in planarian cells due to Norflurazon, a bleaching herbicide (Horvat et al. 2005), and copper sulfate (Guecheva et al. 2001) when compared to the control animals.

These studies have also shown the use of Comet assay in biomonitoring diverse environmental conditions utilizing sentinel species.

Vertebrates

Studies of vertebrate species where the Comet assay is used include fishes, amphibians, birds, and mammals. Cells (blood, gills, kidneys, and livers) of different fishes, tadpoles and adult frogs, as well as rodents have been used for assessing in vivo and in vitro genotoxicity of chemicals, and human biomonitoring has also been carried out employing the Comet assay (Table 1).

Comet assay in fishes

Various fishes (freshwater and marine) have been used for environmental biomonitoring, as they are endemic organisms, which serve as sentinel species for a particular aquatic region, to the adverse effects of chemicals and environmental conditions. The Comet assay has found wide application as a simple and sensitive method for evaluating in vivo as well as in vitro DNA damage in different tissues (gills, liver, blood) of fishes exposed to various xenobiotics in the aquatic environment (Table 1).

Environmental biomonitoring to assess the genotoxic potential of river waters has been carried out in hepatocytes of chub (*Leuciscus cephalus*; Winter et al. 2004), erythrocytes of mullet (*Mugil* sp.), sea catfish (*Netuma* sp.; de Andrade et al. 2004a, b), bullheads (*Ameiurus nebulosus*), and carps (*Cyprinus carpio*; Pandrangi et al. 1995; Buschini et al. 2004). Basal level of DNA damage has been shown to be influenced by various factors, such as temperature of water in erythrocytes of mullet and sea catfish (de Andrade et al. 2004a, b), age and gender in dab (*Limanda limanda*; Akcha et al. 2003), and exhaustive exercise in chub (Aniagu et al. 2006). Therefore, these factors should be accounted for during environmental biomonitoring studies for genotoxicity. The sensitivity of the assay may be affected by high intra-individual variability (Akcha et al. 2003). The protocol and experimental conditions used for the Comet assay for monitoring marine ecosystems may lead to differences in the results obtained (Belpaeme et al. 1998). The use of chemical and mechanical procedures to obtain cell suspension may also lead to DNA damage (Kosmehl et al. 2006). Anesthesia did not contribute towards DNA damage in vivo in methyl methanesulfonate (MMS)-treated fishes, and the anesthetic benzocaine did not alter the DNA damage in erythrocytes after in vitro exposure to MMS or H_2O_2 (de Miranda Cabral Gontijo et al. 2003). Hence, keeping in mind animal welfare, multi-sampling in the same fish can be conducted.

In vitro studies on fish hepatocytes (Risso-de Faverney et al. 2001), primary hepatocytes and gill cells (Schnurstein and Braunbeck 2001), as well as established cell lines (with metabolic competence; Nehls and Segner 2001, 2005) using the Comet assay have also been conducted to assess the genotoxicity of chemicals in water samples. The antioxidant potential of indolinic and quinolinic nitroxide radicals (Villarini et al. 1998), tannins (Fedeli et al. 2004), and low concentrations (<10 μM) of diaryl tellurides and ebselen, an organoselenium compound (Tiano et al. 2000), in oxidative DNA damage has been studied in nucleated trout (*Oncorhynchus mykiss*) erythrocytes for use of these compounds in biological systems. Kammann et al. (2000) demonstrated the Comet assay in isolated leukocytes of carp as an in vitro model for evaluating genotoxicity of marine sediment extracts and increased sensitivity of the method with the use of DNA repair inhibitor, 1-beta-D-arabinofuranosylcyto-

sine (ara C). Comet assay with fish cell lines may be a suitable tool for in vitro screening of environmental genotoxicity; however, the metabolizing capabilities of the cell line need to be taken into account.

Cryopreservation has been shown to induce DNA strand breaks in spermatozoa of trout (Cabrita et al. 2005; Labbe et al. 2001), sea bass (*Dicentrarchus labrax*; Zilli et al. 2003), and gilthead sea bream (*Sparus aurata*; Cabrita et al. 2005). The DNA damage was prevented by the addition of cryopreservants such as bovine serum albumin and dimethyl sulfoxide (Zilli et al. 2003). These studies have demonstrated the sperm Comet assay as a useful model in determining the DNA integrity in frozen samples for commercially cultured species.

These studies have demonstrated the usefulness of the Comet assay in fishes as a model for monitoring genotoxicity of aquatic habitats using these indicator animals.

Comet assay in amphibians

Comet assay in amphibians has been carried out at adult and larval stages for eco-genotoxicity of aquatic environments, and studies until 1999 have been well reviewed by Cotelle and Ferard (1999). The animals chosen for the Comet assay act as sensitive bioindicators of aquatic and agricultural ecosystems (Table 1). The animals were either collected from the site (in situ) or exposed to chemicals under laboratory/natural conditions.

Erythrocytes from tadpole of two sentinal species *Rana clamitans* and *Rana pipiens* have been used for in situ genotoxicity monitoring of water bodies (Ralph and Petras 1997). *R. clamitans* tadpoles collected from agricultural regions showed significantly higher ($P<0.001$) DNA damage than tadpoles collected from sites of little or no agriculture. Similarly, *R. pipiens* tadpoles collected from industrial sites showed significantly higher ($P<0.001$) DNA strand breaks than samples from the agricultural areas. The higher levels of DNA damage may be due to the pesticides used in the agricultural region. Variation in DNA damage due to sampling time (Ralph and Petras 1997) and during various metamorphosis states (Ralph and Petras 1998a) was also observed. Hence, for biomonitoring environmental genotoxicity using the Comet assay, pooling of early tadpole phases could be helpful. Studies have also been conducted on caged tadpoles in areas where indigenous population is not present due to ecological imbalance from pollution. *R. clamitans* and American toad (*Bufo americanus*) tadpoles were caged at polluted reference site and demonstarted significant ($P<0.05$) increases in DNA damage, relative to control tadpoles in the laboratory (Ralph and Petras 1998b). These results demonstrated that caged tadpoles could be used for monitoring genotoxicity of water habitats that do not support the survival of tadpoles, e.g., large lakes and aquatic areas near high industrial activity.

Huang et al. (2007) have shown the genotoxicity of petrochemicals in liver and erythrocytes of toad *Bufo raddeis*. DNA damage was found to be positively correlated to the concentration of petrochemicals in liver, pointing to the fact that liver is the site for metabolism and may be a good marker for studying genotoxicity of compounds which require metabolic activation. Effect of polyploidy on bleomycin-induced DNA damage and repair in *Xenopus laevis* (pseudotetraploid) and *Xenopus tropicalis* (diploid) was studied using Comet assay (Banner et al. 2007). The *X. tropicalis* was more sensitive with lower capacity for repair than *X. laevis*, showing that polyploidy protects DNA damage and allows rapid repair, and hence, these species may be used as a good model for DNA damage and repair studies.

Comet assay in birds

There are few studies involving Comet assay in birds (Table 1). Genetic damage due to a mining accident involving heavy metals has been reported in free-living, nestling white storks (*Ciconia ciconia*) and black kites (*Milvus migrans*) from southwestern Spain (Baos et al. 2006; Pastor et al. 2001a, b, 2004); however, species-specific and intraspecies differences were observed. Faullimel et al. (2005) showed that the neutral Comet assay could be used to study the impact of freezing and thawing on DNA integrity in breast fillets and liver cells of frozen chicken. Frankic et al. (2006) reported that T-2 toxin and deoxynivalenol (DON) induced DNA fragmentation in chicken spleen leukocytes which was abrograted by dietary nucleotides. Kotłowska et al. (2007) have demonstrated increased DNA fragmentation in turkey sperm after 48 h of liquid storage and might be helpful in evaluating the DNA integrity for artificial insemination.

Comet assay in rodents

Mice and rats have been widely used as animal models for the assessment of in vivo genotoxicity of chemicals using the Comet assay (Table 1). The in vivo Comet assay has been accepted by the UK Committee on Mutagenicity testing of chemicals in food, consumer products, and environment (COM 2000) as a test for assessing DNA damage and is recommended for follow-up testing of positive in vitro findings. A positive result in the in vivo Comet assay assumes significance if mutagenic potential of a chemical has already been demonstrated in vitro. Within a battery of tests, Comet assay finds place as a supplemental in vivo test which has been accepted by international guidelines (Brendler Schwaab et al. 2005). There are specific guidelines for the performance of Comet assay in vivo for reliable results (Tice et al. 2000; Hartmann et al. 2003; Burlinson et al. 2007).

Multiple organs of mouse/rat including brain, blood, kidney, lungs liver, and bone marrow have been utilized for the comprehensive understanding of the systemic genotoxicity of chemicals (Meng et al. 2004; Patel et al. 2006; Sasaki et al. 2000; Sekihashi et al. 2002). The most important advantage of the use of Comet assay is that DNA damage in any organ can be evaluated without the need for mitotic activity and DNA damage in target as well as non-target organs can also be seen (Sasaki et al. 2000). A comprehensive data on chemicals representing different classes, e.g., PAHs, alkylating compounds, nitroso compounds, food additives, etc., that caused DNA strand breaks in various organs of mice were compiled by Sasaki et al. (2000, 2002). The mouse or rat organs exhibiting increased levels of DNA damage were not necessarily the target organs for carcinogenicity. Therefore, for the prediction of carcinogenicity of a chemical, organ-specific genotoxicity was necessary but not sufficient (Sasaki et al. 2002). The Comet assay can be used as an in vivo test apart from the cytogenetic assays in hematopoietic cells and also for those compounds which have poor systemic bioavailability.

Different routes of exposure in rodents have been used, e.g., intraperitoneal (Ansari et al. 2004; Patel et al. 2006), oral (Risom et al. 2007; Wang et al. 2006), and inhalation (Meng et al. 2005; Valverde et al. 2002), to study the genotoxicity of different chemicals. The route of exposure is an important determinant of the genotoxicity of a chemical due to its mode of action (Sekihashi et al. 2002). The in vivo comet assay helps in hazard identification and assessment of dose–response relationship as well as mechanistic understanding of a substance's mode of action. Besides being used for testing the genotoxicity of chemicals in laboratory-reared animals, Comet assay in wild mice can be used as a valuable test in pollution monitoring and environmental conservation (Mateos et al. 2008).

In vivo Comet assay in rodents is an important test model for genotoxicity studies, as many rodent carcinogens are also human carcinogens, and hence, this model not only provides an insight into the genotoxicity of human carcinogens but also is suited for studying their underlying mechanisms.

Comet assay in humans

Comet assay is a valuable method for detection of occupational and environmental exposures to genotoxicants in humans and can be used as a tool in risk assessment for hazard characterization (Albertini et al. 2000; Dusinska and Collins 2008; Moller 2005, 2006a; Table 1). DNA damage assessed by the Comet assay gives an indication of recent exposure and at an early stage where it could also undergo repair (Maluf and Erdtmann 2001), and thus, it provides an opportunity for intervention strategies to be implemented timely. The assay can be conducted in the same population after removal of genotoxicant/dietary intervention to detect the extent of reduction in DNA damage. The assay is a noninvasive technique compared to other DNA damage techniques (chromosomal aberrations, micronucleus), which require larger sample (~2–3 ml) as well as proliferating cell population (or cell culture). Human biomonitoring using the Comet assay is advantageous, as it is rapid, cost-effective, easy compilation of data and concordance with cytogenetic assays (Faust et al. 2004a).

