

The Comet Assay in Toxicology

2nd Edition

Issues in Toxicology

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The Comet Assay in Toxicology *2nd Edition*

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Preface

This book is the first of its kind to be devoted exclusively to the Comet assay and its applications as an important tool in current toxicology. This multi-author book will serve as both a reference and a guide for investigators in the biomedical, biochemical and pharmaceutical sciences. Specialists from the fields of genetic toxicology and human epidemiology, with first-hand knowledge of their chosen sub-specialities, have contributed to this peer-reviewed scientific venture.

The simplicity, rapidity, versatility and ease of application of the Comet assay have made it a favourite amongst researchers and it is now also gaining acceptance amongst regulators. It can be used with all single cells from prokaryotes and eukaryotes, plants and animals, including humans, both somatic and germ cells. It is also a relatively inexpensive assay to perform.

The book is divided into different sections, reflecting the range of interest in the exploitation of this assay. It begins with an introductory section reviewing the genesis of the assay for those new to the technique, and details the various fields in which it finds wide acceptance. This sets the scene by explaining why the assay has become the most sensitive and sought after assay in modern toxicology.

There is a section that describes the protocols being followed to assess various types of DNA damage in different cell types. The third section brings together the specific applications of the assay in diverse areas ranging from genetic toxicity testing to human monitoring and environmental toxicology. The last section considers strategies for the conduct of the assay using *in vitro* and *in vivo* systems, based on internationally accepted guidelines. The book draws to be a close with an assessment of image-analysis principles and the statistics used for evaluating the data generated by the assay.

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The Comet Assay in Toxicology, 2nd Edition

Edited by Alok Dhawan and Diana Anderson

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This book is a culmination of over fifteen years of active collaboration and friendship between the editors and provides a good basic understanding of issues relating to the assay.

Alok Dhawan
Diana Anderson

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Section I: Genesis of Comet Assay

CHAPTER 1

The Comet Assay: A Versatile Tool for Assessing DNA Damage

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1.1 Introduction

Toxic substances and newer chemicals being added each year into the environment have led to increasing pollution of ecosystems as well as deterioration of air, water and soil quality. Excessive agricultural and industrial activities also 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 cause deleterious effects in somatic or germ cells of the sentinel species as well as non-target species. Hazard prediction and risk assessment of chemicals, therefore, becomes imperative for assessing the genotoxic potential of chemicals before their release into the environment or for commercial use as well as to evaluate DNA damage in flora and fauna affected by contaminated or polluted

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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 it has increasingly found application in diverse fields ranging from genetic toxicology to human epidemiology.

This review is an attempt to comprehensively examine the use of the Comet assay in diverse cell types from bacteria to humans, to assess the DNA-damaging potential of chemicals and/or environmental conditions. Sentinel species or bioindicator organisms in a particular ecosystem are the first to be affected by adverse changes in their environment. Determination of DNA damage in these organisms provides 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.

Ostling and Johanson¹ (in 1984) were the first to quantify DNA double stranded breaks in cells using a microgel electrophoresis technique, known as the single cell gel electrophoresis (SCGE) or Comet assay. Later, the assay was adapted by Singh *et al.*,² using alkaline conditions, which could assess both double- and single-strand DNA breaks as well as alkali-labile sites expressed as frank strand breaks in the DNA. Since its inception, the assay has been modified at various steps (cell isolation, lysis, electrophoresis, staining) to make it suitable for detecting various kinds of damage in different cells.^{3,4} 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 qualitatively in individual cell populations.⁵ Some other lesions of DNA damage such as DNA crosslinking (*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,³ genotoxicity testing⁶ and human biomonitoring.^{7,8} The field of ecotoxicology also provides a potential for use of Comet assay in natural ecosystems and has recently been reviewed to include the common experimental models used for studies, developments and/or modifications in protocols and improvements for future tests.⁹

Relative to other genotoxicity tests, such as chromosomal aberrations, sister chromatid exchanges, alkaline elution and the micronucleus assay, the advantages of the Comet assay include its demonstrated sensitivity for detecting low levels of DNA damage (one break per 10^{10} Daltons of DNA), requirement for small number of cells ($\sim 10\,000$) 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 the single-cell level allows for robust types of statistical analysis.

A limitation of the Comet assay is that aneugenic effects,¹⁰ and epigenetic mechanisms of indirect 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 humans, used in the Comet assay for assessing DNA damage (Figure 1.1).

1.1.1 Bacteria

Singh *et al.*¹¹ first used the Comet assay to assess the genetic damage in bacteria treated with 12.5–100 rad of X-rays. In the study, DNA double-strand breaks in the single electrostretched DNA molecule of *Escherichia coli* JM101 were determined using the neutral Comet assay. A significant increase in DNA breaks was induced by a dose as low as 25 rad, which was directly correlated to X-ray dosage (Table 1.1). The study supported the hypothesis that the strands of the electrostretched human DNA in the Comet assay represented individual chromosomes.

A modified version of the above Comet assay was used to assess the genotoxicity of antibacterial clay mineral mixture (CB) in *Escherichia coli*. CB leachate caused a significant increase in the double strand breaks in the bacterial cells, showing antimicrobial-mediated genotoxicity and suggesting the use of CB as an alternative bactericidal therapeutic.¹²

1.2 Plant Models

Plant bioassays are important tests which help detect genotoxic contamination in the environment. Plant systems can provide information about a wide range of genetic damage, including gene mutations and chromosome aberrations. Genotoxicity assessment in roots of plants like *Vicia faba*, *Nicotiana* and *Allium cepa*, have been widely conducted.^{14,15} However, during the last decade, the plant Comet assay has been extensively applied to plants (leaves, shoots and roots) to detect DNA damage arising due to chemicals, radiation and heavy metals in polluted soil and comprehensively reviewed¹⁶ (Table 1.1).

