Contents lists available at ScienceDirect

Heliyon

Heliyon

Research article

The importance of understanding the distribution of GSTM1 and GSTT1 genotypes and haplotypes in a region with intense agriculture activity

Alessandro Arruda Alves [a], Fernanda Craveiro Franco [b], Fernanda Ribeiro Godoy [a,c], Jheneffer Sonara Aguiar Ramos [a], Hugo Freire Nunes [a], Thannya Nascimento Soares [d], Daniela de Melo e Silva [a,d,*]

[a] Laboratório de Mutagênese, Programa de Pós-Graduação em Genética e Biologia Molecular, Universidade Federal de Goiás, Goiânia, Goiás, Brasil
[b] Laboratório de Virologia Animal, Instituto de Patologia Tropical, Universidade Federal de Goiás, Goiânia, Goiás, Brasil
[c] Escola de Ciências Biológicas e Agrárias, Campus II, Núcleo de Pesquisas Replicon, Pontifícia Universidade Católica de Goiás, Goiânia, Goiás, Brasil
[d] Laboratório de Genética e Biodiversidade, Programa de Pós-Graduação em Genética e Biologia Molecular. Universidade Federal de Goiás, Goiânia, Goiás, Brasil

ARTICLE INFO

ABSTRACT

Brazil is one of the largest pesticide consumers in the world, mainly due to its intense agricultural activity. The State of Goias, situated in Central Brazil, is a region recognized as an essential producer of soy, corn, beans, sorghum, sugar cane, and cotton. In this study, we evaluated 602 unrelated individuals, distributed in central and southern regions in Goias, presenting combined frequencies (haplotypes) of the GSTT1 and GSTM1 genes. In all municipalities, the frequency of the GSTT1 null genotype was 38.2% and of the GSTM1 null genotype was 50.3%. Goiania, the capital of Goias, presented the highest frequencies of GSTT1 and GSTM1 null genotypes, probably due to a founder effect of non-representative colonizing ancestors. So, the ancestral population adapted to the environment, with the frequencies observed in Goiania. However, nowadays, as there is excessive use of pesticides, the community becomes susceptible to the harmful effects of xenobiotics exposure, mainly due to the high frequency of GSTT1 and GSTM1 null genotypes. As in Goias, the consumption of pesticides has shown considerable growth, haplotypes with null alleles are of high risk for the population. Our results indicated that it is essential to understand the frequencies of the GSTT1 and GSTM1 genes for the monitoring of risk groups, like farmers, who have contact with pesticides, directly or indirectly, as well as assisting in the development of preventive medicine practices.

1. Introduction

Brazil has been the largest consumer of pesticides in the world due to intense agricultural activity and government incentives (Abreu e Alonzo, 2014). Also, until October 2019, the Brazilian government authorized the commercialization of 382 pesticides, many of them banned in other parts of the world, making Brazil one of the countries that uses these products most, increasing the risk to human, animal and environmental health (Ministério da Agricultura, 2019).

The State of Goias is in Central Brazil and is one of the largest producers of corn, beans, cotton, sorghum, sugarcane, and soybeans in the country, with 75% of its exports composed of agricultural products (Instituto Mauro Borges, 2018). This vast production led to the use of 43, 466.3 tons of active pesticide ingredients in 2017. Among the 10 (ten) most used in Goias are: glyphosate (15,486 tons of active ingredient

[AI]); 2,4-D (4,026 tons AI); acephate (2,374 tons AI); mineral oil (1,949 tons AI); atrazine (1,919 tons of AI); mancozeb (1,797 tons of AI); cypermethrin (771 tons of AI); paraquat dichloride (718 tons of AI) and carbendazim (606 tons of AI) (IBAMA, 2019). In this case, Goias became the fourth-highest Brazilian state in commercialized pesticide use (Instituto Mauro Borges, 2018).

In this context, the use of pesticides has intensified, and there is an increase in inadequate handling and excessive use. These situations lead to the presence of pesticides in ponds, rivers, and many other water sources, polluting the environment and damaging both fauna and humans, mainly due to bioaccumulation in food chains (Souza et al., 2011). This may cause respiratory diseases (Ye et al., 2013), non-Hodgkin's lymphoma (Schinasi and Leon, 2014), lung cancer (Bonner et al., 2016), changes in reproductive hormones (Miranda-Contreras et al., 2013), Parkinson's disease (Panov et al., 2002), and Alzheimer's

disease (Richardson et al., 2014).

In 2015, it was underreported that there were 31,900 cases of poisoning by pesticides in Goiás, according to the Minister of Health (Ministério da Saúde, 2016). In 2018, there were 12.74 incidences of poisoning notifications in every 100,000 inhabitants, while Brazil reported only 6.24 (Ministério da Saúde, 2018).

Pesticides are xenobiotics metabolized in two phases in the liver (Coles and Kadlubar, 2003). The superfamily Glutathione S-transferase (GST) is involved in the conjugation phase, making the compounds less toxic and protecting the cell against oxidative damage and electrolyte substrates (Da Fonseca et al., 2010). The subclasses of the GST superfamily studied most in mammals are the Mu (μ), Pi (π), and Theta genes. The gene *GSTM1* (μ) is 4.2 kb, consisting of eight exons and is located on chromosome 1p13.3, with four known alleles. The most frequent is the wild-type allele, and the second one is the null allele characterized by a structural deletion of a region of the gene that causes the loss of the function of the enzyme (θ) (Rodríguez et al., 2014; Strange et al., 2001).

The gene *GSTT1* (θ) is located on chromosome 22p11.2, is composed of six exons, and is flanked by two homologous regions HA3 and HA5. This gene has two alleles known as the most frequent allele (wild) and the null allele. This polymorphism occurs due to the recombination of the homologous regions HA3 and HA5, resulting in the deletion of 5.4 kb and, as a consequence, the loss of function of the enzyme (Rodríguez et al., 2014; Webb et al., 1996).

In this context, individuals with null *GSTM1* genotypes are associated with an increased risk for the development of a variety of diseases, such as arteriosclerosis, Parkinson's disease, and various types of cancer, whereas the null *GSTT1* genotype is associated with the colorectal and central nervous systems (Strange et al., 2001; Wahner et al., 2007; Wang et al., 2010).

Thus, the knowledge of the distribution of *GSTM1* and *GSTT1* polymorphisms in an agricultural population, such as that of Goias, may assist in understanding the frequency of diseases related to poisoning by pesticides or other xenobiotic agents. As Goias is a famous agricultural region, the study of these genes in municipalities of Central Brazil is significant. Therefore, it could be possible to associate the exposure to pesticides, with the null genotypes of *GSTT1* and *GSTM1*, considered to be at risk for the development of chronic diseases, such as cancer.

