Comet assay application in environmental monitoring: DNA damage in human leukocytes and plant cells in comparison with bacterial and yeast tests

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Urban airborne particulate is a complex mixture of air pollutants, many of which have not been identified. However, short-term mutagenesis tests together with chemophysical parameter analysis are able to better assess air quality and genotoxic load. The findings of continuous monitoring (January 1991–August 1998) of urban air genotoxicity of a Po Valley town (Italy) on Salmonella typhimurium and Saccharomyces cerevisiae are reported. During this period, various measures (catalytic devices, unleaded fuels, annual vehicle overhaul, etc.) to improve air-dispersed pollutant control were enforced. However, a continuous presence of genotoxic compounds is shown and more qualitative than quantitative changes are evident. We also demonstrate the ability of the Comet assay to detect DNA-damaging agents in airborne particulate samples. We applied the test to human leukocytes and, with major improvements, to plant cells (Allium cepa roots and epigean tissues of Impatiens balsamina). The first findings on human leukocytes confirm the sensitivity of this assay, its peculiarity and its applicability in assessing genotoxicity in environmental samples. The capability of plants to show the response of multicellular organisms to environmental pollutants largely counterbalances a probable lowering in sensitivity. Moreover, application of the Comet test to epigean tissues could be useful in estimating the bioavailability of and genotoxic damage by air pollutants, including volatile compounds (ozone, benzene, nitrogen oxides, etc.) to higher plants.

Introduction

The ability of chemical substances, extracted from samples of outdoor and indoor airborne particulates, to induce mutagenicity in prokaryotic cells such as Salmonella typhimurium has been widely investigated (most recently by Azuma et al., 1997; Chiang et al., 1997; Kuo et al., 1998; Monarca et al., 1998; Wasserkort et al., 1998; Zhou and Ye, 1998). Some studies have used eukaryotic cell systems, such as the yeast Saccharomyces cerevisiae (Rossi et al., 1995a; Bronzetti et al., 1997). However, human health risk determination associated with air pollutants is not easily extrapolated from these findings. On the other hand, direct monitoring, normally used on professionally exposed workers, cannot be applied to the general population. Furthermore, the international scientific community is encouraging scientists to develop alternative (non-animal) tests for DNA-damaging agents. Particularly appealing is the use of in vitro human cells for better extrapolation to human health (Sasaki et al., 1997; Holian et al., 1998) and of plant material for a more accurate assessment of environmental health (Fomin and Hafner, 1998; Monarca et al., 1998). A good correlation between the data from plant bioassays and those from mammalian systems has been demonstrated. Furthermore, higher plants represent a stable sensor in an ecosystem and hence allow us to follow the evolution of the genotoxic impact.

In this contest the availability of possible alternative/integrative solutions could be of some relevance for environmental genotoxicity assessment.

Our previous reports (Poli et al., 1992; Rossi et al., 1995b) confirmed the complexity of environmental mixtures and the difficulty of assessing the real amount of such genotoxic compound emission in the environment.

The aim of the present research was to compare the genotoxic effects induced by urban airborne particulate mixtures in prokaryotic (Salmonella) and lower eukaryotic (yeast) cells and the DNA damage in human leukocytes and plant cells.

Airborne particulate samples from a mixed residential-commercial area of Parma, a Po valley town, were collected over a long period (April 1990–September 1998) and tested with different assays. The Ames test (Maron and Ames, 1983) and Saccharomyces cerevisiae diploid strain D7 assay (Zimmermann et al., 1975) were performed on all the samples, the latter being able to demonstrate nuclear DNA effects, such as gene conversion and point mutation, and to assess mutation induction in the mitochondrial genome, i.e. respiratory proficiency to respiratory deficiency. Alkaline single-cell gel electrophoresis (SCGE) on in vitro human leukocytes (Fairbairn et al., 1994; Speith et al., 1996; Calderón-Garcidueñas et al., 1997; Malyapa et al., 1997a,b; Mitchelmore and Chipman, 1998; Steinert et al., 1998; Wilson et al., 1998), which can be applied to virtually any eukaryotic cell.

