Mutat Res Gen Tox En 853 (2020) 503170

Contents lists available at ScienceDirect

Mutat Res Gen Tox En

journal homepage: www.elsevier.com/locate/gentox

Increased micronucleus frequencies in reticulocytes of children exposed to industrial pollution: oxidative stress and the *OGG1* S326C polymorphism

Regina D. Montero-Montoya[a,*], Rocío López-Vargas[a], Alejandra Méndez-Serrano[b], Isaías Galicia-Alonso[b], Guadalupe García-Vargas[b], Luis Serrano-García[a], Rosa Beltrán-Portugal[a], Samuel Rosado-Zaidi[c], Arnulfo Albores-Medina[d], Luis Oropeza-Hernández[e], Leticia Hernández-Cadena[f], Francisco Mercado-Calderón[g], Esmeralda Alvarado-Toledo[e], Selene Herrera-Morales[a], Omar Arellano-Aguilar[h]

[a] *Departamento de Toxicología Ambiental, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico*
[b] *Centro Fray Julián Garcés, Derechos Humanos y Desarrollo Comunitario, A.C, Mexico*
[c] *Facultad de Economía, Universidad Nacional Autónoma de México, Mexico*
[d] *Departamento de Toxicología, Centro de Investigación y de Estudios Avanzados, Mexico*
[e] *Laboratorio de Toxicología Industrial, Hospital General de Nanchital, Petróleos Mexicanos, Mexico*
[f] *Instituto Nacional de Salud Pública, Ciudad de México, Mexico*
[g] *Coordinación de Salud en el Trabajo, Facultad de Medicina, Universidad Nacional Autónoma de México, Mexico*
[h] *Facultad de Ciencias, Universidad Nacional Autónoma de México, Mexico*

ARTICLE INFO

Keywords:
erythrocytes
transferrin receptor

ABSTRACT

We examined possible early-effect biomarkers and polymorphisms of susceptibility in primary school children living near the Atoyac River in central México, which receives waste from multiple industries. We observed a significant increase in micronucleated reticulocytes associated with the oxidative stress index (OSI) and the *OGG1 GG* (S326C) genotype, and a significant decrease of reticulocytes carrying the transferrin receptor, inversely correlated with OSI.

1. Introduction

Environmental pollution is associated with adverse health effects, including cancer, cardiovascular disease, kidney disease, reduced fertility, and congenital defects [1]. Rivers may be used as sewage systems into which industries discharge liquid waste containing toxic compounds. Studies have evaluated health risks for people living in communities near such rivers (Table 1). Even when levels of pollutants are within limits considered 'acceptable', for individual compounds, health may be harmed due to simultaneous exposure to multiple compounds.

Communities near the Atoyac River, a small permanent stream in the central plateau of México (Fig. 1), have been affected by its proximity to industrial corridors involved in industrial activities, including petrochemical, automotive, paint, metal-mechanic, textile, porcelain, and pesticides, generating discharges containing VOC, metals, dyes, and phthalates, that, along with the run-off of agricultural pesticides, constitute a toxic mixture that has eradicated most of the river's original wildlife. In 2008, authorities of the National Water Commission [2] conducted a health risk assessment along the river, concluding that the waterway is "highly polluted" and that "at a distance of 2 km from the stream, the adverse consequences, immediate and future, to human populations will manifest in damage to their health, integrity and security as long as the environmental damage continues to provoke alterations in the ecological equilibrium of the basin". People living in the communities bordering the river complain of strong irritating odors emanating from the river every time that there is a discharge, which occurs daily during nighttime.

We have been studying children living in one of the most affected communities, Tepetitla, and regularly attending school, determining their general health status as well as their serum oxidative stress indices (OSI), levels of exposure (measured as BTEX metabolites in urine), and genotoxic damage in bone marrow. A database was generated and, in a

Abbreviations: BTEX, benzene toluene, ethyl-benzene, xylenes; MN, micronucleus; OSI, oxidative stress index; ROS, reactive oxygen species; TO, thiazole orange; VOC, volatile organic compounds

* Corresponding author at: Instituto de Investigaciones Biomédicas, Circuito Exterior, Ciudad Universitaria, C.P. 04510, Ciudad de México, Mexico.
E-mail address: dorinda@iibiomedicas.unam.mx (R.D. Montero-Montoya).

https://doi.org/10.1016/j.mrgentox.2020.503170
Received 5 December 2019; Received in revised form 6 March 2020; Accepted 8 March 2020
Available online 14 March 2020
1383-5718/

Table 1
Studies relating industrial contamination in water bodies with health, in México.

