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REVIEW

Comet assay responses as indicators of carcinogen exposure

Diana Anderson[1,3], Tian-Wei Yu[1] and
Douglas B.McGregor[2]

[1]BIBRA International, Woodmansterne Road, Carshalton, Surrey SM5 4DS, UK and [2]International Agency for Research on Cancer, 150 Cours Albert Thomas, F-69372 Lyon Cedex 08, France

Over 200 agents/factors have been examined in the single cell gel electrophoresis assay, more commonly known as the Comet assay, performed either *in vitro* or *in vivo* in a variety of species. Unequivocal carcinogenicity data are available for 119 of them, amongst which unequivocal Comet assay data exist for 95 agents. Of these 95 agents the prevalence of carcinogens was 88% (84/95). The carcinogens that were Comet positive (sensitivity) formed 88% (74/84), the non-carcinogens that were Comet negative (specificity) formed 64% (7/11). This simple analysis of the Comet assay has not taken account of the difference between *in vitro* and *in vivo* responses, species differences or organ and tissue differences. Also, limitations as to the conduct of the assay have not been examined in any depth. Thus, at the present time the Comet assay has high sensitivity for carcinogens, but its specificity is uncertain because few non-carcinogens have been tested.

Introduction

The following review addresses data generated in the Comet assay. It is an adaptation of a chapter appearing in an IARC Scientific publication, which also includes information relating to DNA repair and alkaline elution assays.

Various methods are used to detect DNA damage in mammalian cells. In the past, DNA strand breakage and alkali-labile sites have been detected by the alkaline elution technique. Its more recent development, the single cell gel electrophoresis (SCGE) assay, can detect DNA damage in any mammalian cells from any tissue or organ at the individual cell level. This review is concerned with Comet assay responses from *in vitro* and *in vivo* studies as indicators of carcinogen exposure.

The Comet assay was first introduced by Östling and Johanson (1984) as a microelectrophoretic technique for direct visualization of DNA damage in individual mammalian cells adapted from other methods, such as nucleotide sedimentation (Cook and Brazell, 1975) and the halo assay (Roti-Roti and Wright, 1987). It is a rapid, simple, visual and sensitive technique for measuring and analysing DNA breakage (Östling and Johanson, 1984; Singh *et al.*, 1988; Olive *et al.*, 1990a), which has been reviewed several times in recent years (e.g. McKelvey-Martin *et al.*, 1993; Fairbairn *et al.*, 1995; Tice, 1995). A special issue of *Mutation Research* on the Comet assay (Schmezer, 1997) and a special section of *Mutagenesis* (Anderson and Plewa, 1998) contain many relevant papers. There is also a report of a Comet workshop (Ross *et al.*, 1995).

Östling and Johanson (1984) performed the original method at close to neutral pH and this was sensitive to the effect of single-strand breaks (SSBs) on DNA supercoiling. The lysis conditions used did not remove all proteins and treatment (ionizing radiation) released a halo of DNA due to a loss of DNA supercoiling which could be used to assay for the presence of SSBs. There was a much greater removal of proteins (up to 95%) under more stringent conditions which allowed broken duplex molecules to migrate. Only the detection of double-strand breaks (DSBs) was permitted with this adaptation, since, at neutral pH, the continuity of the long duplex molecule is not affected by SSBs (McKelvey-Martin *et al.*, 1993). The method was independently modified by two laboratories by applying denaturing conditions to measure DNA SSBs (Singh *et al.*, 1988; Olive, 1989). Singh *et al.* (1988) directed their initial efforts toward the detection of subpopulations varying in drug or radiation sensitivity, while Olive (1989) concentrated upon increasing sensitivity for measurement of low numbers of SSBs.

Rydberg and Johanson (1978) first described the detection of DNA SSBs in individual cells embedded in agarose. Single cells were embedded on microscope slides covered with layers of agarose and lysed under mildly alkaline conditions in order to allow a partial unwinding of the DNA. Acridine orange was used to stain the slides for analysis. After the slides were neutralized, the ratio of DSBs to SSBs was represented by the ratio of green to red fluorescence respectively. As broken ends of the negatively charged particles freely migrated towards the anode in the electric field of the electrophoresis solution, Comet-like tails formed. The size of the DNA fragments and the number of broken ends determined the ability of DNA to migrate. These ends were attached to longer pieces of DNA. Tail length increased with increasing damage, but reached a maximum length. This was defined by electrophoresis conditions and not the size of the fragments. Stretching of attached strands of DNA rather than migration of individual pieces occurred at low levels of damage.

Outline of methods used in the Comet assay

The more commonly adopted SCGE technique is that of Singh *et al.* (1988), in which the procedure of Östling and Johanson is modified by carrying out the assay at a pH > 13, so that alkali-labile sites as well as frank breaks are revealed. Briefly, single cells from any tissue or organ are embedded and immobilized in an agar layer on top of another agar layer on a microscope slide, and sometimes a third layer of agar is added. Slides are placed in lysing buffer, allowing the DNA to unwind, and then they are transferred to an electrophoresis buffer at the desired pH. Broken DNA extends towards the anode from the nucleus. To stain slides, the dyes most frequently used are ethidum bromide (Östling and Johanson, 1984; Östling *et al.*, 1987; Singh *et al.*, 1988), propidium iodide (Olive, 1989, 1994) and 4′,6-diamidino-2-phenylindole (DAPI) (Gedik *et al.*, 1992), and Singh *et al.* (1994) have proposed the use of YOYO-1 (benzoxazolium-4-quinolinum oxazole yellow homodimer), which increases assay sensitivity by giving better visualization. To prevent additional DNA

[3]To whom correspondence should be addressed at: BIBRA International, Woodmansterne Road, Carshalton, Surrey SM5 4DS, UK. Tel: +0181 625 1000; Fax: +0181 661 7029; Email: danderson@bibra.co.uk

Table I. Neutral comet assay, *in vitro* applications

Agents	Cell type	Reference	Response		
			Comet	Cancer	IARC classification (if applicable)
Chemical					
Bleomycin	CHO Chinese hamster ovary cells	Östling and Johanson, 1987	+	+	2B
	V79 Chinese hamster lung cells	Olive and Banath, 1993a	+		
Etoposide	V79 Chinese hamster lung cells	Olive and Banath, 1993a	+	+	
Hydrogen peroxide	L5178Y murine lymphoma	Szumiel *et al.*, 1995	+	+	3
Physical					
Radiation	Human peripheral lymphocytes and mouse lymphoblasts L5178Y	Tronov *et al.*, 1994	+	+	1
	CHO-K1 cells	Kent *et al.*, 1995	+		
X-rays	Various human tumor cell lines	Olive *et al.*, 1994b, Olive and Banath, 1995a	+	+	
	L5178Y murine lymphoma	Szumiel *et al.*, 1995	+		
Sparsely ionizing radiation	CHO Chinese hamster ovary cells	Östling and Johnson, 1984, 1987; Olive *et al.*, 1990b	+	+	
	Human T lymphocytes	Uzawa *et al.*, 1994	+		
	L5178Y mouse lymphoma cells	Olive *et al.*, 1990a	+		
	Mouse macrophages	Olive *et al.*, 1990a	+		
	SCCVII murine tumour cells	Olive *et al.*, 1990a	+		
	TK6 human lymphoblast cells	Evans *et al*, 1993	+		
	Tumour cell lines (MeWo, PECA 4551, PECA 4197)	Muller,W.U. *et al.*, 1994	+		
	V79 Chinese hamster lung cells	Olive 1989; Olive *et al.*, 1990b, 1991, 1992, 1993a, Olive and Banath, 1993a	+		

+, positive; –, negative

damage, all the steps are conducted under dimmed light. The result is a bright fluorescent head and tail, giving the appearance of a Comet. The number of DNA strand breaks induced by the test agent are related to the length of the tail and the intensity of the staining. Undamaged cells appear as intact nuclei. Singh *et al.* (1994) have since reported modifications to the original pH > 13 alkali technique that appear to have increased the sensitivity to the mGy range for ionizing radiation. The application of automated image analysis techniques can be used for quantitation, but simpler, semi-quantitative methods can also be used, e.g. the scoring of numbers of cells in different categories of damage (e.g. no, low, medium and high). Details of the protocols used in the Comet assay are to be found in the reviews cited earlier. An important development was the use of air-dried slides, fixation in methanol after electrophoresis and coating with photographic emulsion (Klaude *et al.*, 1996). This permitted the establishment of a permanent record of the result. These authors also demonstrated that, by using two-dimensional electrophoresis, the Comet tails from neutral and alkaline assays are composed of loops and fragments of DNA respectively.

