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

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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 and is applicable to both eukaryotic and prokaryotic organisms. Although it is mainly used on human cells, the assay has its application in evaluation of DNA damage in bacterial, 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, platodes, planarians, cnidarians, molluscs, annelids, arthropods and oligomers. 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 usage of an invertebrate model and their role from an ecotoxicological point of view will also be discussed as well as the comparison between the usage 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 model 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 imply 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 (Clancy, 2008; De Bont and van Larebeke, 2004; Helleday et al., 2014; Jackson and Bartek, 2009).

There are numerous methods available for the evaluation of DNA damage as well as its repair both *in vitro* and *in vivo* (Garcia-Sagredo, 2008; Mateuca et al., 2006). The most commonly used methods are Ames test (Mortelmans and Zeiger, 2000), alkaline elution assay (Swenberg, 1981), chromosome aberration assay (Natarajan, 2002), sister chromatid exchange (SCE) analysis (Wilson and Thompson, 2007), cytokinesis block micronucleus (CBMN) assay (Fenech, 2007; Fenech et al., 2011) and γ-H2AX test (Gerić et al., 2014; Valdiglesias et al., 2013). 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, visual scoring under the microscope and they often tend to be laborious and rather expensive. Because all of that, 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 was the comet assay, named after the "comet-like" appearance of the cells, which has immediately been widely accepted as a 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 cell. The assay itself can be applied on virtually any cell type derived from different organs and/or tissues and is applicable to both eukaryotic and prokaryotic organisms. 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 bacterial (Singh et al., 1999), plant (Lanier et al., 2015; Santos et al., 2015; Ventura et al., 2013) and animal (Augustyniak et al., 2016a; de Lapuente et al., 2015; Dhawan et al., 2009; Frenzilli and Lyons, 2013; Frenzilli et al., 2009) 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 (Azqueta and Collins, 2013; Collins, 2004; Collins et al., 2008; Cotelle and Férard, 1999; Dhawan et al., 2009; Fairbairn et al., 1995; Garaj-Vrhovac and Gajski, 2010; Gerić et al., 2018; Gunasekarana et al., 2015; McKelvey-Martin et al., 1993; Tice et al., 2000).

Ostling and Johanson (Ostling and Johanson, 1984) 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. Nevertheless, the used neutral conditions allowed only DNA double-strand break detection. Only afterwards was the assay done under alkaline conditions, which was introduced by Singh et al. (Singh et al., 1988) thereby also allowing both double- and single-strand breaks detection, in addition to the alkali labile sites (Singh, 2016).

The assay involves embedding cells into an agarose matrix followed by lysis in neutral or alkaline conditions. Afterwards the cells go through electrophoresis and are subsequently neutralized. For evaluation under fluorescent microscope, the cells are stained with different fluorescent intercalating agents to facilitate visualization and calculation of fluorescence to determine the extent of DNA damage. The concept behind the comet assay is that undamaged DNA remains in the "head" of the comet, while damaged DNA can travel through pores of the agarose gel due to electric field thus displaying a comet like appearance by creating a comet "tail". Therefore, the amount of DNA present in the comet tail corresponds to the actual DNA damage of a certain cell. Although comets can be scored visually and are afterwards classified into different classes according to their appearance that represents a certain amount of DNA damage, more popular and widely used is the 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 fluorescent microscope, which facilitates the evaluation of DNA damage (Collins, 2004; Kumaravel et al., 2009; McArt et al., 2009; Rojas et al., 1999; Singh et al., 1988; Tice et al., 2000).

Besides measuring single- and double-strand breaks and alkali labile sites, other DNA lesions such as DNA crosslinks and oxidative DNA damage can also be evaluated using slight

changes in the comet assay protocol (Collins, 2014, 2009; Wu and Jones, 2012). Although DNA migration can be induced by a wide spectrum of DNA lesions, the standard protocol of the comet assay is not always able to detect 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 slow down DNA migration making the evaluation of the results quite problematic in situations where crosslinks are induced along with other types of DNA damage. In this case the results of the assay will be a combination of inducing *vs.* inhibiting effects, which may underrate induced genotoxicity (Merk and Speit, 1999). 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 as to deliver a fixed level of random strand breaks to the genome (Spanswick et al., 2010).

As for oxidative DNA damage measurements, a modification incorporating a digestion of DNA with a lesion-specific enzymes makes it possible to measure oxidised pyrimidines and purines (Collins, 2014, 2009). There are several enzymes used for the detection of oxidise DNA damage such as *Escherichia coli* Endonuclease III (EndoIII) or formamidopyrimidine-DNA glycosylase (Fpg) and human 8-oxoguanine DNA glycosylase 1 (hOGG1) that catalyses the excision of the numerous forms of DNA damage such as open ring forms of 7-methylguanine, 8-oxoguanine, 5-hydroxycytosine, 5-hydroxyuracil, DNA-containing formamidopyrimidine moieties etc. (Collins, 2014; Dhawan et al., 2009). 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, the Comet-FISH becomes a useful technique for the detection of overall and region-specific DNA damage and repair at the individual cell level (Glei et al., 2009; Spivak et al., 2009).

Because of the above mentioned, 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. The advantages compared to some other assays 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 far more “user friendly” than the rest. 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 or epigenetic effects mostly related to indirect interactions with the DNA molecule. Other limitations include a rather small cell number, variabilities in the procedure between laboratories and evaluation of the gained results (Collins, 2004; Collins et al., 2014, 2008; Dhawan et al., 2009; McArt et al., 2009). 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 intents to discuss the application of the assay through the whole animal kingdom, with Part I covering invertebrate species from protozoans up to oligomers (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 both terrestrial and water ecosystems. A large numbers of species are nowadays included in comet assay assessments such as planarians, cnidarians, molluscs, annelids, arthropods and/or echinoderms. Besides a large number of animal species, the assay is also performed on a large number of cells 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 between the usage of the comet assay in an invertebrate and human model.

2. Protozoans

The comet assay in lower animals is done mainly on a 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 (Dhawan et al., 2009; Nanney and Simon, 2000; Ruehle et al., 2016). *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 (H_2O_2), and formaldehyde, which exhibited concentration-dependent increases in DNA damage (Lah et al., 2004). Afterwards, several toxic compounds were evaluated on *Tetrahymena* using the comet assay such as genotoxic potential of influent and effluent water samples from a

municipal wastewater treatment plant (Lah et al., 2004), water extracts from soil polluted with metals (Pb, Cd, and Zn) from a lead smelter (Vidic et al., 2009), titanium dioxide (TiO_2) particles (Rajapakse et al., 2013), chlorophenols (Li et al., 2014), chlorinated flame retardant (Dou et al., 2015) as well as melamine, a raw material used in the chemical industry (Li et al., 2015). Altogether, these results indicate that the comet assay employing *Tetrahymena* may be used as a cost-effective and reliable tool for genotoxicity assessments.

3. Platodes

Schistosoma mansoni has a complex life cycle, being exposed to a subset of DNA damaging agents, such as those present in the environment as well as to 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 H_2O_2 (Silva et al., 2007).

4. Planarians

There are several studies using planarians for the assessment of DNA damage. Planarians are useful organism for the evaluation of environmental genotoxicity because of their high sensitivity, low cost, high proliferative rate and basal evolutionary position in relation to complex metazoans (Prá et al., 2005). The comet assay was used for the assessment of the model toxicant methyl methanesulfonate (MMS), copper sulphate ($CuSO_4$) as well as for the environmental genotoxicity assessment of an urban stream in the asexual mixoploid (2n/3n) *Girardia schubarti* (Guecheva et al., 2001; Prá et al., 2005). Moreover, the freshwater planarian *Polycelis felina* was used as an aquatic bioindicator species for the assessment of the herbicide norflurazon (Horvat et al., 2005), while *Schmidtea mediterranea* was used to assess the genotoxic activity of tributyltin, an organometallic compound mainly used as a biocide in antifouling paints (Ofoegbu et al., 2016). 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 used for the assessment of DNA damage in 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 toxicant were used, such as H_2O_2, 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 comet assay may prove to be a useful biomarker of stress in cnidarian species (Mitchelmore and Hyatt, 2004). 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 the 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 (Carrozzino et al., 2004). 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 (Rinkevich et al., 2005; Svanfeldt et al., 2014). The coral *Stylophora pistillata* was shown to be a prognostic tool for the evaluation of pollution in the marine environment (Kteifan et al., 2017). Copper and cobalt were used for the evaluation of heavy metal toxicity in both freshwater and marine cnidarians such as *Hydra magnipapillata* (Zeeshan et al., 2017, 2016), the coral *Montastraea franksi* (Schwarz et al., 2013) and sea anemone *Bunodosoma cangicu* (Anjos et al., 2014). 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 (Cotelle and Férard, 1999; de Lapuente et al., 2015; Dhawan et al., 2009; Dixon et al., 2002; Frenzilli and Lyons, 2013; Frenzilli et al., 2009; Martins and Costa, 2015; Mitchelmore and Chipman, 1998), 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 (de Lapuente et al., 2015). 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*) (Dallas et al., 2013; Jaeschke et al., 2015; Lacaze et al., 2015; Lewis et al., 2016; Rank and Jensen, 2003; Sahlmann et al., 2017; Taban et al., 2004), Mediterranean mussel (*Mytilus galloprovincialis*) (Almeida et al., 2013; Banni et al., 2017; Della Torre et al., 2015; Fernández-Tajes et al., 2011; Guidi et al., 2018; Martinović et al., 2016, 2015; Rocha et al., 2014; Toufexi et al., 2016; Zouiten et al., 2016) and bay mussel (*Mytilus trossulus*) (Chelomin et al., 2017; Thomas et al., 2007) although there are studies done on several other mussels such as the Asian green mussel (*Perna viridis*) (Chavan et al., 2016; Juhel et al., 2017; Vasanthi et al., 2017), New Zealand green-lipped mussel (*Perna canaliculus*) (Chandurvelan et al., 2013), brown mussel (*Perna perna*) (Guerreiro et al., 2017) as well as the hydrothermal vent mussel (*Bathymodiolus azoricus*) (Dixon et al., 2004). The comet assay was also done on several other species ranging from oysters, scallops, shells and clams, namely the Pacific oyster (*Crassostrea gigas*) (Behrens et al., 2016; Devos et al., 2015; Mai et al., 2012; Vázquez-Boucard et al., 2014; Xie et al., 2016), eastern oyster (*Crassostrea virginica*) (Bissett et al., 2009; Christl et al., 2004),

marine rock oyster (*Saccostrea cucullata*) (Sarkar et al., 2017; Sarker et al., 2018), Farrer's scallop (*Chlamys farreri*) (Liu et al., 2014; X. Sun et al., 2017), grooved carpet shell (*Ruditapes decussatus*) (Almeida et al., 2013; Florez-Barros et al., 2011; Martins et al., 2013), peppery furrow shell (*Scrobicularia plana*) (Buffet et al., 2014; Châtel et al., 2017; Petridis et al., 2009), pullet carpet shell (*Venerupis pullastra*) (Fernández-Tajes et al., 2011), bean clam (*Donax faba*) (JanakiDevi et al., 2013), manila clam (*Tapes semidecussatus*) (Coughlan et al., 2002; Hartl et al., 2006), clam (*Protothaca staminea*) (Thomas et al., 2007), backwater clam (*Meretrix casta*) (D'costa et al., 2018), surf clam (*Spisula sachalinensis*) (Kim and Hyun, 2006), short neck clam (*Paphia malabarica*) (Praveen Kumar et al., 2014), common cockle (*Cerastoderma edule*) (Dallas et al., 2013; Fernández-Tajes et al., 2011) and inequivalve ark (*Scapharca inaequivalvis*) (Gabbianelli et al., 2006, 2003). 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 (Magni et al., 2017, 2016; Michel and Vincent-Hubert, 2015; Michel et al., 2013; Parolini et al., 2016; Schäfer et al., 2012). Several other freshwater species are also used, such as the quagga mussel (*Dreissena bugensis*) (Schäfer et al., 2012), painter's mussel (*Unio pictorum*) (Gačić et al., 2014; Guidi et al., 2017; Kolarević et al., 2016a, 2016b; Štambuk et al., 2009), swollen river mussel (*Unio tumidus*) (Aborgiba et al., 2016; Gačić et al., 2014; Kolarević et al., 2016a, 2016b; Labieniec et al., 2007), golden mussel (*Limnoperna fortunei*) (Girardello et al., 2016; Villela et al., 2006), Chinese pond mussel (*Sinanodonta woodiana*) (Kolarević et al., 2016b, 2013), Asian clam (*Corbicula fluminea*) (de Oliveira et al., 2016; dos Santos and Martinez, 2014; Fedato et al., 2010), *Lamellidens marginalis* (Mundhe et al., 2016) and paper pondshell (*Utterbackia imbecillis*) (Conners and Black, 2004).

6.2. Gastropods

Several species of both freshwater and marine snails as well as terrestrial snails were 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. Majority of the studies done are on the freshwater snails employing several different species such as *Lymnaea stagnalis* (Clément et al., 2004; A. Y. Koneva, 2013; Koneva, 2014; O. I. Koneva, 2013), *Lymnaea luteola* (Ali et al.,

2017, 2015, 2014, 2012), *Biomphalaria glabrata* (Grazeffe et al., 2008), *Biomphalaria alexandrina* (Ibrahim et al., 2018; Mohamed, 2011), *Marisa cornuarietis* (Osterauer et al., 2011), *Potamopyrgus antipodarum* (Vincent-Hubert et al., 2012), *Bellamya aeruginosa* (Zheng et al., 2013), *Pila globose* (Bhattacharya et al., 2016) and *Heleobia cf. Australis* (Villar et al., 2015). In these studies, several environmental chemicals, insecticides and nanomaterials as well as impact of radiation were evaluated using the comet assay yielding positive results, indicating the DNA damaging effects in different snail cells. Studies were also done using several marine gastropods, namely *Nerita chamaeleon* (J. Bhagat et al., 2017; Sarkar et al., 2015) and *Planaxis sulcatus* (Bhagat and Ingole, 2015). In these studies, the genotoxicity of cadmium chloride ($CdCl_2$), mercuric chloride ($HgCl_2$) 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 (Bhagat and Ingole, 2015; Sarkar et al., 2015). Moreover, the South African abalone (*Haliotis midae*) was used for the evaluation of differential responses to low and high oxygen levels (Vosloo et al., 2013) and H_2O_2 (Vosloo and Vosloo, 2017), whereas the common periwinkle (*Littorina littorea*) was used for the assessment of a wide range of organic pollutants (Noventa et al., 2011). The marine gastropod *Morula granulata* was used for *in situ* evaluation of genotoxic contaminants in the coastal environment (Sarkar et al., 2014) including PAHs (Bhagat et al., 2016; Jacky Bhagat et al., 2017).

