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Review Article

N-Nitrosodimethylamine (NDMA) Synthesis in the Environment, its Toxicity and Modulation by Various Bio-Chemical Approaches: A Review

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Abstract

N-Nitrosodimethylamine (NDMA) or dimethylnitrosamine (DMN) is a well-known hepatotoxin and nephrotoxin with carcinogenic properties. It is an organic compound produced during various industrial processes and as a reaction byproduct of several disinfectants. It is also found in many food items, such as cured or smoked meat, beer and even toiletry and cosmetic products. Severe liver and kidney damage has been linked to NDMA exposure, primarily attributed to mechanisms involving oxidative stress, DNA damage and subsequently liver fibrosis. In this review we have discussed about the sources of NDMA, its synthesis, metabolic breakdown and mechanisms underlying the generation of free radicals. A key aspect of NDMA toxicity involves the induction of oxidative stress. Metabolic activation of NDMA leads to the overproduction of reactive oxygen species (ROS), resulting in lipid peroxidation, protein modification, and DNA damage. The oxidative stress disrupts cellular redox homeostasis and activates inflammatory signaling pathways, further exacerbating tissue damage and contributing to the initiation and progression of carcinogenesis. We have discussed about its toxic effects in various *in vitro* and *in vivo* models, along with the phyto-therapeutic approaches that are being used to suppress the progression of NDMA induced toxicity. Advanced water treatment technologies such as reverse osmosis, activated carbon adsorption and advanced oxidation processes (AOPs) using UV-rays and hydrogen peroxide have shown some promising results. Additionally, studies using biological methods such as microbial degradation and enzymatic treatments are also being tested to break down NDMA in contaminated environments.

Keywords: N-nitrosodimethylamine (NDMA), oxidative stress, ROS, hepatotoxicity and nephrotoxicity

1. Introduction

Toxicity literary means the extent to which something is poisonous or hazardous to living beings. The toxicants could be in the form of gas, solid or liquid. We encounter numerous toxicants in our daily life. A variety of toxicants are present in our food as well as in our surroundings, which is a matter of major concern.

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Toxicants in our food are in the form of food preservatives, pesticides, emulsifiers, colouring agents, flavouring agents and most importantly some toxicants are produced as byproducts of various chemical reactions. Some toxicants are even carcinogenic when encountered frequently by the body in considerable amounts **[1]**. There are a number of well-established parameters employed to evaluate the toxicity of a substance when subjected to the living organisms. These parameters include lethal dose 50 (LD50), lethal concentration (LC50); lowest observed adverse effect level (LOAEL); histopathological analysis in case of hepatotoxicity, nephrotoxicity, neurotoxicity, gastro-intestinal toxicity, teratogenicity; biochemical enzyme markers; oxidative stress markers; genotoxicity and carcinogenicity **[2,3]**.

N-nitrosamines are environmental pollutants which are graded as potent carcinogens by International Agency for Research on Cancer (IARC) and US Environmental Protection Agency (US EPA) **[4]**. Nitrosamines are produced in foundry, leather tanning, rubber and paint manufacturing processes, tobacco smoke, packed or canned food items including cheese, smoked meat and fish, metal industry, toiletry and cosmetic products, and endogenous nitrosation of drugs in the stomach and intestine **[5,6,7,8]**. Nitrite is one of the key components used as an additive during the manufacturing of food are products. At the same time the N-nitrosamines formed as a result of the reaction between nitrites and food components **[9]**. N-nirosamines are even detected in drinking water and beer samples **[10]**. Most common N-nitrosamines found in the food include ethylmethylnitrosamine (EMN), dimethylnitrosamine (DMN), diethylnitrosamine (DEN), dibutylnitrosamine (DBN), nitrosomethylaniline (NMA), nitrososarcosine (NSAR), dipropylnitrosamine (DPN), nitrosomorpholine (NMOR), nitrosopiperidine (NPIP) and N-nitrosopyrrolidine (NPYR) **[4,9]**. In this review we are going to discuss comprehensively about N-nitrosodimethylamine (NDMA) which is also known as dimethylnitrosamine and N-methyl-N-nitrosomethaneamine (**Figure 1**). NDMA is a volatile, yellow coloured, oily liquid, with faint characteristic odour, having molecular formula $C_2H_6N_2O$ and molecular weight of 74.08g/mol **[11].**