Uses of the assay

Processes which introduce single-strand gaps in the DNA, such as incomplete excision repair events, are readily detectable, in addition to direct DNA damage (Gedik *et al.*, 1992; Green,M.H. *et al.*, 1992; Tice *et al.*, 1990). The alkaline assay can also be used to detect DNA cross-linking as demonstrated by a retardation in the extent of DNA migration (Olive *et al.*, 1992). Collins *et al.* (1993) have reported on the use of endonuclease III (EndoIII) to probe for oxidized pyrimidines and formamido pyrimidine glycosylase (FPG) (Collins *et al.*, 1996) for 8-hydroxyguanine lesions. Excision repair detection may be enhanced by including, during incubation, aphidicolin (Gedik *et al.*, 1992) or cytosine arabinoside (Andrews *et al.*, 1990). DNA synthesis is inhibited by aphidicolin and results in delayed completion of the repair sites, while cytotosine arabinoside acts as a chain terminator preventing the sites from being closed.

The Comet assay can be used to quantify apoptotic cells (Olive *et al.*, 1993c) and to discriminate between apoptosis and necrosis (Fairbairn *et al.*, 1995; Gopalokrishna and Khar, 1995). They have suggested that in contrast to necrotic cells, which show signs of plasma membrane permeability and integrity loss prior to late DNA degradation, apoptotic cells maintain their cell surface membrane integrity following nuclear fragmentation. It may also be used to identify genetic toxicity of chemicals, particularly when only very small quantities of chemicals are available for testing. An exogenous activation system (S-9) mix (Anderson *et al.*, 1995) can be used in *in vitro* assays if the chosen target cells have a low potential for the metabolism of xenobiotics.

In this review some of the Comet data have been generated in assays conducted at close to physiological pH (see for example Olive, 1989; Olive and Banath, 1995a), but more commonly they come from assays conducted at pH 12.3 (see for example Olive and Banath, 1992; Higami *et al.*, 1994a,b; Nygren *et al.*, 1994) and pH > 13 (see for example Singh *et al.*, 1988; Tice *et al.*, 1990). The selection of chemicals was probably due to their expected positive effects and only a few for their negative properties (Henderson *et al.*, 1998).

Validation approaches

Because the Comet assay is relatively new, the available data have been generated with genotoxins that can probe the sensitivity of the assay, whereas few non-genotoxins have been tested to assess its specificity. Also, many different protocols

Table II. Alkaline comet assay, *in vitro* applications

Agents	Cell type	Reference	Response		
			Comet	Cancer	IARC classification (if applicable)
Chemical					
2-Acetylaminofluorene	Mouse hepatocytes	Hirai *et al.*, 1991	–	+	
	Rat hepatocytes	Hirai *et al.*, 1991	+		
4-Acetylaminofluorene	Rat hepatocytes	Hirai *et al.*, 1991	+	–	
	Mouse hepatocytes	Hirai *et al.*, 1991	–		
Aloe-emodin	Mouse embryo blastomeres	Muller,S.O. *et al.*, 1996	+	nd	
9-Aminoacridine	TK6 human lymphoblastoid cells	Henderson *et al.*, 1998	+	nd	
2-Aminofluorene	Human peripheral lymphocytes	Plewa *et al.*, 1995	+	+	
PhIP[a]	Human lymphocytes and sperm	Anderson *et al.*, 1997c,e	+	+	2B
	Human colon cells	Pool-Zobel and Leucht, 1997	–		
	Rat colon cells	Pool-Zobel and Leucht, 1997	+		
IQ[a]	Human lymphocytes	Anderson *et al.*, 1997a,c,e	+	+	2A
	Human sperm	Anderson *et al.*, 1997a,e	+		
	Human and rat colon cells	Pool-Zobel and Leucht, 1997	–		
Trp[a]	Human lymphocytes and sperm	Anderson *et al.*, 1997a,c,e	+	+	2B
Apo-transferrin	Human lymphocytes	Anderson *et al.*, 1994	–	nd	
Benzene	Human peripheral lymphocytes	Anderson *et al.*, 1995; Andreoli *et al.*, 1997	+	+	1
1,2,4-Benzenetriol	Human peripheral lymphocytes	Anderson *et al.*, 1995; Andreoli *et al.*, 1997	+	+	
1,4-Benzoquinone	Human peripheral lymphocytes	Anderson *et al.*, 1995; Andreoli *et al.*, 1997	+	+	
Benzo[*a*]pyrene (B[a]P)	Human peripheral leukocytes and fibroblasts cell line MRC5CV1	Hartmann and Speit, 1996	+	+	2A
	Human xeroderma pigmentosum cell line XP12ROSV	Speit and Hartmann, 1995	±		
	Human fibroblast cell line MRC5CV1	Speit and Hartmann, 1995, Speit *et al.*, 1996; Hanelt *et al.*, 1997	+		
	Human and rat colon cells	Pool-Zobel and Leucht, 1997	–		
anti-B[a]P-7,8-diol 9,10-oxide	Human fibroblast cell line MRC5CV1	Speit *et al.*, 1996; Hanelt *et al.*, 1997	+	+	
Bleomycin	Murine Friend erythroleukaemia cells	Sweetman *et al.*, 1995	+	+	2B
	Human lymphocytes	Anderson *et al.*, 1994, 1997i; Tice and Strauss 1995a	+		
	Mouse leukemia L1210 cells	Kasamatsu *et al.*, 1996	+		
Cadmium sulphate	Human gastric mucosa cells	Pool-Zobel *et al.*, 1994	+		
Cadmium sulphate	Human leukocytes	Hartmann and Speit, 1994	+	+	1
Cadmium sulphate (with MMS and B[a]P)	Human peripheral leukocytes and fibroblasts cell line MRC5CV1	Hartmann and Speit, 1996	+		
Carbendazim	Human peripheral lymphocytes	Lebailly *et al.*, 1997	–	+	
Catechol	Human peripheral lymphocytes	Anderson *et al.*, 1995	+	±	3
Chlorinated AH[a]	Human lymphocytes	Tafazoli and Kirsch Volders, 1996	+	+	
1-Chloromethylpyrene	Rat gastric mucosal cell	Kennelly *et al.*, 1993	+	nd	
Chlorothalonil	Human peripheral lymphocytes	Lebailly *et al.*, 1997	+	+	3
Cisplatinum	Human white blood cells	Pfuhler and Wolf, 1996	–	+	2A
Crotonaldehyde	Rat and human primary colon mucosa cells	Golzer *et al.*, 1996	+	±	3
Cycloheximide	Human neuroblastoma SH-SY5Y cells	Maruyama *et al.*, 1997a,b	–	nd	
	TK6 human lymphoblastoid cells	Henderson *et al.*, 1998	–		
Cyclophosphamide	Rat and mouse hepatocytes	Hirai *et al.*, 1991, Tice *et al.*, 1990	+	+	1
	Human blood cells	Hartmann *et al.*, 1995a	+		
Daidzein	Human lymphocytes and sperm	Anderson *et al.*, 1997b,f	+	nd	
Danthron	Mouse embryo blastomeres	Muller,S.O. *et al.*, 1996	+	+	2B
Deferoxamine mesylate	Human lymphocytes	Anderson *et al.*, 1994	–	nd	
Deoxyuridine	HeLa cells	Duthie and McMillan, 1997	+	nd	
DbCEAB	Human leukaemia K562 cells	Ward *et al.*, 1997	+	nd	
DbEAB	Human leukaemia K562 cells	Ward *et al.*, 1997	–	nd	
Diazolidinyl urea	Human white blood cells	Pfuhler and Wolf, 1996	–	+	
Dibromochloropropane	Human lymphocytes and sperm	Anderson *et al.*, 1997b,f	+	+	2B
1,2-Dichloroethylene	Human lymphocytes	Tafazoli and Kirsch Volders, 1996	+	nd	
1,3-Dichloropropane	Human lymphocytes	Tafazoli and Kirsch Volders, 1996	+	nd	
1,2,3,4-Diepoxybutane	Human lymphocytes and sperm	Anderson *et al.*, 1997b	+	+	2B
Diethylstilbestrol	Human lymphocytes and sperm	Anderson *et al.*, 1997b	+	+	1
DMBA	Human cell line MRC5CV1	Speit and Hartmann, 1995	+	+	
	Human xeroderma pigmentosum cell line XP12ROSV	Speit and Hartmann, 1995	±		