Apart from water gastropods, studies were also done on terrestrial ones, such as garden snails *Helix aspersa* and *Helix vermiculata* (Angeletti et al., 2013; de Souza et al., 2015; Ianistcki et al., 2009; Leffa et al., 2010; Pereira et al., 2009; Silva et al., 2013) as well as *Bradybaena fruticum*, *Chondrula tridens*, *Cepaea vindobonensis*, and *Stenomphalia ravergieri* (Snegin, 2014) living in the forest-steppe landscape. *H. aspera* and *H. vermiculata* were used to validate the comet assay and test their suitability as sentinels for primary DNA damage in polluted environments (Angeletti et al., 2013). Afterwards, several environmental pollutants (de Souza et al., 2015; Ianistcki et al., 2009; Leffa et al., 2010) as well as UV irradiation (Pereira et al., 2009) and genotoxicity of *Nicotiana tabacum* leaves (Silva et al., 2013) were evaluated on these species indicating that the comet assay appears to be an appropriate assay and *Helix spp.* populations suitable sentinels to detect 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 few studies employing octopuses and squids as animal models for the evaluation of genome damage. 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. On the contrary, DNA damages in kidney, gills and gonads were lower, reflecting reduced metal accumulation or efficient detoxification (Raimundo et al., 2010). Apart from the effects of environmental pollutants on the DNA integrity of cephalopods, there are studies that tried to use the comet assay to investigate the possible impact of radiation treatment of seafood, such as squids, but were unsuccessful due to the non-isolation of DNA material (Khan et al., 2003).

7. Annelids

Different experiments were done using the comet assay on 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 therefore of increasing importance (Lewis and Galloway, 2008). Consequently, several polychaetes species were used for the assessment of DNA damaging effect upon exposure to different pollutants and the comet assay was done on several different cell types such as spermatozoa, coelomocytes, blood and intestinal cells. King ragworm (*Nereis virens*) and harbour ragworm (*N. diversicolor*) were used for the assessment of genotoxic risk of PAHs and silver nanoparticles (AgNPs) indicating increased DNA damage upon exposure (Cong et al., 2014, 2011; De Boeck and Kirsch-Volders, 1997; Lewis and Galloway, 2008; Palmqvist et al., 2006) while *Hediste diversicolor* was used for the assessment of nanoparticles (Buffet et al., 2014; Saez et al., 2015). Moreover, *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 (Bach

et al., 2005; Palmqvist et al., 2006, 2003). Several other species such as *Perinereis aibuhitensis* (Seo et al., 2005, 2008; L. J. Zhang et al., 2017), *Perinereis cultrifera* (Singh et al., 2017), *Arenicola marina* (Lewis and Galloway, 2009, 2008; Morales-Caselles et al., 2009) and *Laeonereis acuta* (Ferreira-Cravo et al., 2009) displayed a DNA damaging effect upon exposure to marine sediments contaminated with PAHs and heavy metals with observed genotoxicity strongly dependent on cell type used.

7.2. Oligochaete

The comet assay applied to earthworms is a valuable tool for monitoring and detection of genotoxic compounds in terrestrial as well as in aquatic ecosystems. Since they feed on the soil or sediment they live in, they are a good sentinel indicator species of the genotoxic potential of the contaminants present in such areas and their usage in genotoxicity studies with the comet assay has already been extensively reviewed (Andem et al., 2013; Cotelle and Férard, 1999; de Lapuente et al., 2015; Dhawan et al., 2009; Salagovic et al., 1996).

Verschaeve and Gilles (Verschaeve and Gilles, 1995) conducted a pilot study using the comet assay to assess the extent of DNA damage in coelomocytes (lymphocytes) 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. (Di Marzio et al., 2005) 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 (Hertel-Aas et al., 2011; Wang et al., 2018). These species were used for the evaluation of several genotoxic agents present in soil and sediment (Rajaguru et al., 2003; Ramadass et al., 2016) as well as for the genotoxicity of heavy metals (Panzarino et al., 2016), pesticides (Y. Zhang et al., 2017), radionuclides (Lourenço et al., 2011), peloids (natural muds) (Gerencsér et al., 2015), flame retardants (Yang et al., 2016), naphthenic acid (Wang et al., 2016), nanomaterials (Correia et al., 2017; Saez et al., 2015; Yirsaw et al., 2016), phthalates (Ma et al., 2016), PAHs (Sforzini et al., 2012) and organic

compounds (Zhiqun et al., 2017). Moreover, the DNA damaging effects of both ionising (Hertel-Aas et al., 2011; Sowmithra et al., 2015) and non-ionising radiation (Tkalec et al., 2013) were also done. These studies indicated 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.

Besides the above mention species, there are studies done on other terrestrial as well as aquatic species of earthworms such as *Eisenia hortensis* (Ciğerci et al., 2016), several species of lumbricids (*Lumbricus terrestris, L. rubellus, L. castaneous*) (Button et al., 2012, 2010; Šrut et al., 2017), *Amynthas diffringens* (Fourie et al., 2007), *Amynthas gracilis* (Parelho et al., 2017), *Aporrectodea caliginosa* (Fourie et al., 2007; Klobučar et al., 2011), *Branchiura sowerbyi* (Aborgiba et al., 2016), *Dendrodrilus rubidus* (Button et al., 2010; Fourie et al., 2007), *Dichogaster curgensis* (Manerikar et al., 2008; Markad et al., 2016, 2012), *Limnodrilus udekemianus Claparede* (Kračun-Kolarević et al., 2015), *Metaphire posthuma* (Chang et al., 2011), *Microchaetus benhami* (Fourie et al., 2007), *Enchytraeus crypticus* (Maria et al., 2018) and *Pheretima peguana* (Muangphra et al., 2014). Since some studies also showed differences in sensitivity between the tested species in response towards genotoxicants (Button et al., 2010; Fourie et al., 2007; Saez et al., 2015) special attention should be devoted when choosing appropriate species for biomonitoring studies to reduce false negative results.

7.3. Leeches

There are few studies 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 (Khaled et al., 2016). Results showed a dose-dependent increase in DNA damage that was detected in both the ovarian and testicular cells in response towards the tested mixture. Moreover, two species of medicinal leeches *Hirudo medicinalis* (Mihaljević et al., 2011) and *Hirudo verbana* (Mihaljević et al., 2009) were used for the assessment of the genotoxic potential of sulphate-rich surface waters as well as water and sediment contaminated by aluminium compounds. These results indicated increased DNA damaging potential 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*, where results revealed an induction of DNA damage in the ovaries of exposed organisms (Khaled et al., 2017).

7.4. Tardigrades

In order to recover without any apparent damage, tardigrades (*Milnesium tardigradum*) evolved effective adaptations to preserve the integrity of cells and tissues in anhydrobiotic state. The comet assay was thus employed to study the effect of anhydrobiosis on DNA integrity indicating that 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) (Neumann et al., 2009).

8. Arthropods

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

8.1. Hexapods; Entognatha

Although collembolans have a high content of chitin, which does not allow for the mechanical or chemical digestion of organisms, the comet assay was successfully applied to one of the most widely used soil organisms in ecotoxicological studies, *Folsomia candida* (Cardoso et al., 2017). In the study, the genotoxic activity of metallic element (Cd) and a representative of organophosphates, the insecticide dimethoate, was shown, 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 animals such as crabs, lobsters, crayfish and shrimps. The comet assay was carried out in several crustacean species that populate both freshwater and marine environment. Besides their wide distribution, crustaceans can be of very small size belonging to zooplanktonic communities even up to larger

specimens, and are therefore suitable models for both genetic toxicology and environmental biomonitoring on a large scale.

Several zooplanktonic species were used when it comes to DNA damage assessment using the comet assay. Freshwater species like the water flea (*Daphnia magna*) are among the most used in toxicity assessment followed by species such as *Daphnia carinata* and *Ceriodaphnia dubia*. In these studies, DNA damage was assessed using either cells from the haemolymph or cell preparations from whole daphnias. The species were used for the assessment of several physical and chemical agents as well as water pollution (David et al., 2011; den Besten and Tuk, 2000; Lavorgna et al., 2016; Prasath et al., 2016). They were used for the evaluation of heavy metal toxicity (Pellegri et al., 2014), pesticides (Silva et al., 2015), pharmaceuticals (Gómez-Oliván et al., 2014; Parrella et al., 2015) and landfill leachate (Widziewicz et al., 2012) indicating the comet assay as an early warning biomarker for effects of toxicants on Daphnia populations. Besides daphnides, several gammarus species were used as well due to their importance in the food chain, namely amphipod crustaceans *Gammarus fossarum* (Lacaze et al., 2011a, 2011b, 2011c, 2010), *Gammarus elvirae* (Davolos et al., 2015; Di Donato et al., 2016; Ronci et al., 2017, 2015), *Gammarus balcanicus* (Ternjej et al., 2014), *Echinogammarus veneris* (Di Donato et al., 2016) and *Quadrivisio aff. lutzi* (Weber et al., 2013). 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. Moreover, species like freshwater crayfish *Astacus leptodactylus* (Klobučar et al., 2012; Malev et al., 2010) and *Cambarellus montezumae* (Díaz et al., 2015) as well as the prawn *Macrobrachium rosenbergii* (Rani et al., 2015) and *Macrobrachium nipponense* (S. Sun et al., 2017) were used for the assessment of several pesticides and polluted sites as well as different environmental stressors, such as temperature increase, air exposure, hypoxia and food deprivation.

Several species habituating mostly inland saltwater lakes were also used such as the brine shrimp *Artemia salina* (Xu et al., 2015) and *Artemia nauplii* (Arulvasu et al., 2014) for the assessment of antimicrobial agents triclosan and triclocarban toxicity, and AgNPs, respectively. Moreover, differential responses of the sexual *Artemia franciscana* and asexual *Artemia parthenogenetica* to genotoxicity by reference mutagens were also evaluated (Sukumaran and Grant, 2013a, 2013b, 2013c) pointing to the importance of predicting biomarker responses from multigenerational consequences 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 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 different seasons (summer, pre-monsoon, monsoon and post-monsoon) supporting the use of the comet assay as a tool in effectively monitoring genotoxicity in marine plankton communities (Goswami et al., 2014). The comet assay was also used in *Cyclops abyssorum tatricus* populations from clear and turbid alpine lakes, where UV-induced DNA damage and repair processes were studied (Tartarotti et al., 2014). Several species of shrimps and prawns, important species for aquaculture, such as the grass shrimp (*Palaemonetes pugio*) (Sharon E Hook and Lee, 2004; Sharon E. Hook and Lee, 2004; Kim and Lee, 2004; Kuzmick et al., 2007; Lee et al., 2012, 2000, 2008; Lee and Kim, 2002), white shrimp (*Litopenaeus vannamei*) (Chang et al., 2009; Li et al., 2016; Qiu et al., 2011; Wang et al., 2009), seabob shrimp (*Xiphopenaeus kroyeri*) (da Silva Rocha et al., 2012), giant tiger prawn (*Penaeus monodon*) (Jose et al., 2011), decapod shrimp (*Palaemon varians*) (Pavlaki et al., 2016) and marine prawn (*P. serratus*) (Erraud et al., 2018, 2017) 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. Moreover, several species like *Acartia tonsa* (Pavlaki et al., 2016), *Corophium volutator* (Roberts et al., 2013), *Chasmagnathus granulata* (Gouveia et al., 2004), *Callinectus sapidus* (Pie et al., 2015), *Carcinus maenas* (Sahlmann et al., 2017), *Charybdis japonica* (Pan and Zhang, 2006) and *Eriocheir sinensis* (Hong et al., 2017) were also used for assessments of DNA damaging effects in response to 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. The assay showed 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. On the contrary, in synganglia, no significant variation in damage frequency was observed (Freitas et al., 2007).

As for spiders, several species were used for the evaluation of DNA damage in regard 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 Koch*) indicating significant differences in the amount of DNA damage due to pesticide exposure which was higher in the abdomen haemocytes of *P. astrigera* compared to cephalothorax haemocytes (Li et al., 2011). The assay was also used for the evaluation of DNA damaging effect of starvation and dimethoate (organophosphate insecticide) 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 (Wilczek et al., 2016). 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 irrespectively of sex. However, the severity of damage seemed to be sex- and internal organ-dependent (Stalmach et al., 2015).

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 (Bilbao et al., 2002). Since then, interest in the application of the comet assay to studies of insects has been rapidly increasing and its potential for medical and environmental toxicology studies has been greatly acknowledged. Up till now, several papers have given an extensive overview regarding the usage of the comet assay on various insects, describing the methodological approaches on 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 (Andem et al., 2013; Augustyniak et al., 2016a; Dhawan et al., 2009; Gaivão and Sierra, 2014). Thus, we will briefly review their role in the genetic and environmental toxicology.

Insects have become a very interesting model in genetic toxicology and could partially replace vertebrates in toxicological studies due to the ethical issues related to this type of research. It has to be pointed out that 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 such studies 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 (Augustyniak et al., 2016a).

