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Assessments of Mutagenic and Genotoxic Effects of Noodles using *Salmonella typhimurium* and *Caenorhabditis elegans* as a Model Organism

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Abstract

Instant noodles are consumed globally in billions of servings, yet limited attention has been given to their potential genetic and reproductive toxicity. This study aimed to assess the mutagenicity of raw and cooked noodles and their associated seasonings. The study employed both in vitro and in vivo approaches, utilizing the Ames test on Salmonella typhimurium strain TA100 to assess mutagenicity, while Caenorhabditis elegans served as a model organism for in vivo genotoxicity tests. Noodles with their associated seasonings from two frequent brands in Jos North, Nigeria, were tested at various concentrations, with mutagenicity evaluated through revertant colony counts and genotoxicity assessed via the worms' survival and reproduction. Data were analyzed using the Mutagenicity Index (MI) for the Ames test, with a threshold of MI ≥ 2 indicating significant mutagenic potential. The Ames test, conducted on* Salmonella typhimurium *strain TA100, revealed no significant mutagenic activity in the raw noodles or with different cooking methods (Mutagenicity Index [MI] < 1). However, seasonings from the two brands demonstrated weak mutagenic activity at higher concentrations (MI > 1 with metabolic activation). In* C. elegans*, the noodles had no adverse impact on survival or reproduction, but seasonings significantly impaired movement, growth, and reproduction at high doses. These findings suggest that the noodles assessed may be safe for consumption, though high levels of certain seasoning components could pose reproductive or genetic risks. The study underscores the need for further investigations, recommending stricter regulatory scrutiny of food additives and encouraging manufacturers to minimize harmful compounds in seasonings.

Keywords: Noodle, Seasoning, Genotoxic, Mutagenic, Ames test, Caenorhabditis elegans

INTRODUCTION

Instant noodles are a popular and convenient food choice, particularly among busy individuals and college students. These easy-to-prepare meals have gained immense popularity due to their affordability, quick cooking time, and diverse range of flavors. Nigeria is now the tenth-highest consumer of noodles globally. With increasing prices of other carbohydrate diets like rice, Nigerians have turned to noodles, pushing the country's consumption rate up in the global rank to become 10th. Nigeria consumed a total of 3 billion noodles per serving in 2023, up 6.8% from 2.8 billion in 2022 (BusinessDay, 2024). With just four (4) brands in 2006, the Nigerian instant noodles market now boasts up to 16 competing brands (Marketing Edge, 2017). The increasing demand for instant noodles is not limited to Nigeria; globally, they are consumed on a massive scale and rank second only to bread as a staple food item (World Instant Noodles Association, 2016). It has been estimated that one in two Nigerians has tasted noodles and that up to 15 million people in Nigeria eat them regularly (Sanni *et al.*, 2013). Noodles are usually prepared for consumption using their seasoning; therefore, the rate at which noodles are consumed is directly proportional to the rate at which the seasoning is consumed (Alabi *et al.*, 2014). The major constituents of noodles are wheat flour, vegetable oil, iodized salt, sodium polyphosphate, sodium carbonate, potassium carbonate, guargum and tartrazine while that of the seasoning powder are iodized salt, mono-

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sodium glutamate (MSG), hydrolyzed vegetable protein, soy powder, garlic powder, chicken flavor and chili powder (Sanni *et al.*, 2013). Despite the widespread consumption of noodles, especially among students and children of different ages, there is limited scientific research focused on the mutagenic and genotoxic potentials of instant noodles (Joseph *et al.*, 2024). Many studies have concentrated on their nutritional content and general health effects, neglecting the possible genetic risks posed by the chemical constituents used in their production (Joseph *et al.*, 2024).

When humans are directly exposed to a potentially genotoxic substance, as with food, there is a need to evaluate diverse types of DNA alterations in order to thoroughly determine the health hazard (Alabi *et al.*, 2014). This evaluation requires the use of a variety of tests and animal models. The bacterial reverse mutation test, popularly known as the Ames test, is an in vitro genotoxicity test recommended by regulatory agencies to detect genotoxic carcinogens (Alabi *et al.*, 2014). The *Caenorhabditis elegans* model is increasingly being used in toxicology for mechanistic studies, high-throughput screening, and environmental toxicity assessments. In the context of mutagenicity and genotoxicity research, *C. elegans* serves as a useful model for evaluating DNA damage, oxidative stress, and cellular responses to toxicants. Key features of *C. elegans* that contribute to its success as a model organism include its genetic manipulability, well-characterized genome, short and prolific life cycle, clear and transparent morphology, and ease of maintenance (Wu *et al.*, 2024). These characteristics allow researchers to study genetic responses to toxic substances in a controlled environment (Leung *et al.*, 2008; Wu *et al.*, 2024). The Ames test is based on detecting reverse mutations in two histidine auxotrophic mutants of the *Salmonella typhimurium* bacterium, rendering them histidine prototrophs (Ames *et al.*, 1973). One mutant strain, TA100, allows the detection of base substitution mutations, while another strain, TA98, allows the detection of frameshift mutations (Ames *et al.*, 1973). This test, considered a gold standard in chemical mutagenicity detection, has been extensively validated (Kubo *et al.*, 2002).

