

Determination of Carbofuran in Grape-Based Beverages Marketed in the Municipality of Itumbiara, Goiás, Brazil

Determinação de Carbofurano em Bebidas à Base de Uva Comercializadas no Município de Itumbiara, Goiás, Brasil
Determinación de Carbofurano en Bebidas a Base de Uva Comercializadas en el Municipio de Itumbiara, Goiás, Brasil

RESUMO

Introdução: No Brasil, o uso de agrotóxicos se intensificou na década de 1970 com o Plano Nacional de Desenvolvimento da Aquicultura. A Anvisa proibiu o carbofurano em 2017, mas resíduos foram encontrados em amostras de uva, conforme relatórios de dez/2019 e dez/2023 do Programa de Análise de Resíduos de Agrotóxicos. Essa substância é tóxica, podendo causar efeitos no sistema nervoso, como convulsões e depressão respiratória. **Objetivo:** O objetivo deste estudo foi determinar resíduos de carbofurano em bebidas à base de uva comercializadas em Itumbiara-GO. **Método:** Foram analisadas quatro amostras de diferentes marcas utilizando o método Extração Líquido-Líquido com Partição à Baixa Temperatura e Cromatografia Líquida de Autoeficiência com detector UV. Resultados: Os resultados mostraram que as amostras de suco de uva adquiridas em Itumbiara-GO não apresentaram resíduos de carbofurano. **Conclusão:** O estudo contribui para a conscientização sobre a importância do controle rigoroso de resíduos de agrotóxicos em bebidas, pois a ingestão de produtos contaminados pode ameaçar a saúde pública.

DESCRIPTORES: Agrotóxico; Carbofurano; Toxicidade; Bebidas; Uva

ABSTRACT

Introduction: In Brazil, the use of pesticides intensified in the 1970s with the National Aquaculture Development Plan. ANVISA (Brazilian Health Regulatory Agency) banned carbofuran in 2017, but residues were found in grape samples, according to reports from December 2019 and December 2023 from the Pesticide Residue Analysis Program. This substance is toxic and can cause effects on the nervous system, such as seizures and respiratory depression. **Objective:** The objective of this study was to determine carbofuran residues in grape-based beverages sold in Itumbiara-GO. **Method:** Four samples from different brands were analyzed using the Liquid-Liquid Extraction with Low Temperature Partitioning and High Performance Liquid Chromatography with UV detector. Results: The results showed that the grape juice samples acquired in Itumbiara-GO did not present carbofuran residues. **Conclusion:** This study contributes to raising awareness about the importance of rigorous control of pesticide residues in beverages, as the ingestion of contaminated products can threaten public health.

DESCRIPTORS: PESTICIDES; CARBOFURAN; TOXICITY; BEVERAGES; GRAPES

RESUMEN

Introducción: En Brasil, el uso de plaguicidas se intensificó en la década de 1970 con el Plan Nacional de Desarrollo de la Acuicultura. La Anvisa prohibió el carbofurano en 2017, pero se encontraron residuos en muestras de uva, según los informes de dic/2019 y dic/2023 del Programa de Análisis de Residuos de Plaguicidas. Esta sustancia es tóxica y puede causar efectos en el sistema nervioso, como convulsiones y depresión respiratoria. **Objetivo:** El objetivo de este estudio fue determinar residuos de carbofurano en bebidas a base de uva comercializadas en Itumbiara-GO. **Método:** Se analizaron cuatro muestras de diferentes marcas utilizando el método de Extracción Líquido-Líquido con Partición a Baja Temperatura y Cromatografía Líquida de Alta Eficiencia con detector UV. Resultados: Los resultados mostraron que las muestras de jugo de uva adquiridas en Itumbiara-GO no presentaron residuos de carbofurano. **Conclusión:** El estudio contribuye a la concientización sobre la importancia del control riguroso de residuos de plaguicidas en bebidas, ya que la ingestión de productos contaminados puede amenazar la salud pública.

DESCRIPTORES: Plaguicida; Carbofurano; Toxicidad; Bebidas; Uva.