The assay has been widely used in studying DNA damage and repair in healthy individuals (Bajpayee et al. 2002, 2005; Betti et al. 1995; Collins 2004) in clinical studies (Corrie et al. 2005; Wynne et al. 2007; McKenna et al. 2008) as well as in dietary intervention studies (Glei et al. 2005; Moller et al. 2004; Moller and Loft 2002; Porrini et al. 2005; Wilms et al. 2005) and in monitoring the risk of DNA damage resulting from occupational (Güerci et al. 2006; Garaj-Vrhovac and Zeljezic 2002; Piperakis et al. 2006; Srám and Binková

2000), environmental (Gutiérrez-Castillo et al. 2006; Pandey et al. 2005), oxidative DNA damage (Cavallo et al. 2006b; Palus et al. 2005), exposures or lifestyle (Avogbe et al. 2005; Dhawan et al. 2001). White blood cells or lymphocytes are the most frequently used cell type for Comet assay in human biomonitoring studies (reviewed by Angerer et al. 2007; Faust et al. 2004a, b; Moller 2006b); however, other cells have also been used, e.g., buccal cells (Szeto et al. 2005), nasal (Mussali- Galante et al. 2005), sperm (Delbes et al. 2007; Fraser 2004; Schmid et al. 2007; Singh et al. 2003), epithelial (Graham-Evans et al. 2004; Emri et al. 2004; Rojas et al. 2000), and placental cells (Augustowska et al. 2007).

The Comet assay has been used as a test to predict the risk for development of diseases (renal cell carcinoma, cancers of the bladder, oesophagus, and lung) due to susceptibility of the individual to DNA damage (Djuzenova et al. 1999; Lin et al. 2007; Schabath et al. 2003; Shao et al. 2005). The in vitro Comet assay is proposed as an alternative to cytogenetic assays in early genotoxicity/photogenotoxicity screening of drug candidates (Witte et al. 2007) as well for neurotoxicity. Certain factors like age, diet, lifestyle (alcohol and smoking), as well as diseases have been shown to influence the Comet assay parameters, and for interpretation of responses, these factors need to be accounted for during monitoring human genotoxicity (Anderson 2001; Moller et al. 2000).

Human biomonitoring studies using the Comet assay provide an efficient tool for measuring human exposure to genotoxicants, thus helping in risk assessment and hazard identification.

Specificity, sensitivity, and limitations of the Comet assay

The Comet assay has found worldwide acceptance for detecting DNA damage and repair in prokaryotic and eukaryotic cells. However, there are issues relating to the specificity, sensitivity, and limitations of the assay which need to be addressed by the genetic toxicologists before it gets accepted in the regulatory framework including interlaboratory validation of in vitro and in vivo Comet assay.

The variability in the results of the Comet assay is largely due to its sensitivity and minor differences in the conditions of various laboratories as well as the effect of confounding factors in human studies (lifestyle, age, diet, interindividual, and seasonal variation). Prospective cohort studies have not been conducted to find the predictive value of the Comet assay in human biomonitoring, further limiting its application (Moller 2006a). Cell to cell, gel to gel, culture to culture, animal to animal variability as well as use of various image analysis systems or visual scoring (Forchhammer et al. 2008) and use of different Comet parameters, e.g., Olive tail moment and tail (%) DNA, are the other factors contributing to interlaboratory differences in the results.

The limitation of the Comet assay is that it only detects DNA damage in the form of strand breaks. The alkaline (pH>13) version of the assay assesses direct DNA damage or alkali labile sites, while specific classes of DNA damage including base oxidation and DNA adduct formation cannot be measured. The specific and sensitive detection of these lesions requires the use of lesion-specific enzymes (Collins 2004). These enzymes are bacterial glycosylase/endonuclease enzymes, which recognize a particular damage and convert it into a break that can then be measured in the Comet assay. Hence, broad classes of oxidative DNA damage, alkylations, and ultraviolet light-induced photoproducts can be detected as increased amount of DNA in the tail (Moller 2006a). Oxidized pyrimidines are detected with use of endonuclease III, while oxidized purines with formamidopyrimidine DNA glycosylase (FPG). Modifications have been made in the protocol to specifically detect double-strand breaks (neutral Comet assay; Singh 2000), single-strand breaks (at pH 12.1; Miyamae et al. 1997), DNA cross-links (decrease in DNA migration due to cross-links, Singh 2000), and apoptosis (Singh 2000). Neutral comet assay also helps to distinguish apoptosis from necrosis as evidenced by the increased Comet score in apoptotic cells and the almost zero Comet score in necrotic cells (Yasuhara et al. 2003). An adaptation of the Comet assay was also developed which enables the discrimination of viable, apoptotic, and necrotic single cells (Morley et al. 2006). Use of proteinase-K specifically removes DNA–protein cross-links, leading to increased migration but would not affect the DNA–DNA cross-links, thereby indicating a specific type of lesion (Singh 2000).

Tail (%) DNA and Olive tail moment give a good correlation in genotoxicity studies (Kumaravel and

Jha 2006), and as most studies have reported these Comet parameters, it has been recommended that both these parameters should be applied for routine use. Since the OTM is reported as arbitrary units and different image analysis systems give different values, tail (%) DNA is considered a better parameter (Kumaravel and Jha 2006).

It is therefore required that the in vitro and in vivo testing be conducted according to the Comet assay guidelines and appropriately designed multi-laboratory international validation studies be carried out.

Guidelines for the in vitro as well as in vivo Comet assay have been formulated (Hartmann et al. 2003; Tice et al. 2000). Recently, issues relating to study design and data analysis in Comet assay were discussed by the International Workgroup on Genotoxicity Testing where particular attention was given to the alkaline version (pH>13) of the in vivo Comet assay and recommendations were made for a standardized protocol, which would be acceptable to international agencies (Burlinson et al. 2007). It was decided that a single dose should be replaced with multiple dosing to avoid misinterpretation of data, isolated cells or nuclei could be used for the studies, cytotoxicity should be tested in the cells to prevent mechanisms of apoptosis/necrosis from interfering with the results, and scoring of comets could be carried out both manually as well as with image analysis systems. Consensus was also reached on the need for an international validation study to stringently evaluate the reliability and accuracy of the in vivo Comet assay (as well as in vitro versions). These recommendations are also aimed at reducing the variability arising in inter-laboratories studies.

In vivo Comet assay has been accepted as the first-tier screening assay for assessment of DNA damage in rodents by the Committee on Mutagenicity, UK (COM 2000), and international validation studies are underway supported by ECVAM, Japanese Centre for Validation of Alternative Methods (JaCVAM), US Interagency Coordinating Committee on Validation of Alternative Methods (ICCVAM), US National Toxicology Program Interagency Centre for Evaluation of Alternative Toxicological Methods (NICEATM), and Japanese Environmental Mutagen Society (Burlinson et al. 2007).

There has been only one multi-laboratory validation study in the European countries that has been conducted to study the FPG-sensitive sites and background level of base oxidation in DNA using Comet assay in human lymphocytes (Gedik and Collins 2005). It was found that half of the laboratories demonstrated a dose–response effect. However, many laboratories have carried out their own validation studies for DNA damage to optimize their research work (Moller 2006a). Moller (2006b) has critically evaluated the published Comet assay data on human biomonitoring studies using blood cells from 22 countries and has established reference values for DNA damage. The large number of biomonitoring studies has indicated that the Comet assay is a useful tool for detecting exposure, and its validation status as a biomarker in biomonitoring is dependent on its performance in cohort studies (Moller 2006a).

Conclusion

The Comet assay is now well established, and its versatility has imparted a sensitive tool to the toxicologists for assessing DNA damage. This has been demonstrated with its wide applications in assessing genotoxicity in plant and animal models, both aquatic as well as terrestrial, in a variety of organisms, tissues, and cell types. In vitro, in vivo, in situ, and biomonitoring studies using the Comet assay have proven it to be a *Rossetta Stone* in the garden of Genetic Toxicology.

References

Akcha F, Vincent Hubert F, Pfhol-Leszkowicz A. Potential value of the Comet assay and DNA adduct measurement in dab (*Limanda limanda*) for assessment of in situ exposure to genotoxic compounds. Mutat Res. 2003;534 (1–2):21–32.

Albertini RJ, Anderson D, Douglas GR, Hagmar L, Hemminki K, Merlo F, et al. IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. Mutat Res. 2000;463:111–72.

Alink GM, Quik JT, Penders EJ, Spenkelink A, Rotteveel SG, Maas JL, et al. Genotoxic effects in the Eastern mudminnow (*Umbra pygmaea* L.) after exposure to Rhine water, as assessed by use of the SCE and Comet assays: a comparison between 1978 and 2005. Mutat Res. 2007;631 (2):93–100.

Amado LL, Robaldo RB, Geracitano L, Monserrat JM, Bianchini A. Biomarkers of exposure and effect in the Brazilian flounder *Paralichthys orbignyanus* (Teleostei:

Paralichthyidae) from the Patos Lagoon estuary (Southern Brazil). Mar Pollut Bull. 2006;52(2):207–13.

Anderson D. Factors that contribute to biomarker responses in humans including a study in individuals taking vitamin C supplementation. Mutat Res. 2001;480–481:337–47.

Angelis KJ, McGuffie M, Menke M, Schubert I. Adaptation to alkylation damage in DNA measured by the Comet assay. Environ Mol Mutagen. 2000;36(2):146–50.

Angerer J, Ewers U, Wilhelm M. Human biomonitoring: state of the art. Int J Hyg Environ Health. 2007;210(3–4): 201–28.

Aniagu SO, Day N, Chipman JK, Taylor EW, Butler PJ, Winter MJ. Does exhaustive exercise result in oxidative stress and associated DNA damage in the chub (*Leuciscus cephalus*)? Environ Mol Mutagen. 2006;47(8):616–23.

Annas A, Brittebo E, Hellman B. Evaluation of benzo(a) pyrene-induced DNA damage in human endothelial cells using alkaline single cell gel electrophoresis. Mutat Res. 2000;471(1–2):145–55.

Ansari KM, Chauhan LK, Dhawan A, Khanna SK, Das M. Unequivocal evidence of genotoxic potential of argemone oil in mice. Int J Cancer. 2004;112(5):890–5.

Ansari KM, Dhawan A, Khanna SK, Das M. In vivo DNA damaging potential of sanguinarine alkaloid, isolated from argemone oil, using alkaline Comet assay in mice. Food Chem Toxicol. 2005;43(1):147–53.

Aoyama K, Iwahori K, Miyata N. Application of *Euglena gracilis* cells to Comet assay: evaluation of DNA damage and repair. Mutat Res. 2003;538(1–2):155–62.

Augustowska K, Magnowska Z, Kapiszewska M, Gregoraszczuk EL. Is the natural PCDD/PCDF mixture toxic for human placental JEG-3 cell line? The action of the toxicants on hormonal profile, CYP1A1 activity, DNA damage and cell apoptosis. Human Exp Toxicol. 2007;26 (5):407–17.