Polluted water body	Contaminants	Environmental mobility	Health risks studied	References
Coatzacoalcos river: - Discharges from petrochemical complexes	PCB, VOC, PAH, Pb	Transfer to air of VOC, PAH, metals PCB bioaccumulation in fish and seafood Drinking water with VOC	Cancer (USeTox model) Reproductive damage Delayed child development	[43]
Mexican coasts (Ensenada and Coatzacoalcos): - Mine and refinery of Au, Fe, Zn; - Agriculture; - Carbo and thermo-electric industries; - Zn and Pb smelters	Hg	Transfer to the air To the biota To sea birds To sea mammals Transfer to food,animal or vegetable	Teratogenesis Kidney damage	[44]
Santiago river: - discharges from industrial corridors	Al, Hg, Ni, Cr, VOC	Transfer to air and food, animal or vegetable	Increased non-communicable chronic diseases	[45]
Atoyac river: - discharges from industrial corridors	VOC, gasoline, industrial dyes, Hg, Ni, Pt, As	Transfer to air and food, animal or vegetable	Genotoxic damage in peripheral lymphocytes	[21]
Atoyac river: - discharges from industrial corridors	BTEX, industrial dyes, metals	Transfer to air	Oxidative stress & diminished antioxidant capacity	[3]

Fig. 1. The study site in Tepetitla is shown inside the polygon with the Atoyac River crossing transversally. The chaotic expansion of human settlements over agricultural areas is seen as well as industrial settings in the bottom left of the image. On the right side, the town of Ixtenco is seen in the middle of agricultural fields, at the bottom of a volcano covered with forests, whence they receive water for their activities. (Images taken from Google Earth Pro 7.3.2.5776).

first study, we reported that excretion of BTEX metabolites is inversely correlated with the oxidative stress index; i.e., with higher OSI, lesser amounts of metabolites were excreted in urine [3]. This was markedly in contrast with BTEX metabolite excretion by children in a town used as control, where no correlation was found between OSI and metabolite excretion and where OSI was significantly lower. We suggested that increased CYP2E1 activity (involved in VOC biotransformation) generates ROS and electrophilic metabolites, such as quinones and epoxides [4,5,6] leading to increased OSI [3].

Genotoxic damage could be expected to be higher in these children. We have now evaluated genotoxicity in bone marrow, a target tissue for benzene, where levels in the area were similar to those reported for highly polluted cities such as México City (5.3 μg/m^3 [7,3] and supplementary material, Table 3 s). We used peripheral reticulocytes as surrogates of the bone marrow and determined the frequency of micronuclei. In addition, *OGG1* polymorphism S326C was determined, to test whether the damage may be related to oxidative DNA lesions.

2. Material and methods

2.1. Ethics approval

The protocol for the study was approved by the Human Ethics Committee of our Institute. The objectives of the study were explained in school meetings and parents signed an informed consent letter.

2.2. Inclusion criteria

Children to be sampled were chosen from those regularly attending school, one child per family, with no diagnosed chronic diseases and no infections at the time of sampling. The exposed group consisted of children living in two riverside communities of the municipality of Tepetitla and the reference group comprised children living in a rural community of the municipality of Ixtenco (Fig. 1).

The day before drawing blood, a visit was made to the schools to weigh and measure the children and to give them instructions on blood

Table 2
Cell markers employed in the Reticulocyte micronucleus test.

Laser	Detector	Fluorochrome	Cell marker
Blue	BL1-H	Thiazole orange (TO)	RNA
Blue	BL2-H	Phycoerythrin (PE)	CD61
Blue	BL3-H	PE-cyanine7 (PE-Cy7)	TfR (CD71)
Violet	VL1-H	Hoechst 33342	DNA

TfR – transferrin receptor.

sampling the following morning. Next morning, at 7 a.m., venous blood was taken by a pediatric nurse, in two Vacutainer tubes. Samples were kept fresh in an ice chest to be immediately transported to our laboratory for processing. One of the tubes was taken to a certified clinical laboratory for hematologic measurements.

2.3. Reagents

Antibodies for flow cytometric analysis were purchased from BioLegend-CIPQUIM, México (anti-CD61-PE, anti-CD71-CyPE). The fluorochromes thiazole orange (cytoplasmic RNA) and Hoechst 33342 (DNA) were purchased from Sigma México. DNA probes to determine the *OGG1* polymorphism were purchased from Invitrogen-Accesolab, México.