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INTRODUCTION

Pesticides began to be used on a large scale in the 1940s, during World War II, with the aim of protecting soldiers in the tropical and subtropical regions of Africa and Asia against disease-transmitting pests, such as sleeping sickness and malaria¹. The use of pesticides in agriculture began in 1950 in the United States with the so-called Green Revolution, which aimed to modernize agriculture and increase its productivity. In Brazil, this movement arrived in the 1960s and gained momentum in the 1970s with the implementation of the National Program for Agricultural Pesticides (PNDA). The term “pesticide” was officially adopted in Brazil with Federal Law No. 7,802 of 1989, regulated by Decree No. 4,074 of 2002².

In 2001, the National Health Surveillance Agency (ANVISA) created the Program for the Analysis of Pesticide Residues in Food (PARA) with the objective of assessing pesticide residue

levels in plant-based foods and fruits. This program is carried out in partnership with states, municipalities, and public health laboratories³. Based on information found in PARA reports on samples analyzed between 2017 and 2018, published in December 2019, and samples from 2018–2019 and 2022, released in December 2023, carbofuran residues were found in grape samples^{4,5}.

Carbofuran (2,3-dihydro-2,2-dimethylbenzofuran-7-ylmethylcarbamate) belongs to the chemical class of carbamates and is classified as extremely toxic. Until 2017, it was authorized for use on crops such as: cotton, peanuts, rice, bananas, coffee, sugarcane, carrots, beans, corn, cabbage, tomatoes, and wheat⁶.

It is worth noting that the ban on the use of carbofuran in Brazilian agriculture took effect on October 18, 2017, with the aim of mitigating risks to public health. In 2022, the use of carbendazim was also banned, and crops were excluded for the active ingredient

formetanate⁷.

When ingested, the active ingredient inhibits the enzyme acetylcholinesterase, which breaks down acetylcholine. This inhibitory effect facilitates the accumulation of acetylcholine in the synapses and results in uncontrolled movements, paralysis, convulsions, or even death. Carbamate insecticides can be absorbed through the skin and the gastrointestinal tract. Once absorbed, they are immediately distributed to internal tissues, where they can cause oxidative stress⁷⁻⁸.

With the aim of developing a multi-residue method for the determination of 450 types of pesticides, Pang et al.⁹ In their study, 413 pesticides (92% of the 450) showed recovery rates of 70–120%, and 35 pesticides (8% of the 450) showed recovery rates of 59–70%. The method was applied to different types of matrices, including juices made from apples, pears, tangerines, oranges, and grapes, as well as red and white wines.

In the study by Soleas and Gol-

deberg¹⁰, the authors identified 26 pesticides in 1,537 wines and 1,827 unfermented juices from nine major wine-producing countries or regions. In the case of juices, concentrations were 0.1 mg/L. 0.1 mg/L was detected most frequently for Folpet (219 samples, 12%) and Captan (212 samples, 11.6%) and in 0.1% of the samples for Guthion, Imidan, and Carbaryl. Good manufacturing practice guidelines suggest that MRLs for Carbaryl in wine should be set at 0.8 mg/L and 0.1 mg/L for all other pesticides.

In the study conducted by Lima et al.¹¹ in the Campinas-SP region, the results showed that 12 (twelve) of the 27 (twenty-seven) strawberry samples evaluated presented at least one irregularity, with the detection of unauthorized products, such as the active ingredients chlorfenapyr and folpet, affirming the importance of inspection for health safety.

The Liquid-Liquid Extraction by Low-Temperature Partitioning (LLE-LTP) method, which was used in this study, is based on partitioning the sample into two phases—one aqueous and the other organic—followed by freezing the system; it has been widely used to determine carbamates and other pesticides in environmental and food matrices. It is a simple and efficient method and has proven effective in the extraction of pesticides from vegetables, fruits, and beverages¹².

Based on the information cited, the present study aimed to determine carbofuran residues in samples of grape-based beverages sold in Itumbiara, Goiás, Brazil.

MATERIALS AND METHODS

For the analysis of grape-based beverages, the ELL-PBT method was used, followed by HPLC-UV analysis according to the methodology proposed by Goulart et al.¹³. This analytical technique has already been used in different matrices, such as grape juices and choc-

olate milk¹³, water¹⁴, sugarcane juice¹⁽⁵⁾ and orange juice¹⁶.

Sample Collection

Four samples of grape-based beverages were used, from well-known brands sold in the municipality of Itumbiara, Goiás, Brazil. The samples acquired were of different types of grape-based beverages, including one organic beverage, one whole juice, and two nectar-type beverages.

