

Review Article

## A review article on the sweet scandal: The truth behind rhodamine B

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### ABSTRACT

Cotton candy is a popular treat with various names around the world. It debuted toward the end of the 1800s at the St. Louis World's Fair. Different names for it were used in distinct parts of the world. Rhodamine B (Rh-B), a basic dye developed in 1887, serves diverse industrial purposes. During the metabolism of Rh-B, cytochrome 450 breaks down, generating oxidative stress and reactive oxygen species. It should be cautiously used due to its possible mutagenic qualities and negative effects on cellular function, including binding to DNA. The article examines the physical and chemical characteristics of Rh-B and its applications, mechanisms, and impacts on brain function, particularly in the cerebellum and brainstem. Studies suggest that exposure to Rh-B may result in functional abnormalities and mutagenic effects. In this review, Rh-B poses significant risks to human health and the environment, necessitating careful consideration and regulatory measures in its usage and disposal.

**Keywords:** Carcinogen, Cotton candy ban, Mutagenic effect, Oxidative stress, Reactive oxygen species, Rhodamine B, Superoxidase dismutase

### INTRODUCTION

Cotton candy is also known as candy floss, fairy floss, and spun sugar. Cotton candy was created in 1899 by dentist William J. Morrison and confectioner John C. Wharton. Subsequently, it was produced and distributed globally in Australia, it is sold as fairy flows and as candy flows in India, South Africa, Ireland, the United Kingdom, New Zealand, and Sri Lanka. Later, it became a well-liked treat for children in every part of the world.<sup>[1]</sup>

Rhodamine B (Rh-B) is an introductory color developed in the 19<sup>th</sup> century. It belongs to the Rhodamine color family. Rh-B is largely answerable in water, and more constantly Rh-B is used as a coloring agent in a colorful food product substantially in cotton delicacy. The metabolism of Rh-B produces reactive oxygen species (ROS) in the body, which affects the exertion of superoxidase dismutase (SOD), leading to cell injury and apoptosis in the cerebellum and brain stem. Rh-B is also known to bind with DNA and beget genotoxic and mutagenic goods. Latterly, due to the carcinogenic and mutagenic effect of Rh-B, it was banned in some states of India such as Tamil Nadu, Pune, Hyderabad, and Secunderabad.<sup>[2-4]</sup>

### INTRODUCTION TO “FLUORESCENT WONDER”

An introductory color called Rh-B was created by Ceresole in 1887. It is both a color and a chemical substance. It is a cloth coloring substance that food merchandisers use as a coloring

cumulative. Compared to natural food coloring, Rh-B is lustrous and more flexible. Habitual consumption of Rh-B through food may beget cancer and liver damage.<sup>[5]</sup> Tandem mass spectrometry and liquid chromatography have been used to identify the presence of Rh-B in cotton delicacy [Supplementary Figure 1].<sup>[6,7]</sup>

## A LOOK INTO PROPERTIES AND USES OF FLUORESCENT DYE

Rh-B is a green crystal or violet powder which is soluble in water, ethanol, and polar solvents. It is generally used as a dye in industries such as printing, dyeing in textiles, paper paints, and leather.

Rh-B is used for staining in biology as a fluorescent color eventually, it is used along with Auromine O as the Auromine-Rh-B stain to demonstrate acid-fast organisms, especially Mycobacterium. Rh-B is also used as a color (component) in fungicide expression applied to creatures. It is used as a biomarker in oral rabies vaccine for wildlife, similar to raccoons, to identify creatures that have eaten a vaccinee. Rhodamine has operations in biotechnology, including luminescence microscopy, flow cytometry, luminescence correlation spectroscopy, and enzyme-linked immunosorbent assay. It is constantly used as a trace color to measure the water inflow transport rate and analysis. In biochemistry, Rh-B is also used to block the operation of mitochondria.<sup>[8,9]</sup>

## REMOVAL OF RH-B

The removal of dye from water is a challenging task. The removal of Rh-B involves biochemical and physical-chemical methods, such as liquid membrane, ozonation, and adsorption. Semiconductor heterogeneous photocatalysis is one of the most promising applications for the decomposition of organic dyes with superior activity.<sup>[10]</sup>