In previous studies, the effect of Instant noodles on reproductive capacity appears to be mostly unknown. Still, results of a recent study by Khudhur et al. (2021) demonstrate that graded concentrations of Indomie noodles spiced with its seasoning had significant adverse effects on sperm concentration, motility, and viability in rats. Still, the study couldn't trace the source of the effect. In a study by Sanni *et al.* (2013), a rat model was used for mutagenic evaluation, but the study was more focused on one brand of instant noodle spiced with its seasoning. Likewise, a recent study in Nigeria by Alabi *et al.* (2014) used Ames test and SOS Chromotest assays *without an* animal model, and the study focused on seasoning from one brand of instant noodle. There is also no available literature that evaluates the mutagenic potential of cooked noodles from Nigeria. By combining the Ames test with *C. elegans*-based studies, a more comprehensive understanding of the potential risks associated with instant noodle consumption can be obtained. In this study, the genotoxic and mutagenic effects of raw noodles, seasoning, and cooked noodles (Boiled and fried) from two 2 different brands were evaluated using the Ames test and *C. elegans* as a model organism.

MATERIALS AND METHODS

This study was conducted in the Faculty of Pharmaceutical Sciences, University of Jos, Jos North Local Government Area, located in Jos metropolis, the capital of Plateau State, Nigeria. The study population includes; Bacterial Strains (Ames Test): *Salmonella typhimurium* strain TA100 (National Veterinary Research Institute, Vom, Jos). Animal Model (Wild-type *C. elegans*). With test substances, We Used Instant noodle extracts, Seasoning mix, and cooked (boiled and fried) noodles from two frequent brands in the study area.

Preparation of *Salmonella typhimurium* for the Ames Test

Culture bottles and glassware were sterilized at 100°C for 30 minutes. Selenite broth was prepared by dissolving in distilled water, heating for dissolution, and then placing in a water bath at 80°C for 10–20 minutes (autoclaving was not used to preserve media integrity). After cooling,

10 mL of broth was transferred into each sterilized bottle. Salmonella strain TA100 was inoculated using a sterile wire loop and incubated at 37°C for 24 hours.

Histidine Auxotroph Confirmation

Salmonella cultures were treated with sodium azide (0.5 µL, 1 µL, and 1.5 µL) and incubated at 37°C for 24 hours. To confirm the mutation, bacterial cultures were plated on histidine-supplemented nutrient agar and histidine-free minimal media. Only histidine-requiring mutants failed to grow on minimal media, confirming auxotrophy.

Ames Test

The Ames test was carried out according to the method described by Maron and Ames (1983) and Alabi *et al*. (2014). Tests were conducted under aseptic conditions according to the method described by Rao and Lifshitz (1995). Crushed raw and cooked (boiled and fried) noodles, and mixed spices (chili and salt) were weighed using a digital balance. Extracts were prepared at different concentrations of 100 µg/ml, 200 µg/ml, and 300 µg/ml, using distilled water as a solvent, and were sterilized by filtration through a 0.22 µm pore-size cellulose nitrate filter (Millipore). Dose-response relationship was followed throughout the experiments. Mutagenicity tests were performed both with and without metabolic activation with S9 rat liver enzyme (RLE) mix. The S9 Rat Liver Homogenate in KCl (Abdul Bio medicals, Kano). RLE mix was prepared according to the Ames test standard protocol. Relevancy of this in vitro bacterial test to carcinogenesis is increased by adding rat-liver homogenate to the test system, because it contains enzymes which perform several metabolic conversions similar to those of mammalian organs (Stang 1980).

