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METHYL NITRO NITROSOGUANIDINE: A CLOSER LOOK AT ITS CARCINOGENIC IMPACT – A REVIEW

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ABSTRACT

In 1947, McKay and Wright first to synthesis the N-Methyl-N'-nitro-N-nitrosoguanidine as a compound known for its carcinogenic (cancer-causing) and mutagenic (mutation-causing) properties. It has nitro and nitroso-amine group in its chemical structure. It belongs to the nitrosoguanidine family and is widely used in cancer research. MNNG specifically targets guanine and thymine to introduce alkyl groups into DNA. This alkylation can cause subtle mutations that evade detection by DNA repair systems. Since it was first identified as a mutagen in the 1960s, significant research has been conducted to explore its role in cancer mechanisms. Known to induce tumors in various animal models, especially in the gastrointestinal tract. Classified as a Group 2A carcinogen by the International Agency for Research on Cancer (IARC), and animal studies indicate that it may be carcinogenic to humans. Contains a reaction between N-methyl guanidine and nitrite under controlled conditions. Strongly carcinogenic, so strict safety precautions are required when handling. Commonly used models include *rats*, *mice*, *guinea pigs*, *hamsters*, and *dogs* to study the carcinogenic effects of MNNG. Excreted primarily in urine and causes DNA alkylation, causing mutations. Various enzymes, including cytochrome P450 and glutathione S- transferase, are involved in metabolism. It is administered through drinking water, rectal injection, and other routes, causing tumors in various organs. Various routes cause various types of tumors, especially colon cancer when administered rectally. Acute exposure causes immediate DNA damage, while chronic exposure causes tumor development and long-term physiological changes. Major health risks include cancer development, mutagenicity, and possible neurological effects. Proper handling procedures are required to reduce exposure

risk. Due to its instability and potential for releasing toxic compounds, refrigeration, protection from light, and careful handling are required. Detection and quantification of MNNG are mainly performed using chromatographic techniques such as HPLC, fluorescence detection, and comet assay to assess DNA damage.

KEYWORDS: N-Methyl-N'-nitro-N-nitrosoguanidine, MNNG, carcinogenic, DNA alkylation, mutagenicity.

1. INTRODUCTION

N-Methyl-N'-nitro-N-nitrosoguanidine (MNNG) is a potent compound known to be a carcinogen and mutagen. It is a member of the nitrosoguanidine family and is widely used in experimental biology to study the mechanisms of cancer and mutation development. Chemical properties and mechanism of action MNNG is characterized by its ability to introduce alkyl groups into DNA, specifically targeting the O' position of guanine and the O' position of thymine. This alkylation can lead to transition mutations between guanine-cytosine (GC) and adenine-thymine (AT) base pairs. These mutations are subtle and do not significantly distort the DNA double helix, making them difficult to detect by DNA mismatch repair systems. Historical Context and Uses First identified for its mutagenic properties in 1960, MNNG has become one of the most widely used chemical mutagens in research. It has played a key role in studies investigating the link between chemical exposure and cancer development. For example, experiments conducted in 1985 demonstrated its ability to affect tumor growth in animal models.[2]

Stability and Reactivity MNNG is known to be reactive, producing diazomethane, a known DNA methylating agent, in basic solutions and nitrite in acidic environments. This reactivity contributes to its mutagenic properties and its role in a variety of biochemical reactions.[5] Carcinogenicity classification The International Agency for Research on Cancer (IARC) classifies MNNG as a Group 2A carcinogen, indicating that it is probably carcinogenic to humans. This classification is based on animal studies and known DNA damage mechanisms.[4]

N-Methyl-N'-nitro-N-nitrosoguanidine (MNNG) is well known as a source of diazomethane in reactions with aqueous bases and as a good substrate for nucleophilic reagents such as amines and thiols, but this compound has not received much attention. Caution should be exercised until its biological activities are known, including inactivation of transforming

activity, chromosomal aberrations in plant cells, mutagenicity, carcinogenicity, anticarcinogenic activity and prophage-inducing activity. Since then, extensive attempts have been made to link biological activity with chemical reactivity. Therefore, in animal metabolic studies of this compound, methylation and deamination of nucleic acid bases and reactions with proteins have been investigated. However, no clear evidence has been obtained for the association of the chemical with carcinogenic or other biological activities and reactivity MNNG.[5]

2. HISTORY OF MNNG

The history of Methyl-nitro-nitrosoguanidine (MNNG) is marked by its discovery, characterization, and use in cancer research over several decades.

Table 1: Chronicles of MNNG.

Year	Event
1947	When McKay and Wright synthesised MNNG for the first time, they also discovered that it was mutagenic.
1950's	MNNG started to be employed in laboratory research to look at its potential for cancer, especially in animal models.
1970's	The involvement of MNNG in causing stomach and intestinal tumours in other species, such as rats and mice, has been the subject of further research.
1980's	MNNG has been used in carcinogenesis investigations as a result of its growing recognition for its capacity to cause colorectal cancer in experimental animals.
1990's	Research focused on the mechanisms behind MNNG-induced DNA damage, as well as how it affects DNA repair systems.
2000's	MNNG continued to be applied in research to broaden models for reading colorectal cancer and to evaluate capacity preventive techniques.
2010	The use of MNNG in preclinical models for colorectal cancer studies became extra distinguished, with improvements in endoscopic evaluation techniques.
2020	Ongoing studies explores the molecular pathways laid low with MNNG and its capability interactions with different carcinogens.

2.1.Early Detection and Use

1960s: MNNG was first identified as a potent mutagen and carcinogen. Its chemical properties and mechanisms of action began to be studied in a variety of biological contexts.

1985: One of the first important studies involving MNNG was conducted to investigate its effects on tumor growth. In this experiment, MNNG-treated mice were injected with cancer cells from Japanese patients to evaluate its effects on cancer cell proliferation. The results showed that some of the treated mice showed a decrease in the number of cancer cells, highlighting the potential of MNNG as a biochemical tool in cancer research. In the latter half of the 20th century, research focused on the mechanism by which MNNG causes mutations.

In particular, it was found to target the O' position of guanine and the O' position of thymine, thereby alkylating DNA and causing transition mutations between GC and AT base pairs. These mutations are subtle and go undetected by the DNA mismatch repair system, contributing to its role as a carcinogen.

