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The comet assay in animal models: From bugs to whales – (Part 1 Invertebrates)

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Abstract

The comet assay, also called single cell gel electrophoresis, is a sensitive, rapid and low-cost technique for quantifying and analysing DNA damage and repair at the level of individual cells. The assay itself can be applied on virtually any cell type derived from different organs and tissues of eukaryotic organisms. Although it is mainly used on human cells, the assay has applications also in the evaluation of DNA damage in yeast, plant and animal cells. Therefore, the purpose of this review is to give an extensive overview on the usage of the comet assay in animal models from invertebrates to vertebrates, covering both terrestrial and water biota. The comet assay is used in a variety of invertebrate species since they are regarded as interesting subjects in ecotoxicological research due to their significance in ecosystems. Hence, the first part of the review (Part 1) will discuss the application of the comet assay in invertebrates covering protozoans, platyhelminthes, planarians, cnidarians, molluscs, annelids, arthropods and echinoderms. Besides a large number of animal species, the assay is also performed on a variety of cells, which includes haemolymph, gills, digestive gland, sperm and embryo cells. The mentioned cells have been used for the evaluation of a broad spectrum of genotoxic agents both *in vitro* and *in vivo*. Moreover, the use of invertebrate models and their role from an ecotoxicological point of view will also be discussed as well as the comparison of the use of the comet assay in invertebrate and human models. Since the comet assay is still developing, its increasing potential in assessing DNA damage in animal models is crucial especially in the field of ecotoxicology and biomonitoring at the level of different species, not only humans.

Keywords: Comet assay, DNA damage, Invertebrates, *In vitro*, *In vivo*, Biomonitoring

1. Introduction

The DNA molecule is the source of genetic information in each living cell and its integrity and stability are essential to life. However, the DNA molecule is not inert and it is under a constant stream of attack from various physical and/or chemical agents present in the environment both naturally or resulting from the influence of humans. Consequently, if the resulting damage is not repaired, it could easily lead to mutations and afterwards possibly to a number of diseases including cancer. Under the term "DNA damage" we include an alteration in the chemical structure of DNA in the form of a break in a DNA strand, a base missing from the DNA backbone and/or a chemically changed base [1–4].

There are numerous methods available for the evaluation of DNA damage as well as its repair both *in vitro* and *in vivo* [5,6]. Commonly used approaches are the Ames test [7], alkaline elution [8], chromosome aberrations [9], sister chromatid exchanges (SCE) [10], cytokinesis block micronucleus (CBMN) assay [11–13] and γ-H2AX test [14,15]. The above-mentioned methods play an important role for the assessment of environmental pollution and occupational exposure and are used worldwide in laboratories in the fields of genetic and environmental toxicology, human epidemiology and biomonitoring of different populations. Furthermore, these methods are also used to investigate anti-genotoxic, anti-mutagenic and/or anti-carcinogenic properties of different natural and man-made products. Although the above-mentioned methods are very useful in assessing genome damage, they also have various disadvantages such as the need for proliferating cells, and for visual scoring under the microscope, and they often tend to be laborious and rather expensive. As a result, different tests were developed for much simpler, faster and low-cost evaluation of DNA damage and new ones are constantly in development.

The one technique that has changed the scientific world with regard to DNA damage assessment is the comet assay, named after the comet-like appearance of the cellular DNA after electrophoresis, which has immediately been widely accepted as quite simple, sensitive, reliable, rapid and low-cost assay for the detection of DNA damage as well as its repair at the level of individual cells. The assay itself can be applied to virtually any cell type derived from different organs and/or tissues of eukaryotic organisms that can be prepared as a single cell suspension. Although it is mainly used in human cells both *in vivo* (*ex vivo*) and *in vitro* the assay has its application in evaluation of DNA damage in yeast [16,17], plant [18–20] and animal [21–25] cells as well. In line with that, the comet assay has instantly found its

application in different fields; from genetic and environmental toxicology to human epidemiology and biomonitoring [21,26–35].

Östling and Johanson [36] were the first to quantify DNA damage in individual mammalian cells after γ-irradiation using a microgel electrophoresis technique named "single cell gel electrophoresis assay" later known as the comet assay. One of the initial advantages, as concluded by the authors, was that no radioactive labelling and only a small number of cells are required for the described procedure. The neutral conditions allow both DNA single- and double-strand break detection but with less sensitivity than alkaline version [37]. Only afterwards was the assay done under alkaline conditions, by Singh et al. [38] allowing detection of alkali labile sites in addition to double- and single-strand breaks [29,30,39].

The assay involves embedding cells in an agarose matrix followed by lysis in neutral or alkaline conditions. Afterwards the cells go through electrophoresis and are subsequently neutralized. For evaluation under a fluorescence microscope, the cells are stained with different fluorescent agents to facilitate visualization and calculation of fluorescence to determine the extent of DNA damage. The concept behind the comet assay is that undamaged, supercoiled DNA remains in the "head" of the comet, while loops of DNA in which supercoiling is relaxed can travel through pores of the agarose gel attracted to the anode in the electric field, thus creating a "comet tail". Therefore, the relative amount of DNA present in the comet tail corresponds to the actual DNA damage of the cell. Although comets can be scored visually and classified into different categories according to their appearance representing a certain amount of DNA damage, more popular and widely used is the semi-automatic scoring of comet slides. This is done by using appropriate software that enables commercially available image analysing systems to be connected through a camera to a fluorescence microscope, which facilitates the evaluation of DNA damage [29,31,38,40–42].

Besides measuring single- and double-strand breaks and alkali labile sites, other DNA lesions such as DNA crosslinks and DNA base oxidation can also be evaluated using slight changes in the comet assay protocol [43–45]. Although DNA migration can be induced by a wide spectrum of DNA lesions, the standard protocol of the comet assay is not appropriate for detection of DNA damage by crosslinking agents in the form of DNA-DNA-interstrand crosslinks, DNA-DNA intrastrand crosslinks and DNA-protein crosslinks. It has been reported that crosslinking agents physically prevent DNA migration. In this case the results of the assay will be a combination of inducing *vs.* inhibiting effects, which may underrate induced genotoxicity [46]. To overcome this problem, an additional step should be introduced into the protocol such as cell irradiation to induce breaks before performing the comet assay;

the extent to which the tail formation expected from this radiation is decreased is a measure of the crosslinking effect [47].

Regarding the measurements of DNA oxidation damage, a modification incorporating a digestion of DNA with a lesion-specific enzyme makes it possible to measure oxidised pyrimidines and purines [43,44]. There are several enzymes used for the detection of oxidised DNA bases such as *Escherichia coli* endonuclease III (EndoIII) or formamidopyrimidine-DNA glycosylase (Fpg) and human 8-oxoguanine DNA glycosylase 1 (hOGG1) that catalyse the excision of numerous forms of DNA damage such as open ring forms of 7-methylguanine, 8-oxoguanine, 5-hydroxycytosine, 5-hydroxyuracil, DNA-containing formamidopyrimidine moieties etc. [21,43]. Such modifications may give a much more precise insight into the type of DNA damage induced.

Apart from enzymatic modifications of the assay, a combination of the comet assay with fluorescence *in situ* hybridization (FISH) enables the detection of specifically labelled DNA sequences of interest, including whole chromosomes. This combination has been applied for the detection of site-specific breaks in DNA regions relevant for the development of various diseases. In that way, Comet-FISH becomes a useful technique for the detection of overall and region-specific DNA damage and repair at the individual cell level [48–50]. Additionally, several modifications of the comet assay are also introduced for the evaluation of epigenetic changes [51–54] to measure modifications in the global DNA methylation pattern in individual cells under various growth conditions.

Not surprisingly in view of this numerous applications, the comet assay has gained worldwide acceptance as a reliable and sensitive tool in fundamental DNA damage research as well as in epidemiology and biomonitoring with several advantages compared to other genotoxicity tests. These advantages include its sensitivity for low DNA damage detection, small number of cells per sample and/or possibility of using both proliferating as well as non-proliferating cells. All of this coupled with low-costs, easy application and short performance time makes this particular assay relatively very “user friendly”. Although there are many advantages, there are also a few limitations of the assay, mainly related to type of DNA damage that cannot be detected using the comet assay such as aneugenic effects. Other limitations include variations in procedures between laboratories and in evaluation of the gained results [21,29,30,40,55]. Nevertheless, its advantages are far greater than the disadvantages making it very popular in genotoxicity studies using not only human but also animal models.

Since the comet assay has been used for the evaluation of DNA damage in various animal models worldwide, the present review intends to discuss the application of the assay through the whole animal kingdom, with Part I covering invertebrate species from protozoans up to echinoderms (Table 1). The comet assay is used in a variety of invertebrate species since they are regarded as interesting subjects in ecotoxicological research due to their significance in ecosystems. Although the comet assay has been primarily used for genotoxicity assessment in marine and freshwater invertebrates, this was eventually extended to invertebrates inhabiting terrestrial ecosystems. A large number of species are nowadays included in comet assay assessments, including planarians, cnidarians, molluscs, annelids, arthropods and/or echinoderms. Besides the large number of species, the assay is also performed on a wide range of cell types including haemolymph, gills, digestive gland, and embryo cells. These cells have been used for the evaluation of a broad spectrum of genotoxic chemical and physical agents both *in vitro* and *in vivo*. Moreover, the paper will also examine the role of invertebrate species from an ecotoxicological point of view and will also discuss a comparison of the use of the comet assay in invertebrate and human models.

2. Protozoans

The comet assay in lower animals is done mainly on the protozoan *Tetrahymena thermophila*. *Tetrahymena* are unicellular, ciliated eukaryotes that live in fresh water in a wide range of conditions. This protozoan species is widely used in genetic studies due to its well characterized genome [21,56,57]. *Tetrahymena* has been validated as a model organism for the evaluation of DNA damage by a modified comet assay protocol using well known mutagens such as phenol, hydrogen peroxide, and formaldehyde, which exhibited concentration-dependent increases in DNA damage [58]. Afterwards, several materials were evaluated for genotoxic potential on *Tetrahymena* using the comet assay, such as influent and effluent water samples from a municipal wastewater treatment plant [58], water extracts from soil polluted with metals (Pb, Cd, and Zn) from a lead smelter [59], titanium dioxide particles [60], chlorophenols [61], chlorinated flame retardant [62] as well as melamine, a raw material used in the chemical industry [63]. Altogether, these results indicate that the comet assay employing *Tetrahymena* may be used as a cost-effective and reliable tool for genotoxicity assessments.

3. Platyhelminthes (Platodes)

Schistosoma mansoni, a water-borne parasite of humans with an intermediate molluscan host, has a complex life cycle in which it can be exposed to a subset of DNA-damaging agents, such as those that are present in the environment or the ones from the host immune responses. Using the comet assay, it was shown that DNA from adult worms can be damaged by different DNA-damaging agents such as tetramethylammonium chloride (TMA) and hydrogen peroxide [64].

4. Planarians

There are several studies using planarians for the assessment of DNA damage. Planarians are useful organisms for the evaluation of environmental genotoxicity because of their high sensitivity, low cost, high proliferative and regenerative rate and basal evolutionary position in relation to complex metazoans [65]. The comet assay was used to measure effects of the model toxicant methyl methanesulfonate (MMS) and copper sulphate, as well as for the environmental genotoxicity assessment of an urban stream, using the asexual mixoploid (2n/3n) *Girardia schubarti* [65,66]. The freshwater planarian *Polycelis felina* was used as an aquatic bioindicator species for the assessment of the herbicide norflurazon [67], while *Schmidtea mediterranea* was used to assess the genotoxic activity of tributyltin, an organometallic compound mainly used as a biocide in antifouling paints [68]. Based on these studies, it was concluded that planarians are suitable organisms for the *in vivo* detection of chemical genotoxicity in aquatic ecosystems.

5. Cnidarians

The comet assay was also applied to freshwater and marine cnidarian species both *in vitro* and *in vivo*. To optimize the comet assay for cnidarian cells and assess its utility for detecting genotoxic damage, cells were isolated from the North American pacific coast temperate sea anemone *Anthopleura elegantissima*. Several model toxicants were used, such as hydrogen peroxide, ethylmethanesulphonate (EMS) or benzo(a)pyrene (B[a]P) in order to evaluate the degree of DNA damage. Results have shown that in comparison to other marine species, anemone cells exhibited high background values of DNA strand breaks but despite that, these authors were able to observe dose responses for each of the studied chemicals with no reduction in cell viability. This first study demonstrated that anemone cells respond to

known DNA-damaging agents and that the DNA damage measured by the comet assay is a useful biomarker of stress in cnidarian species [69].

Afterwards, several studies were done using both freshwater as well as marine cnidarians for the assessment of environmental toxicants. The sea anemone *Actinia equinae* as a target organism was used for monitoring seawater genotoxicity using the comet assay. Water polluted with several polycyclic aromatic hydrocarbons (PAHs), including B[a]P, which requires the metabolism to exert its genotoxic effect, increased DNA damage in *A. equine* indicating also the capability of cnidarians for pollutant biotransformation [70]. Moreover, the *in vitro* effects of UV irradiation on three cellular compartments of the shallow water coral species *Stylophora pistillata* and scleractinian coral *Seriatopora hystrix* indicated sensitivity towards a physical agents as well [71,72]. The coral *Stylophora pistillata* was shown to be an indicator organism for the evaluation of pollution in the marine environment [73]. Copper and cobalt were used for the evaluation of heavy metal toxicity in both freshwater and marine cnidarians such as *Hydra magnipapillata* [74,75], the coral *Montastraea franksi* [76] and sea anemone *Bunodosoma cangicu* [77]. These organisms displayed significant sensitivity in regard to heavy metal toxicity indicating the use of cnidarians as model organisms for the risk assessment of heavy metal pollution in aquatic ecosystems.

6. Molluscs

The comet assay is done on a range of mollusc species, which includes bivalves, gastropods and cephalopods although the majority of studies are done on mussels and clams as they are regarded important pollution indicator organisms. Moreover, a variety of cells was used in those studies such as embryonic cells and spermatozoa as well as haemocytes, gill cells, hepatopancreas cells and digestive gland cells.

6.1. Bivalves

When it comes to using the comet assay for environmental risk assessments of water pollutants in invertebrates, bivalves are among the most studied marine organisms and there are hundreds of papers dealing with genotoxicity assessment using them as the model. Since genotoxicity assessments in bivalves using the comet assay have been reviewed in several

papers [21–24,33,78–80], here we will briefly review their role in the genetic and environmental toxicology.

Molluscs have long been regarded as the primary species in biomonitoring programmes involving aquatic ecosystems. Bivalves, in particular, receive special attention both as sentinel and toxicity-testing subjects, which can be seen in a large number of published data. Among these, mussels and clams have become one of the most important targets when researching marine genotoxicants using the comet assay owing to their worldwide distribution and known sensitivity to pollutants [22]. Studies were done on several cell types; from embryonic cells and spermatozoa to adult cells such as haemocytes, gill cells and digestive gland cells. Among many marine species, most of the studies were done on the blue mussel (*Mytilus edulis*) [81–88], Mediterranean mussel (*Mytilus galloprovincialis*) [89–100] and bay mussel (*Mytilus trossulus*) [101,102] although there are studies done on several other mussels such as the Asian green mussel (*Perna viridis*) [103–105], New Zealand green-lipped mussel (*Perna canaliculus*) [106], brown mussel (*Perna perna*) [107,108] as well as the hydrothermal vent mussel (*Bathymodiolus azoricus*) [109]. The comet assay was also done on several other species of oysters, scallops, shells and clams, namely the Pacific oyster (*Crassostrea gigas*) [110–114], eastern oyster (*Crassostrea virginica*) [115,116], marine rock oyster (*Saccostrea cucullata*) [117,118], Farrer's scallop (*Chlamys farreri*) [119,120], grooved carpet shell (*Ruditapes decussatus*) [99,121,122], peppery furrow shell (*Scrobicularia plana*) [123–125], pullet carpet shell (*Venerupis pullastra*) [94], bean clam (*Donax faba*) [126], manila clam (*Tapes semidecussatus*) [127,128], Pacific littleneck clam (*Protothaca staminea*) [101], backwater clam (*Meretrix casta*) [129], surf clam (*Spisula sachalinensis*) [130], short neck clam (*Paphia malabarica*) [131], common cockle (*Cerastoderma edule*) [84,94] and inequivalve ark (*Scapharca inaequivalvis*) [132,133]. The use of marine bivalves ranges from substance testing to monitoring of sediment and water bodies both *in situ* and *ex situ*. Research on the genotoxic effects of emerging pollutants, including nanomaterials, is also on the rise.