Materials and methods

Chemicals

Ethidium bromide, L-tryptophan, L-isoleucine and adenine were from Fluka (Röhn Laborchemica GmbH & Co. KG); Tris from ICN Biochemicals (Milan, Italy); yeast extract, bacto peptone and agar from Difco (Detroit, MI); 2,3,5-triphenyl-tetrazolium chloride, hycanthone, EDTA and all other laboratory
Particulate matter was collected using a suction apparatus consisting of a filter (Ø = 47 mm), a filter holder and a low volume sampler (Tecora Bravo 96). The sampling was continuous during a 24 h period (sample flow 1 m³/h) and the daily filters were pooled to obtain the monthly samples. The samples were extracted in a Soxlet apparatus, evaporated with a rotary evaporator and redissolved (50 mg/ml) in dimethyl sulfoxide (DMSO).

Tests on microorganisms

Ames test. The mutagenic activity of airborne particulate extract was studied using the Salmonella typhimurium TA98 and TA100 strains TA98 and TA100, respectively. The cells (10⁴ cells/ml) were added with or without microsomal activation (Maron and Ames, 1983). The data obtained are presented as revertants/m³ of air sample, calculated from the linear portion of the dose–response curves (six doses) by linear regression analysis. DMSO (160 µl/litre) was used as a negative control, whereas hycanthone (50 µg/plate) and 2-aminofluorene (2.5 µg/plate) were used as positive controls without and with S9 mix, respectively (Gatehouse et al., 1994; OECD, 1994). Yeast assays. The diploid strain D7 of *S.cerevisiae* (Zimmermann et al., 1975) was used to determine the reversion frequencies of the *ihl1-92* mutant and of mitotic gene conversion at the trp5 locus with or without endogenous activation. As an alternative system to the microsomal assay, yeast cells were harvested during the logaritmic phase of growth in 20% glucose at maximum activation of the cytochrome P450 complex (Rosti et al., 1997; Poli et al., 1999). Both with and without endogenous metabolic activation, the (10³ cells/ml) were inoculated in 0.1 M phosphate buffer, pH 7.4, in the presence of different (at least five) concentrations of test samples and kept in an alternating shaker (110 r.p.m.) at 37°C for 2 h. A least squares linear regression analysis was used to calculate specific genotoxic activity (mutants or revertants/m³ of air sampled, with reference to the treated population of 10⁷ cells). DMSO (62.5 µl/ml) was used as a negative control; 2-aminofluorene (50 µg/ml) and hycanthone (100 µg/ml) and ethyl methanesulfonate (100 mM) were used as positive controls when cytochrome 450 was induced or not, respectively.

Mitochondrial DNA mutation induction was evaluated in the same strain by determining the frequencies of respiratory-deficient colonies (Poli et al., 1992; Rossit et al., 1995, 1996). The cells (10⁴ cells/ml) were cultured (26°C for 27 h) in the presence of different (at least five) sample concentrations and then plated on a solid complete medium with glucose as the sole carbon source. To detect petite mutants, the plates were overlayed with agar containing tetrazolium after 5 days incubation at 28°C (Ogur et al., 1957). After –1 h, the respiratory-proficient colonies turned red, while the respiratory-deficient colonies remained white. The data are reported as respiratory deficiency increase/m³ of air, with reference to 10⁶ cells. DMSO (12.5 µl/ml) and ethidium bromide (1 µg/ml) were used as negative and positive controls, respectively.

For all the assays the data were analysed using the modified two-fold rule (Chu et al., 1981) in which a response is considered positive if the average response at least twice mixed with low melting point agarose (LMA) and a and placed for the Ames test and for the gene conversion test in strain D7; more than 3-fold for the *ihl1-92* reversion and respiratory deficient mutant tests. The data obtained were subjected to Student’s t-test.