2. Material and methods

2.1. Sample group

This research was approved by the Research Ethics Committee of the Pontifical Catholic University of Goiás, under the protocol number 1978/2011. To determine the power of our sample group, we used the following formula:

$$\frac{\frac{z^2 x\, p\, (1-p)}{e^2}}{1 + \left(\frac{z^2 x\, p\, (1-p)}{e^2 N}\right)}$$

where N = population size; e = *p-value*; z = score z (1.96, considering a confidence interval of 95%). The population size of Goias state is 6,000000 individuals, according to the Brazilian Institute of Geography and Statistics (IBGE, 2017). In this context, the minimal of individuals to be sample should be 384. To this study, we analyzed 602 unrelated individuals, born in Goias, distributed in 25 municipalities of two regions of the state, central (N = 479) and south (N = 123).

The central municipalities of Goias are Abadiania, Anapolis, Aparecida de Goiania, Aragoiania, Bela Vista, Bonfinopolis, Brazabrantes, Caldazinha, Goiania, Goianira, Itapuranga, Leopoldo de Bulhões, Neropolis, Ouro Verde, Roselandia, Santa Terezinha, Santo Antônio de Goiás, Senador Canedo, Trindade, and Turvania. The south municipalities are Itumbiara, Montividiu, Piracanjuba, Rio Verde, Silvania. The

criteria for inclusion in the survey were only those individuals born and live in the State of Goias. All the municipalities presented tomato, corn, sorghum, and soybean crops (the central plants of the state of Goias). We obtained blood samples voluntarily according to the Informed Consent Form (TCLE) Data such as age, sex, and social habits were obtained by a lifestyle questionnaire.

2.2. DNA extraction and samples quantification

We sampled 10 ml of whole blood, stored on ice, and immediately sent it to the Mutagenesis Laboratory at the Federal University of Goias. Genomic DNA samples were obtained from peripheral blood lymphocytes using the Illustra Genomic Blood MS® (GE, USA) extraction kit, according to the manufacturer's protocol. DNA samples were then quantified using the NanoVue Plus™ (GE, USA) equipment, following the manufacturer's recommendations. After quantification, DNA samples were diluted to a final concentration of 10 ng/μL. The samples were then stored in a freezer at -20 °C, until use.

2.3. Real-time PCR

We performed the analyses of *GSTM1* and *GSTT1* genes deletion using the real-time polymerase chain reaction (PCR), with SYBR® Green PCR Master Mix (Applied Biosystems®, USA), and we also evaluated the co-amplification of the gene *RH92600* as an internal control. Marin et al. (2010) previously suggested the primers and the PCR cycling conditions.

For the PCR reaction, the final volume of 25 μL containing 12.5 μL of master mix, 0.5 μL of magnesium chloride (MgCl2), 2.4 μL of forward and reverse primers of the *GSTT1* gene, 3.2 μL of forward and reverse primers of the *GSTM1* gene, 4μL of forward and reverse primers of the *RH92600* gene, and 1μL of DNA at 10 ng/μL. We used the Step One Plus thermal cycler Real-Time PCR Systems (Applied Biosystems®, USA).

2.4. Statistical analysis

The sociodemographic characterization data of the sample group was stored in an Excel 2013 worksheet (Microsoft®), along with the data of the polymorphisms, for further evaluation of Statistica 7 software (StartSoft Inc). We estimated frequencies, genotypes, and haplotypes by direct counting. Differences in allele frequencies of *GSTT1* and *GSTM1* polymorphisms were calculated using the X^2 test, with a 95% confidence interval (p < 0.05).

3. Results

All 602 individuals are born and raised in Goiás. 68.6% are males, and 31.4% are females. The sample group presented a mean age of 38 years ± 13.5 for males and 36 years ± 12.3 for females. We also verified that 51% of males consume alcohol; in contrast, 66.1% of females do not drink alcohol. Finally, 13.6% and 10.6, men and women, respectively, were smokers (Table 1).

The frequency of the *GSTT1* null genotype was 38.2%, and for the municipalities with more than 20 individuals sampled (Table 2), it can be seen in Fig. 1A. Therefore, the frequency of the *GSTM1* null genotype for

Table 1
Social habits of the sample group distributed by sex.

	Men (413)	Women (189)	Total (602)
Age (Mean ± SD*)	38 ± 13.5	36 ± 12.3	37 ± 13.2
Alcohol intake			
Alcoholic drinker	51% (211)	33.9% (64)	45.6% (275)
Non alcoholic drinker	49% (202)	66.1% (125)	54.3% (327)
Smoke habit			
Non smoker	86.4% (357)	89.4% (169)	87.4% (526)
Smoker	13.6% (56)	10.6% (20)	12.6% (76)

* SD = Standard deviation.

Table 2
Absolute number and haplotype composition by Goiás state municipalities.

Municipality	GST T1/M1 +/+	GST T1/M1 −/+	GST T1/M1 −/−	GST T1/M1 +/-	Total number
Abadiânia	2	1	0	0	3
Anápolis	1	1	1	0	3
Aparecida de Goiânia	**79**	**32**	**23**	**31**	**165**
Aragoiânia	0	0	1	0	1
Bela Vista	**15**	**5**	**1**	**14**	**35**
Bomfinópolis	1	0	0	0	1
Brasabrantes	1	1	1	0	3
Caldazinha	0	0	2	0	2
Goianira	0	0	0	1	1
Goiânia	**28**	**6**	**112**	**19**	**165**
Itapuranga	**14**	**3**	**7**	**12**	**36**
Itumbiara	0	0	1	0	1
Leopoldo de Bulhões	4	0	0	11	15
Montividiu	**29**	**9**	**3**	**19**	**60**
Nerópolis	4	1	1	3	9
Ouro Verde	1	0	0	2	3
Piracanjuba	7	2	0	1	10
Rio Verde	1	0	0	3	4
Rozalândia	5	0	0	0	5
Santo António de Goiás	1	0	2	0	3
Santa Terezinha	2	0	0	0	2
Senador Canedo	0	0	2	0	2
Silvânia	**26**	**5**	**3**	**14**	**48**
Trindade	0	0	1	1	2
Turvânia	**12**	**0**	**3**	**8**	**23**
Goiás state municipalities (Total)	233	66	164	139	602

+/+: Genotype *GSTT1* wild type and genotype *GSTM1* wild type. −/+: Genotype *GSTT1* null and genotype *GSTM1* wild type. +/-: Genotype *GSTT1* wild type and genotype *GSTM1* null. −/−: Genotype *GSTT1* null and genotype *GSTM1* null. In bold = municipalities with more than 20 individuals.

Goias was 50.3%, and for the municipalities with more than 20 individuals sampled are in Fig. 1B. Finally, the frequencies of *GSTT1* and *GSTM1* null genotypes (-/-) was 27.24%, −/+ 11%, +/- 23% and +/+ 39% for Goias (Fig. 1C). Fig. 2 illustrated the haplotypes frequencies in municipalities with more than 20 individuals sampled.

