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# Micronucleus Test in Exfoliated Buccal Cells of Female Street Vendors Exposed to Vehicular Exhaust in Iligan City, Philippines

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## Abstract

Vehicular exhaust has been proven by several studies to be genotoxic and deleterious to human health (Health Effects Institute, 2010; IARC, 2012; etc.). In order to make possible health interventions, it is vital to perform bio-monitoring studies among individuals occupationally exposed to clinically high amounts of vehicular exhaust (Holland et.al. 2008 & Tovalin et.al. 2006). The purpose of the study was to assess the DNA damage of the exfoliated buccal cells among urban street vendors exposed to vehicular exhaust. The study utilized a cross-sectional design (n=60). Purposive sampling was used to select thirty (30) street vendors in Iligan City and thirty (30) controls in Marawi City. Only female participants were included to eliminate gender as a confounding factor. Data collection occurred between the months of October 2013 to January 2014. Buccal micronucleus test was used to investigate the genotoxic effects of vehicular exhaust exposure. A total of 1000 cells per test individual were scored for micronucleus frequency. The results showed a highly significant difference (p < 0.001) between the micronuclei frequency of the exposed (9.40±4.46) and control (4.80±3.25). No relationship was established between the micronuclei frequency and the confounding factors such as age, length of vehicular exhaust exposure, smoking, tea drinking, and alcoholism. A weak correlation (p=0.031) was found between coffee drinking and micronuclei frequency. The results of this study suggest that female street vendors occupationally exposed to vehicular exhaust have a significantly higher frequency of micronucleated cells than their minimally-exposed counterparts.

Keywords: Vehicular exhaust; Street vendors; Micronucleus test; Buccal cells

## Introduction

There is an increasing amount of evidence suggesting that exposure to vehicular exhaust increases the rate of DNA damage leading to the development of cancer and many other adverse health effects (Health Effects Institute, 2010, IARC, 2012, etc.) Damage to genome has been

known to be the fundamental cause of many developmental and degenerative diseases (Holland et.al., 2008). Although the exact mechanisms of the damage are still not well understood, the health-damaging effects can be attributed to the components of the exhausts themselves. Huang et al. (2012) stated that particulate matter (PM) of the exhausts, especially PM with diameter less than or equal to 2.5 µm (PM<sub>2.5</sub>), has the ability to penetrate the respiratory and circulatory system easily causing increased risk of having respiratory and cardiovascular diseases as well as adverse pregnancy outcomes depending on the length of exposure (either short-term or long-term). Another component of the exhaust are the polycyclic aromatic hydrocarbons (PAHs). PAHs have carcinogenic and mutagenic properties as they are able adhere easily to the surface of carbon particles and are carried deep into the lungs (Krivoshto et al., 2008). The other components of traffic exhaust, especially those coming from diesel emission sources, have also been proven to have negative health effects. Among the traffic exhaust sources, the diesel powered vehicles are considered to be the most damaging to health. In 2012, the International Agency for Research on Cancer (IARC) has already classified diesel exhaust as carcinogenic to humans. According to IARC, there has been substantive evidence proving that diesel exhaust causes lung cancer. There are also limited evidences showing a positive association of diesel exhaust exposure with an increased risk of bladder cancer. Aside from diesel exhaust, IARC also classified gasoline exhaust, as possibly carcinogenic to humans.

In order to make possible health interventions, it is vital to perform bio-monitoring studies among individuals occupationally exposed to clinically high amounts of vehicular exhaust (Holland et.al., 2008 and Tovalin et.al.,2006). The street vendors are among the occupationally exposed individuals that need to be monitored for DNA damage. Bhowmik (2005) described street vendor as "a person who offers goods for sale to the public without having a permanent built-up structure from which to sell." He further classified street vendors as stationary or mobile. Stationary street vendors are those who occupy spaces on the pavements or other public/private places to sell their goods. Mobile street vendors, on the other hand, are those who move from one place to another by carrying their wares using materials such as push carts and baskets.

In the Philippines, street vendors are prevalent in roads, highways, and city streets near bus and truck routes. They are therefore exposed to high concentrations of traffic-related pollutants having carcinogenic and mutagenic properties (Krivoshto et al., 2008 & Sanderson et al, 2005). Their exposure to these pollutants also increases their risk to develop other health hazards like respiratory diseases and adverse pregnancy birth outcomes as these diseases are proven to occur among street vendors in other countries exposed to traffic exhausts (Amegah & Jakkola, 2013; & Kongtip et al, 2008).