Single cell gel electrophoresis

Human leukocytes. For leucocyte isolation, whole blood of healthy donors was centrifuged twice in lysis buffer (155 mM NaCl, 5 mM KClO₄, 0.005 mM Na₂EDTA, pH 7.4), washed with phosphate-buffered saline (PBS) and resuspended (10⁶ cells/ml) in RPMI-1640 medium. Medium containing cells, appropriate volumes of particulate sample and 0.1 M phosphate buffer (pH 7.4) or of which was added to an Eppendorf tube. S9 was prepared as described by Maron and Ames (1983). S9 was diluted 1:10 in 0.1 M phosphate buffer (pH 7.4) containing NAPD (4 mM), glucose 6-phosphate (5 mM), MgCl₂ (8 mM) and KC1 (33 mM) to constitute S9 mix, 250 µl of which was added to each tube to make a total 1 ml cell suspension. Treatments were for 1 h at 37°C. Negative (DMSO, 50 µl/ml) and positive (Melphalan, 10 µg/ml) controls were also performed.

Aliquots of the cell suspension were used to check viability by the Trypan blue exclusion method and the percentage of apoptotic cells by Tunel assay (Gorzca et al., 1993). Another aliquot was used for SCGE, basically performed according to Singh et al. (1988). Degraded ribbons were previously dipped in 1% normal melting point agarose for the first layer. The cells (~2 × 10⁶ cells) were resuspended with low melting point agarose (LMA) and placed on the first layer. Finally, LMA was added as the top layer. The cells were lysed at 4°C in the dark (2.5 M NaCl, 10 mM Na₂EDTA, 10 mM Tris–HCl, 1% Triton X-100 and 10% DMSO, pH 10) for at least 1 h. The DNA was allowed to unwind for 20 min in alkaline electrophoresis buffer (1 mM Na₂EDTA, 300 mM NaOH, pH 13) and subjected to electrophoresis in the same buffer for 20 min at 0.78 V/cm and 300 mA.

Plant cells. Growth of A.cea was obtained by placing the bulbs at 25°C on a tray in the dark so that only their bases were in contact with tap water. For the experiments, roots larger than 2 cm in length were excised from the bulb and dipped (with the apex resting at the bottom) for 3 h at 26°C in Eppendorf tubes containing H₂O and different concentrations of the substances to be tested.

Seed derived plantlets (3–4 weeks old) of *I. balsamina* were used throughout. Preliminary experiments showed that nuclear suspensions suitable for the SCGE assay may be obtained from root, stem and leaf cuttings and processed essentially as described for *A. cea*. The protocols were maintained in the appropriate solution of the tested compounds for 3 h at 26°C.

The procedure described by Navarrete et al. (1997) for nuclei isolation from root cells of *A. cea* was utilized with major improvements. Roots were cut and gently squeezed, using forcesps, directly into a drop of LMA (0.5% in PBS) resting on the top of the first agarose layer. The slides were placed on a warm surface (37°C) during this stage. The described modifications of the procedure resulted in an increased yield of nuclei and a more uniform distribution of nuclei in the agarose layer. Optimal pre-electrophoresis and electrophoresis conditions were determined testing different voltage and time schedules: 30 and 20 min of pre-electrophoresis and 10 and 20 min of electrophoresis for *A. cea* and *I. balsamina*, respectively, at 230 mA and 0.66 V/cm were selected for the experiments.

All the steps for slide preparation were performed under a yellow light to prevent additional DNA damage. Once electrophoresis had been carried out, the slides were washed in neutralization buffer (0.4 M Tris–HCl, pH 7.5). Immediately before examination, the DNA was stained with 100 µl ethidium bromide (2 µl/ml). The samples were examined under a fluorescent microscope (Leitz Dialux 20) equipped with excitation filter BP 515–560 nm and barrier filter LP 580 nm, using an automatic image analysis system (Cometa Release 2.1; Sarin, Florence, Italy). One hundred cells per sample, selected at random, were analysed under constant sensitivity.

The comet length, measured as the distance between the leading edge of the comet head and the end of the tail, was chosen to estimate DNA damage. Results are presented as frequency distributions of single cell DNA damage as or as box and whisker plots. In this case measured values at the tested concentrations are shown as boxes that include 50% of the data. The top and bottom of the boxes mark the 25th and 75th centiles and the inner line marks the median value; 25% of the data above the 75th centile and 25% below the 25th centile are marked as ‘whiskers’ limited by the maximum or minimum values. Outliers are displayed as points.