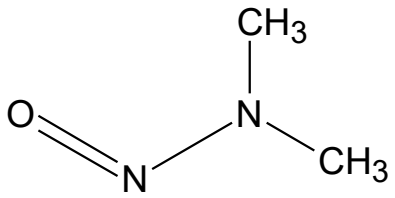


Figure 1: Structure of N- Nitrosodimethylamine (NDMA)

NDMA was first reported as a groundwater contaminant at several sites in California that produced rocket fuel during 1998 and 1999 **[12]**. Lim et al., in 2016 **[13]** demonstrated that NDMA can be produced as a result of ozonization of N,N-dimethylhydrazine compounds such as unsymmetrical dimethylhydrazine (UDMH) and daminozide (DMZ). UDMH is primarily used as a high-energy fuel component in rocket propellants **[12]**. Another study suggests that NDMA can be formed as a byproduct when secondary amines, such as dimethylamine, react with mono- or dichloroamines present in disinfected swimming pool water **[14]**. Possible mechanisms underlying NDMA synthesis are shown in **Figure 2**.

Along with other nitrosamines, NDMA is also found in tobacco smoke, packed or canned food items, smoked meat and fish, cosmetics and as pharmaceutical byproduct of endogenous nitrosation in the stomach and intestine **[7,8,10,15]**. According to some studies chloramination of nitrogenous pharmaceuticals and pesticides can also lead to the synthesis of NDMA **[16,17]**. Infants may be exposed to NDMA from the use of rubber nipples and pacifiers which may contain mild amounts of NDMA, from consumption of processed milk formulas, and even from breast milk of some nursing mothers **[18,19]**. One research has identified 233 potential NDMA precursors that had not been experimentally tested before; interestingly, 60% of these were therapeutics, 13% veterinary therapeutics, and 10% natural products **[17]**.

NDMA intake from dietary sources varies from 0.0004 to 1.02 µg/day for smoked meat, 0.0004 to 0.23 µg/day for cured meat, and 0.0006 to 0.13 µg/day for grilled meat **[20,21]**. Research has established the permissible daily exposures (PDE) for NDMA and NDEA based on available information from rodent cancer bioassays and *in vivo* mutagenicity studies to determine benchmark dose values. These doses were then modified using appropriate uncertainty factors. The identified PDEs for NDMA are 6.2 and 0.6 µg/person/day for cancer risk and mutagenicity, respectively, while the corresponding values for NDEA are 2.2 and 0.04 µg/person/day. Both PDEs surpass the acceptable daily intake limits, which are 96 µg for NDMA as determined by the Carcinogenic Potency Data Base (CPDB) and 26.5 ng for NDEA as established by the European Medicines Agency (EMA). These doses were calculated through simple linear extrapolation from cancer data **[22]**. Based on the FDA reports it is now well established that many drugs with NDMA contamination are circulating in the market without any strict regulations. Valsartan, losartan and irbesartan which are angiotensin receptor binding drugs, formation of NDMA is linked to manufacturing process. Highest NDMA levels were found to be in valsartan being 20.19µg/320mg daily dose. Other drugs like ranitidine and extended-release metformin were found to contain NDMA at 0.86µg/300mg and 0.19 µg/500mg levels. Unlike other drugs which were voluntarily recalled, ranitidine was completely recalled from the market because it was observed that in this drug NDMA concentration tend to increase due to heat exposure. In contrast nizatidine showed much lower levels i.e., 0.02µg/ 150mg, with the same maximum daily usage **[23]**. Although, many studies have detected NDMA in drugs but, very few epidemiological studies have reported cancer risk from their use **[24]**.

2. Health implications associated with NDMA induced toxicity in various *in vitro* and *in vivo* models

NDMA and NDEA have shown to induce hepatic carcinogenesis in animals **[25]**. Barnes and Magee in 1954 **[26]** first reported NDMA induced hepatotoxicity following an industrial accident that resulted in liver cirrhosis in humans. Afterwards, NDMA was categorised as an influential carcino-mutagen and