Regulatory classification in the 2000s: MNNG was classified as a probable human carcinogen (Group 2A) by the International Agency for Research on Cancer (IARC) based on animal study data and established DNA damage mechanisms.

Current research and applications in the 2020s: MNNG continues to be used *in vitro* as a chemical mutagen to study cancer mechanisms, DNA repair processes, and the effects of alkylating agents. Its role in diazomethane formation in organic chemistry remains important, as it is used as a feedstock for methylation reactions in a variety of chemical syntheses.[1] Studies on the detoxification pathways and metabolic interactions of MNNG have expanded to focus on enzymes such as glutathione S-transferase and cytochrome P450, which play a key role in mitigating its toxic effects.

3. SYNONYMS[1]

- Methylnitronitrosoguanidine
- N-Methyl-N'-nitro-N-nitrosoguanidine
- 1-Methyl-3-nitro-1-nitrosoguanidine
- MNNG
- N'-Nitro-N-nitroso-N-methylguanidine (Stabilized with Water) (1.0 mg/mL in Methanol)
- 1-methyl-3-nitro-1-nitrosoguanidine (wetted with ca.50% Water) (unit weight on dry weight basis)

4. PHYSICAL AND CHEMICAL PROPERTIES

4.1.Physical and chemical characters

Appearance – Solid-Liquid Mixture[8] Colour - Pale yellow to pink crystals[7] Molecular formula - C2H5N5O3[1] Molecular Weight – 147.093

Boiling Point – 89-97 °C at 225 °C Melting Point - 118 °C (decomposes) 118-123.5 °C (with decomposition).[9]

Vapour Pressure - 0.00012 [mmHg][1]

Solubility - Slightly soluble in water (less than 0.5%)[7,9]; Soluble in polar organic solvents (often accompanied by decomposition)[7,9]; Soluble in DMSO[9]

Stability - Stable under recommended storage conditions.[1,10]

Pure compound is sensitive to light, changing to orange and green colours. Degradation products arising from prolonged or inadequate storage include N-methyl-N'-nitroguanidine, N-nitroguanidine, nitro-cyanamide and guanidine. MNNG is more stable than comparable alkyl-nitrosoureas and alky nitroso urethanes. Thus, at room temp, the half-life at pH 8 is about 200 hr. At pH 7.0 (phosphate buffer) and 37 °C, the half-life is 170 hr. It was shown that MNNG decomposes much more rapidly in tap water than does deionized water.[1,7]

4.2.Storage Condition

Temperature: MNNG should be refrigerated between 0 and 10Â°C to maintain its stability and potency as a carcinogen.[26,27]

Photosensitivity: The compound is sensitive to light and should be stored in the dark to prevent decomposition.[26]

Heat sensitivity: MNNG is also sensitive to heat, which can cause it to deteriorate and lose activity. Therefore, it is very important to store it in a cool place.[26 27]

Chemical stability: Instability in biological matrices: MNNG is known to be unstable in urine and can react with nitrogenous bases to form adducts that reduce its effectiveness for experimental purposes.[1,27] Toxic Compound Release: MNNG can release nitrite at low pH and produce toxic gases such as diazomethane at high pH in the presence of hydroxyl alkalis.[22,27]

Other Relative Reactions

At acid pH slowly releases nitrous acid to give N-methyl-N'-nitroguanidine; it is converted by concentrated aqueous alkali hydroxide to diazomethane; reactions with several nucleophiles are known, especially with amines.[1,7] Reacts with aqueous KOH to form diazomethane; reacts at acid pH to give methyl nitro guanidine.[1,11]

5. STRUCTURE OF MNNG

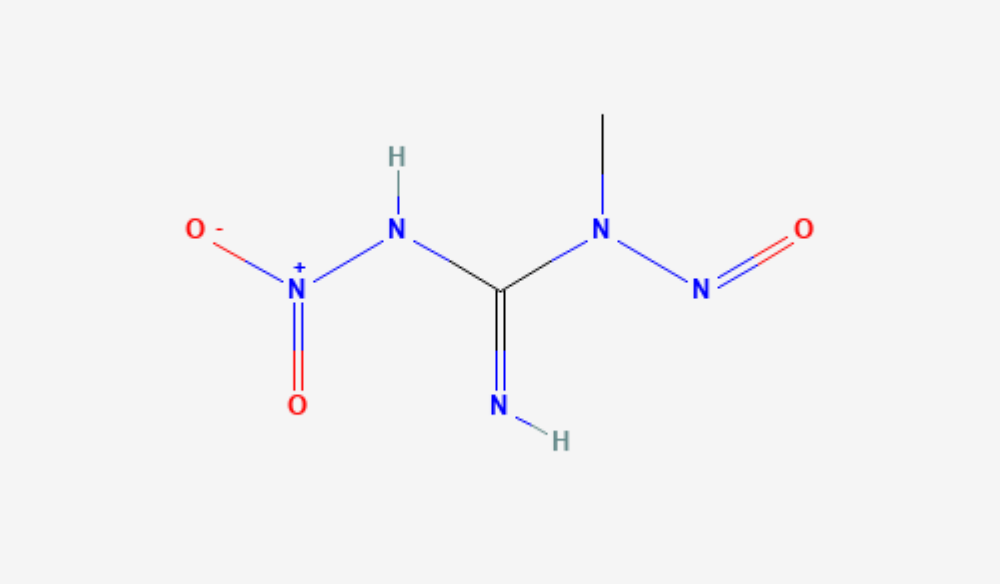

Figure 1: Chemical Structure of MNNG.[1]

5.1.Structural Features

The basic structure of MNNG is based on guanidine, which contains a central carbon atom bonded to two amino groups and a nitrogen atom.

- Nitro Group: MNNG has a nitro group (-NO_2) attached to the guanidine structure, which contributes to its reactivity.
- Nitrosamine Group: The function of the nitrosamine (-N-N=O) is crucial for its alkylation properties, allowing it to interact with DNA.

