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Alkaline comet assay in human biomonitoring studies by the Latin America / LA-COMET group

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ABSTRACT

Technological and chemical advancements have heightened human exposure to synthetic and natural substances, necessitating precise toxicological assessments through sensitive biomarkers. The comet assay has emerged as a robust, minimally invasive tool for biomonitoring. This study reviews 64 articles published between 1997 and 2022 by the LA-COMET group—a collaborative network of 17 research teams across seven Latin American countries (Argentina, Bolivia, Brazil, Colombia, Mexico, Paraguay, and Uruguay). The analysis reveals that research primarily focuses on occupational and environmental exposures, specifically evaluating the impact of pesticides, heavy metals, organic solvents, and particulate matter. The assay proved highly effective in identifying genotoxic effects in exposed populations compared to reference groups, including vulnerable cohorts such as children, women, and patients with clinical conditions like breast cancer and malaria. To enhance future research rigor, this work utilizes and proposes standardized scoring systems, namely QSca (assay quality score) and hmQS (human monitoring quality score). Ultimately, this review underscores the comet assay's pivotal role

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in characterizing regional health risks and seeks to establish evidence-based practices to inform public health policies in Latin America.

1. Background

The LA-COMET group is a Latin American collaborative network, made up of 17 groups from 7 countries; whose publications using the alkaline comet assay are categorized into a) human monitoring studies, b) in vitro studies and c) animal and plant studies [1].

In Latin America, studies have been conducted using the comet assay for human biomonitoring, including those addressing clinical aspects, to investigate various occupational and environmental exposures. The Latin American collaborative group LA-COMET is distinguished by its human monitoring publications that emphasize environmental exposures, in addition to occupational ones, using the alkali comet assay. Furthermore, research cohorts frequently include vulnerable populations, such as women and children, which enriches the collected data.

The aforementioned investigations have primarily focused on agents such as pesticides, heavy metals (e.g., lead and cadmium), organic solvents, and particulate matter. Moreover, there have been specific studies that have explored chemical exposures in contexts such as coal mining and arsenic contamination in drinking water. Research has also been conducted on chemical exposure in particular population groups, such as breast cancer patients undergoing chemotherapy and radiotherapy, as well as individuals with diseases such as ataxia-telangiectasia and malaria. These specialized studies have contributed to a more profound understanding of the health risks associated with chemical exposures in Latin America, offering critical insights for the formulation of public health and disease prevention policy. The comet assay plays a pivotal role in human biomonitoring by enabling the direct assessment of DNA damage at the cellular level. Its significance lies in its ability to detect genetic damage caused by various environmental agents, including chemical pollutants, radiation, and chemicals present in food and medications. Additionally, the comet assay is a valuable tool for monitoring the efficacy of therapeutic interventions and assessing the impact of occupational and environmental exposures on human health.

Revisiting studies published by LA-COMET members with a focus on methodological aspects can significantly contribute to standardization efforts [1]. Through analyze and comparing the methods employed by the group, researchers can identify areas of convergence and discrepancy, which will help establish more uniform guidelines for conducting comet assay to purpose future studies. This collaborative, integrated approach among LA-COMET researchers can improve the validity and reliability of results, promote consistency in experimental procedures, and facilitate cross-study comparisons and data interpretation.

The purpose of this study is to analyze the publications of the LA-COMET group regarding human monitoring in order to relate the quality scores of the assay's methodological procedure and experimental design to the determination of DNA damage caused by a wide range of exposures in occupational, environmental, and lifestyle contexts. This aims to provide information on best practices to ensure the collection of reliable data that support public health policies and interventions designed to mitigate the health risks associated with DNA damage.

2. Methodology

2.1. Literature sources and search strategy

The literature analyzed originated from a validated database of 246 documents published by members of the LA-COMET group between 1996 and 2022 [1], obtained through searches in Web of Science (WOS), and after performing co-citation and bibliographic link analyses. From this database of publications of seventeen research groups of seven countries, sixty-four articles published by LA-COMET members related to human monitoring were selected for the present study. The main criteria for their selection was that they correspond to experimental studies assessing the effects of occupational or environmental exposures, as well as clinical studies using the alkaline comet assay for the assessment of genetic damage exclusively in human samples.

2.2. Study selection and data extraction

Once the 64 publications from LA-COMET members, categorized as human biomonitoring, were selected, the following exclusion criteria were applied: studies conducted *in vitro*, *in vivo*, or *ex vivo* with non-human subjects, as well as in silico research and studies not focused on human populations. Subsequently, information was extracted from the manuscripts regarding the demographic aspects of each study, confirming that they corresponded to research with human participants of any age, sex, or ethnicity, or country of origin. Information regarding lifestyle factors such as smoking or alcohol consumption was also extracted. Likewise, it was considered important to collect the number of participants in control groups of unexposed individuals when possible. Of course, the number of exposed individuals was also collected. Importantly, as much information as possible regarding exposure was extracted, which was classified into: 1) organic solvents, 2) inorganic elements, 3) environmental pollutants, 4) pesticides, and 5) clinical studies.

Detailed descriptions of DNA damage assessment methods were compiled, with particular attention to the comet assay protocol, and the biological sample used (peripheral blood, buccal cells, or tissue samples), especially in relation to the exposure scenario or clinical condition being investigated. The use of additional assays to determine other markers, such as reactive oxygen species levels, GSH levels, polymorphisms, etc., was also noted.

2.3. Quality score of comet assay (QSca) and human monitoring quality score (hmQS) assessment

To establish a methodological quality score for the performance of the alkaline comet assay, the LA-COMET group generated the Comet Assay Quality Score (QSca) based on 21 method parameters (see Table 1 in the Supplement) [1]. The 21 qualitative parameters include information on the cell model, exposure, slide preparation, lysis, unwinding, electrophoresis, neutralization, staining, and nucleoid assessment. Each parameter was assigned one of the following values: 1, information not presented; 2, incomplete information; 3, complete information. QSca scores ranged from 21 to 63 points for each publication. Higher QSca scores indicate higher-quality studies in terms of rigorous implementation of the comet assay [1].

Additionally, in this study, we generated a human monitoring Quality Score (hmQS), which includes eight key parameters that every human monitoring study should include. These parameters encompass demographic and lifestyle aspects, and we also included the QSca, which reflects methodological rigor. Details for assessing the eight parameters were collected on a form for each publication (see Table 2 in the Supplement).

The hmQS system that evaluates eight key criteria, assigns a score of 1, 2, or 3 points to each criteria based on the following parameters:

1. Number of subjects in the control group: studies with fewer than 20 individuals received 1 point; those with 20–50 individuals received 2 points; and studies with more than 50 individuals received 3 points.

2. Number of subjects in the exposed group: studies with fewer than 20 individuals received 1 point; those with 20–50 individuals received 2 points; and studies with more than 50 individuals received 3 points.
3. Age information of groups: studies with no age information received 1 point; incomplete age information received 2 points; and complete age information of groups received 3 points.
4. Gender information of groups: studies with no gender information received 1 point; incomplete gender information received 2 points; and complete gender information received 3 points.
5. Smoking status information of groups: studies with no smoking status information received 1 point; incomplete smoking status information received 2 points; and complete smoking status information received 3 points.
6. Alcohol intake information of groups: studies with no alcohol intake information received 1 point; incomplete alcohol intake information received 2 points; and complete alcohol intake information received 3 points.
7. Chemical exposure assessment: studies based on questionnaire-only assessment received 1 point; those using measurements in the ambient environment received 2 points; and studies using measurements in body fluids received 3 points.
8. Comet assay method quality score (QSca; 21–63): studies based on QSca 21–35 received 1 point; QSca 36–49 received 2 points; and QSca 50–63 received 3 points (Table 1 Supplement).

These criteria were applied to determine a final human monitoring Quality Score, ranging between 8–24 points, allowing for comparative analysis across studies. Establishing that a low score corresponds to values of eight; while a medium score has values between nine and sixteen; and a high score has values greater than sixteen points. The purpose of establishing the hmQS was to identify strengths and weaknesses regarding the rigor applied in previous publications from the LA-COMET group (human monitoring). On the one hand, human monitoring studies are of interest to the seventeen groups that make up the collaborative network, and this category represents 25% of the 246 previous publications [1]. Furthermore, we were interested in identifying whether the hmQS score is related to the exposure category in the studies. Likewise, we sought to determine the relationship between the QSca score, which reflects the methodological rigor used in the publications, and the rigor applied in the design of the monitoring study using the hmQS.

2.4. Data organization

The information was systematically organized and categorized by exposure type (organic solvents, inorganic elements, environmental pollutants, pesticides, and clinical studies) and presented in tables (Tables 1,2,3,4, and 5). These tables summarize key aspects extracted from the original publications regarding study design, which were considered for calculating the hmQS, as well as QSca [1] and the assessment of DNA damage calculated through the odds ratio, regardless of the units reported in the manuscripts [1].

The information contains in each column of the tables is detailed below, as well as an explanation of the calculation of the odds ratio and their propagation of uncertainties.

Parameters related to Quality assessment

hmQS; QSca: This column shows the human monitoring Quality Score (hmQS) for each article. The hmQS, ranging from 8 to 24 points, considers eight parameters described in 2.3 section and Supplemengtary Table 2. The QSca, is the quality score established by LA-COMET group based on 21 methodological parameters of the alkaline comet assay [1]. The score range is 21–63 and is detailed in 2.3 and Supplementary Table 1.

Exposure; country: This column generally describes the context and approach of the type of exposure and the country where the study was conducted, extracted of each original publication.

Subjects (exposed/control): This column shows the total number of subjects involved in the monitoring. It indicates the number of subjects in the exposed group and the number of control or unexposed subject, provided this is indicated in the original publication.

Age: This column indicates the mean average age, expressed as years, of all subjects involved in the monitoring study.