DNA damage among street vendors can be assayed through the use of buccal micronucleus test. Buccal micronucleus test is a minimally invasive method to assay DNA damage caused by lifestyle habits, exposure to environmental pollutants, medical procedures, as well as inherited genetic defects in DNA repair (Fenech et al., 2006). Micronuclei are structures similar to the cell's nucleus that arise from chromosome fragments or whole chromosomes that lag behind at anaphase during nuclear division (Holland et al., 2008). The frequency of micronucleus formation in the cells has been believed to be correlated with the increasing effects of carcinogens (Stitch et. al. as cited by Naderi et. al, 2012). Buccal cells can be used to assay DNA damage caused by carcinogenic substances since they are the first barrier for inhalation or ingestion route. The oral epithelium is maintained by continuous cell renewal through mitotic divisions of the cells of the stratum basale to replace surface cells that are shed. The stem cells of the stratum basale may express genetic damage as micronucleus during karyokinesis upon exposure of the oral epithelium to carcinogenic substances. The daughter cells, which may or may not contain micronucleus, eventually differentiate into the upper epithelial layers and then exfoliate into the buccal cavity (Holland, 2008).

In this study, the DNA damage in urban street vendors with significant exposures to vehicular exhaust was considered. Only female participants were included to eliminate gender as a confounding factor. The place of study was Iligan City since there is no known study about the genotoxicity risk of the female street vendor population in this area. The technique used to assay DNA damage was buccal micronucleus test. Buccal micronucleus test is one of the preferable techniques for measuring DNA damage and cell death biomarkers since it is minimally invasive as it only utilizes the cells of the oral epithelium (Fenech, 2006). Furthermore, it is advantageous over other methods due to its low cost thereby making it suitable for large-scale screening of populations (Holland, 2008).

## Statement of the Problem

The study was conducted to assess the DNA damage of the exfoliated buccal cells among urban female street vendors exposed to vehicular exhaust through the use of micronucleus test. Specifically, it seeks to determine: (1) if there is a significant difference between the frequency of micronuclei between the exposed and control groups, and (2) the relationship of smoking, coffee drinking, tea drinking, alcoholism, age, and length of vehicular exhaust exposure with the frequency of micronuclei in buccal cells.

## Significance of the Study

Much of the air pollution in the Philippines can be attributed to traffic-related air pollutants (The World Bank, 2002). Despite this, the information regarding the DNA damaging effects of trafficrelated exhaust to occupationally exposed individuals in the Philippines, such as the street vendors, is almost nil (Cavite, 2013, & Villarino, 2009). In the report of national air quality status of the Philippines as of 2003-2007, it was evident that there were no data on ambient air quality of some regions in the Philippines, one of which is Region X (Environmental Management Bureau, 2009). Iligan City is one of the urban centers of Region X. According to Environmental Management Bureau (2009), the city has been considered as a model city since it has been a forerunner in traffic management at the local government level. As stated in their report, the construction of north and south bound terminals for bus and jeepney and the road widening projects improved the traffic condition of the city and reduced the traffic-related emissions from inter-city vehicles. Despite these claims, the extent of the improvement of the air quality in the city, whether it is above the normal range or not, is poorly known. This study provides a minimally invasive and cost-effective way of assessing the genotoxic effects of the vehicular exhaust to the female street vendors in the Iligan City. The study may serve as a basis for possible health intervention measures for the benefit of the exposed vendors. Moreover, this study provides useful information that may be helpful for other researchers to evaluate occupational genotoxicity risks of other populations exposed to known carcinogens and mutagens.

## Methods

## A. Study Design

The study utilized a descriptive cross-sectional study to assess the DNA damage of the exfoliated buccal cells among urban female street vendors exposed to vehicular exhaust. The sampling sites for the occupationally exposed group were the female vendors at Southbound Terminal, Pala-o Market and Old Gaisano jeepney and truck routes. The control group were females from Mindanao State University-Main Campus, Marawi City. The locality was chosen for the individuals comprising the control group, since the campus has lesser amount of vehicular exhaust emissions compared to Iligan City. The study period covered the months of October 2013 to January 2014.