1.2.1 The Comet Assay in Lower Plants and Fungi

1.2.1.1 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.³⁹ The authors observed significantly higher ($P < 0.001$) DNA damage in chlorinated water (*i.e.* tap water) when compared with untreated (negative control) or distilled water (laboratory control). Hahn and Hock⁴⁰ used mycelia of *Sordaria macrospora* grown and treated with a variety of DNA-damaging agents directly on agarose

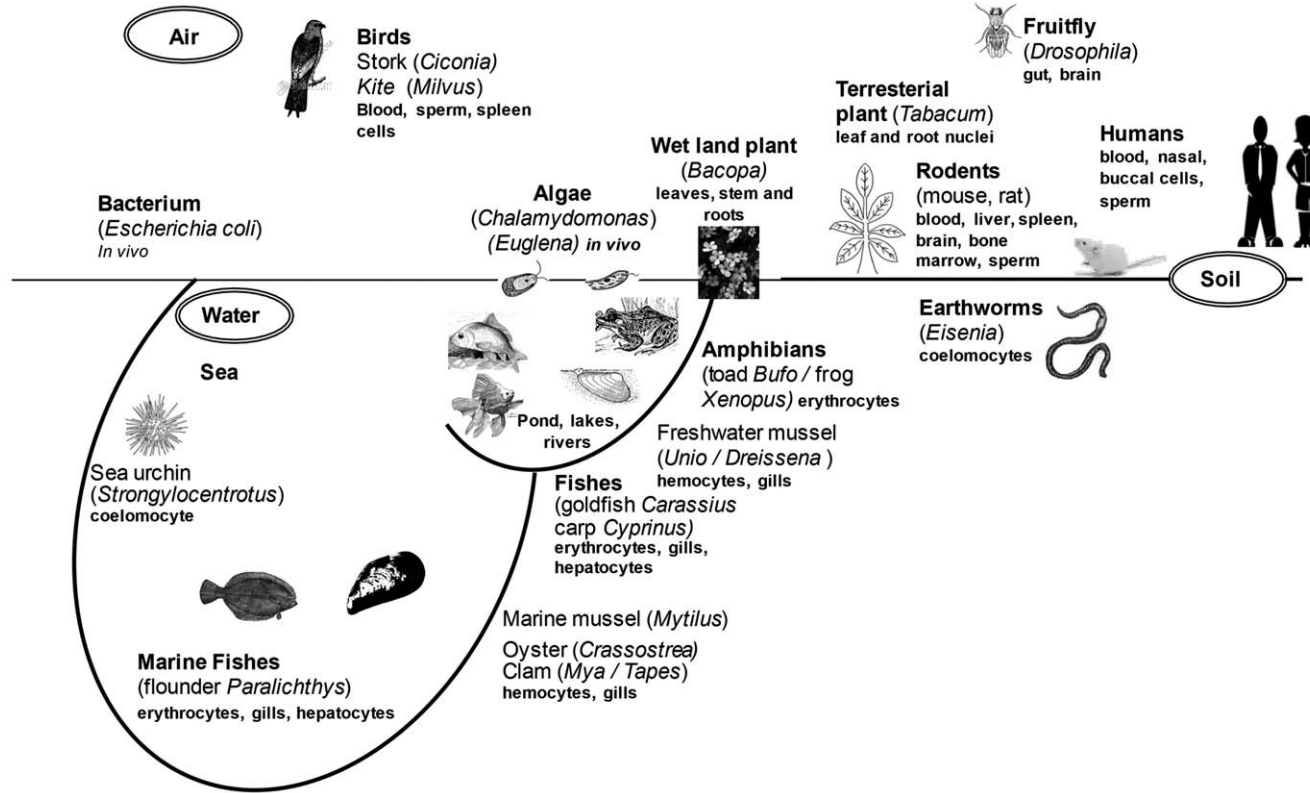


Figure 1.1 Schematic diagram of the use of comet assay in assessing DNA damage in different models from bacteria to humans. Reproduced from A. Dhawan, Comet assay: a reliable tool for the assessment of DNA damage in different models, *Cell Biol. Toxicol.*, 2009, 25(1), 5–32, © Springer Science + Business Media B.V. 2008. With permission of Springer.

Table 1.1 Comet assay for assessment of DNA damage—Bacteria and plants.

Model	Agent tested	Cells used	DNA damage ^a	Ref.
Bacteria				
<i>Escherichia coli</i> JM101	X-rays	Whole organism <i>in vivo</i>	↑	11
	Clay mineral mixture (CB)	Whole organism <i>in vivo</i>	↑	12
	Engineered nanoparticles	Whole organism <i>in vivo</i>	↑	13
Plant models				
<i>Saccharomyces cerevisiae</i>	Engineered nanoparticles	Whole organism <i>in vivo</i>	↑	13
	Cr(III)-citrate	Whole organism <i>in vivo</i>	↑	17
	Amaranth, Allura red azo dyes	Whole organism <i>in vivo</i>	↑	18
	Food additives	Whole organism <i>in vivo</i>	↑	19
<i>Euglena gracilis</i>	Organic pollutants	Whole organism <i>in vivo</i>	↑	20
<i>Chlamydomonas reinhardtii</i>	Chrysoidine	Whole organism <i>in vivo</i>	↑	21
	Paraquat herbicide	Whole organism <i>in vivo</i>	↑	22
<i>Rhodomonas</i>	UV (UVA and UVB) radiation	Whole organism <i>in vivo</i>	↑	23
<i>Vicia faba</i>	Arsenic	Root tip meristematic cells	↑	24
	Lead	Root tip meristematic cells	↑	25
	Organic pollutant	Root tip meristematic cells	↑	26
Tobacco (<i>Nicotiana tabacum</i>)	Ethyl methanesulphonate (EMS) and <i>N</i> -ethyl- <i>N</i> -nitrosourea (ENU), maleic hydrazide (MH). <i>o</i> -Phenylenediamine (<i>o</i> -PDA), hydrogen peroxide and ethyl methanesulphonate (EMS)	Whole roots <i>in vivo</i>	↑	27
		Isolated root nuclei	–	28
		Leaf nuclei	↑	29
	Leaf nuclei	↑	30	
	Leaf nuclei	↑	31	
	Leaf nuclei	↑	32	
	Heavy metal (Cd, Cu, Pb and Zn)	Nuclei from leaf tissue	↑	29
	Potato virus	Nuclei from leaf tissue	↑	33
	Polychlorinated biphenyls	Leaf nuclei	↑	30
	Urban air pollutants	Leaf nuclei	↑	31
Potato plants (<i>Solanum tuberosum</i> var. <i>Korela</i>)	Heavy metal (Cd, Cu, Pb and Zn)	Nuclei from leaf tissue	↑	29
	Potato virus	Nuclei from leaf tissue	↑	33
Castor beans (<i>Ricinus communis</i>)	Air pollution	Leaf cells	Slight ↑	34
<i>Phaseolus vulgaris</i>	Uranium	Root or shoot cells	–	35
<i>Pisum sativum</i>	Cr(VI)	Roots and leaves	↑	36
<i>Bacopa monnieri</i> L.	Ethyl methanesulphonate, methyl methanesulphonate Cadmium	Nuclei isolated from roots and leaves	↑ dose- and time-dependent roots > leaves	37
		Leaves	↑	38

^a ↑ Significant increase in DNA damage; – no DNA damage reported. Data from A. Dhawan, *Cell Biol. Toxicol.*, 2009, 25(1), 5–32.

minigels for the assessment of genotoxicity using the Comet assay. This model allowed for the rapid and sensitive detection of DNA damage by a number of chemicals simultaneously. Few studies of the Comet assay in *Saccharomyces cerevisiae* have been reported, possibly due to the presence of the cell wall and the small amount of cellular DNA, however, it has been optimized as a model system to study oxidative DNA damage and repair,^{41,42} as well as genotoxicity of chemicals^{13,17,18} and food additives.¹⁹