2.4. Micronucleus (MN) frequency in circulating reticulocytes

A dual-laser Attune™ Acoustic Focusing Cytometer (Applied BioSystems of México) was used with a blue/violet configuration and controlled for acquisition and analysis by Attune® Cytometric Software. The methodology has been described in detail elsewhere [8] and is based on the methods developed by Bemis et al. [9], Abramsson et al. [10], Litron Labs Rat Flow Manual [11], and Kasamoto et al. [12]. The parameters determined were: proportion of RNA-containing reticulocytes (TO+ cells) and reticulocytes bearing the transferrin receptor CD71 (CD71+ cells), as well as MN frequencies in TO+ reticulocytes, CD71+ reticulocytes, and mature erythrocytes. Table 2 shows the antibodies and fluorochromes used for these determinations. CD71+ cells were distinguished by their transferrin receptor in an independent channel; however, when evaluating RNA contents, they were also counted. Hence, CD71+ cells are included in the frequency of RNA positive/TO+ cells, which are more numerous, as explained elsewhere [8].

2.5. Genetic polymorphism

OGG1 Ser326Cys was determined by RFLP, based on the method of DeRuyck et al. [13]. Amplification conditions were as follows: one denaturation step at 94 °C for 3 min, and 34 cycles of denaturation at 94 °C for 35 s, annealing at 56 °C for 35 s, and 72 °C for extension for 1 min, with a final extension time of 5 min. Enzymatic digestion was done with Fnu4HI (purchased to New England BioLabs – Valaner, Mexico) for 3 h at 37 °C. Genotypes were identified according to the following patterns: one 553 bp band, wild type; three bands, 553, 399 and 154 bp, heterozygotes; two bands, 399 and 154 bp, homozygous polymorphic. The primers used were: Forward 5'GTGGATTCTCATTGCCTTCG 3'; reverse 5'CTGTTGCTGTCGAGACTGC 3'.

2.6. Oxidative stress index (OSI)

The method used was that developed by Erel [14,15] and previously described in detail [3]. Briefly, two determinations were made on each plasma sample: the total oxidative status (TOS), consisting of the oxidation of the Fe^{2+}/*o*-dianisidine complex to Fe^{3+} by oxidants present in the sample, expressing the results in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 equiv/L); and the total antioxidant capacity (TAC), whereby antioxidants present in the plasma sample suppress oxidation of *o*-dianisidine, induced by ROS generated by a Fenton reaction between the Fe^{2+}/*o*-dianisidine complex and a standardized solution of H_2O_2. Color intensity is inversely related to the amounts of antioxidants in the samples and the results are expressed as millimolar Trolox equivalents per liter (Trolox mmol eqv/L). The oxidative stress index (OSI) is the percent ratio between TOS and TAC: OSI = [(TOS H_2O_2 μmol eqv/L)/(TAC Trolox μmol eqv/L)] × 100. Determinations of TOS and TAC were made 24 h after taking the samples, with plasma samples that had been kept frozen at −80 °C.

2.7. Statistical analyses

Stata 14 statistical software was used. Since all MN have their origin in the bone marrow, and after corroborating that these were highly correlated (supplementary material, Fig. A), only the frequency values of MN-TO+ were used for analysis. MN in CD71+ cells were not used, since these are contained in the MN-TO+ frequency, as already explained, and due to their reduced numbers in the samples from Tepetitla (data not shown). Kruskal-Wallis tests were performed to compare MN-TO+ levels between the municipalities of Tepetitla and Ixtenco. Kruskal-Wallis was also used to compare OSI levels between municipalities. A Pearson chi-squared test was used to determine whether allelic and genotypic frequencies of the *OGG1* polymorphism were comparable among the study sites and to test for Hardy-Weinberg equilibrium. A value $p < 0.05$ was considered significant.

2.7.1. Use of the database to test other correlations

Linear regressions were used to analyze the potential contribution to genotoxicity (log transformed MN-TO+ frequency) of other database variables, including oxidative stress index (OSI, log transformed data), z-scores of BTEX metabolite excretion (previously reported in [3]), age, sex, categorized body mass index, hematocrit, and hemoglobin, in addition to the *OGG1* genetic polymorphism and the *NQO1*2 (C609 T)*, *CYP2E1 RsaI*, *GTM1*, and *GSTT1* metabolic polymorphisms [3]; those showing $p < 0.05$ were included in a robust regression model. This procedure was also used to analyze factors contributing to the percentage of CD71+ cells.