Augustyniak M, Juchimiuk J, Przybyłowicz WJ, Mesjasz-Przybyłowicz J, Babczyńska A, Migula P. Zinc-induced DNA damage and the distribution of metals in the brain of grasshoppers by the Comet assay and micro-PIXE. Comp Biochem Physiol C Toxicol Pharmacol. 2006;144 (3):242–51.

Avogbe PH, Ayi-Fanou L, Autrup H, Loft S, Fayomi B, Sanni A, et al. Ultrafine particulate matter and high-level benzene urban air pollution in relation to oxidative DNA damage. Carcinogenesis. 2005;26(3):613–20.

Bajpayee M, Dhawan A, Parmar D, Pandey AK, Mathur N, Seth PK. Gender-related differences in basal DNA damage in lymphocytes of a healthy Indian population using the alkaline Comet assay. Mutat Res. 2002;520 (1–2):83–91.

Bajpayee M, Pandey AK, Parmar D, Mathur N, Seth PK, Dhawan A. Comet assay responses in human lymphocytes are not influenced by the menstrual cycle: a study in healthy Indian females. Mutat Res. 2005;565(2):163–72.

Bajpayee M, Pandey AK, Zaidi S, Musarrat J, Parmar D, Mathur N, et al. DNA damage and mutagenicity induced by endosulfan and its metabolites. Environ Mol Mutagen. 2006;47(9):682–92.

Bakare AA, Pandey AK, Bajpayee M, Bhargav D, Chowdhuri DK, Singh KP, et al. DNA damage induced in human peripheral blood lymphocytes by industrial solid waste and municipal sludge leachates. Environ Mol Mutagen. 2007;48(1):30–7.

Banerjee P, Talapatra SN, Mandal N, Sundaram G, Mukhopadhyay A, Chattopadhyay D, et al. Genotoxicity study with special reference to DNA damage by Comet assay in fission yeast, *Schizosaccharomyces pombe* exposed to drinking water. Food Chem Toxicol. 2008;46(1):402–7.

Banner SH, Ruben LN, Johnson RO. Bleomycin-induced DNA damage and repair in *Xenopus laevis* and *Xenopus tropicalis*. J Exp Zool Part A Ecol Genet Physiol. 2007;307(2):84–90.

Baos R, Jovani R, Pastor N, Tella JL, Jiménez B, Gómez G, et al. Evaluation of genotoxic effects of heavy metals and arsenic in wild nestling white storks (*Ciconia ciconia*) and black kites (*Milvus migrans*) from southwestern Spain after a mining accident. Environ Toxicol Chem. 2006;25 (10):2794–803.

Basaran N, Shubair M, Undeğer U, Kars A. Monitoring of DNA damage in foundry and pottery workers exposed to silica by the alkaline comet assay. Am J Ind Med. 2003;43 (6):602–10.

Belpaeme K, Delbeke K, Zhu L, Kirsch-Volders M. Cytogenetic studies of PCB77 on brown trout (*Salmo trutta fario*) using the micronucleus test and the alkaline Comet assay. Mutagenesis 1996;11(5):485–92.

Belpaeme K, Cooreman K, Kirsc-Volders M. Development and validation of the in vivo alkaline Comet assay for detecting genomic damage in marine flatfish. Mutat Res. 1998;415(3):167–84.

Benford DJ, Hanley AB, Bottrill K, Oehlschlager S, Balls M, Branca F, et al. Biomarkers as predictive tools in toxicity testing. Altern Lab Anim. 2000;28:119–31.

Betti C, Davini T, Giannessi L, Loprieno N, Barale R. Comparative studies by Comet test and SCE analysis in human lymphocytes from 200 healthy subjects. Mutat Res. 1995;343(4):201–7.

Bian Q, Xu LC, Wang SL, Xia YK, Tan LF, Chen JF, et al. Study on the relation between occupational fenvalerate exposure and spermatozoa DNA damage of pesticide factory workers. Occup Environ Med. 2004;61(12):999–1005.

Bilbao C, Ferreiro JA, Comendador MA, Sierra LM. Influence of mus201 and mus308 mutations of *Drosophila melanogaster* on the genotoxicity of model chemicals in somatic cells in vivo measured with the Comet assay. Mutat Res. 2002;503:11–9.

Bolognesi C, Buschini A, Branchi E, Carboni P, Furlini M, Martino A, et al. Comet and micronucleus assays in zebra mussel cells for genotoxicity assessment of surface drinking water treated with three different disinfectants. Sci Total Environ. 2004;333(1–3):127–36.

Botta C, Iarmarcovai G, Chaspoul F, Sari-Minodier I, Pompili J, Orsière T, et al. Assessment of occupational exposure to welding fumes by inductively coupled plasma-mass spectroscopy and by the alkaline Comet assay. Environ Mol Mutagen. 2006;47(4):284–95.

Brendler Schwaab S, Hartmann A, Pfuhler S, Speit G. The in vivo Comet assay: use and status in genotoxicity testing. Mutagenesis 2005;20(4):245–54.

Buehrlen M, Harréus UA, Gamarra F, Hagen R, Kleinsasser NH. Cumulative genotoxic and apoptotic effects of xenobiotics in a mini organ culture model of human nasal

mucosa as detected by the alkaline single cell microgel electrophoresis assay and the annexin V-affinity assay. Toxicol Lett. 2007;169(2):152–61.

Bürger S, Schindler D, Fehn M, Mühl B, Mahrhofer H, Flentje M, et al. Radiation-induced DNA damage and repair in peripheral blood mononuclear cells from Nijmegen breakage syndrome patients and carriers assessed by the Comet assay. Environ Mol Mutagen. 2006;47(4):260–70.

Burlinson B, Tice RR, Speit G, Agurell E, Brendler-Schwaab SY, Collins AR, et al. In Vivo Comet Assay Workgroup, part of the Fourth International Workgroup on Genotoxicity Testing. Fourth International Workgroup on Genotoxicity testing: results of the In Vivo Comet Assay Workgroup. Mutat Res. 2007;627(1):31–5.

Buschini A, Carboni P, Martino A, Poli P, Rossi C. Effects of temperature on baseline and genotoxicant-induced DNA damage in haemocytes of *Dreissena polymorpha*. Mutat Res. 2003;537(1):81–92.

Buschini A, Martino A, Gustavino B, Monfrinotti M, Poli P, Rossi C, et al. Comet assay and micronucleus test in circulating erythrocytes of *Cyprinus carpio* specimens exposed in situ to lake waters treated with disinfectants for potabilization. Mutat Res. 2004;557(2):119–29.

Bustos-Obregon E, Goicochea RI. Pesticide soil contamination mainly affects earthworm male reproductive parameters. Asian J Androl. 2002;4(3):195–9.

Cabrita E, Robles V, Rebordinos L, Sarasquete C, Herráez MP. Evaluation of DNA damage in rainbow trout (*Oncorhynchus mykiss*) and gilthead sea bream (*Sparus aurata*) cryopreserved sperm. Cryobiology. 2005;50(2):144–53.

Cavallo D, Ursini CL, Carelli G, Iavicoli I, Ciervo A, Perniconi B, et al. Occupational exposure in airport personnel: characterization and evaluation of genotoxic and oxidative effects. Toxicology. 2006a;223(1–2):26–35.

Cavallo D, Ursini CL, Bavazzano P, Cassinelli C, Frattini A, Perniconi B, et al. Sister chromatid exchange and oxidative DNA damage in paving workers exposed to PAHs. Ann Occup Hyg. 2006b;50(3):211–8.

Cavas T, Könen S. Detection of cytogenetic and DNA damage in peripheral erythrocytes of goldfish (*Carassius auratus*) exposed to a glyphosate formulation using the micronucleus test and the Comet assay. Mutagenesis. 2007;22(4):263–8.

Cemeli E, Smith IF, Peers C, Urenjak J, Godukhin OV, Obrenovitch TP, et al. Oxygen-induced DNA damage in freshly isolated brain cells compared with cultured astrocytes in the Comet assay. Teratog Carcinog Mutagen 2003;23(Suppl 2):43–52.

Chemeris NK, Gapeyev AB, Sirota NP, Gudkova OY, Kornienko NV, Tankanag AV, et al. DNA damage in frog erythrocytes after in vitro exposure to a high peak-power pulsed electromagnetic field. Mutat Res. 2004;558 (1–2):27–34.

Chen Z, Lou J, Chen S, Zheng W, Wu W, Jin L, et al. Evaluating the genotoxic effects of workers exposed to lead using micronucleus assay, Comet assay and TCR gene mutation test. Toxicology. 2006;223(3):219–26.

Clements C, Ralph S, Petras M. Genotoxicity of select herbicides in *Rana catesbeiana* tadpoles using the alkaline single-cell gel DNA electrophoresis (Comet) assay. Environ Mol Mutagen. 1997;29(3):277–88.

Collins AR. The Comet assay for DNA damage and repair principles, applications, and limitations. Mol Biotechnol. 2004;26:249–60.

COM (Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment), COM guidance on a strategy for testing of chemicals for mutagenicity, United Kingdom, December 2000.

Conners DE, Black MC. Evaluation of lethality and genotoxicity in the freshwater mussel *Utterbackia imbecillis* (Bivalvia: Unionidae) exposed singly and in combination to chemicals used in lawn care. Arch Environ Contam Toxicol. 2004;46(3):362–71.

Cordelli E, Cinelli S, Lascialfari A, Ranaldi R, Pacchierotti F. Melphalan-induced DNA damage in p53(+/−) and wild type mice analysed by the Comet assay. Mutat Res. 2004;550(1–2):133–43.

Cordelli E, Fresegna AM, D'Alessio A, Eleuteri P, Spano M, Pacchierotti F, et al. ReProComet: a new in vitro method to assess dna damage in mammalian sperm. Toxicol Sci. 2007;99(2):545–52. October 1.

Corrie PG, Shaw J, Spanswick VJ, Sehmbi R, Jonson A, Mayer A, et al. Phase I trial combining gemcitabine and treosulfan in advanced cutaneous and uveal melanoma patients. Br J Cancer. 2005;92(11):1997–2003.

Cotelle S, Ferard JF. Comet assay in genetic ecotoxicology: a review. Environ Mol Mutagen. 1999;34:246–55.

Coughlan BM, Hartl MG, O'Reilly SJ, Sheehan D, Morthersill C, van Pelt FN, et al. Detecting genotoxicity using the Comet assay following chronic exposure of Manila clam *Tapes semidecussatus* to polluted estuarine sediments. Mar Pollut Bull. 2002;44(12):1359–65.

Dailianis S, Piperakis SM, Kaloyianni M. Cadmium effects on ros production and DNA damage via adrenergic receptors stimulation: role of Na^+ /H^+ exchanger and PKC. Free Radic Res. 2005;39(10):1059–70.

da Silva J, Herrmann SM, Heuser V, Peres W, Possa Marroni N, González-Gallego J, et al. Evaluation of the genotoxic effect of rutin and quercetin by comet assay and micronucleus test. Food Chem Toxicol. 2002;40(7):941–7.

de Andrade VM, de Freitas TR, da Silva J. Comet assay using mullet (*Mugil* sp.) and sea catfish (*Netuma* sp.) erythrocytes for the detection of genotoxic pollutants in aquatic environment. Mutat Res. 2004a;560(1):57–67.

de Andrade VM, Silva J, Silva FR, Heuser VD, Dias JF, Yoneama ML, et al. Fish as bioindicators to assess the effects of pollution in two southern Brazilian rivers using the Comet assay and micronucleus test. Environ Mol Mutagen. 2004b;44(5):459–68.