In freshwater environments, the zebra mussel (*Dreissena polymorpha*) is the most common bivalve for genotoxicity assessments using the comet assay [134–141]. Several other freshwater species are also used, such as the quagga mussel (*Dreissena bugensis*) [137], painter's mussel (*Unio pictorum*) [142–146], swollen river mussel (*Unio tumidus*) [142,143,145,147,148], freshwater mussel (*Unio tigridis*) [149], golden mussel (*Limnoperna fortunei*) [150,151], Chinese pond mussel (*Sinanodonta woodiana*) [143,152], Asian clam

(*Corbicula fluminea*) [153–155], *Lamellidens marginalis* [156] and paper pondshell (*Utterbackia imbecillis*) [157].

6.2. Gastropods

Several species of both freshwater and marine snails as well as terrestrial snails have been used for the assessment of DNA damage both *in vitro* and *in vivo*. The studies were done on several cell types from embryonic cells to adult cells such as haemocytes, gill cells, hepatopancreas cells and digestive gland cells. The majority of studies are on freshwater snails employing different species including *Lymnaea stagnalis* [158–161], *Lymnaea luteola* [162–165], *Biomphalaria glabrata* [166], *Biomphalaria alexandrina* [167,168], *Marisa cornuarietis* [169], *Potamopyrgus antipodarum* [170], *Bellamya aeruginosa* [171], *Pila globose* [172], *Viviparous bengalensis* [149] and *Heleobia cf. australis* [173]. In these studies, the effects of several environmental chemicals, insecticides and nanomaterials as well as the impact of radiation were evaluated in various cell types using the comet assay, yielding positive results indicating DNA-damaging effects. Studies were also done using marine gastropods, namely *Nerita chamaeleon* [174,175] and *Planaxis sulcatus* [176]. In these studies, the genotoxicity of cadmium chloride, mercuric chloride and PAHs on gill cells was investigated, showing a significant concentration-dependent increase compared to un-exposed snails. These studies demonstrated the usefulness of the comet assay for detection of DNA damage after exposure and the sensitivity of marine gastropods as a good candidate species for heavy metal pollution monitoring [174,176]. The South African abalone (*Haliotis midae*) was used for the evaluation of differential responses to low and high oxygen levels [177] and hydrogen peroxide [178]; a wide range of organic pollutants were studied with the common periwinkle (*Littorina littorea*) [179]; and the marine gastropod *Morula granulata* proved suitable for *in situ* evaluation of genotoxic contaminants in the coastal environment [180] including PAHs [181,182].

Studies have also been performed on terrestrial gastropods, such as garden snails *Helix aspersa* and *Helix vermiculata* [183–188] as well as *Bradybaena fruticum, Chondrula tridens, Cepaea vindobonensis,* and *Stenomphalia ravergieri* [189] living in a forest-steppe landscape. *H. aspersa* and *H. vermiculata* were used to validate the comet assay and test their suitability

as sentinels for detecting primary DNA damage in polluted environments [188]. Afterwards, several environmental pollutants [183,184,186] as well as UV irradiation [185] and exposure to *Nicotiana tabacum* leaves [187] were evaluated on these species indicating that the comet assay is an appropriate assay and *Helix spp.* populations are suitable sentinels to monitor the genotoxic impact of different pollutants.

6.3. Cephalopods

Although they are the least represented molluscs when it comes to DNA damage assessment using the comet assay, there are a few studies employing octopus and squid as animal models [190,191]. The alkaline comet assay has been employed to estimate basal DNA damage in the digestive gland, gills, kidney and gonads of *Octopus vulgaris* in regard to metal accumulation from contaminated sites. Elevated strand breakages were registered in the digestive gland, recognised for its ability to store and detoxify accumulated metals. In contrast, DNA damages in kidney, gills and gonads were lower, reflecting reduced metal accumulation or efficient detoxification [190].

7. Annelids

The comet assay has been applied to various annelids including polychaetes, oligochaetes, leeches and tardigrades, although the majority of studies were done on several species of earthworms.

7.1. Polychaetes

Since marine sediments are becoming increasingly contaminated by environmental pollutants with the potential to damage DNA, understanding genotoxic responses in sediment-dwelling marine organisms, such as polychaetes, is of increasing importance [192]. Consequently, several polychaete species have been used for the assessment of DNA-damaging effects on exposure to different pollutants, applying the comet assay to different cell types such as spermatozoa, coelomocytes, blood and intestinal cells. King ragworm (*Nereis virens*) and harbour ragworm (*Nereis diversicolor*) showed increased DNA damage upon exposure to PAHs and silver nanoparticles [192–196], while effects of nanoparticles

were also investigated in *Hediste diversicolor* [123,197]. *Capitella capitata*, including *Capitella* sp. S and *Capitella* sp. I, were used for the evaluation of PAHs such as fluoranthene indicating differences in PAH tolerance between *Capitella* species [193,198,199]. Several other species such as *Perinereis aibuhitensis* [200–202], *Perinereis cultrifera* [203], *Arenicola marina* [192,204–206] and *Laeonereis acuta* [207] have displayed DNA-damaging effects on exposure to marine sediments contaminated with PAHs, heavy metals and nanoparticles, with observed genotoxicity strongly dependent on cell type used.

7.2. Oligochaetes

The comet assay applied to oligochaete is a valuable tool for monitoring and detection of genotoxic compounds in terrestrial as well as aquatic ecosystems. Since they feed on the soil or sediment they live in, they are a good sentinel organism for ecogenotoxicology studies with the comet assay – a topic already extensively reviewed [21,22,33,208,209].

Verschaeve and Gilles [210] conducted a pilot study using the comet assay to assess the extent of DNA damage in coelomocytes of earthworms *Lumbricus terrestris* and *Eisenia fetida* exposed to X-rays and mitomycin C and/or maintained in different soil samples as an indicator of soil pollution. Later on, Di Marzio et al. [211] described an improved comet assay for detecting DNA damage in the coelomocytes of earthworms. In their study, extruded coelomocytes contained at least three types of cells, namely eleocytes, amoebocytes and granulocytes. The authors concluded that the comet assay using earthworm eleocytes appears to be a sensitive biomarker for evaluating exposure to genotoxic compounds.

Several species of earthworm were used for the assessment of DNA damage using the comet assay with the most used species being *Eisenia fetida* and *Eisenia andrei* and coelomocytes as the cells of choice. Besides coelomocytes as a somatic type of cells, there are also studies done on spermatogenic cells [212,213]. These species were used for the evaluation of several genotoxic agents present in soil and sediment [214,215] as well as for the genotoxicity of heavy metals [216], pesticides [217,218], radionuclides [219], peloids (natural muds) [220], flame retardants [221], naphthenic acid [222], nanomaterials [197,223,224], phthalates [225], PAHs [226] and organic compounds [227]. The DNA-damaging effects of both ionising [212,228] and non-ionising radiation [229] were also studied, indicating that both types of radiation are able to induce DNA damage and that the comet assay is a sensitive and rapid method for the detection of radiation-induced genotoxicity.

There are many other studies on terrestrial as well as aquatic oligochaete species such as *Eisenia hortensis* [230,231], several species of lumbricids (*Lumbricus terrestris*, *Lumbricus rubellus*, *Lumbricus castaneous*) [232–234], *Amynthas diffringens* [235], *Amynthas gracilis* [236], *Aporrectodea caliginosa* [235,237], *Branchiura sowerbyi* [148], *Dendrodrilus rubidus* [232,235], *Dichogaster curgensis* [238–240], *Limnodrilus udekemianus Claparede* [241], *Metaphire posthuma* [242], *Microchaetus benhami* [235], *Enchytraeus crypticus* [243] and *Pheretima peguana* [244]. Since some studies also showed differences in sensitivity between the tested species in response towards genotoxicants [197,232,235] special attention should be given when choosing appropriate species for biomonitoring studies in order to reduce both false positive and false negative results.

7.3. Leeches

A few studies have been done on both aquatic and medicinal leeches. To determine the association between exposure to a mixture of benzene, toluene, ethylbenzene and xylene (BTEX chemicals), and reproductive toxicity, the freshwater leech (*Limnatis nilotica*) was used as a model [245]. Results showed a dose-dependent increase in DNA damage in both the ovarian and testicular cells. Two species of medicinal leeches *Hirudo medicinalis* [246] and *Hirudo verbana* [247] were used for the assessment of the genotoxic potential of sulphate-rich surface waters as well as water and sediment contaminated by aluminium compounds. An increase in DNA damage was seen in the leeches' haemocytes. The effect on oogenesis due to chronic exposure to organic chemical compounds, including BTEX chemicals, was studied in the freshwater leech *Erpobdella johanssoni*; results revealed an induction of DNA damage in the ovaries of exposed organisms [248].

7.4. Tardigrades

Tardigrades (*Milnesium tardigradum*) have evolved with effective adaptations that protect them from environmental extremes, including radiation damage, preserving the integrity of DNA, cells and tissues in an anhydrobiotic state. The comet assay was therefore employed to study the effect of anhydrobiosis on DNA integrity; the DNA in storage cells was well protected during transition from the active into the anhydrobiotic state. It was also observed that the longer the anhydrobiotic phase lasted, the more damage was inflicted on DNA, probably by oxidative processes mediated by reactive oxygen species (ROS) [249].

8. Arthropods

Arthropods are a large group of invertebrates, which includes insects, arachnids, and crustaceans. They cover all ecological niches from the marine and fresh water to terrestrial environments and can be found in every continent. Therefore, arthropods are regarded as excellent bioindicator species and are used for the genotoxicity assessment of both physical and chemical agents as well as in environmental biomonitoring.

8.1. Hexapods

Although collembolans (springtails) have a high content of chitin, which hinders the mechanical or chemical digestion of the organisms, the comet assay was successfully applied to one of the most widely used soil organisms in ecotoxicological studies, *Folsomia candida*. Since collembolans have a high content of chitin, a new methodology was developed where the head of the collembolans was separated from the rest of the body, allowing the haemolymph to leak out. This procedure allows the cells to be released and after lysis the genetic material is available for the comet assay [250]. In the study, the genotoxic activities of cadmium and a representative of organophosphates, the insecticide dimethoate, were demonstrated, proving that collembolans are sensitive organisms that can be used in the assessment of hazard due to environmental pollution.

8.2. Crustaceans

Crustaceans form a large and very diverse arthropod taxon that includes crabs, lobsters, crayfish and shrimps. The comet assay was carried out in several crustacean species that populate both freshwater and marine environment. Widely distributed, crustaceans can be of very small size belonging to zooplanktonic communities, up to larger specimens, and are therefore suitable models for both genetic toxicology and environmental biomonitoring on a large scale.

Several freshwater zooplanktonic species were used for DNA damage assessment using the comet assay. Species such as the water flea (*Daphnia magna*), *Daphnia carinata* and *Ceriodaphnia dubia* are among the most used in toxicity assessment. In these studies, DNA damage was measured in cells from the haemolymph or in cell preparations from whole daphnides, exposed to various physical and chemical agents as well as to water pollutants

[251–254]. They were used for the evaluation of heavy metal toxicity [255], pesticides [256], pharmaceuticals [257,258] and landfill leachate [259] confirming the comet assay on daphnides as an early warning biomarker for effects of toxicants. Besides daphnides, several amphipod crustaceans have been used due to their importance in the food chain, namely *Gammarus fossarum* [260–263], *Gammarus elvirae* [264–267], *Gammarus balcanicus* [268], *Echinogammarus veneris* [267] and *Quadrivisio aff. lutzi* [269]. Their haemocytes, hepatopancreas cells, oocytes and spermatozoa were used to study the genotoxicity of freshwater ecosystems polluted with several heavy metals and oil as well as wastewater treatment plant effluents. The freshwater crayfish *Astacus leptodactylus* [270,271] and *Cambarellus montezumae* [272], the prawn *Macrobrachium rosenbergii* [273] and the shrimp *Macrobrachium nipponense* [274] were used for the assessment of various pesticides and polluted sites as well as different environmental stressors, such as temperature increase, air exposure, hypoxia and food deprivation.

Several species inhabiting mostly inland saltwater lakes, such as the brine shrimp *Artemia salina* [275] and *Artemia nauplii* [276], were used for the toxicity assessment of antimicrobial agents triclosan and triclocarban, and silver nanoparticles, respectively. Differential responses of the sexual *Artemia franciscana* and asexual *Artemia parthenogenetica* to genotoxicity by reference mutagens were found [277–279], pointing to the importance of considering life history traits and reproductive strategies in ecological risk assessments.

Besides freshwater species, several marine crustaceans were also used for the assessment of DNA integrity by the comet assay. Marine zooplankton species such as *Paracalanus parvus*, *Oithona rigida* and *Euterpina acutifrons* were used for the evaluation of different environmental stressors during four seasons (summer, pre-monsoon, monsoon and post-monsoon) [280]. UV-induced DNA damage and repair processes were studied in *Cyclops abyssorum tatricus* populations from clear and turbid alpine lakes [281]. Several species of shrimps and prawns important for aquaculture, such as the grass shrimp (*Palaemonetes pugio*) [282–289], white shrimp (*Litopenaeus vannamei*) [290–293], seabob shrimp (*Xiphopenaeus kroyeri*) [294], giant tiger prawn (*Penaeus monodon*) [295], decapod shrimp (*Palaemon varians*) [296] and marine prawn (*Palaemon serratus*) [297,298] were also extensively used for genotoxicity assessments. Their embryos, spermatozoa, haemocytes, hepatopancreas and gill cells were assayed for the possible genotoxic effects of heavy metals, coal combustion residues, phototoxicants, PAHs, UV radiation and other environmental stressors. Various species – *Acartia tonsa* [296], *Corophium volutator* [299], *Chasmagnathus*

granulata [300], *Callinectus sapidus* [301], *Carcinus maenas* [87], *Charybdis japonica* [302] and *Eriocheir sinensis* [303] – have been studied for DNA-damaging effects of UV irradiation and contaminants such as heavy metals, herbicides and oil.

8.3. Arachnids

The comet assay was carried out in several arachnid species, namely spiders and ticks. In females of the southern cattle tick (*Boophilus microplus*), the comet assay was used in order to better characterize the cell death process that eliminates unnecessary tissues after detachment from the host. There was a significant increase in DNA breakdown for salivary glands and ovaries during the preoviposition period, when compared with tissues dissected at the time of tick removal. In contrast, in synganglia, no significant variation in damage frequency was observed [304].

Several species of spiders have been used for the evaluation of DNA damage due to environmental stressors and food contaminants. The comet assay was used to assess the effects of two pesticides (acetamiprid and chlorpyrifos) on the DNA of the wolf spider (*Pardosa astrigera*); the amount of DNA damage due to pesticide exposure was higher in the abdomen haemocytes of *P. astrigera* compared to cephalothorax haemocytes [305]. The DNA-damaging effect of starvation and dimethoate (organophosphate insecticide) exposure was studied in female and male wolf spiders (*Xerolycosa nemoralis*) under laboratory conditions in haemocytes and midgut gland cells. In response to the two stressing factors, both cell types showed values higher in males than in females with greater levels of DNA damage in haemocytes than in midgut gland cells [306]. The findings provide valuable information on the potential risks of pesticides to spiders, which are natural enemies of agricultural pests. Moreover, the genotoxic effects of food contaminated with cadmium on haemocytes and midgut gland cells of web-building spiders (*Steatoda grossa*) showed significantly higher DNA-damaging effect under laboratory conditions, irrespective of sex. However, the severity of damage seemed to be sex- and internal organ-dependent [307].

8.4. Insects

The comet assay has only recently been adapted for the evaluation of DNA-damaging effects in insects. The first reports of its use in the fruit fly (*Drosophila melanogaster*) appeared in 2002 [308]. Since then, the interest in the application of the comet assay to insects

has been rapidly increasing, and several papers have given an extensive overview regarding the use of the comet assay on various insects, describing how to prepare a cell suspension, tackling the problem of differences and modifications in research protocols as well as describing various scientific fields where it can be used from a broad spectrum of toxicological and ecotoxicological research [21,25,209,309]. Thus, we will briefly review their role in genetic and environmental toxicology.