1.2.1.2 Algae

Algae are aquatic unicellular plants, which provide information regarding the potential genotoxicity of the water in which they grow. Being single-celled organisms, they can be used as a model for risk assessment monitoring of environmental pollution of aquatic environments using the Comet assay. The freshwater green algae species, *Pseudokirchneriella subcapitata* and *Nannocloris oculata* revealed DNA damage by the insecticide Chlorpyrifos and fungicide Tebuconazole at low concentrations.⁴³ The unicellular green alga *Chlamydomonas reinhardtii* has shown DNA damage due to known genotoxic chemicals^{21,44} and the herbicide paraquat²² and also demonstrated that oxidative stress was better managed by the algal cells under light than dark conditions.⁴⁴ The Comet assay successfully evaluated chemically-induced DNA damage and repair in *Euglena gracilis* and the responses were found to be more sensitive than those of human lymphocytes under the same treatment conditions.⁴⁵ 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 and it can be used as a part of bioassay for ecotoxicology studies. *E. gracilis* demonstrated increased genotoxicity in Comet assay parameters due to organic extracts from Taihu Lake (China), and has thus been selected as a bioindicator organism to provide early warning of organic pollutants.²⁰ 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.²³

1.2.2 The Comet Assay in Higher Plants

Recently there has been an increase in the use of the Comet assay in higher plants to study DNA damage and repair, to understand the effects of genotoxicity of pollutants and the environment. The effect of various stressors on DNA damage in plants, the correlation of the DNA damage with cellular responses¹⁶ and DNA repair^{46,47} have been reviewed and recommendations regarding the method have also been made for increasing the reliability and throughput of the Comet assay in plants.⁴⁸

Vicia faba has been widely used for the assessment of DNA damage using the 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.^{49,50}

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.*⁴⁹ also suggested that the Comet assay was able to detect a phenomenon resembling clastogenic adaptation at molecular level. *Vicia faba* used as a bioindicator plant has shown increased DNA damage due to inorganic arsenic in water (correlated with abnormal molecular changes at 20 and 30 mg l⁻¹ concentration),²⁴ lead (due to oxidative stress at 10 μM concentration),²⁵ and persistent organic pollutant-containing agricultural soils from Tlaxcala, Mexico.²⁶

Gichner and Plewa⁵¹ developed a sensitive method for isolation of nuclei from leaf tissue of *Nicotiana tabacum*, which, due to its high resolution and constant low tail moment values for negative controls, could be incorporated in *in situ* plant environmental monitoring.⁵¹ The Comet assay has been used to study the effect of alkylating agents in tobacco seedlings.⁵² A small but significant increase in DNA damage compared with controls was noted in heterozygous tobacco and potato plants grown on soil contaminated with heavy metals.²⁹ 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. Detection of concentration-dependent genotoxicity of urban air pollutants in leaf nuclei³¹ and titanium dioxide (TiO₂) nanoparticles,³² in *Nicotiana* using the Comet assay has shown it to be useful for environmental monitoring.

No DNA damage was observed in the root or shoot cells of *Phaseolus vulgaris* treated with different concentrations of uranium.³⁵ Cr(VI) showed concentration-dependent increases in DNA damage as detected by Comet assay and complemented by flow cytometry in leaves and roots of *Pisum sativum*, revealing clastogenic action of chromium.³⁶ The alkaline Comet assay was used to measure DNA damage and repair in the model plant *Arabidopsis* and rye grass exposed to X-rays.⁴⁷ Rapid and slow phases of repair were observed for acute exposures of 5 and 15 Gy, and a possible explanation of homologous repair (HR) of double-strand breaks during the slow phase was proposed.⁴⁷ For the first time Comet–fluorescence *in situ* hybridization (FISH) was conducted in the model plant species *Crepis capillaris* following exposure of seedlings to maleic hydrazide (MH), demonstrating 5S rDNA in the tail of the Comets, and suggesting Comet–FISH as a tool for environmental monitoring.⁵³

The major drawback with plant models was the fact that exposure needs to be given through 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. To circumvent this disadvantage, Vajpayee *et al.*,³⁷ 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. monnieri* to 0.001–200 μM cadmium

resulted in significant ($P < 0.05$) levels of DNA damage. Another bioindicator plant duckweed (*Lemna*) was used to study effects of industrial wastewater samples from environmental monitoring sites along the river Sava (Croatia) and showed a marked increase in DNA damage.³⁸

Reviews of the use of Comet assay in higher plants have been recently published which discuss protocols and its use in environmental genotoxicity research,⁵⁴ as well as applications in DNA repair studies and mutation breeding.⁵⁵ These studies revealed that DNA damage measured in plants using the Comet assay is a good model for *in situ* monitoring and screening of genotoxicity of polluted environments. Higher plants can also be used as an alternative first-tier assay system for the detection of possible genetic damage resulting from polluted waters or effluents due to industrial activity or agricultural run offs.

1.3 Animal Models

Animal models have long been used to assess the safety or toxicity of chemicals and finished products. With the advancements in technology, use of knockouts and transgenic models has become common for mimicking the effects in humans. The Comet assay has globally been used for assessment of DNA damage in various animal models.

1.3.1 Lower Animals

The Comet assay has been used in a unicellular protozoan and invertebrates for establishing the safety of the environment in which these species are found (Table 1.2)

1.3.1.1 Protozoan

Tetrahymena thermophila is a unique unicellular protozoan, with both somatic and germ nucleus present in the same cell, and is widely used for genetic studies due to its well characterized genome. Therefore it was 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.⁵⁶ The method was then used for the assessment of genotoxic potential of influent and effluent water samples from a local municipal wastewater treatment plant.⁵⁶ The method provided an excellent, low level detection of genotoxicants and proved to be a cost-effective and reliable tool for genotoxicity screening of waste water. Ecological risk assessment of the organic pollutant dechlorane plus (DP) was conducted in *Tetrahymena* using the Comet assay, which showed its potential genotoxicity at high levels.⁵⁷ Melamine was found to be highly toxic to the *Tetrahymena* genome which also caused apoptosis.⁵⁸ An acellular Comet assay in *Tetrahymena* has also been used to study the genotoxicity of TiO₂ nanoparticles.⁵⁹

1.3.1.2 Invertebrates

Various aquatic (marine and freshwater) and terrestrial invertebrates have been used for genotoxicity studies employing the Comet assay (Table 1.2) which have also been reviewed.^{9,93,125,126} Cells from haemolymph, embryos, gills, digestive glands and coelomocytes from mussels (*Mytilus edulis*), 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 and fruit fly (*Drosophila*). 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 used as a mechanistic tool to determine pollutant effects and mechanisms of DNA damage.⁷⁸