After acquisition, the samples were refrigerated and analyzed at the Chromatography and Spectroscopy Laboratory of the Federal Institute of Goiás – Itumbiara *campus*, where they were prepared and analyzed in duplicate.

Reagents and Solutions

The stock standard solution at a concentration of 100.0 mg L⁻¹ of carbofuran (99.9% w/w – Sigma Aldrich) was prepared in acetonitrile (J.T. Baker, HPLC-UV grade), methanol (J.T. Baker), and stored in a freezer.

Chromatographic Analysis

In the preparation of the samples, following the methodology of Goulart et al.¹³, 2 mL samples of grape-based beverages were prepared and placed in 22 mL clear glass vials, to which 1.5% NaCl (w/v) and 4 mL of acetonitrile were added. The mixture was *vortexed* for 60 seconds and left in a freezer at

-20°C for approximately 3 hours to allow for freezing and phase separation. Subsequently, 1.0 mL of the supernatant was collected, transferred to an injection *vial*, and analyzed by High-Performance Liquid Chromatography with Ultraviolet Detection (HPLC-UV).

To verify the selectivity of the technique in grape beverage samples, a sample free of carbofuran was spiked with a carbofuran standard solution at a concentration of 5.0 mg L⁻¹ following the methodology described above.

Chromatographic Conditions

A liquid chromatograph equipped with an ultraviolet detector (HPLC-UV) was used under the following chromatographic conditions: C18 column; mobile phase: purified water: acetonitrile 65:35 v:v, mobile phase flow rate: 0.8 mL min⁻¹, column temperature: 35 °C, and injection volume: 20 µL. UV detector (λ: 195 nm).

RESULTS AND DISCUSSION

Regarding the characteristics of the samples used in the study, Table 1 provides definitions of the types of grape-based beverages, and we emphasize that information from the labels was used; the presence of information regarding the grape variety used in juice production was also analyzed.

Table 1. Definitions of Grape-Based Beverage Types: Organic, Whole, and Nectar

Type of Beverage	Definition
Organic	This is a type of beverage that does not use pesticides, seeks to use a minimum of organic fertilizers in agricultural production, and employs management practices aimed at mitigating environmental impacts ¹⁷⁻¹⁹ .
Whole	The only industrialized juice that is 100% fruit juice is the one labeled "Whole Juice." This juice retains the original concentration of juice extracted from the fruit, with no added water or sugar ^{20,21} .
Nectar	It is a non-fermented beverage obtained by diluting the edible part of the plant or its extract with drinking water. The basic difference is that nectar is not required to retain all the original characteristics of natural fruit juice ^{20,21} .

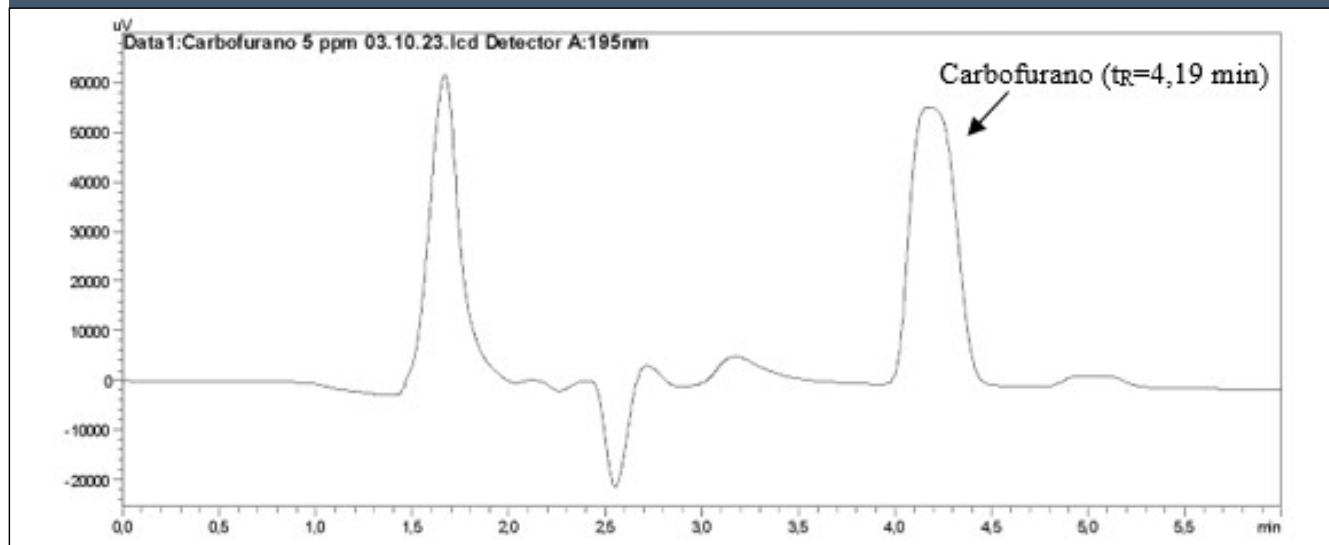
Source: Prepared by the authors, 2025.