Insects could partially replace vertebrates in toxicological studies, avoiding the ethical issues related to this type of research. While the extrapolation of the data obtained in such models to higher animals could be problematic and sometimes impossible, nevertheless, there are many advantages that insects as a model can provide in this type of study such as inexpensive breeding that does not require much space or time, the possibility of large-scale experiments at a low cost and minimization of inter-individual variability leading to more reliable statistical analyses. As insects are the largest group of invertebrates, they can be widely utilized in both toxicological and ecotoxicological research [25].

The comet assay has been applied to several insect species belonging to various systematic groups and inhabiting different ecological niches. The most often used insect in DNA damage research is undoubtedly *D. melanogaster* [21,309–313], although there are studies conducted also on *Drosophila simulans* [314]. The presence of numerous repair deficient/efficient mutants of *D. melanogaster*, allows for the design of complex experimental models that can be used to understand DNA repair mechanisms [25] and *D. melanogaster* was successfully developed as a model organism in toxicological studies [315]; a new term "Drosophotoxicology" was proposed [316]. The comet assay has been performed mainly *in vivo* using different larval cell types derived from the brain, midgut, haemolymph, and imaginal disk. In addition, *in vitro* tests are also done using the *Drosophila* S2 cell line. The Drosophila comet assay has been used to analyse the genotoxicity and mechanisms of action of different chemicals with good sensitivity and reproducibility. Besides, it is the only assay that can be used to analyse DNA repair in somatic cells *in vivo*, comparing the effects of chemicals in different repair strains, and quantitating repair activities *in vitro*. Additionally, the Drosophila comet assay, both *in vivo* and *in vitro*, has been applied to study the influence of protein overexpression on genome integrity and degradation [309].

Insects other than Drosophila have also been widely studied in genotoxicity assessment studies. Short life span, easy maintenance, the production of a large number of offspring in a single generation and tissues with appropriate cell populations make them ideal for studies in developmental biology, diseases, genetics, genetic toxicology and stress

biology. Besides, their cosmopolitan presence makes them suitable candidates for ecological biomonitoring [317].

Up until now, several terrestrial species of insects including economically relevant species were examined using the comet assay [25]. These include Diptera – the American serpentine leafminer (*Liriomyza trifolii*) [318]; Coleoptera - chestnut weevil (*Curculio sikkimensis*) [319], maize weevil (*Sitophilus zeamais*) [320,321], yellow fever mosquito (*Aedes aegypti*) [322] and cigarette beetle (*Lasioderma serricorne*) [323]; Lepidoptera - Indian meal moth (*Plodia interpunctella*) [324], diamondback moth (*Plutella xylostella*) [325], gypsy moth (*Lymantria dispar*) [326], common Mormon (*Papilio polytes*) [327], Oriental leafworm moth (*Spodoptera litura*) [328], beet armyworm (*Spodoptera exigua*) [329], Mediterranean flour moth (*Ephestia kuehniella*) [330], cotton bollworm (*Helicoverpa armigera*) [331,332] and corn stalk borer (*Sesamia nonagrioides*) [333]; Orthoptera - common field grasshopper (*Chorthippus brunneus*) [334–336], bow-winged grasshopper (*Chorthippus biguttulus*) [337], grasshopper (*Aiolopus thalassinus*) [338], desert locust (*Schistocerca gregaria*) [339], cave crickets (*Dolichopoda laetitiae* and *D. geniculate*) [340], house cricket (*Acheta domesticus*) [341]; Hemiptera – red cotton stainer (*Dysdercus cingulatus*) [327], and Hymenoptera - black garden ant (*Lasius niger*) [342] and honeybee (*Apis mellifera*) [343,344]. Besides terrestrial species, several aquatic species were assayed as well. These include aquatic midges such as *Chironomus riparius* [345–350], *Chironomus kiiensis* [351], and *Chironomus tentans* [352]. It is expected that the number of insect species will rise with the application of the comet assay procedure in genotoxicity, especially since these animals are of great importance for humans in terms of agriculture and ecology. In the particular case of species as important as the honeybee (*A. mellifera*), whose populations are experiencing a significant decline, the comet assay could provide valuable information to help in understanding the potential negative effect of certain chemicals, such as pesticides, on their genome [25].

The comet assay was used on many species listed above for the assessment of DNA damage after irradiation, confirming that irradiation (electron beam and γ-rays) can cause DNA-damaging effects in economically important agricultural pests. DNA damage affects the ability of a pest to survive and reproduce and in that way may be considered as a tool for grain and vegetable disinfestations instead of resorting to chemical treatment. Insects have also been employed for the evaluation of effects on DNA integrity of a wide range of environmental pollutants, especially different agrochemicals. The assay can also be used to examine the impact of stress induced by starvation or extreme temperatures on DNA damage,

as well as the repair efficiency under limited energy conditions. Moreover, the assay could be used for studying the key phases of life, such as metamorphosis, moulting, diapause or quiescence; for investigating DNA damage in insects during extensive physical activity, aging mechanisms or DNA stability in relation to age and sex [25].

9. Echinoderms

Several species of echinoderms such as sea stars and urchins have been used for the assessment of DNA damage in the marine environment resulting from both physical and chemical agents. They are valuable organisms to study the relationship between DNA repair and resistance to genotoxic stress due to their history and use as ecotoxicological models, little evidence of senescence, and few reported cases of neoplasia [353]. The DNA-damaging effects of direct- and indirect-acting genotoxins such as hydrogen peroxide [87], MMS and cyclophosphamide [354] were evaluated in the coelomocyte of the sea star (*Asterias rubens*) exposed to a range of concentrations indicating a strong genotoxic effect. Apart from exposure to chemicals, the comet assay was also used as a marker of cell aging, to detect single- and double-stranded DNA damage in nuclei from coelomic epithelia cells in regenerating and intact arms of the *A. rubens*. Analysis of nuclear DNA damage showed a small but significant reduction in damage in coelomic epithelia preparations from regenerating arms, compared with those from normal arms indicating that the ‘‘new’’ arms do not form from ageing cells but rather from physiologically young cells [355].

In the coelomocytes of sea urchins (*Strongylocentrotus droebachiensis*) exposed to dispersed crude oil, a significant concentration-dependent increase in the percentage of DNA in comet tail was observed indicating that the comet assay can be used for biomonitoring of DNA damage in marine invertebrates following oil contamination [85]. It was also noted that ocean acidification increases copper toxicity in purple sea urchins (*Paracentrotus lividus*) where an increase in DNA damage was observed [86]. DNA strand breaks were increased in coelomocytes and sperm cells from *P. lividus* exposed to zinc oxide nanoparticles, common contaminants of marine environment via sunscreens lotion [356]. El-Bibany et al. [353] reported that coelomocytes from four echinoderm species (sea urchins *Lytechinus variegatus*, *Echinometra lucunter lucunter*, and *Tripneustes ventricosus*, and a sea cucumber *Isostichopus badionotus*) can repair both UV-C and hydrogen peroxide-induced DNA damage; however, differences in repair capacities between species were noticed.

Moreover, since gametes and embryos of broadcast spawners are exposed to a wide range of chemical and physical stressors, which may alone, or in conjunction, have serious consequences on reproductive outcomes, Mediterranean echinoid species, such as *P. lividus* and *Sphaerechinus granularis*, were chosen as models to study the genotoxicity of UV radiation on sea urchin eggs and spermatozoa. The results demonstrated that the genetic material of sea urchin eggs and sperm is susceptible to UV exposure, which can induce structural and chromatin damage, suggesting that UV-impairment of the genetic integrity of the eggs and sperm might have a role in post-fertilization failures and abnormal embryonic development [357,358]. Present studies indicate that the comet assay could be used for the routine screening of substances for genotoxicity in marine systems following environmental exposure.

10. Conclusions and future prospects

Since its first introduction in 1988 by Singh and colleagues [38], the use and the applications of the alkaline comet assay have dramatically increased. Its use in genetic toxicology, either *in vitro* or *in vivo*, has extended to both laboratory and field work, either aquatic or terrestrial. Invertebrates are a large group of animals and their application in genetic toxicology is also increasing. Hence, the comet assay is currently performed on a large number of animals including platyhelminthes, planarians, cnidarians, molluscs, annelids, arthropods and echinoderms, and these species are especially used in the field of ecotoxicology due to their significance in ecosystems.

A large number of new chemicals are synthesized each year and they can be regarded as potential emerging pollutants that may possess significant biological effects if and when released into the environment. The presence in the environment of biologically active and slowly degradable xenobiotics represents a degree of stress often unacceptable for living organisms and the entire ecosystem. Both direct and indirect toxic activities of such chemicals can be important risk factors not only for animals but for the human population as well. Therefore, for proper ecotoxicological testing it is necessary to use well-defined tests, in which a range of selected species representing the main trophic levels are exposed to a single pollutant or complex mixtures under controlled laboratory conditions. However, one should have in mind that the extrapolation of data obtained in such way does not always reflect the reality and/or severity of the situation. Another approach would be based on the use of native

species from designated areas, assessing the degree of toxicity and evaluating pollution levels after collection from the environment – bearing in mind that extrapolation is not always possible in terms of human exposure. Moreover, reproduction stress or stress caused by handling of animals could be important sources of stress, especially in wildlife populations. Intrinsic biological variations such as animal size, tissue specificity, biochemical and enzymatic responses related to growth and reproduction cycles have to be considered in biomonitoring programs in areas characterized by low or sub lethal concentrations of pollutants [359].

In this kind of assessment, the comet assay has become the method of choice, allowing a fast and efficient screening of a large number of physical and/or chemical agents on a variety of species, with invertebrates being more frequently used both *in vitro* and *in vivo*, as well as for the *in situ* evaluation of genotoxic threats. The comet assay presents several significant advantages over other commonly used genotoxicity assays. Not only is the assay applicable to both eukaryotic and prokaryotic organisms, but the other great achievement is that it can be done using almost any cell type, as can be verified from the literature reviewed in the present paper. For many reasons, namely scientific, practical and/or technical, blood/haemolymph is the most commonly used biological matrix; however, tissues and/or cells such as gills, liver, early larval stages, spermatocytes or coelomocytes have also been frequently used. Moreover, the data obtained by the comet assay can be gathered relatively quickly, are quite reliable and (to a certain extent) reproducible. The relatively high variability observed in some cases between laboratories as well as from experiment to experiment in the same laboratory has to be taken into account when interpreting the results, but it can be largely avoided if critical steps in the assay are recognised and standardized. These steps include agarose concentration, duration of alkaline incubation, and electrophoresis conditions (time, temperature, and voltage gradient); but even when they are controlled, some variation seems to be inevitable. In line with that, it is recommended to include in experiments reference standards, i.e., cells with a known amount of specific damage to the DNA in order to control variation both within one laboratory and between different laboratories [55,360]. Problems may also arise by using different species for genotoxicity assessments in complex environments, since there can be large inter-species, not to mention inter-individual differences. Therefore, the choice of the optimal species for a genotoxicity assessment based on the designated environmental conditions, as well as chemical and/or physical agents under evaluation, is crucial.

Obviously, there are several invertebrate species that are more commonly used in comparison with others, and especially relevant are those from the aquatic environment. Regarding the environmental risk assessment of water pollutants in invertebrates, bivalves are among the most studied organisms both in marine and freshwater environments. They have long been seen as primary species in biomonitoring programmes involving aquatic ecosystems both as sentinel and toxicity-testing subjects since they are filter-feeding organisms. Among them, mussels and clams have become one of the most important targets when researching genotoxicants using the comet assay owing to their worldwide distribution and known sensitivity to pollutants. The most assayed marine species are *Mytilus edulis* and *Mytilus galloprovincialis*, while in a freshwater environment *Dreissena polymorpha* is among the most studied ones. Moreover, since marine sediments are becoming increasingly contaminated by environmental pollutants with the potential to damage DNA, understanding genotoxic responses in sediment-dwelling marine organisms, such as polychaetes, is also receiving increasing attention. Regarding annelids, the comet assay applied to earthworms (oligochaetes) is also a valuable tool for the monitoring and detection of genotoxic compounds in both aquatic and terrestrial environments since they feed on the soil or sediment they live in. Among the most studied ones are certainly *Lumbricus terrestris* and *Eisenia fetida*.

Arthropods, especially crustaceans, are also one of the most used subgroups of invertebrates with respect to genotoxicity testing using the comet assay. They cover ecological niches from the marine to freshwater and terrestrial environments and are regarded as excellent bio-indicator species. Crustaceans are widely distributed, and range from very small members of zooplanktonic communities up to large specimens, which makes them suitable model organisms for both genetic toxicology and environmental biomonitoring on a large scale. Among the most studied are *Daphnia* and *Gammarus* species. Insects, relatively recently adopted for the evaluation of DNA-damaging effects with the comet assay [308], have also become an increasingly used model. Although the above-mentioned animals are more and more frequently used in toxicological studies and the comet assay is readily applied on them, it has to be remembered that extrapolation from data obtained in such models to higher animals, not to mention humans, could be problematic and sometimes impossible.

Regarding the need for standardization of the comet assay protocol to ensure more reliable results, this can be problematic, especially when using invertebrate species, in view of the large number of different protocols specifically designed – perhaps in a single laboratory – for use with a particular specie and/or cell type. Hence, the development of guidelines at least

for the common steps in the comet assay procedure should be addressed, and adherence to such guidelines should be encouraged. This is also critical issue if the assay itself is to be recognized as an efficient environmental monitoring tool and for its eventual incorporation into regulatory guidelines.

Conflict of interest

None declared.

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Table 1. The comet assay for the evaluation of DNA damage in animal models (invertebrates; from protozoans to echinoderms).