Male %: This column indicates the percentage of men involved in each study.

Current Smokers%: The column indicates the % of subjects identified as smokers in each stidy, based on data from the original publications.

Table 1
Summary of the LA-COMET group studies corresponding to the organic solvents' category.

hmQS[a] (QSca)[b]	Exposure/ Country	Subjects (Exposed/ Control)	Age (mean years)	Male (%)	Current Smokers (%)	DNA damage Parameter (Odd ratio)*	Other Biomarkers[c]	Chemical Exposure Assessment	Ref.
16 (58)	Dyes + Organic solvents/ Colombia	61 (33/28)	(32,8)	(44,3)	(65)	Tail Length, Total blood (1,08 ± 0,29)*; Lymphocytes (1,05 ± 0,24)*	MN lymphocytes (↑)	Organic solvents Urinary metabolites: Phenol, hippuric acid and methyl hippuric acid (↑)	[3]
16 (60)	Organic solvents + metals (footwear manufacture)/ Brazil	62 (45/25)	(26)	(100)	(6,2)	Damage index (2,42 ± 4,55); SBA Damage index (0,62± 1,82)* WBA	MN Buccal cells (↑)	Hippuric acid (↑)	[4]
19 (60)	Organic solvents + metals (footwear manufacture)/Brazil	94 (39/55)	(28)	(79)	(2,6)	Damage index (3 ± 6,74); Damage frequency (2.13 ± 10,43)	MN lymphocytes (↑) and in buccal cells	Hippuric acid (↑) and hemoglobin	[5]
23 (56)	Organic solvents (Phenol, toluene, Xylene) / Colombia	90 (30/60)	(32)	(72,2)	(28,9)	Damage frequency (0.81 + 0.11)*; Tail length (1.04 ± 0.14)*	GSTM1, GSTT1 and CYP2E1	Benzene, toluene and xylene in blood phenol, ortho and meta methyl hippuric acids in urine	[6]

[a] hmQS: human monitoring Quality Score (ranging of 8-24) described in Supplementary Table 2;
[b] QSca: Quality Score comet assay (ranging of 21-63) described in Supplementary Table 1; * negative result (odds ratio<1.2); SBA: solvent based adhesive; WBA: water-based adhesives;
[c] Other biomarkers: MN: micronuclei; *GSTT1: Glutathione S-Transferase Theta 1*; GSTM1: *Glutathione-Transferase Mu 1; CYP2E1: Cytochrome P450 2E1*.

Table 2
Summary of the LA-COMET group studies corresponding to inorganic elements category.

hmQS[a] (QSca[b])	Exposure/ Country	Subjects (Exposed/ Control)	Age (Mean years)	Male (%)	Current Smokers (%)	DNA Damage parameter (Odd ratio)	Other Biomarkers[c]	Chemical Exposure Assessment	Ref.
22 (57)	Chronic occupational exposure to chemical contaminants in a landfill/ Paraguay	84 (50 exposed / 34 non-exposed)	Exposed (33); Non-exposed (29)	(0)	Smokers were excluded from the study	% Tail DNA (2.80 ± 1.75)	Frequency of BMN (↑)	Presence of mercury, arsenic, and hexavalent chromium	[7]
17 (60)	Copper smelters/ Brazil	22 (11/11)	(43.4)	(100)	(55)	Damage index, All individuals (4.11 ± 0.91) Nonsmokers (3.44 ± 1.76) Smokers (4.86 ± 5.16) Age < /= 40 years (3,05 ± 4.4) Age > 40 years (3,30 ± 2.56)		PIXE analysis: copper, iron, other metals	[8]
22 (57)	Children of three communities exposed to Arsenic and Lead	60 (Community A 20 Community B 28/ Community C control 12)	(8.1)	NI	NA	Olive Tail Moment Community A (1.23 ± 0.10) Community B (1.38 ± 0.08)	Metals accumulation in liver, lung and kidney of sentinel rodents	As in urine Pb in blood As, Pb, Cd, Cu, Zn in soil	[9]
20 (56)	Children exposed to Arsenic, Lead, PAHs, DDT/ Mexico	(276)	(7.8)	(40)	NA	Olive Tail moment (3.57 ± 2.38)		As in urine Pb blood 1-OHP in urine Total DDT in blood	[10]
23 (56)	Children of three communities environmentally exposed to different levels of As and Pb/ Mexico	85 (48 Mining site 12 Urban population 25 Agricultural area)	(7.3)	(50)	Passive smokers (33)	Tail moment High exposition (2.08 ± 0.41) Low Exposition (1.4 ± 0.27)	DNA repair capacity	Arsenic in urine and Lead in blood	[11]
23 (56)	Arsenic/Indigenous Children/ Mexico	120 (80/40)	(11)	(37)	NA	Tail moment Community A (3.6 ± 0.36) Community B (1.07 ± 0.2)		Arsenic	[12]
19 (63)	Chronic, environmental exposure to arsenic through drinking water / Mexico.	42 (22 / 20)	Exposed (50) Controls (48)	(100)	NI	Tail Length (5.05 ± 0.31)	CA (↑) Acentric fragments; (↑) Terminal deletions MI and RI (↑)	Arsenic levels in drinking water	[13]
20 (59)	Children exposed to As and Pb/ Mexico	55 (20/35)	(4.9)	(45)	NA	Tail length (1.62 ± 1.13); Tail moment (2.12 ± 2.2)		Levels of As and Pb	[14]
18 (55)	Chronic occupational lead exposure in a battery recycling plant/ Mexico.	53 (37/16)	Exposed (31.65) Non-exposed (37.15)	(81,2)	Exposed (16) Non-exposed (18.7)	Tail length 3 Gy gamma radiation (1.43 ± 0.30) 60 min posttreatment (1.1 ± 0.23) Pb exposed 3 Gy gamma radiation (1,42 ± 0.43) 60 min post treatment (1.38 ± 0.5)	DNA Repair Capacity cDNA Expression Array Ape1 Functional Assay	Blood Pb levels measured by voltammetry	[15]
18 (47)	Children environmentally exposed to different levels of As and Pb/ Mexico	65	(8.5)	(53.8)	NA	Tail length[d] Gómez Palacio (Farther) (4.17 ± 2.56) Heroes de Nacozari (Intermediate) (3.61 ± 2.2) Pedro Garcia (Nearest) (4.08 ± 2.43)	DNA Repair Capacity	Exposure to smelter emissions; arsenic and lead	[16]

[a] hmQS: human monitoring Quality Score (ranging of 8-24) described in Supplementary Table 2;

DNA Damage Parameters and Other Biomarkers DNA damage parameter;Odds ratio: This column indicates the comet assay parameter used to measure DNA damage (Damage index, %Tail DNA, Tail length or Tail intensity), which was extracted from each original publication. The Odds ratio was calculated from the DNA damage value determined in the exposed group/ DNA damage value of the control group.This ratio was defined in the previous publication of the collaboration network [1], were we indicates that values > 1 indicate and identified genotoxic effect in the exposed group, while values < 1 indicate decreased DNA damage in the exposed group. The margin of error is also included for these ratios and was calculated based on the following function and formula [2]:

FUNCTION $y = x_1/x_2$

Expression giving standard uncertainty: $(u(y)/y)^2 = [(u(x_1)/x_1)^2 + (u(x_2)/x_2)^2]$

Where "y" is the ratio of DNA damage of the exposed group; "x_1" is the average DNA damage value in the exposed group; and "x_2" is the average DNA damage value in the control or unexposed group; and "u" is the uncertainty.

Other Biomarkers: This column indicates, for each study, whether biomarkers in addition to the alkaline comet assay were determined, specifying which ones and the trend of their results, whenever possible, from the original publication.

Chemical exposure assessment: This column describes which assay was used to quantify the exposure, provided the information could be obtained from the original publications. Therefore, Table 3 have partial data and Table 5 do not have this column.

3. Results and discussion

From a total of 246 publications by LA-COMET group members, we selected 64 documents of the topic human monitoring, as described in Sections 2.1 and 2.2. These 64 studies were categorized into five groups based on the exposure type, and their distribution is shown in Fig. 1. The "organic solvents" category, which also includes complex chemical mixtures, comprises 4 articles (6%). The "inorganic elements" category contains 9 articles (14%) addressing exposure to metals, fluoride, and arsenic. The "environmental pollutants" consists of 13 articles (20%), which include studies of exposure to dust, ozone, particulate matter, and diesel emissions. The "pesticides" category includes 24 articles (38%), conducted in various contexts, including rural versus urban scenarios, as well as occupational exposure for those who spray crops and those who carry out vector control. Lastly, the "clinical studies" category consist of 14 articles (22%) which study various disorders and lifestyle factors. Regarding the categorization of human monitoring publications, most studies fall under the pesticide category (Fig. 1A), reflecting the area in which the LA-COMET group has the most expertise. Odds ratio analysis of the publications indicates that all studies in the pesticide and inorganic element categories were positive (Fig. 1B). In the organic solvent category, half of the studies had positive ratios in the DNA damage assessment (Fig. 1B). Some articles address more than one exposure category, reflecting the complexity and interconnectedness of environmental and occupational factors. The distribution of studies by country where the 64 monitoring studies were conducted indicates that Brazil and Mexico have the most environmental monitoring studies, each with 23 publications. Next is Colombia with 6 studies; 5 were conducted in Argentina, 4 in Bolivia, and 3 in Paraguay. Excluding clinical studies, the analysis of the publications reveals that 35/64 studies were conducted in an occupational context, while 15/64 were related to environmental exposure, and 5/64 publications covered both contexts, occupational and environmental, involving exposure to various agents. It is worth noting that 25% of the LA-COMET group's publications have focused on human monitoring studies, and to summarize their overall content, we will briefly describe them by category based on the type of exposure. This study is a descriptive analysis of the body of work generated by current LA-COMET group members, with the aim of validating effective working methods for implementation in future studies.