## EXPERIMENTAL STUDIES OF RH-B WITH DNA

Rh-B, a xanthene dye, attaches itself to DNA to form a complex. Commercial Rh-B dye is mutagenic after being activated *in vitro* systems, according to the results of mutagenicity investigations. However, a large amount of this effect may be due to (unidentified) impurities. When Rh-B is coupled with peptides, it has metabolic and pharmacological effects in addition to its anti-bacterial activity. Human and rabbit urine samples showed no carcinogenic activity, according to Elliott *et al.*'s investigation into the mutagenic potential of urinary metabolites of Rh-B.<sup>[11]</sup>

By using the Ames test, assessed two samples of Rh-B and discovered that one was significantly weaker than the other. The reason for this discrepancy appears to be an unexplained

impurity. Activation revealed that both samples were positive. (The Ames test is a biological assay that determines if a chemical can cause mutations in the DNA of the test organism using bacteria to examine the mutagenic potential of chemical compounds).

Furthermore, Nestman and his team conducted another Ames experiment in which Rh-B was reported to be positive. The amount of DNA damage was significantly decreased by the dye's purification. Comparing their results to those of the control group, they discovered that all concentrations and time intervals indicated a rise in the frequency of chromosomal abnormalities.<sup>[12]</sup>

The higher doses also result in increased chromosomal abnormalities. Ames test on strains TA98 and TA100 using Rh-B to assess DNA fragmentation in rat liver *in vivo* has been performed. Both results were not good. Research on *Drosophila* has shown that rhodamine is genotoxic to germline and somatic cells. Based on what is already known, it is plausible to anticipate that Rh-B may be genotoxic and rhodamine B may form bonds with DNA sequences.<sup>[13,14]</sup>

We can study the molecular interactions between Rh-B and DNA using spectrophotometry, fluorimetry, circular dichroic, calorimetric, and computer docking procedures. These investigations show the structural characteristics and binding affinity of the Rh-B-DNA complex.

## MECHANISM AND PHYSIOLOGICAL EFFECT OF RH-B

The Rh-B is a xenobiotic substance that gets broken down by cytochrome P450 in the body, resulting in the production of six ROS, which are free radicals. It affects the activity of SOD which leads to oxidative stress, and cell injury, this increases apoptosis in cerebellum tissue and brain stem. Enzyme SOD is the most important natural antioxidant that can mitigate the consequences of oxidative stress. It catalyzes superoxide's reaction with oxygen and hydrogen peroxide. Moreover, it is crucial for shielding bodily cells from damage and reducing inflammation brought on by free radicals.

Dewi Ratna Sulistina *et al.*<sup>[15]</sup> performed a test to determine the effect of Rh-B on the cerebellum and brain stem tissue of *Rattus norvegicus*. The cerebellum is in the posterior cerebral fossa, where the fourth ventricle divides the medulla oblongata and pons. It is essential for the precision and synchronization of motor functions. Numerous issues arise from its degradation, such as walking difficulties, limb cramps, and body tremors. The subcortical components are connected to the spinal cord by the brainstem. It is linked to several vital functions, such as the circadian cycle, awareness, respiratory and cardiovascular control, maintenance of the cranial nerve core, and the creation of neural connections

that facilitate information transmission between the brain, spinal cord, and cerebellum.

## CONCLUSION

Rh-B has potential mutagenic properties and adverse effects on cellular function, including its binding to DNA and generation of ROS during metabolism, raising concern about its safety. This review suggests caution in the usage of Rh-B due to its potential health risks particularly its impact on brain function, including oxidative stress, cell damage, and apoptosis in the cerebellum and brain stem, which causes a variety of abnormal activities, such as functional abnormality, affecting motor coordination, learning, sleep-wake cycle, and respiratory and cardiovascular control.

Considering these findings, regulatory measures and careful consideration are warranted in the utilization of Rh-B to mitigate its potential adverse effects on human health and the environment. Due to its carcinogenic effects, it has been banned in several states of India.

## Ethical approval

Institutional Review Board approval is not required.

## Declaration of patient consent

Patient's consent not required as there are no patients in this study.

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## Conflicts of interest

There are no conflicts of interest.

## Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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