Table 3
Geographic and socio-demographic data of the municipalities studied.

Data	*Ixtenco*	*Tepetitla*
No. of samples	93	91
Boys:girls (%)	52:48	48:52
Ages (years ± s.d.) (minimum–maximum)	9.6 ± 1.4 (7–12)	10.5 ± 1.4 (7–13)
Degree of marginalization [CONAPO, 2010]	Medium	Medium
Ethnicity	Mestizos	Mestizos
Nutrition type	Mixed (varied)	Mixed (varied)
Home distance to the Atoyac River or water channels	More than 7 km	No more than 500 m
Geographical coordinates	19°15'N; 97°53'W	19°17'N; 98°24'W
Altitude above sea level	2,500 m	2,230 m

Table 4
Allele and genotype frequencies of *OGG1 Ser326Cys*, in each municipality.

Polymorphism	Frequency in Tepetitla	Frequency in Ixtenco	*P**	All
OGG1Ser326Cys allele C	0.53	0.54		0.54
OGG1Ser326Cys allele G	0.47	0.46	0.76	0.46
CC genotype (wild type)	0.26	0.24	0.73	0.25
CG genotype	0.54	0.61		0.58
GG genotype	0.20	0.15		0.17

* Pearson, chi-square test.

3. RESULTS

3.1. Sociodemographic and anthropometric characteristics of the donors

Community data (Table 3) show that the two selected sites are comparable. The degree of marginalization was obtained from the National Population Council [16], which uses this parameter to identify social groups lacking opportunities and public services. Although Ixtenco is a rural community primarily dedicated to agriculture and Tepetitla is located in the middle of an industrial zone and agricultural fields (Fig. 1), both are classified in the medium level of marginalization.

3.2. OGG1 polymorphism S326C

Genotype frequencies did not differ between the municipalities, as also found for metabolic polymorphisms [3]. The *G* allele frequencies for this polymorphism were 0.46 and 0.47 for Ixtenco and Tepetitla, respectively (p = 0.76). Genotype frequencies were also similar between sites and no statistical difference was found, chi-square, p = 0.73 (Table 4); they are in Hardy-Weinberg equilibrium, as are other polymorphisms determined (Table 2s, supplementary material). Despite the intention to sample only one child per family, we had some siblings in the study: three pairs in Tepetitla and five pairs in Ixtenco; one pair in Tepetitla showed the same genotype (CG), and four pairs in Ixtenco (three CG and one, CC). Data of all of the children were considered for analysis.

3.3. Oxidative stress index (OSI)

The index was calculated as the ratio between TOS and TAC. Values were significantly higher in Tepetitla, p = 0.0001 (Kruskal-Wallis). Values in Tepetitla/ Ixtenco were, respectively: mean 5.2/ 2.6, s.d. 3.4/ 1.5, median 4.9/2.3, min–max 0.96–19.6/ 0.21–7.5. Results for the variables TOS and TAC have been reported previously [3].

3.4. Proportion of reticulocytes carrying cytoplasmic RNA and the transferrin receptor (CD71)

The method used provides the proportion of reticulocytes, both those containing detectable cytoplasmic RNA and those carrying the transferrin receptor CD71 [8]. As explained in that publication, cells containing cytoplasmic RNA and stained with TO are identified as among those with the brightest signal in the respective channel, according to the rationale of Hayashi et al. [17]. The proportions found are presented in Table 5, top panel, showing a significant difference in the amounts of CD71+ cells between the municipalities (lower in Tepetitla, p = 0.0001), whereas no difference was found regarding the more abundant RNA-containing TO+ cells.

3.5. Micronucleus (MN) frequencies in circulating reticulocytes and mature erythrocytes

A difference in MN levels was observed between the municipalities (Kruskal-Wallis, p = 0.0001): higher in Tepetitla (Fig. 2a). This difference was observed in CD71+ and TO+ reticulocytes as well as in mature erythrocytes (Table 5, lower panel). Although the frequency was low, we could identify increased MN in mature erythrocytes in the samples from the polluted site, and the difference was significant with respect to the non-polluted site. Also, a clear correlation between MN in TO+ and CD71+ cells was observed, so we chose to use MN-TO+ only. This parameter was logarithmically transformed (ln(MN)) for further analysis.

Table 5
Reticulocyte and micronucleus frequencies per site of study.