3.1. Organic solvent exposure

Publications on organic solvent exposure from the LA-COMET group are still limited, with only four articles summarized in Table 1, which outlines parameters related to quality assessment and DNA damage. The studies compiled in Table 1 assessed DNA damage in individuals exposed to various organic solvents, dyes, and complex chemical mixtures, primarily in occupational settings such as dye factories and footwear manufacturing plant [3–6]. Only two of the four studies in this category had positive odds ratios, considering a cutoff point of 1.2. These studies used the damage index as the assessment parameter for damage and were conducted primarily with male subjects. It is important to note that one of these studies evaluated both solvent-based adhesives (SBA) and water-based adhesives (WBA); the latter showed negative damage in the assessment. The remaining studies had negative odds ratios. It is also important to mention that all studies achieved high quality scores for monitoring parameters (hmQS 16–23) and methodological parameters (QSca 56–60). This is further reflected in the fact that the studies determined additional biomarkers, in addition to measuring chemical exposure by more than one metabolite.

3.2. Inorganic elements exposure

The inorganic elements category comprises ten publications, the summary of whose quality assessment parameters is shown in Table 2 [7–16]. Specifically, the studies included in this exposure category evaluated mercury [7], arsenic [7,9–14,16], hexavalent chromium [7], copper [8,9], iron [8], lead [9–12,15,16], and PAHs [10]. These studies covered diverse exposure scenarios, such as mining, industrial smelting, battery recycling, and landfill work. They highlight the impact of chronic exposure to heavy metals in both occupational and environmental settings. In this category, we note that the studies vary significantly in the proportion of male participants. Two of the ten studies in this exposure category were conducted exclusively on men, one in an occupational setting at a copper smelter [8] and the other on subjects exposed to arsenic (As) through drinking water [13]. Interestingly, these same studies also yielded the highest odds ratios in the category: 4.11 ± 0.91 [8] and 5.05 ± 0.31 [12]. Tovar-Sánchez et al. [13] analyzed 22 people with chronic exposure to drinking water contaminated with arsenic (0.240 ± 0.008 μg/mL, exceeding national standards). They found significant effects through other biomarkers, including a significant increase in chromosomal aberrations, such as acentric fragments and terminal deletions, along with elevated tail length values in the comet assay, which allowed them to discuss the mechanism of action caused by the exposure. The study published by Duarte et al. [8] also provides relevant information regarding the increased odds ratio of DNA damage caused by smoking, showing a mean value of 4.86 ± 5.16, while in non-smokers, the mean value was 3.44 ± 1.76, suggesting a synergistic effect between tobacco and the genotoxicity generated by metal exposure. In contrast, only one study was conducted in a landfill occupational setting, exclusively on women chronically exposed to mercury, arsenic, and hexavalent copper, where the study showed a positive odds ratio with an average value of 2.8 ± 1.75 [7]. This same study, in addition to being designed only for women, also excluded smokers in

[b] QSca: Quality Score comet assay (ranging of 21-63) described in Supplementary Table 1; NA: non-applied because there are child's; NI: non-information;
[c] Other biomarkers: BMN: Buccal micronuclei; 1-OHP: 1-Hydroxy pyrene; CA: Chromosomal aberrations; MI: Mitotic index; RI: Replication index;
[d] The data were obtained from children attending schools located at different distances from a smelter in Region Lagunera, Mexico.

Table 3
Summary of the LA-COMET group studies corresponding to environmental pollutants category.

hmQs[a] (QSCA[b])	Exposure/ Country	Subjects (Exposed/ controls)	Age (mean years)	Male (%)	Current Smokers (%)	DNA damage parameter (Odd ratio)*	Other biomarkers[c]	Chemical Exposure Assessment	Ref.
21 (60)	Coal exposure/ Colombia, Brazil	200 Coal mining general (100/ 100) Transport coal (50/100) Coal Equipment maintenance (18/100) Coal stripping (17/100) Coal embarking (15/100)	(43.91 ± 7.8)	(100)	NI	Tail length (1.63 ± 0.53) % Tail DNA (4.51 ± 3.58) Damage index (6.66 ± 6.46) Tail length (1.60 ± 0.56) % Tail DNA (4.13 ± 3.46) Damage index (6.21 ± 6.27) Tail length (1.65 ± 0.50) % Tail DNA (4.65 ± 3.58) Damage index (6.96 ± 6.52) Tail length (1.75 ± 0.58) % Tail DNA= 5.68 ± 4.37 Damage index (8.22 ± 7.75) Tail length (1.53 ± 0.37) % Tail DNA (4.06 ± 2.97) Damage index (5.97 ± 5.47)	MN (↑) in exposed people		[17]
21 (60)	Coal exposure/ Brazil	128 (71/57)	(42 ± 11.05)	(100)	(0)	Damage index (2.16 ± 2.21) Damage frequency (2.21 ± 2.21)	MN (↑) and TBARS, SOD CAT (negative)		[18]
21 (60)	Coal exposure/ Brazil	139 (98/41)	(32.8 ± 12.4)	(29.49)	(0)	% Tail DNA (1.79 ± 2.26) FPG = 4 ± 1.45 Endo III = 4.36 ± 1.24	MN (↑)	Si,Al,Cu,Cr, K_2O, Ti_2O P_2O_5 SO_3, Quartz, Keolinite	[19]
21 (60)	Coal Exposure in four cities Bage, Candiota, Machado, Acegua/ Brazil	284 (69/93) (72/93) (50/93)	(36.5 ± 13.3)	(45)	(17.95)	Bage vs Candiota DI= 0.96 ± 0.24* Bage vs P.Machado DI= 1.21 ± 0.28 Bage vs Acegua DI= 0.42 ± 0.11*	MN (↓)	Si,S,Cu,Zn	[20]
22 (45)	Diesel engine exhaust/ Colombia	220 (120/100)	(34.6 ± 12)	(100)	(NI)	% Tail DNA (1.32 ± 0.91) Damage index, (1.22 ±.55)	MN and BMN (↑)	PM	[21]
14 (46)	Diesel engine exhaust/ Colombia	123 (43/80)	(34.6 ± 12)	(100)	(NI)	GSST1non null/ null %Tail DNA, (1.31 ± 0.21)	MN and BMN negative GSST1 polymorphism		[22]
16 (55)	Environmental pollution/ Mexico	43 (28/15)	(19 ± 2)	(43)	(4.2)	Leukocytes Tail length (1.59 ± 1.26) Nasal cells Tail length (1.73 ± 1.19) Buccal cells Tail length (1.12 ± 0.71)		Environmental Ozone	[23]
19 (42)	Environmental pollution/ Mexico	55 (28/27)	(35.6 ± 8)	(100)	NI	Mexico City Tail length (1.71 ± 1.44) Mexico City Street vendor Tail length (3.61 ± 2.06) Mexico City Taxi drivers Tail length		Environmental VOCs, $PM_{2.5}$ and ozone	[24]

(*continued on next page*)

Table 3 (*continued*)

hmQs[a] (QSCA[b])	Exposure/ Country	Subjects (Exposed/ controls)	Age (mean years)	Male (%)	Current Smokers (%)	DNA damage parameter (Odd ratio)*	Other biomarkers[c]	Chemical Exposure Assessment	Ref.
						(1.70 ± 0.96) Puebla city Tail length (1.12 ± 0.87)*			
21 (55)	Environmental pollution / Mexico	38 (11/27)	(20)	(37)	(21)	Tail length (1.29 ± 0.06)	Exfoliated tear duct epithelial cells were used	Environmental Ozone	[25]
20 (58)	Environmental pollution/ Mexico	207 (157/50)	(19 ± 2)	(47)	(30)	Nasal cells Tail length (1.80 ± 0.12) Leukocytes Tail length (2.36 ± 0.16) Nasal cells females Tail length (1.77 ± 0.09) Nasal cells males Tail length (1.83 ± 0.14) Leukocytes females Tail length (1.88 ± 0.17) Leukocytes males Tail length (2.77 ± 0.14)	Metaplasia increase	Environmental Ozone	[26¡
19 (55)	Environmental pollution / Mexico	59 Arsenic (31/28) 31 Gasoline (16/15) 10 Fluor (3/7)	NI (28.6 ± 14) NI	(47) (100) (100)	NI NI NI	Arsenic Tail length (1.32 ± 0.76) Gasoline Tail length (1.22 ± 0.38) Fluor Tail length (1.24 ± 0.45)	-	Environmental VOCs, $PM_{2.5}$ and ozone	[27]
20 (59)	Photocopier operators exposed to PM / Argentina	78 (26/52)	23	(46,2)	9,6	Damage index (1,34±0,07)	GSH/GSSG (↓) CAT (↓) TBARS (↑) MN in buccal cells (↑)	$PM_{0.45}$	[28]

[a] hmQS: human monitoring Quality Score (ranging of 8-24) described in Supplementary Table 2;
[b] QSca: Quality Score comet assay (ranging of 21-63) described in Supplementary Table 1; * negative result (odds ratio<1.2);
[c] Other biomarkers: BMN: buccal micronuclei, CAT: catalase; GSH: glutathione reduced; GSSG: glutathione disulfide; GSTT1: glutathione-S-transferase polymorphism T1; MN: micronuclei; PM $_{2.5}$: particulate matter 2.5 um; SOD: superoxide dismutase; TBARS: thiobarbituric acid reactive substances; VOCs: Volatile Organic compounds. NI: Not information provided.

order to eliminate this confounding factor. Even so, the relationship with DNA damage was important, but not between the highest in this category. The last study of this type in adults was that of Hernández-Franco et al. [15], which evaluated 37 workers at a battery recycling plant with chronic lead exposure. DNA repair capacity was significantly reduced in the exposed workers, as demonstrated by the results of the comet assay before and after exposure to gamma radiation (increase of 1.42 ± 0.43 vs. 1.38 ± 0.5 after exposure in lead-exposed workers, compared to 1.40 ± 0.30 vs. 1.1 ± 0.23 in unexposed individuals).