The comet assay has been used for the evaluation of DNA damage in several insect species that belong to various systematic groups and inhabit different ecological niches. The most often used insect in DNA damage research is undoubtedly *D. melanogaster* (Demir and Marcos, 2017; Dhawan et al., 2009; Gaivão and Sierra, 2014; Katanić et al., 2017; Rajak et al., 2017; Verçosa et al., 2017), although there are studies conducted also on *D. simulans* (Brennan et al., 2012). This could be explained by the fact that *D. melanogaster* is a model organism that is perfectly suited for genetic studies with the presence of numerous repair deficient/efficient mutants, which allows for the design of complex experimental models that can be used to understand DNA repair mechanisms (Augustyniak et al., 2016a). As such, *D. melanogaster* was successfully developed as a model organism in toxicological studies (Ong et al., 2015) and a new term "Drosophotoxicology" was proposed (Rand, 2010). *D. melanogaster* is a valuable model for all kind of processes related to human health, including DNA damage response. The comet assay has been performed mainly *in vivo* using different larvae cell types derived from the brain, midgut, haemolymph, and imaginal disk. Moreover, *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 to quantitate repair activities *in vitro*. Additionally, Drosophila comet assay, both *in vivo* and *in vitro*, has been applied to study the influence of protein overexpression on genome integrity and degradation (Gaivão and Sierra, 2014).

Studies in dipterans, other than Drosophila have also been widely used 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 these flies ideal for studies in developmental biology, diseases, genetics, genetic toxicology and stress biology. Besides, their cosmopolitan presence makes them suitable candidates for ecological biomonitoring (Mishra et al., 2017).

Up until now, several terrestrial species of insects including economically relevant species were examined using the comet assay (Augustyniak et al., 2016a). These include

Diptera – the American serpentine leafminer (*Liriomyza trifolii*) (Koo et al., 2012); Coleoptera - chestnut weevil (*Curculio sikkimensis*) (Todoriki et al., 2006), maize weevil (*Sitophilus zeamais*) (Hasan et al., 2008, 2012), yellow fever mosquito (*Aedes aegypti*) (Shetty et al., 2017) and cigarette beetle (*Lasioderma serricorne*) (Kameya et al., 2012); Lepidoptera - Indian meal moth (*Plodia interpunctella*) (Imamura et al., 2004), diamondback moth (*Plutella xylostella*) (Koo et al., 2011), gypsy moth (*Lymantria dispar*) (Matić et al., 2016), common Mormon (*Papilio polytes*) (Ravi et al., 2017), Oriental leafworm moth (*Spodoptera litura*) (Yun et al., 2014), beet armyworm (*Spodoptera exigua*) (Augustyniak et al., 2016b), Mediterranean flour moth (*Ephestia kuehniella*) (Güven et al., 2015), cotton bollworm (*Helicoverpa armigera*) (Kim et al., 2015; Packiam et al., 2015) and corn stalk borer (*Sesamia nonagrioides*) (Avan Aksoy et al., 2017); Orthoptera - common field grasshopper (*Chorthippus brunneus*) (Augustyniak et al., 2015, 2014, 2006), bow-winged grasshopper (*Chorthippus biguttulus*) (Karpeta-Kaczmarek et al., 2016b), grasshopper (*Aiolopus thalassinus*) (Abdelfattah et al., 2017), desert locust (*Schistocerca gregaria*) (Yousef et al., 2010), cave crickets (*Dolichopoda laetitiae* and *D. geniculate*) (Gustavino et al., 2014), house cricket (*Acheta domesticus*) (Karpeta-Kaczmarek et al., 2016a); Hemiptera – red cotton stainer (*Dysdercus cingulatus*) (Ravi et al., 2017), and Hymenoptera - black garden ant (*Lasius niger*) (Lucas et al., 2017) and honeybee (*Apis mellifera*) (Pavelić, 2014; Silva de Moraes and Bowen, 2000). Besides terrestrial species, several aquatic species were assayed as well. These include aquatic midges such as *Chironomus riparius* (Aquilino et al., 2018; Bernabò et al., 2017; Lee et al., 2009; Martínez-Paz et al., 2013; Morales et al., 2013; Park and Choi, 2009), *C. kiiensis* (Al-Shami et al., 2012), and *C. tentans* (Park and Choi, 2007). It is expected that the number of insect species will arise 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. They could be especially relevant for species as important as the honeybee (*A. mellifera*), whose populations are experiencing a significant decline (Augustyniak et al., 2016a).

The comet assay was used on many species listed above for the assessment of DNA damage after irradiation. The assay confirmed that irradiation (electron beam and γ-rays) can cause DNA damaging effects in the investigated agricultural pests. These studies are useful especially for those insects that are undesirable from an economic point of view since DNA damage provoked by irradiation is related to 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 using chemical treatment. Moreover, the impact of chemical agents on DNA integrity using the comet assay was also employed for the evaluation of a wide range of environmental pollutants,

especially different agrochemicals. Besides the evaluation of the impact of physical and chemical agents on the DNA integrity of insects, the assay could also be used to explain 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, molting, diapause or quiescence; for investigating DNA damage in insects during extensive physical activity, aging mechanisms or DNA stability in relation to age and sex (Augustyniak et al., 2016a).

9. Oligomers

9.1. Echinoderms

Several species of echinoderms such as sea stars and urchins were used for the assessment of DNA damage in the marine environment 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 (El-Bibany et al., 2014). The DNA damaging effects of direct and indirect acting genotoxins such as H_2O_2 (Sahlmann et al., 2017), MMS and cyclophosphamide (Canty et al., 2009) 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 (Hernroth et al., 2010).

In the coelomocytes of sea urchins (*Strongylocentrotus droebachiensis*) exposed to dispersed crude oil, a significant concentration-related 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 (Taban et al., 2004). It was also noted that ocean acidification increases copper toxicity in purple sea urchins (*Paracentrotus lividus*) where an increase in DNA damage was observed (Lewis et al., 2016). The % of DNA strand breaks was provoked in coelomocytes and sperm cells from *P. lividus* exposed to ZnO nanoparticles, common contaminants of marine environment via sunscreens lotion (Manzo et

al., 2017). El-Bibany et al. (El-Bibany et al., 2014) 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 H_2O_2-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 (Nahon et al., 2008; Pruski et al., 2009). 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 it was first introduced in 1988 by Singh and colleagues (Singh et al., 1988), the usage and application of the comet assay has drastically increased. Its usage in genetic toxicology, either *in vitro* or *in vivo*, has extended to both laboratory work as well as field work, either aquatic or terrestrial. Invertebrates are a quite large group of animals and their usage in genetic toxicology is also increasing. Hence, the comet assay is currently done on a large number of animals including platodes, planarians, cnidarians, molluscs, annelids, arthropods and oligomers, and these species are especially used in the field of ecotoxicology due to their significance in ecosystems.

Overproduction in the chemical industry has led to a large number of new chemicals synthesized each year and these chemicals can be regarded as new and emerging pollutants that may possess significant biological effects if and when released into the environment. The presence of xenobiotics in environment, which are biologically active and difficult to break down, represents a degree of stress often unacceptable for living organisms and the entire ecosystem. As such, both direct and indirect toxic activity of such chemicals can be an important risk factor 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 under controlled laboratory conditions. However, one should have in mind that the extrapolation of data obtained in such a way does not always reflect the reality and/or severity of the situation. Another approach would be based on the usage of native species from designated areas, assessing the degree of toxicity after collection from the environment. One could evaluate pollution as a complex situation that is present in the environment but should also bear in mind that extrapolation is not always possible in terms of human exposure.

In this kind of assessment the comet assay became the method of choice by providing 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, which can be verified from the literature reviewed in the present paper. It has to be pointed out that for many reasons, namely scientific, practical and/or technical, blood is the most commonly used biological matrix, however tissues and/or cells such as haemolymph, gills, liver, early larval stages, sperm cells or coelomocytes have also been more frequently used. Moreover, data obtained by the comet assay can be gathered relatively fast, they are quite reliable and to some extent reproducible. Problems may 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 to chemical and/or physical agents under evaluation, should be carefully chosen.

Obviously, there are several invertebrate species that are more commonly used in comparison with others, and especially relevant are those from the aquatic environment. When it comes to 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 regarded as primary species in biomonitoring programmes involving aquatic ecosystems both as sentinel and toxicity-testing subjects. 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 polymorfa* 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. When it comes to 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 when it comes to genotoxicity testing using the comet assay. They cover ecological niches from the marine to freshwater environment and are regarded as excellent bio-indicator species. Besides their wide distribution, crustaceans can be of very small size belonging to zooplanktonic communities even up to larger specimens, which makes them suitable model organisms for both genetic toxicology and environmental biomonitoring on a large scale. Among the most studied ones are the *Daphnia* and *Gammarus* species. Moreover, although they have been only recently adapted, in 2002 by Bilbao and colleagues (Bilbao et al., 2002), for the evaluation of DNA damaging effects, insects have also become an increasingly used model when it comes to the comet assay. There are several key advantages that insects as a model can provide in toxicological studies such as inexpensive breeding that does not require much space or time, possibility of large scale experiments at a low cost and minimization of inter-individual variability leading to more reliable statistical results. 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 pointed out that extrapolation from data obtained in such models to higher animals, not to mention humans, could be problematic and sometimes impossible.

Another point that should also be addressed concerns the standardization of the comet assay protocol in order to get more reliable and more reproducible results. This is very hard to obtain, especially when using invertebrate species since there is a variety of cells that can be used for the DNA damage evaluation and as such there are large numbers of different protocols for each cell type disabling the standardization of the protocol. There are of course a wide variety of internal protocols optimised in each laboratory where the comet assay is performed and those protocols were made for a particular species and/or cell type. Hence, the development of guidelines for the standardization of the comet assay procedure should be addressed in order to achieve comparable results. This is also one of the critical issues if the assay itself is

recognized as an efficient environmental monitoring tool and for its eventual implementation into regulatory guidelines. Nevertheless, 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 in humans, but also in invertebrates.

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 oligomers).

	Animal	Cell type	Type of study	Agent/Stressor	Concentration range	Parameters	Response	Reference
Protozoans	*Tetrahymena thermophila*	*T. thermophila* cells	*in vivo*	H2O2, phenol, formaldehyde, WW (heavy metals)	H2O2 (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	↑ (H2O2), ↑ (phenol), ↑ (Formaldehyde), ↑ (WW)	Lah et al., 2004
		T. thermophila cells	*in vivo*	polluted water (heavy metals), H2O2 as PC	H2O2 (100 µM)	OTM	↑	Vidic et al., 2009
		T. thermophila cells	*in vivo*	Dechlorane Plus	2.4, 12, 60, 300, 1500 µg/L	% tail DNA, TM, OTM	↑ (> 300 µg/L)	Dou et al., 2015
		T. thermophila cells	*in vivo*	chlorophenol (2,4-DCP, 2,4,6-TCP and PCP)	1.2, 2.4, 3.6 mg/L	OTM	↑, ↑, ↑ (> 2.4 mg/L)	Li et al., 2014#
		T. thermophila cells	*in vivo*	melamine	1, 2, 4 g/L	VS, AU	↑ (> 2 g/L)	Li et al., 2015
		free cells, cells embedded in gel or nuclei embedded in gel	acellular exposure, *in vitro*, *in vivo*	bulk- or nano-TiO2	bulk-TiO2 (1 - 0.1 µg/mL), nano-TiO2 (2 - 100 µg/mL)	% tail DNA	↑ (after alkaline lysis except for 100 µg/mL after acellular exposure)	Rajapakse et al., 2013
Platodes	*Schistosoma mansoni*	*S. mansoni* cells (different cells)	*in vitro*	colchicin, TMA, H2O2	colchicin (50 µM), TMA (0.06%), H2O2 (50 µM)	TL, CD (ratio)	Ø (colchicin), ↑ (TMA), ↑ (H2O2),	Silva et al., 2007

Planarians	*Girardia schubarti*	*G. schubarti* cells	*in vivo*	urban pollution (poluted water), MMS as PC	Diluvio's Basin (Brazil), MMS (8×10−5 M)	VS, DI, DF	↑ (some sites), ↑ (MMS)	Prá et al., 2005
		G. schubarti cells (neoblasts, nerve, epidermal and fixed parenchyma cells)	*in vivo*	CuSO4, MMS	CuSO4 (1, 2, 3, 4, 5×10-5 M), MMS (4, 8, 12, 16×10-5 M)	VS, AU, DF, TL	↑ (> 3×10-5 M), ↑	Guecheva et al., 2001
	Polycelis felina	*P. felina* cells	*in vivo*	norflurazon	0.2 and 2 µM	TL, % tail DNA, TM	↑	Horvat et al., 2005
	Schmidtea mediterranea	*S. mediterranea cells*	*in vivo*	tributyltin	0.25, 1 and 4 µg/L	AU	↑ (103 ng/L Sn only)	Ofoegbu et al., 2016
Cnidarians	*Anthopleura elegantissima*	isolated aposymbiotic *A. elegantissima* cells	*in vitro*	H2O2, EMS, B[a]P	H2O2 (50, 100, 200 uM), EMS (50, 100, 200 µg/mL), B[a]P (50, 100, 200 µM)	TL, % tail DNA, TM	↑ (H2O2 200 µM), ↑ (EMS > 100 µg/mL), ↑ (B[a]P > 100 µM)	Mitchelmore and Hyatt, 2004
	Actinia equine	cellular suspension (cells from a single foot fragment)	*in vivo*	polluted water (PAHs), ENU, B[a]P	polluted water (PAHs), ENU (200 ppm), B[a]P (300 ppm)	TL, TM	↑ polluted water, ↑ ENU, ↑ B[a]P	Carrozzino et al., 2004
	Bunodosoma cangicum	cell suspension (explants of pedal disk tissue fragments)	*in vitro*	CuCl2	7.8 and 15.6 µg/L	TL, % tail DNA, TM	↑	Anjos et al., 2014