De Boeck M, Kirsch-Volders M. *Nereis virens* (Annelida: Polychaeta) is not an adequate sentinel species to assess the genotoxicrisk (Comet assay) of PAH exposure to the environment. Environ Mol Mutagen. 1997;30:82–90.

Delbes G, Hales BF, Robaire B. Effects of the chemotherapy cocktail used to treat testicular cancer on sperm chromatin integrity. J Androl. 2007;28(2):241–51.

de Miranda Cabral Gontijo AM, Barreto RE, Speit G, Valenzuela Reyes VA, Volpato GL, Favero Salvadori DM. Anesthesia of fish with benzocaine does not interfere with comet assay results. Mutat Res. 2003;534 (1–2):165–72.

Dhawan A, Mathur N, Seth PK. The effect of smoking and eating habits on DNA damage in Indian population as measured in the Comet assay. Mutat Res. 2001;474(1–2):121–8.

Dhawan A, Anderson D, de Pascual-Teresa S, Santos-Buelga C, Clifford MN, Ioannides C. Evaluation of the antigenotoxic potential of monomeric and dimeric flavanols, and black tea polyphenols against heterocyclic amine-induced DNA damage in human lymphocytes using the Comet assay. Mutat Res. 2002;515(1–2):39–56.

Dhawan A, Taurozzi JS, Pandey AK, Shan W, Miller SM, Hashsham SA, et al. Stable colloidal dispersions of C60 fullerenes in water: evidence for genotoxicity. Environ Sci Technol. 2006;40(23):7394–401.

Di Marzio WD, Saenz ME, Lemière S, Vasseur P. Improved single-cell gel electrophoresis assay for detecting DNA damage in *Eisenia foetida*. Environ Mol Mutagen. 2005;46(4):246–52.

Dixon DR, Pruski AM, Dixon LR. The effects of hydrostatic pressure change on DNA integrity in the hydrothermal-vent mussel *Bathymodiolus azoricus*: implications for future deep-sea mutagenicity studies. Mutat Res. 2004;552 (1–2):235–46.

Djuzenova CS, Schindler D, Stopper H, Hoehn H, Flentje M, Oppitz U. Identification of ataxia telangiectasia heterozygotes, a cancer-prone population, using the single-cell gel electrophoresis (Comet) assay. Lab Invest. 1999;79 (6):699–705.

Djuzenova CS, Mühl B, Fehn M, Oppitz U, Müller B, Flentje M. Radiosensitivity in breast cancer assessed by the Comet and micronucleus assays. Br J Cancer. 2006;94 (8):1194–203.

Dusinská M, Collins A, Kazimírová A, Barancoková M, Harrington V, Volkovová K, et al. Genotoxic effects of asbestos in humans. Mutat Res. 2004;553(1–2):91–102.

Dusinska M, Collins AR. The comet assay in human biomonitoring: gene–environment interactions. Mutagenesis. 2008 (March 7, in press) DOI 10.1093/mutage/gen007.

Emri G, Schaefer D, Held B, Herbst C, Zieger W, Horkay I, et al. Low concentrations of formaldehyde induce DNA damage and delay DNA repair after UV irradiation in human skin cells. Exp Dermatol. 2004;13(5):305–15.

Erbes M, Wessler A, Obst U, Wild A. Detection of primary DNA damage in *Chlamydomonas reinhardtii* by means of modified microgel electrophoresis. Environ Mol Mutagen. 1997;30(4):448–58.

Eren K, Ozmeriç N, Sardaş S. Monitoring of buccal epithelial cells by alkaline Comet assay (single cell gel electrophoresis technique) in cytogenetic evaluation of chlorhexidine. Clin Oral Investig. 2002;6(3):150–54.

Faullimel C, Ennahar S, Aoude-Werner D, Guterl P, Marchioni E. DNA Comet assay for the detection of time–temperature abuse during the storage of poultry. J Food Prot. 2005;68(7):1414–20.

Faust F, Kassie F, Knasmüller S, Boedecker RH, Mann M, Mersch-Sundermann V. The use of the alkaline Comet assay with lymphocytes in human biomonitoring studies. Mutat Res. 2004a;566(3):209–29.

Faust F, Kassie F, Knasmüller S, Kevekordes S, Mersch-Sundermann V. Use of primary blood cells for the assessment of exposure to occupational genotoxicants in human biomonitoring studies. Toxicology. 2004b;198(1–3):341–50.

Fedeli D, Berrettini M, Gabryelak T, Falcioni G. The effect of some tannins on trout erythrocytes exposed to oxidative stress. Mutat Res. 2004;563(2):89–96.

Feng S, Kong Z, Wang X, Zhao L, Peng P. Acute toxicity and genotoxicity of two novel pesticides on amphibian, Rana N. Hallowell. Chemosphere 2004;56(5):457–63.

Flamand N, Marrot L, Belaidi JP, Bourouf L, Dourille E, Feltes M, et al. Development of genotoxicity test procedures with Episkin, a reconstructed human skin model: towards new tools for in vitro risk assessment of dermally applied compounds? Mutat Res. 2006;606(1–2):39–51.

Folkmann JK, Loft S, Møller P. Oxidatively damaged DNA in aging dyslipidemic ApoE−/− and wild-type mice. Mutagenesis. 2007a;22(2):105–10.

Folkmann JK, Risom L, Hansen CS, Loft S, Møller P. Oxidatively damaged DNA and inflammation in the liver of dyslipidemic ApoE−/− mice exposed to diesel exhaust particles. Toxicology. 2007b;237(1–3):134–44.

Forchhammer L, Brauner EV, Folkmann JK, Danielsen PH, Nielsen C, Jensen A, et al. Variation in assessment of oxidatively damaged DNA in mononuclear blood cells by the comet assay with visual scoring. Mutagenesis. 2008 (March 7, in press) DOI 10.1093/mutage/gen006.

Fourie F, Reinecke SA, Reinecke AJ. The determination of earthworm species sensitivity differences to cadmium genotoxicity using the Comet assay. Ecotoxicol Environ Saf. 2007;67(3):361–8.

Fracasso ME, Doria D, Franceschetti P, Perbellini L, Romeo L. DNA damage and repair capacity by Comet assay in lymphocytes of white-collar active smokers and passive smokers (non- and ex-smokers) at workplace. Toxicol Lett. 2006;167(2):131–41.

Frankic T, Pajk T, Rezar V, Levart A, Salobir J. The role of dietary nucleotides in reduction of DNA damage induced by T-2 toxin and deoxynivalenol in chicken leukocytes. Food Chem Toxicol. 2006;44(11):1838–44.

Fraser L. Structural damage to nuclear DNA in mammalian spermatozoa: its evaluation techniques and relationship with male infertility. Pol J Vet Sci. 2004;7(4):311–21.

Frenzilli G, Nigro M, Scarcelli V, Gorbi S, Regoli F. DNA integrity and total oxyradical scavenging capacity in the Mediterranean mussel, *Mytilus galloprovincialis*: a field study in a high eutrophicated coastal lagoon. Aquat Toxicol. 2001;53:19–32.

Frenzilli G, Scarcelli V, Del Barga I, Nigro M, Förlin L, Bolognesi C, et al. DNA damage in eelpout (*Zoarces viviparus*) from Göteborg harbour. Mutat Res. 2004;552 (1–2):187–95.

Frenzilli G, Scarcelli V, Fornai F, Paparelli A, Nigro M. The comet assay as a method of assessment of neurotoxicity: usefulness for drugs of abuse. Ann N Y Acad Sci. 2006;1074:478–81.

Gabbianelli R, Lupidi G, Villarini M, Falcioni G. DNA damage induced by copper on erythrocytes of gilthead sea bream *Sparus aurata* and mollusk *Scapharca inaequivalvis*. Arch Environ Contam Toxicol. 2003;45(3):350–6.

Gabbianelli R, Moretti M, Carpenè E, Falcioni G. Effect of different organotins on DNA of mollusk (*Scapharca*

inaequivalvis) erythrocytes assessed by the comet assay. Sci Total Environ. 2006;367(1):163–69.

Gambelunghe A, Piccinini R, Ambrogi M, Villarini M, Moretti M, Marchetti C, et al. Primary DNA damage in chrome-plating workers. Toxicology. 2003;188(2–3):187–95.

Garaj-Vrhovac V, Zeljezic D. Assessment of genome damage in a population of Croatian workers employed in pesticide production by chromosomal aberration analysis, micronucleus assay and Comet assay. J Appl Toxicol. 2002;22 (4):249–55.

García Sar D, Montes-Bayón M, Aguado Ortiz L, Blanco-González E, Sierra LM, Sanz-Medel A. In vivo detection of DNA adducts induced by cisplatin using capillary HPLC-ICP-MS and their correlation with genotoxic damage in Drosophila melanogaster. Anal Bioanal Chem. 2008;390(1):37–44.

Gedik C, Ewen S, Collins A. Single-cell gel electrophoresis applied to the analysis of UV-C damage and its repair in human cells. Int J Radiat Biol. 1992;62:313–20.

Gedik CM, Collins A, ESCODD (European Standards Committee on Oxidative DNA Damage). Establishing the background level of base oxidation in human lymphocytes DNA: results of an interlaboratory validation study. FASEB J 2005;19(1):82–4.

Gichner T. Differential genotoxicity of ethyl methanesulphonate, *N*-ethyl-*N*-nitrosourea and maleic hydrazide in tobacco seedlings based on data of the Comet assay and two recombination assays. Mutat Res. 2003a;538(1–2):171–9.

Gichner T. DNA damage induced by indirect and direct acting mutagens in catalase-deficient transgenic tobacco. Cellular and acellular Comet assays. Mutat Res. 2003b;535 (2):187–93.

Gichner T, Plewa MJ. Induction of somatic DNA damage as measured by single cell gel electrophoresis and point mutation in leaves of tobacco plants. Mutat Res. 1998;401 (1–2):143–52.

Gichner T, Ptácek O, Stavreva DA, Plewa MJ. Comparison of DNA damage in plants as measured by single cell gel electrophoresis and somatic leaf mutations induced by monofunctional alkylating agents. Environ Mol Mutagen. 1999;33(4):279–86.

Gichner T, Ptácek O, Stavreva DA, Wagner ED, Plewa MJ. A comparison of DNA repair using the Comet assay in tobacco seedlings after exposure to alkylating agents or ionizing radiation. Mutat Res. 2000;470(1):1–9.

Gichner T, Patková Z, Száková J, Demnerová K. Toxicity and DNA damage in tobacco and potato plants growing on soil polluted with heavy metals. Ecotoxicol Environ Saf. 2006;65(3):420–6.

Gichner T, Lovecká P, Kochánková L, Macková M, Demnerová K. Monitoring toxicity, DNA damage, and somatic mutations in tobacco plants growing in soil heavily polluted with polychlorinated biphenyls. Mutat Res. 2007;629(1):1–6.