The plate incorporation method was used. Sodium azide (NaN_3) was used as a positive control for TA100, while distilled water was used as a negative control. Minimal Agar plates were prepared, and tubes containing 3ml of molten top agar were placed in a 45°C water bath. Using a pipette and tips, 150µl of *Salmonella typhimurium* strain was added to the test tubes. 300 µl of histidine was also added to the tubes that required histidine. 0.5 ml of S9 mix was added to the test tubes and control tubes, and 1.25 µg/plate of sodium azide solution was added to positive control tubes. Test extract was added to the test tubes at different concentrations: 100 µg/ml, 200 µg/ml, and 300 µg/ml, respectively. To mix, the tubes were rolled between palms and then poured immediately on top of the glucose minimal agar plate. It was then allowed to settle for several minutes. Plates were incubated at 37°C for 48h, and revertant colonies were counted. The presence of a background lawn was confirmed. The number of revertants counted on the negative control plates is directly related to the mutagenicity of the test extract. A chemical is considered to be mutagenic if the number of induced revertants is two or more times greater than the number of spontaneous revertants (i.e., mutagenic index (MI)). Mutagenicity Index (MI) for the 3 test levels was calculated (Raw noodle, Spices, and Cooked (boiled and fried)). Using the formula: MI = Rt/Rc, where Rt = mean number of revertant colonies with test substance, and Rc = mean number of spontaneous revertant colonies obtained with a negative control. A significant positive mutagenic effect is determined by a calculated MI ≥ 2 and a linear relationship between dosage and number of revertant colonies (Stang 1980).

***C. elegans* Assay**

Wild-type *C. elegans* was isolated from fresh ripe fruit samples (banana, orange, mango, and watermelon). The fruits were homogenized and incubated in sterile containers for four days in an open environment. The resulting liquid was filtered through fine sterile muslin cloth, and *C. elegans* was isolated using a warm needle for microscopic confirmation. Adult *C. elegans* were inoculated on control and test plates. Sodium azide and E. coli were used on the positive control plates at 0.5, 1.0, and 1.5 µg/ml, respectively, while distilled water and E. coli were used on the negative control plates. Raw noodles and spice extracts at 100, 200, and 300 mg/ml were used on test plates. Worms (10 per plate) were monitored at 20°C for three days, with observations on survival,

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reproduction, movement, and morphology. Survival and reproduction rates were calculated by: Survival rate (%) = Number of survivors/Total number of initial organisms' X 100,

Reproduction rate = Total number of offspring/Total number of reproducing organisms.

To carry out data analysis for the Ames test, mutagenicity was analyzed using MI (MI ≥ 2 considered significant). For the C. elegans assay, survival and reproduction rates were calculated and compared across treated and control groups. Statistical analysis was performed using SPSS version 27.0, employing descriptive statistics.

RESULTS

The Ames Test

The mutagenicity profiles assessed by the Ames test consistently show that both raw and cooked instant noodles from the two tested brands had MI values well below the threshold of 2.0, with most MI scores ranging from 0.67 to 0.96. These values remained consistently low regardless of metabolic activation using S9 mix, indicating the absence of DNA-damaging compounds or their metabolic precursors in these products.

Table 1: Mutagenicity Results for Raw Noodles (Brands A and B) in *Salmonella typhimurium* TA100 with and without S9 Mix

	Without S9 RLE		With S9 RLE	
Dose (µg/plate)	Revertants (M ± SD)	MI	Revertants (M ± SD)	MI
Raw Noodle A				
0.0	96.00 ± 7.21	-	139.00 ± 9.00	-
100.0	81.00 ± 13.90	0.84	93.67 ± 5.51	0.67
200.0	79.00 ± 14.80	0.82	101.00 ± 7.55	0.73
300.0	88.67 ± 10.50	0.92	110.67 ± 5.03	0.79
Ctrol+	889.67 ± 11.93	-	1269.33 ± 73.66	-
Raw Noodle B				
0.0	102.00 ± 4.00	-	153.33 ± 4.16	-
100.0	80.00 ± 1.00	0.78	108.33 ± 4.73	0.71
200.0	85.33 ± 2.52	0.83	111.00 ± 3.61	0.72
300.0	83.00 ± 6.08	0.81	114.67 ± 11.93	0.75
Ctrol+	895.00 ± 12.53	-	1401.33 ± 24.09	-

Key: Negative Control (0.0): distilled water, Positive Control (Ctrol+); sodium azide (1.25 µg/plate—TA100). RLE: Rat liver extract, MI: Mutagenicity Index.