Reticulocyte frequencies

%TO+ cells	*Mean*	*s.d.*	*Median*	*Min*	*Max*	*P**
Tepetitla	1.6	1.6	1.1	0.2	8.1	0.71
Ixtenco	1.5	1.4	0.8	0.13	5.2	
%CD71+cells						
Tepetitla	0.03	0.02	0.02	0.008	0.1	0.0001
Ixtenco	0.06	0.04	0.05	0.01	0.21	

Micronucleus frequencies

%MN-TO+ cells	*Mean*	*s.d.*	*Median*	*Min*	*Max*	*P**
Tepetitla	3.9	3.1	3.4	0.18	17.9	0.0001
Ixtenco	2.3	2.3	1.5	0.38	10.4	
%MN-CD71+ cells						
Tepetitla	9.3	7.3	7.9	0.65	52.6	0.0001
Ixtenco	5.3	4.8	3.9	0.09	23.0	
%MN-erythrocytes						
Tepetitla	0.08	0.1	0.02	0.002	0.41	0.00001
Ixtenco	0.03	0.04	0.01	0.0001	0.22	

* Kruskal-Wallis.

The environmental genotoxicity risk was calculated with these data, based on the rationale of Baršienė et al. [18] according to which the upper 10% of reference values in Ixtenco would imply elevated MN frequencies, whereas the values < 90th percentile would correspond to less exposed or more resistant individuals. The number of individuals in Tepetitla presenting a ln(MN) value > 90th percentile of Ixtenco were counted and the numbers compared to calculate the odds ratio, obtaining a value of 3.9, p = 0.0006 (C.I. 95% 1.6–10.1). Calculations (Table 1s) are presented in the Supplementary Material.

3.6. Other parameters contributing to genotoxicity

ln(MN) was used to explore the contribution of all the other parameters determined in the study, as stated in the Methods section, other than municipality, which was the main factor explaining MN levels. Linear regressions were made with individual factors, and those showing p < 0.05 were included in a robust regression model. Results are presented in Table 6, showing that the OSI was another important factor contributing to genotoxicity, as well as the *OGG1 GG* genotype. The same strategy was used to determine factors affecting the presence of the transferrin receptor (CD71) in reticulocytes, other than municipality, and we found, again, that the oxidative stress index showed a significant correlation, albeit a negative one (Fig. 3).

With regard to the OGG1 polymorphism, carriers of the *GG* genotype showed a significantly higher MN frequency than the other genotypes (p = 0.001, Kruskal-Wallis). When we applied the same test by community (Fig. 2b), we found a significant difference in Tepetitla, i.e., *GG* genotypes showed higher MN frequencies, p = 0.001, whereas no significant difference was seen in Ixtenco (p = 0.27). When each genotype was compared between communities, the CC homozygotes showed no difference (p = 0.17) in MN levels, whereas CG heterozygotes were significantly different (p = 0.001) as were GG homozygotes (p = 0.003), being higher in Tepetitla (Fig. 2b).