The relationship between comet length and dose was calculated as mean comet length increase/m³ air equivalent from the dose–response curves by linear regression analysis.

A SPSS 8 statistical package was used. Variance analysis was by one or multiple pairwise comparisons; the data obtained were also subjected to Student’s t-test.

The samples were coded and evaluated blind. All the tests were usually performed once, since the amounts of our environmental samples were insufficient to repeat the tests in independent trials.

Results

Assays on microorganisms

The mutagenicity data on the two strains of *S. typhimurium* (Table 1), with or without S9, show a seasonal trend with negligible values during the summer. The highest induction of revertants occurred during the year 1992. A decrease during the following years is evident, with special regard to the annual mean values. This fact could be explained by the decreased concentration of some compounds in the air due to the enforcement of catalytic converters in new vehicles since the year 1993. However, this hypothesis is not confirmed by the data on *S. cerevisiae* (Table 1). In this organism, with endogenous metabolic activation (i.e. a high cytochrome P450 cellular content), induction of both convertants and revertants is increased by the particulates collected after the introduction of the catalytic device. Respiratory deficiency induction does not appear to correlate with any measure against air pollution or exogenous factors such as seasonal meteorologic conditions. The disagreement between the tests could be ascribed
### Table I. Urban air particulate genotoxicity effects

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Monthly mean value per m$^3$ air equivalent in different genetic targets: reversion in *Salmonella* strains TA98 and TA100 with (+) and without S9; gene conversion (GC) and point mutation (PM), with (+) and without cytochrome P450 induction; respiratory deficiency (RD) in *S.cerevisiae* strain D7. Total = sum of effects. Annual means are reported. Italics represent the four months tested with the Comet assay.

to the different sensitivity of the two organisms toward different classes of chemicals.

**SCGE on human leukocytes**

To evaluate the sensitivity of the Comet assay on human leukocytes in vitro for detecting genotoxicity in urban airborne particulates we tested samples from four different months, collected in the centre of Parma. The four samples, previously assayed on strains TA98 and TA100 of *S.typhimurium* (Figure 1) and on the diploid strain D7 of *S.cerevisiae* (Figure 2), were selected since they showed different effectiveness on the various targets.

**July 1996.** Very low revertant induction in *Salmonella* (~4 revertants/m$^3$ for the two strains); a low increase in convertant (1- to 2-fold) and revertant (1- to 2.5-fold) frequencies; an increase (~8 times) in respiratory deficiency (Table I).

**February 1997.** Very high effectiveness on TA100 (~20 and 35 revertants/m$^3$ with and without S9, respectively), on TA98 (~30 and 34 revertants/m$^3$ with and without S9, respectively) and on strain D7 when induced for cytochrome P450 (~50 and 15 times the spontaneous frequencies of gene conversion and reversion, respectively). A toxic effect was observed at 3.2 m$^3$ air equivalent. Effects on mitochondria are not evident. April 1997. Medium effects on *Salmonella* (~13 and 9 revertants/m$^3$ in TA100, with or without S9 and ~8 and 11 partilcates we ... 3 in TA98, with or without S9); convertant induc-collected in the centre of Parma. The four samples, previously assayed on strains TA98 and TA100 of *S.typhimurium* (Figure 1) and on the diploid strain D7 of *S.cerevisiae* (Figure 2), were selected since they showed different effectiveness on the various targets.

**November 1997.** Medium-high effects on *Salmonella* (~18 and 7 revertants/m$^3$ in TA100, with or without S9 and ~17 and 15 revertants/m$^3$ in TA98, with or without S9); the effects on *S.cerevisiae* are low or negligible.

These differences could be explained not only by the different genotoxic load in the four samples but also by the...
effectiveness on different targets of compounds present in the four mixtures. The different cellular organization of Salmonella and S. cerevisiae can affect the response to xenobiotic compounds. Furthermore, in yeast recombinational events are evaluated together with reverse mutation. Recombinational events are increased by exposure to genetically active chemicals in a non-specific manner while the responses of the two Salmonella strains are specific enough towards some classes of compounds.