In Table 1 above, without S9 (no metabolic activation), both Raw Noodle Brands, A and B, show a slight decrease in the number of revertant colonies with increasing concentrations (100 µg/plate to 300 µg/plate). However, these reductions are not drastic, and the Mutagenicity Index (MI) remains below 1 across all concentrations, indicating that neither brand induces significant mutagenic effects without metabolic activation. While with S9 (With metabolic activation), the revertant counts are higher in both brands when S9 is introduced, indicating that metabolic activation enhances mutagenesis. However, the MI values are still below 1, suggesting that even with metabolic activation, neither noodle brand exhibits potent mutagenic activity. But in Table 2, where the result for Spices is tested, it shows; Without S9, Spices Brand A and B show a consistent number of revertant colonies across increasing doses, with the MI values close to 1 but never exceeding it. This indicates that the spices have limited mutagenic activity without metabolic activation. But with S9, Spice Brand A at higher doses (300 µg/plate) shows an MI of 1.05, while Spice B at the same concentration shows an MI of 1.02. Although these values fall short of the MI ≥ 2 mutagenicity threshold, the trend indicates potential for low-level DNA damage under conditions of metabolic conversion. These findings suggest the presence of pro-mutagenic compounds requiring enzymatic activation within seasoning formulations, which may include known additives like MSG or hydrolyzed proteins.

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Table 2: Mutagenicity Results for Seasoning Powders (Brands A and B) in *Salmonella typhimurium* TA100 with and without S9 Mix

Dose/µg/plate	Without S9 RLE Revertants (M ± SD)	MI	With S9 RLE Revertants (M ± SD)	MI
Spices A				
0.0	113.33 ± 5.86	-	133.67 ± 4.16	-
100.0	90.67 ± 4.04	0.80	122.67 ± 5.03	0.92
200.0	102.00 ± 5.29	0.90	133.00 ± 3.61	0.99
300.0	119.67 ± 3.22	1.00	134.33 ± 5.03	1.05
Ctrol+	688.67 ± 11.02	-	1106.67 ± 10.41	-
Spices B				
0.0	112.33 ± 5.86	-	156.67 ± 6.11	-
100.0	101.67 ± 2.52	0.91	112.67 ± 5.03	0.72
200.0	104.33 ± 2.08	0.93	126.00 ± 5.29	0.80
300.0	111.33 ± 8.08	0.99	159.33 ± 7.02	1.02
Ctrol+	962.33 ± 13.05	-	1408.33 ± 15.37	-

Key: Negative Control (0.0): distilled water, Positive Control (Ctrol+); sodium azide (1.25 µg/plate—TA100). RLE: Rat liver extract, MI: Mutagenicity Index.

Table 3: Mutagenicity Results for Cooked Noodles (Boiled and Fried) in *Salmonella typhimurium* TA100 with and without S9 Mix

Dose/µg/plate	Without S9 RLE Revertants (M ± SD)	MI	With S9 RLE Revertants (M ± SD)	MI
Fried				
0.0	116.67 ± 3.51	-	150.00 ± 3.61	-
100.0	101.00 ± 2.65	0.87	125.33 ± 3.05	0.84
200.0	103.67 ± 4.73	0.89	125.33 ± 3.05	0.84
300.0	103.00 ± 3.61	0.88	125.33 ± 3.51	0.84
+Control	1105.33 ± 11.72	-	1406.00 ± 15.10	-
Boiled				
0.0	98.67 ± 5.13	-	135.00 ± 2.65	-
100.0	80.00 ± 5.00	0.81	104.33 ± 6.66	0.77
200.0	89.00 ± 1.00	0.90	114.33 ± 3.51	0.84
300.0	95.00 ± 3.00	0.96	115.67 ± 3.51	0.86
+Control	1088.00 ± 19.67	-	1438.33 ± 7.64	-

Key: Negative Control (0.0): distilled water, Positive Control (Ctrol+); sodium azide (1.25 µg/plate—TA100). RLE: Rat liver extract, MI: Mutagenicity Index.

Table 4: Effects of Positive Control (Sodium Azide) and Negative Control(*E. coli*)On *C. elegans*

Mutagen Concentration (Sodium azide)	Survival Rate (%)	Movement	Morphology	Reproduction Rate (offspring/adult)
1.5 µg/plate	45%	Dull locomotion	No growth, black	0/adult
1.0 µg/plate	30%	Dull locomotion	No growth, black	0/adult
0.5 µg/plate	20%	Dull locomotion	No growth, black	0/adult
Negative Plates (*E.coli*)				
	100	Normal	Transparent, grown	35/adult
	100	Normal	Transparent, grown	50/adult
	100	Normal	Transparent, grown	55/adult

Table 3 above shows the results for cooking methods. Without S9 activation, Both Fried and boiled noodles exhibit low numbers of revertant colonies across increasing concentrations, with MI values below 1. This indicates that neither cooking method significantly increases the mutagenic potential of the noodles without metabolic activation. The presence of S9 does not significantly affect the mutagenic activity of either cooking method, with MI values remaining below 1 across all doses. The slight variations between Fried and Boiled noodles do not suggest any significant mutagenic risk associated with either cooking method.