The central carbon (C) is bonded to an amino group and a nitrosamino group (N-N=O). The nitro group (-NO_2) is attached to the carbon adjacent to the nitrogen in the guanidine structure. The structure reflects the atomic arrangement that determines the chemical activity of MNNG, particularly its ability to alkylate DNA.[11] Once the reaction is complete, isolate MNNG from the reaction mixture using standard organic chemistry techniques such as recrystallization or chromatography, separating it from unreacted starting materials and byproducts.[28,29]

6. SYNTHESIS OF MNNG

MNNG is a potent carcinogen and mutagen. Thus, it should be handled with extreme care in a well-ventilated fume hood, using appropriate personal protective equipment (PPE) such as gloves and safety goggles.

Proper waste disposal methods should be employed for any residues or byproducts generated

during the synthesis. There are many methods used for synthesis of MNNG and here the two methods of it shown as.

6.1. Method of preparation

Method I

Chemical material

- N-Methyl urea: This serves as the primary nitrogen source.
- Sodium Nitrite ($NaNO_2$): This is used to generate nitrous acid (HNO_2) in situ.
- Hydrochloric Acid (HCl): This acid is used to create an acidic environment necessary for the reaction.

Steps involved in synthesis

Step 1: Preparation of Nitrous Acid in Situ

$NaNO_2 + HCl \rightarrow HNO_2 + NaCl$

Step 2: Nitrosation of Methyl Urea

$CH_3NHCONH_2 + HNO_2 \rightarrow CH_3\text{-}N(NO)\text{-}CONH_2$

Step 3: Nitration

$CH_3\text{-}N(NO)\text{-}CONH_2 + HNO_3 \rightarrow CH_3\text{-}N(NO)\text{-}C(=NH)\text{-}NHNO_2$

Maintain the reaction temperature at low levels (0 to 5 °C) to control the reaction rate and minimize side reactions. Stir the mixture gently during this step.[28]

Method II

The synthesis of N-Methyl-N'-nitro-N-nitrosoguanidine (MNNG) typically involves the reaction between N-methylguanidine and nitrous acid (HNO_2) in an acidic medium.

Chemicals Required

- N- methylguanidine
- Sodium Nitrate
- Hydrochloric Acid Steps involved in synthesis:

Step 1: Preparation of Nitrous Acid (HNO_2)

$NaNO_2 + HCl \rightarrow HNO_2 + NaCl$

Step 2: Nitrosation of N-Methyl guanidine

CH 3 -NH-C(=NH)-NH 2 + HNO_2 → CH_3-N-N=NO-C(=NH)-NH-NO_2

After the reaction is complete, isolate MNNG from the reaction mixture using standard organic chemistry techniques such as recrystallization or chromatography to separate it from any unreacted starting materials and byproducts.[28,29]

7. QUANTIFICATION AND DETECTION METHODS OF MNNG

The methods used for the quantification and detection of 1-Methyl-3-nitro-1-nitrosoguanidine (MNNG) primarily involve chromatographic techniques which is given in table 2.

Table 2: Methods used for detection and quantification of MNNG.

Analytical Method	Description	Sensitivity
High-Performance Liquid Chromatography (HPLC)[22,23]	For analysis of MNNG in biological samples using a reversed-phase column with UV detection.	Effective separation and quantification Detection Limit: Feces - 2.5 µg/g Gastric Contents - 2.0 µg/g Urine - 5.0 µg/mL
Fluorescence Detection[22]	In detection of low concentration of MNNG in biological samples, it used in conjunction with HPLC to enhance sensitivity.	Increased Sensitivity
Single cell gel electrophoresis (SCGE)[24]	A comet assay variation that enables high-throughput evaluation of DNA damage in individual cells.	High throughput
Long Amplification PCR[24]	Evaluates the effectiveness of long DNA fragment PCR amplification, which is negatively correlated with damage, in order to quantify DNA damage.	Not specified
Alkaline Elution Technique[19]	Performed to determine the degree of DNA damage brought on by MNNG and provide information on its mutagenic effects.	Detects at 1 µg/mL
Next-Generation Sequencing (NGS)[25]	Used for mapping specific DNA lesions and assessing the impact on gene expression and mutations.	High Sensitivity and precision
Comet Assay[22,19]	Assesses the DNA damages that MNNG causes in mammalian cells sensitive to small amounts and capable of identifying breaks in DNA strands.	Detects as low as 0.1 µg/mL
DNA Unwinding Techniques[19]	Measures the unwinding of DNA strands following MNNG exposure to evaluate DNA damage.	Detects at 1-2 µg/mL

8. INDUSTRIAL APPLICATIONS

N-Methyl-N-nitro-N'-nitrosoguanidine is primarily used in research as a biochemical tool to induce tumor or cancer on living beings.

Table 3: Industrial applications of MNNG.

Application	Description
Research and Experiments	In Laboratory, MNNG used extensively to study cancer mechanism and also as a potent carcinogen and mutagen.[21]
Carcinogenic Studies	MNNG induces tumor in animal models, especially for gastric and intestinal cancers.[20]
Genetic Research	Employed to understand mutation process and the effects of DNA alkylation.[21]
Organic Chemistry	In preparation of Diazomethane, MNNG reacted with Potassium hydroxide in aqueous solutions.[21]
Drug development	In screening of anticancer properties of Compounds and in mutation mechanism studies.[20]