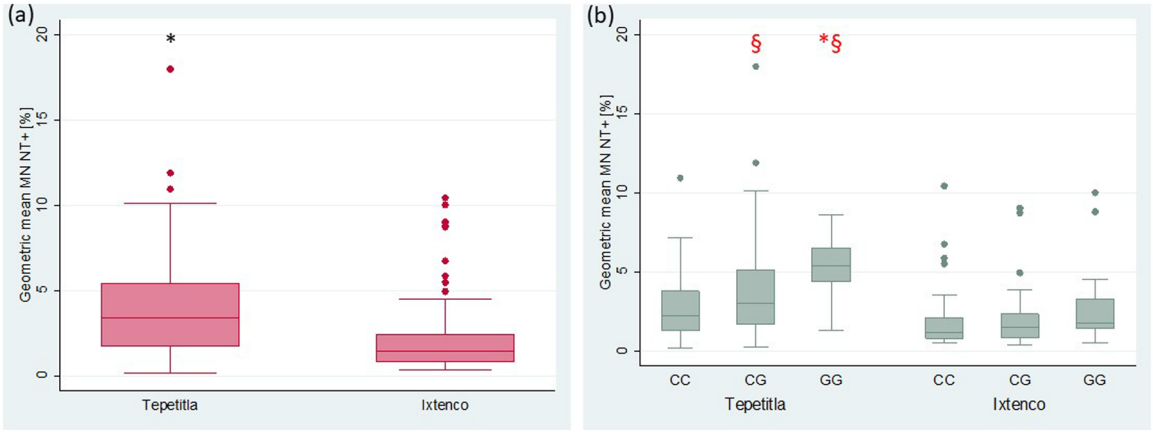

Fig. 2. (a) % MN-TO+ were significantly increased in Tepetitla, compared to Ixtenco (Kruskal-Wallis, p = 0.0001). (b) When stratified by the *Ser326Cys OGG1* polymorphism, *GG* homozygotes in Tepetitla showed significantly higher MN frequencies compared to wild type homozygotes and heterozygotes in the same town (* Kruskal-Wallis, p = 0.001), and to individuals in Ixtenco (p < 0.0006). Furthermore, when each genotype was compared between communities, there was no difference between CC homozygotes, but heterozygotes and GG homozygotes in Tepetitla showed significantly more MN damage than their counterparts in Ixtenco, p < 0.003, thus suggesting a gene-dosage effect (§).

4. Discussion

Genotoxic damage in reticulocytes was studied in children exposed to contaminants discharged with industrial wastewater into a nearby river (without treatment and without warning about risks to health). These people live within 500 m of the riverbank, in communities that have suffered environmental deterioration for decades. Genotoxic events were more frequent among children living near the river (Tepetitla) than among children in the agricultural community (Ixtenco). Children in Tepetitla were four times more likely to show genotoxic damage in erythroid cells than were children in Ixtenco. The oxidative stress index (OSI) was another factor contributing to genotoxicity, which suggests that toxic chemicals induce genotoxicity not only via activated metabolites but also via the production of ROS during their metabolism, as suggested by Belmont et al. [19] and by López et al. [3].

MN were significantly higher in individuals carrying the *OGG1 GG* genotype, particularly in the Tepetitla children. We previously reported the frequencies of several metabolic polymorphisms [20,21,22] in different populations in Mexico, finding that numerous alleles of susceptibility are more frequent compared to other world populations [3]. The *OGG1* polymorphism was no exception, with the *G* allele reaching a high frequency of 0.46, distributed between heterozygous (58%) and homozygous genotypes (17%). *OGG1* frequency had been reported for Mexican-Americans [23] but the frequency of the variant allele found in our study is higher than in those groups (0.46 vs 0.36), as well as the levels reported for Europeans (0.17–0.25) [24,25] and Brazilians (0.19) [26], although similar to Asian populations (0.36–0.45) [27,28]. This

Table 6
Cofactors contributing to the MN levels found and to the proportion of CD71+ reticulocytes.

Variable	Cofactors	P	Coefficient	t	F of the model	P of the model
ln(MN)	Oxidative stress index	.04	0.21	2.1	6.5	0.002
	OGG1 polymorphism	.005	0.32	2.9		
CD71+ reticulocytes	Oxidative stress index	.016	−0.20	−2.43	10.9	0.00001
	Body mass index	.0001	0.28	3.67		

Robust regression models.

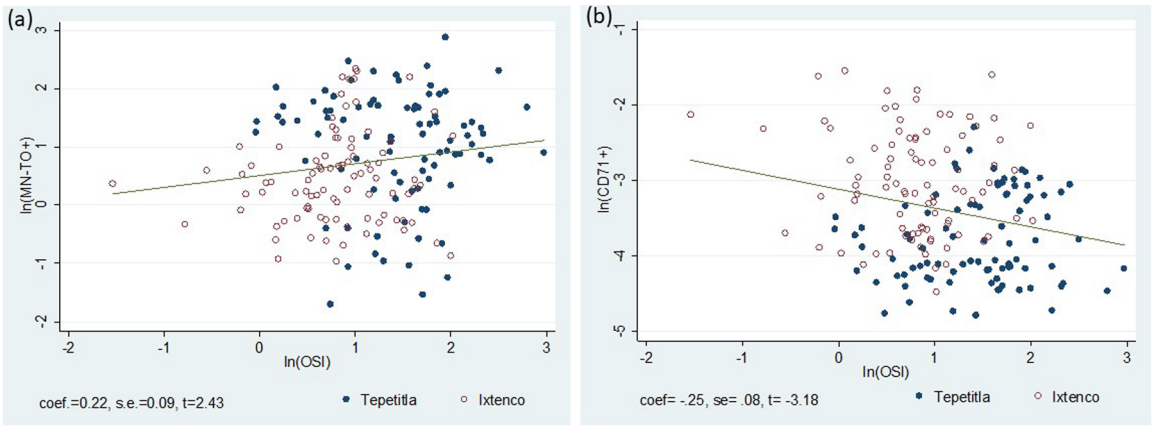

Fig. 3. Robust regressions of lnMN versus lnOSI in (a), and lnCD71+ vs lnOSI in (b), both significant with p < 0.05. Notice that higher lnMN and lnOSI values are found in Tepetitla (a), whereas the lowest lnCD71+ are also found in this municipality (b).