It is worth noting that most studies in children comprise this exposure category [9–12,14,16], highlighting their vulnerability to the effects of mixtures of inorganic elements. Several studies have examined DNA damage in children exposed primarily to mixtures of metals, predominantly arsenic (As) and lead (Pb), due to their residence in areas near mines and their use and consumption of contaminated water. Jasso-Pineda et al. [9–11] have published several studies in children from different environmental settings in Mexico and reported higher tail moment odds ratio (3.57 ± 2.38) in children residing in areas contaminated by complex mixtures of metals and DDT [10]. In one study, lead concentrations reached 11.40 μg/dL, far exceeding international safety limits.Maldonado et al. [12] examined 120 indigenous children exposed to arsenic, reporting a 3.6 ± 0.36-fold increase in comet assay tail length in the most contaminated community compared to 1.07 ± 0.20 in the lower-exposure group.

Mining-related contamination affected not only workers but also surrounding communities, particularly children. Yanez et al. [14] reported a 2.12 ± 2.2-fold increase in tail moment values in children exposed to arsenic and lead in mining regions. Méndez-Gómez et al. [16] examined 65 children in smelting-affected areas and found an important reduction of the DNA repair capacity according with the exposure to lead and arsenic. All reviewed studies reported significant increases in DNA damage in exposed groups, as assessed through comet assay parameters such as tail length, tail DNA percentage, and Olive tail moment (Table 2). Some studies incorporated additional biomarkers to validate genotoxic effects. Micronucleus (MN) assays confirmed an increased frequency of nuclear anomalies in metal-exposed populations, with exposed individuals showing a frequency of 12.51% compared to 3.38% in controls. DNA repair capacity tests, particularly in occupational studies, revealed that chronic metal exposure impairs DNA repair mechanisms, making individuals more susceptible to accumulating genetic damage.

The findings published so far by the LA-COMET group emphasize the urgent need for continuous biomonitoring and the implementation of preventive measures in populations at risk of exposure to complex mixtures, both in occupational settings and in environments where children spend time. They also underscore the importance of monitoring

Table 4
Summary of the LA-COMET group studies corresponding to pesticides exposure category.

hmQS[a] (QSca[b])	Exposure/ Country	Subjects (Exposed/ Control)	Age mean years)	Male (%)	Current Smokers (%)	DNA damage parameter (Odd Ratio)	Other Biomarkers[c]	Chemical Exposure Assessment	Ref.
Environmental exposure									
11 (61)	DDT pesticide spraying for malaria/ México In three cities	118	(9.2)	(NI)	(NI)	Tail moment (2.0 ± 0.72)	Tunnel assay: apoptosis (↑) correlated with increase of DDT (↑) in follow-up after one year for the city with higher exposure	Blood concentrations of DDT (↑), DDE (↑), DDD, and total DDT (↑) (higher exposure compared to lower exposure	[31]
17 (51)	DDT pesticide spraying for malaria/México	100 (50 from mothers and 50 umbilical cords/0)	(23.4)	(0)	(0)	Olive Tail Moment (1.2 ± 1.25)	(↑) MN for mothers in relation to umbilical cords BUD and NPB (no difference)	Organochlorines determinations[d]: (↑) for umbilical cords in relation to mothers (except DDE)	[32]
20 (56)	Mainly malaria community (12 cities: mining, urban and rural area)/Mexico	256 from 12 cities, with 111 from rural area /0)	(7.8)	(51.4)	(0)	Olive Tail Moment (3.6 ± 5.9)		Arsenic (no difference) and 1-hydroxypyrene (1-OHP) in urine (↑children from rural cities in relation to others); lead (no difference) and total DDT in blood (↑ children from rural cities in relation to others)	[10]
16 (53)	Monocultures including transgenic soybean and sunflower/ Paraguay	84 (43/41)	(7.8)	(54.8)	(0)	Tail moment, (2.3 ± 0.31)	Micronucleus in buccal cells (↑ for MN, BUD, BN, and death cells); blood parameters (cholinesterase and hemoglobin, no difference; eosinophilia and proteinemia ↑)		[33]
Occupational exposure									
14 (61)	Multiple cropping/Bolivia In three communities	297 (297/0)	(42.4)	(55.9)	(41.8)	% Tail DNA (2.4 ± 0.22)	Micronucleus test in lymphocyte (difference between individuals from city with higher exposure compared to those with lower exposure) Confounders: age, alcohol consumption, and type of water source influenced levels of genotoxic damage Relation with exposure to tebuconazole, 2,4-D, or cyfluthrin.	Pyrethroid, oganophosphate, chlorophenoxy, and more (questionnaire) Pesticides in urine: OH-PYR, TEB-OH, 5-OH-TBZ, CFCA, TCP, 4F3PBA, 3-PBA, DCCA, 2,4-D, MCPA	[34]
14 (61)	Multiple cropping/Bolivia	198 (118/80)	(40)	(58.6)	(8.1)	Tail moment (↑3.2 ± 0.39) Olive tail moment (2.61 ± 0.33)	Micronucleus test in buccal cells (↑)	Organophosphate, pyrethroid, carbamate, dithiocarbamates (questionnaire)	[35]
15 (63)	Multiple crops/ Bolivia	81 (48/33)	(37.3)	(74.1)	(19.7)	% Tail DNA (1.63 ± 1.43)	Chromosomal aberrations (↑) People who live at high altitudes have more DNA damage	Organophosphate, pyrethroid (questionnaire)	[36]
15 (61)	Multiple crops/ Bolivia	208 (131/77)	(NI)	(70)	(18.1)	% Tail DNA (3.3 ± 0.82)	Micronucleus test in lymphocyte (↑) Sister chromatid exchanges (SCEs) (↑) Chromosomal aberrations (↑)	Organophosphate, Pyrethroid (questionnaire)	[37]
20 (63)	Multiple crops/ Mexico	171 (111/60)	(45.7)	(100)	(0)	Tail length (1.80 ± 0.10)	Micronucleus test in buccal cells: MN (↑) Binucleate (↑) Pyknosis (↑) Con. Chromatin (↑) Nuclear buds Karyolitic (no difference) Karyorrhexis	Organochlorines, organophosphorus, carbamates, pyrethroids (questionnaire)	[38]
15 (49)	Rice crops (three cities)/ Colombia	223 (223/0)	(45.8)	(97.7)	(42)	% Tail DNA (2.9 ± 0.40) Tail length (3.1 ± 0.44)	Two groups of mixtures—a-benzene hexachloride hexachlorobenze and b-BHC and pirimiphos-methyl malathion bromophos-methyl and bromophos-ethyl	Organophosphorus, carbamates, dithiocarbamates; organochlorines (questionnaire) Pesticides in blood, serum and urine (15 organochlorines, 10 organophosphorus, 5 carbamates, and ethylene thiourea	[39]
20 (60	Vineyard workers/ Brazil	173 (108/65)	(39.7)	(100)	(0)	Damage index (4.58 ± 6.62)	MN (↑) PON1, GSTM, GSTT1, GSTP1, CYP1A1, CYP2E1, XRCC1 and OGG1	Organophosphorus, carbamates, pyrethroids,	[40–42]

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Table 4 (*continued*)