	Animal	Cell type	Type of study	Agent/Stressor	Concentration range	Parameters tested	Response	Reference
Protozoans	*Tetrahymena thermophila*	*T. thermophila* cells	*in vivo*	H_2O_2, phenol, formaldehyde, WW (heavy metals)	H_2O_2 (0.1, 0.2 and 0.5 mM), phenol (0.05, 0.1 and 0.2 mM), formaldehyde (0.05, 0.1 and 0.2 mM)	OTM	↑ (H_2O_2), ↑ (phenol), ↑ (formaldehyde), ↑ (WW)	[58]
		T. thermophila cells	*in vivo*	polluted water (heavy metals), H_2O_2 as PC	H_2O_2 (100 µM)	OTM	↑	[59]
		T. thermophila cells	*in vivo*	Dechlorane Plus	2.4, 12, 60, 300 and 1500 µg/L	% tail DNA, TM, OTM	↑ (≥ 300 µg/L)	[62]
		T. thermophila cells	*in vivo*	chlorophenol (2,4-DCP, 2,4,6-TCP and PCP)	1.2, 2.4 and 3.6 mg/L	OTM	↑, ↑, ↑ (≥ 2.4 mg/L)	[61][#]
		T. thermophila cells	*in vivo*	melamine	1, 2 and 4 g/L	VS, AU	↑ (≥ 2 g/L)	[63]
		free cells, cells embedded in gel or nuclei embedded in gel	acellular exposure, *in vitro*, *in vivo*	bulk-TiO_2, nano-TiO_2	0.1 and 100 µg/mL	% tail DNA	↑ (after alkaline lysis except for 100 µg/mL after acellular exposure)	[60]
Platyhelminthes (Platodes)	*Schistosoma mansoni*	*S. mansoni* cells (different cells)	*in vitro*	colchicin, TMA, H_2O_2	colchicin (50 µM), TMA (0.06%), H_2O_2 (50 µM)	TL, CD (ratio)	Ø (colchicin), ↑ (TMA), ↑ (H_2O_2)	[64]

Planarians	*Girardia schubarti*	*G. schubarti* cells	*in vivo*	urban pollution (polluted water), MMS as PC	Diluvio's Basin (Brazil), MMS (8×10^{-5} M)	VS, DI, DF	↑ (some sites), ↑ (MMS)	[65]
		G. schubarti cells (neoblasts, nerve, epidermal and fixed parenchyma cells)	*in vivo*	$CuSO_4$, MMS	$CuSO_4$ (1, 2, 3, 4 and 5×10^{-5} M), MMS (4, 8, 12 and 16×10^{-5} M)	VS, AU, DF, TL	↑ ($CuSo_4$, ≥ 3×10^{-5} M), ↑ (MMS)	[66]
	Polycelis felina	*P. felina* cells	*in vivo*	norflurazon	0.2 and 2 µM	TL, % tail DNA, TM	↑	[67]
	Schmidtea mediterranea	*S. mediterranea cells*	*in vivo*	tributyltin	0.25, 1 and 4 µg/L	AU	↑ (10^3 ng/L tin (Sn) only)	[68]
Cnidarians	*Anthopleura elegantissima*	isolated aposymbiotic *A. elegantissima* cells	*in vitro*	H_2O_2, EMS, B[a]P	H_2O_2 (50, 100 and 200 uM), EMS (50, 100 and 200 µg/mL), B[a]P (50, 100 and 200 µM)	TL, % tail DNA, TM	↑ (H_2O_2; 200 µM), ↑ (EMS; ≥ 100 µg/mL), ↑ (B[a]P; ≥ 100 µM)	[69]
	Actinia equine	cellular suspension (cells from a single foot fragment)	*in vivo*	polluted water (PAHs), ENU, B[a]P	Genova (Italy), ENU (200 ppm), B[a]P (300 ppm)	TL, TM	↑ polluted water, ↑ ENU, ↑ B[a]P	[70]
	Bunodosoma cangicum	cell suspension (explants of pedal disk tissue fragments)	*in vitro*	$CuCl_2$	7.8 and 15.6 µg/L	TL, % tail DNA, TM	↑	[77]

	Stylophora pistillata	cell suspension (animal cells, algal cells, holobiont entities)	*in vitro*	UVB	4.05, 8.1 and 12.2 kJ/m^2	TE, TEM	↑ (different cell response - holobiont entity more sensitive)	[71]
		S. pistillata cells	*in situ, ex situ*	crude oil, phosphate dust	500 ppm	VS	↑	[73]
	Seriatopora hystrix	*S. hystrix* cells	*in vitro*	UVA, UVB	UVB (3.55 W/s), UVA (8.09 W/s)	TEM	↑	[72]
	Montastraea franksi	*M. franksi* cells	*in vivo*	Cu_2SO_4	1, 8 and 30 µg/L	TD	↑ (30 µg/L)	[76]
	Hydra magnipapillata	*Hydra* cells	*in vivo*	$CuSO_4$	0.06 and 0.1 mg/L	VS, TL	↑	[74]
		Hydra cells	*in vivo*	$CoCl_2$	8 and 16 mg/L	VS, % tail DNA	↑	[75]
Molluscs								
Bivalves	*Mytilus edulis**	haemocytes	*in vivo*	radioactive particles	137Cs, 241Am, 90Sr/90Y	% tail DNA	↑ (dependent on the particle)	[82]
		haemocytes	*in vitro*	fluoxetine, paroxetine, venlafaxine, carbamazepine, sulfamethoxazole, trimethoprim, erythromycin, DMSO as PC	0.001 mg/L - 150 mg/L	VS, AU	↑ (V ≥ 15 mg/L, P ≥ 0.0015 mg/L, F ≥ 10 mg/L, T ≥ 0.2 mg/L and E ≥ 100 mg/L), Ø (C and S)	[83]
		haemocytes	*in situ*	polluted sediment (heavy metals)	Tamar Estuary, South West England (UK)	TL, TM	↑ (dependent on the site)	[84]

		haemocytes	*in vivo*	Cu, ocean acidification	effect of ocean acidification (pH 7.71, pCO_2 1480 µatm) on Cu toxicity (~0.1 µM)	% tail DNA	↑ (combined exposure)	[86]
		haemocytes	*in vivo*	crude oil	0.015, 0.06 and 0.25 mg/L	% tail DNA	↑	[85]
		haemocytes	*in vitro*	Ag_2S and CdS nanoparticles, MMS as PC	0.01, 0.1, 1 and 10 mg/L	% tail DNA	↑ (≥ 10 mg/L)	[88]
		haemocytes, gill cells	*in vitro, in vivo, in situ*	MMS, (UV and H_2O_2 as PC), polluted area	H_2O_2 (22.5, 45 and 90 µM, *in vitro*), UV (253.7 nm, 15 W, 33 cm, *in vitro*), MMS (0.01 - 2 mg/L, *in vitro*), MMS (1 - 33 mg/L, *in vivo*/gills), MMS (1 - 33 mg/L, *in vivo*, haemocytes)	TM	↑ (H_2O_2, dose response), ↑ (UV, dose response, except the highest), ↑ (MMS, *in vitro*, ≥ 0.01 mg/L), ↑ (MMS, *in vivo*, gills, ≥ 1 mg/L), ↑ (MMS, *in vivo*, haemocytes, ≥ 1 mg/L), ↑ (polluted area, dependent on the site)	[81]
		haemocytes, coelomocytes	*ex vivo*	H_2O_2, reference sites	25 and 250 µM	% tail DNA	↑	[87]

	*Mytilus galloprovincialis**	haemocytes	*in vivo*	QDs, CdTe QDs, $Cd(NO_3)_2$, H_2O_2	CdTe QDs, $Cd(NO_3)_2$ at 10 µg/L	% tail DNA, VS	↑	[89]
		haemocytes	*in vitro*	diclofenac	5 and 10 ng/L	% tail DNA, TM, OTM	↑ (10 ng/L)	[98]
		haemocytes	*in vivo*	TBT, B[a]P as PC	TBT (10, 100 and 1000 µg/L), B[a]P (50 µg/L)	% tail DNA, HH	↑ (TBT ≥ 10 µg/L), ↑ (B[a]P)	[95]
		haemolymph	*ex situ*	superdispersant-25 (S-25), diesel oil, dispersed diesel oil mixtures, $CdCl_2$ as PC	diesel oil (100 µL/L and 1 mL/L), S-25 (5 and 50 µL/L), dispersed diesel oil mixtures M1 (diesel oil 100 µL/L + S-25 5 µL/L) and M2 (diesel oil 1 mL/L + S-25 50 µL/L), $CdCl_2$ 40 µM	% tail DNA, ACS, HDC, HH	↑ (S-25), Ø (diesel oil alone), ↑ ($CdCl_2$)	[96]
		haemolymph	*in situ*	environmental pollution (metals: Cu, Zn, Cd, Ni, Pb and PAHs)	Ria Formosa lagoon (Portugal)	% tail DNA	↑	[97]
		haemocytes, gill cells	*in situ*	PAHs (in sediment)	Corcubión estuary (Spain)	% tail DNA	↑ (compared to reference site)	[92]

		haemocytes, gill cells	*in vitro*	CdS quantum dots (QDs), H_2O_2 as PC	ionic Cd (0.1, 0.25, 0.5, 1 and 2 mg Cd/L), bulk CdS (0.62, 1.25, 2.5, 5 and 10 mg Cd/L), CdS QDs (0.31, 0.62, 1.25, 2.5 and 5 mg Cd/L), H_2O_2 (50 µM)	% tail DNA	↑ (ionic Cd ≥ 1 mg Cd/L), ↑ (bulk CdS ≥ 10 mg Cd/L), ↑ (CdS QDs ≥ 2.5 mg Cd/L), ↑ (H_2O_2)	[100]
		gill cells	*in vitro*, *in vivo*	nanoparticles (TiO_2, 2,3,7,8-TCDD + mixture)	*in vitro* (TiO_2 (0.1 µg/mL), 2,3,7,8-TCDD (0.1 ng/mL)), *in vivo* (TiO_2 (100 µg/L), 2,3,7,8-TCDD (0.25 µg/L))	% tail DNA	Ø (*in vitro*, TiO_2), ↑ (*in vitro*, TCDD, mixture), Ø (*in vivo*)	[99]
		gill cells	*in vivo*	TiO_2, $CdCl_2$, + mixture	nano-TiO_2 and $CdCl_2$ at 0.1 mg/L (nominal conc. level)	% tail DNA	↑ Cd, Ø (TiO_2), ↓ (TiO_2 reduced Cd genotoxicity, Ø)	[90]
		gill cells	*in vivo*	pharmaceutical wastewater (antibiotic pollution)	Sidi Thabet city (Tunisia)	VS, TDD, % tail DNA	↑	[91]
		gill cells	*in situ*	dioxin-like compounds	Gulf of Follonica (Italy)	% tail DNA	Ø	[94]
		gills and digestive glands	*in vivo*	B[a]P	5, 50 and 100 µg/L	% tail DNA	↑	[93]
	Mytilus trossulus	haemocytes	*in situ*	PAHs	Exxon Valdez spill (Alaska, USA)	TM	↑	[101]

		gill and digestive gland cells	*in vivo*	CuO-NP, dissolved Cu	CuO-NP (0.02 mg/L), Cu^{2+} ($CuCl_2$ solution - 0.02 mg/L)	% tail DNA, GDI	↑ gill cells, Ø digestive gland cells	[102]
	Perna viridis	haemocytes	*in situ, ex situ, in vivo*	chlorination	0.2 and 0.5 mg/L (chlorine in lab)	% tail DNA	↑	[104]
		haemocytes	*in vivo*	carbamazepine, bisphenol A, atrazine, + mixture	low, medium and high concentrations	% tail DNA	↑ BPA, ↑ ATZ, Ø CBZ, ↑ mixture	[103]
		gill and hepatopancreatic cells	*in situ*	heavy metals	Ennore estuary (India)	HL, CL, TL, % head DNA, % tail DNA, TM, OTM	↑	[105]
	Perna canaliculus	haemocytes	*in vivo*	Cd	acute (2000 and 4000 µg/L Cd), subchronic (200 and 2000 µg/L Cd)	% tail DNA	↑ (subchronic exposure)	[106]
	Perna perna	haemocytes	*in vivo*	antifouling biocide (chlorothalonil)	0.1 and 10 µg/L	TL	Ø	[107]
		haemocytes	*in vivo*	offshore petroleum exploration	coastal zone (Brazil)	% tail DNA	↑ (dependent on the site)	[108]
	Bathymodiolus azoricus	haemocytes, gill cells	*in vitro, in vivo*	hydrostatic pressure change, H_2O_2, MMC	H_2O_2 (20, 40 and 60 µM), MMC (6, 12 and 60×10^{-6} M)	% tail DNA	↑, ↓ (with time)	[109]
	Crassostrea gigas	haemocytes	*in situ*	pesticides, heavy metals	Sinaloa and Sonora (Mexico)	VS, AU	↑ (dependent on the site)	[111]
		haemocytes	*in situ*	heavy metal pollution (Pb, Co, Ni, As, Cd, Zn, Fe, Cu)	Shandong Peninsula, Bohai Sea (China)	% tail DNA	↑	[112]

		haemocytes	*in vivo*	tritiated water (ionizing radiation), H_2O_2 as PC	0.9 and 13.8 MBq/L, H_2O_2 (10, 50 and 100 µM)	% tail DNA	↑	[113]
		larvae cells	*in vivo*	herbicide diuron (+ metabolites DCPMU, DCPU and 3,4-DCA)	0.002 to 2.5 ug/L	% tail DNA	↑	[110]
		embryos-larvae	*in vivo*	Cu, Cd, irgarol and metolachlor	Cu (0.1 µg/L), Cd (10 µg/L), irgarol and metolachlor (0.01 µg/L)	% tail DNA	↑	[114]
	Crassostrea virginica	hematocytes	*in situ*	pollution	Lavaca Bay (Texas, USA)	% tail DNA, TL, OTM, TotI	↑	[115]
		haemocytes	*in vivo*	atrazine	20 and 200 ppb (µg/L)	OTM	↑	[116]
	Saccostrea cucullata	gill	*in situ*	PAHs and PCBs	Arabian Sea coast, Goa (India)	% tail DNA	↑	[117]
		gill	*in vivo, in situ*	B[a]P (*in vivo*), PAHs and heavy metals (*in situ*)	B[a]P (2.5, 5, 10 and 20 µg/L, *in vivo*) and PAHs and heavy metals (Pb, Cd, Cu, Fe and Mn, *in situ*)	% tail DNA, DNA integrity	↑	[118]
	Chlamys farreri	digestive gland	*in vivo*	B[a]P	50 ng/L	% tail DNA, VS	↑	[119]
		haemocytes	*in vivo*	CuO nanoparticles	CuO NPs (NPtotal) and Cu^{2+}(NPion)	TM	↑	[120]

	Ruditapes decussatus	haemocytes, gill cells	*in vitro, in vivo*	OA	*in vitro* (exposing haemocytes to different concentrations of OA - 10, 50 and 100 nM) and *in vivo* (feeding clams with toxic dinoflagellate *P. lima* - the max OA body burden detected was 44.65 ng/g and 1452 ng/g for low- and high-OA *P. lima* cultures)	% tail DNA	↑ (*in vitro* ≥ 10 nM), ↑ (*in vivo*, dependent on the concentration of OA and cell type evaluated)	[122]
		gill cells	*in vivo*	PAHs	sediment and water samples	% tail DNA	↑ (dependent on the exposure time)	[121]
		haemolymph	*in situ*	environmental pollution (Cu, Zn, Cd, Ni and Pb and PAHs)	Ria Formosa lagoon (Portugal)	% tail DNA	↑	[97]
	Scrobicularia plana	gills, digestive glands	*in vivo*	silver nanoparticles	Ag at 10 µg/L in nanoparticulate (Ag NPs) or soluble salt ($AgNO_3$) forms	% tail DNA	↑	[123]

		haemocytes	*In vitro, in vivo*	H_2O_2, natural oestrogen 17β-oestradiol (E2) and synthetic (xeno)oestrogens (ethinyloestradiol (EE2) and nonylphenol (NP)), EMS	*in vitro* (H_2O_2 (10, 50 and 100 uM), E2 and EE2 (1, 10 and 100 ng/L, 1 and 10 µg/L), NP (1, 10 and 100 µg/L, 1 and 10 mg/L), *in vivo* (E2 (1, 10 and 100 ng/L, 1 µg/L) NP (1, 10 and 100 ng/L, 1 mg/L), EMS (32 µg/L))	% tail DNA, TL, OTM	↑ (*in vitro*, H_2O_2, E2 ≥100 ng/L, EE2 ≥1 µg/L, NP ≥100 µg/L; ↑ (*in vivo*, E2 1 µg/L, NP 1 mg/L)	[124]
		spermatozoa	*in vitro*	B[a]P	B[a]P (10 and 100 µg/L)	% tail DNA	↑	[125]
	Venerupis pullastra	haemocytes, gill cells	*in situ*	PAHs (in sediment)	Corcubión estuary (Spain)	% tail DNA	↑ (compared to reference site)	[92]
	Donax faba	gill, body, foot tissues	*in vivo*	chlorpyrifos, carbendazim	chlorpyrifos (79.08, 158.16, 316,32 and 1265.31 µg/L), carbendazim (52.65, 105.32, 210.65, 421.3 and 842.6 µg/L)	% tail DNA	↑ (dependent on the concentration and cell type)	[126]
	Tapes semidecussatus	haemocytes, gill cells, digestive gland	*in vivo*	polluted sediment	Douglas Estuary and Ballymacoda Estuary (Ireland)	TM	↑	[128]
	Tapes semidecussatus	haemocytes, gill cells, digestive gland	*in vivo*	polluted sediment	Cork Harbour and Ballymacoda Estuary (Ireland)	% tail DNA	↑	[127]