	Stylophora pistillata	cell suspension (animal cells, algal cells, holobiont entities)	*in vitro*	UVB	4.05, 8.1 and 12.2 kJ/m2	TE, TEM	↑ (different cell response - holobiont entity more sensitive)	Rinkevich et al., 2005
		S. pistillata cells	*in situ, ex situ*	crude oil, phosphate dust	500 ppm	VS	↑	Kteifan et al., 2017
	Seriatopora hystrix	*S. hystrix* cells	*in vitro*	UVA, UVB	UVB (3.55 W/s), UVA (8.09 W/s)	TEM	↑	Svanfeldt et al., 2014
	Montastraea franksi	*M. franksi* cells	*in vivo*	Cu2SO4	1, 8 and 30 µg/L	TD	↑ (30 µg/L)	Schwarz et al., 2013
	Hydra magnipapillata	*Hydra* cells	*in vivo*	CuSO4	0.06 and 0.1 mg/L	VS, TL	↑	Zeeshan et al., 2016
		Hydra cells	*in vivo*	CoCl2	8 and 16 mg/L	VS, % tail DNA	↑	Zeeshan et al., 2017
Molluscs								
Bivalves	*Mytilus edulis**	haemocytes	*in vivo*	radioactive particles	137Cs, 241Am, 90Sr/90Y	% tail DNA	↑ (dependent on the particle)	Jaeschke et al., 2015
		haemocytes	*in vitro*	fluoxetine, paroxetine, venlafaxine, carbamazepine, sulfamethoxazole, trimethoprim, erythromycin, DMSO as PC	from 0.001 mg/L to 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)	Lacaze et al., 2015
		hematocytes	*in situ*	polluted sediment (heavy metals)	Tamar Estuary, South West	TL, TM	↑ (depending on the site)	Dallas et al., 2013

					England, UK			
		haemocytes	*in vivo*	Cu, ocean acidification	effect of ocean acidification (pH 7.71, pCO2 1480 µatm) on Cu toxicity (~0.1 µM)	% tail DNA	↑ (combined exposure)	Lewis et al., 2016
		haemocytes	*in vivo*	crude oil	0.015, 0.06 and 0.25 mg/L	% tail DNA	↑	Taban et al., 2004
		hemocytes, gill cells	*in vitro, in vivo, in situ*	MMS, (UV and H2O2 as PC), polluted area	H2O2 (22.5, 45, 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	↑ (H2O2, 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, hemocytes, > 1 mg/L), ↑ (polluted area, depending on the site)	Rank and Jensen, 2003
		hemocytes, coelomocytes	*ex vivo*	H2O2, reference sites	25 and 250 µM	% tail DNA	↑	Sahlman et al, 2017
	*Mytilus galloprovincialis**	haemocytes	*in vivo*	QDs, CdTe QDs, Cd(NO3)2, H2O2	CdTe QDs, Cd(NO3)	% tail DNA, VS	↑	Rocha et al., 2014

					2 at 10 µg/L			
		haemocytes	*in vitro*	diclofenac	5 and 10 ng/L	% tal DNA, TM, OTM	↑ (10 ng/L)	Toufexi et al., 2016
		haemocytes	*in vivo*	TBT, B[a]P as PC	TBT (10, 100 and 1000 µg/L), B[a]P (50 ug/L)	% tail DNA, HH	↑ (TBT > 10 ug/L), ↑(B[a]P)	Martinovic et al., 2016
		haemolymph	*ex situ*	superdispersant-25 (S-25), diesel oil, dispersed diesel oil mixtures, CdCl2 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), CdCl2 40 µM	% tail DNA, ACS, HDC, HH	↑ (S-25), Ø (diesel oil alone), ↑ (CdCl2)	Martinovic et al., 2015
		haemolymph	*in situ*	environmental pollution (metals: Cu, Zn, Cd, Ni, Pb and PAHs)	Ria Formosa lagoon (Portugal)	% tail DNA	↑	Almeida et al., 2013
		haemocytes, gill cells	*in situ*	PAHs (in sediment)	Corcubión estuary (Spain)	% tail DNA	↑ (compared to reference site)	Fernández-Tajes et al., 2011
		haemocytes, gill cells	*in vitro, in vivo*	okadaic acid (OA) and dinophysistoxins	OA (10, 50, 100, 200 and 500 nM, in vitro), P. lima (1000 and	% tail DNA	↑ (OA > 200 nM),↑ (P. lima > 100000)	Prego-Faraldo et al., 2016

					100000 cells/L)			
		gill cells	*in vivo*	TiO2, CdCl2, + mixture	nano-TiO2 and CdCl2 at 0.1 mg/L (nominal conc. level)	% tail DNA	↑Cd, Ø (TiO2), ↓ (TiO2 reduced Cd genotoxicity, Ø)	Della Torre et al., 2015
		gill cells	*in vivo*	pharmaceutical wastewater (antibiotic pollution)	Sidi Thabet city (Tunisia)	VS, TDD, % tail DNA	↑	Zouiten et al., 2016
		gill cells	*in situ*	dioxin-like compounds	Gulf of Follonica (Italy)	% tail DNA	Ø	Guidi et al., 2018
		gills and digestive glands	*in vivo*	B[a]P	5, 50, and 100 µg/L	% tail DNA	↑	Banni et al., 2017
	Mytilus trossulus	haemocytes	*in situ*	PAHs	Exxon Valdez spill (Alaska, USA)	TM	↑	Thomas et al., 2007
		gill and digestive gland cells	*in vivo*	CuO-NP, dissolved Cu	CuO-NP (0.02 mg/L), Cu2+ (CuCl2 solution - 0.02 mg/L)	% tail DNA, GDI	↑ gill cells, Ø digestive gland cells	Chelomin et al., 2017
	Perna viridis	haemocytes	*in situ, ex situ, in vivo*	chlorination	0.2 and 0.5 mg/L (clorine in lab)	% tail DNA	↑	Chavan et al., 2016
		haemocytes	*in vivo*	carbamazepine, bisphenol A, atrazine, + mixture	low, medium and high concentrations	% tail DNA	↑ BPA, ↑ ATZ, Ø CBZ, ↑ mixture	Juhel et al., 2017
		gill and hepatopancreatic cells	*in situ*	heavy metals	Ennore estuary (India)	HL, CL, TL, % head DNA, % tail DNA,	↑	Vasanti et al., 2017

						TM,OTM,		
	Perna canaliculus	haemocytes	*in vivo*	Cd	acute (2000 and 4000 µg/L Cd), subchronic (200 and 2000 µg/L Cd)	% tail DNA	↑ (subchronic exposure)	Chandurvelan et al., 2013
	Perna perna	haemocytes	*in vivo*	antifouling biocide (chlorothalonil)	0.1 and 10 µg/L	TL	Ø	Guerreiro et al., 2017
	Bathymodiolus azoricus	haemocytes, gill cells	*in vitro, in vivo*	hydrostatic pressure change, H2O2, MMC	H2O2 (20, 40, 60 uM), MMC (6, 12, 60×10-6 M)	% tail DNA	↑, ↓(with time)	Dixon et al., 2004
	Crassostrea gigas	haemocytes	*in situ*	pesticides, heavy metals	Sinaloa and Sonora (Mexico)	VS, AU	↑ (depending on the site)	Vázquez-Boucard et al., 2014
		haemocytes	*in situ*	heavy metal pollution (Pb, Co, Ni, As, Cd, Zn, Fe, Cu)	Shandong Peninsula, Bohai Sea (China)	% tail DNA	↑	Xie et al., 2016
		haemocytes	*in vivo*	tritiated water (ionizing radiation), H2O2 as PC	0.9 and 13.8 MBq/L, H2O2 (10, 50 and 100 µM)	% tail DNA	↑	Devos et al., 2015
		larvae cells	*in vivo*	herbicide diuron (+ metabolites DCPMU, DCPU, 3,4-DCA)	0.002 to 2.5 ug/L	% tail DNA	↑	Behrens et al., 2016
		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	↑	Mai et al., 2012

	Crassostrea virginica	hematocytes	*in situ*	pollution	Lavaca Bay (Texas, USA)	% tail DNA, TL, OTM, TotI	↑	Bissett et al., 2009
		haemocytes	*in vivo*	atrazine	20 and 200 ppb (µg/L)	OTM	↑	Christl et al., 2004
	Saccostrea cucullata	gill	*in situ*	PAHs and PCBs	Arabian Sea coast, Goa (India)	% tail DNA	↑	Sarker et al., 2017
		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	↑	Sarker et al., 2018
	Chlamys farreri	digestive gland	*in vivo*	B[a]P	50 ng/L	% tail DNA, VS	↑	Liu et al., 2014
		hemocytes	*in vivo*	CuO nanoparticles	CuO NPs (NPtotal) and Cu2+(NP ion)	TM	↑	Sun et al., 2017
	Ruditapes decussatus	haemocytes, gill cells	*in vitro, in vivo*	okadaic acid	in vitro (exposing hemocytes to different concentrations of OA - 10, 50 and 100 nM) and in vivo (feeding clams with toxic dinoflagellate Prorocentrum lima - The max OA	% tail DNA	↑ (in vitro >10 nM), in vivo (dependending upon the concentration of OA and cell type evaluated)	Flórez-Barrós et al., 2011

					body burden detected was 44.65 ng/g and 1452 ng/g for low- and high-OA P. lima cultures)			
		gill cells	*in vivo*	PAHs	sediment and water samples	% tail DNA	↑ (depending on the exposure time)	Martins et al., 2013
		Haemolymph	*in situ*	environmental pollution (Cu, Zn, Cd, Ni and Pb and PAHs)	Ria Formosa lagoon (Portugal)	% tail DNA	↑	Almeida et al., 2013
	Scrobicularia plana	gills, digestive glands	*in vivo*	silver nanoparticles	Ag at 10 µg/ L in nanoparticulate (Ag NPs) or soluble salt (AgNO3) forms	% tail DNA	↑	Buffet et al., 2014
		haemocytes	*In vitro, in vivo*	H2O2, natural oestrogen 17β-oestradiol (E2) and synthetic (xeno)oestrogens (ethinyloestradiol (EE2) and nonylphenol (NP)), EMS	in vitro (H2O2 (10, 50, 100 uM), E2 and EE2 (1, 10, 100 ng/L, 1, 10 µg/L), NP (1, 10, 100 µg/L, 1, 10 mg/L), in vivo (E2 (1, 10, 100 ng/L, 1 µg/L) NP	% tail DNA, TL, OTM	in vitro ↑H2O2, E2 >100 ng/L, EE2 >1 ug/L, NP >100 ug/L; in vivo E2 1 ug/L, NP 1 mg/L	Petridis et al., 2009

					(1, 10, 100 ng/L, 1 mg/L), EMS (32 µg/L))			
		spermatozoa	in vitro	B[a]P	B[a]P (10 and 100 µg/L)	% tail DNA	↑	Châtel et al. 2017
	Venerupis pullastra	haemocytes, gill cells	in situ	PAHs (in sediment)	Corcubión estuary (Spain)	% tail DNA	↑ (compared to reference site)	Fernández-Tajes et al., 2011
	Donax faba	gill, body, foot tissues	in vivo	chlorpyrifos, carbendazim	chlorpyrifos (79.08, 158.16, 316,32, 1265.31 µg/L), carbendazim (52.65, 105.32, 210.65, 421.3, 842.6 µg/L)	% tail DNA	↑ (depending on the concentration and cell type)	Janaki Devi et al., 2013
	Tapes semidecussatus	haemocytes, gill cells, digestive gland	in vivo	polluted sediment	Douglas Estuary and Ballymacoda Estuary (Ireland)	TM	↑	Coughlan et al., 2002
	Tapes semidecussatus	haemocytes, gill cells, digestive gland	in vivo	polluted sediment	Cork Harbour and Ballymacoda Estuary (Ireland)	% tail DNA	↑	Hartl et al., 2006
	Protothaca staminea	haemocytes	in situ	PAHs	Exxon Valdez spill (Alaska, USA)	TM	↑	Thomas et al., 2007

	Meretrix casta	gill cells	in situ	pollution (petroleum hydrocarbons and trace metals)	Vasco and Palolem, Goa (India)	% tail DNA	↑ (compared to unpolluted site)	D'costa et al., 2018
		haemolymph	in vivo	γ-radiation, EMS	γ-radiation (2, 4, 6, 8 and 10 Gy), EMS (18, 32 and 56 mg/L)	% tail DNA	↑, ↑	Praveen Kumar et al., 2014
	Spisula sachalinensis	gills and digestive glands	in vivo	B[a]P, MNNG	0.005% of final concentration	TL	↑	Kim and Hyun, 2006
	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	↑, ↑	Praveen Kumar et al., 2014
	Cerastoderma edule	haemocytes, gill cells	in situ	PAHs (in sediment)	Corcubión estuary (Spain)	% tail DNA	↑ (compared to reference site)	Fernández-Tajes et al., 2011
		hematocytes	in situ	heavy metals (sediment)	Tamar Estuary (England)	TL, TM	↑ (compared to reference site)	Dallas et al., 2013
	Scapharca inaequivalvis	erythrocytes	in vivo	copper (Cu^{2+})	0.1 ppm	TL, % tail DNA, TM	↑ (TL)	Gabbianelli et al., 2003
		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)	Gabbianelli et al., 2006
	Dreissena polymorpha	hematocytes	in vivo	opioids (morphine)	0.05 ug/L and 0.5 ug/L	% tail DNA	↑ (0.5 ug/L)	Magni et al., 2016
		hematocytes	in vivo	antidepresants (fluoxetine, citalopram)	500 ng/L alone or in mixture	% tail DNA	Ø	Magni et al., 2017

		hematocytes	in vivo	amphetamine	500 ng/L and 5000 ng/L	% tail DNA	↑ (5000 ng/L)	Parolini et al., 2016
		gill cells	in situ	seasonal variations, PAHs	Seine River basin (France)	% tail DNA	↑↓ (based on te season), ↑ (PAHs)	Michel et al., 2013
		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	↑	Michel et al., 2015
		gill cells	in vivo	polluted sediment	River Elbe in Dessau and River Havel in Havelberg (Germany)	% tail DNA, TME	Ø (specie differences)	Schafer et al., 2012
	Dreissena bugensis	gill cells	in vivo	polluted sediment	River Elbe in Dessau and River Havel in Havelberg (Germany)	% tail DNA, TME	Ø (specie differences)	Schafer et al., 2012
	Unio pictorum	hematocytes	in vitro, in vivo	5-fluorouracil, CdCl2 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 (CdCl2, 100 µM), in vivo (CdCl2, 4, 40 and 100 µM)	% tail DNA	↑ (in vivo 5-FU > 0.4 µM), Ø (in vitro), ↑ (in vitro CdCl2), ↑ (in vivo CdCl2 > 40 µM)	Gačić et al., 2014
		hematocytes	in situ	pollution	Danube River (Serbia)	% tail DNA	↑	Kolarević et al., 2016b