Gielazyn ML, Ringwood AH, Piegorsch WW, Stancyk SE. Detection of oxidative DNA damage in isolated marine bivalve hemocytes using the Comet assay and formamidopyrimidine glycosylase (Fpg). Mutat Res. 2003;542(1–2):15–22.

Glei M, Habermann N, Osswald K, Seidel C, Persin C, Jahreis G, et al. Assessment of DNA damage and its modulation by dietary and genetic factors in smokers using the Comet assay: a biomarker model. Biomarkers. 2005;10(2–3):203–17.

Graham-Evans B, Cohly HH, Yu H, Tchounwou PB. Arsenic-induced genotoxic and cytotoxic effects in human keratinocytes, melanocytes and dendritic cells. Int J Environ Res Public Health. 2004;1(2):83–9.

Grant WF. Higher plant assays for the detection of chromosomal aberrations and gene mutations—a brief historical background on their use for screening and monitoring environmental chemicals. Mutat Res. 1999;426:107–12.

Guecheva T, Henriques JA, Erdtmann B. Genotoxic effects of copper sulphate in freshwater planarian in vivo, studied with the single-cell gel test (comet assay). Mutat Res. 2001;497(1–2):19–27.

Güerci AM, Grillo CA, Dulout FN, Seoane AI. Assessment of genotoxic damage in lymphocytes of hospital workers exposed to ionizing radiation in Argentina. Arch Environ Occup Health. 2006;61(4):163–9.

Guo L, Wang LH, Sun B, Yang JY, Zhao YQ, Dong YX, et al. Direct in vivo evidence of protective effects of grape seed procyanidin fractions and other antioxidants against ethanol-induced oxidative DNA damage in mouse brain cells. J Agric Food Chem. 2007;55(14):5881–91.

Gutiérrez-Castillo ME, Roubicek DA, Cebrián-García ME, De Vizcaya-Ruíz A, Sordo-Cedeño M, Ostrosky-Wegman P. Effect of chemical composition on the induction of DNA damage by urban airborne particulate matter. Environ Mol Mutagen. 2006;47(3):199–211.

Gwo JC, Wu CY, Chang WS, Cheng HY. Evaluation of damage in Pacific oyster (*Crassostrea gigas*) spermatozoa before and after cryopreservation using Comet assay. Cryo Letters. 2003;24(3):171–80.

Hahn A, Hock B. Assessment of DNA damage in filamentous fungi by single cell gel electrophoresis, Comet assay. Environ Toxicol Chem. 1999;18:1421–24.

Hamouten D, Payne JF, Rahimtula A, Lee K. Use of the Comet assay to assess DNA damage in hemocytes and digestive gland cells of mussels and clams exposed to water contaminated with petroleum hydrocarbons. Mar Environ Res. 2002;54(3–5):471–4.

Hartl MG, Coughlan BM, Sheehan D, Mothersill C, van Pelt FN, O'Reilly SJ, et al. Implications of seasonal priming and reproductive activity on the interpretation of Comet assay data derived from the clam, *Tapes semidecussatus* Reeves 1864, exposed to contaminated sediments. Mar Environ Res. 2004;57(4):295–310.

Hartl MG, Kilemade M, Sheehan D, Mothersill C, O'Halloran J, O'Brien NM, et al. Hepatic biomarkers of sediment-associated pollution in juvenile turbot, *Scophthalmus maximus* L. Mar Environ Res. 2007;64(2):191–208.

Hartmann A, Agurell E, Beevers C, Brendler-Schwaab S, Burlinson B, Clay P, et al. Recommendations for conducting the in vivo alkaline comet assay. Mutagenesis. 2003;18:45–51.

Heuser VD, Erdtmann B, Kvitko K, Rohr P, da Silva J. Evaluation of genetic damage in Brazilian footwear-

workers: biomarkers of exposure, effect, and susceptibility. Toxicology. 2007;232(3):235–47.

Hoffmann H, Högel J, Speit G. The effect of smoking on DNA effects in the Comet assay: a meta-analysis. Mutagenesis. 2005a;20(6):455–66.

Hoffmann H, Isner C, Högel J, Speit G. Genetic polymorphisms and the effect of cigarette smoking in the Comet assay. Mutagenesis. 2005b;20(5):359–64.

Hook SE, Lee RF. Interactive effects of UV, benzo[alpha] pyrene, and cadmium on DNA damage and repair in embryos of the grass shrimp *Paleomonetes pugio*. Mar Environ Res. 2004;58(2–5):735–39.

Horvat T, Kalafatić M, Kopjar N, Kovacević G. Toxicity testing of herbicide norflurazon on an aquatic bioindicator species —the planarian *Polycelis felina* (Daly). Aquat Toxicol. 2005;73(4):342–52.

Huang D, Zhang Y, Wang Y, Xie Z, Ji W. Assessment of the genotoxicity in toad *Bufo raddei* exposed to petrochemical contaminants in Lanzhou Region, China. Mutat Res. 2007;629(2):81–8.

Jacobsen NR, Saber AT, White P, Møller P, Pojana G, Vogel U, et al. Increased mutant frequency by carbon black, but not quartz, in the lacZ and cII transgenes of muta mouse lung epithelial cells. Environ Mol Mutagen. 2007;48 (6):451–61.

Jha AN, Dogra Y, Turner A, Millward GE. Impact of low doses of tritium on the marine mussel, Mytilus edulis: genotoxic effects and tissue-specific bioconcentration. Mutat Res. 2005;586(1):47–57.

Kamer I, Rinkevich B. In vitro application of the Comet assay for aquatic genotoxicity: considering a primary culture versus a cell line. Toxicol In Vitro. 2002;16(2):177–84.

Kammann U, Riggers JC, Theobald N, Steinhart H. Genotoxic potential of marine sediments from the North Sea. Mutat Res. 2000;467(2):161–8.

Kang MH, Park YK, Kim HY, Kim TS. Green vegetable drink consumption protects peripheral lymphocytes DNA damage in Korean smokers. Biofactors. 2004;22(1–4):245–7.

Kassie F, Parzefall W, Knasmüller S. Single cell gel electrophoresis assay: a new technique for human biomonitoring studies. Mutat Res. 2000;463(1):13–31.

Kim YD, Eom SY, Ogawa M, Oyama T, Isse T, Kang JW, Zhang YW, Kawamoto T, Kim H. Ethanol-induced oxidative DNA damage and CYP2E1 expression in liver tissue of Aldh2 knockout mice. J Occup Health. 2007;49(5):363–9.

Kleinsasser NH, Juchhoff J, Wallner BC, Bergner A, Harréus UA, Gamarra F, et al. The use of mini-organ cultures of human upper aerodigestive tract epithelia in ecogenotoxicology. Mutat Res. 2004;561(1–2):63–73.

Klobucar GI, Pavlica M, Erben R, Papes D. Application of the micronucleus and Comet assays to mussel *Dreissena polymorpha* haemocytes for genotoxicity monitoring of freshwater environments. Aquat Toxicol. 2003;64 (1):15–23.

Kopjar N, Garaj-Vrhovac V. Assessment of DNA damage in nuclear medicine personnel—comparative study with the alkaline Comet assay and the chromosome aberration test. Int J Hyg Environ Health. 2005;208(3):179–91.

Koppen G, Toncelli LM, Triest L, Verschaeve L. The Comet assay: a tool to study alteration of DNA integrity in developing plant leaves. Mech Ageing Dev. 1999;110(1–2):13–24.

Kosmehl T, Hallare AV, Reifferscheid G, Manz W, Braunbeck T, Hollert H. A novel contact assay for testing genotoxicity of chemicals and whole sediments in zebrafish embryos. Environ Toxicol Chem. 2006;25(8):2097–106.

Kotłowska M, Dietrich G, Wojtczak M, Karol H, Ciereszko A. Effects of liquid storage on amidase activity, DNA fragmentation and motility of turkey spermatozoa. Theriogenology. 2007;67(2):276–86.

Kumaravel TS, Jha AN. Reliable Comet assay measurements for detecting DNA damage induced by ionising radiation and chemicals. Mutat Res. 2006;605(1–2):7–16.

Kuzmick DM, Mitchelmore CL, Hopkins WA, Rowe CL. Effects of coal combustion residues on survival, antioxidant potential, and genotoxicity resulting from full-life-cycle exposure of grass shrimp (*Palaemonetes pugio* Holthius). Sci Total Environ. 2007;373(1):420–30.

Labbe C, Martoriati A, Devaux A, Maisse G. Effect of sperm cryopreservation on sperm DNA stability and progeny development in rainbow trout. Mol Reprod Dev. 2001;60 (3):397–404.

Labieniec M, Gabryelak T. Response of DNA, proteins and membrane bilayer in the digestive gland cells of freshwater mussel *Unio tumidus* to tannins exposure. Toxicol In Vitro. 2004;18(6):773–81.

Labieniec M, Gabryelak T. Oxidatively modified proteins and DNA in digestive gland cells of the fresh-water mussel *Unio tumidus* in the presence of tannic acid and its derivatives. Mutat Res. 2006;603(1):48–55.

Labieniec M, Biernat M, Gabryelak T. Response of digestive gland cells of freshwater mussel *Unio tumidus* to phenolic compound exposure in vivo. Cell Biol Int. 2007;31 (7):683–90.

Laffon B, Fraga-Iriso R, Pérez-Cadahía B, Méndez J. Genotoxicity associated to exposure to Prestige oil during autopsies and cleaning of oil-contaminated birds. Food Chem Toxicol. 2006a;44(10):1714–23.

Laffon B, Teixeira JP, Silva S, Roma-Torres J, Pérez-Cadahía B, Méndez J, et al. Assessment of occupational genotoxic risk in the production of rubber tyres. Ann Occup Hyg. 2006b;50(6):583–92.

Lah B, Malovrh S, Narat M, Cepeljnik T, Marinsek-Logar R. Detection and quantification of genotoxicity in wastewater-treated *Tetrahymena thermophila* using the Comet assay. Environ Toxicol. 2004;19(6):545–53.

Large AT, Shaw JP, Peters LD, McIntosh AD, Webster L, Mally A, et al. Different levels of mussel (*Mytilus edulis*) DNA strand breaks following chronic field and acute laboratory exposure to polycyclic aromatic hydrocarbons. Mar Environ Res. 2002;54(3–5):493–7.

Lee RF, Steinert S. Use of the single cell gel electrophoresis/ Comet assay for detecting DNA damage in aquatic (marine and freshwater) animals. Mutat Res. 2003;544:43–64.

Lee RF, Maruya KA, Bulski K. Exposure of grass shrimp to sediments receiving highway runoff: effects on reproduction and DNA. Mar Environ Res. 2004;58(2–5):713–7.