Table II. *Cont*

Agents	Cell type	Reference	Response		
			Comet	Cancer	IARC classification (if applicable)
DMDHQ	Human neuroblastoma SH-SY5Y cells	Maruyama *et al.*, 1997a,b	–	nd	
Dimethylmercury	Human and rat lymphocytes, rat gastric mucosa	Betti *et al.*, 1993	+	+	2B
Dimethylnitrosamine	Rat hepatocytes	Ashby *et al.*, 1995	+	+	2A
	Rat and mouse hepatocytes	Hirai *et al.*, 1991	+		
Dimethylol urea	Human white blood cells	Pfuhler and Wolf, 1996	–	+	
Dimetridazole	Human lymphocytes	Re *et al*, 1997	+	±	
Dinitrosocaffeidine	Human colon cells	Pool-Zobel and Leucht, 1997	+	nd	
Doxorubicin	Human lymphocytes	Anderson *et al.*, 1997h	+	+	2A
	Chinese hamster V79 cells and murine SCCVII cells	Olive *et al.*, 1997a	+		
Emodin	Mouse embryo blastomeres	Muller,S.O. *et al.*, 1996	–	nd	
Epichlorohydrin	Human VH-10 fibroblasts	Kolman *et al.*, 1997	+	+	2A
1,2-Epoxybutene	Human lymphocytes and sperm	Anderson *et al.*, 1997b,f	+	+	3
β-Estradiol	Human lymphocytes and sperm	Anderson *et al.*, 1997b,f	+	+	1
EGMME	Human lymphocytes	Anderson *et al.*, 1997b	±	nd	
	Human sperm	Anderson *et al.*, 1997b	+		
Ethylene oxide	Human fibroblast cell line VH-10	Nygren *et al.*, 1994	+	+	1
Ethylmethanesulphonate	HUT-78 T-lymphocyte cell culture	Shafer *et al.*, 1994	+	+	2B
	Rat and mouse hepatocytes	Hirai *et al.*, 1991	+		
	TK6 human lymphoblastoid cells	Henderson *et al.*, 1998	+		
N-Ethyl-*N*-nitrosourea	Lymphocytes from healthy donors and chronic lymphatic leukemia	Buschfort *et al.*, 1997	+	+	2A
	Human peripheral lymphocytes	Anderson *et al.*, 1997i	+		
	Human TK6 lymphoblastoid cells	Henderson *et al.*, 1998	+		
	CHO cells	Fortini *et al.*, 1996	+		
ENNG	CHO cells	Fortini *et al.*, 1996	+	+	
Etoposide	Chinese hamster V79 cells	Olive and Banath 1992, 1993b, Olive *et al.*, 1993a, 1997a	+	+	
	?	Olive *et al*, 1994c	+		
	Murine SCCVII cells	Olive *et al.*, 1997a	+		
	Human peripheral lymphocytes	Lebailly *et al.*, 1997	+		
Medicinal herbs extracts	Human peripheral lymphocytes	Basaran *et al.*, 1996	+	?	
Fluoroquinolones with simulated solar light	Mouse lymphoma cells	Chetelat *et al*, 1996	+	+	1
5-Fluorouracil	Mouse leukemia L1210 cells	Kasamatsu *et al.*, 1996	+/–	±	3
Formaldehyde	Human white blood cells	Pfuhler and Wolf, 1996	–	+	2A
Glutathione	Human peripheral lymphocytes	Thomas *et al.*, 1998	+		
Genistein	Human lymphocytes and sperm	Anderson *et al.*, 1997b	+	nd	
Hard metals (Co+W carbide)	Human lymphocytes	Anard *et al.*, 1997	+	?	
	Human leukocytes	Van Goethem *et al.*, 1997	+		
(*E*)-2-Hexenal	Rat and human primary colon mucosa cells	Golzer *et al.*, 1996	+	nd	
Hydrogen peroxide	Fresh and cryopreserved lymphocytes	Visvardis *et al.*, 1997	+	+	3
	Rat mesothelial and tracheal epithelial cells	Churg *et al.*, 1995	+		
	Intact, isolated intestinal crypts	Brooks and Winton, 1996	+		
	Bovine lens epithelial cells	Kleiman and Spector, 1993	+		
	HeLa	Collins *et al.*, 1993; O'Neill *et al.*, 1993	+		
	HL-60 cell line	Fairbairn *et al.*, 1993	+		
	Human lymphocytes	Singh *et al*, 1988, 1991, Tice *et al.*, 1990, 1991; Anderson *et al.*, 1994, 1995, 1997d Collins *et al.*, 1997	+		
	Mouse splenocytes	Grigsby *et al.*, 1993	+		
	Raji B cells	Meyers *et al.*, 1993	+		
	Murine lymphoma cell line L5178Y	Kruszewski *et al.*, 1994, Bouzyk *et al.*, 1997	+		
	Various human cell lines	Duthie and Collins, 1997	+		
	Mouse leukemia L1210 cells	Kasamatsu *et al.*, 1996	+		
	?	Olive *et al.*, 1994c	+		
	Raji lymphoblastoid cells	Sweetman *et al.*, 1997	+		
	Human TK6 lymphoblastoid cells	Henderson *et al.*, 1998	+		
	Human lymphocytes and sperm	Anderson *et al.*, 1997b	+		
	Human bladder-carcinoma cell lines	Ward *et al.*, 1993	+		
	Rat hepatocytes	Higami *et al*, 1994a,b	+		
	Friend erythroleukaemia cells	McCarthy *et al.*, 1997	+		