In order to determine the presence in these environmental mixtures of chemical classes able to induce DNA strand breaks and alkali-labile sites and also to compare the DNA effects on prokaryotic or eukaryotic microorganisms and on human cells, an SCGE assay on human leukocytes was performed, with and without addition of the hepatic microsomal fraction (S9) of induced rats.

The samples were tested at different concentrations. Neither cell viability (95–100%) nor apoptosis (0–2%) were affected. An analysis of the genotoxic effects, without (Figure 3) and with S9 (Figure 4), shows the different effectiveness of the four samples on the induction of DNA damage ($P < 0.001$, univariate analysis of variance). Without S9, February is the more genotoxic sample with respect to the negative control ($P < 0.001$, Student’s t-test), with effects at the highest dose comparable with the positive control effects. The addition of S9 mix appears to partially detoxify some compounds in the February sample ($P < 0.01$, univariate analysis of variance). The differential genotoxic action of the four monthly extracts is further confirmed by the mean comet length increase/m$^2$ air equivalent (Figure 5). Without S9 addition, February ($P < 0.001$, Student’s t-test) and April ($P < 0.01$, Student’s t-test) are more genotoxic than July and November. In the presence of S9 mix, February and April appear more effective on DNA than July, although the differences are not significant. November does not show a significant effect on DNA.

**SCGE on A. cepa root cells**

Preliminary experiments were performed to test the reliability and sensitivity of the overall procedure. The Comet assay in A. cepa was originally used (Navarrete et al., 1997) to test DNA damage induced by irradiation of the bulbs, before root growth; no data were reported by the authors on the effect of chemical mutagens on the roots.

At first, for the sake of convenience, we wanted to ascertain the possibility of using roots detached from the bulbs; this would have provided us with the opportunity to use a single bulb (or the least possible number of bulbs) for all the treatments required for an experiment. To perform this test, H$_2$O$_2$, a DNA-damaging agent, was used on roots detached from or connected to the bulb. High concentrations (100 mM) of H$_2$O$_2$ (30 min at 26°C) were used in order to obtain significant effects on the nuclei, since a considerable amount of the H$_2$O$_2$ may be inactivated by a specific enzymatic apparatus or by the presence of protective agents such as flavonoids (see for example Duthie et al., 1997) in A. cepa root cells. The observation that comparable results were obtained under both conditions (data not shown) prompted us to adopt the detached root system for further experiments.

We then assayed the effect on this system of Cr$^{6+}$, a heavy
Fig. 3. Genotoxic effects of four airborne particulate monthly samples on human leukocytes. DNA breaks as measured by the Comet assay. Cells were treated without S9. Negative (DMSO, 50 µl/ml) and positive (Melphalan, 10 µg/ml) controls are reported. The data are displayed as frequency distribution of comet length values. Values >70 µm are not reported.

metal for which genotoxic effects have been assessed in *Vicia faba* with the use of SCGE (Koppen and Verschaeve, 1996). K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> at concentrations ranging from 10 to 80 mM was effective in increasing the comet length of *A.capa* root cells (Figure 6) as compared with the negative control (*P* < 0.001, Student’s *t*-test). Thus, the responsiveness of our modified plant SCGE assay to Cr<sup>6+</sup> treatment as a positive control prompted us to analyse the genotoxic potential of the previously described four samples of airborne particulates from Parma.

Three concentrations corresponding to 1.25, 2.50 and 5.00 m<sup>3</sup> air equivalent/ml were tested for their genotoxic effect (Figure 7). A concentration of DMSO equivalent to that present at the highest dose of particulate was used as a control. No effects were observed in the samples treated with the July 1996, April 1997 and November 1997 samples, whereas a significant dose-dependent increase in DNA damage was observed in those treated with the February 1997 airborne particulate (*P* < 0.001, Student’s *t*-test). The DNA damage at the highest dose was comparable with that observed when the roots were treated with 40 mM Cr<sup>6+</sup>.