## B. Study Subjects

Purposive sampling was used in selecting the study participants. The occupationally exposed group (n=30) was chosen based on the following criteria: (1) must be a stationary street vendor in the selected sampling sites, and (2) must be occupationally exposed to vehicular exhaust at least six to eight hours a day. The control group (n=30) are aged-matched individuals with no known exposure to occupational or environmental carcinogens like students, office workers, instructors, and other residents of MSU-Main Campus, Marawi City. Only female participants were included to eliminate gender as a confounding factor. A total of sixty (60) individuals participated in the study.

Prior to data collection, the study participants were informed of the purpose of the study. This was done to ensure that the participants understood the data collection procedure. Those who agreed to participate were asked to sign an informed consent form and to fill out a questionnaire to obtain necessary information on their lifestyle and personal factors (age, length of exposure, smoking habits, drinking habits, etc.). The questionnaire was in accordance with the protocol published by the International Commission for Protection against Environmental Mutagens and Carcinogens (Carrano and Natarajan, 1988 as cited by Cavite 2013). The participants were coded to ensure their anonymity.

## C. Buccal Micronucleus Test

The assay protocol was adapted from Sellappa et.al (2011). Prior to buccal cell sample collection, the participants rinsed their mouths with distilled water to remove any unwanted debris. Buccal cells were collected by rubbing an extra soft toothbrush in the inner sides of both cheeks for 30seconds and then rinsing the mouth with 20 ml of 0.9% saline solution. The gargled solution was expectorated into sterile test tubes and was transferred into centrifuge tubes containing Phosphate Buffered Saline (PBS) at pH 7.0, creating a cell suspension. The cells were washed three times by centrifugation at 1500 rpm for 10 mins in the buffer solution. After discarding the supernatant, the pellet was smeared on clean microscope slides and allowed to air dry for 5-10 min. The slides were fixed in cold methanol: acetic acid (3:1) for 15 minutes, air-dried, and stained with 5%Giemsa for 20 minutes. The slides were prepared per sample. A total of 1000 cells per test individual were scored for micronuclei frequency.

The buccal micronuclei (MN) were scored based on the criteria established by Tolbert et.al. (1992): (1) MN must be less than one third the diameter of the main nucleus; (2) must be on the same focal plane; (3) must have the same color, texture, and refraction as the main nucleus; (4) must have a smooth oval or round shape; and (5) must be clearly separated from the main nucleus. A micronucleated cell rate over 8% is considered abnormal. Only non-fragmented, non-

accumulated, non-overlaid cells, as well as those cells containing an intact nucleus were considered for scoring. The frequency of other degenerative nuclear changes such as binucleated cells, nuclear bud, karyolysis, pyknosis, and karyorrhexis were not included in the analysis.

#### D. Confounding factors

To account for the confounding factors of this study, the experimental and control groups were classified as smokers and non-smokers; alcoholics and non-alcoholics; tea drinker and non-tea drinker; and coffee drinker and non-coffee drinker respectively. The aforementioned factors were considered since a number of studies suggest that these factors may affect the frequency of micronuclei formation and may contribute to the occurrence of cancer (Bolukbas et.al., 2006 and Majer et.al., 2001 as cited by Cavite 2013; IARC 1997). The subjects who smoke >5 cigarettes/day for 1 year were considered as smokers and those who consumed >120 mg of alcohol/day were considered as alcoholics in both groups (Vasuvedan et. al, 2011). The subjects who drink at least 1 cup of coffee and tea everyday were considered as coffee drinkers and tea drinkers respectively. The length of vehicular exhaust exposure was considered for the exposed group. On the other hand, the length of vehicular exhaust exposure for the controls was set to zero. The basis for the length of vehicular exhaust exposure was the number of years of selling goods in the streets of Iligan City.

#### Treatment of Data

The results of this study were statistically analyzed using Statistical Package for the Social Sciences (SPSS) 17.0. Mean and standard deviation (SD) of the micronucleus frequency were calculated for both exposed and control groups. Student's T test was used to evaluate the difference between the exposed and control groups with the level of significance set at 0.05. Furthermore, the relationship of the confounding variables to micronucleus formation within the exposed and within the control groups was analyzed using Spearman's rho correlation.