Table II. *Cont.*

Agents	Cell type	Reference	Response		
			Comet	Cancer	IARC classification (if applicable)
	Human and rat colon cells	Pool-Zobel and Leucht, 1997	+		
	Human sperm	McKelvey-Martin *et al.*, 1997	+		
Hydroquinone	Human peripheral lymphocytes	Anderson *et al.*, 1995, 1997g; Andreoli *et al.*, 1997	+	±	3
Kaempferol	Human lymphocytes and sperm	Anderson *et al.*,1997a	+	+	3
Kaempferol-3-rutinoside	Human lymphocytes	Anderson *et al.*,1997a	+	nd	
Lead acetate	Human lymphocytes and sperm	Anderson *et al.*,1997b	+	+	2B
Lead nitrate	Human lymphocytes and sperm	Anderson *et al.*,1997b	+	+	2B
Lead sulphate		Anderson *et al.*,1997b	+	+	2B
Lindane	Human lymphocytes	Pool-Zobel *et al.*, 1993b	–	+	2B
	Rat gastric and nasal mucosa cells	Pool-Zobel *et al.*, 1993b	+		
	Rat gastric cells	Pool-Zobel *et al.*, 1994	+		
Lithocholic acid	Human and rat colon cells	Pool-Zobel and Leucht, 1997	+	–	
Manganese chloride	Human lymphocytes	De Méo *et al.*, 1991	+	nd	
Menadione	Human lymphocytes	Woods *et al.*, 1997	+	nd	
D-Menthol	V79 Chinese hamster cells, human WBC	Hartmann and Speit, 1997	–	–	
Methotrexate	Mouse leukemia L1210 cells	Kasamatsu *et al.*, 1996	+/–	+	3
Methyl methanesulphonate	Human peripheral leukocytes and fibroblasts cell line MRC5CV1	Hartmann and Speit, 1996	+	+	2B
	Human white blood cells	Pfuhler and Wolf, 1996	+		
	Chinese hamster cell lines V-E5 and XR-V15B	Helbig and Speit, 1997	+		
Methylmercury chloride	Human and rat lymphocytes, rat hepatocytes and gastric mucosa	Betti *et al.*, 1993	+	+	2B
MNNG[a]	Human and rat lymphocytes, rat hepatocytes and gastric mucosa	Pool-Zobel *et al.*, 1993b; Betti *et al.*, 1993	+	+	2A
	Rat gastric mucosa	Kennelly *et al.*, 1993	+		
	Human VH10 and Hep G2 cells, hamster V79 cells	Slamenova *et al.*, 1997	+		
	Chinese hamster V79 cells and murine SCCVII cells	Olive *et al.*, 1997a	+		
	Mouse skin keratinocytes	Yendle *et al.*, 1997	+		
	Human gastric and gastric mucosal cells	Pool-Zobel *et al.*, 1994	+		
	Human and rat colon cells	Pool-Zobel and Leucht, 1997	+		
N-Methyl-*N*-nitrosourea	Mouse leukemia L1210 cells	Kasamatsu *et al.*, 1996	+	+	2A
N-Methyl-(*R*)-salsolinol	Human neuroblastoma SH-SY5Y cells	Maruyama *et al.*, 1997a,b	+	nd	
Metronidazole	Human lymphocytes	Re *et al.*, 1997	+	+	2B
Mitomycin C	Human TK6 lymphoblastoid cells	Henderson *et al.*, 1998	±	+	2B
	Human white blood cells	Pfuhler and Wolf, 1996	–		
Morphine	HUT-78 T cell culture	Shafer *et al.*, 1994	+	nd	
3-Morpholinosydnonimine	Rat islets of Langerhans, HIT-T15 cells	Delaney *et al.*, 1993; Green,I.C. *et al.*, 1994	–	nd	
	HIT-T15, HLCO, MRC5 SV-1 cells	Green,M.H. *et al.*, 1996			
	HIT-T15 cells	Delaney *et al.*, 1997a	+		
Muconic acid	Human peripheral lymphocytes	Anderson *et al.*, 1995	+	+	
Myricetin	Human sperm	Anderson *et al.*, 1997a,e	+	nd	
	Human lymphocytes	Anderson *et al.*, 1997c	+		
	Human Caco-2, HepG2, HeLa cells and lymphocytes	Duthie *et al.*, 1997	–		
Neocarzinostatin	Chinese hamster cell lines V-E5 and XR-V15B	Helbig and Speit, 1997	+	nd	
Nickel sulphate	Human gastric and nasal mucosal cells	Pool-Zobel *et al.*, 1994	+		
Nickel (crystalline nickel subsulfide)	Human embryo lung fibroblast cell line (MRC-5)	Zhuang *et al.*, 1996	+	+	1
Nitric oxide	Rat and human pancreatic β-cells	Delaney and Eizirik, 1996	+	±	
	Human and rat pancreatic islet cells	Eizirik *et al.*, 1996	+		
Nitrogen mustard	Chinese hamster lung cells	Olive *et al.*, 1992	+	+	2A
Nitromonomethyl arginine	Rat islets of Langerhans, HIT-T15 cells	Delaney *et al.*, 1993	–	nd	
p-Nitrophenol	V79 Chinese hamster cells, human WBC	Hartmann and Speit, 1997	–	–	
4-Nitroquinoline-1-oxide	Human cell line MRC5CV1	Speit and Hartmann, 1995	+	+	
	Human xeroderma pigmentosum cell line XP12ROSV	Speit and Hartmann, 1995	±		

Table II. *Cont.*

Agents	Cell type	Reference	Response		
			Comet	Cancer	IARC classification (if applicable)
	TK6 human lymphoblastoid cells	Henderson *et al.*, 1998	+		
	Mouse leukemia L1210 cells	Kasamatsu *et al.*, 1996	+/–		
4-Nitroquinoline-*N*-oxide	Chinese hamster V79 cells and murine SCCVII cells	Olive *et al.*, 1997a	+	nd	
S-Nitrosoglutathione	Rat islets of Langerhans	Delaney *et al.*, 1993	+	nd	
	HIT-T15 cells	Delaney *et al*, 1997a	+		
	Human peripheral lymphocytes	Thomas *et al.*, 1998	+		
Oxygen (hyperbaric)	Blood leukocytes	Dennog *et al.*, 1996	±	nd	
m-Phenylenediamine	Human peripheral lymphocytes	Plewa *et al.*, 1995	+	+	3
Polychlorinated biphenyls	Human peripheral lymphocytes	Belpaeme *et al.*, 1996b	–	+	2A
Potassium permanganate	Human lymphocytes	De Méo *et al.*, 1990	+	nd	
Potassium cyanide	Human TK6 lymphoblastoid cells	Henderson *et al.*, 1998	±	nd	
β-Propiolactone	Mouse skin keratinocytes	Yendle *et al.*, 1997	+	+	2B
Propylene oxide	Human VH-10 fibroblasts	Kolman *et al.*, 1997	+	+	2B
Quercetin	Human lymphocytes and sperm	Anderson *et al.*,1997a,e	+	±	3
	Human Caco-2, HepG2, HeLa cells and lymphocytes	Duthie *et al.*,1997	–		
Roussin's black salt	HIT-T15 cells	Delaney *et al*, 1997a	+	nd	
Rutin	Human lymphocytes	Anderson *et al.*, 1997a	+	nd	
(*R*)-Salsolinol	Human neuroblastoma SH-SY5Y cells	Maruyama *et al.*, 1997a,b	–	nd	
(*S*)-Salsolinol	Human neuroblastoma SH-SY5Y cells	Maruyama *et al.*, 1997a,b	–	nd	
Silymarin	Human lymphocytes	Anderson *et al.*, 1994, 1997a	–	–	
	Human lymphocytes	Anderson *et al.*, 1997c	+/–		
	Human sperm	Anderson *et al.*, 1997a,e	+		
	Human Caco-2, HepG2, HeLa cells and lymphocytes	Duthie *et al.*, 1997	–		
Sodium arsenite	Human leukocytes	Hartmann and Speit, 1994	+	+	1
Sodium arsenite (with MMS and BaP)	Human peripheral leukocytes and fibroblasts cell line MRC5CV1	Hartmann and Speit, 1996	+		
Sodium dichromate	Rat and human gastric and nasal mucosal cells	Pool-Zobel *et al.*, 1994	+		
Sodium lauroyl sarcosine	V79 Chinese hamster cells, human WBC	Hartmann and Speit, 1997	–	nd	
Sodium lauroyl sulphate	Human TK6 lymphoblastoid cells	Henderson *et al.*, 1998	±	–	
Styrene-7,8-oxide	Cultured human lymphocytes	Bastlova *et al.*, 1995	+	+	2A
Sulfonated anthraquinones	? (also anthraquinone derivatives)	Barnard *et al.*, 1995	±	?	
Nitrosyl iron-sulphur cluster	HIT-T15 cells	Delaney *et al.*, 1997a	+	nd	
Tirapazamine	murine (SCCVII, EMT6, RIF-1) and human (HT1080, A549, HT29) tumour cell lines	Siim *et al.*, 1996, 1997	+	nd	
	Murine spheroid and tumour cells	Olive, 1995a	+		
	Chinese hamster V79 cells and murine SCCVII cells	Olive *et al.*, 1997a	+		
1,1,2-Trichloroethane	Human lymphocytes	Tafazoli and Kirsch Volders, 1996	+	+	3
1,1,3-Trichloropropene	Human lymphocytes	Tafazoli and Kirsch Volders, 1996	+	nd	
1,2,3-Trichloropropane	Human lymphocytes	Tafazoli and Kirsch Volders, 1996	+	+	2A
Vanadium pentoxide	Human peripheral leukocytes and lymphocytes	Rojas *et al.*, 1996a	+	nd	
Various antitumour agents	Murine leukemia L1210 cells	Kasamatsu *et al.*, 1995	+	?	
Vitamin C	Human lymphocytes	Anderson *et al*, 1994; Green,M.H. *et al.*, 1994	+	–	
Vitamin E	Human lymphocytes	Anderson *et al.*, 1994	–	–	
Physical					
Densely ionizing radiation	CHO Chinese hamster ovary cells A_L	Jostes *et al.*, 1993	+	+	1
Hyperthermia	Human myeloid leukaemia K562	McNair *et al.*, 1997	–	nd	
Ionizing radiation	Human nucleated blood cells	Green,M.H. *et al.*, 1994, Plappert *et al*, 1997	+	+	
	Human peripheral lymphocytes	Wojcik *et al.*, 1996	+		
	Lymphocytes from children with systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis and dermatomyositis	McCurdy *et al.*, 1997	+		
Light	Human myeloid leukaemia K562	McNair *et al.*, 1997	–	–	
Laser pulses (308 nm) or dye laser (312–640 nm)	Cultured human lymphocytes	de With and Greulich, 1995	+	?	
Radiation	Human sperm	Hughes *et al.*, 1997	–	+	
	Various CHO cell lines	Hu and Hill, 1996	+		
	CHO-K1 cells	Kent *et al.*, 1995	+		
	Murine tumour cell lines KHT-LP1, B16F1, RIF-1 and SCCVII	Hu *et al*, 1995	+		
	Human peripheral lymphocytes	Malcolmson *et al.*, 1995	+		
Neutron radiation	Human melanoma cell line MeWo	Poller *et al*, 1996	+	+	