	Protothaca staminea	haemocytes	*in situ*	PAHs	Exxon Valdez spill (Alaska, USA)	TM	↑	[101]
	Meretrix casta	gill cells	*in situ*	pollution (petroleum hydrocarbons and trace metals)	Vasco and Palolem, Goa (India)	% tail DNA	↑ (compared to unpolluted site)	[129]
		haemolymph	*in vivo*	γ-radiation, EMS	γ-radiation (2, 4, 6, 8 and 10 Gy), EMS (18, 32 and 56 mg/L)	% tail DNA	↑, ↑	[131]
	Spisula sachalinensis	gills and digestive glands	*in vivo*	B[a]P, MNNG	0.005% of final concentration	TL	↑	[130]
	Paphia malabarica	haemolymph	*in vivo*	γ-radiation, EMS	γ-radiation (2, 4, 6, 8 and 10 Gy), EMS (18, 32 and 56 mg/L)	% tail DNA	↑, ↑	[131]
	Cerastoderma edule	haemocytes, gill cells	*in situ*	PAHs (in sediment)	Corcubión estuary (Spain)	% tail DNA	↑ (compared to reference site)	[92]
		hematocytes	*in situ*	heavy metals (sediment)	Tamar Estuary (England)	TL, TM	↑ (compared to reference site)	[84]
	Scapharca inaequivalvis	erythrocytes	*in vivo*	copper (Cu^{2+})	0.1 ppm	TL, % tail DNA, TM	↑	[132]
		erythrocytes	*in vitro*, *in vivo*	organotin compounds (MBTC, DBTC and TBTC)	10 µM of organotin compounds (*in vitro*), 50 ppb of TBTC (*in vivo*)	TL, % tail DNA	↑ (*in vitro*), ↑ (*in vivo*)	[133]
	*Dreissena polymorpha**	hematocytes	*in vivo*, *in situ*	pentachlorophenol (PCP), polluted sites	PCP (10, 80, 100, 150 µg/L), River Sava (Croatia)	TL, % tail DNA, TM	↑ (*in vivo*, PCP ≥ 80 µg/L), ↑ (*in situ*)	[140]

		hematocytes	*in situ*	polluted sites	River Sava (Croatia)	% tail DNA, TM	↑ (compared to reference site)	[141]
		hematocytes	*in vivo*	opioids (morphine)	0.05 and 0.5 µg/L	% tail DNA	↑ (0.5 µg/L)	[135]
		hematocytes	*in vivo*	antidepressants (fluoxetine, citalopram)	500 ng/L alone + mixture	% tail DNA	Ø	[138]
		hematocytes	*in vivo*	amphetamine	500 and 5000 ng/L	% tail DNA	↑ (5000 ng/L)	[134]
		gill cells	*in situ*	seasonal variations, PAHs	Seine River Basin (France)	% tail DNA	↑↓ (based on the season), ↑ (PAHs)	[136]
		gill cells	*in vivo*	B[a]P, Cd	B[a]P (7, 12 and 18 µg/L), Cd (3, 32 and 81 µg/L)	OTM, AU	↑	[139]
		gill cells	*in vivo*	polluted sediment	River Elbe in Dessau and River Havel in Havelberg (Germany)	% tail DNA, TME	Ø (specie differences)	[137]
	Dreissena bugensis	gill cells	*in vivo*	polluted sediment	River Elbe in Dessau and River Havel in Havelberg (Germany)	% tail DNA, TME	Ø (specie differences)	[137]
	Unio pictorum	hematocytes	*in vitro*, *in vivo*	5-FU, $CdCl_2$ as PC	*in vitro* (5-FU, 0.04, 0.4, 4 and 40 µM), *in vivo* (5-FU, 0.04, 0.4, 4, 40 and 100 µM), *in vitro* ($CdCl_2$, 100 µM), *in vivo* ($CdCl_2$, 4, 40 and 100 µM)	% tail DNA	↑ (*in vivo*, 5-FU ≥ 0.4 µM), Ø (*in vitro*), ↑ (*in vitro*, $CdCl_2$), ↑ (*in vivo*, $CdCl_2$ ≥ 40 µM)	[142]

		hematocytes	*in situ*	pollution	Danube River (Serbia)	% tail DNA	↑	[143]
		haemocytes	*in vivo*	metalloid and other trace element polluted river sediments	River Cecina (Italy)	% tail DNA	↑	[146]
		haemocytes	*in vitro, in vivo*	cytostatic drugs (ETO, VIN, CDDP)	ETO (4, 40, and 100 µM), VIN (0.004, 0.04, 0.4, and 4 µM), and CDDP (0.04, 0.4, and 4 µM)	% tail DNA	ETO (↑ *in vitro*, ≥ 4 µM, ↑ *in vivo*, ≥ 40 µM), VIN (Ø *in vitro*, ↑ *in vivo* ≥ 0.04 µM); CDDP (Ø *in vitro*, *in vivo*, ↑ after post-treatment with H_2O_2 (20 µM))	[145]
		hematocytes	*in situ*	polluted freshwaters	Sava and Drava River (Croatia)	% tail DNA	↑	[144]
	Unio tumidus	hematocytes	*in vitro, in vivo*	5-FU, $CdCl_2$ as PC	*in vitro* (5-FU, 0.04, 0.4, 4 and 40 µM), *in vivo* (5-FU, 0.04, 0.4, 4, 40 and 100 µM), *in vitro* ($CdCl_2$, 100 µM), *in vivo* ($CdCl_2$, 4, 40 and 100 µM)	% tail DNA	↑ (*in vivo*, 5-FU at 0.4 and 40 µM), Ø (*in vitro*), ↑ (*in vitro*, $CdCl_2$), ↑ (*in vivo*, $CdCl_2 \geq$ 40 µM)	[142]
		haemocytes	*in situ*	pollution (river water)	Sava River (Croatia)	OTM	↑	[148]

		hematocytes	*in situ*	pollution	Danube River (Serbia)	% tail DNA	↑	[143]
		haemocytes	*in vitro*, *in vivo*	cytostatic drugs (ETO, VIN, CDDP)	ETO (4, 40, and 100 µM), VIN (0.004, 0.04, 0.4, and 4 µM), and CDDP (0.04, 0.4, and 4 µM)	% tail DNA	ETO (↑ *in vitro*, ≥ 4 µM, ↑ *in vivo*, ≥ 40 µM), VIN (Ø *in vitro*, ↑ *in vivo*, ≥ 0.04 µM); CDDP (Ø *in vitro*, *in vivo*, ↑ after post-treatment with H_2O_2 (20 µM))	[145]
		digestive gland cells	*in vivo*	phenolic compounds (tannic, ellagic and gallic acid)	60, 200 and 500 µM	TM	↑ (≥ 60 µM)	[147]
	Unio tigridis	digestive gland cells	*in vivo*	domestic heating oil (DHO)	5.8, 8.7 and 17.4 mL/L	CL, TL, TM	↑ (≥ 8.7 mL/L)	[149]
	Limnoperna fortunei	hematocytes	*in vivo*	TiO_2-NP	1, 5, 10 and 50 µg/mL	% tail DNA, OTM	↑ (≥ 1 µg/mL)	[150]
		haemolymph cells	*in vitro*, *in vivo*	UV, PCP, $CuSO_4$, environmental sample	UVC (*in vitro*, 0.7, 2.5, 3.3, 4.5 and 5 J/m^2), PCP (*in vivo*, 10, 80, 100 and 150 µg/L), $CuSO_4$ (*in vivo*, 3.75, 7.5, 15 and 20 µg/mL), Diluvio stream (Brazil)	CL, VS, DI, DF	↑ (UVC ≥ 0.7 j/m^2, PCP ≥ 100 µg/L, $CuSO_4$ ≥ 3.75 µg/mL), ↑ (environmental sample)	[151]

	Sinanodonta woodiana	hematocytes	*in situ*	pollution, seasonal variations	Velika Morava River (Serbia)	OTM	↑	[152]
		hematocytes	*in situ*	pollution	Danube River (Serbia)	% tail DNA	↑	[143]
	Corbicula fluminea	hematocytes	*in situ*	landfill leachate discharge	Periquitos stream (Brazil)	VS	↑	[153]
		hematocytes	*in vivo*	ATZ, Roundup, + mixture	ATZ (2 and 10 ppb), RD (2 and 10 ppm), AZT+RD	VS	Ø (AZT and RD alone), ↑ (AZT+RD)	[154]
		haemocytes and gill cells	*in vivo*	Gasoline water-soluble fraction	diluted to 5%	CS	↑	[155]
	Lamellidens marginalis	gill cells	*in vivo*	organophosphate pesticide (monocrotohpos)	5.25 mg/L	OTM	↑	[156]
	Utterbackia imbecillis	hematocytes		Cu, ATZ, glyphosate, carbaryl, diazinon, 4-nitroquinoline as PC	Cu (3.12 and 6.30 µg/L), ATZ (11.28 and 22.55 mg/L), glyphosate (2.5 and 5 mg/L), carbaryl (0.88 and 1.75 mg/L), diazinon (0.28 and 0.55 mg/L)	TM	↑ (Cu ≥ 3.12 µg/L, ATZ at 22.55 mg/L, diazinon at 0.28 mg/L), Ø (glyphosate, carbaryl, 4-nitroquinoline)	[157]
Gastropods	*Lymnea stagnalis*	hematocytes	*in vivo*	sediment (heavy metals), PAHs, PCBs	740 mg Cu/kg, 1220 mg Zn/kg, PAHs (< 10 mg/kg), PCBs (< 0.6 mg/kg)	TM	↑ (dependent on the site)	[159]
		hematocytes	*in situ*	radiation	Chernobyl region (Ukraine)		↑	[158]#

		hematocytes	*in situ*	environmental pollution (heavy metals, Sr)	inlet of Pripyat River and Perstok Lake (Belarus)	TM, TL, % tail DNA, CDNA, CA	↑	[161]
	Lymnaea luteola	digestive gland cells	*in vivo*	ZnONPs	10, 21 and 32 µg/mL	% tail DNA, OTM	↑ (≥ 10 µg/mL)	[164]
		digestive gland cells	*in vivo*	AgNPs	4, 12 and 24 µg/L	% tail DNA, OTM	↑ (≥ 4 µg/L)	[163]
		hepatopancreas cells	*in vivo*	single walled carbon nanotubes (SWCNTs)	0.05, 0.15, 0.30 mg/L	% tail DNA, OTM	↑ (≥ 0.05 mg/L)	[162]
		haemocytes	*in vivo*	$Pb(NO_3)_2$	10, 20 and 40 µg/mL	% tail DNA, OTM	↑ (≥ 20 µg/mL)	[165]
	Biomphalaria glabrata	hematocytes	*in vivo*	γ-radiation (^{60}Co)	2.5, 5, 10 and 20 Gy	VS	↑ (≥ 2.5 Gy)	[166]
	Biomphalaria alexandrina	hematocytes	*in vivo*	Roundup (48% Glyphosate)	10 mg/L	VS	↑	[167]
		haemocytes	*in vivo*	insecticide Match	Match 5% EC (its active ingredient is lufenuron 5% EC)	OTM	↑	[168]
	Marisa cornuarietis	hatched embryos cells	*in vivo*	platinum ($PtCl_2$)	0.1, 1, 10, 50, 100 and 200 µg/L	TM	↑ (≥ 1 µg/L)	[169]
	Potamopyrgus antipodarum	embryonic cells, adult gill cells, whole neonate cells	*in vitro, in vivo*	H_2O_2, MMS, Cd, BPA	H_2O_2 (0.1, 1, 10 and 50 µM), MMS (1, 3 and 6 mg/L), (Cd, 1, 10 and 100 µg/L), BPA (2, 10 and 50 µg/L)	% tail DNA, TEM	↑ (H_2O_2 ≥ µM, MMS ≥ 1 mg/L, BPA ≥ 10 µg/L, Cd ≥1 µg/L)	[170]
	Bellamya aeruginosa	hepatopancreas cells	*in vivo*	ethylbenzene	5, 45, 100, 450 and 1000 µg/L	OTM	↑ (≥ 5 µg/L)	[171]
	Pila globosa	haemolymph cells	*in vivo*	composite tannery effluent	effluent treatment plant of Kolkata (India)	VS	↑	[172]

	Viviparous bengalensis		*in vivo*	domestic heating oil (DHO)	6.5, 9.7 and 19.5 mL/L	CL, TL, TM	↑ (≥ 6.5 mL/L)	[149]
	Heleobia cf. australis	*Heleobia cf. australis* cells	*in situ*	pollution (Cr, Pb)	Montevideo Bay and Laguna Garzón (Uruguay)	% tail DNA	↑ (compared to reference site)	[173]
	Nerita chamaeleon	gill cells	*in vitro, in vivo*	H_2O_2, $CdCl_2$	H_2O_2 (1, 10, 25 and 50 µM), $CdCl_2$ (10, 25, 50 and 75 µg/L)	% tail DNA, TL, OTM	↑ ($H_2O_2 \geq 1$ µM), ↑ ($CdCl_2 \geq 10$ µg/L)	[174]
		cells from soft tissue	*in situ*	pollution (PAHs)	Arambol, Anjuna, Sinquerim, Dona Paula, Velsao, Betul and Palolem, Goa (India)	% tail DNA	↑ (dependent on the site)	[175]
	Planaxis sulcatus	gill cells	*in vitro, in vivo*	H_2O_2, $HgCl_2$	*in vitro* H_2O_2 (1, 10, 20, 50 µM), *in vivo* $HgCl_2$ (10, 20, 50, and 100 µg/L)	% tail DNA, OTM	↑ ($H_2O_2 \geq 1$ µM), ↑ ($HgCl_2 \geq 10$ µg/L)	[176]
	Haliotis midae	hematocytes	*in vivo*	oxygen levels	low and high oxygen levels	% tail DNA, OTM	Ø (juveniles), ↑ (adult)	[177]
		haemolymph cells, germ cells (oocytes and sperm)	*in vivo*	H_2O_2	5.5 mmol/L	% tail DNA, OTM, DI	↑	[178]
	Littorina littorea	haemolymph cells	*in situ*	PAHs, OTCs, PCBs, OCPs	South coast of England (England)	% tail DNA	↑	[179]
	Morula granulata	gill cells	*in situ*	marine pollution	Goa (India)	HD, % tail DNA, OTM, TL	↑	[180]

		cells from soft tissues	*in vitro, in vivo*	H_2O_2, phenanthrene	H_2O_2 (1, 10, 25 and 50 µM), phenanthrene (10, 20, 50 and 100 µg/L)	% tail DNA	↑ (H_2O_2 ≥1 µM), ↑ (phenanthrene ≥ 25 µg/L)	[182]
		cells from soft tissues	*in vivo*	PAH (benzo(k)fluoranthene)	1, 10, 25 and 50 µg/L	% tail DNA	↑ (≥ 1 µg/L)	[181]
	Helix aspersa	haemolymph cells	*in vivo*	soil contaminated with mineral coal tailings (PAHs)	Charqueadas (Brazil)	VS, DI, DF	↑	[183]
		haemolymph cells	*in vivo*	PAHs	Porto Alegre (Brazil)	VS, DI, DF	↑ (dependent on the site)	[186]
		haemolymph cells	*in vivo*	coal waste (mineral coal tailings - coal pyrite tailings)	Santa Catarina Coal Basin (Brazil)	VS, DI, DF	↑	[184]
		haemolymph cells	*in vitro*	UVC	UVC 4.5 J/m^2	VS, DI, DF	↑	[185]
		haemolymph cells	*in vivo*	*Nicotiana tabacum* leaves	fed on tobacco leaves	VS, DI, DF	↑	[187]
		hematocytes	*in vitro, in situ*	validation study, H_2O_2, different sites (polluted (coal-fired power station) and reference)	H_2O_2 (75 and 150 µM), coal-fired power station (Italy)	TL, % tail DNA, TM	↑ (H_2O_2 ≥ 75 µM), ↑ (compared to reference site)	[188]
	Helix vermiculata	hematocytes	*in vitro, in situ*	validation study, H_2O_2, different sites (polluted (coal-fired power station) and reference)	H_2O_2 (75 and 150 µM), coal-fired power station (Italy)	TL, % tail DNA, TM	↑ (H_2O_2 ≥ 75 µM), ↑ (compared to reference site)	[188]