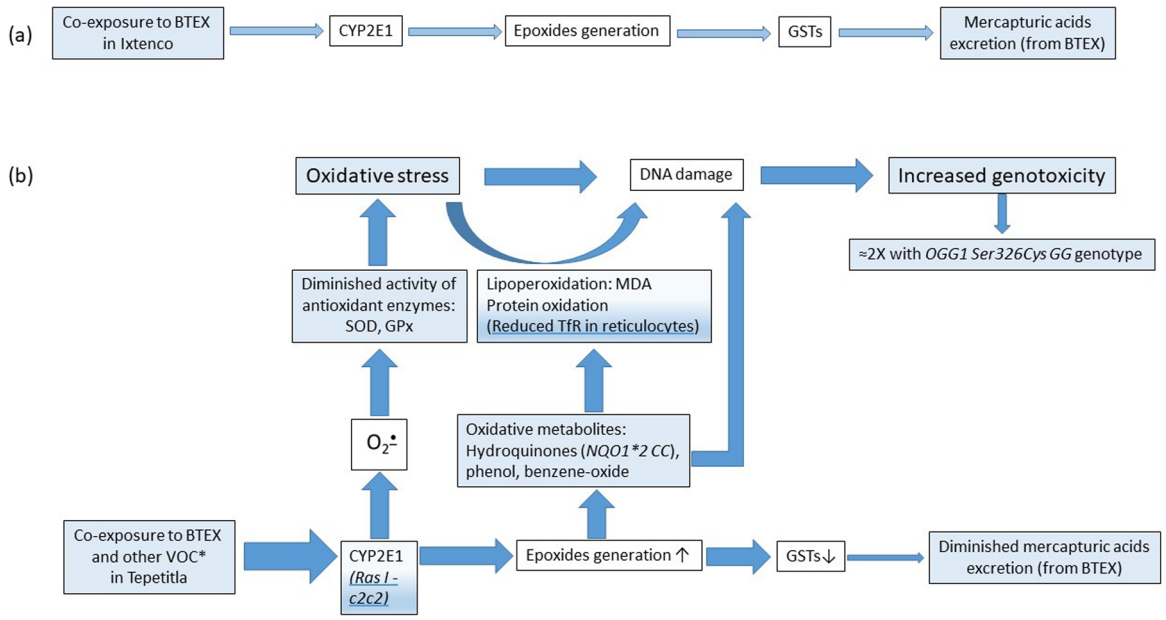

Fig. 4. Conceptual model (a) In Ixtenco, normal elimination of contaminants was observed, with a basal level of genotoxicity (not represented), (b) in Tepetitla, the co-exposure to higher levels of BTEX and other VOC contaminants could induce the activity of CYP2E1 (more with the *c2c2* genotype of the RasI polymorphism), with increased generation of ROS and oxidative metabolites (some as the result of the more active *NQO1*2 CC* genotype), probably saturating both CYP2E1 and GST activities, diminishing hippuric acid metabolites, leading to an increased oxidative state, damaging the antioxidant response, resulting in oxidative stress that could damage macromolecules, such as the TfR, with greater generation of DNA damage and genotoxicity further increased by the *OGG1 Ser326Cys GG* genotype. (Squares filled in blue correspond to the parameters measured in this study and in [3]).

polymorphism decreases OGG1 enzyme activity [29,30] and has been associated with two-fold higher risk of lung cancer [31] and breast cancer in Asian populations [32]. Determining genetic polymorphisms in exposed populations may allow researchers better to predict the health impacts of pollution. In addition, the greater risk of the *GG* genotype for exposed populations suggests a low penetrance of this polymorphism in low pollution level conditions (as in Ixtenco). No gene-dose effect was observed among the children at the exposed site (no difference in genotoxicity between *CC* homozygotes and *CG* heterozygotes). The difference between MN levels between communities for each genotype carrying the *G* allele (Fig. 2b), suggests that there may be a gene-dose; this should be further explored in future studies.