Lehmann J, Pollet D, Peker S, Steinkraus V, Hoppe U. Kinetics of DNA strand breaks and protection by antioxidants in UVA- or UVB-irradiated HaCaT keratinocytes using the

single cell gel electrophoresis assay. Mutat Res. 1998;407 (2):97–108.
Lin X, Wood CG, Shao L, Huang M, Yang H, Dinney CP, et al. Risk assessment of renal cell carcinoma using alkaline Comet assay. Cancer. 2007;110(2):282–8.
Lu Y, Morimoto K, Takeshita T, takeuchi T, Saito T. Genotoxic effects of a-endosulfan and b-endosulfan on human HepG2 cells. Environ Health Perspect. 2000;108:559–61.
Ma T, Xu Z, Xu C, McConnell H, Rabago EV, Arreola GA, et al. The improved Allium/Vicia root tip micronucleus assay for clastogenicity of environmental pollutants. Mutat Res. 1995;334:185–95.
Machella N, Battino M, Pisanelli B, Regoli F. Influence of the SCGE protocol on the amount of basal DNA damage detected in the Mediterranean mussel, *Mytilus galloprovincialis*. Environ Mol Mutagen. 2006;47(8):579–86.
Mamaca E, Bechmann RK, Torgrimsen S, Aas E, Bjørnstad A, Baussant T, et al. The neutral red lysosomal retention assay and Comet assay on haemolymph cells from mussels (*Mytilus edulis*) and fish (*Symphodus melops*) exposed to styrene. Aquat Toxicol. 2005;75(3):191–201.
Maluf SW, Erdtmann B. Genomic instability in Down syndrome and Fanconi anemia assessed by micronucleus analysis and single-cell gel electrophoresis. Cancer Genet Cytogenet. 2001;124(1):71–5.
Maluszynska J, Juchimiuk J. Plant genotoxicity: a molecular cytogenetic approach in plant bioassays. Arh Hig Rada Toksikol. 2005;56(2):177–84.
Martin FL, Cole KJ, Muir GH, Kooiman GG, Williams JA, Sherwood RA, et al. Primary cultures of prostate cells and their ability to activate carcinogens. Prostate Cancer Prostatic Dis. 2002;5(2):96–104.
Martin FL, Pearce TG, Hewer A, Phillips DH, Semple KT. A biomarker model of sublethal genotoxicity (DNA single-strand breaks and adducts) using the sentinel organism *Aporrectodea longa* in spiked soil. Environ Pollut. 2005;138(2):307–15.
Mastaloudis A, Yu TW, O'Donnell RP, Frei B, Dashwood RH, Traber MG. Endurance exercise results in DNA damage as detected by the Comet assay. Free Radic Biol Med. 2004;36(8):966–75.
Masuda S, Deguchi Y, Masuda Y, Watanabe T, Nukaya H, Terao Y, et al. Genotoxicity of 2-[2-(acetylamino)-4-[bis (2-hydroxyethyl)amino]-5-methoxyphenyl]-5-amino-7-bromo-4-chloro-2H-benzotriazole (PBTA-6) and 4-amino-3,3¢-dichloro-5,4¢-dinitro-biphenyl (ADDB) in goldfish (*Carassius auratus*) using the micronucleus test and the Comet assay. Mutat Res. 2004;560(1):33–40.
Mateos S, Daza P, Domínguez I, Cárdenas JA, Cortés F. Genotoxicity detected in wild mice living in a highly polluted wetland area in south western Spain. Environ Pollut 2008 (in press) DOI 10.1016/j.envpol.2007.09.008.
McKelvey-Martin VJ, Green MH, Schmezer P, Pool-Zobel BL, De Méo MP, Collins A. The single cell gel electrophoresis assay (Comet assay): a European review. Mutat Res. 1993;288(1):47–63.
McKelvey-Martin VJ, Melia N, Walsh IK, Johnston SR, Hughes CM, Lewis SE, et al. Two potential clinical applications of the alkaline single-cell gel electrophoresis assay: (1). Human bladder washings and transitional cell carcinoma of the bladder; and (2). Human sperm and male infertility. Mutat Res. 1997;375(2):93–104.
McKenna DJ, McKeown SR, McKelvey-Martin VJ. Potential use of the comet assay in the clinical management of cancer. Mutagenesis. 2008 (February 5, in press) DOI 10.1093/mutage/gem054.
Meng Z, Qin G, Zhang B, Bai J. DNA damaging effects of sulfur dioxide derivatives in cells from various organs of mice. Mutagenesis. 2004;19(6):465–8.
Meng Z, Qin G, Zhang B. DNA damage in mice treated with sulfur dioxide by inhalation. Environ Mol Mutagen. 2005;46 (3):150–5.
Menke M, Meister A, Schubert I. N-Methyl-N-nitrosourea-induced DNA damage detected by the Comet assay in *Vicia faba* nuclei during all interphase stages is not restricted to chromatid aberration hot spots. Mutagenesis. 2000;15 (6):503–6.
Migliore L, Fontana I, Trippi F, Colognato R, Coppedè F, Tognoni G, et al. Oxidative DNA damage in peripheral leukocytes of mild cognitive impairment and AD patients. Neurobiol Aging. 2005;26(5):567–73.
Mitchelmore CL, Chipman JK. DNA strand breakage in aquatic organisms and the potential value of the Comet assay in environmental monitoring. Mutat Res. 1998a;399:135–47.
Mitchelmore CL, Birmelin C, Livingstone DR, Chipman JK. Detection of DNA strand breaks in isolated mussel (*Mytilus edulis* L.) digestive gland cells using the "Comet" assay. Ecotoxicol Environ Saf. 1998b;41(1):51–8.
Mitchelmore CL, Hyatt S. Assessing DNA damage in cnidarians using the Comet assay. Mar Environ Res. 2004;58(2–5):707–11.
Miyamae Y, Iwasaki K, Kinae N, Tsuda S, Murakami M, Tanaka M, et al. Detection of DNA lesions induced by chemical mutagens by the single cell gel electrophoresis (Comet) assay: relationship between DNA migration and alkaline conditions. Mutat Res. 1997;393:107–13.
Moller P. Genotoxicity of environmental agents assessed by the alkaline Comet assay. Basic Clin Pharmacol Toxicol. 2005;96:1–42.
Moller P. The alkaline Comet assay: towards validation in biomonitoring of DNA damaging exposures. Basic Clin Pharmacol Toxicol. 2006a;98(4):336–45.
Moller P. Assessment of reference values for DNA damage detected by the Comet assay in human blood cell DNA. Mutat Res. 2006b;612(2):84–104.
Moller P, Loft S. Oxidative DNA damage in human white blood cells in dietary antioxidant intervention studies. Am J Clin Nutr. 2002;76(2):303–10.
Moller P, Knudsen LE, Loft S, Wallin H. The Comet assay as a rapid test in biomonitoring occupational exposure to DNA-damaging agents and effect of confounding factors. Cancer Epidemiol Biomarkers Prev. 2000;9(10):1005–15.
Moller P, Viscovich M, Lykkesfeldt J, Loft S, Jensen A, Poulsen HE. Vitamin C supplementation decreases oxidative DNA damage in mononuclear blood cells of smokers. Eur J Nutr. 2004;43(5):267–74.
Morley N, Rapp A, Dittmar H, Salter L, Gould D, Greulich KO, et al. UVA-induced apoptosis studied by the new apo/necro-Comet-assay which distinguishes viable, apoptotic and necrotic cells. Mutagenesis. 2006;21(2):105–14.

Mouchet F, Gauthier L, Mailhes C, Ferrier V, Devaux A. Comparative study of the Comet assay and the micronucleus test in amphibian larvae (*Xenopus laevis*) using benzo(a)pyrene, ethyl methanesulfonate, and methyl methanesulfonate: establishment of a positive control in the amphibian Comet assay. Environ Toxicol. 2005a;20 (1):74–84.

Mouchet F, Gauthier L, Mailhes C, Jourdain MJ, Ferrier V, Devaux A. Biomonitoring of the genotoxic potential of draining water from dredged sediments, using the Comet and micronucleus tests on amphibian (*Xenopus laevis*) larvae and bacterial assays (Mutatox and Ames tests). Toxicol Environ Health A. 2005b;68(10):811–32.

Mouchet F, Gauthier L, Mailhes C, Ferrier V, Devaux A. Comparative evaluation of genotoxicity of captan in amphibian larvae (*Xenopus laevis* and *Pleurodeles waltl*) using the Comet assay and the micronucleus test. Environ Toxicol. 2006;21(3):264–77.

Mouchet F, Gauthier L, Baudrimont M, Gonzalez P, Mailhes C, Ferrier V, et al. Comparative evaluation of the toxicity and genotoxicity of cadmium in amphibian larvae (*Xenopus laevis* and *Pleurodeles waltl*) using the Comet assay and the micronucleus test. Environ Toxicol. 2007;22 (4):422–35.

Mukhopadhyay I, Saxena DK, Bajpai VK, Kar Chowdhuri D. Argemone oil induced cellular damage in the reproductive tissues of transgenic *Drosophila melanogaster*: protective role of 70 kDa heat shock protein. J Biochem Mol Toxicol. 2003;17:223–33.

Mukhopadhyay I, Chowdhuri DK, Bajpayee M, Dhawan A. Evaluation of in vivo genotoxicity of cypermethrin in *Drosophila melanogaster* using the alkaline Comet assay. Mutagenesis. 2004;19(2):85–90.

Mussali-Galante P, Avila-Costa MR, Piñón-Zarate G, Martínez-Levy G, Rodríguez-Lara V, Rojas-Lemus M, et al. DNA damage as an early biomarker of effect in human health. Toxicol Ind Health. 2005;21(7–8):155–66.

Nazir A, Saxena DK, Kar Chowdhuri D. Induction of hsp70 in transgenic Drosophila: biomarker of exposure against phthalimide group of chemicals. Biochim Biophys Acta. 2003;1621:218–25.

Nehls S, Segner H. Detection of DNA damage in two cell lines from rainbow trout, RTG-2 and RTL-W1, using the Comet assay. Environ Toxicol. 2001;16(4):321–9.

Nehls S, Segner H. Comet assay with the fish cell line rainbow trout gonad-2 for in vitro genotoxicity testing of xenobiotics and surface waters. Environ Toxicol Chem. 2005;24(8):2078–87.

Nigro M, Frenzilli G, Scarcelli V, Gorbi S, Regoli F. Induction of DNA strand breakage and apoptosis in the eel *Anguilla anguilla*. Mar Environ Res. 2002;54(3–5):517–20.

O'Donovan M. An evaluation of chromatin condensation and DNA integrity in the spermatozoa of men with cancer before and after therapy. Andrologia. 2005;37(2–3):83–90.

Olive PL, Banath JP. The Comet assay: a method to measure DNA damage in individual cells. Nature Protocols 2006;1 (1):23–9.

Ostling O, Johanson KJ. Microelectrophoretic study of radiation-induced DNA damages in individual mammalian cells. Biochem Biophys Res Commun. 1984;123(1):291–8.

Palus J, Rydzynski K, Dziubaltowska E, Wyszynska K, Natarajan AT, Nilsson R. Genotoxic effects of occupational exposure to lead and cadmium. Mutat Res. 2003;540(1):19–28.

Palus J, Lewinska D, Dziubaltowska E, Stepnik M, Beck J, Rydzynski K, et al. DNA damage in leukocytes of workers occupationally exposed to arsenic in copper smelters. Environ Mol Mutagen. 2005;46(2):81–7.

Pandey AK, Bajpayee M, Parmar D, Rastogi SK, Mathur N, Seth PK, et al. DNA damage in lymphocytes of rural Indian women exposed to biomass fuel smoke as assessed by the Comet assay. Environ Mol Mutagen. 2005;45(5):435–41.