		haemocytes	in vivo	metalloid and other trace element polluted river sediments	River Cecina (Italy)	% tail DNA	↑	Guidi et al., 2017
		haemocytes	in vitro, in vivo	cytostatic drugs (etoposide, vincristine sulfate, cisplatin)	etoposide (4, 40, and 100 µM), vincristine sulfate (0.004, 0.04, 0.4, and 4 µM), and cisplatin (0.04, 0.4, and 4 µM)	% tail DNA	ETO (↑in vitro, > 4 uM, ↑in vivo, > 40 uM), VIN (Ø in vitro, ↑in vivo > 0.04); CP (Ø in vtro, in vivo, ↑ after post-treatment with H2O2 (20 uM))	Kolarević et al., 2016a
		hematocytes	in situ	polluted freshwaters	Sava and Drava River (Croatia)	% tail DNA	↑	Štambuk et al., 2009
	Unio tumidus	hematocytes	in vitro, in vivo	5-fluorouracil, CdCl2 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 (CdCl2, 100 µM), in vivo (CdCl2, 4, 40 and 100 µM)	% tail DNA	↑ (in vivo 5-FU at 0.4 and 40 µM), Ø (in vitro), ↑ (in vitro CdCl2), ↑ (in vivo CdCl2 > 40 µM)	Gačić et al., 2014
		haemocytes	in situ	pollution (river water)	Sava River (Croatia)	OTM	↑	Aborgiba et al., 2016
		hematocytes	in situ	pollution	Danube River (Serbia)	% tail DNA	↑	Kolarević et al., 2016b

		haemocytes	in vitro, in vivo	cytostatic drugs (etoposide (4, 40, and 100 µM), vincristine sulfate (0.004, 0.04, 0.4, and 4 µM), and cisplatin (0.04, 0.4, and 4 µM))	etoposide (4, 40, and 100 µM), vincristine sulfate (0.004, 0.04, 0.4, and 4 µM), and cisplatin (0.04, 0.4, and 4 µM)	% tail DNA	ETO (↑in vitro, > 4 uM, ↑in vivo, > 40 uM), VIN (Ø in vitro, ↑in vivo > 0.04); CP (Ø in vitro, in vivo, ↑ after post-treatment with H2O2 (20 uM))	Kolarević et al., 2016a
		digestive gland cells	in vivo	phenolic compounds (tannic, ellagic and gallic acid)	60, 200 and 500 µM	TM	↑ (>60 µM)	Labieniec et al., 2007
	Limnoperna fortunei	hematocytes	in vivo	TiO2-NP	1, 5, 10 and 50 µg/mL	% tail DNA, OTM	↑ (>1 ug/mL)	Girardello et al., 2016
		haemolymph cells	in vitro, in vivo	UV radiation, pentachlorophenol (PCP), copper sulphate (CuSO4), enviromental sample	UVC (in vitro 0.7, 2.5, 3.3, 4.5, 5 J/m2), PCP (in vivo 10, 80, 100, 150 µg/L), CuSO4 (in vivo 3.75, 7.5, 15, 20 µg/mL), Diluvio stream (Brasil)	CL, VS, DI, DF	↑ (UVC >0.7 j/m2, PCP >100 ug/L, CuSO4 >3.75 ug/mL), ↑ (environmental sample)	Vilella et al., 2006
	Sinanodonta woodiana	hematocytes	in situ	pollution, seasonal variations	Velika Morava River (Serbia)	OTM	↑	Kolarević et al., 2013
		hematocytes	in situ	pollution	Danube River (Serbia)	% tail DNA	↑	Kolarević et al., 2016b

	Corbicula fluminea	hematocytes	in situ	landfill leachate discharge	Periquitos stream (Brasil)	VS	↑	de Oliveira et al., 2016
		hematocytes	in vivo	atrazine, Roundup	ATZ (2 and 10 ppb), RD (2 and 10 ppm), AZT+RD	VS	Ø (AZT and RD alone), ↑ (AZT+RD)	dos Santos and Martinez, 2014
		hemocytes and gill cells	in vivo	Gasoline water-soluble fraction	diluted to 5%	CS	↑	Fedato et al., 2010
	Lamellidens marginalis	gill cells	in vivo	oganophosphate pesticide (monocrotohpos)	5.25 mg/L	OTM	↑	Mundhe et al., 2016
	Utterbackia imbecillis	hematocytes		copper, atrazine, glyphosate, carbaryl, diazinon, 4-nitroquinoline	copper (3.12 and 6.30 µg/L), atrazine (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), 4-nitroquinoline ()	TM	↑ (Cu >3.12 ug/L, ATZ at 22.55 mg/L, diazinon at 0.28 mg/L), Ø (glyphosate, carbaryl, 4-nitroquinoline)	Conners and Black, 2004
Gastropods	*Lymnea stagnalis*	hematocytes	in vivo	sediment (heavy metals), PAHs, PCBs	740 mg copper/kg, 1220 mg zinc/kg, PAHs (< 10 mg/kg), PCBs (< 0.6 mg/kg)	TM	↑ (dependent on the site)	Clement et al., 2004
		hematocytes	in situ	radiation	near Chernobyl region (Ukraine)		↑	Koneva, 2014#

		hematocytes	in situ	environmental pollution (heavy metals, Sr)	inlet of Pripyat River and Perstok Lake (Belarus)	TM, TL, % tail DNA, CDNA, CA	↑	Koneva, 2013
	Lymnaea luteola	digestive gland cells	in vivo	ZnONPs	10, 21, 32 µg/mL	% tail DNA, OTM	↑ (> 10 µg/mL)	Ali et al., 2012
		digestive gland cells	in vivo	AgNPs	4, 12, 24 µg/L	% tail DNA, OTM	↑ > 4 µg/L	Ali et al., 2014
		hepatopancreas cells	in vivo	single walled carbon nanotubes (SWCNTs)	0.05, 0.15, 0.30 mg/L	% tail DNA, OTM	↑ >0.05 mg/L	Ali et al., 2015
		haemocytes	in vivo	lead nitrate	10, 20 and 40 µg/mL	% tail DNA, OTM	↑ >20 µg/mL	Ali et al., 2017
	Biomphalaria glabrata	hematocytes	in vivo	60Co gamma radiation	2.5, 5, 10 and 20 Gy	VS	↑ >2.5 Gy	Grazeffe et al., 2008
	Biomphalaria alexandrina	hematocytes	in vivo	Roundup (48% Glyphosate)	10 mg/L	VS	↑	Mohamed, 2011
		hemocytes	in vivo	insecticide Match	Match 5% EC (its activeingredient is lufenuron 5% EC)	OTM	↑	Ibrahim et al., 2018
	Marisa cornuarietis	hatched embryos cells	in vivo	platinum (PtCl2)	0.1, 1, 10, 50, 100 and 200 µg/L	TM	↑ > 1 µg/L	Osterauer et al., 2011
	Potamopyrgus antipodarum	embryonic cells, adult gill cells, whole neonate cells	in vitro, in vivo	H2O2, MMS, cadmium (Cd), bisphenol A	H2O2 (0.1, 1, 10, 50 µM), MMS (1, 3, 6 mg/L), (Cd, 1, 10, 100 µg/L), bisphenol A (2, 10, 50 µg/L)	% tail DNA, TEM	↑ (H2O2 > uM, MMS >1 mg/L, BPA >10 ug/L, Cd >1 ug/L)	Vincent-Hubert et al., 2012
	Bellamya aeruginosa	hepatopancreas cells	in vivo	ethylbenzene	5, 45, 100, 450, 1000 µg/L	OTM	↑ > 5 µg/L	Zheng et al., 2013

	Pila globose	haemolymph cells	in vivo	composite tannery effluent	effluent treatment plant of Kolkata (India)	VS	↑	Bhattacharya et al., 2016
	Heleobia cf. australis	Heleobia cf. australis cells	in situ	pollution (chromium, lead)	Montevideo Bay and Laguna Garzón (Uruguay)	% tail DNA	↑ (compared to reference site)	Villar et al., 2015
	Nerita chamaeleon	gill cells	in vitro, in vivo	H2O2, cadmium chloride (CdCl2)	H2O2 (1, 10, 25, and 50 µM), CdCl2 (10, 25, 50, and 75 µg/L)	% tail DNA, TL, OTM	↑ H2O2 > 1 uM, CdCl2 > 10 µg/L	Sarkar et al., 2015
		cells from soft tissue	in situ	pollution (PAHs)	Arambol, Anjuna, Sinquerim, Dona Paula, Velsao, Betul and Palolem, Goa (India)	% tail DNA	↑ (depending on the site)	Bhagat et al., 2017
	Planaxis sulcatus	gill cells	In vitro, in vivo	H2O2, mercuric chloride (HgCl2)	in vitro H2O2 (1, 10, 20, 50 µM), in vivo HgCl2 (10, 20, 50, and 100 µg/l)	% tail DNA, OTM	↑ H2O2 > 1 µM, HgCl2 > 10 µg/L	Bhagat and Ingole, 2015
	Haliotis midae	hematocytes	in vivo	oxygen levels	low and high oxygen levels	% tail DNA, OTM	Ø (juveniles), ↑ (adult)	Vosloo et al., 2013
		hemolymph cells, germ cells (oocytes and sperm)	in vivo	H2O2	5.5 mmol/L	% tail DNA, OTM, DI	↑	Vosloo and Vosloo, 2017
	Littorina littorea	haemolymph cells	in situ	PAHs, OTCs, PCBs, OCPs	South coast of England (England)	% tail DNA	↑	Noventa et al., 2011

	Morula granulata	gill cells	in situ	marine pollution	Goa (India)	HD, % tail DNA, OTM, TL	↑	Sarkar et al., 2014
		cells from soft tissues	in vitro, in vivo	H2O2, phenanthrene	H2O2 (1, 10, 25 and 50 µM), phenanthrene (10, 20, 50 and 100 µg/L)	% tail DNA	↑ H2O2 >1 µM, phenanthrene >25 µg/L	Bhagat et al., 2016
		cells from soft tissues	in vivo	PAH (benzo(k)fluoranthene)	1, 10, 25 and 50 µg/L	% tal DNA	↑ > 1 µg/L	Bhagat et al., 2017
	Helix aspersa	haemolymph cells	in vivo	soil contaminated with mineral coal tailings (PAHs)	Charqueadas (Brazil)	VS, DI, DF	↑	de Souza et al., 2015
		haemolymph cells	in vivo	PAHs	Porto Alegre (Brazil)	VS, DI, DF	↑ (depending on the site)	Ianistcki et al., 2009
		haemolymph cells	in vivo	coal waste (mineral coal tailings - coal pyrite tailings)	Santa Catarina Coal Basin (Brasil)	VS, DI, DF	↑	Leffa et al., 2010
		haemolymph cells	in vitro	UVC	UVC 4.5 J/m2	VS, DI, DF	↑	Pereira et al., 2009
		haemolymph cells	in vivo	Nicotiana tabacum leaves	fed on tobacco leaves	VS, DI, DF	↑	da Silva et al., 2013
		hematocytes	in vitro, in situ	validation study, H2O2, diffent sites (polluted (coal-fired power station) and reference)	H2O2 (75 and 150 µM), coal-fired power station (Italy)	TL, % tal DNA, TM	↑ (H2O2 > 75 µM), ↑ (compared to reference site)	Angeletti et al., 2013
	Helix vermiculata	hematocytes	in vitro, in situ	validation study, H2O2, diffent sites (polluted (coal-fired	H2O2 (75 and 150 uM), coal-fired power	TL, % tal DNA, TM	↑ (H2O2 > 75 µM), ↑ (compared to	Angeletti et al., 2013

				power station) and reference)	station (Italy)		reference site)	
	Bradybaena fruticum	hepatopancreatic cells	in situ	habitat (biotope) differences	Mid Russian Upland (Belgorod fouling)	VS, DCI, % tail DNA, TM	↑ (depending on the site and age)	Snegin, 2014
	Chondrula tridens	hepatopancreatic cells	in situ	habitat (biotope) differences	Mid Russian Upland (Belgorod fouling)	VS, DCI, % tail DNA, TM	↑ (depending on the site and age)	Snegin, 2014
	Cepaea vindobonensis	hepatopancreatic cells	in situ	habitat (biotope) differences	Mid Russian Upland (Belgorod fouling)	VS, DCI, % tail DNA, TM	↑ (depending on the site and age)	Snegin, 2014
	Stenomphalia ravergieri	hepatopancreatic cells	in situ	habitat (biotope) differences	Mid Russian Upland (Belgorod fouling)	VS, DCI, % tail DNA, TM	↑ (depending on the site and age)	Snegin, 2014
Cephalopods	*frozen squid (Loligo vulgaris)*	N/A	N/A	radiation	10 MeV electron beam	N/A	N/A	Khan et al., 2003
	Octopus vulgaris	digestive gland cells, gill cells, kidney cells, gonad cells	in situ	heavy metals	Matosinhos and Olhao (Portugal)	% tail DNA	↑ ↓(depending on the site and cell type)	Raimundo et al., 2010
Annelids								
Polychaetes	*Nereis virens*	coelomocytes	in vivo	BaP, EMS, DMSO	BaP (0.3, 0.6, 10, 20, 35 and 45 mg/mL), EMS (12.1 mg/ml), DMSO (98.9%)	TL, TM	↑BaP at 45 mg/ml , ↑ EMS, ↑ DMSO	De Boeck and Kirsch-Volders, 1997
		intestinal cells	in vivo	PAHs (fluoranthene), H2O2 as PC	fed with Capitella capitata	TEM	Ø (Flu), ↑H2O2	Palmqvist et al., 2006