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***Caenorhabditis elegans* Assay**

In Table 4 below, the Positive Control shows the survival rates decrease as the concentration increases (45% at 0.5 µg, 30% at 1.0 µg, and 20% at 1.5 µg), indicating that the substance used is toxic to the test organisms at higher concentrations. There is also zero reproduction across all concentrations, which suggests that the positive control not only affects survival but also inhibits reproduction, movement, and growth of *C. elegans*.

For the Negative Control, 100% survival across all replicates was shown, indicating that the standard conditions (feeding the worms with E. coli) do not harm the test organisms. The reproduction rate is high, with an average of 46.7 offspring per adult. This indicates that the negative control provides optimal conditions for reproduction, movement, and growth.

In Table 5 above, both Noodles A and B show high survival rates (96–100%) across all concentrations, with normal movement, normal growth (transparent bodies), and consistent reproduction rates (40–60 offspring per adult). This indicates that these noodle brands do not adversely affect *C. elegans* survival, growth, or reproduction.

Table 5: Effects of Noodles(Brand A and B) on *C. elegans*

Noodles (Brand A)	Concentration (mg/mL)	Survival Rate(%)	Movement	Morphology	Reproduction Rate (offspring/adult)
	100	96	Normal	Transparent, grown	50/adult
	200	98	Normal	Transparent, grown	40/adult
	300	100	Normal	Transparent, grown	55/adult
Noodles (Brand B)	100	97	Normal	Transparent, grown	50/adult
	200	95	Normal	Transparent, grown	50/adult
	300	100	Normal	Transparent, grown	60/adult

Table 6: Effects of Seasoning Powders (Brand A and B) on *C. elegans*

Spices (Brand A)	Concentration (mg/mL)	Survival Rate (%)	Movement	Morphology	Reproduction Rate (offspring/adult)
	100	80	Sluggish after 12hr	Dark, no growth	0/adult
	200	75	Sluggish after 24hr	Dark, no growth	0/adult
	300	70	Sluggish after 24hr	Dark, no growth	0/adult
Spices (Brand B)	100	85	Sluggish after 12hr	Dark, no growth	10/adult
	200	70	Sluggish after 24hr	Dark, no growth	0/adult
	300	60	Sluggish after 24hr	Dark, no growth	0/adult

Looking at the results for spices in the C. elegans assay in Table 6. Spice Brand A shows reduced survival rates (80%, 75%, 70%) with slow movement and impaired growth (dark, no increase in size). No reproduction was observed at any concentration. Spice Brand B also shows reduced survival rates (85%, 70%, 60%) with similar slow movement and impaired growth. Reproduction was observed only at the lowest concentration (10 offspring/adult), with no reproduction at higher doses.

The *C. elegans* model provides insights into possible *in vivo* toxic effects (Tables 4 to 6). Noodles, regardless of brand or dose, did not impact nematode survival, morphology, movement, or reproductive capacity. Reproduction rates remained high (40–60 offspring per adult), and survival was >95% across all concentrations, reinforcing the conclusion that the tested noodles themselves are not genotoxic or toxic to whole organisms. Conversely, both seasoning brands tested produced dose-dependent adverse effects in *C. elegans*. At concentrations of 200–300 mg/mL, survival dropped to 60–75%, locomotion became sluggish, and reproduction was either absent or sharply reduced.

DISCUSION

The findings of this study provide important insights into instant noodles and their associated seasonings. The results align with previous

literature in several key areas, while also presenting unique patterns that suggest further avenues for investigation. The Ames test results indicate that none of the three (noodle, seasoning powder, and cooking method) tested fit the three USEPA (United States Environmental Protection Agency) criteria for classification as a mutagenic compound. That is, that none of the instant noodle or it seasoning tested exhibited (1) a significant relationship between the number of revertant colonies and dose concentration and (2) a two-fold or more increase in the number of revertant colonies over the number of spontaneous revertants seen in the control and (3) a Mutagenicity Index (MI) value greater than 2.0. This aligns with prior studies suggesting that most food items without significant additives have limited mutagenicity (Fardet, 2016). The observed results in this study further clarifies the study of Alabi *et al.*, (2014) where the result showed mutagenicity and genotoxicity at 0.0175 and 0.008125 g/mL of the spice from Indomie noodle in the Ames and SOS Chromo tests respectively, which is lower than the quantity present in the indomie smallest pack (7g seasoning). This is an indication of the high mutagenicity and genotoxicity of indomie seasoning and a possible effect on the general populace who are exposed to it.