**SCGE on *I.balsamina***

We wanted to verify the possibility of extending the plant Comet assay to different plant systems that may prove more suitable for *in situ* monitoring of the genotoxic potential of air pollutants. *Impatiens balsamina* was chosen as the result of a preliminary screening within ornamental plant species.

Plant roots, stems and leaves can represent different targets for the various environmental pollutants (in the soil, water or air). With a view to improving our understanding of the different bioavailability of a pollutant to roots, stems and leaves depending on the means of uptake, we evaluated the DNA damage in these different parts of *I.balsamina* treated with Cr<sup>6+</sup> through a radical apparatus dipping. Whole plantlet roots were exposed to different Cr<sup>6+</sup> concentrations and then washed with water. The cuts were performed on the main root, on the stem and on the first leaf. The data (Table II) show an increase in DNA migration in all the treated samples with respect to the controls (*P* < 0.001, Student’s *t*-test). Increasing Cr<sup>6+</sup> concentrations resulted in a decrease in the comet length values. This can be related to the simultaneous increase in the number of ‘clouds’ of DNA fragments, with disintegration of the head region, identified as ‘ghost’ cells (e.g. at 50 mM, ghost cells were only 3% for root, whereas they were 44% for stem and 28% for leaf). These cells were not considered in the analysis.

Three concentrations of a different airborne particulate sample from Parma, January 1998, were tested for their genotoxic effect on stem cuttings of *Impatiens* (Figure 8). The particulate induces a significant increase in DNA migration with respect to the control (Student’s *t*-test, *P* < 0.001). The 2.5 m<sup>3</sup> sample is different from the 1.25 m<sup>3</sup> sample (*P* <
Comet assay in environmental monitoring

Fig. 5. Dose–effect relationships between four airborne particulate monthly samples and mean values of comet length in human leukocytes, with (+S9) or without (−S9) exogenous metabolic activation. Regression lines are reported.

![Graph of dose–effect relationships]

Fig. 6. Genotoxic effects on *A. cepa* roots of K₂Cr₂O₇. DNA breaks as measured by the Comet assay. Results are reported as comet length and displayed as box plots (see Materials and methods).

![Box plots of DNA breaks]

0.002, *t* test) whereas the 5.0 m³ sample does not show any significant differences from the 2.5 m³ sample. This could be due to the simultaneously increased induction of ‘ghost cells’ (not considered in the analysis) at the highest dose.

**Discussion**

Improvements in air quality is one of the main goals of The European Community and, in this context, airborne particulate genotoxicity appears to be one of the more promising indices of human genotoxic/cancerogenic risk assessment.

The time series of the data allowed us to describe the Parma urban air genotoxic load over a long period of time (1990–1998), in the course of which various measures (catalytic devices, unleaded fuels, annual vehicle overhaul, etc.) to improve air-dispersed pollutant control were enforced. Some chemico-physical parameters, such as lead and sulphur dioxide, decreased with increased unleaded petrol use (data not reported). However, the findings on airborne particulate genotoxicity, assessed over the period by short-term mutagenicity

![Graphs of DNA damage]

Fig. 7. DNA damage induced by four different airborne particulate extracts on *A. cepa* root cells. DNA breaks as measured by the Comet assay. Results are reported as comet length and displayed as box plots.
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<tr>
<td>20</td>
<td>34.2 ± 6.4</td>
</tr>
<tr>
<td>50</td>
<td>43.8 ± 9.6</td>
</tr>
<tr>
<td>80</td>
<td>30.1 ± 9.2</td>
</tr>
</tbody>
</table>

*Ghost cells >80%.

The approach proves to be very helpful in environmental monitoring since these biological assays appear more sensitive and effective than the chemophysical parameter measure alone. Nevertheless, further investigations are necessary for more complete genotoxic compound detection in the urban environment.

In this context, in order to evaluate the sensitivity of the Comet assay for environmental mixture genotoxicity detection, we applied the test to human leukocytes and to plant cells, previously treated with some urban airborne particulate samples presenting not only a different quantitative mutagenic load but also a differential action on the considered DNA targets in microorganisms. The Comet assay was used on human and plant cells in comparison with results on *Salmonella* and *S. cerevisiae*.