The frequencies for the null *GSTT1* were 43.2% (207) in the central region and 18.7% (23) in the south region, and for the null *GSTM1* we found frequencies of 54% (259) in the central region and 35.8% (44) in

the south region. The distribution of *GSTT1* and *GSTM*1 haplotypes by region was as follows: central region +/- 35.5% (170), -/+ 10.4% (50), -/- 32.8% (157), +/- 21.3 % (102) and in the south +/+ 51.2% (63), -/+ 13% (16), -/- 5.7% (7), +/- 30% (37) (Table 3).

4. Discussion

In all municipalities of Goias, the frequency of the *GSTT1* null genotype was 38.2%, and of the *GSTM1* null genotype 50.3%. In the Northeast of India, some authors (Thoudam et al., 2010) demonstrated a frequency of 32.7% for null *GSTT1* and 41.9% for the null *GSTM1* genotype. Klautau-Guimarães et al. (2005) evaluated 120 individuals, distributed in two Amerindian tribes of Brazil, the Munduruku tribe of the village of Missão Cururu, and the tribe of Kayabi. The frequency was 27% for null *GSTT1* and 0% for null *GSTM1* in Munduruku samples. In the Kayabi tribe, those authors detected frequencies of 29% for *GSTT1* and 27% for *GSTM1* null genotypes. Hiragi et al. (2007) analyzed 91 samples from the Federal District, Brazil, and 273 samples of Brazilian Quilombolas (African offspring) from the states of Bahia, Sergipe, and Goias. Those authors observed frequencies for *GSTT1* null genotype in the Federal District of 22% for *GSTM1* null genotype of 34% and *GSTT1/M1* null genotypes of 11%.

Chiurillo et al. (2013) analyzed 120 urban samples from a Venezuelan population and 188 Amerindian samples, showing a null *GSTT1* genotype frequency of 11% and a null *GSTM1* genotype of 51% in the urban population. The Amerindian samples were divided into five populations. They presented the following frequencies for the null *GSTT1* and *GSTM1* null genotypes, respectively: Bari with 11.4% and 54.3%, Panare with 6.5% and 15.2%, Pemon with 0% and 40%, Warao with 0% and 51.7% and Wayuu with 7.9% and 44.7%.

Magno et al. (2009) found lower frequencies for null *GSTM1* genotypes (33.2%) and the null *GSTT1* genotype (30.2%) in 203 samples from the city of Ilheus, Bahia state, Brazil. Berthou et al. (2001) analyzed 174 samples from Santiago, Chile, and found frequencies for the *GSTM1* null genotype of 24%. Rossini et al. (2002) observed frequencies of 42.1% of the *GSTM1* null genotype and frequencies of 25.4% of the *GSTT1* genotype in 591 individuals from the state of Rio de Janeiro. However, no significant differences were observed between the genotypes *GSTT1* and *GSTM1* concerning the population of Goias and the populations of Kalunga (Goias, Brazil), suggesting a genetic similarity between the communities (Table 4).

We observed that the city of Goiania has a different distribution of *GSTM*1 and *GSTT1* frequencies (p < 0.01) regarding all the
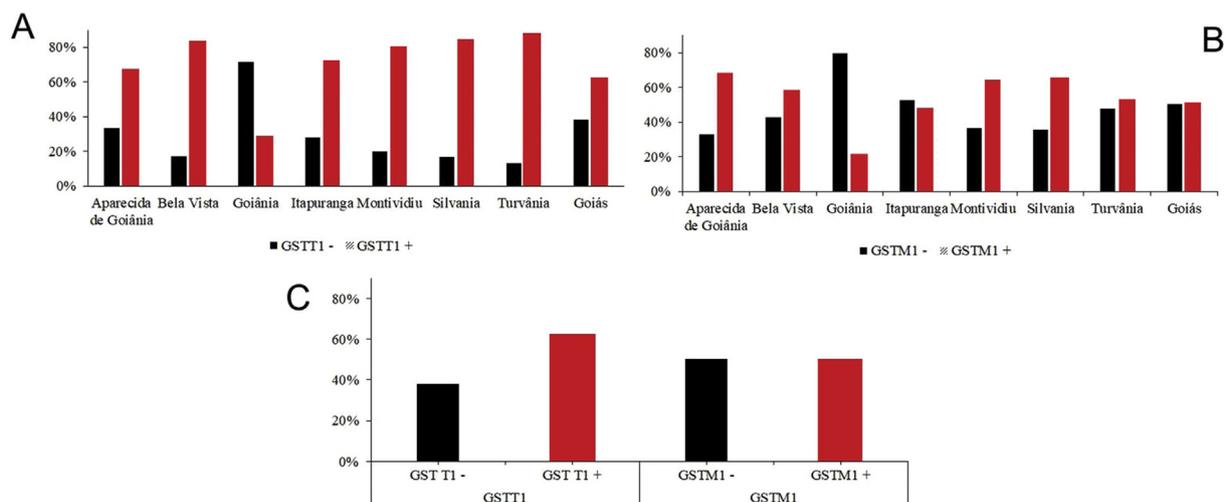