1.3.1.2.1 The Comet Assay in Mussels. Adverse effects of contaminants in the aquatic environment have been studied in freshwater and marine mussels as they are important pollution indicator organisms. These sentinel species provide the potential for environmental biomonitoring of aquatic environments which they inhabit. The Comet assay in mussels can be used to detect a reduction in water quality caused by chemical pollution.^{75,127} *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),⁷⁰ and oil spills with petroleum hydrocarbons.⁹² 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.⁷⁰ Repairable DNA damage with B[*a*]P was also observed with *Mytilus galloprovincialis* and the green lipped mussels (*Perna viridis*).⁸⁵ Effects of ionizing radiation, due to anthropogenic addition of radionuclides in aquatic environment, have been found to alter DNA damage and RAD 1 genes in *Mytilus* tissues.⁷³ Since the biomonitoring of the indicator organisms *in situ* may cause time constraints and not all samples may be processed at the same time, the cryopreservation of samples for later analysis in laboratory would be beneficial. Kwok *et al.*¹²⁸ used different media for this study and found that preserved haemocytes samples of *Mytilus* may be stored at cryogenic temperatures for a month without change in DNA damage for analysis in Comet assay.¹²⁸

Inter-individual variability, including seasonal variations in DNA damage have been reported from some studies, both in laboratory and field,^{71,130,131} hence baseline monitoring has to be carried out over long time intervals. Haemocytes of freshwater Zebra mussel *Dreissena polymorpha* have shown temperature-dependent DNA damage showing that the mussels are sensitive to changes in water temperatures,⁶⁴ and monitoring ecogenotoxicity with these species should account for variations in temperatures. The Comet assay in haemocytes of *D. polymorpha* was used as a

Table 1.2 Comet assay for assessment of DNA damage—Animal models (Invertebrates).

Model	Agent tested	Cell used	DNA damage ^a	Ref.	
<i>Tetrahymena thermophila</i>	Phenol, hydrogen peroxide and formaldehyde, influent and effluent water samples	Whole animal <i>in vivo</i>	↑	56	
	Dechlorane plus (DP)	Whole animal <i>in vivo</i>	↑	57	
	Melamine	Whole animal <i>in vivo</i>	↑	58	
	Titanium dioxide nanoparticles	Acellular	↑	59	
Invertebrates bivalves					
Freshwater bivalve zebra mussel (<i>Dreissena polymorpha</i>)	Polybrominated diphenyl ethers (PBDEs)	Haemocytes	↑↑	60	
	Sodium hypochlorite and chlorine dioxide and peracetic acid	Haemocytes	↑	61	
	NSAIDS (diclofenac, ibuprofen and paracetamol)	Haemocytes	↑	62	
	Pentachlorophenol	Haemocytes	↑	63	
	Varying temperatures	Haemocytes	↑	64	
	Polluted waters	Haemocytes	↑	65	
	Cadmium (Cd)	Gills	–	66	
<i>Mytilus edulis</i>	Styrene	Haemolymph cells	↑	67	
	Tritium	Haemocytes	↑	68	
	Marine waters (Denmark), French Atlantic Coast	Haemocytes	↑	69	
	Polycyclic aromatic hydrocarbons	Gill and haemolymph	↑	70	
	Seasonal variation	Gill and haemocytes	↑	71	
	C60 fullerene and fluoranthene	Haemocytes	Concentration-dependent ↑ alone and ↑↑ together	72	
	Ionizing radiation	Haemocytes	↑	73	
	Tamar estuary waters (England)	Haemocytes	↑ at site of high Cr concentration	74	
	<i>Mytilus galloprovincialis</i>	Environmental stress	Haemocytes	↑	75
		Copper oxide and silver nanoparticles	Haemolymph cells	↑	76
Titanium dioxide nanoparticles		Haemocytes	↑	77	
Freshwater mussels <i>Unio tumidus</i>	Polyphenols	Digestive gland cells	↑	78, 79	
	Base analogue 5-Fluorouracil (FU)	Haemocytes	↑	80	

<i>Unio pictorum</i>	Base analogue 5-Fluorouracil (FU)	Haemocytes	↑	80
Golden mussel (<i>Limnoperna fortunei</i>)	Guaíba Basin water	Haemocytes	↑	81
Bivalve mollusc (<i>Scapharca inaequivalvis</i>)	Organotin compounds (MBTC, DBTC and TBTC)	Erythrocytes	↑	82
Vent mussels (<i>Bathymodiolus azoricus</i>)	Hydrostatic pressure change	Haemocytes and gill tissues	↑	83, 84
Green-lipped mussels <i>Perna viridis</i>	Benzo[<i>a</i>]pyrene	Haemocytes	↑	85
<i>Perna canaliculus</i>	Cadmium	Haemocytes	↑	86
Freshwater mussel (<i>Utterbackia imbecillis</i>)	Chemicals used in lawn care (atrazine, glyphosate, carbaryl and copper)	Glochidia	↑	87
Oyster (<i>Crassostrea gigas</i>)	Cryopreservation	Spermatozoa	↑	88
	Diuron (0.05 µg l ⁻¹), glyphosate	Spermatozoa	↑, -	89
Manila clam (<i>Tapes semidecussatus</i>)	Sediment-bound contaminants	Haemolymph, gill and digestive gland	↑	90, 91
Clams <i>Mya arenaria</i>	Petroleum hydrocarbons	Haemocytes and digestive gland cells	-	92
<i>Ruditapes decussatus</i>	PAH	Gills	↑	93
Earthworms				
<i>Eisenia foetida</i>	Soil from industrialized contaminated areas	Coelomocytes	↑	94
	Sediment from polluted river	Coelomocytes	↑	95
	Waste water irrigated soil	Coelomocytes	↑	96
	Commercial parathion	Sperm cells	↑	97
	Imidacloprid and RH-5849	Coelomocytes	↑	98
	PAH contaminated soil and hydrogen peroxide, Cadmium (<i>in vitro</i>)	Eleocytes	↑	99
	Nickel chloride	Coelomocytes	↑	100
	Dechlorane plus	Coelomocytes and Spermatogenic cells	↑	101
	Ionizing radiation (<i>in vivo</i> and <i>in vitro</i>)	Coelomocytes	↑	102
	Radiation and mercury	Coelomocytes	↑ synergistic effect	103
	Nickel and deltamethrin, with humic acid	Coelomocytes	↑, synergistic effect, damage ↓ with humic acid	104

Table 1.2 (Continued)