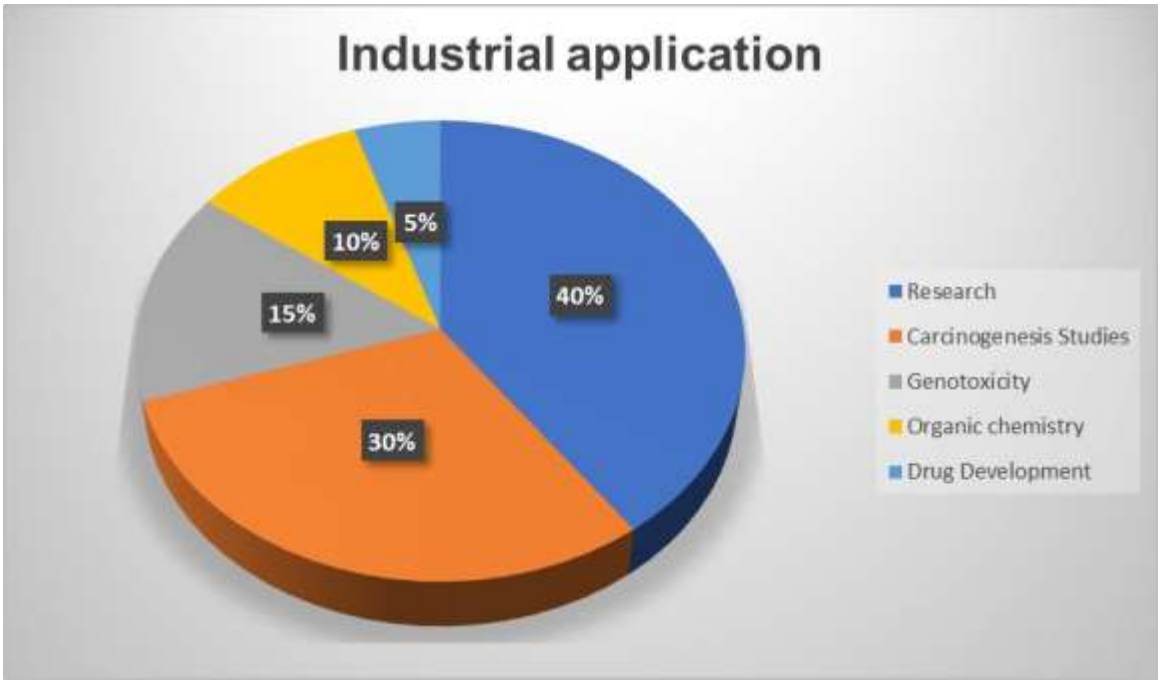

Figure 2: Diagrammatic representation of Industrial application of MNNG.

9. PHARMACOKINETICS

9.1. Absorption, Distribution, and Excretion

After oral administration of (14) C-labelled N-methyl-N'-nitro-N-nitrosoguanidine, most of the radioactivity was excreted in the urine within 24 hours, and less than 3% was excreted in the feaces. Less than 3% of the radioactivity remained in the body as acid-insoluble material for 24 to 48 hours.[30]

9.2. Metabolism

9.2.1. DNA Alkylation

MNNG acts by adding an alkyl group to the O' position of guanine and the O' position of thymine in DNA. This can lead to transition mutations between GC and AT base pairs.[2] These subtle mutations do not significantly distort the DNA double helix, making them difficult to detect by the DNA mismatch repair system.

9.2.2. Formation of diazomethane

In basic aqueous solutions, MNNG can produce diazomethane, a known DNA methylating agent.[2] This reaction suggests that MNNG can promote DNA methylation and potentially alter gene expression patterns.

9.2.3. Formation of nitrite

MNNG can also produce nitrite in acidic environments.[2] Nitrite is a known mutagen that can deaminate DNA bases, causing additional mutations.

Potential Metabolic: Interactions Although not directly studied with MNNG, it has been suggested that alcohol metabolism by dehydrogenases may result in a decrease in cellular nicotinamide adenine dinucleotide (NAD), a substrate for DNA repair enzymes. This could potentially impair the ability of cells to repair DNA damage caused by MNNG.

9.3. Enzyme involved in metabolism of MNNG

The metabolism of Methylnitrosoguanidine (MNNG) includes many enzymes that initiate its activation and further result in DNA damage. However, there is scant detailed information on the individual enzymes in the metabolism of MNNG, the following summarizes the general insights of metabolic interactions. Key Enzymes Involved Cytochrome P450 enzymes: This group of enzymes is reported to be centrally involved in bioactivating various nitrosamines and most notably MNNG. They are able to mediate the process of producing reactive metabolites that have the ability to form DNA adducts.[13]

Alkyl transferases: These enzymes are responsible for the reversal of the DNA damage created by MNNG and other alkylating agents. They can repair this damage by transferring the alkyl group buried in the damaged DNA back to the cysteine main chain of the protein.[13] DNA repair enzymes: In most such cases, base excision repair (BER) and nucleotide excision repair (NER) which are some of the segments of DNA repair processes become activated

after MNNG induced DNA damage. These pathways which include DNA glycosylase and DNA polymerase that are responsible for removal of vigilant substitutes also involve modulation of hydrolyzed iodide bases.[13]

Nitro-reductase: It is also reported that this enzyme would take part in reduction of the nitro functional group that is present in nitrosamines which is associated with the generation of electro positive atoms which are aggressive and can further attack the DNA structure.[13]

9.4.Enzyme involved in detoxification of MNNG

9.4.1. Glutathione S-transferase (GST)

GST is a family of enzymes that catalyzes the binding of glutathione to a variety of electrophilic and hydrophobic compounds, including carcinogens and oxidative stress products. This binding promotes their removal from the body, thereby reducing their toxicity.[14]

9.4.2. Glutathione peroxidase (GPX)

GPX is an enzyme that uses glutathione as a cofactor to catalyze the reduction of hydrogen peroxide and organic peroxides to water and alcohol, respectively. By reducing these reactive oxygen species, GPX helps protect cells from oxidative damage that may result from MNNG exposure.[14]

9.4.3. Superoxide dismutase (SOD)

SOD is a metalloenzyme that catalyzes the conversion of superoxide radicals to oxygen and hydrogen peroxide. This is an important protective mechanism against the toxicity of superoxide radicals that may be generated by exposure to MNNG.[14]

9.4.4. Catalase

Catalase is an enzyme that catalyzes the breakdown of hydrogen peroxide into water and oxygen. By removing hydrogen peroxide, catalase helps prevent the formation of highly reactive hydroxyl radicals that can damage cellular components.[14]

These enzymes work together to detoxify MNNG and its reactive metabolites, thereby reducing DNA damage and carcinogenic potential. However, it is important to note that prolonged or high exposure to MNNG can suppress these detoxification systems, which can lead to cellular damage and disease.