Fig. 1. Distribution of *GSTT1* and *GSTM1* null genotypes among Goias state. A. Distribution of *GSTT1* null genotype in municipalites with more than 20 individuals sampled. B. Distribution of *GSTM1* null genotype for Goias state in municipalities with more than 20 individuals sampled. C. *GSTT1* and *GSTM1* null genotypes (-/-) for the Goias state.
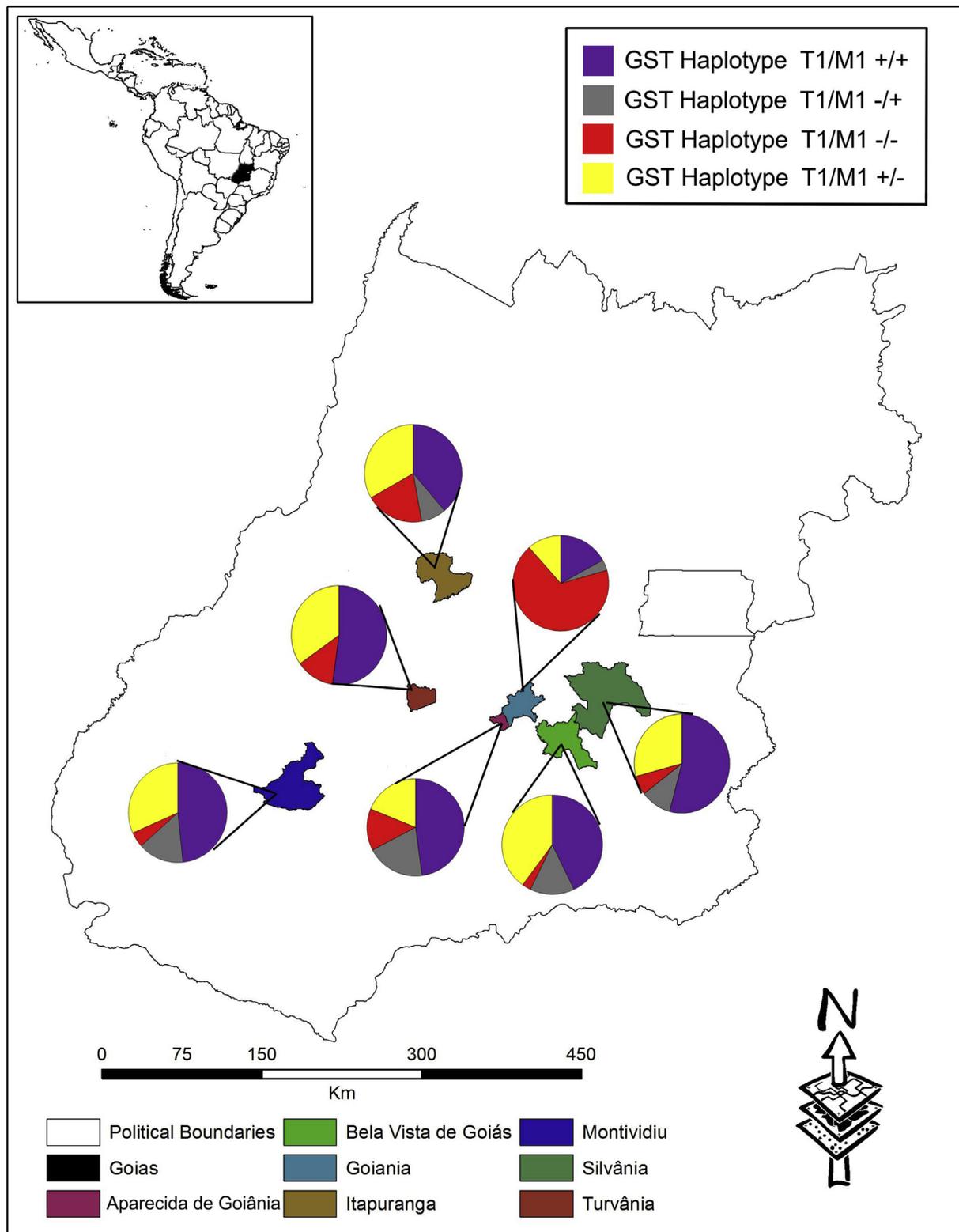
Fig. 2. Map illustrating the distribution of *GSTT1* and *GSTM1* haplotype frequencies in Goias state municipalities with more than 20 individuals sampled.

municipalities and the other groups and countries (Table 4). Goiania is only like the populations of Riacho de Sucutiaba (Bahia, Brazil) (Hiragi et al., 2007) (p = 0.891) and Kayabu (Mato Grosso, Brazil) (Klautau-Guimarães et al., 2005) (p = 0.103) (Table 5). Considering the distribution of the null *GSTM1* genotype, the other municipalities of our study do not present a significant difference from the Kalunga population and the Federal District, except Goiania. Only the cities of Aparecida de

Goiania and Goiania showed substantial differences in the distribution of the null *GSTM1* genotype when compared to Rio de Janeiro (Table 6). The municipality of Goiania also differed from all other cities of this study (Aparecida de Goiania, Bela Vista de Goiás, Itapuranga, Montividiu, Silvania, Turvania).

The high prevalence of *GSTT*1 and *GSTM1* null genotypes in the municipality of Goiania may reflect a founder effect of non-

Table 3

Frequency of genotypes *GST T1* and *GST M1* in the south and central regions of Goias state.

Region	Genotype			
	GSTT1 -	*GSTT1 +*	*GSTM1 -*	*GSTM1 +*
Central	43.2% (207)	56.8% (272)	54.% (259)	46% (220)
South	18.7% (23)	81.3% (100)	35.8% (44)	64.3% (79)

+: Genotype GSTT1 wild type and genotype GSTM1 wild type. -: Genotype GSTT1 null and genotype GSTM1 null.

Table 4

GSTT1 and *GSTM1* genotypes distribution in worldwide populations compared to our population.

		GSTT1	GSTM1
Country	References	*p-value**	*p-value***
Mexican	(Mejia-Sanchez et al., 2017)	< 0.0001	0.5838
North of Indian	(Thoudam. et al., 2010)	0.08172	**0.009758**
Riacho de Sacutiaba (Bahia, Brazil)	(Hiragi et al., 2007)	0.2333	**< 0.0001**
Rio das Rãs (Bahia, Brazil)	(Hiragi et al., 2007)	**0.002535**	**< 0.0001**
Mocambo (Sergipe, Brazil)	(Hiragi et al., 2007)	0.3621	0.08033
Kalunga (Goiás, Brazil)	(Hiragi et al., 2007)	0.1281	0.07782
Distrito Federal (Brazil)	(Hiragi et al., 2007)	**0.01328**	**0.02819**
Kayabu (Mato Grosso, Brazil)	(Klautau-Guimarães et al., 2005)	**< 0.0001**	0.07798
Munduruku (Pará, Brazil)	(Klautau-Guimarães et al., 2005)	0.5679	**< 0.0001**
Venezuela	(Chiurillo et al., 2013)	**< 0.0001**	0.9998
Bari (Venezuela)	(Chiurillo et al., 2013)	**0.002576**	0.7788
Panare (Venezuela)	(Chiurillo et al., 2013)	**< 0.0001**	**< 0.0001**
Pernon (Venezuela)	(Chiurillo et al., 2013)	**< 0.0001**	0.2703
Warao (Venezuela)	(Chiurillo et al., 2013)	**< 0.0001**	1
Wayuu (Venezuela)	(Chiurillo et al., 2013)	**0.000328**	0.6158
Ilhéus (Bahia, Brazil)	(Magno et al., 2009)	**0.04471**	**< 0.0001**
Rio de Janeiro (Brazil)	(Rossini et al., 2002)	**< 0.0001**	**0.005401**

Bold values are significant values demonstrating differences in the populations compared to our sample group.

* p-values associated to the x^2.test referred to the GSTT1 polymorphism distribution.

** p-values associated to the x^2.test referred to the GSTM1 polymorphism distribution.

Table 5

Pairwise comparison of *GSTT1* frequencies between our population and other authors worldwide.