Model	Agent tested	Cell used	DNA damage ^a	Ref.
	Lead and BDE209	Coelomocytes	↑ alone, antagonistic effect	105
<i>Eisenia hortensis</i>	Cobalt chloride	Coelomocytes	↑ dose-dependant	106
<i>Aporrectodea longa</i> (Ude)	Soil samples spiked with benzo[<i>a</i>]pyrene (B[<i>a</i>]P) and/or lindane	Intestine and crop or gizzard cells	↑ intestine > crop	107
Other invertebrates				
Fruit fly (<i>Drosophila melanogaster</i>)	Ethyl methanesulfonate (EMS), methyl methanesulfonate (MMS), <i>N</i> -ethyl- <i>N</i> -nitrosourea (ENU) and cyclophosphamide (CP)	Gut and brain cells of first instar larvae	↑	108, 109
	Cypermethrin	Brain and anterior midgut cells	↑	110
	Leachates of industrial waste	Gut and brain cells of first instar larvae	↑	108
	Cisplatin	Midgut cells	↑	111
	Hexavalent chromium	Larval haemocytes	↑↑	112
	Zinc oxide nanoparticles	Larval haemocytes	↑ at high dose.	113
	Copper oxide nanoparticles,	Larval haemocytes	↑	114
	Cadmium selenium (CdSe) quantum dots	Larval haemocytes	↑	115
Grasshoppers (<i>Chorthippus brunneus</i>)	Different polluted sites	Larval brain cells	↑↑ in heavy polluted site	116
	Paraquat (<i>in vitro</i> , <i>in vivo</i>)	Larval brain cells	↑ time dependent	117
Sea urchins (<i>Strongylocentrotus droebachiensis</i>)	Dispersed crude oil	Coelomocytes	↑ concentration-dependent	118
Grass shrimp, (<i>Palaemonetes pugio</i>)	UV, benzo[<i>a</i>]pyrene, and cadmium	Embryos	↑ damage and decreased repair	119
	Estuarine sediments	Hepatopancreas	↑	120
	Coal combustion residues	Hepatopancreas	↑	121
Sea anemone (<i>Anthopleura elegantissima</i>)	Hydrogen peroxide ethylmethanesulphonate (EMS) or benzo[<i>a</i>]pyrene (B[<i>a</i>]P)	Blood cells	↑ dose response	122
Marine invertebrate (<i>Donax faba</i>)	Pesticide Chlorpyrifos and fungicide Carbendazime	Gill, body and foot cells	↑	123
Polychaete (<i>Nereis diversicolor</i>)	Nano-, micro- and ionic-Ag	Coelomocytes	↑↑ Nano >micro >ionic	124

^a↑ Significant increase in DNA damage, ↑↑ highly significant increase in DNA damage; ↓ decrease in DNA damage; - no DNA damage reported.

tool in determining the potential genotoxicity of water pollutants,^{60–63} and Klobucar *et al.*⁶⁵ suggested that haemocytes from caged, non-indigenous mussels could be used for Comet assay for monitoring genotoxicity of freshwater. The hOGG1 enzyme was used in the Comet assay to evaluate 8-oxo-2'-deoxyguanosine (8-oxo-dG) as a marker of oxidative DNA damage in *D. polymorpha*.¹²⁹

DNA damage and repair studies in vent mussels, *Bathymodiolus azoricus*, have been carried out to study the genotoxicity of naturally contaminated deep-sea environment.^{83,84} The vent mussels demonstrated similar sensitivity to environmental mutagens to that of coastal mussels and thus could be used for ecogenotoxicity studies of deep sea waters using the Comet assay. Villela *et al.*¹³² used the golden mussel (*Limnoperna fortunei*) as a potential indicator organism for freshwater ecosystems due to its sensitivity to water contaminants.

In vitro Comet assay has also been used in cells of mussels, which can be used to screen genotoxic agents destined for release or disposal into the marine environment. Dose-responsive increases in DNA strand breakages were recorded in digestive gland cells¹³³ haemocytes¹³⁴ and gill cells¹³⁴ of *M. edulis* exposed to both direct-acting (hydrogen peroxide and 3-chloro-4-(dichloromethyl)-5-hydroxy-2[5H]-furanone) and indirect-acting (B[a]P, 1-nitropyrene, nitrofurantoin and *N*-nitrosodimethylamine) genotoxicants. Digestive gland cells^{78,135} and haemocytes⁸⁰ of *Unio tumidus* were also used for *in vitro* studies of DNA damage and repair by different compounds.

1.3.1.4 The Comet Assay in Other Bivalves

Coughlan *et al.*⁹⁰ 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.^{90,91} 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.⁸⁸ The Comet-FISH assay, conducted in haemocytes of *C. gigas*, was shown to have potential for detecting DNA damage of target genes, induced by toxicant exposure and to allow better understanding of the impact of genotoxicity on animal physiology and fitness.¹³⁶ Gielazyn *et al.*¹³⁷ 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 haemocytes from oyster (*Crassostrea virginica*) and clam (*Mercenaria mercenaria*). The herbicide diuron induced significant DNA damage in oyster spermatozoa at 0.05 $\mu\text{g l}^{-1}$ upwards while its environmental concentrations significantly affected embryo-larval development, showing deleterious effects of herbicide in non-target organisms.⁸⁹

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.¹³⁸

1.3.1.5 The Comet Assay in Earthworms

The Comet assay applied to earthworms is a valuable tool for monitoring and detection of genotoxic compounds in terrestrial ecosystems^{94–105} (Table 1.2). Since 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.

Coelomocytes from *Eisenia foetida* have been used for biomonitoring purposes, to assess DNA damage in worms exposed to soil samples from industrialized contaminated areas⁹⁴ and sediment samples from polluted river systems.⁹⁵ Ecogenotoxicity studies have shown dose dependent DNA strand breaks caused by insecticide⁹⁷ and pesticides⁹⁸ in *E. foetida* as well as *Pheretima* species¹³⁹ demonstrating that pesticides could also have adverse effects on non-target species. Ionizing radiation affects the soil ecology, as it induced oxidative damage in spermatogenic cells of *E. foetida* and also reduced reproduction at dose rates at or >4 mGy h⁻¹.¹⁰² Radiation with exposure to mercury produced synergistic effects and increased damage to DNA.¹⁰³ Humic acid was found to alleviate nickel- and deltamethrin-induced toxicity in earthworms, and could be used to reduce oxidative damage to DNA, lipids and proteins.¹⁰⁴ Medicinal therapy using peloids (natural mud), despite usually being beneficial, may also pose a risk of toxic effects as was seen in a study with *E. foetida* exposed to peloids.¹⁴⁰

In vitro exposure of primary cultures of coelomocytes to nickel chloride as well as exposure of whole animals either in spiked artificial soil water or in spiked cattle manure substrates exhibited increased DNA strand breaks due to the heavy metal.¹⁰⁰ The leucocytes cells, a subset of coelomocytes produced increased DNA strand breaks under both *in vitro* and *in vivo* conditions and could be used a sensitive biomarker for genotoxicity in earthworms.⁹⁹ Another earthworm *Aporrectodea longa* (Ude), when exposed to soil samples spiked with B[a]P and/or lindane demonstrated genotoxicity in the intestinal cells to be more sensitive to the effect of the toxicants than the crop or gizzard cells.¹⁰⁷

Fourie *et al.*¹⁴¹ used five earthworm species (*Amyntas diffringens*, *Aporrectodea caliginosa*, *Dendrodrilus rubidus*, *Eisenia foetida* and *Microchaetus benhami*) to study genotoxicity of cadmium sulphate, with significant DNA damage being detected in *E. foetida* followed by *D. rubidus* and *A. caliginosa*. The study showed the 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.