10. PHARMACOLOGICAL EFFECTS MNNG

1. Tumors of the stomach: Adenocarcinoma: MNNG has been specifically associated with the development of adenocarcinoma of the stomach. This has been observed in several studies using various animal models, including rats and Mongolian gerbils.[4,19]

Squamous cell carcinoma: In mice, MNNG has been shown to induce squamous cell carcinoma of the anterior chamber.[3,4]

2. Colon tumors: Colon adenomas and adenocarcinomas: MNNG administration via rectal injection has been associated with the development of several colon tumors, particularly adenomas and adenocarcinomas, in rats.[17]

3. Oesophageal tumor: Papilloma and squamous cell carcinomas: In several animal studies, oral administration of MNNG has resulted in papillomas and squamous cell carcinomas of the oesophagus and prostate.[19]

4. Other types of tumors: Lung tumors: MNNG has been reported to induce lung tumors in mice after subcutaneous injection.[19] Liver tumors: As with lung tumors, liver tumors have also been observed following exposure to MNNG via the subcutaneous route.[19]

Sarcomas: MNNG can induce sarcomas in the gastrointestinal tract, further demonstrating its broad carcinogenic potential in a variety of tissue types.[19]

10.1. Carcinogenic Effect of MNNG in various Animals models

Common animal models used to study the carcinogenic effects of 1-methyl-3-nitro-1-nitrosoguanidine include a variety of species that are sensitive to its carcinogenic properties.

The main models include.

- *Rat*

- *Sprague-Dawley rat*: Frequently used in studies involving colon cancer and other gastrointestinal tumors. MNNG is administered orally, rectally, or subcutaneously to induce tumors in the stomach and intestine.[19]
- *F344 rat*: This strain is also used in a variety of carcinogenicity studies, including studies examining the effects of MNNG on the gastrointestinal tract.[19]

- *Mice*

- *C57BL/6 mice*: This strain is commonly used in studies related to colorectal cancer development. MNNG is administered by rectal injection to study its effects on colon tumor development.
- *Neonatal mice*: MNNG has been shown to induce tumors in *neonatal mice* following

subcutaneous injection, making it a useful model for studying early carcinogen exposure.[19]

- *Guinea pigs*

- These animals were used to study colon carcinoma induced by intrarectal administration of MNNG. This model is helpful in understanding carcinogenic effects in various mammalian systems.[19]

- *Hamsters*

- *Syrian hamsters*: MNNG is known to induce gastric carcinoma in *hamsters*, making them a suitable model for studying gastric carcinogenesis.[19]

- *Dogs*

- **Beagles**: MNNG has been administered orally to induce gastric tumors in *dogs*, providing an opportunity to study its carcinogenic effects in larger mammals.[19]

10.2. Route of administration

MNNG is often administered through drinking water, allowing for prolonged, sustained exposure. Studies have shown that this route can induce several tumors in the gastrointestinal tract, including the prostate and prostate glands of rats and mice.

Rectal administration of MNNG has been shown to induce a number of colon tumors in laboratory animals.[17] This route is particularly relevant to the study of colon cancer, as it specifically targets the colon.

MNNG can also be administered by subcutaneous injection. This method has been used to induce tumors in a variety of tissues, including the liver and skin, in both rats and mice. Subcutaneous administration has been shown to induce benign tumors, such as Hemangioendothelioma in *mice*.

Gastrostomy or direct gastric administration is another method used to deliver MNNG. This method allows for precise administration and has been used in studies to evaluate tumor induction in the gastrointestinal tract.[2]

MNNG can also be administered intraperitoneally, leading to systemic exposure and tumor development in various organs, including the liver and peritoneum.[15]

Type Tumor induction using MNNG: MNNG has been shown to induce a variety of tumor

types, including adenomas, adenocarcinomas, and sarcomas, primarily in the gastrointestinal tract. The specific tumor type varies depending on the route of administration and the species used in the study.[16]

Comparative effectiveness: Different routes of administration can result in different tumor incidence and types. For example, rectal administration has been particularly effective in inducing colon tumors, while exposure to drinking water has been used for more extensive gastrointestinal evaluation.[15]

Influence of host factors: The tumor-inducing effect of MNNG may vary depending on host factors such as the genetic background of the animal model and the presence of other oncogenic agents such as infections (e.g., *Helicobacter pylori*), which may synergistically enhance tumor formation.[2]

10.3. Preparation and dose induce carcinogenicity

Table 4: Route of administration or formulation of MNNG with species, dose and its effect.

Formulation / Route of administration	Species	Dose	Effect/Tumor type
Drinking Water [19,15]	*Rats*	0.1% in drinking water	Induction of tumors in the forestomach and glandular stomach.
Intrarectal Injection[19,15]	*Rats*	0.5 mg MNNG per rat	Induction of colonic tumors (adenomas, carcinomas).
Subcutaneous Injection[19,15]	*Mice*	10 mg/kg	Induction of liver tumors (hemangioendotheliomas).
Oral Administration (Gavage)[19,15]	*Mice*	20 mg/kg	Induction of small intestine tumors (adenocarcinomas).
	Hamsters	25 mg/kg	Induction of stomach tumors (adenocarcinomas).
Intraperitoneal Injection [19,15]	*Mice*	5 mg/kg	Induction of lung tumors.
Dermal Application [19,15]	*Mice*	10 mg/application	Induction of skin tumors (papillomas, carcinomas).
Intrauterine Application [19]	*Rats*	0.1 mg	Local tumors in reproductive tissues.

10.4. Body response difference between acute and chronic exposure to MNNG

Table 5: Response of acute and chronic exposure.[15,31,32]

Aspect	Acute exposure	Chronic exposure
Duration	Short term exposure (secs to hours).	Long term exposure (Months to yrs).

Immediate effects	Rapid onset of DNA damage and cytotoxicity.	Development of tumors and precancerous lessions.
Tissue targeting	Primarily affect the site of administration.	Systemic effects lead to tumors in organs.
Carcinogenic potential	Limited evidence of carcinogenicity.	Strong evidence of carcinogenicity.
Physiological changes	May cause symptoms like nausea, vomiting, respiratory distress depend on route of exposure.	Long term changes include tumor development, organ damage and alterations in cell signaling pathways.
Cellular response	Induce cell death and DNA repair.	Alters cellular processes such as proliferation and autophagy.
Dose-Response relationship	Dose-dependent cytotoxic effects observed.	Dose-dependent increase in tumor incidence and severity over time.
Recovery potential	Generally reversible effects.	Long lasting effects with potential for incomplete recovery even after cessation.

10.4.1. Safety Considerations using MNNG

- Carcinogenicity: MNNG is a potent carcinogen and mutagen; therefore, it should be handled with extreme care.
- Personal Protective Equipment (PPE): Always use appropriate PPE such as gloves, safety goggles, and lab coats when conducting the synthesis.[28]
- Ventilation: Perform all reactions in a well-ventilated fume hood to avoid inhalation of toxic fumes.
- Waste Disposal: Proper waste disposal methods should be employed for any residues or byproducts generated during the synthesis. Follow your institution's guidelines for hazardous waste disposal.[29]
- Containers: Refrigerator containers were used which maintain the stable temperature of the MNNG. In some instances, Intermediate Bulk Containers (IBCs) and Safety cabinets were used for storage. All containers should be clearly labeled with appropriate hazard warnings.