A standard solution of carbofuran with a concentration of 5 mg L^{-1} in acetonitrile was prepared and then

injected into the chromatograph to determine the retention time (t_R); the result for this pesticide under these

analytical conditions was 4.19 min, as shown in Figure 1.

Figure 1: Carbofuran standard solution in acetonitrile with a concentration of 5 mg L^{-1} .



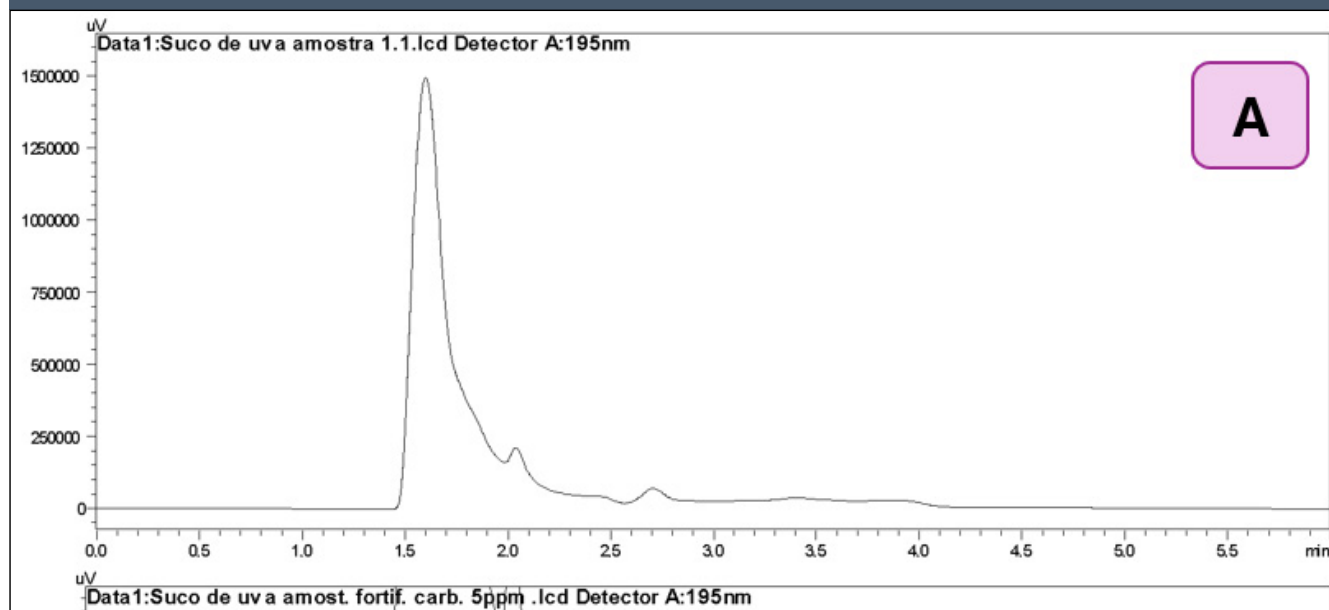
Source: Prepared by the authors, 2025.