Table II. *Cont.*

Agents	Cell type	Reference	Response		
			Comet	Cancer	IARC classification (if applicable)
γ-Radiation	Fresh and cryopreserved lymphocytes	Visvardis *et al.*, 1997	+	+	
	Ataxia telangiectasia lymphoblasts	Humar *et al.*, 1997	+		
	Plant root cell from *Allium cepa* L.	Navarrete *et al.*, 1997	+		
	Intact, isolated intestinal crypts	Brooks and Winton, 1996	+		
	Bloom's syndrome cell lines YBL6 and GM 1492, normal human 1BR/3 fibroblasts	Nocentini, 1995	+		
Nonylphenol	Human lymphocytes and sperm	Anderson *et al.*, 1997b	+	nd	
	Human lymphocytes	Wojewodzka *et al.*, 1997	+		
Solar radiation	Human lymphocytes	Arlett *et al.*, 1993	+	+	1
Sparsely ionizing radiation	CHO Chinese hamster ovary cells A_L	Olive *et al.*, 1992	+	+	
	Human fibroblasts	Singh *et al.*, 1991	+		
	Human granulocytes	Vijayalaxmi *et al.*, 1993	+		
	Human leukocytes	Vijayalaxmi *et al.*, 1992; Green,M.H. *et al.*, 1994	+		
	Human lymphocytes	Singh *et al.*, 1988, 1990, 1994; Tice *et al.*, 1990; Vijayalaxmi *et al.*, 1992, 1993; Strauss *et al.*, 1994; Tice and Strauss, 1995a,b	+		
	V79 Chinese hamster lung cells	Olive *et al.*, 1992	–		
Ultrasound	CHO cells	Miller *et al.*, 1995	+	?	
Ultrasound (also lithotripter shock wave)	CHO cells	Miller and Thomas, 1996	+		
UV	Cultured CHO cells	Miller *et al.*, 1996	+	+	2A
	Bloom's syndrome cell lines YBL6 and GM 1492, normal human 1BR/3 fibroblasts	Nocentini, 1995	+		
	Human lymphocytes	Tice *et al.*, 1990	+		
	Human cell line MRC5CV1	Speit and Hartmann, 1995	+		
	Human xeroderma pigmentosum cell line XP12ROSV	Speit and Hartmann, 1995	±		
UV and γ-radiation	Human peripheral granulocytes and lymphocytes	Lankinen *et al.*, 1996	+		
γ-irradiation	Human bone marrow cells	Lankinen and Vilpa, 1997	+	+	2A
UVA	Hamster fibroblasts V79	Reavy *et al.*, 1997	+	+	2A
UVA+furonaphthopyranone	CHO AS52 cells	Adam *et al.*, 1997	+		
UVA, B, C and γ-rays	Human fibroblasts and lymphocytes	Alapetite *et al.*, 1996	+	+	2A
UVB	Cultured melanocytes	Noz *et al.*, 1996	+	+	2A
	Human lymphocytes and fibroblasts (normal, XP)	Arlett *et al.*, 1993	+		
UVB laser	Cultured human lymphocytes	de With *et al.*, 1994	+		
UVC	Intact, isolated intestinal crypts	Brooks and Winton, 1996	+	+	2A
	HeLa cells (+ aphidicolin)	Gedik *et al.*, 1992	+		
	Human lymphocytes (normal, XP)	Green,M.H. *et al.*, 1992	+		
X-rays	Human lymphocytes	Wojewodzka *et al.*, 1996	+	+	1
	Canine hemopoietic cells and stromal cells	Kreja *et al.*, 1996b	+		
	Peripheral blood cells	Plappert *et al.*, 1995	+		
	Raji lymphoblastoid cells	Sweetman *et al.*, 1997	+		
	Various tumour cell lines	Muller,W.U. *et al.*, 1994	+		
	Human sperm	McKelvey-Martin *et al.*, 1997	+		
	Human TK6 lymphoblast	Olive and Banath, 1995	+		
	Murine tumours and normal tissues	Olive *et al.*, 1994c	+		
	Mouse testicular cells	Zheng and Olive, 1997	+		
	Human melanoma cell line MeWo	Poller *et al.*, 1996	+		
Other					
Ageing	Rat hepatocytes	Higami *et al.*, 1994a,b	+	?	
Folate deficiency	Human lymphocytes and HeLa cells	Duthie and McMillan, 1997	+	nd	
Apoptosis (TGF-β-induced)	Retinal pigment epithelial cells	Esser *et al.*, 1997	+	×	
Apoptosis (1-methyl-2-nitroimidazole-induced)	CHO cells	Brezden *et al.*, 1997	+		
Cell cycling	Human lymphocytes	Salagovic *et al.*, 1997	+	×	
Cytokines (interleukin-1β + TNF-α + interferon-γ)	Human pancreatic islet cells	Delaney *et al.*, 1997b	+	nd	
Interferon-γ	Rat islet cell	Dunger *et al.*, 1996	+	nd	

Table II. *Cont.*

Agents	Cell type	Reference	Response		
			Comet	Cancer	IARC classification (if applicable)
Interleukin-1β	Rat islets of Langerhans cells	Delaney *et al*, 1993	+	nd	
	HIT-T15 cells	Green,I C *et al.*, 1994	+		
Light+haematoporphyrin derivative	Human myeloid leukaemia K562	McNair *et al.*, 1997	+	×	
Light + methylene blue	Human myeloid leukaemia K562	McNair *et al.*, 1997	+	×	
Light+ meso-tetrahydroxyphenylchlorin	Human myeloid leukaemia K562	McNair *et al*, 1997	–	×	
Oxygen radicals	Rat hepatocytes	Higami *et al.*, 1994a,b	+	–	
ROS generated by neutrophils	Cells from ataxia-telangiectasia and normal persons	Ward *et al.*, 1994	–	–	
Serum, novobiocin	Cultured CHO cells	Miller *et al.*, 1996	–	?	
Tobacco smoking	Blood samples	Tates *et al.*, 1996	±	+	1
Trypsin	Human TK6 lymphoblastoid cells	Henderson *et al.*, 1998	–	–	
Tumor necrosis factor α	Rat islet cell	Dunger *et al.*, 1996	+	?	