Environmental exposure									
hmQS[a] (QSca[b])	**Exposure/ Country**	**Subjects (Exposed/ Control)**	**Age mean years)**	**Male (%)**	**Current Smokers (%)**	**DNA damage parameter (Odd Ratio)**	**Other Biomarkers[c]**	**Chemical Exposure Assessment**	**Ref.**
							genotypes (no effect for comet assay, but with effect for MN frequency)	organochlorines, glyphosate, and more (questionnaire)	
18 (60)	Soybean cultivation/ Brazil	127 (81/46)	(49)	(57)	(0)	Damage index (1.96 ± 1.44)	Micronucleus in buccal cells: MN (↑) Nuclear buds (↑) Binucleated (↑) Condensed chromatin (↑) Karyorrhectic (↑) Karyolitic (↑) Pyknotic (no difference)	organophosphorus, carbamates, pyrethroids, organochlorines (questionnaire). Inorganic elements in buccal cells (no difference; BChE (no difference)	[43]
16 (60)	Tobacco cultivation;/ Brazil	60 (30/30)	(41.1)	(53)	(0)	Damage index (1.89 ± 2.53)	Micronucleus in lymphocytes (no difference) No influence of gender or PPE[e] use for DNA damage. ↑off season and harvest	Organophosphate, carbamate, dithiocarbamate pyrethroid and glyphosate (questionnaire) BChE (no difference) Cotinine (↑ harvest)	[44]
14 (59)	Tobacco cultivation;/ Brazil	137 (77/60)	(89.6)	(63.5)	(25)	Damage index (2.9 ± 3.13)	Micronucleus in buccal cells (↑) PON1 genotype (↑ MN) Hemogram: Band neutrophils and monocytes (↑) SOD (↑)	Organophosphate, carbamate, pyrethroid and more (questionnaire). Inorganic elements: zinc and magnesium (↑)	[45]
22 (61)	Soybean cultivation/ Brazil	220 (137/83)	(46)	(100)	(0)	Damage index (1.29 ± 0.062) Damage index Net hOGGss (7.0 ± 1.14)	BCMN (↑) Nuclear buds (↑) Binucleated cells (↑) Karyorrhectic cells (↑) Pyknotic cell (↑) Karyolytic cells (↑) Global DNA methylation (↑) Hematological markers and biochemical parameters (no difference) No influence of gender or PPE[e] use for DNA damage	Organophosphorus, carbamates, pyrethroids, organochlorines (questionnaire). Inorganic elements: aluminium and phosphorus (↑); BChE (no difference)	[46]
13 (58)	Tobacco cultivation/ Brazil	110 (110/0)	(42.3)	(60)	(12.7)	Damage index Folate intake (3.7 ± 3.32) B12 intake (3.9 ± 3.27) B6 consumption (1.8 ± 2.23)	MN (no difference) BCMN: folate intake and B12 intake (no difference) B6 consumption (↑) MTHFR C677T genotypes (no difference)	Glyphosate, flumetralin, clomazone, imidacloprid and sulfentrazone (questionnaire)	[47]
20 (60)	Tobacco cultivation (harvest and grading seasons)/ Brazil	241 (156/85	(41.1)	(50.6)	(0)	Damage index Harvest (2.23 ± 2.76) Grading (1.66 ± 1.89)	BCMN: (↑ and binucleated cells) TBARS (↑ harvest) TEAC (↑) No influence of gender	Organophosphates, carbamates, pyrethroids and more (questionnaire) Nicotine (natural pesticide): Cotinine (↑)	[48]
15 (59)	Multiple cropping/ Argentina	123 (41 /82)	(37.4)	(54.5)	(21.1)	Damage index (1.9 ± 0.31)	BChE; AChE (↓) CAT (↓) TBARS (↑ only for sprayers)	Captan, copper, mancozeb, chlorpyrifos, cypermethrin, dimethoate, endosulfan, imidacloprid, malathion, methamidophos, parathion, permethrin, and glyphosate (questionnaire)	[49]
16 (59)	Multiple cropping/ Argentina	145 (95 /50)	(44.6)	(55.2)	(18.6)	Damage index (1.8 ± 0.26)	AChE (↓) CAT (↓) TBARS (↑ only for sprayers)	Captan, copper, mancozeb, chlorpyrifos, carbofuran, cypermethrin, dimethoate, endosulfan, imidacloprid, malathion, methamidophos, parathion, permethrin, and glyphosate (questionnaire)	[50]
20 (60)	Tobacco cultivation/ Brazil	242 (121/ 121)	(42.4)	(43.8)	(0)	Damage index (4.8 ± 0.54)	PON1, SOD2, OGG1, XRCC1, and XRCC4; Telomere length (↓) TBARS (↑) TEAC (↑)	Carbamates, organophosphates, pyrethroids and organochlorines (questionnaire) Inorganic elements: zinc, phosphorus, sulphur, and chlorine (↑), and cotinine (↑)	[51]

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Table 4 (*continued*)

Environmental exposure									
hmQS[a] (QSca[b])	**Exposure/ Country**	**Subjects (Exposed/ Control)**	**Age mean years)**	**Male (%)**	**Current Smokers (%)**	**DNA damage parameter (Odd Ratio)**	**Other Biomarkers[c]**	**Chemical Exposure Assessment**	**Ref.**
22 (59)	Tobacco Farmers (harvest season)/ Brazil	80 (40/40)	(42)	(50)	(0)	Damage index (2.9 ± 3.04)	TEAC (↑) TBARS (no difference)	Nicotine (natural pesticide): Cotinine (↑)	[52]
18 (60)	Tobacco cultivation/ Brazil	130 (56/74)	(42)	(26)	(28)	Damage index (4.0 ± 0.44)	Telomere length (↓) Global DNA methylation (↓) p16 methylation (↓) Cotinine (↑) TEAC (no difference) TBARS (↑) Influence of PPE[e]: incomplete PPE[e] (↑)	Glyphosate, flumetralin, clomazone, imidacloprid and sulfentrazone (questionnaire) Inorganic elements: phosphorus, sulphur, and chlorine (↑)	[53]

[a] hmQS: human monitoring Quality Score (ranging of 8-24) described in Supplementary Table 2;
[b] QSca: Quality Score comet assay (ranging of 21-63) described in Supplementary Table 1;
[c] Other biomarkers: AChE: acetylcholinesterase; BChE: Butyrylcholinesterase; BCMN: buccal cells micronuclei; BUD: chromatin buds; CAT: catalase activity; DDE: 1,1-dichloro-2,2-bis(4-chlorophenyl)- ethylene; DDT: Dichlorodiphenyltrichloroethane; MN: micronuclei; NPB: nucleoplasmic bridges; SOD: superoxide dismutase; TBARS: thiobarbituric acid reactive substances; TEAC: total antioxidant capacity;
[d] p'p'-DDT (1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane), p'p'-DDE (1,1-bis-(4-chlorophenyl)-2,2-dichloroethene), a-endosulfan, b-endosulfan, a-hexachlorocyclohexane (HCH), b-HCH, g-HCH, bexachlorobenzene (HCB), aldrin, heptachlor epoxide, oxychlordane, trans chlordane, cis-chlordane, cis-nonachlor, and mirex;
[e] PPE, personal protective equipment;

with robust study designs to influence local and regional public policies; this is one of the objectives of the collaborative network to be implemented in the future.

3.3. Environmental pollutant exposure

Publications by LA-COMET group that employed the comet assay to assess DNA damage in populations exposed diverse environmental pollutants such as coal, diesel, ozone, colatile organic compounds (VOC's), and particulate matter (PM), were summarized un Table 3 [17–28]. The table features a total of 12 articles from Colombia, Brazil, and Mexico, examine various exposure scenarios, including coal mining, diesel engine exhaust fumes, and environmental contaminants such as ozone, arsenic and fluoride.

The monitoring quality parameters used to calculate the hmQS in this exposure category, shown in Table 3, indicate specific scenarios, which we list below. Regarding sample sizes, 67% of the publications involved 100 or more subjects [17–22,26,27]; while the studies involving fewer than 100 subjects [23–25,28] evaluated the effects of various pollutants, primarily air pollutants. It was noteworthy that all studies were conducted in young adult populations, ranging in age from 19 to 43 years. Fifty percent of the studies deliberately focused on men, given that the exposure was occupational, particularly in industries such as coal mining and diesel engine repair [17–22]. Surprisingly, smoking was a poorly represented parameter in the studies of this exposure category, as five out of twelve studies did not report on it [17,21,22,24,27]. While the studies in which two were reported considered smoking an exclusion criterion [18,19], and the remaining five studies included percentages of smokers between 10 and 30% [20,23,25,26,28]. Regarding the assessment of DNA damage through damage ratios, with the exception of the monitoring by Souza et al. [20], the remaining eleven studies had positive results. The only study with a negative result [20] assessed carbon exposure in four Brazilian cities and, unlike the rest of the carbon studies, this one included smoking subjects in almost 18%; a parameter to which we attribute a confounding effect between those exposed and controls. In general, all the positive odds ratios showed discrete ranges (between 1.5 and 3.5) (Table 3). However, it was noteworthy that the studies involving subjects working in the coal mining and diesel manufacturing industries showed the most significant changes. In particular, the manuscript by León-Mejía et al. [17] was the study that showed that subjects exposed to coal had more than six times greater damage than control subjects. Additionally, this same study identified a gradient in damage in relation to subgroups that performed specific functions. In other words, coal miners have more DNA damage than maintenance workers, who in turn have more damage than coal loading workers, and these latter have more damage than the rest of the coal mine workers.

We consider it important to highlight that this exposure category is the only one in which epithelial bioarrays were used to evaluate the effect of environmental pollution [23,26]. Furthermore, these same studies were the first to report the use of the alkaline comet assay in non-invasively obtained human cells from the nasal and oral epithelium. Both studies showed that ozone causes DNA damage in a cell-specific manner within the same individual, which is advantageous for human monitoring studies, allowing the identification of the cell type most susceptible to damage from a given exposure.

Several studies also incorporated additional biomarkers to validate genotoxic effects. For instance, MN assays were used to confirm DNA damage, showing an increase in MN induction in the exposed populations [17–22,28]. Biomarkers of oxidative stress, such as thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD), and catalase (CAT) levels, further supported the presence of oxidative damage [18,28]. Moreover, genetic polymorphisms, such as GSTT1 null, were explored in some studies to assess individual susceptibility to DNA damage [22].

This DNA damage, assessed using the comet assay, was associated with markers of oxidative stress, including reduced TBARS and catalase levels, and increased SOD activity [18,28]. These findings suggest that reactive oxygen species (ROS) play a significant role in the observed damage. Additional validation came from the use of restriction enzymes ENDO III and FPG to identify oxidative lesions [19]. Individuals with the GSTT1 null polymorphism were found to be more susceptible to the effects of coal exposure, exhibiting greater DNA damage [22].

In another study, we examined 120 mechanics who were exposed to diesel engine exhaust fumes, containing toxic gases, PAHs, and particulate matter. Results indicated that exposed individuals experienced significantly more DNA damage compared to the control group, as confirmed by MN induction. Genetic polymorphisms involved in DNA repair and metabolic detoxification, particularly the GSTT1 null variant, were associated with increased levels of damage in these individuals [22].