hepatotoxin **[27,28,29,30,31].** NDMA does not cause harm in its native form, rather its toxicity is mediated by highly reactive intermediate compounds **[32]. George et al., 2019** extensively investigated molecular, biochemical and histopathological aspects of NDMA induced pathogenesis in rodents. Several group of academicians comprehensively examined the molecular mechanisms associated with the development of hepatic fibrosis caused by N-nitrosodimethylamine (NDMA) **[33]**. There has been extensive studies on various molecular aspects attributed to NDMA toxicity such as glycoprotein and collagen metabolism **[28,34]**; hyaluronic acid and hyaluronidase **[33,35]**; LDH isoenzymes, osteopontin and oxidative stress **[36,37]**; lysosomal fragility **[38]**; metalloproteinases **[39]**; antioxidants and gene therapy **[40]**. **George and Tsutsumi, 2007** have well demonstrated that rats when administered with serial intra-peritoneal injections of NDMA induced severe hepatic fibrosis by upregulating the expression of connective tissue growth factor (CTGF) and transforming growth factor-1β (TGF-1β). CTGF being profibrogenic molecule plays an important role in the synthesis of connective tissue proteins. Subsequently, when these rats were exposed to siRNA complementary to CTGF a significant downregulation in the expression of CTGF and TGF-1β was observed. siRNA were capable of silencing the genes in the hepatic stellate cells at transcriptional level **[40]**.

NDMA induced hepatic fibrosis is associated with alterations in major trace elements in serum and liver of rats. Consequently, variations in these trace elements play a key role in the development of hepatic fibrosis. These changes arise due to metabolic imbalances, biochemical abnormalities, reduced serum albumin levels, and ascites following NDMA-induced liver injury **[33].** In 2020, George et al., **[41]** comprehensively examines the biochemical and histopathological abnormalities caused by N-nitrosodimethylamine in the liver and kidneys of a rodent model. Adeleke and Adaramoye, 2021 **[42]** reported that NDMA administration in Wistar rats induced oxidative stress, disrupted lipid metabolism, and altered drug-metabolizing enzyme activities. It leads to liver fibrosis, kidney degeneration, and histological changes, including hepatic infiltration and fibroplasia, as well as cortical degeneration in the kidney. Another study demonstrated that repeated NDMA administration in rats creates a reliable model of hepatic fibrosis, cirrhosis, and portal hypertension, similar to human conditions **[43]**. Jezequel and colleagues further studied the pathophysiological and biochemical aspects of NDMA induced hepatic fibrosis, confirming its effectiveness as a reproducible model for investigating early events in human liver fibrosis **[44,45,46]**. Recently, this model has been extensively utilized to explore the molecular mechanisms underlying the pathogenesis of hepatic fibrosis **[47,48]**.

Early deposition of collagen in the liver is a hallmark of fibrosis and early cirrhosis. NDMA induced model of chronic liver injury has been employed to examine the inhibition of hepatic stellate cells (HSCs) activation and to investigate various therapeutic strategies aimed at preventing the progression of fibrosis to liver cirrhosis **[49].** A more recent study has demonstrated that stellate cells in the rat liver are activated during the progression of NDMA induced hepatic fibrosis. Upon activation, HSCs transform into myofibroblast like cells that begin to express extracellular matrix components in significantly higher amounts, contributing to fibrosis. NDMA-induced liver injury is associated with increased serum enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), osteopontin, malondialdehyde, collagen type 4 and hyaluronic acid. In addition quantification of mRNA levels of α-smooth muscle actin (α-SMA), a marker of HSC activation, 4-hydroxy nonenal (4-HNE), osteopontin, collagen type 1 and type 3 via immunohistochemistry and real time PCR (RT-PCR) showed increased expression of these markers **[50]**. Moreover, administration of NDMA to rats leads to a significant reduction in body weight gain compared to the control group, along with an increase in the relative weights of the liver and kidney **[25,42].** Comparable findings were observed with the use of N-nitrosodiethylamine (NDEA), a related toxin **[51].** The reduction in weight gain among rats treated with NDMA could be linked to digestive issues, malabsorption, and loss of appetite **[25].**