	APA	BEL	GYN	ITA	MON	SIL	TUR
MEX	0.000	0.000	0.253	0.000	0.000	0.000	0.000
NEI	0.961	0.086	0.000	0.674	0.066	0.035	0.081
RSB	0.100	0.008	0.025	0.096	0.005	0.003	0.010
RRB	0.083	0.608	0.000	0.724	0.783	0.481	0.427
MSE	0.145	0.011	0.001	0.140	0.007	0.004	0.014
KGO	0.043	0.004	0.001	0.066	0.001	0.001	0.006
DIS	0.166	0.541	0.000	0.846	0.688	0.420	0.384
KYM	0.000	0.000	1	0.000	0.000	0.000	0.000
MUP	1	0.104	0.000	0.641	0.099	0.053	0.090
VEN	0.000	0.479	0.000	0.025	0.148	0.439	1
BAV	0.017	0.733	0.000	0.152	0.428	0.723	1
PAV	0.001	0.250	0.000	0.021	0.091	0.227	0.650
PEV	0.000	0.021	0.000	0.001	0.007	0.020	0.084
WAV	0.001	0.056	0.000	0.006	0.024	0.053	0.160
WUV	0.003	0.399	0.000	0.052	0.182	0.376	0.833
ILB	0.574	0.172	0.000	0.939	0.173	0.091	0.141
RIO	0.053	0.372	0.000	0.902	0.446	0.241	0.273
APA	1	0.092	0.000	0.653	0.077	0.041	0.083
BEL	0.092	1	0.000	0.431	0.943	1	0.959
GYN	0.000	0.000	1	0.000	0.000	0.000	0.000
ITA	0.653	0.431	0.000	1	0.531	0.337	0.313
MON	0.077	0.943	0.000	0.531	1	0.846	0.676
SIL	0.041	1	0.000	0.337	0.846	1	0.965
TUR	0.083	0.959	0.000	0.313	0.676	0.965	1

MEX = Mexican; NEI = North of Indian; RSB = Riacho de Sacutiaba (Bahia, Brazil); RRB = Rio das Rãs (Bahia, Brazil); MSE = Mocambo (Sergipe, Brazil); KGO = Kalunga (Goiás, Brazil); DIS = Distrito Federal (Brazil); KYM = Kayabu (Mato Grosso, Brazil); MUP = Munduruku (Pará, Brazil); VEN = Venezuela; BAV = Bari (Venezuela); PAV = Panare (Venezuela); PEV = Pernon (Venezuela); WAV = Warao (Venezuela); WUV = Wayuu (Venezuela); ILB = Ilhéus (Bahia, Brazil); RIO = Rio de Janeiro (Brazil); APA = Aparecida de Goiânia; BEL = Bela Vista de Goiás; GYN = Goiânia; ITA = Itapuranga; MON = Montividiu; SIL = Silvânia; TUR = Turvânia.

representative colonizing ancestors. However, as other enzymes are part of the xenobiotic detoxification pathway (Nebert et al., 2004; Nebert and Russell, 2002; Norppa, 1997; Singh et al., 2011), the ancestral population adapts to the environment with the frequencies observed in Goiania. However, as in the present day there is excessive use of pesticides, the community becomes susceptible to the harmful effects of exposure to such xenobiotics, probably due to the high frequencies of null genotypes. In this context, *GSTT1* null and *GSTM1* null genotypes, alone or in combination, should identify individuals with some detoxification capacity deficiency and, therefore, more likely to undergo carcinogen formation, DNA adducts, and mutations. Some authors (Strange et al., 2001) found the null *GSTM1* associated with increased chromosomal aberrations in smokers.

Wang et al., (2010) in a case-control study in the Han Chinese population, found a contribution of *GSTT1* null and *GSTM1* null for the development of respiratory bronchial dysplasia. Malik et al. (2017) also associated the null genotypes of *GSTT*1 and *GSTM1* to the development of lung cancer. Tacca et al., 2019 observed that null *GSTT1* and *GSTM1* were not associated with the development of cervical cancer in smokers but *GSTT1* null genotype was significantly associated with worse prognosis of this disease. In another study conducted in Brazil (Kubiszeski et al., 2015), there was a higher prevalence of *GSTT1* null with the onset of endometriosis. Singh et al., (2011) found higher values of DNA damage in workers with *GSTT1* null exposed to organophosphates.

The central and south regions of Goias showed frequencies of 43.2% and 18.7% for *GSTT*1 null and 54% and 35.8% for *GSTM1* null (Fig. 3A). These regions together are responsible for the production of 18,356,659 tons of grain in the state of Goias, which comprises 80.46% of the state's total production (Instituto Mauro Borges, 2018). As Goias is the fourth-largest producer of grains and is also the fourth-largest user of pesticides (Instituto Mauro Borges, 2018), the null genotypes represent a risk to the population due to high agricultural production and the high handling of pesticides in the state. The mean population age (37 ± 13.2) may represent the basal exposure to pesticides of the people of Goias. However, another important fact is the number of grains produced by the state; 22,814,803 tons (Instituto Mauro Borges, 2018) can be representative of the number of rural workers (95,745 by 2017) (Instituto Mauro Borges, 2018). For those who have direct contact with the pesticide, the null genotypes represent an even higher risk. In this case, the south region is the most representative region for grain production (17,572,323 tons) (Fig. 3B).

Exposure to pesticides can produce abnormal amounts of free radicals, leading to overproduction of oxidants or an inadequate supply of antioxidants (Corsini et al., 2013). In this context, occupational exposure to pesticides increases DNA damage, as Franco et al. (2016) noted in their study of public state agents who handle pesticides to combat the mosquito *Aedes aegypti*, which is responsible for the transmission of arboviruses. Those authors observed that recently intoxicated agents had an increased frequency of translocation between chromosomes 14 and 18. In addition, Roulland et al. (2004) concluded that the BCL2-IGH translocation (14:18) is linked to non-Hodgkin's lymphoma, since it may be a measure of instability caused by the genotoxic effect of the pesticide in a relevant lymphomagenesis. Ventura et al. (2019) demonstrated that 100-day exposure to chlorpyrifos promoted the development of mammary tumors in addition to relieving a hypo-expression of stromal cell receptors.

Table 6

Pairwise comparison of *GSTM1* frequencies between our population and other authors worldwide.

	APA	BEL	GYN	ITA	MON	SIL	TUR
MEX	0.009	0.755	0.000	0.698	0.198	0.190	1
NEI	0.050	1	0.000	0.278	0.524	0.474	0.734
RSB	0.000	0.000	0.891	0.007	0.000	0.000	0.007
RRB	0.191	0.063	0.000	0.003	0.140	0.233	0.047
MSE	0.000	0.086	0.030	0.416	0.007	0.008	0.297
KGO	0.514	0.809	0.000	0.224	1	0.909	0.573
DIS	0.542	0.717	0.000	0.165	1	0.967	0.498
KYM	0.000	0.075	0.103	0.350	0.007	0.008	0.253
MUP	0.000	0.000	0.000	0.000	0.000	0.000	0.000
VEN	0.003	0.523	0.000	0.988	0.101	0.101	0.971
BAV	0.027	0.473	0.004	1	0.145	0.137	0.831
PAV	0.033	0.012	0.000	0.001	0.025	0.045	0.009
PEV	0.494	0.988	0.000	0.376	0.900	0.825	0.734
WAV	0.078	0.648	0.003	1	0.262	0.243	1
WUV	0.226	1	0.000	0.646	0.560	0.512	1
ILB	1	0.347	0.000	0.037	0.710	0.882	0.236
RIO	0.037	1	0.000	0.280	0.496	0.450	0.744
APA	1	0.342	0.000	0.038	0.694	0.862	0.233
BEL	0.342	1	0.000	0.549	0.705	0.646	0.919
GYN	0.000	0.000	1	0.002	0.000	0.000	0.002
ITA	0.038	0.549	0.002	1	0.183	0.171	0.917
MON	0.694	0.705	0.000	0.183	1	1	0.497
SIL	0.862	0.646	0.000	0.171	1	1	0.458
TUR	0.233	0.919	0.002	0.917	0.497	0.458	1