In addition, LA-COMET has investigated genotoxic damage from air

Table 5
Summary of the LA-COMET group studies corresponding to clinical studies and lifestyle factors category.

hmQS[a] (QSca[b])	Clinical assessment, Country	Treatment	Subjects (Exposed/ Control)	Age (Mean years)	Male (%)	Current Smokers (%)	DNA Damage (Odd Ratio)*	Other Biomarkers	Ref.
20 (61) (i)	Breast Cancer/ Brazil	4 weeks (chemotherapy)	150 (100 Before and after / 50 controls)	Exposed (50) Control (47)	(0)	(10)	Damage frequency (6.53 ± 3.73)	BMN	[57]
20 (61) (ii)	Breast Cancer/ Brazil	3 weeks (radiotherapy)	150 (100 Before and after / 50 controls)	Exposed (50) Control (47)	(0)	(10)	Damage frequency (6.21 ± 3.62)	BMN	[57]
19 (61) (i)	Breast Cancer/ Brazil	4 weeks (chemotherapy)	100 (50 Before and after / 50 controls)	Exposed (49.8 ± 10.6) Control (48.3 ± 13.4)	(0)	(46)	Damage index 20–40 years (9.10 ± 4.84) 41–60 years (5.35 ± 2.87) 61–80 years (8.52 ± 4.88) Frequency of damage 20–40 years (6.25 ± 2.14) 41–60 years (5.36 ± 1.75) 61–80 years (5.9 ± 2.5)	BMN	[58]
19 (61) (ii)	Breast Cancer/Brazil	3 weeks (radiotherapy)	150 (100 Before and after / 50 controls)	Exposed (50.4 ± 11.9) Control (848.3 ± 13.4)	(0)	(48)	Damage index 20–40 years (10.97 ± 4.8) 41–60 years (5.9 ± 3.1) 61–80 years (10.24 ± 0.51) Frequency of damage 20–40 years (6.5 ± 2.2) 41–60 years (5.26 ± 2.4) 61–80 years (6.5 ± 8.5)	BMN	[58]
19 (61)	Breast Cancer (untreated)/ Mexico	N/A	100 (40/60)	NI	(0)	(0)	Frequency damage SSB (1.34 ± 0.44) DSB (1.64 ± 0.85) Mean tail moment: SSB (1.9 ± 0.6) DSB (2.7 ± 1.5)		[59]
20 (57)	Breast Cancer/ Mexico	80 days (chemotherapy)	101 (41/60)	43	(0)	(0)	Frequency Damage SSB (8.9 ± 2.96) DSB (9.85 ± 4.9)		[60]
15 (59)	Ataxia-telangiectasia/ Brazil	N/A	40 (AT 14, parents 12/ 14 controls)	Patients AT (16.93) Parents (40.25) Controls (26.93)	Patients (85) Parents (8) Controls No info	Patients No info Parents (8) Controls (0)	Damage index, Unaffected parents (0.73 ± 0.82)* AT patients (2.03 ± 3.03)	CBMN DNA repair	[61]
20 (61)	Systemic Lupus Erythematosus/ Argentina		196 (89/107)	(40)	(9)	(12)	Damage index, (1.86 ± 0.34)	AChE, BChE, CAT, TBARS, GSH/GSSG, SOD	[62]
18 (57)	Hemodialysis diabetic patients/ Paraguay	6 months	80 (40 Before and after treatment/ 40 controls)	(65.3)	(77.5)	NI	% Tail DNA, after Hemodialysis (1.57 ± 0.79) before Hemodialysis (2.35 ± 1.17)	Oxidative DNA damage	[63]
18 (57)	Chronic obstructive pulmonary disease/ Mexico	3 weeks	129 (89 Before and after /40 controls	(>70)	(44)	(18.8)	Damage index (1.11 ± 0.43)	IL-6 levels	[64]

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Table 5 (*continued*)

hmQS[a] (QSca[b])	Clinical assessment, Country	Treatment	Subjects (Exposed/ Control)	Age (Mean years)	Male (%)	Current Smokers (%)	DNA Damage (Odd Ratio)*	Other Biomarkers	Ref.
17 (56)	Smokers/ Mexico	N/A	20 (11/9)	(27)	(40)	(55)	Image length (1.4 ± 0.46)		[65]
14 (50)	Smokers/ Mexico	N/A	82 (39/43)	Smokers (40.6) Former smokers (55.3) Non-smokers (47.2)	(35)	(35)	Multiparameter Current smokers 340 (0.38*/ 0.01*) Former smokers 450 (0.45*/ 0.01*)	-	[66]
14 (58)	Asthmatics/ Mexico	N/A	77 (11/66)	(20)	(34)	NI	Tail length Nasal epithelial cells (1.9 ± 0.29) Leucocytes (0.86 ± 0.19)	Cytological evaluation for nasal epithelial cells (neutrophils and eosinophils proportion)	[67]
18 (59)	Elderly individuals (normal CD4:CD8 and inverted CD4: CD8 ratio)/ Brazil	N/A	16 (8/8)	(65.68 ± 4.35)	(50)	(0)	Damage index (0.94) *	Peripheral frequency of CD3 + / CD4 + CD3 + / CD8 + CD8 + /CD25 + CD8 + / CD28 + CD8 + / CD45ro+	[68]
12 (52)	Metronidazole treatment/ Mexico	10 days	20 (10 Before treatment/10 After treatment)	(23.5)	NI	(0)	Tail length (1.22 ± 0.24)		[69]
23 (57)	Malaria/ Colombia		200 (100/100)		(55)	(13)	Tail length (1.81 ± 0.46)		[70]

[c] Other biomarkers:): AChE: acetylcholinesterase; BChE: butyrylcholinesterase;; BMN: buccal micronuclei; CAT: catalase activity; CBMN: Cytokinesis blocked micronucleus assay; GSH: Glutathione; GSSG: Glutathione Disulfide; SOD: Superoxide dismutase; CD3 + : Protein complex for all mature T-cells; CD4 + : Co-receptor protein for helper T-cells; CD8 + : Co-receptor protein for cytotoxic T-cells; CD25 + : Alpha subunit of the interleukin-2 (IL-2) receptor; CD28 + : Co-stimulatory receptor found on the surface of T-cells; CD45ro+ : Isoform expressed on memory T-cells; IL-6: Interleukin-6.

[a] hmQS: human monitoring Quality Score (ranging of 8-24) described in Supplementary Table 2;

[b] QSca: Quality Score comet assay (ranging of 21-63) described in Supplementary Table 1; * negative result (odds ratio<1.2);

pollution in large urban centers, where pollutants such as volatile organic compounds (VOCs), particulate matter, and ozone are prevalent. Studies revealed a strong correlation between exposure to these pollutants and increased DNA damage in lymphocytes, buccal, nasal, and tear cells, as detected by the comet assay. Cellular markers, such as squamous metaplasia in the nasal epithelium, further supported these findings. Additionally, the comet assay was highly sensitive in detecting DNA damage induced by other environmental pollutants, including arsenic and fluoride.

These observations led us to understand that monitoring studies with more than 200 subjects, along with the ability to classify the roles they perform and quantify the level of exposure, are extremely relevant for obtaining valuable data that can be incorporated into risk assessment decision-making. Therefore, we consider these conditions essential for the LA-COMET group to incorporate into its future work when conducting human monitoring studies.

3.4. Pesticides exposure

Pesticides are widely used throughout the world, and Latin America is no exception, given the region's high level of agri-food activity. This situation poses a public health problem due to the risks associated with exposure to these compounds. This exposure can occur through occupational activities related to their production or dispersal, as well as through the persistence of pesticides in the environment, contaminating both water bodies and food. Furthermore, it is important to note that the IARC has classified several pesticides as possible or probable human carcinogens. This classification is based on studies conducted in humans and animals that link pesticide exposure to the incidence of multiple types of cancer, with lung, prostate, lymphatic system, and hematopoietic cancers being the most frequent. This has been documented by Blair et al. [29] and Adad et al. [30].

Twenty-four studies published by the LA-COMET group on the effects of pesticide exposure reflect that this is a topic of interest to several members of the collaborative network [10,31–53]. A summary of these studies is presented in Table 4. Of these, 20 articles investigated occupational exposures, representing 81%, 57% of which were publications from Brazil. Only four publications (19%) addressed environmental pesticide exposure, and of these, 80% were conducted in Mexico. In three of the four studies on environmental pesticide exposure, participants were children aged 6–13 years, and the fourth study used umbilical cord and maternal samples (average maternal age 23.4 years). Overall, the environmental studies showed a significant increase in DNA damage compared to individuals from less exposed or control regions. It is worth noting that in the study of mothers and umbilical cord blood, the times increase in damage was the lowest (1.20 ± 1.25).

According to Alavanja and Bonner [54], two main routes of exposure to pesticides are identified: inhalation, which involves breathing air containing pesticide aerosols or small particles with adsorbed pesticides; and dermal absorption, which can occur during mixing, handling, spraying, and harvesting processes. In the review by Bolognesi and Holland [55], it was also reported that most studies on pesticide exposure demonstrate a significant increase in DNA damage; which we observe from the publications of the LA-COMET group in this context. Also, a meta-analysis by Pinto et al. [56] confirmed that occupational exposure to pesticides increases DNA damage, evaluated by both the comet assay and the micronucleus assay, as we observed in this work (Table 4). According to the hmQS scores, 8% of the publications received a low score; 54.5% received an intermediate hmQS score, and 37.5% of the publications received a high score. In general, the design of the pesticide effects monitoring studies was well executed; however, we would like to mention aspects that we identified as opportunities to

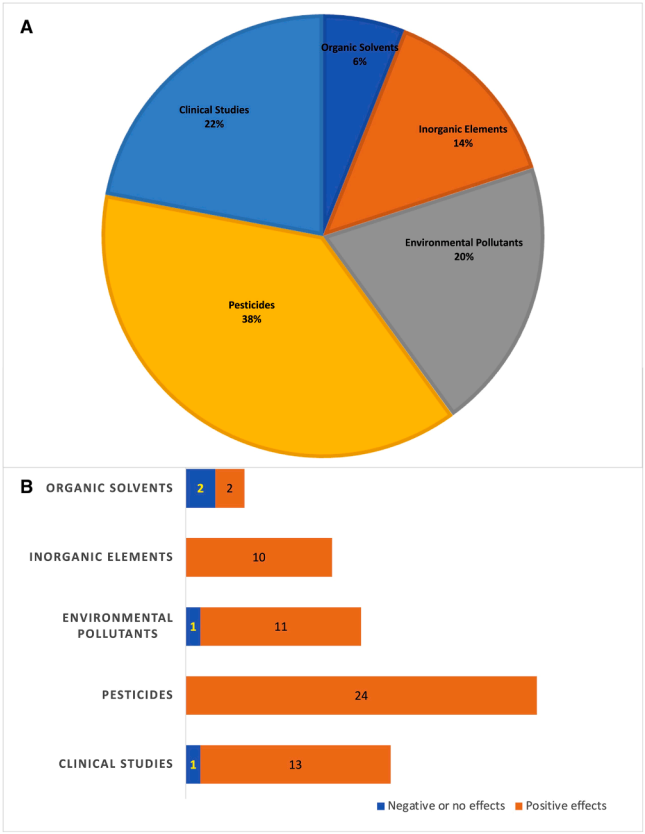


Fig. 1. A) Distribution of LA-COMET group publications on human monitoring according to the source of exposure: organic solvents, inorganic elements, environmental pollutants, pesticides, and clinical studies. B) Number of publications with positive and negative results based on the odds ratio calculated for each exposure type category (the cutoff value for considering a result positive or negative was greater or less than 1.2 odds ratio, respectively).

improve the performance of the collaborative network in future studies. On the one hand, several studies negatively impacted their hmQS scores, given that the number of subjects in the control group was around 20–50 (see Supplementary Table 2), and five studies did not have a control group [10,31,34,36,40]. Therefore, for future studies, efforts should be made to include control groups of subjects matched for sex and age.