Morphologically, affected worms displayed darkened pigmentation and stunted growth, hallmarks of systemic toxicity. These effects mirror studies in which food-related additives, including dyes and flavor enhancers, induced oxidative stress, reduced motility, and shortened lifespan in C. elegans, via pathways implicated in neurodegeneration and germline apoptosis (Li *et al.*, 2024; Ning *et al.*, 2024). Numerous mechanisms may function in the reproductive toxic influences of noodle seasoning on *C. elegans*. Potential mechanisms were discussed in studies by Jewett et al. (2022) and Wu et al. (2024); the primary mechanisms are predominantly related to germ cell apoptosis, spermatogenesis, and epigenetic modifications. The outcome of the study by Sanni *et al.* (2013) also shows that indomie noodles spiced with the seasoning reduced the activities of alanine aminotransferase, an important antioxidant enzyme. This observed genotoxicity and mutagenicity of indomie seasoning in rats is believed to be due to its constituents that might have worked synergistically to cause the observed genetic damage.

Indomie noodles' seasoning, one of the tested brands, contains Monosodium glutamate, a major constituent of indomie seasoning, which has been reported to have a toxic effect on the testes of male Wistar rats by causing a significant oligozoospermia and increased abnormal sperm morphology in a dose-dependent manner (Onakewhor *et al.*, 1998). It has also been implicated in male infertility by causing testicular hemorrhage, degeneration, and alteration of sperm cell population and morphology (Oforofuo *et al.*, 1997). The outcome of the study by Khudhur et al. (2021) supported this finding. The study showed that significant increases in platelet count, AST, ALP, and blood urea values were determined, while significant decreases in MCH, MCHC, total protein, and serum uric acid values were observed in rats treated with Indomie noodles. However, with the consumption of Indomie noodles, a significant decrease in sperm count, sperm motility, and sperm viability was determined in rats treated with Indomie noodles. More studies have examined other metabolic and toxic effects of MSG, with a number of the reports showing the induction of oxidative stress in different tissues of experimental animals after administration of chronic doses of MSG (Onyema *et al.*, 2006; Diniz *et al.*, 2004; Singh *et al.*, 2003).

Given the established findings that seasoning powders can harbor heavy metals like Pb and Ni above acceptable limits shown to carry potential carcinogenic and mutagenic risks (Obi *et al.*, 2023; Uti *et al.*, 2023) it is plausible the weak mutagenic signals and nematode reproductive toxicity are due to such contaminants, either alone or in combination with additive-induced oxidative stress. As documented with environmental contaminants like flame retardants and plasticizers, even low-level chronic exposures can lead to trans-generational reproductive effects in *C. elegans* (Li *et al.*,

2024; Ning *et al.*, 2024), underscoring that repetitive, high-volume seasoning intake may carry risk.

LIMITATIONS

Although the *in vivo* (Caenorhabditis elegans Assay) and *in vitro* (Ames Test) experiments show promising results, future investigations should integrate:

1. More brands of instant noodles.
2. Additive content should be chemically quantified.
3. Long-term or multigenerational assays.

CONCLUSION

Based on the Ames test and *C. elegans* assays, both Raw Noodles and Cooked Noodles show negligible mutagenicity and toxicity, supporting their safety for consumption. While Spices A and B do not show significant mutagenicity, their toxic effects on *C. elegans*, particularly in terms of reproduction, highlight the need for further investigation into their safety at higher concentrations. As a model organism, *C. elegans* has certain limitations that should not be overlooked. It lacks specialized organs, and its evolutionary distance from humans may present challenges when studying complex conditions. Future research on the effect of seasonings and food additives on reproduction should integrate *C. elegans* with other animal models for a more comprehensive assessment. These findings suggest that the consumption of seasonings in high quantities may carry genetic risks, highlighting the need for stricter regulatory oversight on food additives and the reformulation of potentially harmful components in these seasonings. The overall message emphasizes the non-toxicity of noodles but calls for caution regarding the additives, urging both manufacturers and regulatory bodies to take action to ensure consumer safety.

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