## Findings

The descriptive characteristics of the study participants were summarized in Table 1. There is a similarity in the basal characteristics of the exposed and control. The exposed individuals were matched with the controls in terms of age and smoking habit. In order to test the effect of vehicular exhaust to the street vendors, the length of exposure of the controls was set to zero (0) years. The data about the recent illness and drinking habits of both exposed and control groups were also included. No data was accounted for alcohol drinking since none of the test subjects consumed >120 mg of alcohol/day. There was also no tea drinker among the control individuals since none of them consumed at least 1 cup of tea every day.

Variable	Exposed (n=30)	Control (n=30)
Age:		
Minimum	12	12
Maximum	63	63
Mean±SD	37.5 <b>±</b> 14.0	37.5±14.5
Length of Exposure:		
Minimum	1 month	0
Maximum	39 years	0
Mean±SD	11.1 <b>±</b> 10.5	0
Smoking Habits:		
Yes	1 (3.33 %)	1 (3.33 %)
No	29 (96.67 %)	29 (96.67 %)
Recently Ill:	8 (26.67%)	5 (16.67%)
Dermatologic disease Respiratory-related disease CNS-related disease Cardiovascular-related disease Fertility related disease Fever	$\begin{array}{c} 0 \ (0.00\%) \\ 3 \ (37.5\%) \\ 1 \ (12.5\%) \\ 1 \ (12.5\%) \\ 1 \ (12.5\%) \\ 2 \ (25.00\%) \end{array}$	$\begin{array}{c}1\ (20.00\%)\\2\ (40.00\%)\\0(00.00\%)\\1\ (20.00\%)\\0\ (0.00\%)\\1\ (20.00\%)\\1\ (20.00\%)\end{array}$
Coffee Drinker:		
Yes	20 (66.67 %)	13 (43.33 %)
No	10 (33.33 %)	17 (56.67 %)
Tea Drinker:		
Yes	1 (3.33 %)	0 (0.00 %)
No	29 (96.67 %)	30 (100 %)
Alcoholic:		
Yes	0 (0.00 %)	0 (0.00 %)
No	30 (100 %)	30 (100 %)

Table 1	Descri	ntive	characteristics	of the	study	nonul	ation
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CNS- Central Nervous System

Micronuclei frequencies (MN) in the exfoliated buccal cells were scored for both exposed and control groups (Figure 1). The results showed that the MN frequency of the street vendors was almost two times higher than the control ( $9.40\pm4.46$  vs.  $4.80\pm3.25$  per 1000 cells) and the difference was highly significant (p < 0.001).



Figure 1: Micronucleated buccal cells (1000x magnification)

The correlation of the confounding factors with the micronuclei frequency was analyzed (Table 2). Among the confounding variables tested for the exposed group, only coffee drinking showed a statistically significant relationship with micronuclei frequency (p=0.031). However, the strength of correlation between coffee drinking and micronuclei frequency is relatively weak.

Confounding Variables	Exposed		Control	
	Correlation Coefficient	p value	Correlation Coefficient	p value
Smoking	0.108	0.570	0.162	0.393
Coffee Drinking	0.394	0.031*	-0.098	0.608
Tea Drinking	0.108	0.570	-	-
Length of Exposure	-0.028	0.884	-	-
Age	-0.038	0.842	0.096	0.614
Medicinal Intake for a Recent Illness	-0.219	0.245	0.120	0.529
Alcohol Drinking	-	-	-	-

Table 2. Relationshi	p of the confour	nding variables to	o micronuclei frequency

\*Statistically significant (p<0.05)

The relationship of the confounding variables to the micronucleus frequency of the control group was also investigated. Smoking, coffee drinking, age, and medicinal intake did not show a significant relationship with micronucleus frequency (p>0.05).