10.5. Potential risk associated with MNNG during experiment

N-Methyl-N'-nitro-N-nitrosoguanidine is a potent chemical mutagen and carcinogen that can cause serious health risks when exposed.

The main potential health effects include.

Cancer: MNNG is classified by the International Agency for Research on Cancer (IARC) as a

possible human carcinogen (Group 2A) based on animal study data and known DNA damage mechanisms. [2] Exposure to MNNG can cause a variety of cancers.

Mutagenicity

MNNG is a highly mutagenic compound that can specifically target guanine and thymine residues to introduce alkyl groups into DNA. [2] This can cause subtle mutations in DNA that are difficult for mismatch repair systems to detect, resulting in permanent genetic changes.

Neurological Effect

The primary routes of exposure are inhalation and ingestion, but MNNG can also cause neurological effects when absorbed through the skin. Symptoms may include tremors, stiffness, slowed motor skills, and potentially severe depression, anxiety, and hostility.[2]

Respiratory Effects

Inhalation of MNNG can cause pneumonia, pneumonia, and decreased lung function. [18] Occupational exposure to MNNG and related nitrosoguanidines during chemical production or research should be strictly controlled.

Gastrointestinal Effects

MNNG ingestion can potentially cause gastrointestinal upset, but data on specific effects are limited. Proper handling and containment procedures are important to prevent accidental exposure.[18]

11. SPECTRAL ASSESSMENT[19,26]

- UV-Vis Spectroscopy
- It used to identify electronic transitions in MNNG with characteristic absorbance peaks typically in range 200-300nm.
- Infrared (IR) Spectroscopy
- It identifies functional groups by measuring molecular vibrations,
  N-H stretching (~3300 cm^{-1}); C=O stretching (~1650 cm^{-1}); NO stretching (~1500 cm^{-1})
- Nuclear Magnetic Resonance (NMR) Spectroscopy
- The Chemical shifts in the MNNG indicating the environment of protons in the guanidine structure.

12. CLINICAL TRIALS ON MNNG

In clinical trials of MNNG, several number or trials were done based on the carcinogenicity impact on the humans.

Table 6: Clinical trials of MNNG.

Year	Study Type	Description
1966[33]	Preclinical	For E.coli, MNNG was identified as a potent mutagen, inducing chromosomal aberrations.
1973[19]	Preclinical	When MNNG given through Rectal administration in rats, it induce colorectal carcinoma.
1974[19]	Preclinical	When given orally, MNNG induce stomach cancer in hamsters.
	Preclinical	When given orally, MNNG induce carcinoma and related lesions in dog stomach.
1976[19]	Preclinical	Leiomyosarcomas of the small intestine induced in dogs by MNNG.
	Preclinical	Large bowel carcinoma in Guinea pig when administered through intrarectal instillation of MNNG.
1983[19]	Preclinical	When given orally, MNNG induce uterine cancer in rats.
1993[33]	Clinical	In rats, Inhibition of Gastric tumorigenesis by α-di-fluoro methylornithine treated with MNNG
2004	Preclinical	When treated with MNNG, it induce senescence like growth arrest in colon cancer cells.
2020	Preclinical	Enhanced NF-κB signaling linked to precancerous lesions induced by MNNG exposure in gastric models.

13. CURRENT RESEARCH TRENDS IN MNNG

MNNG continues to be widely used in a variety of animal models, particularly in the gastrointestinal tract, to induce tumors.[15,17,26]

A 2024 study showed that injection of MNNG into TGES1 cells resulted in the growth of subcut aneous tumors in nude mice. MNNG has been known to induce tumors at the site of administration, primarily in the gastrointestinal tract, including tumors of the acinar, adenomatous, small intestine, and colon in *rats*, *mice*, *hamsters*, and *dogs*.[15]

A 1997 study showed that MNNG could induce gastric adenocarcinoma in ferrets infected with *Helicobacter-must-ellie*, with nine out of ten ferrets developing gastric cancer after a single oral dose of MNNG.[26] MNNG has been shown to induce tumors in several other sites in laboratory animals, including the liver, peritoneum, injection site, liver, lungs, blood vessels, and skin in rats. [15] As shown in studies on ferrets, the carcinogenic effect of MNNG is enhanced when combined with other factors, such as *Helicobacter* infection.[26]

14. EFFICIENCY OF CARCINOGENS

While comparing the efficiency of carcinogen N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) with other carcinogens such as N-methyl-N-nitrosourea (MNU), Azoxymethane (AOM) and Dimethylhydrazine (DMH) which all are used in induction of Colorectal Cancer for Research aspect.

Table 7: Effectiveness of MNNG, DMH, MNU and AOM.

Characteristic Property	MNNG	DMH	MNU	AOM
Type[34,35]	Direct alkylating	Requires metabolic activation	Direct alkylating agent	Requires metabolic activation
Efficiency[35]	100% tumor induction in rats	50-70% tumor induction	78% tumor induction	60-90% tumor induction
ear of Detection[36]	1967	1970	1970	1970
Sensitivity[34,36]	Very sensitive & effective at low doses.	Mild sensitivity, higher dose required.	Effective even at low doses and very sensitive.	High dose required and has mild sensitivity.
Dose unit[34,37]	1-3 mg/rat/week (IR).	20mg/kg body weight (Oral or SC).	0.3 mg/mouse three times/week for 10 weeks (IR).	10-20 mg/kg body weight (IP).
Tumor types induced[34,35,36]	In distal colon and rectum, it primarily induce adenocarcinomas and adenomas.	In the colon, primarily induce adeno-carcinomas.	Induce tumors in distal colon, rectum and auns.	In the distal colon, primarily induce adenocarcinomas.
Mechanism of action[34,35]	Direct DNA alkylation without metabolic activation.	Metabolically activated to form DNA adducts.	Direct DNA alkylation without metabolic activation.	Metabolically activated to form DNA adducts.
Duration of study[34,36]	Effective over a period of 20 weeks.	Multiple administration over weeks require.	Usually evaluated over a few weeks.	Usually evaluated over a few weeks.
Species used in Studies[34,35,37]	Commonly used in rats.	Commonly used in rats and mice.	Commonly used in mice.	Commonly used in mice and rats.