					exposed to Flu			
		coelomocytes (eleocytes, amoebocytes, spermatozoa)	in vitro, in vivo	MMS, BaP	MMS (18, 32 and 52 mg/L), B[a]P (0.1, 1.0, and 10 mg/L)	% tail DNA	↑↓ (specie and cell type dependent)	Lewis and Galloway, 2008
	Nereis diversicolor	coelomocytes	in vivo	silver nanoparticles (Ag NPs, AgNO3), 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	Cong et al., 2011
		coelomocytes	in vivo	silver nanoparticles (Ag NPs, AgNO3), 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	Cong et al., 2014
		coelomocytes (eleocytes, amoebocytes, spermatozoa)	in vitro, in vivo	MMS, BaP	MMS (18, 32 and 52 mg/L), B[a]P (0.1, 1.0, and 10 mg/L)	% tail DNA	↑↓ (specie and cell type dependent)	Lewis and Galloway, 2008
	Hediste diversicolor	coelomocytes	in vivo	lipid-coated CdSe/ZnS quantum dots and CdCl2	0.001, 0.01, 0.1 and 1 ng/g	OTM	↑ (>0.001 ng/g)	Saez et al., 2015
		coelomic fluid (coelomocytes)	in vivo	AgNPs, H2O2 as PC (200 uM)	Ag at 10 µg/L in nanoparticulate (Ag NPs) or soluble salt (AgNO3) forms	% tail DNA	↑	Buffet et al., 2014
	Capitella capitata	cell suspension	in vivo	PAHs (Flu), H2O2 (differences in PAH tolerance between Capitella species)	21 and 26 g Flu/g dry weight	TEM	↑↓ (depending on the specie)	Bach et al., 2005

		cell suspension	in vivo	PAHs (fluoranthene (Flu))	~30 mg Flu/g dry-weight sediment or 50 mg Flu/L seawater	VS, AU	↑	Palmqvist et al., 2003
	Perinereis aibuhitensis	blood cells	in vitro	Cd, Pb, Pyrene, BaP, H2O2	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), BaP (0.0001, 0.001, 0.01, 0.1 and 1 µg/L), H2O2 (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), ↑BaP (> 0.01 µg/L), ↑H2O2 (> 0.1 µM)	Seo et al., 2005#
		blood cells	in vitro	sediment extracts, PAHs, total organic carbon (TOC)	Masan Bay (Korea)	TM	↑	Seo et al., 2008
		coelomocytes	in vivo	mercuric chloride (HgCl2; 0.05 mg/L and 0.5 mg/L)	(HgCl2; 0.05 mg/L and 0.5 mg/L)	% tail DNA	↑ (> 0.05 mg/L)	Zhang et al., 2017
		coelomocytes	in vitro, in vivo	lead nitrate (Pb(NO3)2), cobalt chloride (CoCl2), H2O2 as PC	in vitro Pb(NO3)2 (100, 300, and 500 µg/l), CoCl2 (100, 300, and 500 µg/l) H2O2 (50	OTM, % tail DNA	↑ (in vitro >100 µg/L), ↑ (H2O2), ↑ (in vivo >100 µg/L)	Singh et al., 2017

					ug/L); in vivo Pb(NO3)2 (100, 300, and 500 µg/l) and CoCl2 (100, 500, and 1000 µg/l)			
	Arenicola marina	coelomocytes (eleocytes, amoebocytes, spermatozoa)	in vitro, in vivo	MMS, BaP	MMS (18, 32 and 52 mg/L), B[a]P (0.1, 1.0, and 10 mg/L)	% tail DNA	↑↓ (specie and cell type dependent)	Lewis and Galloway, 2008
		coelomocytes	in vivo	oil-contaminated sediments, PAHs, PCBs, heavy metals	Bay of Algeciras and Galician Coast (Spain)	% tail DNA	↑	Morales-Caselles et al., 2009
	Laeonereis acuta	cell suspension	in vivo	copper (Cu)	62.5 mg/L	VS, DS	↑, Ø (dependent on the body region)	Ferreira-Cravo et al., 2009
Oligochaete	*Eisenia fetida**	coelomocytes	in vivo	soil pollution	illegal dumping ground	TL	↑	Verschaeve and Gilles, 1995
		coelomocytes (eleocytes, amoebocytes, granulocytes)	in vitro, in vivo	soil contamination (PAHs), H2O2, CdCl2	in vitro (H2O2 37 to 300 µM, CdCl2 0.5, 5 and 50 µM), in vivo (contaminated soil)	HD, % tail DNA, TEM, OTM, TL, L/H	↑ (H2O2 >37 µM), ↑ (CdCl2 at 50 µM),↑ (soil)	Di Marzio et al., 2005
		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	% tail DNA	↑	Hertel-Aas et al., 2011

					(41.9 Gy/h), in vitro (0.5, 1, 2, 3, 6 or 10 Gy X-rays)			
		coelomocytes	in vivo	polluted river system (sediment samples)	Noyyal River (India)	TL, L/W	↑	Rajaguru et al., 2003
		coelomocytes	in vivo	imidaclothiz	(0.3 and 1 mg/kg)	OTM	↑ (> 0.3 mg/kg)	Zhang et al., 2017
		coelomocytes	in vivo	peloids (natural muds)	peloid samples (Kolop and Hevız (Hungary))	TM	↑ (dependent on the mud)	Gerencsér et al., 2015
		coelomocytes	in vivo	dechlorane plus (DP)	0.1, 0.5, 6.25 and 12.5 mg/kg	% tail DNA, TL, OTM	↑ (> 0.1 mg/kg)	Yang et al., 2016
		coelomocytes	in vivo	naphthenic acids (NAs)	5, 10, 50, 100 mg/kg dry weight	% tail DNA, OTM	↑ (> 10 mg/kg dry weight)	Wang et al., 2016
		coelomocytes	in vivo	zero valent iron nanoparticles (C-nZVI)	60, 150, 500 and 1500 mg/kg soil	% tail DNA, TL, OTM	↑(>150 mg/kg)	Yirsaw et al., 2016
		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	Ma et al., 2016
		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	TL, % tail DNA, OTM	↑ (dependent on the compound)	Zhiqun et al., 2017

					heptane and 2,4-di tert buyl phenol			
		coelomocytes	in vivo	γ-radiation	1, 5, 10, 20, 30, 40 and 50 Gy	% tail DNA	↑ (>5 Gy)	Sowmithra et al., 2015
		coelomocytes	in vivo	radiofrequency electromagnetic field (RF-EMF)	900 MHz (field levels of 10, 23, 41 and 120 V/m)	% tail DNA	↑	Tkalec et al., 2013
		sperm	in vivo	arsenite	5, 10, 20, 40, and 80 mg As/kg	OTM	↑ (>5 mg As/kg)	Wang et al., 2018
		coelomocytes	in vivo	olive mill waste (OMW), dimethoate as PC	raw Two-Phase Olive Mill Waste (TPOMW) and raw Olive Mill Waste Waters (OMWW), dimethoate (0.6 mg/kg dry soil)	% tail DNA	↑ (OMW), ↑ dimethoate	Campani et al., 2017
		coelomocytes	in vivo	CdSO4	20 mg/L	% tail DNA	↑	Fourie et al., 2007
		coelomocytes	in vivo	copper, cadmium and pentachlorophenol	Cu (0,25, 0.75 and 2.25 µg/cm2), Cd (1.32, 6.6 and 13.2 µg/cm2), PCP (0.05,	% tail DNA	↑ (Cu >2.25 µg/cm2), ↑ (Cd >1.32 µg/cm2), ↑ (PCP >0.125 µg/cm2)	Klobučar et al., 2011

					0.125 and 0.25 µg/cm2)			
		coelomocytes	in vivo	lipid-coated CdSe/ZnS quantum dots and CdCl2	0.001, 0.01, 0.1 and 1 ng/g	OTM	↑ (QDNs > 0.1 ng/g), ↑(CdCl2 > 0.01 ng/g)	Saez et al., 2015
	Eisenia andrei	coelomocytes	in vivo	soil pollution (petroleum hydrocarbon (PH))	520, 750, 1040, 1170, 1390, 1450 mg hydrocarbons/kg soil	TL, OTM, TM, % tail DNA	↑	Ramadass et al., 2016
		coelomocytes	in vivo	Cd (Cd contaminated artificial soils)	10 or 100 µg/g	% tail DNA	Ø	Panzarino et al., 2016)
		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, 2×10−3 ppm)	% tail DNA	↑ (B[a]P > 0.1 ppm), TCDD (1x10-5 ppm)	Sforzini et al., 2012
		coelomocytes	in vivo	soils pullution (heavy metals, radionuclides)	Cunha Baixa uranium mine (Portugal)	VS, AU	↑	Lourenço et al., 2011
		coelomocytes	in vivo	nanomaterial (inorganic (TiSiO4), organic (nano-vesicles of sodium sodecyl sulfate/ didodecyl dimethylammonium bromide –	TiSO4 (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	VS, AU	↑ TiSO4 (> 444.4 mg/kg dw), ↑ SDS/DDAB (at 1000 mg/kg dw)	Correia et al., 2017

				SDS/DDAB)	mg/kg dw)			
		coelomocytes	in vivo	neonicotinoids	<200 ng/g dry weight, nominal concentration	VS	↑	Chevillot et al., 2017
	Eisenia hortensis	coelomocytes	in vivo	cobalt chloride (CoCl2)	113, 226 and 452 ppm	VS	↑ at 452 ppm	Ciğerci et al., 2016
	Lumbricus terrestris	coelomocytes	in vivo	X-rays, mitomycin C	X-rays (5, 10 and 15 cGy), MMC (12.5, 25 and 50 ng/mL)	TL	↑	Verschaeve and Gilles, 1995
		cell suspension (from coelomic fluid)	in vivo	soil poluttion, As, heavy metals	former mine site of Devon Great Consols (DGC), 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)	Button et al., 2010
		tissue homogenates	in vivo	Cd	10 mg/kg Cd in soli	% tail DNA	↑	Šrut et al., 2017
	Lumbricus rubellus	cell suspension (from coelomic fluid)	in vivo	soil poluttion, As, heavy metals	former mine site of Devon Great Consols (DGC, UK) (203 to 9025 mg/kg	% tail DNA	↑ (As >98 mg/kg), ↑↓ (dependent on the soil and species)	Button et al., 2010

					As), As (98, 183, 236, 324 and 436 mg/kg)			
	Lumbricus castaneous	coelomocytes		soil poluttion, As, heavy metals, H2O2	former gold mine in Nova Scotia (Canada), As (880 to 2700 mg/kg), H2O2 (500 uM)	% tail DNA	↑ (dependent on the soil), ↑ H2O2	Button et al., 2012
	Amynthas diffringens	coelomocytes	in vivo	CdSO4	20 mg/L	% tail DNA	Ø	Fourie et al., 2007
	Amynthas gracilis	coelomocytes	in vivo	livestock pollutants (heavy metals), H2O2 as PC	São Miguel Island (Azores archipelago, Portugal), H2O2 (50 mM)	VS, GDI	↑	Parelho et al., 2017
	Aporrectodea caliginosa	coelomocytes	in vivo	CdSO4	20 mg/L	% tail DNA	↑	Fourie et al., 2007
		coelomocytes	in vivo, in situ	copper, polluted sites	Cu (0,25, 0.75 and 2.25 µg/cm2), Zagreb (Croatia)	% tail DNA	↑ (Cu >0.25 µg/cm2), ↑ (dependent on the site)	Klobučar et al., 2011
	Branchiura sowerbyi	haemocytes, coelomocytes	in situ	pollution (river water)	Sava River (Serbia)	OTM	↑	Aborgiba et al., 2016
	Dendrodrilus rubidus	coelomocytes	in vivo	CdSO4	20 mg/L	% tail DNA	↑	Fourie et al., 2007
		cell suspension (from coelomic fluid)	in vivo	soil poluttion, As, heavy metals	former mine site of Devon Great Consols (DGC, UK) (203	% tail DNA	↑ (As >98 mg/kg), ↑↓ (dependent on the	Button et al., 2010

					to 9025mg/kg As), As (98, 183, 236, 324 and 436 mg/kg)		soil and species)	
		coelomocytes		soil poluttion, As, heavy metals, H2O2	former gold mine in Nova Scotia (Canada), As (880 to 2700 mg/kg), H2O2 (500 uM)	% tail DNA	↑ (dependent on the soil), ↑ H2O2	Button et al., 2012
	Dichogaster curgensis	coelomocytes	in vitro, in vivo	Cr(VI), H2O2 as PC	Cr(VI) (1, 3,10, 30, 70 and 100 ppm), H2O2 (70.4 µM)	VS, AU	↑ (in vitro Cr(VI) >1 ppm), ↑ (H2O2)	Manerikar et al., 2008
		coelomocytes	in vivo	fly ash, heavy metals	Nashik district, Maharashtra (India) (0–40%, w/w)	OTM	↑	Markad et al., 2012
		coelomocytes	in vivo	fly ash, heavy metals	fly ash (40%)	% tail DNA	↑	Markad et al., 2016
	Limnodrilus udekemianus Claparede	coelomocytes	in vivo	5-fluorouracil, etoposide, CdCl2	5-FU (0.004, 0.04, 0.4, 4 and 40 µM), ETO (0.004. 0.04, 0.4 and 4 µM), CdCl2 (0.004, 0.04, 0.4, 4 and 40 µM)	% tail DNA	↑ 5-FU (> 0.004 µM), ↑ ETO (> 0.04 µM), ↑ CdCl2 (> 0.004 µM)	Kračun-Kolarević et al., 2015
	Metaphire posthuma	testis cells	in vitro	UV radiation, H2O2	UVC (2, 4 and 6 J/m2),	CM	↑ U(UVC >2	Chang et al., 2011