MEX = Mexican; NEI = North of Indian; RSB = Riacho de Sacutiaba (Bahia, Brazil); RRB = Rio das Rãs (Bahia, Brazil); MSE = Mocambo (Sergipe, Brazil); KGO = Kalunga (Goiás, Brazil); DIS = Distrito Federal (Brazil); KYM = Kayabu (Mato Grosso, Brazil); MUP = Munduruku (Pará, Brazil); VEN = Venezuela; BAV = Bari (Venezuela); PAV = Panare (Venezuela); PEV = Pernon (Venezuela); WAV = Warao (Venezuela); WUV = Wayuu (Venezuela); ILB = Ilhéus (Bahia, Brazil); RIO = Rio de Janeiro (Brazil); APA = Aparecida de Goiânia; BEL = Bela Vista de Goiás; GYN = Goiânia; ITA = Itapuranga; MON = Montividiu; SIL = Silvânia; TUR = Turvânia.

Therefore, risk factors for a variety of diseases, such as smoking, sun exposure, obesity, alcohol consumption, and lack of physical activity, in addition to pesticide exposure, have all gained widespread repercussions due to their impact on public health (Khayat et al., 2013; Donkor et al., 2016; Godoy et al., 2019). Ingestion of contaminated water and food is a significant source of human exposure to pesticide residues in the general population (Sharma et al., 2010; Donkor et al., 2016), although occupational and para-occupational exposures are the most worrying (Deziel et al., 2017; Godoy et al., 2019).

Neurological, reproductive, and developmental disorders have already been associated with occupational exposure to pesticides; however, the most recognized factor is the risk of developing specific cancers (Blair and Freeman, 2009; Bolognesi et al., 2011; Stojanovic et al., 2018; Zeng et al., 2017). It has also become essential to determine if people living in areas of high agricultural intensity where pesticides are widely used show a higher prevalence and risk of cancer and, ultimately, to elucidate the mechanisms by which these environmental contaminants relate to the emergence of disease (Alavanja et al., 2013; Parrón et al., 2014). Additionally, along with daily pesticide exposures, people living in areas of intense agricultural activity may interact with other products to which the general population is exposed, such as pollution, dyes, preservatives, and various products that, for example, can affect the immune system, where malfunction leads to the susceptibility of multiple diseases. Genetic factors, such as the presence of polymorphisms capable of increasing the risk of cancer, should be added to the relevant aspects. In general, the increasing trend in cancer incidence in recent years can be attributed not only to aging but also to the spread of carcinogenic agents that lead to daily exposure. This makes it very important to study the factors related to the risks to which different populations are exposed (Tebourbi et al., 2011).

In Goias, the use of pesticides has grown considerably, with 43,466 kg consumed in 2017 alone (IBAMA, 2019). In this context, knowing the critical role of GST enzymes for detoxification pathways and that haplotypes with null alleles are of high risk for the population, this use reached 61% of the Goias population. Finally, our results indicated that it is essential to know the frequencies of the *GSTT1* and *GSTM1* genes for the monitoring of risk groups; for example, farmers who have contact with pesticides directly or indirectly, as well as assisting in the development of preventive medicine practices.

5. Conclusions

The use of glyphosate, 2,4-D, acephate, and other pesticides totaling 43,663 tons is related to the high agricultural productivity of Goias, leaving Goias in four places in underreporting numbers of intoxication in Brazil. However, with the new Brazilian government authorizing the use of banned pesticides worldwide, this situation may increase the number of poisonings, especially in Brazilian regions involved with agriculture.

With the high frequency of null genotypes in the south and central regions of Goias, and also with these regions being the first and third in the ranking of grain production respectively, it is of great importance that the government take measures to prevent cases of intoxication, given the risk that the null *GSTT1* and *GSTM1* genotypes offer for people exposed to pesticides.

In this context, *GSTT1* and *GSTM1* genotypes may be essential biomarkers of susceptibility to poisoning, mainly in populations with pesticide contact, due to the role of GST enzymes. However, it is necessary to associate *GSTT1* and *GSTM1* genotyping with other biomarkers such as comet assay, micronucleus test, oxidative stress enzyme measurements, for confirmation, quantification of biological damage and better elucidation of a given natural process.

This study presented a combined frequency (haplotypes) of *GSTT1* and *GSTM1* genes in Central Brazil. Future studies to monitor the toxicity of occupationally exposed workers and planting areas in which pesticides are used, as well as non-target animals, are necessary to verify human and environmental health, against the use of pesticides in this Brazilian
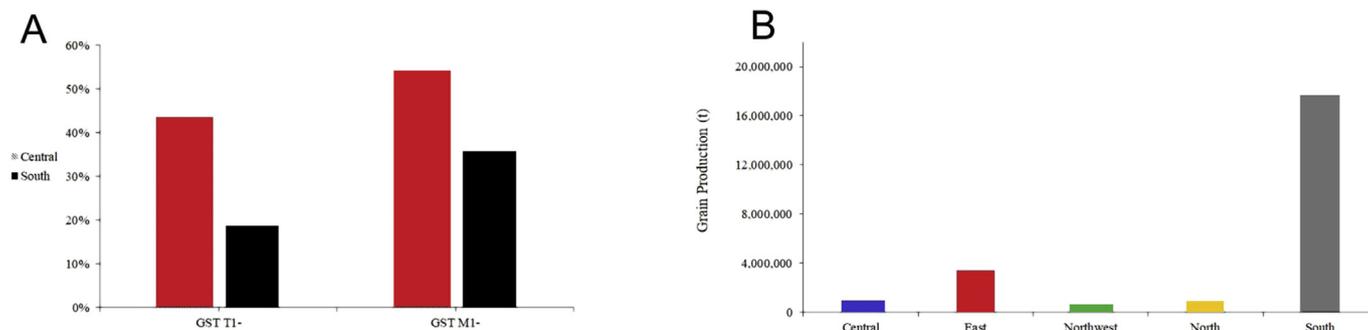
Fig. 3. A. Distribution of *GSTT1* and *GSTM1* in central and south regions of Goias state. B. Amount of grain production in the five regions (central, east, northwest, north and south) of Goias state.

region, which is characterized by intense agricultural activity.

Declarations

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

References

Abreu, P.H.B. De, Alonzo, H.G.A., 2014. Trabalho rural e riscos à saúde: uma revisão sobre o "uso seguro" de agrotóxicos no Brasil. Ciência Saúde Coletiva 19, 4197–4208.

Alavanja, M.C.R., Ross, M.K., Bonner, M.R., 2013. Increased cancer burden among pesticide applicators and others due to pesticide exposure. CA. Cancer J. Clin. 63, 120–142.