Biochemical enzymatic markers in serum are commonly used to assess liver damage. Elevations in serum levels of these enzymes are indicative of liver injury. These enzymes enter the bloodstream after liver cell damage occurs, acting as indicators of liver toxicity. Administration of NDMA leads to a significant increase in serum ALT (alanine aminotransferase), AST (aspartate aminotransferase), and GGT (gamma-glutamyltransferase), indicating hepatic damage in rats **[52,53]**. Similar elevations in these enzymatic markers have been observed with other toxicants such as NDEA, NDBA, and N-butyl-N-(4-hydroxybutyl) nitrosamine **[54,55,56].** This increase may be attributed to NDMA induced hepatocyte damage, which alters membrane permeability, resulting in the leakage of these enzymes into the serum **[25].** Impairment of the liver function can also be estimated by measuring the total bilirubin level in serum. NDMA intoxication results in an increased total bilirubin level in the blood and plasma of rats, indicating that NDMA may induce hyperbilirubinemia in rats **[25,43,51,57].** NDMA is metabolized in the liver by the cytochrome P450 dependent enzyme system, resulting in the generation of reactive intermediates that cause cellular damage. Studies have demonstrated that NDMA administration in rat and mice models leads to a significant increase in lipid peroxidation and a significant decrease in the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S

transferase (GST) and glutathione in the liver and kidney tissues. Serum inflammatory markers like interleukin-6 and tumor necrosis factor alpha (TNF-α) were also elevated significantly. Histopathology revealed cortical degeneration in the kidneys, while infiltration and fibroplasia was observed in the liver **[42]**. NDMA is well known to cause cancers and even asthma **[14]**. From *in vitro* and *in vivo* studies on mice and rats it is evident that NDMA requires activation in order to become proximate mutagenic or carcinogenic metabolite. It has been reported that oxidative demethylation, denitrosation and hydroxylation of alpha carbon of NDMA gives rise to reactive intermediates such as formaldehyde, methylating agents and free radicals which are considered as potent carcinogens **[58]**. Methylation of guanine to 7-methylguanylic acid and cytosine to 3-methylcytosine by methylating agents brings about structural changes in the DNA which has been thought to underlie the molecular basis of NDMA induced carcinogenesis **[59,60]**. In another study, it was observed that when the nuclei of mouse hepatocytes treated with NDMA were digested with endonucleases under controlled conditions, the chromatin areas that were more impacted by dimethylnitrosamine exhibited greater fragmentation compared to the overall chromatin. This finding indicates that the effect of the hepatotoxin on chromatin fibers is non-random **[61]**.

3. Mitigation of NDMA induced toxicity through Phytotherapeutics and Bio-chemical approaches

Several studies have been conducted on the protective effect of various plant products and extracts against the toxicity induced by NDMA. **Kiziltas et al, 2017 [62]** evaluated the hepatoprotective effects of *Ferulago angulata* flower extract against NDMA-induced liver damage in Wistar albino rats, assessing antioxidant enzyme activity and histopathological parameters. The study demonstrated the potential of the extract in mitigating NDMA-induced liver toxicity. Betulinic acid, a pentacyclic lupane-type triterpene isolated from the bark of the birch tree (*Betula pendula* Roth), showed protection against NDMA induced redox imbalance via the antioxidative pathways **[25]**. Rats exposed to NDMA once in 48 hours at 30 mg/kg bw (body weight) induced renal toxicity as well as testicular abnormalities in rats. When the treated rats were subjected to *Cnidoscolus aconitifolius* leaf extract (CALE) at a dose of 400 mg/kg bw a significant improvement in the histopathological architecture of kidneys and testes was observed. The ameliorating effect of CALE may be attributed to its antioxidant properties which resulted in a significant reduction of antioxidant enzyme markers such as GST, GPx, SOD and CAT **[63]**. Ascorbic acid N-acetylcysteine (NAC), which is a prodrug of L-cysteine and serves as a precursor to glutathione (GSH), is well-known as a safe treatment for paracetamol overdose and acts as a mucolytic agent. Additionally, NAC has been found to provide protective effects on the liver and kidneys against toxicity induced by NDMA. **[64].**

Dimethylnitrosamine (DMN) is generated via the nitrosation of DMA by nitrite, which causes oxidative stress, cell proliferation, and DNA damage in the liver of rats. This damage can be mitigated by the leaf extract of *Diospyros chloroxylon* **[65]** and *Vernonia amygdalina* **[66]**. NDMA toxicity is ameliorated by the therapeutic potential of zinc oxide nanoparticles (ZnONPs), which may induce zinc metallothionein (Zn-MT) and contribute to the reduction of NDMA-induced toxic effects **[67]**. Similar group has also demonstrated nephroprotective effect of (ZnONPs) against NDMA induced toxicity in rats. The tissue homogenate of both the organs showed a significant increase in GSH and a decrease in lipid peroxidation, GPx, GST and NO. Modulation in these enzyme markers may have resulted in decreased oxidative stress consequently DNA damage. However, the potential liver toxicity due ZnONPs also must not be ignored **[67,68]**. Ethanolic root extract from *Operculina turpethum* (OTE) has shown to ameliorate NDEA induced renal carcinogenesis when administered in male mice at a concentration of 300 mg/Kg body weight. These results were based on the observations that when the NDMA exposed mice were treated with OTE they showed a significant decrease in the serum levels of low density lipoprotein (LDL), cholesterol and triglycerides. On the other hand the serum levels of high density lipoprotein (HDL) were found to have increased **[69]**.