+, positive; –, negative; +/–, sometimes positive, sometimes negative; ±, equivocal, nd, no data found, ?, not sure or not chemically defined; ×, unspecified results for interest only.
[a]PhIP, 2-amino-1-methyl-6-phenyl-imadazo[4,5-*b*]pyridine; IQ, 2-amino-3-methylimidazo-[4,5-*f*]quinoline, Trp, 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole; AH, aliphatic hydrocarbons; DbCEAB, 2,5-diaziridinyl-3,6-bis(carboethoxyamino)-1,4-benzoquinone; DbEAB, 2,5-diaziridinyl-3,6-bis(ethanolamino)-1,4-benzoquinone: DMBA, 7,12-dimethyl-benz[*a*]anthracene; DMDHQ, 1,2-dimethyl-6,7-dihydroxyisoquinolinium ion; EGMME, ethyleneglycol monomethyl ether, ENNG, *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, ROS, reactive oxygen species.

have been followed, using different cell types, exposure regimens, DNA repair inhibitors, lysis and electrophoresis techniques and scoring criteria. How these factors affect the outcome of this genotoxicity assay is unknown. For the conduct of the assay, critical factors such as appropriate toxicity limits and criteria for a positive response have not been systematically investigated (unlike the usual alkaline elution assay; Storer *et al.*, 1996). In addition, the intra- and inter-laboratory variability in detecting standard genotoxins in the assay have not been studied. The impact of this potentially important assay upon carcinogen exposure evaluation will remain insignificant, until this situation changes. The problem, however, has been receiving attention.

Whether cytotoxins may give rise to Comets has been investigated by Hartman and Spiet (1995, 1997). They studied four cytotoxic compounds and concluded that the assay was not likely to give false positives due to extreme cytotoxicity, since cells with DNA 'clouds' that were assumed to represent dead cells were excluded from the evaluation.

Several compounds were examined by Henderson *et al.* (1999) which are toxic by different mechanisms in the Comet assay in TK6 human lymphoblastoid cells. At a top dose of 5 mg/ml, cycloheximide and trypsin gave a negative Comet response and no toxicity was observed, as measured by trypan blue exclusion immediately after exposure. Potassium cyanide and sodium lauryl sulphate produced a positive Comet response at cell survival levels of 75% or lower. The distribution of damaged cells indicated cells at various stages of necrotic cell death. Ethylmethane sulphonate, hydrogen peroxide, 4-nitroquinoline oxide, 9-aminoacridine, *N*-nitroso-*N*-ethylurea and glyoxal produced Comet tails. Mitomycin C was only weakly positive at survival levels of ~70%. The results indicated that the maximum concentration of test substance tested should produce viability >75% in order to avoid false positive responses due to cytotoxicity, according to prejudiced criteria. DNA damage was detected in the assay after induction by an alkylating agent, an intercalating agent and an agent causing oxidative damage. The cross-linking agent mitomycin C was not positive if a cut-off point of 75% viability was used as a criterion of a positive response.

Publications are available for 212 agents/factors that have been examined in the Comet assay performed either *in vitro* or *in vivo* in a variety of species. Individual agents are shown in the neutral and alkaline Comet assays (Tables I–III) and a summary table of responses is shown in Table IV. Unequivocal carcinogenicity data are available for 119 agents, amongst which unequivocal Comet assay data exist for 95 agents. The responses shown (plus or minus) are those stated by the authors of the papers cited. The cancer classification comes from the IARC database, the National Toxicology Program or from the general literature. Based on these 95 agents, the proportion of carcinogens in the population of chemicals, i.e. the prevalence, was 88% (84/95) and the assay characteristics with regard to carcinogen identification (after Cooper *et al.*, 1979) were: carcinogens that were Comet positive (sensitivity), 88% (74/84); non-carcinogens that were Comet negative (specificity), 64% (7/11); proportion of Comet-positive compounds that are carcinogens (positive predictivity), 95% (74/74 + 4); proportion of Comet-negative compounds that are non-carcinogens (negative predictivity), 41% (7/7 + 10). The number of carcinogens (74) and non-carcinogens (7) predicted by the Comet assay (accuracy/concordance) is 85% (81/95). This simple analysis of the Comet assay data has not taken account of the differences between *in vitro* and *in vivo* responses, species differences or organ and tissue differences. Twenty chemicals have, consequently, been judged as equivocal which are known carcinogens, such as 2-acetylaminofluorene, which has a different response in the Comet assay with rat and mouse hepatocytes, and benzene, which has a different response in different organs.

At the present time, the exact biological significance of the Comet positive response is not clear. It is known that the integrity of the genome is affected after treatment with an agent, but what proportion of the DNA damage is biologically relevant is uncertain. As is the case with DNA adducts, some of the DNA damage can be repaired and during repair mutation