Berthou, F., Varela, N., Lee, K., 2001. CYP1A1, CYP2E1 and GSTM1 genetic polymorphisms . The effect of single and combined genotypes on lung cancer susceptibility in Chilean people. Cancer Lett. 174, 35–44.

Blair, A., Freeman, L.B., 2009. Epidemiologic studies in agricultural populations: observations and future directions. J. Agromed. 14, 125–131.

Bolognesi, C., Creus, A., Ostrosky-Wegman, P., Marcos, R., 2011. Micronuclei and pesticide exposure. Mutagenesis 26, 19–26.

Bonner, M.R., Freeman, L.E.B., Hoppin, J.A., Koutros, S., Sandler, D.P., Lynch, C.F., Hines, C.J., Thomas, K., Blair, A., Alavanja, M.C.R., 2016. Occupational exposure to pesticides and the incidence of lung cancer in the agricultural health study. Environ. Health Perspect. 19, 388–395.

Chiurillo, M.A., Griman, P., Santiago, L., Torres, K., Moran, Y., Borjas, L., 2013. Distribution of GSTM1, GSTT1, GSTP1 and TP53 disease-associated gene variants in native and urban Venezuelan populations. Gene 531, 106–111.

Coles, B.F., Kadlubar, F.F., 2003. Detoxification of electrophilic compounds by glutathione S-transferase catalysis: determinants of individual response to chemical carcinogens and chemotherapeutic drugs? Biofactors 17, 115–130.

Corsini, E., Sokooti, M., Galli, C.L., Moretto, A., Colosio, C., 2013. Pesticide induced immunotoxicity in humans: a comprehensive review of the existing evidence. Toxicology 307, 123–135.

Da Fonseca, R.R., Johnson, W.E., O'Brien, S.J., Vasconcelos, V., Antunes, A., 2010. Molecular evolution and the role of oxidative stress in the expansion and functional diversification of cytosolic glutathione transferases. BMC Evol. Biol. 10.

Deziel, N.C., Freeman, L.E.B., Graubard, B.I., Jones, R.R., Hoppin, J.A., Thomas, K., Hines, C.J., Blair, A., Sandler, D.P., Chen, H., Lubin, J.H., Andreotti, G., Alavanja, M.C.R., Friesen, M.C., 2017. Relative contributions of agricultural drift,

para-occupational, and residential use exposure pathways to house dust pesticide concentrations: meta-regression of published data. Environ. Health Perspect. 125, 296–305.

Donkor, A., Osei-Fosu, P., Dubey, B., Kingsford-Adaboh, R., Ziwu, C., Asante, I., 2016. Pesticide residues in fruits and vegetables in Ghana: a review. Environ. Sci. Pollut. Res. Int. 23, 18966–18987.

Franco, F.C., Alves, A.A., Godoy, F.R., Avelar, J.B., Rodrigues, D.D., Pedroso, T.M.A., da Cruz, A.D., Nomura, F., de Melo e Silva, D., 2016. Evaluating genotoxic risks in Brazilian public health agents occupationally exposed to pesticides: a multi-biomarker approach. Environ. Sci. Pollut. Res. 23, 19723–19734.

Godoy, F.R., Nunes, H.F., Alves, A.A., Carvalho, W.F., Franco, F.C., Pereira, R.R., da Cruz, A.S., da Silva, C.C., Bastos, R.P., de Melo e Silva, D., 2019. Increased DNA damage is not associated to polymorphisms in OGGI DNA repair gene, CYP2E1 detoxification gene, and biochemical and hematological findings in soybeans farmers from Central Brazil. Environ. Sci. Pollut. Res. Int. 1–10.

Hiragi, C. de O., Oliveira, S.F. De, Hatagima, A., Ferreira, L.B., Grisolia, C.K., Klautau, M.D.N., 2007. Glutathione S-transferase M1 and T1 polymorphisms in Brazilian African descendants. Hum. Biol. 79, 131–140.

IBAMA, 2019. Relatórios de comercialização de agrotóxicos [WWW Document]. URL https://www.ibama.gov.br/agrotoxicos/relatorios-de-comercializacao-de-agrotoxicos.

Instituto Brasileiro De Geografia E Estatística (IBGE), 2017. https://www.ibge.gov.br.

Instituto Mauro Borges, 2018. Estatísticas Básicas Quadrimestrais Estado de Goiás.

Khayat, C.B., Costa, E.O.A., Gonçalves, M.W., da Cruz e Cunha, D.M., da Cruz, A.S., de Araújo Melo, C.O., Bastos, R.P., da Cruz, A.D., de Melo e Silva, D., 2013. Assessment of DNA damage in Brazilian workers occupationally exposed to pesticides: a study from Central Brazil. Environ. Sci. Pollut. Res. Int. 20, 7334–7340.

Klautau-Guimarães, M. de N., Hiragi, C. de O., D'Ascenção, R.F., Oliveira, S.F., Grisolia, C.K., Hatagima, A., Ferrari, Í., 2005. Distribution of glutathione S-transferase GSTM1 and GSTT1 null phenotypes in Brazilian Amerindians. Genet. Mol. Biol. 28, 32–35.

Kubiszeski, E.H., de Medeiros, S.F., Seidel, J.A. da S., Barbosa, J.S., Galera, M.F., Galera, B.B., 2015. Glutathione S-transferase M1 and T1 gene polymorphisms in Brazilian women with endometriosis. J. Assist. Reprod. Genet. 32, 1531–1535.

Magno, L.A.V., Talbot, J., Talbot, T., Borges Santos, A.M., Souza, R.P., Marin, L.J., Moreli, M.L., De Melo, P.R.S., Corrêa, R.X., Rios Santos, F., Di Pietro, G., 2009. Glutathione S-transferase variants in a Brazilian population. Pharmacology 83, 231–236.

Malik, S.S., Masood, N., Baig, M., Yasmin, A., 2017. The association of *GSTM1* and *GSTT1* deletion polymorphisms with lung cancer risk: evidence from an updated meta-analysis. Meta. Gene. 11, 111–116.

Marin, F., Garcia, N., Munoz, X., Capella, G., Gonzalez, C.A., Agudo, A., Sala, N., 2010. Simultaneous genotyping of GSTT1 and GSTM1 null polymorphisms by melting curve analysis in presence of SYBR Green I. J. Mol. Diagn. 12, 300–304.

Ministério da Agricultura, 2019. DIÁRIO OFICIAL DA UNIÃO, 24/06/2019.

Ministério da Saúde, 2016. Vigilância em Saúde de Populações Expostas a Agrotóxicos – VSPEA.

Ministério da Saúde, 2018. Relatório Nacional de Vigilância em Saúde de Populações Expostas a Agrotóxicos.

Miranda-Contreras, L., Gómez-Pérez, R., Rojas, G., Cruz, I., Berrueta, L., Salmen, S., Colmenares, M., Barreto, S., Balza, A., Zavala, L., Morales, Y., Molina, Y., Valeri, L., Contreras, C.a., Osuna, J.a., 2013. Occupational exposure to organophosphate and carbamate pesticides affects sperm chromatin integrity and reproductive hormone levels among venezuelan farm workers. J. Occup. Health 55, 195–203.