					H2O2 (0-80 µM)		J/m2), ↑ H2O2	
	Microchaetus benhami	coelomocytes	in vivo	CdSO4	20 mg/L	% tail DNA	Ø	Fourie et al., 2007
	Enchytraeus crypticus	cells from the whole organism	in vivo	silver nanomaterial (Ag NM300K), silver nitrate (AgNO3), H2O2 as PC	Ag NM300K (60, 170 and225 mg Ag/kg dw), AgNO3 (45, 60 and 96 mg Ag/kg dw), H2O2 (75 µM)	VS, AU, GDI	↑	Maria et al., 2018
	Pheretima peguana	coelomocytes	in vivo	glyphosate, paraquat	glyphosate (0.02, 0.25, 2.51, 25.15 and 251.50 µg/cm2), paraquat (39 x 10-5 to 10-1 µg/cm2)	% tail DNA, TL, TM	Ø (glyphosate), ↑ (paraquat >39 x 10-4)	Muangphra et al., 2014
Leeches	*Limnatis nilotica*	ovarian cells, testicular cells	in vivo	oil-related environmental pollutants (mixture - benzene, toluene, ethylbenzene, xylene (BTEX))	BTEX (1.4 and 2.8 mg/L)	VS, AU	↑	Khaled et al., 2016
	Hirudo verbana	haemocytes	in vivo	water, sediment pollution (Al compounds, heavy metals)	Lake Njivice (Krk Island, Croatia)	TL, % tail DNA, TM, AST	↑	Mihaljević et al., 2009
	Hirudo medicinalis	haemocytes	in vivo	sulfate-rich surface waters (SO4), heavy metals	two sites near a gypsum factory (Knin, Croatia)	TL, % tail DNA, AST	↑	Mihaljević et al., 2011

	Erpobdella johanssoni	ovary cells	in vivo	oil-related environmental pollutants (benzene, toluene, ethyl benzene and xylene (BETX))	BETX (25 µg/L)	VS, AU	↑	Khaled et al., 2017
Tardigrades	*Milnesium tardigradum*	storage cells	in vivo	effect of anhydrobiosis, UVB radiation	UVB (20.75 J/sm2 as PC)	% tail DNA	↑	Neumann et al., 2009
Arthropods								
	Folsomia candida	hemolymph	in vivo	cadmium (Cd), the organophosphate insecticide 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)	Cardoso et al., 2017
Crustaceans	*Daphnia magna**	daphnid cells (neonates)	in vivo	sodium dichromate, chrysoidine, BaP	chrysoidine (0.1, 0.5, 1, 2 and 3 uM), sodium dichromate (0.25, 0.5, 0.75, 1, 2, 3 and 4 uM), BaP + sodium dichromate (0.01 + 0.25, 0.05 + 0.5, 0.1 + 0.75 and 0.2 + 1 uM)	% tail DNA	Ø (non-statistically significant response)	David et al., 2011
		whole daphnias	in vivo	CdCl2, K2Cr2O7, lindane, PCP, EMS, 4-NQO, H2O2	LOEC	TL	↑	den Besten and Tuk, 2000
		whole neonates cells	in vivo	benzalkonium chloride (BAC)	0.04, 0.4, 4, 40 and 400 ng/L	% tail DNA	↑>0.4 ng/L	Lavorgna et al., 2016

		whole daphid cells	in vivo	DOC, UVA +mixture	DOC (2.03, 5, 10 and 20 mg/ L), UVA (10.8, 21.7 and 43.4 µmol/m2/ s at 390 nm) +mixture	% tail DNA	↑ (DOC), Ø (UVA), ↑ (mixture)	Wolf et al., 2017
		haemocytes (granulocytes, amoeboid cells)	in vivo	CdCl2, H2O2	CdCl2 (5, 10 and 20 ug Cd2+/L), H2O2 (1, 2, 5 and 10 uM)	% tail DNA, VS	↑ Cd (10 ug), H2O2 (5 uM)	Pellegri et al., 2014
		daphnid cells	in vivo	triclosan, carbendazim , +mixture	triclosan (120, 160 and 206 ug/L), carbendazim (5, 20 and 25 ug/L)	VS	↑	Silva et al., 2015
		whole neonates cells	in vivo	diclofenac, ibuprofen, naproxen, H2O2 as PC	IBP (2.9 mg/L), NPX (0.018 mg/L), DCF (9.7 mg/L), H2O2 (10 uM)	% tail DNA	↑	Gómez-Oliván et al., 2014
		whole neonates cells	in vivo	5-FU, CDDP, ET, DOX, IM, 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), ET (0.03, 3, 30, 300 and 3000 µg/L), DOX (0.002, 0.02, 0.2,	% tail DNA	↑5-FU >0.5 µg/L, ↑CDDP >0.01 µg/L, ↑ET >0.3 µg/L, ↑DOX >0.02 µg/L, ↑IM >2 µg/L, ↑CAP >22.5 µg/L	Parrella et al., 2015

					2 and 20 µg/L), IM (0.2, 2, 20 and 200 µg/L), CAP (2.25, 22.5, 225 and 2250 µg/L)			
		whole neonates cells	in vivo	carbendazim	5 mg/L	VS, CS	↑	Silva et al., 2017
		homogenate daphnid cells	in vivo	quantum dots, quantum dots coated with indolicidin	0.3, 0.6, 1.2, 2.5, 5 and 10 nM	TM	↑	Galdiero et al., 2016
		daphnid somatic cells	in vivo	landfill leachate	Zabrze (Poland)	OTM	↑	Widziewicz et al., 2012
	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	↑	Prasath et al., 2016
	Ceriodaphnia dubia	whole neonates cells	in vivo	BAC	0.4, 4, 40, 400 and 4000 ng/L	% tail DNA	↑>4 ng/L	Lavorgna et al., 2016
		whole neonates cells	in vivo	5-FU, CDDP, ET, DOX, IM, 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), ET (0.01, 0.1, 1, 10 and 100 µg/L), DOX (0.005,	% tail DNA	↑5-FU > 0.06 µg/L, ↑CDDP > 0.3 µg/L, ↑ET > 0.1 µg/L, ↑DOX > 0.05 µg/L, ↑IM > 0.3 µg/L, ↑CAP > 1.2x102 µg/L	Parrella et al., 2015

					0.05, 5 and 50 µg/L), IM (0.03, 0.3, 3 and 30 µg/L), CAP (12, 1.2x102, 1.2x103, 1.2x104 and 1.2x105 µg/L)			
	Gammarus fossarum	haemocytes, oocytes, spermatozoa	in vitro, in vivo	MMS, environmetal contaminats (CdCl2, $K_2Cr_2O_7$, paraquat, AMPA and BaP)	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)	Lacaze et al., 2010
		spermatozoa	in vivo	MMS	0.8, 2.4, 7 and 22 mg/L	% tail DNA	↑ >2.4 mg/L	Lacaze et al., 2011a
		spermatozoa	in vivo	temperature, conductivity		% tail DNA	Ø	Lacaze et al., 2011a
		oocytes, spermatozoa	in vivo, in situ	MMS, K2Cr2O7, waste water treatment plant effluent output	in vivo MMS (0.44, 2.2 and 11 mg/L) and K2Cr2O7 (0.0625, 0.25 and 1 mg/L), in situ (Bourbre River and Bion River , France)	% tail DNA	↑	Lacaze et al., 2011b
		spermatozoa	in situ	wastewater treatment plant effluent (WWTP)	Rhône-Alpes rivers (France)	% tail DNA	↑	Lacaze et al., 2011c

		haemocytes, oocytes	in situ	wastewater treatment plant effluent (WWTP)	Rhône-Alpes rivers (France)	% tail DNA	Ø	Lacaze et al., 2011c
	Gammarus elvirae	hemocytes	in vivo	arsenic-contaminated freshwater (heavy metals)	Latium region (Italy)	TL, % tail DNAI, TM	↑	Davolos et al., 2015
		hemocytes	in vivo	contaminated water (heavy metals)	Latium region (Italy)	% tail DNA	↑	Ronci et al., 2015
		hemocytes	in vivo	arsenic-contaminated freshwater	Latium region (Italy), As (5, 10 and 50 µ/L)	% tail DNA	↑ >5 ug/L	Ronci et al., 2017
		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)	Donato et al., 2016
	Gammarus balcanicus	hemocytes	in vivo	gypsum mine water (heavy metals)	Kosovčica River (Croatia)	TL, % tal DNA, TM, Tail Migration	↑	Ternjej et al., 2014
	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)	Donato et al., 2016
	Quadrivisio aff. lutzi	hemocytes (granulocytes, adipohemocytes, plasmatocytes)	in vivo	water-soluble fraction of heavy oil, MMS	North Fluminense region (Rio de Janeiro, Brazil)	% tail DNA	↑	Weber et al., 2013
	Astacus leptodactylus	hemocytes	in vivo	environmental stressors	temperature increase, air exposure, food	% tail DNA	↑ (temp), Ø	Malev et al., 2010

					deprivation			
		hemocytes	in situ	polluted sites (PAHs, mineral-oils, heavy metals)	Sava River (Zagreb, Sisak, Krapje, Croatia)	% tail DNA	↑	Klobučar et al., 2012
	Cambarellus montezumae	brain cells, hepatopancreas cells	in vivo	dieldrin, chlorpyrifos	0.05 and 0.5 mg/L	T/N index (length to width index)	↑	Díaz et al., 2015
	Macrobrachium rosenbergii	spermatozoa	in vivo	tributyltin (TBT)	1, 2 and 4 mg/L	TL, % tail DNA D, OTM	↑ >2 mg/L	Rani et al., 2015
	Macrobrachium niponnense	gill cells	in vivo	acute hypoxia and reoxygenation	1.5±0.1 mg O2/L	OTM	↑	Sun et al., 2017
	Artemia salina	coelomocytes	in vivo	triclosan, triclocarban	TCS (171 µg/L), TCC (18 µg/L)	% tail DNA, OTM	↑	Xu et al., 2015
	Artemia nauplii	nauplii cells (cell suspension)	in vivo	silver nanoparticles (AgNPs)	2, 10 and 12 nM	HL, TL, CL, HD, % tail DNA, tail movement, OTM	↑	Arulvasu et al., 2014
	Artemia franciscana, Artemia parthenogenetica		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)	Sukumaran and Grant, 2013a
	Paracalanus parvus	whole body cell suspension	in situ	environmental stressors (heavy metals)	Ennore estuary (India)	% tail DNA	↑	Goswami et al., 2014
	Oithona rigida	whole body cell suspension	in situ	environmental stressors (heavy metals)	Ennore estuary (India)	% tail DNA	↑	Goswami et al., 2014

	Euterpina acutifrons	whole body cell suspension	in situ	environmental stressors (heavy metals)	Ennore estuary (India)	% tail DNA	↑	Goswami et al., 2014
	Cyclops abyssorum tatricus	whole body homogenate	in vivo	UV	84 J/m2/min	% tail DNA	↑	Tartarotti et al., 2013
	Palaemonetes pugio	embryo cells	in vivo	BaP, Cr(VI), H2O2	BaP (37.5, 75 and 225 nM), Cr(VI) (0.5, 1 and 2 µM), H2O2 (8.8, 17.7 and 44.2 µM)	% tail DNA	↑BaP (>37.5 nM), Cr(VI) (>0.5 µM), H2O2 (>8.8 µM)	Hook and Lee, 2004a
		embryo cells	in vivo	UV, BaP, Cd (+mixture)	BaP (0.2 µM), Cd (5 µM), UV (330 kJ/m2)	% tail DNA	↑	Hook and Lee, 2004b
		embryo cells	in vivo	2-methyl-1,4-naphthoquinone (MNQ), 4-nitroquinoline-N-oxide (NQO)	MNQ (1, 5, 10, 20 and 50 µM), NQO (1, 2, 3, 4 and 5 µM)	TM	↑ MNQ (> 5 uM), NQO (> 2 uM)	Kim and Lee, 2004
		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	↑	Lee and Kim, 2002
		hepatopancreas cells	in vivo	coal combustion residues (CCR) (heavy	H2O2 (25, 50 and 100 µM)	% tail DNA, TL, TM	↑ (CCR), ↑ H2O2 (>25 µM)	Kuzmick et al., 2007

				metals), H2O2 as PC				
		embryo cells	in vivo	chromium(III) chloride (CrCl3), sodium chromate (Na2CrO4), mercuric chloride (HgCl2), 2-methyl-1,2-naphthoquinone (MNQ)	CrCl3 (1000 µg/L), Na2CrO4 (1000 µg/L), HgCl2 (1 and 10 µg/L), MNQ (86, 172 and 430 µg/L)	TM	↑	Lee et al., 2000
		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	↑	Lee et al., 2008
		embryo cells	in vivo	brominated flame retardant PBDEs and UV-exposed PBDEs	PBDEs (5 and 50 µg/L), UV (270 w/m2)	TM	↑	Lee et al., 2012
	Litopenaeus vannamei	haemocytes, hepatopancreas cells, gill cels	in vivo	Cd (CdCl2)	4.25 and 8.50 µmol/L	OTM	↑	Chang et al., 2009
		hepatopancreas cells	in vivo	hypoxia (dissolved oxygen levels)	oxygen levels 6.5 ppm, 3.0 ppm and 1.5 ppm and then reoxygen	OTM	↑	Li et al., 2016