Table III. Alkaline comet assay, *in vivo* applications

Agents	Cell type	Reference	Species	Response		
				Comet	Cancer	IARC classification (if applicable)
Chemical						
Acetochlor	Nasal cells	Ashby *et al.*, 1996	rat	–	+	
2-Acetylaminofluorene	Liver, lung, kidney, spleen, and bone marrow cells	Sasaki *et al.*, 1997c	mouse	+	+	
Acrylamide	Blood leucocytes, bone marrow, brain, liver, lung, ovary, skin, spleen and testis cells	Tice *et al.*, 1990, 1991; Friend *et al.*, 1993	mouse	+	+	2A
p-Aminoazobenzene	Liver, lung, kidney, spleen, and bone marrow cells	Sasaki *et al.*, 1997a	mouse	+	+	2B
Amsol	Tadpole erythrocytes	Clements *et al.*, 1997	*Rana catesbeiana*	–	?	
Atrazine	Tadpole erythrocytes	Clements *et al.*, 1997	*Rana catesbeiana*	+	+	2B
Auramine	Liver, lung, kidney, spleen, and bone marrow cells	Sasaki *et al.*, 1997a	mouse	+	+	2B
Antioxidants (vitamins C, E + β-carotene)	Peripheral lymphocytes	Duthie *et al.*, 1996	human	–	nd	
Benzene	Peripheral blood lymphocytes and bone marrow nucleated cells	Tuo *et al.*, 1996	mouse	+	+	1
	Blood, bone marrow, spleen, liver cells	Plappert *et al.*, 1994a,b	mouse	+/–		
BhC[a]	Liver, lung, kidney, spleen, and bone marrow cells	Sasaki *et al.*, 1997a	mouse	–	+	2B
Benzo[*a*]pyrene	Peripheral lymphocytes, bone marrow and liver cells	Vaghef *et al.*, 1996	mouse	+/–	+	2A
	Liver, lung, kidney, spleen, and bone marrow cells	Sasaki *et al.*, 1997c	mouse	+		
	Coelomocytes	De Boeck and Kirsch-Volders, 1997	*Nereis virens*	–		
MAOEAHAD[a]	T50/80 murine tumours	Hejmadi *et al.*, 1996	mouse	+	nd	
Bleomycin	Bone marrow and testicular cells	Anderson *et al.*, 1996	rat	–	+	2B
1,3-Butadiene	Bone marrow, liver and testicular cells	Anderson *et al.*, 1997d	mouse	–	+	2A
	Blood cells	Tates *et al.*, 1996	human	–	+	
Cadmium chloride	Plant root cells	Koppen and Verschaeve, 1996	*Vicia faba*	+	+	1
1-Chloromethylpyrene	Gastrointestinal tract cells	Kennelly *et al.*, 1993	rat	+	nd	
Cisplatin	Peripheral blood cells	Tice *et al.*, 1992	Human	+	+	2A
Colchicine	Liver, lung, kidney, spleen, and bone marrow cells	Sasaki *et al.*, 1997c	mouse	–	nd	
Cycloheximide	Plant root cells	Koppen and Verschaeve, 1996	*Vicia faba*	–	nd	
Cyclophosphamide	Peripheral blood cells	Hartmann *et al.*, 1995a	human	±	+	1
	Peripheral blood cells	Tice *et al.*, 1992	human	+		
	Peripheral lymphocytes, bone marrow and liver cells	Vaghef *et al.*, 1996	mouse	+		
	Lymphocytes	Hellman *et al.*, 1997	mouse	+		
	Bone marrow and testicular cells	Anderson *et al.*, 1996	rat	+		
2,4-Diaminotoluene	Liver, lung, kidney, spleen, and bone marrow cells	Sasaki *et al.*, 1997a	mouse	+	+	2B
p-Dichlorobenzene	Liver, lung, kidney, spleen, and bone marrow cells	Sasaki *et al.*, 1997a	mouse	+	+	2B
CPMMU[a]	Liver and testicular cells	Scassellati-Sforzolini *et al.*, 1997	rat	+/–	nd	
1,2,3,4-Diepoxybutane	Bone marrow and testicular cells	Anderson *et al.*, 1997i	mouse and rat	+/–	+	2B
p-Dimethylaminoazobenzene	Liver, lung, kidney, spleen and bone marrow cells	Sasaki *et al.*, 1997d	mouse	±	+	2B
1,2-Dimethylhydrazine	Colon cells	Pool-Zobel *et al.*, 1996; Rowland *et al.*, 1996; Hambly *et al.*, 1997	rat	+	+	2B
Dimethylnitrosamine	Blood leucocytes, ovary cells	Croom *et al.*, 1991	mouse	+	+	2A
1,2-Epoxybutene	Bone marrow and testicular cells	Anderson *et al.*, 1997d	mouse and rat	+/–	+	3
N-Ethyl-*N*-nitrosourea	Liver, lung, kidney, spleen and bone marrow cells	Sasaki *et al.*, 1997d	mouse	+	+	2A
EGMME[a]	Bone marrow and testicular cells	Anderson *et al.*, 1996	rat	+	nd	
Ethylene thiourea	Liver, lung, kidney, spleen, and bone marrow cells	Sasaki *et al.*, 1997a	mouse	+	+	2B
Ethylmethanesulphonate	Plant root cells	Koppen and Verschaeve, 1996	*Vicia faba*	+	+	2B

Table III. *Cont.*

Agents	Cell type	Reference	Species	Response		
				Comet	Cancer	IARC classification (if applicable)
	Erythrocytes	Belpaeme *et al*, 1996a	fish	+		
	Blood leucocytes, ovary cells	Croom *et al*., 1991	mouse	+		
	Bone marrow and testicular cells	Anderson *et al*., 1996	rat	±		
	Liver, lung, kidney, spleen, and bone marrow cells	Sasaki *et al*., 1997c	mouse	+		
	Coelomocytes	De Boeck and Kirsch-Volders, 1997	*Nereis virens*	+		
5-Fluorouracil	Liver, lung, kidney, spleen, and bone marrow cells	Sasaki *et al*, 1997c	mouse	–	±	3
Glyphosate	Tadpole erythrocytes	Clements *et al*., 1997	*Rana catesbeiana*	+	–	
Lindane	Gastrointestinal tract, nasal epithelial cells	Pool-Zobel *et al*, 1993b	rat	+	+	2B
Metalochlor	Tadpole erythrocytes	Clements *et al*., 1997	*Rana catesbeiana*	+	±	
Methyl methanesulphonate	Erythrocytes in tadpoles	Ralph *et al*., 1996	*Rana clamitans* and *Bufo americanus*	+	+	2B
	Plant root cells	Koppen and Verschaeve, 1996	*Vicia faba*	+		
	Liver, lung, kidney, spleen, and bone marrow cells	Sasaki *et al*, 1997c	mouse	+		
Methylmercury chloride	Blood leucocytes,	Betti *et al*., 1993	rat	–	+	2B
MNNG[a]	Blood leucocytes	Betti *et al*., 1993; Pool-Zobel *et al*, 1993a,b	rat	–	+	2A
	Colon cells	Pool-Zobel *et al*., 1996	rat	+		
	Gastrointestinal tract cells	Betti *et al*, 1993; Pool-Zobel *et al*, 1993a,b	rat	+		
	Liver, lung, kidney, spleen, and bone marrow cells	Sasaki *et al*., 1997c	mouse	+		
	Skin keratinocytes	Yendle *et al*., 1997	mouse	+		
MNAPB[a]	Blood leucocytes	Pool-Zobel *et al*., 1992	rat	–	nd	
	Nasal epithelial cells	Pool-Zobel *et al*., 1992	rat	+		
Mitomycin C	Plant root cells	Koppen and Verschaeve, 1996	*Vicia faba*	+	+	2B
	Liver, lung, kidney, spleen, and bone marrow cells	Sasaki *et al*., 1997c	mouse	+		
Metribuzin	Tadpole erythrocytes	Clements *et al*., 1997	*Rana catesbeiana*	+	–	
Nicotinamide (synergy with radiation)	SCCVII murine tumors and normal tissues	Zheng and Olive, 1996	mouse	+	?	
2-Nitropropane	Bone marrow cells	Deng *et al*, 1997	rat	+	+	2B
N-Nitrosodimethylamine	Blood leucocytes, nasal epithelial cells	Pool-Zobel *et al*., 1992	rat	+	+	2A
Phenacetin	Liver, lung, kidney, spleen, and bone marrow cells	Sasaki *et al*., 1997c	mouse	+	+	2A
Phenobarbital sodium	Liver, lung, kidney, spleen, and bone marrow cells	Sasaki *et al*., 1997c	mouse	+	+	2B
PCBP[a]	Erythrocyte	Pandrangi *et al*., 1995	bullheads and carp	+	+	2A
PAH[a]	Erythrocyte	Pandrangi *et al*., 1995	bullheads and carp	+	+	
Potassium bromate	Liver, lung, kidney, spleen, and bone marrow cells	Sasaki *et al*., 1997c	mouse	+	+	2B
Potassium chromate	Liver, lung, kidney, spleen, and bone marrow cells	Sasaki *et al*., 1997c	mouse	+	+	1
Potassium dichromate	Plant root cells	Koppen and Verschaeve, 1996	*Vicia faba*	+	+	1
β-Propiolactone	Skin keratinocytes	Yendle *et al*., 1997	mouse	+	+	2B
Pyrimethamine	Liver, lung, kidney, spleen, and bone marrow cells	Sasaki *et al*., 1997b	rat	+	±	3
RSU 1069	Tumour cells, spleen and marrow cells	Olive, 1995	mouse	+	nd	
Sodium arsenite	Blood leucocytes, bladder, liver, lung and skin cells	Tice *et al*., 1994; Yager *et al*, 1994	mouse	±	+	1
Streptozotocin	Kidney, liver cells	Schmezer *et al*., 1994	mouse	+	+	2B
Styrene	T-lymphocytes	Vodicka, 1995	human	+	+	2B
Styrene-7,8-oxide	Liver, lung, kidney, spleen, and bone marrow cells	Sasaki *et al*., 1997c	mouse	+	+	2A
Tirapazamine	Tumour cells, pleen and marrow cells	Olive, 1995b	mouse	+	nd	
	Multicell spheroids and tumors	Olive *et al*, 1996	mouse	+		