## Discussion

Numerous studies suggest that personal and occupational exposure to vehicular exhaust cause DNA damage and a wide variety of adverse health effects (Huang et. al., 2012; Krivoshto et. al., 2008; Tovalin et. al., 2006; etc.). The exhaust can be from diesel or gasoline with the former being classified by International Agency for Research on Cancer (IARC) as carcinogenic to humans and the latter being classified as possibly carcinogenic to humans (IARC, 2012). The genotoxicity of vehicular exhaust can be attributed to its hazardous chemical components. Particulate matter from vehicular exhausts, specifically diesel exhaust particles, have been proven to generate reactive oxygen species, which in excess causes oxidative stress that may lead to DNA damage (Krivoshto et. al., 2008 and Li & Nel, 2006). In addition, the reactive metabolites of polycyclic aromatic hydrocarbons (PAHs) from vehicular exhausts have the ability to bind to cellular proteins and DNA resulting in increased frequency of mutation and DNA strand breakage (Lah, 2011 and Li & Nel, 2006). Furthermore, other components of vehicular exhaust such as formaldehyde, monocyclic aromatic hydrocarbons, and benzene have been shown to be carcinogenic (Krivoshto et. al., 2008).

The genotoxicity of vehicular exhaust among frequently exposed populations can be examined through buccal micronucleus assay. Exfoliated cells of the buccal mucosa are at greater risk to DNA damage due to their frequent exposure to genotoxic substances (Holland et. al., 2008 and Li & Nel, 2006). Moreover, the cells of the oral cavity are capable of metabolizing carcinogens to reactive forms (Zhang & Mock, 1989 and Zhang, 1994 as cited by Cerqueira & Meireles, n.d.). The assay protocol utilized micronuclei as a bio-marker of DNA damage. Micronuclei are minute structures similar to the cell's nucleus which have arisen at anaphase from chromosome fragments or whole lagging chromosomes that fail to be integrated in the daughter nuclei and were then eventually enclosed by a nuclear membrane (Holland et. al, 2008 and Fenech et. al., 2011).

The molecular mechanism of micronuclei formation was thoroughly illustrated by Fenech et.al (2011). Micronuclei arise from chromosome fragments which may be caused by misrepair or unrepaired DNA breaks. Chromosome fragments may arise from misrepair due to defects in genes such as BRCA1 and BRCA2 leading to the dysfunction of the homologous recombinational DNA repair pathway. The DNA breaks may be left unrepaired DNA breaks cause micronucleus formation when the cell fails to repair the DNA because the DNA damage load is too much for the cell to handle within the specified time frame. Another mechanism of micronuclei formation due to chromosome fragments is the simultaneous excision repair of damaged or inappropriate bases incorporated in DNA that are in proximity and on opposite complementary DNA strands. Aside from chromosome fragments, micronuclei can also arise from whole lagging chromosomes. This may happen due to a wide array of mechanisms including hypomethylation of repeat sequences in centromeric and pericentrometic DNA, defects in kinetochore proteins or assembly, dysfunctional spindle, and defective anaphase checkpoint genes.

In this study, the DNA damage of the exfoliated buccal cells of thirty (30) urban female street vendors occupationally exposed to vehicular exhaust was evaluated using buccal micronucleus assay. Most of the street vendors in the Philippines, whether stationary or mobile, are

occupationally exposed to high amounts of vehicular exhaust. They are usually situated in areas (i.e. roads, highways, and bus terminals) which are among the highest sources of clinically significant exposure to diesel exhaust and other traffic related pollutants (Bhowmik, 2005; Krivoshto et. al., 2008; and Tovalin et. al., 2006). The results of this study showed that vehicular exhaust exposure has a highly significant effect on the micronuclei frequency of the street vendors (p<0.001). This is in agreement with other studies which also assayed the effects of traffic-related exhaust exposure to occupationally exposed individuals such as jeepney drivers, taxi drivers, engine-repair workers, gasoline station attendants, road construction workers, and traffic enforcers (Cavite, 2013; Celik et. al., 2013; Hallare et. al., 2009; and Karahalil et. al., 1999).

Aside from exposure to vehicular exhaust, the combination of exogenous and endogenous factors may influence the frequency of micronuclei formation (Bolukbas et al., 2006 as cited by Rickes et al., 2010). Among the exogenous factors are chemicals, physical agents, seasonal changes, viruses, and lifestyle factors such as smoking, drinking habits and nutrition (Major et. al., 1998). Smoking and alcohol drinking are among the factors that have been reported to be correlated with several forms of cancer (Majer et. al., 2001 as cited by Cavite, 2013). Moreover, endogenous factors such as age and gender may also influence the results of any cytogenetic monitoring (Major et. al., 1998).