Table 8: Percentage of efficiency of various carcinogens.

Carcinogen	Efficiency (%)	Year of Detection
MNNG	100	1967
AOM	60-90	1970
MNU	78	1970
DMH	50-70	1970

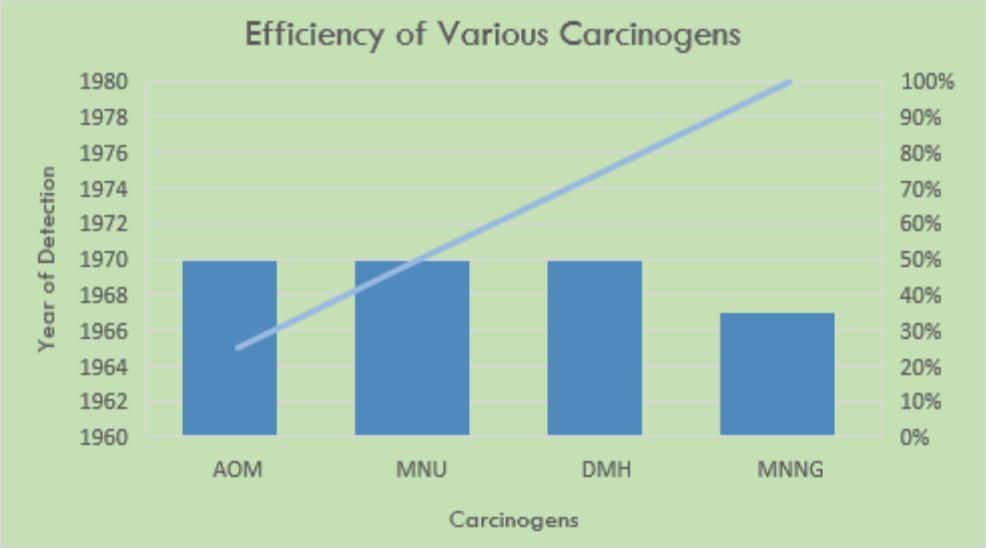

Figure 3: Graphical representation of Efficiency of Various Carcinogens.

15. CONCLUSION

In this review, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) is a potent carcinogen inducing Colorectal cancer among experimental animals. MNNG considered as the most potent cancer inducing chemical carcinogens which is cheap, effective and less risk on exposure during experiments among other chemical carcinogens. In this, the involved molecular mechanism for induction of Colorectal cancer, Liver cancer and several types of tumors shown clearly. Hence, using MNNG to animals/any living beings leads to induction of carcinogenicity or mutagenicity especially in their genetic materials.

16. REFERENCE

1. National Center for Biotechnology Information (2024). PubChem Compound Summary for CID 135436526, 1-Methyl-3-nitro-1-nitrosoguanidine. Retrieved September 25, 2024 from https://pubchem.ncbi.nlm.nih.gov/compound/1-Methyl-3-nitro-1-nitrosoguanidine.
2. Gichner T, Velemínský J. Genetic effects of N-methyl-N'-nitro-N-nitrosoguanidine and its homologs. Mutat Res, 1982 Sep; 99(2): 129-242. PMID: 6750393.
3. Hayakawa, Y., Fox, J. G., Gonda, T., Worthley, D. L., Muthupalani, S., & Wang, T. C. (2013). Mouse models of gastric cancer. *Cancers*, *5*(1): 92–130.
4. Zhang, W., Wang, S., Zhang, H., Meng, Y., Jiao, S., An, L., & Zhou, Z. (2024). Modeling human gastric cancers in immunocompetent mice. *Cancer biology & medicine*, *21*(7): 553–570.
5. Chikayoshi nagata, Masahiro nakadate, Yoshikazu ioki, and Akira imamura. Electron Spin Resonance Study on the free radical production from N-methyl-N'-nitro-

nitrosoguanidine. Gann, August, 1972; 63: 471-481.

6. A. F. McKay and George F Wright. Preparation and properties of N-Methyl-N-nitroso-N'-nitroguanidine. J. Am. Chem. Soc, 1947; 69, 12, 3028–3030.
7. International Agency for Research on Cancer: IARC. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Geneva: World Health Organization, International Agency for Research on Cancer, 1972-PRESENT. V4: 183-184: ISBN-13 978-92-832-1204-1
8. Zhang, Yue et al., The role of m6A modified circ0049271 induced by MNNG in precancerous lesions of gastric cancer. Heliyon, 2024; 10(16): e35654.
9. National Center for Biotechnology Information. PubChem Annotation Record for, N-methyl-N'-nitro-N-nitrosoguanidine, Source: Hazardous Substances Data Bank (HSDB). Accessed, Sept. 9, 2024.
10. Sigma-Aldrich; Safety Data Sheet for 1-Methyl-3-nitro-1-nitrosoguanidine, Product Number: 129941, Version 3.13 (Revision Date 10/12/2018). Available from, as of, December 21, 2018: https://www.sigmaaldrich.com/safety-center.html
11. O'Neil, M.J. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Cambridge, UK: Royal Society of Chemistry, 2013; 1131.
12. A. P. Corfield, i. Stewart, p. W. J. Houghton, n. J. Mcc. Mortensen, r. C. N. Williamson, j. R. Clamp; Changes in enzymes involved in sialic acid and hexosamine metabolism in a gastric cancer model. *Biochem Soc Trans*, 1 December 1987; 15(6): 1052.
13. Kurama EE, Fenille RC, Rosa VE Jr, Rosa DD, Ulian EC. Mining the enzymes involved in the detoxification of reactive oxygen species (ROS) in sugarcane. Mol Plant Pathol, 2002 Jul 1; 3(4): 251-9.
14. National Toxicology Program. 15th Report on Carcinogens [Internet]. Research Triangle Park (NC): National Toxicology Program; 2021 Dec 21. N-Nitrosamines: 15 Listings.
15. Lee SH, Lee SJ, Kim JH, Park BJ. Chemical carcinogen, N-methyl-N'-nitro-N-nitrosoguanidine, is a specific activator of oncogenic Ras. Cell Cycle, 2007 May 15; 6(10): 1257-64. PMID: 17495535.
16. Mishra R, Rajsiglová L, Lukáč P, Tenti P, Šima P, Čaja F, Vannucci L. Spontaneous and Induced Tumors in Germ-Free Animals: A General Review. Medicina (Kaunas), 2021 Mar 11; 57(3): 260. PMID: 33799911; PMCID: PMC3732107.
17. Williams M, Todd GD, Roney N, et al. Toxicological Profile for Manganese. Atlanta (GA): Agency for Toxic Substances and Disease Registry (US); 2012 Sep. 3, Health effects.

18. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42. Lyon (FR): International Agency for Research on Cancer; 1987. (IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS, No. Supplement 7).
19. Yanan Li, Jingqi Xin, Yongbing Sun, Tao Han, Hui Zhang, Feifei An. Magnetic resonance imaging-guided and targeted theranostics of colorectal cancer. Cancer Biology & Medicine, May 2020; 17(2): 307-327.
20. Hollaender A. Chemical Mutagens: Principles and Methods for Their Detection Volume 1. Springer US, 1971.
21. Slamenová D, Gábelová A, Ruzeková L, et al. Detection of MNNG-induced DNA lesions in mammalian cells; validation of comet assay against DNA unwinding technique, alkaline elution of DNA and chromosomal aberrations. *Mutat Res*, 1997; 383(3): 243-252.
22. W.T. Brashear, P.P. Lu, M.M. Ketcha. Purity analysis of MNG and chemical analysis of MNG and MNNG in Biological samples. AL-TR-1992-0060.
23. Sykora, P., Witt, K.L., Revanna, P. *et al.* Next generation high throughput DNA damage detection platform for genotoxic compound screening. *Sci Rep*, 2018; **8:** 2771.
24. Hedlich-Dwyer, J., Allard, J. S., Mulgrave, V. E., Kisby, G. E., Raber, J., & Gassman, N. R. Novel Techniques for Mapping DNA Damage and Repair in the Brain. *International journal of molecular sciences*, 2018; 25(13): 7021.
25. Fox, J. G., Wishnok, J. S., Murphy, J. C., Tannenbaum, S. R., & Correa, P. MNNG-induced gastric carcinoma in ferrets infected with Helicobacter mustelae. *Carcinogenesis*, 1993; *14*(9): 1957–1961.
26. Jaiswal, A.S., Multani, A.S., Pathak, S. *et al.* N-Methyl-N'-nitro-N- nitrosoguanidine-induced senescence-like growth arrest in colon cancer cells is associated with loss of adenomatous polyposis coli protein, microtubule organization, and telomeric DNA. *Mol Cancer*, 2004; **3:** 3.
27. William Lijinsky; Reaction of Drugs with Nitrous Acid as a Source of Carcinogenic Nitrosamines. *Cancer Res*, 1 January 1974; 34(1): 255–258.
28. Sidney S. Mirvish, Formation of N-nitroso compounds: Chemistry, kinetics, and *in vivo* occurrence, Toxicology and Applied Pharmacology, 1975; 31(3): 325-351, ISSN 0041-008X.
29. Kleihues, P., & Wiestler, O. D. Involvement of thiols in gastric cancer induced by N-methyl-N'-nitro-N-nitrosoguanidine: biochemical and autoradiographic studies. *IARC*

scientific publications, 1984; 57: 603–608.

30. Kandimalla, R., Das, M., Bhattacharjee, S., Choudhury, P., Devi, R., Talukdar, N. C., & Samanta, S. K. Effect of multiple doses of N-methyl-N-nitrosourea, an end product of methylguanidine (found in processed food), on the fertility of female Swiss albino mice. *Heliyon*, 2021; *7*(4): e06738.
31. SUGIMURA, T., NAGAO, M. & OKADA, Y. Carcinogenic Action of *N*-Methyl- *N*′-nitro-*N*-nitrosoguanidine. *Nature*, 1966; 210: 962–963.
32. Nascimento-Gonçalves, E., Mendes, B. A. L., Silva-Reis, R., Faustino-Rocha, A. I., Gama, A., & Oliveira, P. A. Animal Models of Colorectal Cancer: From Spontaneous to Genetically Engineered Models and Their Applications. *Veterinary sciences*, 2021; *8*(4): 59.
33. Daniel W. Rosenberg, Charles Giardina, Takuji Tanaka, Mouse models for the study of colon carcinogenesis, *Carcinogenesis*, February 2009; 30(2): 183–196.
34. Machado, V. F., Parra, R. S., Leite, C. A., Minto, S. B., Cunha, T. M., Cunha, F. Q., Garcia, S. B., Feitosa, M. R., da Rocha, J. J. R., & Feres, O. (2020). Experimental Model of Rectal Carcinogenesis Induced by N-Methyl-N-Nitrosoguanidine in Mice with Endoscopic Evaluation. *International journal of medical sciences*, 2020; 17(16): 2505–2510.
35. Zhang, Jianping et al., Effectiveness of Screening Modalities in Colorectal Cancer: A Network Meta-Analysis. Clinical Colorectal Cancer, 16(4): 252–263.
36. Weisburger J.H., Reddy B.S., Narisawa T., Wynder E.L. Germ-free status and colon tumor induction by *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine. *Proc. Soc. Exp. Biol. Med*, 1975; 148: 1119–1121.
37. Sumi Y., Miyakawa M. Gastrointestinal carcinogenesis in germ-free rats given *N*- methyl-*N*′-nitro-*N*-nitrosoguanidine in drinking water. *Cancer Res*, 1979; 39 Pt 1: 2733–2736.
38. Evans I.A., Mason J. Carcinogenic activity of bracken. *Nature*, 1965; 208: 913– 914.
39. Pamukcu A.M., Price J.M. Induction of intestinal and urinary bladder cancer in rats by feeding bracken fern (*Pteris aquilina*) *J. Natl. Cancer Inst,* 1969; 43: 275–281.
40. Price J.M., Pamukcu A.M. The induction of neoplasms of the urinary bladder of the cow and the small intestine of the rat by feeding bracken fern (*Pteris aquilina*) *Cancer Res*, 1968; 28: 2247–2251.