Table III. *Cont.*

Agents	Cell type	Reference	Species	Response: Comet	Cancer	IARC classification (if applicable)
3,3′,4,4′-tetrachlorobiphenyl	Erythrocytes	Belpaeme *et al.*, 1996a	fish	–	+	2A
Toluene	Blood leucocytes, bone marrow and liver cells	Plappert *et al.*, 1994b	mouse	–	±	3
Vanadium pentoxide	Testicular cells	Altamirano-Lozano *et al.*, 1996	mouse	+	nd	
Vitamin C	Human nucleated blood cells	Green,M.H. *et al.*, 1994	human	–	–	
Vitamin E	Peripheral white blood cells	Hartmann *et al.*, 1995b	human	–	–	
Physical						
Combination of radiation	T50/80 murine tumours	Hejmadi *et al.*, 1996	mouse	+	+	1
Ionizing radiation	SCCVII murine tumors and normal tissues	Zheng and Olive, 1996	mouse	+	+	1
	Human nucleated blood cells	Plappert *et al.*, 1997	human	+		
γ-Radiation	Lymphoid cells from the peripheral blood, spleen and thymus	Sirota *et al.*, 1996	mouse	+		
X-rays	Hypoxic cells in solid tumors and normal tissues	Olive *et al.*, 1994a	mouse	+		
	Testicular cells	Zheng and Olive, 1997	mouse	+		
	Peripheral blood and bone marrow cells	Kreja *et al.*, 1996a	dogs	+		
Other						
Autosomal recessive diseases: xeroderma pigmentosum and trichothiodystrophy, to UV challenge	Amniotic or chorionic villus cells	Alapetite *et al.*, 1997	human pregnancy	–	?	
Cancer	Bladder transitional cell carcinoma	McKelvey-Martin *et al.*, 1992, 1997	human	+	×	
Carotenoid-rich foods	Peripheral blood lymphocytes	Pool-Zobel *et al.*, 1997	human	–	?	
Cellular metabolism	Kidney cells	Fairbairn *et al.*, 1994	mouse	+	?	
Coke oven pollution	Coelomic leucocytes	Salagovic *et al.*, 1996	earthworm	+	+	
Diabetes (IDDM)	Peripheral leukocytes	Collins *et al.*, 1997	human	+	×	
Diabetes (IDDM and NIDDM)	Peripheral leukocytes	Anderson *et al.*, 1998	human	–	×	
Exhaustive exercise	Peripheral leukocytes	Hartmann *et al.*, 1995b	human	+	×	
Fly ash (coal)	Blood leucocytes, bladder, liver, lung and skin cells	Andrews *et al.*, 1994	mouse	±	?	
Hazardous waste	Blood, brain, liver and bone marrow cells	Nascimben *et al.*, 1991	mouse	+	×	
Infection, malnutrition	Peripheral leukocytes	Betancourt *et al.*, 1995	human	+	?	
Ischemia/reperfusion/ surgical trauma	Peripheral leukocytes	Dahouk *et al.*, 1997	human	+	×	
Male infertility	Human sperm	McKelvey-Martin *et al.*, 1997	human	–	×	
Physical activity	Blood leucocytes	Hartmann *et al.*, 1994	human	±	×	
Pollution (air containing PAH)	Peripheral blood cells	Binkova *et al.*, 1996	human	+	+	
Pollution (air)	Peripheral leukocytes, buccal and nasal epithelial cells	Valverde *et al.*, 1997	human	+	?	
Pollution (water containing agrochemicals)	Tadpole erythrocytes	Ralph and Petras, 1997	*Rana clamitans*, *Rana pipiens*	+	?	
Rubber manufacture	Peripheral lymphocytes	Moretti *et al.*, 1996	human	±	+	1
Oral squamous cell carcinoma	Peripheral leukocytes	Rao *et al.*, 1997	human	+	×	
Tobacco smoking	Peripheral lymphocytes	Duthie *et al.*, 1996	human	+	+	1
	Exfoliated buccal mucosa cells	Rojas *et al.*, 1996b	human	+		
	Peripheral lymphocytes	Betti *et al.*, 1995	human	±		
	Human lymphocytes	Betti *et al.*, 1994	human	–		
	Peripheral blood leukocytes	Frenzilli *et al.*, 1997	human	+		
Tumour oxygenation and ionizing radiation	C3H mammary tumours, SCCVII squamous cell carcinoma	Olive *et al.*, 1994, 1997b	mouse	+	×	
Vitamin C supplementation	Peripheral lymphocytes	Anderson *et al.*, 1997g	human	–	–	

+, positive; –, negative; +/–, sometimes positive, sometimes negative; ±, equivocal; nd, no data found; ?, not sure or not chemically defined; ×, unspecified results for interest only.

[a]BhC, benzene-1,2,3,4,5,6-hexachloride; MAOEAHAD, (1,4-bis-([2-(dimethylamino-*N*-oxide)ethyl]amino)5,8-dihydroxyanthracene-9,10-dione); CPMMU, 3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea; EGMME, ethyleneglycol monomethyl ether; MNNG, *N-methyl-N′*-nitro-*N*-nitrosoguanidine; MNAPB, 4-(*N*-Methyl-*N*-nitrosoamino-1-(3-pyridyl)-1-butanone; PCBP, polychlorinated biphenyl; PAH, polycyclic aromatic hydrocarbons.
IDDM, insulin dependent; NIDDM, non-insulin dependent diabetes mellitus.

Table IV. Comet assay and cancer responses for the 212 agents/factors cited

Cancer	Comet
Carcinogens (104)	–10
	+74
	±20
Non-carcinogens (15)	–7
	+4
	±4
Equivocal (9)	–1
	+6
	±2
No data found (53)	–13
	+33
	±2
Not chemically defined (19)	–4
	+13
	±7
Other factors (lifestyle, excercise) (12)	–2
	+9
	±1

+, positive; –, negative, ±, equivocal.

could occur. When daidzen, a phytoestrogen, produces damage in human lymphocytes and sperm, does this mean that this compound is a human carcinogen and germ cell mutagen? At present there is no answer to this question and, in the absence of human cancer and germ cell data for this compound, this cannot be confirmed. In fact, no adequate response is available to this type of question for any genotoxicity assay. It is now known that vitamin C has been shown to be positive in this assay and so has the flavonoid rutin. Both compounds are known to be anti-genotoxins, but, under certain conditions, vitamin C can act as a pro-oxidant, not only in the Comet assay, but also in other assays (Anderson *et al.*, 1994). Other factors, such as physical exercise and ageing, can cause an increase in Comet damage and have also been shown to affect other genotoxicity assays. Such lifestyle effects have to be taken into account in study design. The significance of such findings for any genotoxicity assay remains undetermined. Also, limitations as to the conduct of the Comet assay do not appear to have been examined in any depth. However, within an assay there should always be concurrent controls and damage observed will be over and above control levels. The assay itself takes less than a day to achieve a result and can be used with single mammalian cells from any organ or tissue from any species. It is currently used for many functions, not only for genotoxicity assays *in vitro* and *in vivo*, but for clinical purposes and human monitoring, and new applications are continuously being found (Anderson and Plewa, 1998).

In conclusion, at the present time the Comet assay has high sensitivity for carcinogens, but its specificity is uncertain because few non-carcinogens have been tested.

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