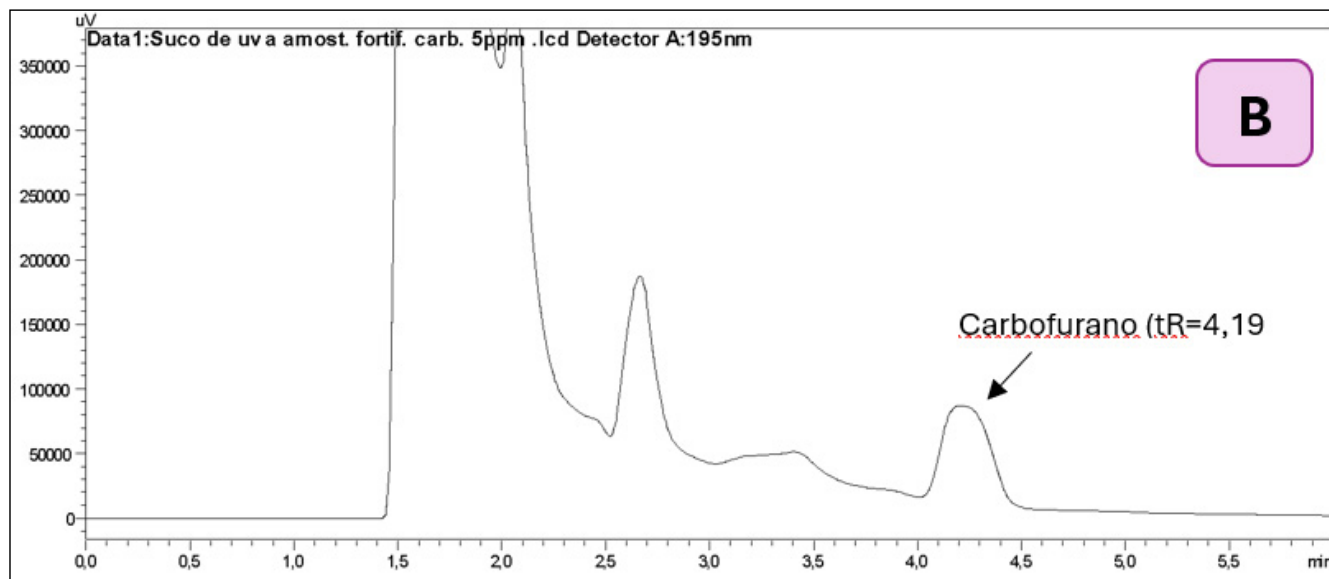
The method used demonstrated adequate selectivity, as shown in Figure 2, through a comparative analysis of the chromatograms of the blank

extract from the grape-based beverage and the blank extract from the beverage fortified with a 5 mg L^{-1} solution. It is noted in the analyses that no interferences were detected at the

same retention time as carbofuran, thus confirming the selectivity of the method²².

Figure 2: (A) Chromatogram of an extract obtained from a carbofuran-free grape-based beverage and (B) Chromatogram of a sugarcane juice extract containing 5.0 mg L^{-1} of carbofuran





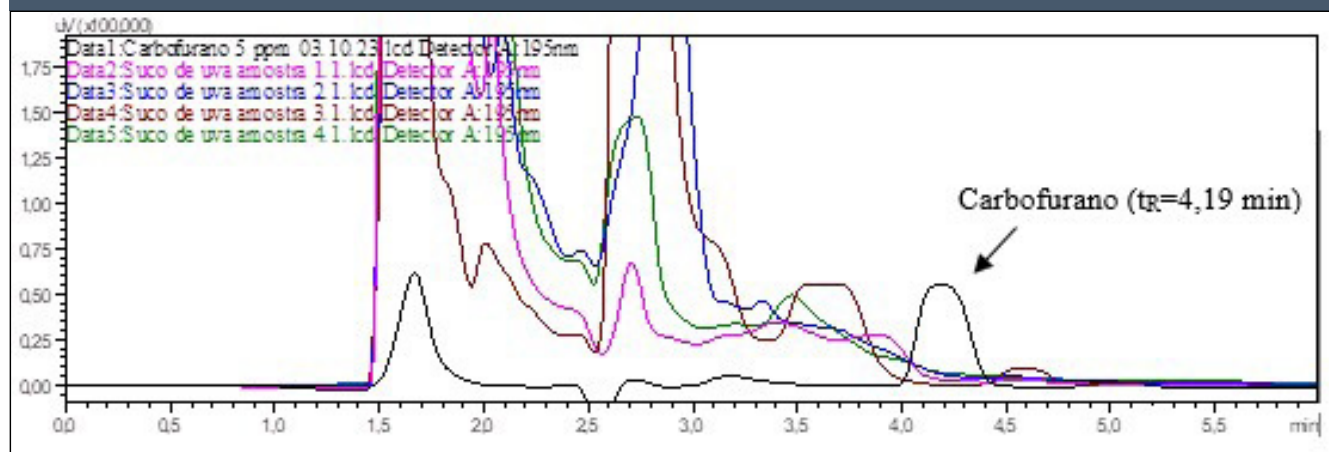
Source: Prepared by the authors, 2025.