In addition to the above, in the case of pesticide exposure, this is a factor that deserves much discussion; for example, the control subjects in these monitoring studies generally come from the same region. Therefore, although they are not occupationally exposed, they live in sprayed regions and are thus also environmentally exposed to some extent, given the persistence of pesticides. The quality of the methodology is well positioned for this exposure category given that the average QSca score is 58 points.

A positive self-assessment of the LA-COMET group's previous publications regarding pesticide exposure is based on the fact that all publications evaluated chemical exposure to pesticides and/or their metabolites, as shown in Table 4 [10,31–53]. Elevated blood concentrations of DDT, DDE, DDD, and total DDT were observed in individuals with higher exposure. Arsenic in urine and lead in blood showed no significant differences, while organophosphate levels were higher in umbilical cords compared to maternal levels, except for DDE. Increased levels of 1-hydroxypyrene (1-OHP) were found in children from rural areas, with higher lead and total DDT in their blood. Pesticide exposure was assessed through questionnaires, covering substances like captan, copper, mancozeb, chlorpyrifos, and glyphosate. Urine samples revealed pesticides including OH-PYR and 2,4-D. Blood, serum, and urine analyses identified 15 organochlorines, 10 organophosphates, 5 carbamates, and ethylene thiourea. No significant differences were found for inorganic elements in buccal cells or BChE levels, although zinc and magnesium levels were higher. Increased cotinine levels indicated nicotine exposure.

One of the challenges facing investigators has been to identify the individual compounds that may be responsible for the possible adverse effects associated with exposure to environmental agents. While the magnitude of increased genotoxic effects is difficult to predict for each individual, positive results from biomonitoring studies suggest a genotoxic risk at the group level, as a group of exposed subjects. To improve risk assessment of occupational and environmental exposures, some chemical exposure assessment should be carried out (both environmental and body fluids), the exposed and control groups should be adequately matched, and potential confounders of response (e.g. gender, age, lifestyle factors, and diet) should be addressed. Genotoxic damage caused by chemical compounds can also be influenced by the individual inheritance of variant polymorphic genes involved in the metabolism of chemical compounds and in DNA repair mechanisms, so it is very important to evaluate the genetic differences.

3.5. Clinical studies and lifestyle factors

In this review, 22% of the articles, as shown in Table 5, focus on clinical evaluations using various approaches [57–70]. These studies examine diverse health conditions, including breast cancer, ataxia-telangiectasia (AT) [61], systemic lupus erythematosus (SLE) [62], diabetes (DBT) [63], chronic obstructive pulmonary disease (COPD) [64], asthma, and malaria [70]. Additionally, two articles investigate lifestyle factors, particularly smoking [65,66], and one study focuses on elderly individuals [68]. Notably, six out of the fourteen clinical studies evaluate the effects of treatments, such as metronidazole, hemodialysis, pulmonary rehabilitation, chemotherapy, and radiotherapy.

Breast cancer emerges as a significant public health concern in LA-COMET group, characterized by high morbidity and mortality rates. The comet assay has proven valuable for assessing genomic damage in breast cancer patients. One study involving 100 patients undergoing chemotherapy reported a significantly higher frequency of cells with DNA damage (94.83% ± 6.80) compared to controls (14.53% ± 8.24). Similarly, patients receiving radiotherapy showed a frequency of 90.20% ± 12.23 of cells with DNA damage, which was also significantly elevated relative to the control group. These findings underscore the adverse genotoxic effects of cancer treatments and highlight the importance of monitoring genomic integrity in these patients.

Ataxia-telangiectasia, a rare genetic disorder characterized by defective DNA repair mechanisms, renders affected individuals highly sensitive to radiation and predisposes them to cancer development. In one study, 14 patients with AT, along with 12 of their parents and 14 control individuals, underwent DNA damage assessment using both the comet assay and the Cytokinesis-Block Micronucleus (CBMN) test. While baseline levels of DNA damage did not differ significantly among the groups, AT patients exhibited delayed DNA repair following radiation exposure, emphasizing the disorder's impact on genomic stability [61].

Systemic lupus erythematosus (SLE), a complex autoimmune disease influenced by genetic and environmental factors, was also examined. In a study involving 89 SLE patients, approximately half of whom were exposed to pesticides, rural patients exhibited 3.52 times more oxidative DNA damage compared to their urban counterparts. This observation suggests a possible link between environmental exposures and disease progression in SLE [62].

Diabetes, a condition whose global prevalence has quadrupled over the past three decades, was addressed in a study that evaluated DNA damage in hemodialyzed patients with type 2 diabetes mellitus. The results showed significantly higher baseline DNA damage in diabetic patients with chronic kidney disease compared to controls, with further increases in DNA damage observed following hemodialysis treatment

[63].

Chronic obstructive pulmonary disease, a progressive lung disorder often aggravated by environmental exposures and comorbidities like diabetes, was evaluated in a study involving 89 patients participating in a three-week pulmonary rehabilitation program. Following the intervention, a significant increase in DNA damage was observed, particularly among non-responders and patients who required oxygen therapy [64].

Regarding lifestyle factors, two studies analyzed in this review consistently reported higher DNA damage indices in smokers [65,66]. Asthma patients also showed increased DNA damage levels, as determined by the comet assay, with significant oxidative stress detected in nasal epithelial cells [67].

In relation to pharmacological treatments, one study investigated the genotoxic effects of metronidazole, a commonly used antiprotozoal and bactericidal agent [70]. The findings revealed a significant increase in DNA strand breaks observed one day after treatment completion, likely attributable to metronidazole metabolites rather than the parent compound.

Finally, malaria, a disease caused by *Plasmodium* protozoa and affecting millions worldwide, was associated with increased DNA damage in infected individuals. One study reported significantly higher tail length values in malaria patients (26.9 ± 9.8) compared to controls (14.8 ± 3.2), suggesting that malaria infection is linked to enhanced genotoxicity, independent of smoking status or treatment factors [70].

Overall, these clinical studies demonstrate the broad applicability of the comet assay for detecting genomic instability across a range of diseases, environmental exposures, and therapeutic interventions. The findings reinforce the assay's sensitivity in identifying DNA damage associated with both endogenous disease processes and external factors, highlighting its relevance for clinical monitoring, risk assessment, and evaluation of treatment-related genotoxic effects.

3.6. Global analysis of the LA-COMET group's human monitoring publications

One of the objectives of the LA-COMET collaboration network is to analyze how its members have been publishing over the past 26 years, in order to identify limitations that can be addressed in future collaborations. The first step of this initiative was to provide a general overview of the group [1].

In previous sections of this study, we have described the published works by exposure category, noting some aspects that can be improved for future monitoring studies. However, we also consider it important to comment on the cell types used, comparison of the DNA damage assessment parameters, and the relationship between the two types of quality scores, QSca and hmQS.

3.6.1. Cell types

Regarding the cell types used in human monitoring studies, 85% were performed on peripheral blood samples and 5% on isolated lymphocytes. Ten percent of the publications employed alternative cell types (5% buccal epithelial cells, 3% nasal exfoliated cells, and 2% ocular exfoliated cells). Our group's pioneering work in exploring alternative biological matrices to determine DNA damage using the alkaline comet assay is a strength. The use of exfoliated buccal, nasal, and ocular exfoliated cells obtained from tears is relevant when dealing with agents that interact directly with them [23,25,26]. However, we also identify their limited use as a weakness. Therefore, we consider their use an opportunity to promote them among members of our collaborative network. Peripheral blood remains a dominant model in genetic toxicology and is widely validated for human biomonitoring. Since the sampling is minimally invasive, these cells act as effective "sentinels" to monitor systemic effects, and they also have a long lifespan.

3.6.2. DNA damage assessment parameters

The relevance of DNA damage assessment using the comet assay depends heavily on the parameter employed. Historically, comet tail length was the parameter used in the methodology. Subsequently, with the implementation of image analysis, tail intensity, tail moment, and Olive tail moment emerged as parameters. It's worth noting that, as an alternative to tail length determined with a graduated eyepiece, damage index and damage frequency were also considered. Thus, based on a thorough analysis of publications from the LA-COMET group, we identified that most studies used visual assessment parameters to evaluate DNA damage [1]. We believe this is primarily due to limited access to image analyzers in several of our studies. Therefore, the analysis of alternative damage parameters has been identified as a priority for the group's future research, as it is the way to avoid bias in intergroup evaluation.