The first data on human leukocytes by SCGE confirm the sensitivity of the assay and its applicability in assessing genotoxicity in environmental samples such as airborne particulates. A peculiarity of the Comet assay is evident. Our knowledge concerning the xenobiotic metabolizing capacity of human blood cells is rather limited, however, blood monocytes have been shown to constitutively express P450 isoenzymes (Baron et al., 1998). On the other hand, addition of the rat hepatic microsomal fraction allowed partial detoxification of the February 1997 sample. A comparison of the data shows a different response of human cells with respect to the tests on *Salmonella*: leukocytes treated in the presence of S9 mix show a behaviour similar to gene conversion in *S. cerevisiae* when not induced for cytochrome P450 complex (*P* = 0.05, Spearman *r*); on the other hand, leukocytes without S9 correlate with gene conversion in yeast induced for cytochrome P450 (see Table I and Figures 1, 2 and 5). Nevertheless, when the sum of the two (±S9) mean comet length increases/m³ in human cells (July, 1.9; February, 4.9; April, 2.8; November, 1.1) were compared with the sum of the effects in *Salmonella* and *S. cerevisiae* (see Table I), respectively, a similar trend (*P* = 0.01, Spearman *r*) was obtained between the effects on leukocytes and on the eukaryotic microorganism.

These preliminary findings suggest a good correlation between genotoxic effects in yeast and DNA damage in human cells.

The measurement of DNA damage in the nuclei of higher plant tissues is a new area of study with SCGE (Koppen and Verschaeeve, 1996; Gichner and Plewa, 1988; Koppen and Angelis, 1998). This assay could be incorporated into *in situ* plant monitoring of the atmosphere, water and soil: the Comet assay allows fast detection of DNA damage without any need to wait for progression into mitosis. However, the presence of a cell wall causes technical difficulties for SCGE. This cell wall proved to be a resistant barrier to cell lysis when using a lysis buffer sufficient to degrade animal cells. In this context, we improved previously described protocols (Navarrete et al., 1997) in *A. cepa*, increasing the nuclear yield and widening its application to different experimental conditions.

The protocol was validated by using a genotoxic compound (Cr⁶⁺): a dose–effect response was observed. The first findings on airborne particulate extracts confirm the high genotoxic load of the February 1997 sample.

Plant systems represent a complex multicellular environment where the efficiency of different protection or repair mechanisms can be modulated by cellular homeostasis. However, the capability of the system to show a multicellular organism response against environmental pollutants widely counterbalances a probable lowering in sensitivity, for example, to compounds such as free radicals (Duthie et al., 1997).

Furthermore, our revised Comet assay was applied to different tissues (root, stem and leaf) of *I. balsamina* to verify whether plant SCGE could be used as a suitable tool for monitoring primary DNA damage induced by environmental pollutants.

In order to improve our understanding of the different bioavailability of a pollutant to roots, stems and leaves
depending on uptake, we evaluated the DNA damage in these different parts of *Ibalsamina* treated with Cr⁶⁺ through a radical apparatus dipping. Cr⁶⁺ was able to induce DNA damage in all the tissues. The different sensitivity (root < leaf < stem) could be ascribed to the different cell types but also to the different genotoxic action (i.e. Cr⁶⁺ and/or generated free radicals). DNA damage was also induced by airborne particulate samples (January 1998). These preliminary findings suggest that application of the test to different plant tissues could be useful in estimation of the bioavailability and genotoxic damage due to environmental pollutants, including volatile compounds such as ozone, benzene, nitrogen oxides, etc., in higher plants.

In conclusion, the results of this study confirm that SCGE is applicable to genotoxic load assessment of environmental mixtures such as urban airborne particulates in comparison with classical short-term mutagenesis tests. The use of human cells could give further information regarding environmental genotoxic load. Higher plants have a long tradition of use in mutation research and the inclusion of SCGE in plant systems (roots and epigean tissues) could extend their utility in *in situ* environmental mutagenesis.

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References


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