1.3.1.6 The Comet Assay in *Drosophila*

The simple genetics and developmental biology of *Drosophila melanogaster* has made it the most widely used insect model. It has been recommended as an alternate animal model by the European Centre for the Validation of Alternative Methods¹⁴² and evolved into a model organism for toxicological studies.^{143,144} *D. melanogaster* has been used as an *in vivo* model (Table 1.2) for assessment of genotoxicity^{108–115} and oxidative DNA damage¹⁴⁵ as well as for *in vitro* studies¹⁴⁶ using the Comet assay. Cisplatin induced adducts in *D. melanogaster* are influenced by conditions of nucleotide excision repair, and this correlates well with DNA damage as seen in Comet assay.¹⁴⁷ Recently, the Comet assay in *Drosophila* as an *in vivo* model has been used to assess the genotoxicity of zinc, copper and cadmium nanomaterials, which have demonstrated oxidative DNA damage.^{113–115}

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

1.3.1.7 The Comet Assay in Other Invertebrates

Nereis virens, a polychaete, plays an important role in the distribution of pollutants in sediments due to its unique property of bioturbation. These worms are similar to earthworms in soil and can be used for genotoxicity assessment of sediments. They have been used to study sediment-associated toxicity of silver nanoparticles, and bioaccumulation in the body was also shown.¹²⁴ Genotoxicity of intracoelomically injected B[a]P was assessed in worm coelomocytes using Comet assay, however, *Nereis* species was not found to be suitable for assessing PAH genotoxicity due to their lack of metabolic capability to convert B[a]P to its toxic metabolite.¹⁴⁸

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 the insects due to industrial pollutants.¹⁴⁹ Comet assay parameters in brain cells of larvae originating from eggs of grasshoppers from different polluted sites have shown an association between increased DNA damage and heavy environmental pollution.¹¹⁶ Paraquat caused increased DNA damage in brain cells in both *in vitro* and *in vivo* administrations.¹¹⁷

Chronic exposure to coal combustion residues from coal-fired electrical generation in estuarine grass shrimp, *Palaemonetes pugio*, caused DNA damage in hepatopancreatic cells of adult shrimps as compared with the reference shrimp as seen in the Comet assay.¹²¹ The Comet assay in planarians is an important test for environmental monitoring studies since these are simple organisms with high sensitivity, low cost and a high proliferative rate.¹⁵⁰ The genotoxic potential of polluted waters from Diluvio's Basin, Norflurazon, a bleaching herbicide¹⁵¹ and copper sulfate¹⁵² was evaluated in planarians, where, significant increases in primary DNA damage were

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

1.4 Higher Animals

1.4.1 Vertebrates

Studies of vertebrate species where the Comet assay is used have included 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 1.3).

1.4.1.1 The 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 and blood) of fishes exposed to various xenobiotics in the aquatic environment (Table 1.3).

The basal level of DNA damage detected in the Comet assay has been shown to be influenced by various factors, such as the temperature of water in erythrocytes of mullet and sea catfish,^{156,157} age and gender in dab (*Limanda limanda*¹⁷⁹), exhaustive exercise¹⁵⁴ and seasonal changes¹⁵⁵ in chub. Therefore, these factors should be accounted for during environmental biomonitoring studies. The high intra-individual variability may also affect the sensitivity of the assay.¹⁷⁹ The protocol and experimental conditions used for the Comet assay for monitoring marine ecosystems may lead to differences in the results obtained. Also, chemical and mechanical procedures to obtain cell suspensions may lead to additional DNA damage.³¹⁸ Anaesthesia did not contribute towards DNA damage *in vivo* in methyl-methanesulfonate (MMS)-treated fishes and the anaesthetic benzocaine did not alter the DNA damage in erythrocytes after *in vitro* exposure to MMS or H₂O₂.³¹⁹ Hence keeping in mind animal welfare, multi-sampling of the same fish can be conducted. Recently, nanomaterials toxicity has gained importance in aquatic toxicology as nanomaterials synthesis and use has increased. Its impact on the aquatic environment and on fishes needs to be elucidated and this calls for development and implementation of protocols for nanomaterial genotoxicity in ecotoxicology.^{320–322}

In vitro studies on fish hepatocytes,^{182,185} primary hepatocytes and gill cells¹⁸⁶ as well as established cell lines (with metabolic competence^{189–191}) using the Comet assay have also been conducted to assess the genotoxicity of

Table 1.3 Comet assay for assessment of DNA damage–Animal models (Vertebrates).

Model	Agent tested	Cell used	DNA damage ^a	Ref.
Fishes				
Chub (<i>Leuciscus cephalus</i>)	PAHs, PCBs, organochlorine pesticides (OCPs), and heavy metals	Hepatocytes	↑	153
	Exhaustive exercise	Erythrocytes	↑	154
	Seasonal change at polluted sites.	Gills, liver, blood	↑ in spring/autumn, gills and liver > blood	155
Estuarine mullet (<i>Mugil</i> sp.) and sea catfish (<i>Netuma</i> sp.)	Organochlorine pesticides and heavy metals	Erythrocytes	↑	156
	High temperature	Erythrocytes	↑	157
Fresh water teleost (<i>Mystus vittatus</i>)	Endosulfan	Gill, kidney and erythrocytes	↑ in all cells	158
Fresh water murrel (<i>Channa punctatus</i>)	Tannery effluent in Ganges, India	Gills	↑	159
Tilapia (<i>Oreochromis niloticus</i>)	Antibiotics Florfenicol (FLC) and oxytetracycline (OTC)	Blood erythrocytes	↑	160
Eastern mudminnow (<i>Umbra pygmaea</i> L.)	Rhine water for 11 days	Blood erythrocytes	↑	161
Neotropical fish <i>Prochilodus lineatus</i>	Diesel water soluble fraction acute (6, 24 and 96 h) and subchronic (15 days) exposures, Cypermethrin, <i>in vivo</i>	Erythrocytes	↑	162
	Ethyl methanesulfonate, hydrogen peroxide (<i>in vitro</i>)	Epithelial gill cells	↑ <i>in vivo</i> and <i>in vitro</i>	163
Freshwater goldfish (<i>Carassius auratus</i>)	Technical herbicide Roundup (glyphosate)	Erythrocytes	↑↑ dose-dependent	164
	ADDB and PBTA-6	Erythrocytes	↑ dose-dependent	165
Turbot (<i>Scophthalmus maximus</i> L.)	Sediment collected from polluted sites in Cork Harbour (Ireland)	Hepatocytes	↑	166
	PAH by different routes	Erythrocytes	↑ by all routes	167
Zebra fish (<i>Danio rerio</i>)	Methyl methanesulphate	Gill, gonads and liver cells	↑ in all cells	168
Brazilian flounder (<i>Paralichthys orbignyanus</i>)	Contaminated estuary waters	Blood cells	↑↑	169