					ated (6.5 ppm)			
		hemocytes	in vivo	low temperature stress	from 23±2 °C to 12±2 °C	OTM	↑	Qiu et al., 2011
		haemocytes, hepatopancreas cells	in vivo	pH stress	pH 5.6, 7.4 and 9.3	OTM	↑	Wang et al., 2009
		hemocytes	in vivo	Cu	0.101, 0.351 and 0.878 mg/L	TL, HW	↑	Frias-Espericueta et al., 2011
	Xiphopenaeus kroyeri	hemocytes	in vivo	BaP	100, 200, 400 and 800 µg/L	VS, DDI	↑	da Silva Rocha et al., 2012
	Penaeus monodon	hemocytes	in vitro	heavy metals (CdCl2 and HgCl2), pesticides (malathion and monocrotophos)	CdCl2 (140 mM), HgCl2 (17 mM), malathion (60 mg/L), monocrotophos (186 mg/L)	% tail DNA, TL, TM, % Cells with tail	↑	Jose et al., 2011
	Palaemon varians	larval and postlarval stage cell suspension	in vivo	Cd	14, 27 and 54 µg/L	VS	↑	Pavlaki et al., 2016
	Palaemon serratus	spermatozoa	in vitro	optimisation (H2O2, UVC, MMS)	UVC (13.3, 26.5 and 79.5 J/m2), H2O2 (5, 25 and 100 µM), MMS (0.5, 1	VS, AU	↑	Erraud et al., 2017

					and 5 mM)			
		sperm	in situ	abiotic factors (water temperature), environmental pollution	Seine Bay (Normandy, France)	VS, AU	↑	Erraud et al., 2018
	Acartia tonsa	cell suspension, eggs	in vivo	Cd	0.59, 2.39 and 9.57 µg/L	VS	↑	Pavlaki et al., 2016
	Corophium volutator	cell suspension	in vivo	sediment pollution (heavy metals)	West Inner Tees dredged material disposal location (UK)	% tail DNA	↑	Roberts et al., 2013
	Chasmagnathus granulata	epidermis cells (epidermis cells of the dorsal region of cephalothorax and pereiopods cells of intact and eyestalkless crabs)	in vivo	UV-B	8.6 J/cm2	VS	↑, Ø (dependent on the body region)	Gouveia et al., 2005
	Callinectus sapidus	haemocytes, hepatopancreas cells	in vivo	sediment pollution (oil, PAHs)	Mississippi River (Louisiana, USA)	% tail DNA, TL, OTM	Ø, ↓ (depending on the site)	Pie et al., 2015
	Carcinus maenas	coelomocytes, hemocytes	ex vivo	H2O2	25 and 250 µM	% tail DNA	↑	Sahlmann et al., 2017
	Charybdis japonica	gills, hepatopancreas	in vivo	Cd, CdCl2	0.025 and 0.05 mg/L	% tail DNA	↑	Pan and Zhang, 2006

	Eriocheir sinensis	haemocytes	in vivo	glyphosate	4.4, 9.8, 44 and 98 mg/L	% tail DNA, comet ratio	↑	Hong et al., 2017
Arachnids	*Boophilus microplus*	salivary gland cells, ovaries cells, synganglia cells	in vivo	cell death process		TL, AU	↑ (salivary gland cells, ovaries cells), Ø (synganglia)	Freitas et al., 2007
	Pardosa astrigera Koch	hemocytes	in vivo	acetamiprid, chlorpyrifos		TL, cells with tails	↑	Li et al., 2011#
	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)	Wilczek et al., 2016
	Steatoda grossa	haemocytes, midgut gland cells	in vivo	Cd (contaminated food)	0.25 mM CdCl2 fed Drosophila hydei flies	% tail DNA, TL, OTM	↑ (sex and cell type dependent)	Stalmach et al., 2015
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	Bilbao et al., 2002
		haemocytes and midgut cells from larvae	in vivo	4-oxo-2-nonenal (4-ONE), 4-hydroxy-hexenal (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	Demir and Marcos, 2017
		midgut	in vivo	plant extracts rich in phenolic compounds, EMS as PC	*Digitalis ferruginea* and *Digitalis lamarckii*, EMS (1 mM)	VS, CS	↑	Katanic et al., 2017
		hemocytes (larvae	in vivo	acephate	5 µg/mL	TL, % tail	↑	Rajak et al., 2017

		and adults)				DNA, TM		
		hemocytes	in situ	radioactive environment	Lajes Pintadas city (Brasil)	VS, DI, DF	↑	Jorge Verçosa et al., 2017
	Drosophila simulans	spermatocytes	in vivo	Wolbachia-infection (ROS)		VS, % tal DNA	↑	Brennan et al., 2012
	Liriomyza trifolii	whole body cell suspension (adults)	in vivo	electron beam irradiation	30, 50, 70, 100, 150 and 200 Gy	tail migration, TL	↑	Koo et al., 2012
	Curculio sikkimensis	larvae cells	in vivo	electron beam irradiation	1 and 4 kGy	TL, TM, OTM, %DNA damage	↑	Todoriki et al., 2006
	Sitophilus zeamais	larvae, pupae and adults whole body cells	in vivo	γ-radiation	0.5 and 1 kGy	TD, TL, %DNA damage	↑	Hasan et al., 2008
		adult cells	in vivo	γ-radiation	0.5 and 1 kGy	% tail DNA, OTM, TM, HD	↑	Hasan et al., 2012
	Aedes aegypti	adult cells	in vivo	γ-radiation	1, 5, 10, 20, 30, 40 and 50 Gy	% tail DNA	↑ >5 Gy	Shetty et al., 2017
	Lasioderma serricorne	whole body cell suspension	in vivo	γ-radiation	1 kGy	TM, TotL, Ratio	↑	Kameya et al., 2012
	Plodia interpunctella	larvae cells	in vivo	‘‘soft-electron’’ (low-energy electron)	170 kV	VS	↑	Imamura et al., 2004
	Plutella xylostella	larvae cells	in vivo	electron beam irradiation	30, 50 and 100 Gy	TM	↑ (>30 Gy)	Koo et al., 2011
	Lymantria dispar	haemocytes of larvae	in vitro	Cd	50 and 100 mg Cd/g dry food	% tail DNA	↑	Matic et al., 2016

	Spodoptera litura	whole body cell suspension (adults)	in vivo	electron beam irradiation	30, 50, 100, 150, 200 and 250	TM	↑	Yun et al., 2014
	Spodoptera exigua	hemocytes	in vitro	Cd, Cd+H2O2	H2O2 (50 γM)	% tail DNA, TL, OTM	↑	Augustyniak et al., 2016
	Ephestia kuehniella	larvae homogenate	in vivo	UV radiation	254 and 365 nm	% tail DNA, TL	↑	Güven et al., 2015
	Helicoverpa armigera	adult cells	in vivo	γ-radiation (60Co)	400 Gy	TM	↑	Kim et al., 2015
		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	↑	Packiam et al., 2015
	Sesamia nonagrioides	larvae, pupae and adults	in vivo	X-ray irradiation	50, 100, 150 and 200 Gy	CL, TL, TM	↑	Avan Aksoy et al., 2017
	Papilio polytes	5th instar caterpillars	in vivo	γ-radiation	10, 30, 40, 50 and 70 Gy	TL, TM	↑ >30 Gy	Ravi et al., 2017
	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	↑	Augustyniak et al., 2006
		larvae cells (brain cells)	in situ, in vivo	site pollution (heavy metals), H2O2	Olkusz, Szopienice (Poland), H2O2 (20 µM)	% tail DNA, TL, OTM	↑	Augustyniak et al., 2014
		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	↑	Augustyniak et al., 2015

	Schistocerca gregaria	hemocytes		Cd, Pb (CdCl2, PbCl2)	contaminated food with CdCl2 and PbCl2 (25 and 50 mg/kg)	TL, % tail DNA, TM	↑	Yousef et al., 2010
	Dolichopoda laetitiae	haemocytes, brain cells	in situ	radioactive radon exposure	Six caves in Central Italy (221–26,000 Bq/m3)	TL, % tail DNA, TM	↑	Gustavino et al., 2014
	Dolichopoda geniculate	haemocytes, brain cells	in situ	radioactive radon exposure	Six caves in Central Italy (221–26,000 Bq/m3)	TL, % tail DNA, TM	↑	Gustavino et al., 2014
	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,	↑	Abdelfattah et al., 2017
	Chorthippus biguttulus	hemocytes	in vitro	dimethoate, H2O2 as PC	dimethoate (0.16 µg of active substance), H2O2 (50 µM)	% tail DNA	↑	Karpeta-Kaczmarek et al., 2016
	Dysdercus cingulatus	5th instar nymphs	in vivo	γ-radiation	10, 30, 40, 50 and 70 Gy	TL, TM	↑ >40 Gy	Ravi et al., 2017
	Acheta domesticus	hemocytes	in vivo	nanodiamonds	20 and 200 mg/g food	% tail DNA, TL, OTM	↑ at 200 mg/g food	Karpeta-Kaczmarek et al., 2016
	Lasius niger	head (brain) cells, leg cells	in vivo	age, caste (workers, queens)	lifespan differences	% tail DNA	Ø	Lucas et al., 2017
	Apis mellifera	hypopharyngeal	in vivo	nurse and forager worker bees	modes of cell death	TL	↑, Ø	Silva de Moraes

		gland cells						and Bowen, 2000
		larvae cells	in vivo	non-ionizing radiation	900 MHz	N/A	Ø	Pavelić, 2014
	Chironomus riparius	larvae	in vivo	Cu, H2O2 as PC	Cu (0.05, 1 and 25 mg/L), H2O2 (20 mM)	% tail DNA, OTM	↑ >1 mg/L, ↑H2O2	Bernabo et al., 2017
		4th instar larvae	in vivo	vinclozolin	20 and 200 µg/L	TA, OTM, TM, % tail DNA	↑	Aquilino et al., 2018
Oligomers								
Echinoderms	*Asterias rubens*	coelomocytes	in vivo	MMS, cyclophosphamide	MMS (18, 32 and 56 mg/L), CP (18, 32 and 56 mg/L)	% tal DNA	↑ (MMS at 18 mg/L), ↑(CP >18 mg/L)	Canty et al., 2009
		coelomic epithelia cells (cells in intact and regenerating arm)	in vivo	aging proces		% tal DNA	↑↓ (denendent on the cell type)	Hernroth et al., 2010
		coelomocytes, hemocytes	in vitro	H2O2	H2O2 (25 and 250 µM)	% tal DNA	↑ >25 µM	Sahlmann et al., 2017
	Strongylocentrotus droebachiensis	coelomocytes	in vivo	crude oil	0.06 and 0.25 mg/L dispersed crude oil	% tal DNA	↑ >0.06 mg/L	Taban et al., 2004
	Paracentrotus lividus	coelomocytes	in vivo	copper toxicity, ocean acidification (OA)	Cu (~0.1 µM), OA (pH 7.71; pCO2 1480 µatm)	% tal DNA	↑ (under OA compared to control conditions, pH 8.14; pCO2 470 µatm)	Lewis et al., 2016

		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	↑	Manzo et al., 2017
		eggs	in vitro	UV radiation, H2O2	UV radiation (UVA fluence of 18.2 W m22 and UVB fluence of 2.1 W m22 for 60 min), H2O2 (250, 500 and 750 uM)	% tal DNA	↑ UV, ↑ (H2O2 >250 uM)	Nahon et al., 2008
	Sphaerechinus granularis	eggs	in vitro	UV radiation, H2O2	UV radiation (UVA fluence of 18.2 W m22 and UVB fluence of 2.1 W m22 for 60 min), H2O2 (250, 500 and 750 uM)	% tal DNA	↑ UV, ↑ (H2O2 >250 uM)	Nahon et al., 2008
		spermatozoa	in vitro	UVB radiation, H2O2	UVB radiation (2.2 and 5 kJ/m2), H2O2 (100, 25, 500 and 1000 uM)	% tal DNA	↑ (UVB >2.2 kJ/m2), ↑ (H2O2 >100 µM)	Pruski et al., 2009

	Lytechinus variegatus	oelomocytes	in vitro	H2O2, UVC	H2O2 (0.1, 1, 10 and 100 mM) and UVC (2000, 4000, 6000, 8000 and 10000 J/m2)	SSF	↑ (H2O2 and UVC)	El-Bibany et al., 2014
	Echinometra lucunter	oelomocytes	in vitro	H2O2, UVC	H2O2 (0.1, 1, 10 and 100 mM) and UVC (2000, 4000, 6000, 8000 and 10000 J/m2)	SSF	↑ (H2O2 and UVC)	El-Bibany et al., 2014
	Tripneustes ventricosus	oelomocytes	in vitro	H2O2, UVC	H2O2 (0.1, 1, 10 and 100 mM) and UVC (2000, 4000, 6000, 8000 and 10000 J/m2)	SSF	↑ (H2O2 and UVC)	El-Bibany et al., 2014
	Isostichopus badionotus	oelomocytes	in vitro	H2O2, UVC	H2O2 (0.1, 1, 10 and 100 mM) and UVC (2000, 4000, 6000, 8000 and 10000 J/m2)	SSF	↑ (H2O2 and UVC)	El-Bibany et al., 2014

*, commonly used species; #, non-English communication; ↑, significant increase; ↓, significant decrease; Ø, no effect; % 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; GDI, genetic damage ; ndicator; 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; AMPH, amphetamine; ATZ, atrazine; B[a]P, benzo(a)pyrene; BAC,

benzalkonium chloride; BPA, bisphenol A; CBZ, carbamazepine; Cd(NO3)2, cadmium nitrate; CdTe, cadmium telluride ; CH4, methane; CoCl2, cobalt chloride; CoCl2, cobaltous chloride; CP, cisplatin; CrCl3, chromium(III) chloride; Cu2SO4, copper sulphate; CuCl2, copper chloride; CuSO4, copper sulphate; DBTC, dibutyltin-chloride; DCF, diclofenac; DMSO, dimethyl sulfoxide; DNAN, 2, 4-dinitroanisole; DOC, dissolved organic carbon; EMS, ethylmethanesulphonate; ENU, N-ethyl-N-nitrosourea; ENU, N-ethyl-N-nitrosourea; ETO, etoposide; H2O2, hydrogen peroxide; H2S, hydrogen sulphide; HgCl2, 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; Na2CrO4, sodium chromate; 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; PCBs, polychlorinated biphenyl; PCBs, polychlorinated biphenyls; PCP, pentachlorophenol; QDs, quantum dots; 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; UV, ultra violet; VIN, vincristine; WW, wastewater; WWTP, wastewater treatment plant effluent; ZnO, zinc oxide