As indicated in the methodology, we calculated an odds ratio for each study (damage parameter value of the exposed group/damage parameter value of the control group). This allows us to standardize damage values, enabling comparison between studies, regardless of the parameter involved (see 2.4). Considering this, we were interested in establishing whether this ratio applied between different damage parameters revealed a more sensitive parameter. To this end, in Fig. 2A, we show the percentage of human monitoring publications from the LA-COMET group as a function of the damage parameter used. We found that the damage index and tail length were used most frequently; the latter parameter could be evaluated using a graduated eyepiece or an image digitizer. Furthermore, in Fig. 2A, we also show the average odds ratio for each of the damage parameters. After statistical analysis, the damage index (DI) and damage frequency (DF) differ significantly from the parameters associated with tail length, tail DNA percentage, and Olive tail moment (OTM). Based on these results, and taking advantage of the fact that some studies evaluated several parameters in the same samples, we performed a percentage increase analysis (Fig. 2B) using the following formula:

$$\Delta\% = (m1 - m2) / m2 \times 100$$

Where m1 and m2 represent the slopes of the damage index and the percentage of tail DNA, respectively. Our analysis revealed that the damage index values are, on average, 48% higher than those obtained for the percentage of tail DNA (Fig. 2B). This discrepancy could be attributed to the method used to calculate the damage index, in which the value for the least damaged cells is multiplied by zero. This is derived from the characteristic damage distribution in comet assay data, where undamaged or slightly damaged cells are the most frequent, while the most damaged cells are usually the least frequent, and this value is multiplied by four to calculate the damage index. This weighted calculation likely explains the observed discrepancy between the two parameters.

3.6.3. Correlation between quality scores

We aimed to determine whether the quality of the monitoring study design, as measured by the hmQS, was related to the quality of the methodological process, as measured by the QSca score. To this end, we performed a correlation between the two QS scores and found no statistically significant relationship between these two parameters (Fig. 3A). Similarly, we examined the correlation between the quality of our biomonitoring study design (hmQS) and the odds ratio obtained for each manuscript. As illustrated in Fig. 3B, no relationship was found, indicating that the DNA damage results were not influenced by the study design. However, it is worth noting that our studies were rated as "good" to "very good" for these parameters; therefore, further evaluation is required to determine whether this lack of correlation persists when the parameters are rated at a lower level.

A

	%Tail DNA	Olive tail moment	Tail length	Damage index	Damage frequency
Publications %	20	12	24	33	11
Mean	2.25± 1.09	2.41 ± 0.82	2.01 ± 1.14	3.80 ± 2.8•••	4.9 ± 2.46***

Statistical significance determined by One-way ANOVA with Tukey´s comparison test with respect to %Tail DNA, Olive tail moment and Tail length. *** p< 0.001.

B

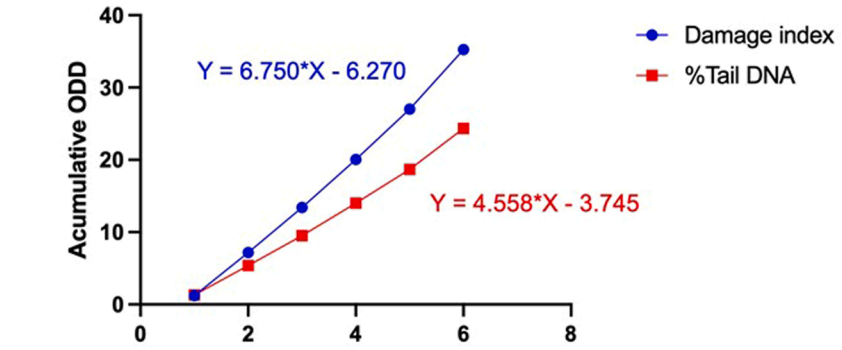


Fig. 2. Comparison of DNA damage assessment parameters. A) Table showing the percentage of use of each DNA damage assessment parameter in the LA-COMET group's human monitoring publications (statistical analysis). B) Relationship between the damage parameters, damage index and % Tail DNA, with respect to the evaluation of the oods ratio, in the publications that reported both in the same study.

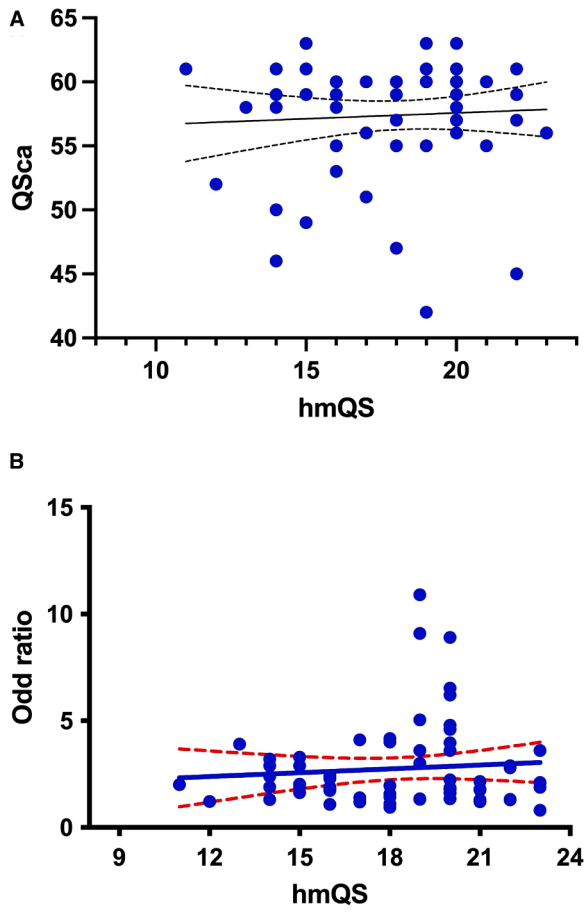


Fig. 3. Correlation between QSca and hmQS quality scores. A) Correlation between the two QS scores (hmQS vs QSca) and found no statistically significant relationship between these two parameters. B) hmQS influence into DNA damage evaluation by Odds ratio.

4. Conclusion

As part of the LA-COMET group's description, within the context of publications on human monitoring studies, the collaborative network has demonstrated expertise in assessing DNA damage caused by organic solvents, inorganic elements, environmental pollutants, pesticides, clinical studies, and lifestyle factors that reflect the region's vulnerabilities. Through the analysis of sixty-four studies, the use of the COMET assay, complemented by additional biomarkers of oxidative stress and cellular damage, has been fundamental in demonstrating the genotoxic risks faced by specific occupational groups and diverse communities in Latin America, highlighting their vulnerability to various health conditions. By proposing quality scores for methodological performance and the design of monitoring studies, LA-COMET identified factors that allow for the improvement of population studies, aiming to transform them into multidisciplinary studies that can impact local and regional decision-making. This, in turn, contributes to improving environmental quality and individual health, thus supporting global initiatives such as "One Health".

It is crucial to recognize that optimizing protocols is fundamental to ensuring the consistency and reliability of results across the various studies planned for the collaborative network in the short, medium, and long term. Genetic disorders such as ataxia-telangiectasia also showed impaired DNA repair. Conditions such as systemic lupus erythematosus, diabetes, chronic obstructive pulmonary disease, and asthma were also associated with increased DNA damage, often linked to oxidative stress and environmental factors.

Lifestyle factors, particularly smoking, further amplified DNA damage, as observed in both smokers and individuals exposed to air pollutants and pesticides. These findings underscore the importance of addressing modifiable risk factors and strengthening public health initiatives to reduce genotoxicity in vulnerable populations. It is also important to note the need to incorporate exposomal analysis to provide a more targeted approach to genotoxicity assessment. Overall, this review highlights DNA damage and its associated health risks, according to 17 teams from seven-member countries of the LA-COMET group. As can be seen, the Latin American region faces very similar problems regarding human population exposure. However, collaboration among the different country groups within the LA-COMET group is very limited,

representing an opportunity to generate broader and more robust studies that facilitate decision-making and, ultimately, contribute to protecting the health of people exposed to hazardous chemicals and living with chronic diseases.

CRediT authorship contribution statement

Tovar-Sanchez Efrain: Writing – original draft, Validation, Data curation. **Guillermo Espinosa-Reyes:** Writing – original draft, Data curation. **Sonia Soloneski:** Writing – original draft, Validation, Data curation. **Yañez Leticia:** Writing – review & editing, Formal analysis, Data curation. **Emilio Rojas:** Writing – review & editing, Writing – original draft, Validation, Formal analysis, Conceptualization. **Gomez-Arroyo Sandra:** Writing – original draft, Validation, Data curation. **Mahara Valverde:** Writing – review & editing, Writing – original draft, Validation, Data curation, Conceptualization. **Flores-Marquez Ana Rosa:** Writing – original draft, Validation, Data curation. **Celeste Ruiz de Arcaute:** Writing – original draft, Validation, Data curation. **Poletta Gisella:** Writing – original draft, Validation, Data curation. **Simoniello Maria Fernanda:** Writing – review & editing, Data curation, Conceptualization. **Narvaez Diana M.:** Writing – original draft, Validation, Data curation. **Elda Leonor Pacheco-Pantoja:** Writing – review & editing, Methodology, Data curation, Conceptualization. **Noemi Tirado:** Writing – review & editing, Data curation. **Laura Lafon-Hughes:** Writing – original draft, Validation, Data curation. **Juliana Da Silva:** Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization. **Ilizarrituri-Hernandez Cesar:** Writing – original draft, Validation, Data curation. **Patricia Mussali-Galante:** Writing – original draft, Validation, Data curation. **Garcia Ana Leticia:** Writing – original draft, Data curation, Conceptualization. **Leon-Mejia Grethel:** Writing – original draft, Validation, Data curation. **Deidamia Franco:** Writing – review & editing, Formal analysis.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mrgentox.2026.503940.

Data availability

All the data analyzed during this study have already been published and can therefore be reviewed in the original journals.

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