Pandey AK, Bajpayee M, Parmar D, Rastogi SK, Mathur N, Seth PK, et al. DNA damage in lymphocytes of Indian rickshaw pullers as measured by the alkaline Comet assay. Environ Mol Mutagen. 2006;47(1):25–30.

Pandrangi R, Petras M, Ralph S, Vrzoc M. Alkaline single cell gel (Comet) assay and genotoxicity monitoring using bullheads and carp. Environ Mol Mutagen. 1995;26 (4):345–56.

Park YK, Park E, Kim JS, Kang MH. Daily grape juice consumption reduces oxidative DNA damage and plasma free radical levels in healthy Koreans. Mutat Res. 2003;529(1–2):77–86.

Pastor N, López-Lázaro M, Tella JL, Baos R, Forrero MG, Hiraldo F, et al. DNA damage in birds after the mining waste spill in southwestern Spain: a Comet assay evaluation. J Environ Pathol Toxicol Oncol. 2001a;20 (4):317–24.

Pastor N, López-Lázaro M, Tella JL, Baos R, Hiraldo F, Cortés F. Assessment of genotoxic damage by the Comet assay in white storks (*Ciconia ciconia*) after the Doñana ecological disaster. Mutagenesis. 2001b;16(3):219–23.

Pastor N, Baos R, López-Lázaro M, Jovani R, Tella JL, Hajji N, et al. A 4 year follow-up analysis of genotoxic damage in birds of the Doñana area (south west Spain) in the wake of the 1998 mining waste spill. Mutagenesis. 2004;19(1):61–5.

Patel S, Pandey A, Bajpayee M, Parmar D, Dhawan A. Cypermethrin-induced DNA damage in organs and tissues of the mouse: evidence from the comet assay. Mutat Res. 2006;607(2):176–83.

Patel S, Bajpayee M, Pandey A, Parmar D, Dhawan A. In vitro induction of cytotoxicity and DNA strand breaks in CHO cells exposed to cypermethrin, pendimethalin, and dichlorovous. Toxicol In vitro. 2007;21:1409–12.

Pavlica M, Klobucar GI, Mojas N, Erben R, Papes D. Detection of DNA damage in haemocytes of zebra mussel using Comet assay. Mutat Res. 2001;490(2):209–14.

Paz-y-Mino C, Arévalo M, Sanchez ME, Leone PE. Chromosome and DNA damage analysis in individuals occupationally exposed to pesticides with relation to genetic polymorphism for CYP 1A1 gene in Ecuador. Mutat Res. 2004;562(1–2):77–89.

Picada JN, Flores DG, Zettler CG, Marroni NP, Roesler R, Henriques JA. DNA damage in brain cells of mice treated with an oxidized form of apomorphine. Brain Res Mol Brain Res. 2003;114(1):80–5.

Piperakis SM, Kontogianni K, Siffel C, Piperakis MM. Measuring the effects of pesticides on occupationally

exposed humans with the Comet assay. Environ Toxicol. 2006;21(4):355–9.

Pitozzi V, Giovannelli L, Bardini G, Rotella CM, Dolara P. Oxidative DNA damage in peripheral blood cells in type 2 diabetes mellitus: higher vulnerability of polymorphonuclear leukocytes. Mutat Res. 2003;529(1–2):129–33.

Poli P, Buschini A, Restivo FM, Ficarelli A, Cassoni F, Ferrero I, et al. Comet assay application in environmental monitoring: DNA damage in human leukocytes and plant cells in comparison with bacterial and yeast tests. Mutagen. 1999;14:547–55.

Popanda O, Ebbeler R, Twardella D, Helmbold I, Gotzes F, Schmezer P, et al. Radiation-induced DNA damage and repair in lymphocytes from breast cancer patients and their correlation with acute skin reactions to radiotherapy. Int J Radiat Oncol Biol Phys. 2003;55(5):1216–25.

Porrini M, Riso P, Brusamolino A, Berti C, Guarnieri S, Visioli F. Daily intake of a formulated tomato drink affects carotenoid plasma and lymphocyte concentrations and improves cellular antioxidant protection. Br J Nutr. 2005;93(1):93–9.

Prá D, Lau AH, Knakievicz T, Carneiro FR, Erdtmann B. Environmental genotoxicity assessment of an urban stream using freshwater planarians. Mutat Res. 2005;585(1–2):79–85.

Pruski AM, Dixon DR. Effects of cadmium on nuclear integrity and DNA repair efficiency in the gill cells of *Mytilus edulis* L. Aquat Toxicol. 2002;57(3):127–37.

Pruski AM, Dixon DR. Toxic vents and DNA damage: first evidence from a naturally contaminated deep-sea environment. Aquat Toxicol. 2003;64(1):1–13.

Qiao M, Chen Y, Wang CX, Wang Z, Zhu YG. DNA damage and repair process in earthworm after in-vivo and in vitro exposure to soils irrigated by wastewaters. Environ Pollut. 2007;148(1):141–7.

Radyuk SN, Michalak K, Rebrin I, Sohal RS, Orr WC. Effects of ectopic expression of Drosophila DNA glycosylases dOgg1 and RpS3 in mitochondria. Free Radic Biol Med. 2006;41(5):757–64.

Rajaguru P, Kalpana R, Hema A, Suba S, Baskarasethupathi B, Kumar PA, et al. Genotoxicity of some sulfur dyes on tadpoles (*Rana hexadactyla*) measured using the Comet assay. Environ Mol Mutagen. 2001;38(4):316–22.

Rajaguru P, Suba S, Palanivel M, Kalaiselvi K. Genotoxicity of a polluted river system measured using the alkaline Comet assay on fish and earthworm tissues. Environ Mol Mutagen. 2003;41(2):85–91.

Ralph S, Petras M. Genotoxicity monitoring of small bodies of water using two species of tadpoles and the alkaline single cell gel (Comet) assay. Environ Mol Mutagen. 1997;29(4):418–30.

Ralph S, Petras M. Comparison of sensitivity to methyl methanesulphonate among tadpole developmental stages using the alkaline single-cell gel electrophoresis (Comet) assay. Environ Mol Mutagen. 1998a;31(4):374–82.

Ralph S, Petras M. Caged amphibian tadpoles and in situ genotoxicity monitoring of aquatic environments with the alkaline single cell gel electrophoresis (Comet) assay. Mutat Res. 1998b;413(3):235–50.

Rank J, Jensen K. Comet assay on gill cells and hemocytes from the blue mussel *Mytilus edulis*. Ecotoxicol Environ Saf. 2003;54(3):323–9.

Rank J, Jensen K, Jespersen PH. Monitoring DNA damage in indigenous blue mussels (*Mytilus edulis*) sampled from coastal sites in Denmark. Mutat Res. 2005;585(1–2):33–42.

Reinecke SA, Reinecke AJ. The Comet assay as biomarker of heavy metal genotoxicity in earthworms. Arch Environ Contam Toxicol. 2004;46(2):208–15.

Ribeiro DA, Pereira PC, Machado JM, Silva SB, Pessoa AW, Salvadori DM. Does toxoplasmosis cause DNA damage? An evaluation in isogenic mice under normal diet or dietary restriction. Mutat Res. 2004;559(1–2):169–76.

Ribeiro DA, Calvi SA, Picka MM, Persi E, de Carvalho TB, Caetano PK, et al. DNA damage and nitric oxide synthesis in experimentally infected Balb/c mice with *Trypanosoma cruzi*. Exp Parasitol. 2007;116(3):296–301.

Risom L, Møller P, Dybdahl M, Vogel U, Wallin H, Loft S. Dietary exposure to diesel exhaust particles and oxidatively damaged DNA in young oxoguanine DNA glycosylase 1 deficient mice. Toxicol Lett. 2007;175(1–3):16–23.

Risso-de Faverney C, Devaux A, Lafaurie M, Girard JP, Bailly B, Rahmani R. Cadmium induces apoptosis and genotoxicity in rainbow trout hepatocytes through generation of reactive oxygene species. Aquat Toxicol. 2001;53(1):65–76.

Riva C, Binelli A, Cogni D, Provini A. Evaluation of DNA damage induced by decabromodiphenyl ether (BDE-209) in hemocytes of Dreissena polymorpha using the Comet and micronucleus assays. Environ Mol Mutagen. 2007;48(9):735–43.

Rojas E, Valverde M, Lopez MC, Naufal I, Sanchez I, Bizarro P, et al. Evaluation of DNA damage in exfoliated tear duct epithelial cells from individuals exposed to air pollution assessed by single cell gel electrophoresis assay. Mutat Res. 2000;468(1):11–7.

Roy LA, Steinert S, Bay SM, Greenstein D, Sapozhnikova Y, Bawardi O, et al. Biochemical effects of petroleum exposure in hornyhead turbot (*Pleuronichthys verticalis*) exposed to a gradient of sediments collected from a natural petroleum seep in CA, USA. Aquat Toxicol 2003;65(2):159–69.

Salagovic J, Gilles J, Verschaeve L, Kalina I. The Comet assay for the detection of genotoxic damage in the earthworms: a promising tool for assessing the biological hazards of polluted sites. Folia Biol (Praha). 1996;42(1–2):17–21.

Sánchez P, Peñarroja R, Gallegos F, Bravo JL, Rojas E, Benítez-Bribiesca L. DNA damage in peripheral lymphocytes of untreated breast cancer patients. Arch Med Res. 2004;35(6):480–3.

Sardas S, Izdes S, Ozcagli E, Kanbak O, Kadioglu E. The role of antioxidant supplementation in occupational exposure to waste anaesthetic gases. Int Arch Occup Environ Health. 2006;80(2):154–9.

Sasaki YF, Sekihashi K, Izumiyama F, Nishidate E, Saga A, Ishida K, et al. The comet assay with multiple mouse organs: comparison of comet assay results and carcinogenicity with 208 chemicals selected from the IARC monographs and U.S. NTP Carcinogenicity Database. Crit Rev Toxicol. 2000;30(6):629–799.

Sasaki YF, Kawaguchi S, Kamaya A, Ohshita M, Kabasawa K, Iwama K, et al. The comet assay with 8 mouse organs:

results with 39 currently used food additives. Mutat Res. 2002;519:103–98.

Sastre MP, Vernet M, Steinert S. Single-cell gel/Comet assay applied to the analysis of UV radiation-induced DNA damage in *Rhodomonas* sp. (Cryptophyta). Photochem Photobiol. 2001;74(1):55–60.

Schabath MB, Spitz MR, Grossman HB, Zhang K, Dinney CP, Zheng PJ, et al. Genetic instability in bladder cancer assessed by the Comet assay. J Natl Cancer Inst. 2003;95 (7):540–7.

Schmid TE, Eskenazi B, Baumgartner A, Marchetti F, Young S, Weldon R, et al. The effects of male age on sperm DNA damage in healthy non-smokers. Hum Reprod. 2007;22 (1):180–7.

Schnurstein A, Braunbeck T. Tail moment versus tail length —application of an in vitro version of the Comet assay in biomonitoring for genotoxicity in native surface waters using primary hepatocytes and gill cells from zebrafish (*Danio rerio*). Ecotoxicol Environ Saf. 2001;49 (2):187–96.

Sekihashi K, Yamamoto A, Matsumura Y, Ueno S, Watanabe-Akanuma M, Kassie F, et al. Comparative investigation of multiple organs of mice and rats in the Comet assay. Mutat Res. 2002;517(1–2):53–75.