Bone marrow MN may increase with oxidative events, as suggested by the correlation between OSI and the *OGG1* variant genotype. In our previous study, the excretion of BTEX metabolites by exposed children was inversely correlated with OSI, indicating that co-exposure to toxic VOC may alter their normal excretion and increases oxidative status. Our investigations indicate that elevated oxidative status may result in increased DNA damage and may affect expression of the transferrin receptor in reticulocytes, although it did not affect the entire reticulocyte population, identified by their cytoplasmic RNA (TO + cells). This finding should be taken into account when evaluating reticulocyte MN, since CD71 could be a target of toxicity for some chemicals. Benzene metabolites inhibit expression of CD71 in erythroid cell lines [33], and this could affect heme/ hemoglobin synthesis, due to reduced iron transfer into cells. Nonetheless, hemoglobin levels were normal in the children studied (mean 14.8 ± 0.8), except for one Tepetitla boy (12.2).

The conceptual model shown in Fig. 4 describes our findings. Exposure in Tepetitla includes BTEX and other VOC discharged by industries, such as formaldehyde, vinyl chloride, trichloroethylene, and benzene derivatives [3]. This complex mixture may cause the increased oxidative status and genotoxicity we observed, increasing the risks of cancer and cardiovascular disease [34–39]. When these children reach adolescence and sexual maturity, other deleterious effects may become evident [1]. There is mounting evidence that polluted environments harm human health. Landrigan et al. [40] reported that air pollution is becoming the leading cause of premature death worldwide, particularly for children and the elderly. Air pollution has been declared to be mutagenic and carcinogenic to humans [41].

Many small communities and cities in Mexico, particularly in industrialized areas, lack air-quality monitoring systems. The widespread view of residents is that chronic diseases are increasing, but lack of adequate public records on chronic diseases and the reluctance of health authorities to recognize problems make it very difficult to study health outcomes. Mortality, however, is registered by an independent Mexican government agency, the Institute of Statistics and Geography. Using the datasets of this agency, and cross-referencing that information with the Transfer and Emission of Contaminants Registry and the National Water Board, Rosado [42] established that mortality due to cancer (2012–16) was significantly higher in the communities closest to the Atoyac River than in more distant communities in the state of Tlaxcala (Fig. B, supplementary material). Rosado [42] also determined that living less than 25 km from polluted water bodies in Mexico increases the probability of dying from cancer (Fig. C, supplementary material). These findings coincide with the report by Landrigan et al. [40] that environmental pollution contributes to premature death around the world.

Our results show that early biological effects related to the development of cancer and other non-transmissible diseases can be detected in individuals who live in polluted environments, without occupational exposure, and whose genetic susceptibility is enhanced by such exposure, long before the onset of such diseases, when preventative and remedial measures might still be taken.

5. Conclusions

Industrialization of developing economies has brought with it environmental pollution and chronic, non-communicable health problems. Non-governmental organizations such as Centro Fray Julián Garcés, in the area under study, and others in similarly polluted locations, are working to raise public awareness of health risks posed by the

discharge of toxic chemical wastes directly into the environment. Our study provides further data on biological effects attributable to the presence of toxic pollutants in the environment. Levels of pollutants should be monitored by environmental authorities and public health programs for surveillance of health outcomes should be implemented, in coordination with governmental authorities as well as industries, with the goal of controlling and reducing population exposures.

Funding

This research received support from the Universidad Nacional Autónoma de México (UNAM) and from the Children's Environmental Health Network (CONACYT - 251229/271626). CONACYT provided a fellowship for Rocío López-Vargas (number 259598) and a fellowship for Antonio Araujo-Soto (number 372619) students in the UNAM PhD Program in Biological Sciences (Programa de Doctorado en Ciencias Biológicas).

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

We thank the enthusiastic staff of Centro Fray Julián Garcés, the Coordinator for a Living Atoyac, and the Network of Youth for the Defense of the People for their assistance in applying questionnaires and recruiting volunteers for the study in Tepetitla and Ixtenco, as well as the children and their parents who participated in this study; the LABNALCIT-UNAM (CONACYT) for its technical support in the acquisition of flow cytometry samples; Laboratorios Xotepingo for its advice in the transportation of samples and their support in receiving them anytime in the morning; to nurse Manuela González-Rojas, whose expertise permitted us to obtain blood samples without pain from the children; and Antonio Araujo-Soto for his technical participation in MN data acquisition.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.mrgentox.2020.503170.

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