	Bradybaenafruticum	hepatopancreatic cells	*in situ*	habitat (biotope) differences	Mid Russian Upland (Belgorod fouling)	VS, DCI, % tail DNA, TM	↑ (dependent on the site and age)	[189]
	Chondrula tridens	hepatopancreatic cells	*in situ*	habitat (biotope) differences	Mid Russian Upland (Belgorod fouling)	VS, DCI, % tail DNA, TM	↑ (dependent on the site and age)	[189]
	Cepaea vindobonensis	hepatopancreatic cells	*in situ*	habitat (biotope) differences	Mid Russian Upland (Belgorod fouling)	VS, DCI, % tail DNA, TM	↑ (dependent on the site and age)	[189]
	Stenomphalia ravergieri	hepatopancreatic cells	*in situ*	habitat (biotope) differences	Mid Russian Upland (Belgorod fouling)	VS, DCI, % tail DNA, TM	↑ (dependent on the site and age)	[189]
Cephalopods	*Octopus vulgaris*	digestive gland cells, gill cells, kidney cells, gonad cells	*in situ*	heavy metals	Matosinhos and Olhao (Portugal)	% tail DNA	↑ (dependent on the site and cell type)	[190]
Annelids								
Polychaetes	*Nereis virens*	coelomocytes	*in vivo*	B[a]P, EMS, DMSO	B[a]P (0.3, 0.6, 10, 20, 35 and 45 mg/mL), EMS (12.1 mg/ml), DMSO (98.9%)	TL, TM	↑(B[a]P ≥ 45 mg/ml), ↑ (EMS), ↑ (DMSO)	[196]
		intestinal cells	*in vivo*	PAHs (Flu), H_2O_2 as PC	fed with *Capitella capitata* exposed to Flu	TEM	Ø (Flu), ↑ (H2O2)	[193]
		coelomocytes (eleocytes, amoebocytes, spermatozoa)	*in vitro, in vivo*	MMS, B[a]P	MMS (18, 32 and 52 mg/L), B[a]P (0.1, 1.0, and 10 mg/L)	% tail DNA	↑, Ø (specie and cell type dependent)	[192]

	Nereis diversicolor	coelomocytes	*in vivo*	Ag NPs, $AgNO_3$, UV as PC	1, 5, 10, 25, and 50 µg Ag/g dry weight sediment	TM, % tail DNA	↑ (≥ 25 µg Ag/g dry weight), ↑ (UV)	[194]
		coelomocytes	*in vivo*	AgNPs, $AgNO_3$, UV as PC	1, 5, 10, 25, 50 and 100 µg Ag/g dry weight sediment	TM, % tail DNA	↑ (≥ 25 µg Ag/g dry weight), ↑ (UV)	[194]
		coelomocytes (eleocytes, amoebocytes, spermatozoa)	*in vitro, in vivo*	MMS, B[a]P	MMS (18, 32 and 52 mg/L), B[a]P (0.1, 1.0, and 10 mg/L)	% tail DNA	↑, Ø (specie and cell type dependent)	[192]
	Hediste diversicolor	coelomocytes	*in vivo*	lipid-coated CdSe/ZnS quantum dots and $CdCl_2$	0.001, 0.01, 0.1 and 1 ng/g	OTM	↑ (≥ 0.001 ng/g)	[197]
		coelomic fluid (coelomocytes)	*in vivo*	AgNPs, H_2O_2 as PC (200 µM)	Ag at 10 µg/L in nanoparticulate (Ag NPs) or soluble salt ($AgNO_3$) forms	% tail DNA	↑	[123]
	Capitella capitata	cell suspension	*in vivo*	PAHs (Flu), H_2O_2 (differences in PAH tolerance between *Capitella* species)	21 and 26 g Flu/g dry weight	TEM	↑, Ø (dependent on the specie)	[198]
		cell suspension	*in vivo*	PAHs (Flu))	~30 mg Flu/g dry-weight sediment or 50 mg Flu/L seawater	VS, AU	↑	[199]

	Perinereis aibuhitensis	blood cells	*in vitro*	Cd, Pb, Pyrene, B[a]P, H_2O_2	Cd (0.001, 0.01, 0.1, 1 and 10 µg/L), Pb (0.01, 0.1, 1, 10 and 100 µg/L), Pyrene (0.001, 0.01, 0.1, 1 and 10 µg/L), B[a]P (0.0001, 0.001, 0.01, 0.1 and 1 µg/L), H_2O_2 (0.01, 0.1, 1 and 10 µM)	TM	↑ (Cd ≥ 0.1 µg/L), ↑ (Pb at 1 and 10 µg/L), ↑ (Pyrene ≥ 0.001 µg/L), ↑ (B[a]P ≥ 0.01 µg/L), ↑ (H_2O_2 ≥ 0.1 µM)	[200]#
		blood cells	*in vitro*	sediment extracts, PAHs, TOC	Masan Bay (Korea)	TM	↑	[201]
		coelomocytes	*in vivo*	$HgCl_2$	0.05 and 0.5 mg/L	% tail DNA	↑ (≥ 0.05 mg/L)	[202]
		coelomocytes	*in vitro*, *in vivo*	$Pb(NO_3)_2$, $CoCl_2$, H_2O_2 as PC	*in vitro* ($Pb(NO_3)_2$ 100, 300, and 500 µg/L), ($CoCl_2$ 100, 300, and 500 µg/L), (H_2O_2, 50 µg/L); *in vivo* ($Pb(NO_3)_2$, 100, 300, and 500 µg/L), ($CoCl_2$ 100, 500 and 1000 µg/L)	OTM, % tail DNA	↑ (*in vitro*, ≥ 100 µg/L), ↑ (H_2O_2), ↑ (*in vivo*, ≥ 100 µg/L)	[203]
	Arenicola marina	coelomocytes (eleocytes, amoebocytes, spermatozoa)	*in vitro*, *in vivo*	MMS, B[a]P	MMS (18, 32 and 52 mg/L), B[a]P (0.1, 1.0, and 10 mg/L)	% tail DNA	↑, Ø (specie and cell type dependent)	[204]

		coelomocytes	*in vivo*	oil-contaminated sediments, PAHs, PCBs, heavy metals	Bay of Algeciras and Galician Coast (Spain)	% tail DNA	↑	[205]
	Laeonereis acuta	cell suspension	*in vivo*	Cu	62.5 mg/L	VS, DS	↑, Ø (dependent on the body region)	[207]
Oligochaete	*Eisenia fetida**	coelomocytes	*in vivo*	soil pollution	illegal dumping ground	TL	↑	[210]
		coelomocytes (eleocytes, amoebocytes, granulocytes)	*in vitro*, *in vivo*	soil contamination (PAHs), H_2O_2, $CdCl_2$	*in vitro* (H_2O_2 37 - 300 µM, $CdCl_2$ 0.5, 5 and 50 µM), *in vivo* (contaminated soil)	HD, % tail DNA, TEM, OTM, TL, L/H	↑ ($H_2O_2 \geq 37$ µM), ↑ (CdCl*2* at 50 µM), ↑ (soil)	[211]
		coelomocytes, spermatogenic cells	*in vitro*, *in vivo*	γ-radiation, X-rays	*in vivo* (60Co γ-radiation (dose rates 0.18–43 mGy/h)), X-rays (41.9 Gy/h), *in vitro* (0.5, 1, 2, 3, 6 or 10 Gy X-rays)	% tail DNA	↑	[212]
		coelomocytes	*in vivo*	polluted river system (sediment samples)	Noyyal River (India)	TL, L/W	↑	[215]
		coelomocytes	*in vivo*	imidaclothiz	(0.3 and 1 mg/kg)	OTM	↑ (≥ 0.3 mg/kg)	[217]
		coelomocytes	*in vivo*	peloids (natural muds)	peloid samples (Kolop and Hevız (Hungary))	TM	↑ (dependent on the mud)	[220]
		coelomocytes	*in vivo*	Dechlorane Plus	0.1, 0.5, 6.25 and 12.5 mg/kg	% tail DNA, TL, OTM	↑ (≥ 0.1 mg/kg)	[221]

		coelomocytes	*in vivo*	naphthenic acids (NAs)	5, 10, 50 and 100 mg/kg dry weight	% tail DNA, OTM	↑ (≥ 10 mg/kg dry weight)	[222]
		coelomocytes	*in vivo*	zero valent iron nanoparticles (C-nZVI)	60, 150, 500 and 1500 mg/kg soil	% tail DNA, TL, OTM	↑ (≥ 150 mg/kg)	[223]
		coelomocytes	*in vivo*	di-n-butyl phthalate (DnBP)	1, 2.5, 5 and 10 mg DnBP/kg soil	TL, % tail DNA, TM, OTM	↑ (≥ 5mg/kg)	[225]
		coelomocytes	*in vivo*	Eucalyptus volatile organic compounds (VOCs)	octane, undecane, decane, 3-methyl heptane, 2,4-dimethyl heptane, 3,3-dimethyl octane, 2,2,4,6,6-pentamethyl heptane and 2,4-di tert butyl phenol	TL, % tail DNA, OTM	↑ (dependent on the compound)	[227]
		coelomocytes	*in vivo*	γ-radiation	1, 5, 10, 20, 30, 40 and 50 Gy	% tail DNA	↑ (≥ 5 Gy)	[228]
		coelomocytes	*in vivo*	radiofrequency electromagnetic field (RF-EMF)	900 MHz (field levels of 10, 23, 41 and 120 V/m)	% tail DNA	↑	[229]
		sperm	*in vivo*	arsenite	5, 10, 20, 40, and 80 mg As/kg	OTM	↑ (≥ 5 mg As/kg)	[213]
		coelomocytes	*in vivo*	$CdSO_4$	20 mg/L	% tail DNA	↑	[235]

		coelomocytes	*in vivo*	Cu, Cd and PCP	Cu (0,25, 0.75 and 2.25 µg/cm^2), Cd (1.32, 6.6 and 13.2 µg/cm^2), PCP (0.05, 0.125 and 0.25 µg/cm^2)	% tail DNA	↑ (Cu ≥ 2.25 µg/cm^2), ↑ (Cd ≥ 1.32 µg/cm^2), ↑ (PCP ≥ 0.125 µg/cm^2)	[237]
		coelomocytes	*in vivo*	lipid-coated CdSe/ZnS QDs and $CdCl_2$	0.001, 0.01, 0.1 and 1 ng/g	OTM	↑ (QDNs ≥ 0.1 ng/g), ↑ ($CdCl_2$ ≥ 0.01 ng/g)	[197]
	Eisenia andrei	coelomocytes	*in vivo*	soil pollution (petroleum hydrocarbon (PH))	520, 750, 1040, 1170, 1390 and 1450 mg hydrocarbons/kg soil	TL, OTM, TM, % tail DNA	↑	[214]
		coelomocytes	*in vivo*	Cd (Cd contaminated artificial soils)	10 and 100 µg/g	% tail DNA	Ø	[216]
		coelomocytes	*in vivo*	B[a]P, 2,3,7,8-tetrachloro-dibenzo-para-dioxin (TCDD) spiked soils	B[a]P (0.1, 10 and 50 ppm), TCDD (1×10^{-5}, 1×10^{-4} and 2×10^{-3} ppm)	% tail DNA	↑ (B[a]P ≥ 0.1 ppm), TCDD (1×10^{-5} ppm)	[226]
		coelomocytes	*in vivo*	soils pollution (heavy metals, radionuclides)	Cunha Baixa uranium mine (Portugal)	VS, AU	↑	[219]

		coelomocytes	*in vivo*	nanomaterial (inorganic ($TiSiO_4$), organic (nano-vesicles of sodium sodecyl sulphate/ didodecyl dimethylammonium bromide – SDS/DDAB)	$TiSO_4$ (197.5, 296.3, 444.4, 666.7 and 1000 mg/kg dw), SDS/DDAB (24.6, 370.4, 555.6, 833.3 and 1000 mg/kg dw)	VS, AU	↑ ($TiSO_4 \geq$ 444.4 mg/kg dw), ↑ (SDS/DDAB at 1000 mg/kg dw)	[224]
		coelomocytes	*in vivo*	triclosan and its transformation product methyl-triclosan	50 ng/g dry weight soil, nominal concentration	VS	Ø	[218]
	Eisenia hortensis	coelomocytes	*in vivo*	$CoCl_2$	113, 226 and 452 ppm	VS	↑ at 452 ppm	[230]
		coelomocytes	*in vivo*	iron oxide nanoparticles (IONPs) and ionic iron (Fe_2O_3)	100, 125, 200, 250 and 500 µg/mL	VS	↑	[231]
	Lumbricus terrestris	coelomocytes	*in vivo*	X-rays, MMC	X-rays (5, 10 and 15 cGy), MMC (12.5, 25 and 50 ng/mL)	TL	↑	[210]
		cell suspension (from coelomic fluid)	*in vivo*	soil pollution, As, heavy metals	former mine site of Devon Great Consols (UK) (203 to 9025mg/kg As), As (98, 183, 236, 324 and 436 mg/kg)	% tail DNA	↑ (As ≥ 98 mg/kg), ↑, ↓ (dependent on the soil and species)	[232]

		tissue homogenates	*in vivo*	Cd	10 mg/kg Cd in soli	% tail DNA	↑	[234]
	Lumbricus rubellus	cell suspension (from coelomic fluid)	*in vivo*	soil pollution, As, heavy metals	former mine site of Devon Great Consols (DGC, UK) (203 to 9025 mg/kg As), As (98, 183, 236, 324 and 436 mg/kg)	% tail DNA	↑ (As ≥ 98 mg/kg), ↑, Ø (dependent on the soil and species)	[232]
	Lumbricus castaneous	coelomocytes	*in vivo*	soil pollution, As, heavy metals, H_2O_2	former gold mine in Nova Scotia (Canada), As (880 to 2700 mg/kg), H_2O_2 (500 µM)	% tail DNA	↑ (dependent on the soil), ↑ H_2O_2	[233]
	Amynthas diffringens	coelomocytes	*in vivo*	$CdSO_4$	20 mg/L	% tail DNA	Ø	[235]
	Amynthas gracilis	coelomocytes	*in vivo*	livestock pollutants (heavy metals), H_2O_2 as PC	São Miguel Island (Azores, Portugal), H_2O_2 (50 mM)	VS, GDI	↑	[236]
	Aporrectodea caliginosa	coelomocytes	*in vivo*	$CdSO_4$	20 mg/L	% tail DNA	↑	[235]
		coelomocytes	*in vivo, in situ*	Cu, polluted sites	Cu (0,25, 0.75 and 2.25 µg/cm^2), Zagreb (Croatia)	% tail DNA	↑ (Cu ≥0.25 µg/cm^2), ↑ (dependent on the site)	[237]
	Branchiura sowerbyi	haemocytes, coelomocytes	*in situ*	pollution (river water)	Sava River (Serbia)	OTM	↑	[148]
	Dendrodrilus	coelomocytes	*in vivo*	$CdSO_4$	20 mg/L	% tail DNA	↑	[235]

	rubidus	cell suspension (from coelomic fluid)	*in vivo*	soil pollution, As, heavy metals	former mine site of Devon Great Consols (UK) (203 to 9025mg/kg As), As (98, 183, 236, 324 and 436 mg/kg)	% tail DNA	↑ (As ≥ 98 mg/kg), ↑, Ø (dependent on the soil and species)	[232]
		coelomocytes	*in vivo*	soil pollution, As, heavy metals, H_2O_2	former gold mine in Nova Scotia (Canada), As (880 to 2700 mg/kg), H_2O_2 (500 µM)	% tail DNA	↑ (dependent on the soil), ↑ H_2O_2	[233]
	Dichogaster curgensis	coelomocytes	*in vitro*, *in vivo*	Cr(VI), H_2O_2 as PC	Cr(VI) (1, 3,10, 30, 70 and 100 ppm), H_2O_2 (70.4 µM)	VS, AU	↑ (*in vitro*, Cr(VI) ≥1 ppm), ↑ (H_2O_2)	[240]
		coelomocytes	*in vivo*	fly ash, heavy metals	Nashik district, Maharashtra (India) (0–40 %, w/w)	OTM	↑	[239]
		coelomocytes	*in vivo*	fly ash, heavy metals	fly ash (40 %)	% tail DNA	↑	[238]
	Limnodrilus udekemianus Claparede	coelomocytes	*in vivo*	5-FU, ETO, $CdCl_2$	5-FU (0.004, 0.04, 0.4, 4 and 40 µM), ETO (0.004. 0.04, 0.4 and 4 µM), $CdCl_2$ (0.004, 0.04, 0.4, 4 and 40 µM)	% tail DNA	↑ (5-FU ≥ 0.004 µM), ↑ (ETO ≥ 0.04 µM), ↑ ($CdCl_2$ ≥ 0.004 µM)	[241]
	Metaphire posthuma	testis cells	*in vitro*	UV radiation, H_2O_2	UVC (2, 4 and 6 J/m^2), H_2O_2 (0-80 µM)	CM	↑ (UVC ≥2 J/m^2), ↑ H_2O_2	[242]