Shadnia S, Azizi E, Hosseini R, Khoei S, Fouladdel S, Pajoumand A, et al. Evaluation of oxidative stress and genotoxicity in organophosphorus insecticide formulators. Hum Exp Toxicol. 2005;24(9):439–45.

Shao L, Lin J, Huang M, Ajani JA, Wu X. Predictors of esophageal cancer risk: assessment of susceptibility to DNA damage using Comet assay. Genes Chromosomes Cancer. 2005;44(4):415–22.

Sharma S, Nagpure NS, Kumar R, Pandey S, Srivastava SK, Singh PJ, et al. Studies on the genotoxicity of endosulfan in different tissues of fresh water fish *Mystus vittatus* using the Comet assay. Arch Environ Contam Toxicol. 2007;53 (4):617–23.

Shaw JP, Large AT, Chipman JK, Livingstone DR, Peters LD. Seasonal variation in mussel Mytilus edulis digestive gland cytochrome P4501A- and 2E-immunoidentified protein levels and DNA strand breaks (Comet assay). Mar Environ Res. 2000;50(1–5):405–9.

Shaw JP, Large AT, Donkin P, Evans SV, Staff FJ, Livingstone DR, et al. Seasonal variation in cytochrome P450 immunopositive protein levels, lipid peroxidation and genetic toxicity in digestive gland of the mussel Mytilus edulis. Aquat Toxicol. 2004;67(4):325–36.

Shen H, Ong C. Detection of oxidative DNA damage in human sperm and its association with sperm function and male infertility. Free Radic Biol Med. 2000;28(4):529–36.

Siddique HR, Chowdhuri DK, Saxena DK, Dhawan A. Validation of *Drosophila melanogaster* as an in vivo model for genotoxicity assessment using modified alkaline Comet assay. Mutagenesis. 2005a;20(4):285–90.

Siddique HR, Gupta SC, Dhawan A, Murthy RC, Saxena DK, Chowdhuri DK. Genotoxicity of industrial solid waste leachates in *Drosophila melanogaster*. Environ Mol Mutagen. 2005b;46(3):189–97.

Singh NP. Microgels for estimation of DNA strand breaks, DNA protein crosslinks and apoptosis. Mutat Res. 2000;455(1–2):111–27.

Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res. 1988;175(1):184–91.

Singh NP, Stephens RE, Singh H, Lai H. Visual quantification of DNA double-strand breaks in bacteria. Mutat Res. 1999;429:159–68.

Singh NP, Muller CH, Berger RE. Effects of age on DNA double-strand breaks and apoptosis in human sperm. Fertil Steril. 2003;80(6):1420–30.

Siu WH, Cao J, Jack RW, Wu RS, Richardson BJ, Xu L, et al. Application of the Comet and micronucleus assays to the detection of B[*a*]P genotoxicity in haemocytes of the green-lipped mussel (*Perna viridis*). Aquat Toxicol. 2004;66(4):381–92.

Speit G, Witton-Davies T, Heepchantree W, Trenz K, Hoffmann H. Investigations on the effect of cigarette smoking in the Comet assay. Mutat Res. 2003;542(1–2):33–42.

Speit G, Hartmann A. The Comet assay: a sensitive genotoxicity test for the detection of DNA damage. Methods Mol Biol. 2005;291:85–95.

Srám RJ, Binková B. Molecular epidemiology studies on occupational and environmental exposure to mutagens and carcinogens, 1997–1999. Environ Health Perspect. 2000;108:57–70.

Steinert SA, Streib-Montee R, Leather JM, Chadwick DB. DNA damage in mussels at sites in San Diego Bay. Mutat Res. 1998;399:65–85.

Struwe M, Greulich KO, Suter W, Plappert-Helbig U. The photo Comet assay—a fast screening assay for the determination of photogenotoxicity in vitro. Mutat Res. 2007;632(1–2):44–57.

Sul D, Lee D, Im H, Oh E, Kim J, Lee E. Single strand DNA breaks in T- and B-lymphocytes and granulocytes in workers exposed to benzene. Toxicol Lett. 2002;134(1–3):87–95.

Szeto YT, Benzie IF, Collins AR, Choi SW, Cheng CY, Yow CM, et al. A buccal cell model Comet assay: development and evaluation for human biomonitoring and nutritional studies. Mutat Res. 2005;578(1–2):371–81.

Taban IC, Bechmann RK, Torgrimsen S, Baussant T, Sanni S. Detection of DNA damage in mussels and sea urchins exposed to crude oil using Comet assay. Marine Environ Res. 2004;58:701–5.

Tiano L, Fedeli D, Santroni AM, Villarini M, Engman L, Falcioni G. Effect of three diaryl tellurides, and an organoselenium compound in trout erythrocytes exposed to oxidative stress in vitro. Mutat Res. 2000;464(2):269–77.

Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, et al. The single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. Environ Mol Mutagen. 2000;35:206–21.

Tovalin H, Valverde M, Morandi MT, Blanco S, Whitehead L, Rojas E. DNA damage in outdoor workers occupationally exposed to environmental air pollutants. Occup Environ Med. 2006;63(4):230–6.

Ursini CL, Cavallo D, Colombi A, Giglio M, Marinaccio A, Iavicoli S. Evaluation of early DNA damage in healthcare workers handling antineoplastic drugs. Int Arch Occup Environ Health. 2006;80(2):134–40.

Vajpayee P, Dhawan A, Shanker R. Evaluation of the alkaline Comet assay conducted with the wetlands plant *Bacopa*

monnieri L. as a model for ecogenotoxicity assessment. Environ Mol Mutagen. 2006;47(7):483–9.

Valverde M, Fortoul TI, Díaz-Barriga F, Mejía J, del Castillo ER. Genotoxicity induced in CD-1 mice by inhaled lead: differential organ response. Mutagenesis. 2002;17(1):55–61.

Vandenhove H, Cuypers A, Van Hees M, Koppen G, Wannijn J. Oxidative stress reactions induced in beans (*Phaseolus vulgaris*) following exposure to uranium. Plant Physiol and Biochem. 2006;44:795–805.

Vanzella TP, Martinez CB, Cólus IM. Genotoxic and mutagenic effects of diesel oil water soluble fraction on a neotropical fish species. Res. 2007;631(1):36–43.

Verschaeve L, Gilles J, Schoeters J, Van Cleuvenbergen R, De Fre' R. The single cell gel electrophoresis technique or Comet test for monitoring dioxin pollution and effects. In: Fiedler H, Frank H, Hutzinger O, Parzefall W, Riss A, editors. Organohalogen compounds 11. Austria: Federal Environmental Agency; 1993. p. 213–6.

Villarini M, Moretti M, Damiani E, Greci L, Santroni AM, Fedeli D, et al. Detection of DNA damage in stressed trout nucleated erythrocytes using the Comet assay: protection by nitroxide radicals. Free Radic Biol Med. 1998;24(7–8):1310–5.

Villela IV, de Oliveira IM, da Silva J, Henriques JA. DNA damage and repair in haemolymph cells of golden mussel (*Limnoperna fortunei*) exposed to environmental contaminants. Mutat Res. 2006;605(1–2):78–86.

Villela IV, de Oliveira IM, Silveira JC, Dias JF, Henriques JA, da Silva J. Assessment of environmental stress by the micronucleus and Comet assays on *Limnoperna fortunei* exposed to Guaíba hydrographic region samples (Brazil) under laboratory conditions. Mutat Res. 2007;628(2):76–86.

Wang XF, Xing ML, Shen Y, Zhu X, Xu LH. Oral administration of Cr(VI) induced oxidative stress, DNA damage and apoptotic cell death in mice. Toxicology. 2006;228(1):16–23.

Wang H, Chen W, Zheng H, Guo L, Liang H, Yang X, et al. Association between plasma BPDE-Alb adduct concentrations and DNA damage of peripheral blood lymphocytes among coke oven workers. Occup Environ Med. 2007;64(11):753–58.

Wilms LC, Hollman PC, Boots AW, Kleinjans JC. Protection by quercetin and quercetin-rich fruit juice against induction of oxidative DNA damage and formation of BPDE-DNA adducts in human lymphocytes. Mutat Res. 2005;582(1–2):155–62.

Wilson JT, Pascoe PL, Parry JM, Dixon DR. Evaluation of the Comet assay as a method for the detection of DNA damage in the cells of a marine invertebrate, *Mytilus edulis* L. (Mollusca: Pelecypoda). Mutat Res. 1998;399(1):87–95.

Winter MJ, Day N, Hayes RA, Taylor EW, Butler PJ, Chipman JK. DNA strand breaks and adducts determined in feral and caged chub (*Leuciscus cephalus*) exposed to rivers exhibiting variable water quality around Birmingham, UK. Mutat Res. 2004;552(1–2):163–75.

Wirnitzer U, Gross-Tholl N, Herbold B, von Keutz E. Photochemically induced DNA effects in the comet assay with epidermal cells of SKH-1 mice after a single oral administration of different fluoroquinolones and 8-methoxypsoralen in combination with exposure to UVA. Mutat Res. 2006;609(1):1–10.

Witte I, Plappert U, de Wall H, Hartmann A. Genetic toxicity assessment: employing the best science for human safety evaluation part III: the comet assay as an alternative to in vitro clastogenicity tests for early drug candidate selection. Toxicol Sci. 2007;97(1):21–6.

Wynne P, Newton C, Ledermann JA, Olaitan A, Mould TA, Hartley JA. Enhanced repair of DNA interstrand cross-linking in ovarian cancer cells from patients following treatment with platinum-based chemotherapy. Br J Cancer. 2007;97(7):927–33.

Xiao RY, Wang Z, Wang CX, Yu G, Zhu YG. Genotoxic risk identification of soil contamination at a major industrialized city in northeast China by a combination of in vitro and in vivo bioassays. Environ Sci Technol. 2006;40(19):6170–75.

Yasuhara S, Zhu Y, Matsui T, Tipirneni N, Yasuhara Y, Kaneki M, et al. Comparison of comet assay, electron microscopy, and flow cytometry for detection of apoptosis. J Histochem Cytochem. 2003;51(7):873–85.

Yusuf AT, Vian L, Sabatier R, Cano JP. In vitro detection of indirect acting genotoxins in the Comet assay using HepG2 cells. Mutat Res. 2000;468:227–34.

Zang Y, Zhong Y, Luo Y, Kong ZM. Genotoxicity of two novel pesticides for the earthworm, Eisenia fetida. Environ Pollut. 2000;108(2):271–8.

Zeljezic D, Garaj-Vrhovac V. Chromosomal aberration and single cell gel electrophoresis (Comet) assay in the longitudinal risk assessment of occupational exposure to pesticides. Mutagenesis. 2001;16(4):359–63.

Zeljezic D, Garaj-Vrhovac V. Genotoxicity evaluation of pesticide formulations containing alachlor and atrazine in multiple mouse tissues (blood, kidney, liver, bone marrow, spleen) by comet assay. Neoplasma. 2004;51(3):198–203.

Zilli L, Schiavone R, Zonno V, Storelli C, Vilella S. Evaluation of DNA damage in *Dicentrarchus labrax* sperm following cryopreservation. Cryobiology. 2003;47(3):227–35.