When applying the ELL-PBT method to grape-based beverage samples, Figure 3 shows that no car-

bofuran residues were identified in the samples collected from stores in Itumbiara, Goiás, when compared to the substance standard, or that these

residues were present in concentrations below the instrument's detection limit of $12 \mu\text{g L}^{-1}$.

Figure 3: Chromatograms obtained from grape-based beverages: Sample 1 (___); Sample 2 (___); Sample 3 (___) and Sample 4 (___) compared to the carbofuran standard 5.0 mg L^{-1} (___)



Source: Prepared by the authors, 2025.

The data obtained in this study are important, since carbofuran is subject to strict restrictions imposed by PARA, coordinated by ANVISA.

The absence of carbofuran is important because it causes overstimulation of the central nervous system by inhibiting enzymes called cholinesterases, which are responsible

for the breakdown of acetylcholine, a neurotransmitter responsible for transmitting impulses in the nervous system. When cholinesterase is inhibited, it cannot break down acetylcholine, leading to a disorder known as a cholinergic crisis, which causes symptoms of poisoning, such as nausea, vomiting, weakness, and pain²³.

According to the authors Pezzini et

al.²⁴ carbamates are among the most widely used classes of pesticides in agricultural production and domestic settings, ranking among the primary toxic agents associated with cases of acute human poisoning, whether accidental or not—such as in cases of homicide and suicide—given the high toxicity of some of these compounds, which are often mixed with

dark beverages to mask the presence of the “poison.”

Kegley et al.²⁵ state that carbofuran is highly toxic and can cause damage to the central nervous system, with potential developmental toxicity in humans under real-world exposure conditions, including functional and behavioral teratogenic effects, as well as posing a risk of chronic toxicity with prolonged exposure. Carbofuran also exhibits high toxicity regarding its adverse effects on reproduction and the endocrine system, as well as neurotoxicity, as demonstrated by delayed neuropathy.

Exposure to pesticides is a major public health problem, posing significant risks in both the short and long term. The consequences range from immediate effects, such as acute poisoning, to chronic conditions, including the development of serious diseases. Studies show associations between such exposure and cellular changes^{26,27}, brain neoplasms²⁸, non-Hodgkin lymphoma^{29,30}, cutaneous melanoma^{31,32}, cancer of the digestive system, male and female genital

systems, urinary system, respiratory system, breast cancer⁽³³⁾, and esophageal cancer³⁴. These correlations, compiled in the systematic review by Lopes and Albuquerque¹, underscore the complexity of the impacts on human health, highlighting the urgency of public policies aimed at reducing occupational and environmental exposure to these compounds.

In the context of men's health, a systematic review examined data from twenty studies exploring the influence of organophosphates and carbamates. The study identified evidence supporting the relationship between exposure to these pesticides and impairments in sperm quality and function^{35,36}.

Therefore, it is essential that efficient analytical methods, such as the one applied in this study, be consistently used and refined to monitor pesticides in various samples and ensure consumer health.

CONCLUSION

The analyses of grape-based bev-

erage samples sold in the municipality of Itumbiara, GO, using the ELL-PBT technique, did not detect residues of the pesticide carbofuran under the analytical conditions used.

This study highlights the importance of monitoring pesticides in food products, especially given the impact that contamination by these substances can have on public health. Carbofuran, a highly toxic insecticide, can cause acute and chronic health effects in humans, including neurological and endocrine disorders.

The study contributes to the strict control of pesticide residues in beverages, since the consumption of grape juice or other contaminated products can pose a threat to public health, as it is a frequent problem in the emergency departments of large hospitals, whether due to accidental ingestion by children or suicide attempts.

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APPENDIX 1



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