	Microchaetus benhami	coelomocytes	*in vivo*	$CdSO_4$	20 mg/L	% tail DNA	Ø	[235]
	Enchytraeus crypticus	cells from the whole organism	*in vivo*	silver nanomaterial (Ag NM300K), $AgNO_3$, H_2O_2 as PC	Ag NM300K (60, 170 and225 mg Ag/kg dw), $AgNO_3$ (45, 60 and 96 mg Ag/kg dw), H_2O_2 (75 µM)	VS, AU, GDI	↑	[243]
	Pheretima peguana	coelomocytes	*in vivo*	glyphosate, paraquat	glyphosate (0.02, 0.25, 2.51, 25.15 and 251.50 µg/cm^2), paraquat (39×10^{-5} to 10^{-1} µg/cm^2)	% tail DNA, TL, TM	Ø (glyphosate), ↑ (paraquat ≥ 39×10^{-4})	[244]
Leeches	*Limnatis nilotica*	ovarian cells, testicular cells	*in vivo*	oil-related environmental pollutants (BTEX)	BTEX (1.4 and 2.8 mg/L)	VS, AU	↑	[245]
	Hirudo verbana	haemocytes	*in vivo*	water, sediment pollution (Al compounds, heavy metals)	Lake Njivice (Krk, Croatia)	TL, % tail DNA, TM, AST	↑	[247]
	Hirudo medicinalis	haemocytes	*in vivo*	sulphate-rich surface waters (SO4), heavy metals	two sites near a gypsum factory, Knin (Croatia)	TL, % tail DNA, AST	↑	[246]
	Erpobdella johanssoni	ovary cells	*in vivo*	oil-related environmental pollutants (BETX)	BETX (25 µg/L)	VS, AU	↑	[248]
Tardigrades	*Milnesium tardigradum*	storage cells	*in vivo*	effect of anhydrobiosis, UVB radiation	UVB (20.75 J/s m^2 as PC)	% tail DNA	↑	[249]
Arthropods								

Hexapods	*Folsomia candida*	haemolymph	*in vivo*	Cd, dimethoate	Cd (13.42, 26.85 and 53.7 mg/kg), dimethoate (0.4, 0.8 and 1.6 mg/kg)	VS, AU, TotCS	↑ (Cd ≥ 26.85 mg/kg), ↑ (dimethoate ≥0.4 mg/kg)	[250]
Crustaceans	*Daphnia magna**	daphnid cells (neonates)	*in vivo*	$Na_2Cr_2O_7$, chrysoidine, B[a]P	chrysoidine (0.1, 0.5, 1, 2 and 3 µM), $Na_2Cr_2O_7$ (0.25, 0.5, 0.75, 1, 2, 3 and 4 µM), B[a]P + $Na_2Cr_2O_7$ (0.01 + 0.25, 0.05 + 0.5, 0.1 + 0.75 and 0.2 + 1 µM)	% tail DNA	Ø (non-statistically significant response)	[252]
		whole daphnias	*in vivo*	$CdCl_2$, $K_2Cr_2O_7$, lindane, PCP, EMS, 4-NQO, H_2O_2	LOEC	TL	↑	[251]
		whole neonates cells	*in vivo*	BAC	0.04, 0.4, 4, 40 and 400 ng/L	% tail DNA	↑ (≥ 0.4 ng/L)	[253]
		haemocytes (granulocytes, amoeboid cells)	*in vivo*	$CdCl_2$, H_2O_2	$CdCl_2$ (5, 10 and 20 µg Cd^{2+}/L), H_2O_2 (1, 2, 5 and 10 µM)	% tail DNA, VS	↑ (Cd ≥ 10 µg), ↑ (H_2O_2 ≥ 5 µM)	[255]
		daphnid cells	*in vivo*	triclosan, carbendazim, + mixture	triclosan (120, 160 and 206 µg/L), carbendazim (5, 20 and 25 µg/L)	VS	↑	[256]
		whole neonates cells	*in vivo*	DCF, IBP, NPX, H_2O_2 as PC	IBP (2.9 mg/L), NPX (0.018 mg/L), DCF (9.7 mg/L), H_2O_2 (10 µM)	% tail DNA	↑	[258]

		whole neonates cells	*in vivo*	5-FU, CDDP, ETO, DOX, IMA, CAP	5-FU (0.05, 0.5, 5, 50, 500 and 5000 µg/L), CDDP (0.001, 0.01, 0.1, 1, 10 and 100 µg/L), ETO (0.03, 3, 30, 300 and 3000 µg/L), DOX (0.002, 0.02, 0.2, 2 and 20 µg/L), IMA (0.2, 2, 20 and 200 µg/L), CAP (2.25, 22.5, 225 and 2250 µg/L)	% tail DNA	↑ (5-FU ≥ 0.5 µg/L), ↑ (CDDP ≥ 0.01 µg/L), ↑ (ETO ≥ 0.3 µg/L), ↑ (DOX ≥ 0.02 µg/L), ↑ (IMA ≥ 2 µg/L), ↑ (CAP ≥ 22.5 µg/L)	[257]
		daphnid somatic cells	*in vivo*	landfill leachate	Zabrze (Poland)	OTM	↑	[259]
	Daphnia carinata	whole neonates cells	*in vivo*	2, 4-dinitroanisole and its metabolites (DNAN), 2,4,6-trinitrotoluene (TNT)	DNAN (1, 8 and 15 mg/L), TNT (0.5, 1 and 2.5 mg/L)	% tail DNA, OTM	↑	[254]
	Ceriodaphnia dubia	whole neonates cells	*in vivo*	BAC	0.4, 4, 40, 400 and 4000 ng/L	% tail DNA	↑ (≥ 4 ng/L)	[253]

		whole neonates cells	*in vivo*	5-FU, CDDP, ETO, DOX, IMA, CAP	5-FU (0.006, 0.06, 0.6, 6 and 60 µg/L), CDDP (0.03, 0.3, 3, 30 and 300 µg/L), ETO (0.01, 0.1, 1, 10 and 100 µg/L), DOX (0.005, 0.05, 5 and 50 µg/L), IMA (0.03, 0.3, 3 and 30 µg/L), CAP (12, 1.2×10^2, 1.2×10^3, 1.2×10^4 and 1.2×10^5 µg/L)	% tail DNA	↑ (5-FU ≥ 0.06 µg/L), ↑ (CDDP ≥ 0.3 µg/L), ↑ (ETO ≥ 0.1 µg/L), ↑ (DOX ≥ 0.05 µg/L), ↑ (IMA ≥ 0.3 µg/L), ↑ (CAP ≥ 1.2×10^2 µg/L)	[257]
	Gammarus fossarum	haemocytes, oocytes, spermatozoa	*in vitro*, *in vivo*	MMS, environmental contaminants ($CdCl_2$, $K_2Cr_2O_7$, paraquat, AMPA and B[a]P)	*in vitro* (1, 2, 10 and 20 mmol/L), *in vivo* (4, 20 and 100 mol/L)	% tail DNA	↑ (*in vitro* ≥ 1 mmol/L), ↑ (*in vivo* ≥ 4 mol/L), ↑ ($K_2Cr_2O_7$, paraquat, AMPA)	[260]
		spermatozoa	*in vivo*	MMS	0.8, 2.4, 7 and 22 mg/L	% tail DNA	↑ (≥ 2.4 mg/L)	[263]
		spermatozoa	*in vivo*	temperature, conductivity		% tail DNA	Ø	[263]
		oocytes, spermatozoa	*in vivo*, *in situ*	MMS, $K_2Cr_2O_7$, WWTP	*in vivo* MMS (0.44, 2.2 and 11 mg/L) and $K_2Cr_2O_7$ (0.0625, 0.25 and 1 mg/L), *in situ* (Bourbre River and Bion River (France))	% tail DNA	↑	[261]

		spermatozoa	*in situ*	WWTP	Rhône-Alpes Rivers (France)	% tail DNA	↑	[262]
		haemocytes, oocytes	*in situ*	WWTP	Rhône-Alpes Rivers (France)	% tail DNA	Ø	[262]
	Gammarus elvirae	haemocytes	*in vivo*	As-contaminated freshwater (heavy metals)	Latium region (Italy)	TL, % tail DNAI, TM	↑	[264]
		haemocytes	*in vivo*	contaminated water (heavy metals)	Latium region (Italy)	% tail DNA	↑	[265]
		haemocytes	*in vivo*	As-contaminated freshwater	Latium region (Italy), As (5, 10 and 50 µ/L)	% tail DNA	↑ (≥ 5 µg/L)	[266]
		haemocytes, spermatozoa	*in vivo*	Hg, Pb	Hg (0.1, 0.5 and 1 µg/L), Pb (25, 50 and 100 µg/L)	% tail DNA	↑ (Hg ≥ 0.5 µg/L), ↑ (Pb ≥ 50 µg/L)	[267]
	Gammarus balcanicus	haemocytes	*in vivo*	gypsum mine water (heavy metals)	Kosovčica River (Croatia)	TL, % tail DNA, TM, Tail Migration	↑	[268]
	Echinogammarus veneris	haemocytes, spermatozoa	*in vivo*	Hg, Pb	Hg (0.1, 0.5 and 1 µg/L), Pb (25, 50 and 100 µg/L)	% tail DNA	↑ (Hg ≥ 0.5 µg/L), ↑ (Pb ≥ 50 µg/L)	[267]
	Quadrivisio aff. lutzi	haemocytes (granulocytes, adipohemocytes, plasmatocytes)	*in vivo*	water-soluble fraction of heavy oil, MMS	North Fluminense region (Rio de Janeiro, Brazil)	% tail DNA	↑	[269]
	Astacus leptodactylus	haemocytes	*in vivo*	environmental stressors	temperature increase, air exposure, food deprivation	% tail DNA	↑ (temp), Ø	[271]

		haemocytes	*in situ*	polluted sites (PAHs, mineral-oils, heavy metals)	Sava River (Zagreb, Sisak, Krapje (Croatia))	% tail DNA	↑	[270]
	Cambarellus montezumae	brain cells, hepatopancreas cells	*in vivo*	dieldrin, chlorpyrifos	0.05 and 0.5 mg/L	T/N index (length to width index)	↑	[272]
	Macrobrachium rosenbergii	spermatozoa	*in vivo*	TBT	1, 2 and 4 mg/L	TL, % tail DNAD, OTM	↑ (≥ 2 mg/L)	[273]
	Macrobrachium niponnense	gill cells	*in vivo*	acute hypoxia and reoxygenation	1.5±0.1 mg O_2/L	OTM	↑	[274]
	Artemia salina	coelomocytes	*in vivo*	TCS, TCC	TCS (171 µg/L), TCC (18 µg/L)	% tail DNA, OTM	↑	[275]
	Artemia nauplii	nauplii cells (cell suspension)	*in vivo*	AgNPs	2, 10 and 12 nM	HL, TL, CL, HD, % tail DNA, tail movement, OTM	↑	[276]
	Artemia franciscana, Artemia parthenogenetica	whole animal cells	*in vivo*	EMS (differential responses of sexual and asexual Artemia)	0.78, 1.01, 1.24 and 1.48 mM	% tail DNA	↑ (≥ 0.78 mM, differential responses)	[277]
	Paracalanus parvus	whole body cell suspension	*in situ*	environmental stressors (heavy metals)	Ennore estuary (India)	% tail DNA	↑	[280]
	Oithona rigida	whole body cell suspension	*in situ*	environmental stressors (heavy metals)	Ennore estuary (India)	% tail DNA	↑	[280]
	Euterpina acutifrons	whole body cell suspension	*in situ*	environmental stressors (heavy metals)	Ennore estuary (India)	% tail DNA	↑	[280]

	Cyclops abyssorum tatricus	whole body homogenate	*in vivo*	UV	84 J/m^2/min	% tail DNA	↑	[281]
	Palaemonetes pugio	embryo cells	*in vivo*	B[a]P, Cr(VI), H_2O_2	B[a]P (37.5, 75 and 225 nM), Cr(VI) (0.5, 1 and 2 µM), H_2O_2 (8.8, 17.7 and 44.2 µM)	% tail DNA	↑(B[a]P ≥ 37.5 nM), ↑ (Cr(VI) ≥ 0.5 µM), ↑ (H_2O_2 ≥ 8.8 µM)	[286]
		embryo cells	*in vivo*	UV, B[a]P, Cd, + mixture	B[a]P (0.2 µM), Cd (5 µM), UV (330 kJ/m^2)	% tail DNA	↑	[284]
		embryo cells	*in vivo*	MNQ, NQO	MNQ (1, 5, 10, 20 and 50 µM), NQO (1, 2, 3, 4 and 5 µM)	TM	↑ (MNQ ≥ 5 µM), ↑ (NQO ≥ 2 µM)	[285]
		embryo cells	*in vivo*	phototoxicants (solar exposure), chemicals (anthracene, pyrene, alpha-terthienyl, methylene blue)	solar exposure (2 h), anthracene (3 µg/L), pyrene (10 µg/L), alpha-terthienyl (50 µg/L), methylene blue (1000 µg/L)	TM	↑	[283]
		hepatopancreas cells	*in vivo*	coal combustion residues (CCR) (heavy metals), H_2O_2 as PC	H_2O_2 (25, 50 and 100 µM)	% tail DNA, TL, TM	↑ (CCR), ↑ (H_2O_2 ≥ 25 µM)	[287]
		embryo cells	*in vivo*	$CrCl_3$, Na_2CrO_4, $HgCl_2$, MNQ	$CrCl_3$ (1000 µg/L), Na_2CrO_4 (1000 µg/L), $HgCl_2$ (1 and 10 µg/L), MNQ (86, 172 and 430 µg/L)	TM	↑	[282]

		embryo cells	*in vivo*	highway runoff sediments (PAHs), sediments with coal fly ash (heavy metals)	estuary in Hilton Head (South Carolina, USA), coal fly ash from power plants in Augusta (GA, USA), Candiota, Rio Grande do Sul (Brazil)	% tail DNA	↑	[288]
		embryo cells	*in vivo*	brominated flame retardant PBDEs and UV-exposed PBDEs	PBDEs (5 and 50 µg/L), UV (270 w/m^2)	TM	↑	[289]
	Litopenaeus vannamei	haemocytes, hepatopancreas cells, gill cells	*in vivo*	Cd ($CdCl_2$)	4.25 and 8.50 µmol/L	OTM	↑	[290]
		hepatopancreas cells	*in vivo*	hypoxia (dissolved oxygen levels)	oxygen levels (6.5, 3.0 and 1.5 ppm) and then reoxygenated (6.5 ppm)	OTM	↑	[293]
		haemocytes	*in vivo*	low temperature stress	from 23±2 to 12±2 °C	OTM	↑	[292]
		haemocytes, hepatopancreas cells	*in vivo*	pH stress	pH 5.6, 7.4 and 9.3	OTM	↑	[291]
	Xiphopenaeus kroyeri	haemocytes	*in vivo*	B[a]P	100, 200, 400 and 800 µg/L	VS, DDI	↑	[294]

	Penaeus monodon	haemocytes	*in vitro*	heavy metals ($CdCl_2$ and $HgCl_2$), pesticides (malathion and monocrotophos)	$CdCl_2$ (140 mM), $HgCl_2$ (17 mM), malathion (60 mg/L), monocrotophos (186 mg/L)	% tail DNA, TL, TM, % cells with tail	↑	[295]
	Palaemon varians	larval and post larval stage cell suspension	*in vivo*	Cd	14, 27 and 54 µg/L	VS	↑	[296]
	Palaemon serratus	spermatozoa	*in vitro*	optimisation (H_2O_2, UVC, MMS)	UVC (13.3, 26.5 and 79.5 J/m^2), H_2O_2 (5, 25 and 100 µM), MMS (0.5, 1 and 5 mM)	VS, AU	↑	[297]
		sperm	*in situ*	abiotic factors (water temperature), environmental pollution	Seine Bay (Normandy, France)	VS, AU	↑	[298]
	Acartia tonsa	cell suspension, eggs	*in vivo*	Cd	0.59, 2.39 and 9.57 µg/L	VS	↑	[296]
	Corophium volutator	cell suspension	*in vivo*	sediment pollution (heavy metals)	West Inner Tees dredged material disposal location (UK)	% tail DNA	↑	[299]