Table 1.3 (Continued)

Model	Agent tested	Cell used	DNA damage ^a	Ref.
European flounder (<i>Platichthys flesus</i>)	Different estuaries, seasons and genders	Blood cells	↑	170
Carp (<i>Cyprinus carpio</i>).	Disinfectants	Erythrocyte	↑	171
	NSAID-manufacturing plant effluent	Erythrocyte	↑	172
Armoured catfish (<i>Pterygoplichtys anisitsi</i>)	Diesel and biodiesel	Erythrocytes	↑	173
Trout (<i>Oncorhynchus mykiss</i>)	Cryopreservation (Freeze-thawing)	Spermatozoa	Slight ↑	174
European eel (<i>Anguilla anguilla</i>)	Benzo[<i>a</i>]pyrene, Arochlor 1254, 2-3-7-8-tetrachlorodibenzo- <i>p</i> -dioxin and beta-naphthoflavone	Erythrocytes	↑	175
	Herbicides-Roundup, Garlon	Erythrocytes	↑	176
Eelpout (<i>Zoarces viviparus</i>)	Oil spill (PAH)	Nucleated erythrocytes	↑	177
Gilthead sea bream (<i>Sparus aurata</i>)	Copper	Erythrocytes	↑↑	178
Dab (<i>Limanda limanda</i>)	PAHs and PCBs polluted waters of English channel Gender and age	Blood cells	↑ in adults and males	179
Hornyhead turbot (<i>Pleuronichthys verticalis</i>)	Sediments collected from a natural petroleum seep (pahs)	Liver cells	↑	180
In vitro				
Carp (<i>Cyprinus carpio</i>)	Organic sediment extracts from the North Sea (Scotland)	Leukocytes	↑	181
Trout (<i>Oncorhynchus mykiss</i>)	Cadmium	Hepatocytes	↑	182
	Tannins	Erythrocytes	↓	183
	Diaryl tellurides and ebselen (organoselenium)	Erythrocytes	↓	184
Zebrafish (<i>Danio rerio</i>)	Oil sands processed water, (PAH and naphthnic acids)	Hepatocytes (<i>in vitro</i>)	↑	185
	Surface waters of German rivers, Rhine and Elbe	Hepatocytes and gill cells	↑	186
<i>Danio rerio</i> (ZFL) hepatocyte cell line	Biodiesel	Hepatocytes	↑	187
Rainbow trout hepatoma cell line (RTH-149)	Water samples from the polluted Kishon river (Israel)	Liver	↑	188

Rainbow trout gonad (RTG-2) cell line	4-nitroquinoline- <i>N</i> -oxide <i>N</i> -methyl- <i>N'</i> -nitro- <i>N</i> -nitrosoguanidine, benzo[<i>a</i>]pyrene, nitrofurantoin, 2-acetylaminofluorene, dimethylnitrosamine, and surface waters	Gonad	↑ dose dependent response	189
Rainbow trout liver (RTL-W1) cell line	2,4,7,9-tetramethyl-5-decyne-4,7-diol (TMDD) Coal tar run off water	Epitheloid liver Epitheloid liver	Slight ↑ ↑	190 191
Amphibians				
Amphibian larvae (<i>Xenopus laevis</i> and <i>Pleurodeles waltl</i>)	Cadmium (CdCl ₂)	Erythrocytes	↑ concentration and time dependent	192
	Captan (<i>N</i> -trichloromethylthio-4-cyclohexene-1,2-dicarboximide)	Erythrocytes	↑ concentration and time dependent	193
Amphibian larva (<i>Xenopus laevis</i>)	Benzo[<i>a</i>]pyrene, ethyl and methyl methanesulfonate	Erythrocytes	–	194
	Aqueous extracts of five sediments from French channels	Erythrocytes	↑	195
Toad (<i>Bufo raddei</i>)	Petrochemical (mainly oil and phenol) polluted area	Liver cells and erythrocytes	↑	196
Southern toad (<i>Anaxyrus terrestris</i>)	Low-dose-rate ionizing radiation	Red blood cells	↓ at ≥21 mGy	197
Toad (<i>Xenopus laevis</i> , and <i>Xenopus tropicalis</i>)	Bleomycin induced DNA damage and repair	Splenic lymphocytes	↑ DNA damage in <i>X. tropicalis</i> > <i>X. laevis</i>	198
<i>Xenopus laevis</i> , and <i>Xenopus tropicalis</i>			DNA repair in <i>X. laevis</i> > <i>X. tropicalis</i>	
Tadpoles of <i>Rana N. Hallowell</i>	Imidacloprid [1-(6-chloro-3-pyridylmethyl)- <i>N</i> -nitro-imidazolidin-2-ylideneamine] and RH-5849 [2'-benzoyl-1'- <i>tert</i> -butylbenzoylhydrazine]	Erythrocytes	↑	199
Tadpoles (<i>Rana hexadactyla</i>)	Sulfur dyes (Sandopel Basic Black BHLN, Negrosine, Dermapel Black FNI, and Turquoise Blue) used in the textile and tannery industries	Erythrocytes	↑↑	200
Tadpoles of Bullfrog (<i>Rana catesbeiana</i>)	Herbicides AAtrex Nine-O (atrazine), Dual-960E (metalochlor), Roundup (glyphosate), Sencor-500F (metribuzin), and Amsol (2,4-D amine)	Erythrocytes	↑↑	201

Table 1.3 (Continued)