Many active compounds produced as secondary metabolites in plants such as flavonoids, alkaloids, terpenoids and glycosides have been extensively examined against a number of toxicants. Flavonoids were found to be most effective in overcoming the oxidative stress induced due to the administration of toxicants **[70]**. Intraperitoneal administration of NDMA at a concentration of 10 mg/kg body weight for three consecutive days per week over a period of four weeks induced pulmonary fibrosis, apoptosis, inflammation, and oxidative stress in Wistar rats. However, supplementing NDMA along with syringic acid (at 50mg/kg bw) and ascorbic acid (at 100mg/kg bw) for 4 weeks resulted in preventing pulmonary fibrosis, apoptosis, inflammation and oxidative stress by modulating PI3K-Akt/PKB-mTOR-PTEN pathway **[71]**.

NDMA is not only a threat to animals or humans but also to aquatic plants. In a recent study metabolomic insights from *Vallisneria natans* leaves which were exposed to different concentrations of NDMA (0.1, 1.0 and 10 μg L^{-1}) revealed disruptions in the composition and metabolism of fatty acids, nucleotides, lipids, cofactors, amino acids, vitamins, and specific antioxidants, which ultimately impacted plant growth **[72]**. Another team of researchers have found that epigallocatechin-3-gallate can ameliorate hepatic fibrosis by inhibiting the expression of osteopontin.

Osteopontin plays plethora of complex functions in liver, affecting both normal and pathological processes. In the liver osteopontin is implicated to induce fibrosis, inflammation and even carcinogenesis **[50]**.

Current studies are focusing on degrading nitrosamines in food items, using different bacterial strains such as *Paenibacillus provencensis, Stenotrophomonas rhizophila, Microbacterium oxydans, Bacillus subtilis, Lactiplantibacillus plantarum, Paenibacillus polymyxa, Staphylococcus xylosus and Lactobacillus plantarum.* The results evident from the studies are quite promising **[73,74]**. Recent advancements in water treatment have highlighted the efficacy of zero-valent iron (ZVI)-assisted biological activated carbon (BAC) systems in degrading nitrosamines—potent carcinogens prevalent in aquatic environments. A 2021 study published in the Journal of Environmental Engineering demonstrated that integrating ZVI with BAC significantly enhances the removal efficiency of various nitrosamines, achieving degradation rates between 49.8% and 99.0%. This improvement is attributed to the synergistic action of ZVI-induced reductive reactions and the metabolic activities of nitrosamine-reducing bacteria immobilized on the activated carbon. The degradation kinetics adhered to a pseudo-second-order model, with rate constants ranging up to 6.9 × 10^5 $M^{-1} \cdot s^{-1}$, indicating a strong correlation with the molecular weight and hydrophobicity of the nitrosamines. Optimal degradation occurred under acidic and anaerobic conditions, while the presence of humic substances was found to inhibit the process. The primary degradation products included secondary amines, methylamine, formic acid, nitrate, and nitrite, suggesting a comprehensive breakdown of the nitrosamine compounds **[75]**. Further research in 2023 expanded on this approach by developing immobilized beads combining powdered activated carbon, ZVI, and nitrosamine-degrading bacteria. This composite system not only achieved high removal rates for nitrosamines (up to 98.8%) but also effectively reduced hexavalent chromium levels by 81.8%, addressing the challenge of co-contaminants in water sources. Interestingly, the presence of nitrosamines enhanced chromium removal, whereas chromium exhibited an inhibitory effect on nitrosamine degradation, underscoring the complex interactions in multi-contaminant system. These findings underscore the potential of ZVI-assisted BAC systems as a robust and versatile solution for mitigating nitrosamine pollution in water treatment processes **[76]**.