	Chasmagnathus granulata	epidermis cells	*in vivo*	UVB	8.6 J/cm^2	VS	↑, Ø (dependent on the body region)	[300]
	Callinectus sapidus	haemocytes, hepatopancreas cells	*in vivo*	sediment pollution (oil, PAHs)	Mississippi River (Louisiana, USA)	% tail DNA, TL, OTM	Ø, ↓ (dependent on the site)	[301]
	Carcinus maenas	coelomocytes, haemocytes	*ex vivo*	H_2O_2	25 and 250 µM	% tail DNA	↑	[87]
	Charybdis japonica	gills, hepatopancreas	*in vivo*	Cd, $CdCl_2$	0.025 and 0.05 mg/L	% tail DNA	↑	[302]
	Eriocheir sinensis	haemocytes	*in vivo*	glyphosate	4.4, 9.8, 44 and 98 mg/L	% tail DNA, comet ratio	↑	[303]
Arachnids	*Boophilus microplus*	salivary gland cells, ovaries cells, synganglia cells	*in vivo*	cell death process		TL, AU	↑ (salivary gland cells, ovaries cells), Ø (synganglia)	[304]
	Pardosa astrigera	haemocytes	*in vivo*	acetamiprid, chlorpyrifos		TL, cells with tails	↑	[305]#
	Xerolycosa nemoralis	haemocytes, midgut gland cells	*in vivo*	starvation, dimethoate	dimethoate (0.16 µg/specimen/day)	% tail DNA, TL, OTM	↑ (sex and cell type dependent)	[306]
	Steatoda grossa	haemocytes, midgut gland cells	*in vivo*	Cd (contaminated food)	0.25 mM $CdCl_2$ fed *Drosophila hydei* flies	% tail DNA, TL, OTM	↑ (sex and cell type dependent)	[307]

Insects	*Drosophila melanogaster*	neuroblast cells from larvae	*in vivo*	MMS, EMS, ENU	MMS (0.5 and 1 mM), EMS (1 and 2 mM), ENU (0.5 and 1 mM)	TL, % tail DNA, TM, tailed cells	↑ MMS, EMS, ENU	[308]
		haemocytes and midgut cells from larvae	*in vivo*	4-ONE, 4-HHE, EMS	4-ONE, 4-HHE (0.01, 0.1, 0.5, and 1 mM), EMS (4 mM)	% tail DNA	↑ (4-ONE and 4-HHE ≥ 0.5 mM), ↑ (EMS)	[310]
		midgut	*in vivo*	plant extracts rich in phenolic compounds, EMS as PC	*Digitalis ferruginea* and *Digitalis lamarckii*, EMS (1 mM)	VS, CS	↑	[311]
		haemocytes (larvae and adults)	*in vivo*	acephate	5 µg/mL	TL, % tail DNA, TM	↑	[312]
		haemocytes	*in situ*	radioactive environment	Lajes Pintadas city (Brazil)	VS, DI, DF	↑	[313]
	Drosophila simulans	spermatocytes	*in vivo*	Wolbachia-infection (ROS)		VS, % tail DNA	↑	[314]
	Liriomyza trifolii	whole body cell suspension (adults)	*in vivo*	electron beam irradiation	30, 50, 70, 100, 150 and 200 Gy	tail migration, TL	↑	[318]
	Curculio sikkimensis	larvae cells	*in vivo*	electron beam irradiation	1 and 4 kGy	TL, TM, OTM, %DNA damage	↑	[319]
	Sitophilus zeamais	larvae, pupae and adults whole body cells	*in vivo*	γ-radiation	0.5 and 1 kGy	TD, TL, %DNA damage	↑	[320]
		adult cells	*in vivo*	γ-radiation	0.5 and 1 kGy	% tail DNA, OTM, TM, HD	↑	[321]

	Aedes aegypti	adult cells	*in vivo*	γ-radiation	1, 5, 10, 20, 30, 40 and 50 Gy	% tail DNA	↑ (≥ 5 Gy)	[322]
	Lasioderma serricorne	whole body cell suspension	*in vivo*	γ-radiation	1 kGy	TM, TotL, Ratio	↑	[323]
	Plodia interpunctella	larvae cells	*in vivo*	‘‘soft-electron’’ (low-energy electron)	170 kV	VS	↑	[324]
	Plutella xylostella	larvae cells	*in vivo*	electron beam irradiation	30, 50 and 100 Gy	TM	↑ (≥ 30 Gy)	[325]
	Lymantria dispar	haemocytes of larvae	*in vitro*	Cd	50 and 100 mg Cd/g dry food	% tail DNA	↑	[326]
	Spodoptera litura	whole body cell suspension (adults)	*in vivo*	electron beam irradiation	30, 50, 100, 150, 200 and 250	TM	↑	[328]
	Spodoptera exigua	hemocytes	*in vitro*	Cd, Cd+ H_2O_2	H_2O_2 (50 µM)	% tail DNA, TL, OTM	↑	[329]
	Ephestia kuehniella	larvae homogenate	*in vivo*	UV	254 and 365 nm	% tail DNA, TL	↑	[330]
	Helicoverpa armigera	adult cells	*in vivo*	γ-radiation (^{60}Co)	400 Gy	TM	↑	[331]
		3rd instar larva	*in vivo*	phytopesticidal formulations from pongam and neem oils, EMS as PC	5, 10, 15, and 20 ppm, EMS (5 mM)	TL, % tail DNA, TM	↑	[332]
	Sesamia nonagrioides	larvae, pupae and adults	*in vivo*	X-rays	50, 100, 150 and 200 Gy	CL, TL, TM	↑	[333]
	Papilio polytes	5th instar caterpillars	*in vivo*	γ-radiation	10, 30, 40, 50 and 70 Gy	TL, TM	↑ (≥ 30 Gy)	[327]

	Chorthippus brunneus	brain cells (neuroblasts)	*in situ, in vivo*	polluted site (heavy metals), zinc	Olkusz site, Poland, additional Zn (100 and 1000 µg Zn/g dry mass of sand)	% tail DNA, TL, OTM, VS	↑	[334]
		larvae cells (brain cells)	*in situ, in vivo*	site pollution (heavy metals), H_2O_2	Olkusz, Szopienice (Poland), H_2O_2 (20 µM)	% tail DNA, TL, OTM	↑	[335]
		brain cells (hatchlings)	*in vitro, in vivo*	paraquat	*in vitro* (10, 50 and 250 µM), *in vivo* (50, 250 and 1250 µM)	% tail DNA, TL, OTM	↑	[336]
	Schistocerca gregaria	hemocytes		Cd, Pb ($CdCl_2$, $PbCl_2$)	contaminated food with $CdCl_2$ and $PbCl_2$ (25 and 50 mg/kg)	TL, % tail DNA, TM	↑	[339]
	Dolichopoda laetitiae	haemocytes, brain cells	*in situ*	radioactive radon exposure	Six caves in Central Italy (221–26,000 Bq/m^3)	TL, % tail DNA, TM	↑	[340]
	Dolichopoda geniculate	haemocytes, brain cells	*in situ*	radioactive radon exposure	Six caves in Central Italy (221–26,000 Bq/m^3)	TL, % tail DNA, TM	↑	[340]
	Aiolopus thalassinus	brain, thoracic muscles and gut cells	*in situ*	atmospheric pollutants	Abu-Zaabal Company for Fertilizers and Chemical Industries (Egypt)	TL, % tail DNA, TM, OTM,	↑	[338]
	Chorthippus biguttulus	hemocytes	*in vitro*	dimethoate, H_2O_2 as PC	dimethoate (0.16 µg of active substance), H_2O_2 (50 µM)	% tail DNA	↑	[337]

	Dysdercus cingulatus	5th instar nymphs	*in vivo*	γ-radiation	10, 30, 40, 50 and 70 Gy	TL, TM	↑ (≥ 40 Gy)	[327]
	Acheta domesticus	haemocytes	*in vivo*	nanodiamonds	20 and 200 mg/g food	% tail DNA, TL, OTM	↑ (≥ 200 mg/g food)	[341]
	Lasius niger	head (brain) cells, leg cells	*in vivo*	age, caste (workers, queens)	lifespan differences	% tail DNA	Ø	[342]
	Apis mellifera	hypopharyngeal gland cells	*in vivo*	nurse and forager worker bees	modes of cell death	TL	↑, Ø	[343]
		larvae cells	*in vivo*	non-ionizing radiation	mobile phone radiofrequency (900 MHz and field levels of 10, 23, 41 and 120 V/m)	% tail DNA	↑ (modulated (80% AM 1 kHz sinus) field at 23 V/m)	[344]
	Chironomus riparius	larvae	*in vivo*	Cu, H_2O_2 as PC	Cu (0.05, 1 and 25 mg/L), H_2O_2 (20 mM)	% tail DNA, OTM	↑ (≥ 1 mg/L), ↑ (H_2O_2)	[349]
		4th instar larvae	*in vivo*	vinclozolin	20 and 200 µg/L	TA, OTM, TM, % tail DNA	↑	[350]
Echinoderms	*Asterias rubens*	coelomocytes	*in vivo*	MMS, CP	MMS (18, 32 and 56 mg/L), CP (18, 32 and 56 mg/L)	% tail DNA	↑ (MMS ≥ 18 mg/L), ↑ (CP ≥ 18 mg/L)	[354]
		coelomic epithelia cells (cells in intact and regenerating arm)	*in vivo*	aging process		% tail DNA	↑, ↓ (dependent on the cell type)	[355]
		coelomocytes, haemocytes	*in vitro*	H_2O_2	H_2O_2 (25 and 250 µM)	% tail DNA	↑ (≥ 25 µM)	[87]

	Strongylocentrotus droebachiensis	coelomocytes	*in vivo*	crude oil	0.06 and 0.25 mg/L dispersed crude oil	% tail DNA	↑ (≥ 0.06 mg/L)	[85]
	Paracentrotus lividus	coelomocytes	*in vivo*	Cu toxicity, ocean acidification (OA)	Cu (~0.1 µM), OA (pH 7.71; pCO2 1480 µatm)	% tail DNA	↑ (under OA compared to control conditions, pH 8.14; pCO2 470 µatm)	[86]
		coelomocytes, sperm cells	*in vivo*	ZnO NP	exposed through the diet to different sizes (100 and 14 nm) ZnONPs (1 and 10 mg Zn/kg ZnONPs 100 nm and 1 and 10 mg Zn/kg ZnONPs 14 nm)	% DN	↑	[356]
		eggs	*in vitro*	UV, H_2O_2	UV radiation (UVA fluence of 18.2 W/m^2 and UVB fluence of 2.1 W/m^2 for 60 min), H_2O_2 (250, 500 and 750 µM)	% tail DNA	↑ UV, ↑ (H_2O_2 ≥ 250 µM)	[357]
	Sphaerechinus granularis	eggs	*in vitro*	UV, H_2O_2	UV radiation (UVA fluence of 18.2 W/m^2 and UVB fluence of 2.1 W/m^2 for 60 min), H_2O_2 (250, 500 and 750 µM)	% tail DNA	↑ UV, ↑ (H_2O_2 ≥ 250 µM)	[357]

		spermatozoa	*in vitro*	UVB, H_2O_2	UVB radiation (2.2 and 5 kJ/m^2), H_2O_2 (100, 25, 500 and 1000 µM)	% tail DNA	↑ (UVB ≥ 2.2 kJ/m^2), ↑ (H_2O_2 ≥ 100 µM)	[358]
	Lytechinus variegatus	oelomocytes	*in vitro*	H_2O_2, UVC	H_2O_2 (0.1, 1, 10 and 100 mM) and UVC (2000, 4000, 6000, 8000 and 10000 J/m^2)	SSF	↑ (H_2O_2 and UVC)	[353]
	Echinometra lucunter	oelomocytes	*in vitro*	H_2O_2, UVC	H_2O_2 (0.1, 1, 10 and 100 mM) and UVC (2000, 4000, 6000, 8000 and 10000 J/m^2)	SSF	↑ (H_2O_2 and UVC)	[353]
	Tripneustes ventricosus	oelomocytes	*in vitro*	H_2O_2, UVC	H_2O_2 (0.1, 1, 10 and 100 mM) and UVC (2000, 4000, 6000, 8000 and 10000 J/m^2)	SSF	↑ (H_2O_2 and UVC)	[353]
	Isostichopus badionotus	oelomocytes	*in vitro*	H_2O_2, UVC	H_2O_2 (0.1, 1, 10 and 100 mM) and UVC (2000, 4000, 6000, 8000 and 10000 J/m^2)	SSF	↑ (H_2O_2 and UVC)	[353]

*, commonly used species (only few examples are given); #, non-English communication; ↑, significant increase; ↓, significant decrease; Ø, no effect; ≥, at and above; % tail DNA; ACS, atypically sized comets; AU, arbitrary units; CA, comet area; CD, cell diameter; CDNA, comet DNA; CL, comet length; CM, comet moment; CS, comet score; DF, damage frequency; DI, damage index; DN, damaged nuclei; GDI, genetic damage index; HDC, highly damaged comets; HH, hedgehogs; HL, head length; OTM, Olive tail moment; TA, tail area; TDD, total DNA damage; TE, tail extent; TEM, tail extent moment; TL, tail length; TM, tail moment; TME, tail moment extent; TotI, total intensity; VS, visual scoring; 4-HHE, 4-hydroxy-hexenal; 4-ONE, 4-oxo-2-nonenal; 5-FU, 5-fluorouracil; $AgNO_3$, silver nitrate; AMPH, amphetamine; ATZ, atrazine; BAC, benzalkonium chloride; B[a]P, benzo(a)pyrene; BAC, benzalkonium chloride; BPA, bisphenol A; BTEX, benzene, toluene, ethylbenzene and xylene; CBZ, carbamazepine; $CdCl_2$, cadmium chloride; CDDP, cisplatin; $Cd(NO_3)_2$, cadmium nitrate; $CdSO_4$, cadmium sulfate; CdTe, cadmium telluride ; CH4, methane; $CoCl_2$, cobalt chloride; CP, cyclophosphamide; $CrCl_3$, chromium(III) chloride; Cu_2SO_4, copper sulphate; $CuCl_2$, copper chloride; DBTC, dibutyltin-chloride; DCF, diclofenac; DMSO, dimethyl sulfoxide; DNAN, 2, 4-dinitroanisole; DOC,

dissolved organic carbon; EMS, ethylmethanesulphonate; ENU, N-ethyl-N-nitrosourea; ETO, etoposide; Flu, fluoranthene; H2O2, hydrogen peroxide; H2S, hydrogen sulphide; $HgCl_2$, mercuric chloride; IBP, ibuprofen; $K_2Cr_2O_7$, potassium dichromate; MBTC, monobutyltin-chloride; MMC, mitomycin C; MMS, methylmethanesulfonate; MNNG, N-methyl-N0-nitro-N-nitrosoguanidine; MNQ, 2-methyl-1,4-naphthoquinone; Na_2CrO_4, sodium chromate; $Na_2Cr_2O_7$, sodium dichromate; NP, nanoparticles; NPX, naproxen; NQO, 4-nitroquinoline-N-oxide; OA, okadaic acid; OCPs, organochlorine pesticides; OMW, olive mill waste; OTCs, organotin compounds; PAHs, polynuclear aromatic hydrocarbons; PBDEs, polybrominated diphenyl ethers; PC, positive control; PCB, polychlorinated biphenyl; PCP, pentachlorophenol; $Pd(NO_3)_2$, lead nitrate; QDs, quantum dots; QDs-Ind, quantum dots coated with indolicidin; RD, Roundup; ROS, reactive oxygen species; SDS/DDAB, sodium sodecyl sulfate/ didodecyl dimethylammonium bromide; SWCNTs, single walled carbon nanotubes; TBT, tributyltin chloride; TBTC, tributyltin-chloride; TCC, triclocarban; TCS, triclosan; TiO2, titanium dioxide; TMA, tetramethylammonium; TNT, 2, 4, 6-trinitrotoluene; TOC, total organic carbon; UV, ultra violet; VIN, vincristine; WW, wastewater; WWTP, wastewater treatment plant effluent; ZnO, zinc oxide