Model	Agent tested	Cell used	DNA damage ^a	Ref.
Tadpole	Agricultural regions	Erythrocytes	↑ industrial regions > agricultural regions	202
<i>Rana clamitans</i>	Industrial regions			
<i>Rana pipiens</i>				
Tadpoles (<i>Rana limnocharis</i>)	Cadmium (CdCl ₂)	Erythrocytes	↑	203
	Sodium arsenite	Whole blood	↑	204
Eurasian marsh frog (<i>Pelophylax ridibundus</i>)	Pollution in the different lakes in central Anatolia, Turkey.	Blood cells	↑	205
Anuran amphibian (<i>Hypsiboas faber</i>)	Heavy metal, in coal open-cast mine	Blood cells	↑	206
Frog tadpoles (<i>Dendropsophus minutes</i>)	Agrochemicals	Blood cells	↑	207
In vitro				
<i>Xenopus laevis</i>	high peak-power pulsed electromagnetic field	Erythrocytes	↑ due to rise in temperature	208
Birds				
Wild nestling white storks (<i>Ciconia ciconia</i>)	Heavy metals and arsenic	Blood cells	↑ correlated with arsenic	209
Black kites (<i>Milvus migrans</i>)	Toxic acid mining waste rich in heavy metals	Blood cells	↑↑	210–212
	Heavy metals and arsenic	Blood cells	↑ correlated with copper and cadmium	209
Turkey	Toxic acid mining waste rich in heavy metals	Blood cells	↑ (2–10 fold)	210, 212
	Short term storage	Sperm	↑	213
Green finches	Paraquat	Blood	↑ oxidative damage	214
Broiler chicken	Deoxynivalenol (DON) and mycotoxin	Blood lymphocytes	↑ by DON, ↓ by mycotoxin	215
Turkey and chicken	Aflatoxin B1	Foetal liver cells	↑	216
Chicken	T-2 toxin and deoxynivalenol (DON)	Spleen leukocytes	↑	217
Chicken	Storage conditions (4 °C)	Liver and breast muscle cells	↑ liver cells > breast muscle cells	218
Japanese quails	GSM 900 MHz cellular phone radiation	Embryo cells	↑	219

Rodents

Aldh2 knockout mice	Ethanol	Hepatic cells	↑ oxidative damage	220
B6C3F1 mice	Vanadium pentoxide	Lung cells	–	221
C57Bl/6 mice	Straight and tangled multi-walled carbon nanotubes	Lung cells	↑ dose dependent	222
<i>p53</i> ^{+/-} mice	Melphalan	Liver, bone marrow, peripheral blood and the distal intestine	DNA crosslinks in all cells tested	223
SKH-1 mice	UV A + Fluoroquinolones (clinafloxacin, lomefloxacin, ciprofloxacin) UVA + 8-methoxypsoralene (8-MOP) Age dynamics	Epidermal cells	↑↑ for fluoroquinolones ↓ for MOP	224
Dyslipidemic <i>ApoE</i> ^{-/-} mice	Ageing	Aorta, liver and lung	↑ Oxidative damage in liver, – in lung or aorta	225
	Diesel exhaust particles	Aorta, liver and lung	↑ Oxidative damage in liver, – in lung or aorta	226
Balb/c mice	<i>Trypanosoma cruzi</i> infection	Peripheral blood, liver, heart and spleen cells	↑ in heart and spleen	227
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	228
Swiss albino mice	Sanguinarine alkaloid, argemone oil	Blood, bone marrow cells and liver	↑ dose dependent in blood and bone marrow	229, 230
	Cypermethrin	Brain, liver, kidney, bone marrow, blood, spleen, colon	↑	231
	Steviol	Stomach cells, hepatocytes, kidney and testicle cells	↑	232
	Apomorphine	Brain cells	–	233

Table 1.3 (Continued)

Model	Agent tested	Cell used	DNA damage ^a	Ref.
	8-oxo-apomorphine-semiquinone	Brain cells	↑	233
	Ethanol, grape seed oligomer and polymer procyanidin fractions	Brain cells	↓ ethanol-induced protection by grape seed	234
	Nonylphenol and/or ionizing radiation	Liver, spleen, femora, lungs and kidneys	↑ in all organ of males, kidney only in females. ↓ with radiation in males, ↑ in female mice	235
Male CBA mice	Pesticide formulations (Bravo and Gesaprim)	Hepatic cells, bone marrow cells spleen cells	↑↑	236
Isogenic mice	Sulfonamide, protozoan parasite <i>Toxoplasma gondii</i>	Peripheral blood cells, liver cells and brain cells	↑ in peripheral blood cells	237
Cirrhotic rats	Rutin and quercetin	Bone marrow cells	↑↑	238
Male Sprague–Dawley rats	<i>N</i> -butyl- <i>N</i> -(4-hydroxybutyl)nitrosamine (BBN), glycidol, 2,2-bis(bromomethyl)-1,3-propanediol (BMP), 2-nitroanisole (2-NA), benzyl isothiocyanate (BITC), uracil, and melamine	Urinary bladders	↑ with BBN, glycidol and BMP, – with 2-NA, BITC, uracil and melamine	239
<i>In vitro</i>				
FE1 Muta Mouse lung epithelial cell line.	Carbon black	Lung epithelial cell line.	↑	240
Rat Alveolar type II epithelial cells	Cigarette smoke	Lung cells	↑	241
L5178Y mouse lymphoma cells	Ketoprofen, promazine, chlorpromazine, dacarbazine, acridine, lomefloxacin, 8-methoxypsoralen, chlorhexidine, titanium dioxide, octylmethoxycinnamate	Lymphoma cells	Positive with phototoxic compound	242

Murine primary cultures of brain cells and a continuous cell line of astrocytes	Xanthine and xanthine oxidase, hydrogen peroxide, Superoxide dismutase, catalase, or ascorbic acid.	Brain cells	↓ by antioxidants	243
Chinese hamster ovary (CHO) cell line	Endosulfan	Ovary cells	↑	244
	Cypermethrin, pendimethalin, dichlorovous	Ovary cells	↑	245
Humans clinical				
Breast cancer patients and controls	Radiosensitivity	Peripheral blood mononuclear cells	↑↑ and reduced DNA repair	246, 247
Breast cancer patients and controls	Radiotherapy and/or chemotherapy treatment	Peripheral blood mononuclear cells	↓ post treatment	248
Papillary thyroid cancer (PTC) patients	Basal DNA damage	Peripheral blood lymphocytes	↑	249
Children	Exposed to air pollution	Oral mucosa cells	↑	250
Normal individuals	Chlorhexidine	Buccal epithelial cells and peripheral blood lymphocytes	↑	251
Non-small cell lung cancer (NSCLC) patients	Chemotherapy, Platinum based derivatives for therapy	Lung cells	↑ in patients	252
Ataxia telangiectasia heterozygote	X-irradiation	Peripheral leukocytes	↑ (~3 times higher) in patients	253
Nijmegen breakage syndrome (NBS) patients	X-irradiation	Peripheral blood mononuclear cells	↑ in patients	254
Alzheimer disease patients	–	Peripheral blood mononuclear cells	↑ in patients	255
Breast cancer patients	–	Peripheral blood mononuclear cells	↑ in patients	256
Type 2 diabetes mellitus and healthy males	Oxidative DNA damage	Peripheral blood cells	↑	257
	Exercise training	Peripheral blood cells	↓ in patients	258
Cancer (testicular cancer, lymphoma and leukaemia) patients	DNA integrity	Spermatozoa	Decreased DNA integrity	259