Nebert, D.W., Russell, D.W., 2002. Clinical importance of the cytochromes P450. Lancet 360, 1155–1162.

Nebert, D.W., Dalton, T.P., Okey, A.B., Gonzalez, F.J., 2004. Role of aryl hydrocarbon receptor-mediated induction of the CYP1 enzymes in environmental toxicity and cancer. J. Biol. Chem. 279, 23847–23850.

Norppa, H., 1997. Cytogenetic markers of susceptibility: influence of polymorphic carcinogen-metabolizing enzymes. Environ. Health Perspect. 105 (Suppl), 829–835.

Panov, A.V., Greenamyre, J.T., Sherer, T.B., Betarbet, R., MacKenzie, G., Garcia-Osuna, M., 2002. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. Nat. Neurosci. 3, 1301–1306.

Parrón, T., Requena, M., Hernández, A.F., Alarcón, R., 2014. Environmental exposure to pesticides and cancer risk in multiple human organ systems. Toxicol. Lett. Environ. Contam. Target Organ Toxicities 230, 157–165.

Richardson, J.R., Roy, A., Shalat, S.L., Von Stein, R.T., Hossain, M.M., Buckley, B., Gearing, M., Levey, A.I., German, D.C., 2014. Elevated serum pesticide levels and risk for Alzheimer disease. JAMA Neurol 71, 284–290.

Rodríguez, M., Mejía, F., Lecourtois, M., Domínguez, V., Castillo, J., 2014. Influence of GSTT1, GSTM1 and GSTP1 polymorphisms on the development of breast cancer. J. Cancer Ther. 5, 552–559.

Rossini, A., Rapozo, D.C.M., Amorim, L.M.F., Macedo, J.M.B., Medina, R., Neto, J.F.N., Gallo, C.V.M., Pinto, L.F.R., 2002. Frequencies of GSTM1, GSTT1, and GSTP1 polymorphisms in a Brazilianpopulation. Genet. Mol. Res. 1, 233–240 [pii].

Roulland, S., Lebailly, P., Lecluse, Y., Briand, M., Potter, D., Gauduchon, P., 2004. Characterization of the t(14;18) BCL2-IGH translocation in farmers occupationally exposed to pesticides. Cancer Res. 64, 2264–2269.

Schinasi, L., Leon, M.E., 2014. Non-hodgkin lymphoma and occupational exposure to agricultural pesticide chemical groups and active ingredients: a systematic review and meta-analysis. Int. J. Environ. Res. Public Health 11, 4449–4527.

Sharma, D., Nagpal, A., Pakade, Y.B., Katnoria, J.K., 2010. Analytical methods for estimation of organophosphorus pesticide residues in fruits and vegetables: a review. Talanta 82, 1077–1089.

Singh, S., Kumar, V., Thakur, S., Banerjee, B.D., Rautela, R.S., Grover, S.S., Rawat, D.S., Pasha, S.T., Jain, S.K., Ichhpujani, R.L., Rai, A., 2011. Paraoxonase-1 genetic

polymorphisms and susceptibility to DNA damage in workers occupationally exposed to organophosphate pesticides. Toxicol. Appl. Pharmacol. 252, 130–137.

Souza, A. De, Medeiros, A.D.R., Souza, A.C. De, Wink, M., Siqueira, I.R., Ferreira, M.B.C., Fernandes, L., Loayza Hidalgo, M.P., Torres, I.L.D.S., 2011. Evaluation of the impact of exposure to pesticides on the health of the rural population: vale do Taquari, State of Rio Grande do Sul (Brazil). Ciência Saúde Coletiva 16, 3519–3528.

Stojanovic, J., Milovanovic, S., Pastorino, R., Iavicoli, I., Boccia, S., 2018. Occupational exposures and genetic susceptibility to urinary tract cancers: a systematic review and meta-analysis. Eur. J. Cancer Prev. Off. J. Eur. Cancer Prev. Organ. ECP 27, 468–476.

Strange, R.C., Spiteri, M.A., Ramachandran, S., Fryer, A.A., 2001. Glutathione-S-transferase family of enzymes. Mutat. Res. Fundam. Mol. Mech. Mutagen. 482, 21–26.

Tacca, A., Lopes, A., Vilanova-Costa, C., Silva, A., Costa, S., Nogueira, N., et al., 2019. Null polymorphisms in *GSTT1* and *GSTM1* genes and their associations with smoking and cervical cancer. Genetics and molecular research: GMR. 25 de fevereiro de 18, 18067.

Tebourbi, O., Sakly, M., Rhouma, K.B., 2011. Molecular mechanisms of pesticide toxicity. Pestic. Mod. World - Pests Control Pestic. Expo. Toxic. Assess.

Thoudam, R.D., Yadav, D.S., Mishra, A.K., Kaushal, M., Ihsan, R., Chattopadhyay, I., Chauhan, P.S., Sarma, J., Zomawia, E., Verma, Y., Nandkumar, A., 2010. Distribution of glutathione S-transferase T1 and M1 genes polymorphisms in north east Indians: a potential report. Genet. Test. Mol. Biomark. 14, 163–169.

Ventura, C., Zappia, C.D., Lasagna, M., Pavicic, W., Richard, S., Bolzan, A.D., Monczor, F., Núñez, M., Cocca, C., 2019. Effects of the pesticide chlorpyrifos on breast cancer disease. Implication of epigenetic mechanisms. J. Steroid Biochem. Mol. Biol. 186, 96–104.

Wahner, A.D., Glatt, C.E., Bronstein, J.M., Ritz, B., 2007. Glutathione S-transferase mu, omega, pi, and theta class variants and smoking in Parkinson's disease. Neurosci. Lett. 413, 274–278.

Wang, T., Yu, H.T., Wang, W., Pan, Y.Y., He, L.X., Wang, Z.Y., 2010. Genetic polymorphisms of cytochrome P450 and glutathione S-transferase associated with antituberculosis drug-induced hepatotoxicity in Chinese tuberculosis patients. J. Int. Med. Res. 38, 977–986.

Webb, G., Vaska, V., Coggan, M., Board, P., 1996. SHORT COMMUNICATION chromosomal localization of chromosomal localization of the gene for the human theta class glutathione transferase (GSTT1). Genet. Med. 33, 121–123.

Ye, M., Beach, J., Martin, J.W., Senthilselvan, A., 2013. Occupational pesticide exposures and respiratory health. Int. J. Environ. Res. Public Health 10, 6442–6471.

Zeng, F., Lerro, C., Lavoué, J., Huang, H., Siemiatycki, J., Zhao, N., Ma, S., Deziel, N.C., Friesen, M.C., Udelsman, R., Zhang, Y., 2017. Occupational exposure to pesticides and other biocides and risk of thyroid cancer. Occup. Environ. Med. 74, 502–510.