In this study, the relationship of the possible confounding variables such as age, length of vehicular exhaust exposure, smoking, drinking habits and medicinal intake to the frequency of micronuclei formation was investigated. The relationship of the micronuclei frequency and alcoholism for both the exposed and control groups was not examined since none of the test individuals qualified as an alcoholic. One probable reason may be the low incidence of alcoholism of women in the Philippines. The results of this study showed that, except for coffee drinking, there was no significant relationship between the micronuclei frequency of the street vendors and the confounding variables considered in this study. For the control group, there was no significant correlation between micronuclei frequency and the confounding factors of smoking, coffee drinking, age, and medicinal intake for recent illness.

Smoking did not show any significant relationship to the micronucleus frequency for both the exposed and control groups. Although many studies suggest that smoking leads to the development of some cancers, there has been conflicting information about the relationship of smoking with the frequency of micronuclei in the buccal cells (Holland et.al, 2008). The results of this study agree with the majority of studies which also showed a non-significant effect of smoking on the micronuclei frequency of the buccal cells (Celik et.al., 2013; Hallere et. al., 2009; Holland et.al, 2008; Karahalil et.al, 1999; Naderi et.al., 2012; etc.).

The length of vehicular exhaust exposure did not also show any significant relationship with micronuclei frequency of the exposed individuals. This finding is consistent with the results of other micronucleus assays about the genotoxic risks of occupational exposure to vehicular exhaust (Cavite, 2013; Celik et.al., 2013; Karahalil et.al., 1999; etc.). The lack of correlation between micronuclei frequency and length of vehicular exhaust exposure may be attributed to a wide array of factors such as differences of the individuals in terms of genetic composition and lifestyle which could have affected the rate of micronuclei formation (Martino-Roth et al., 2002). The individual differences in terms of exogenous and endogenous factors may also be the underlying reason why age did not show any correlation with micronuclei frequency for both the exposed and control groups (Martino-Roth et al., 2002).

Coffee drinking has been shown to have a weak correlation with micronuclei frequency in this study (p < 0.05). Many volatile organic compounds such as glyoxal and methylglyoxal have been

characterized in coffee (IARC, 1997). These compounds are known to be carcinogenic. However, the International Agency for Research on Cancer (IARC) had found a weak association between coffee drinking and cancer. Coffee drinking has not been shown to cause cancer in almost all human organs except the urinary bladder; thereby classifying it as only possibly carcinogenic to human urinary bladder (IARC, 1997).

Lastly, the recent illness of the street vendors and controls were documented in this study. Although the results showed that there was no association between the recent illness and medicinal consumption to the frequency of micronucleus formation, it was noticeable that the recent illnesses of the street vendors were similar with the ones described by the studies of Amegah & Jakkola (2013) and Kongtip et.al. (2008) about the health risks of street vendors. However, there is no sufficient data in this study that would correlate the adverse health outcomes and the exposure of the street vendors to vehicular exhaust.

#### Conclusion and Implication of the Study

Urban street vendors are individuals in need for genotoxicity assessment due to their highly clinically significant exposures to vehicular exhaust (Bhowmik, 2005; Krivoshto et. al., 2008; and Tovalin et. al., 2006). Vehicular exhaust is a complex mixture of substances that have been proven by a wide array of studies to be genotoxic and deleterious to human health (Health Effects Institute, 2010 and IARC, 2012). This study assessed the DNA damage of street vendors exposed to vehicular exhaust through the use of the buccal micronucleus test. The test is a cheap and minimally invasive method that is widely used to bio-monitor populations exposed to known carcinogens and mutagens (Holland et.al, 2008). The results of the study showed that exposure of street vendors to vehicular exhaust have a highly significant effect (p<0.001) on the frequency of micronuclei in the buccal cells compared to the controls ( $9.40\pm4.46$  vs.  $4.80\pm3.25$  per 1000 cells). No significant relationship was found among the confounding factors except coffee drinking which showed a weak correlation with micronuclei frequency. Since the micronuclei are indicators of genotoxicity, the findings of this study suggest that the occupational vehicular exhaust exposure of urban female street vendors in Iligan City predisposes them to more DNA damage compared to their minimally-exposed counterparts.

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