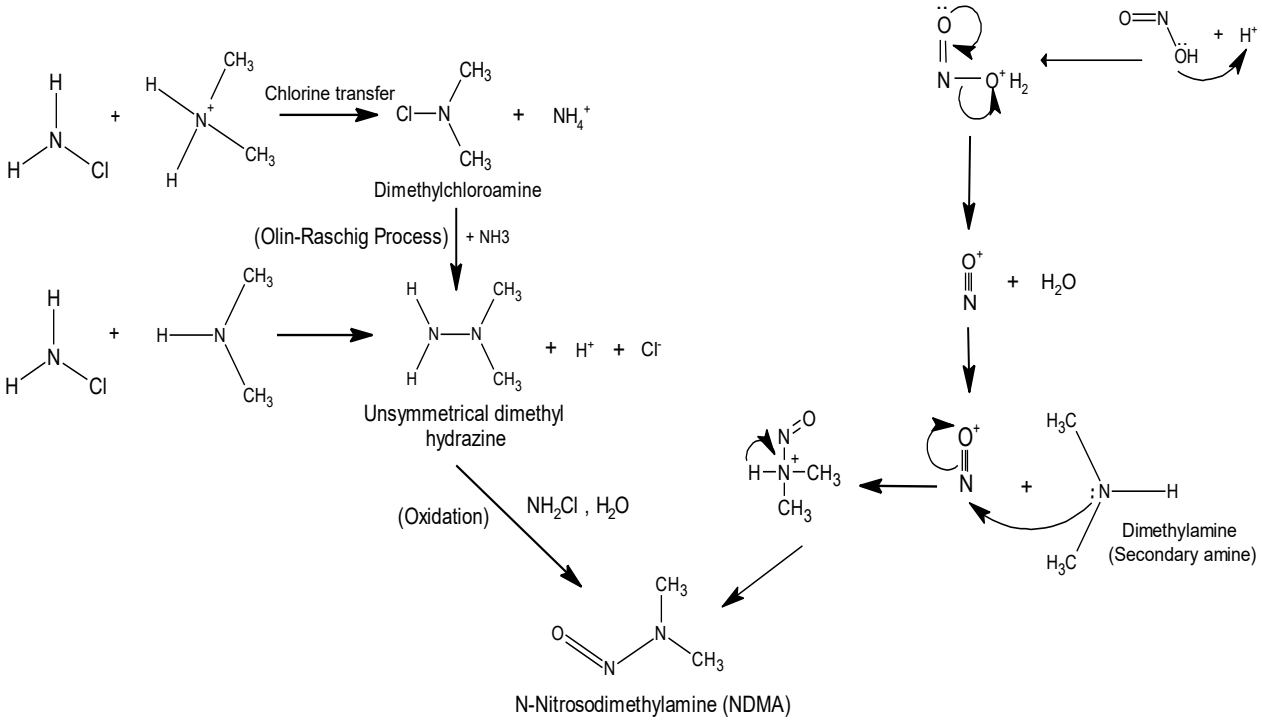
4. Conclusion

NDMA is a potential carcinogen present in the environment in significant amounts that are sufficient to cause hepatotoxic and nephrotoxic effects. Due to its ability to cause oxidative stress, DNA damage, liver fibrosis and kidney damage, NDMA has gained attention to be monitored and regulated for its presence in the environment and consumer products. In order to develop effective therapeutic strategies against its harmful effects there is a serious need for the search of protective agents and mechanisms underlying its toxicity. The prevention and remediation of NDMA pollution are the two main focuses of efforts to reduce its negative consequences. The degradation of NDMA in water has shown encouraging results when using sophisticated water treatment methods such reverse osmosis, activated carbon adsorption, and advanced oxidation processes (AOPs) that use hydrogen peroxide and ultraviolet (UV) radiation. Several nations are enforcing or proposing regulatory limits for NDMA in drinking water in an effort to reduce exposure. Furthermore, studies are being conducted to examine biological techniques including enzymatic treatments and microbial degradation to degrade NDMA in contaminated environments. Although the studies have shown consistent results but, there are chances of interaction of bacterial enzymes with other chemicals in the food which may interfere with its shelf-life, flavor, texture, colour, odour and other properties. Therefore, the application of such bacterial strains in food must be thoroughly tested before introducing them in the food industry.

Ground water treatment with membrane bioreactors inoculated with *Rhodococcus ruber* ENV425 (a bacterial strain) can be a viable option provided that the water is not contaminated with trichloroethene (TCE). TCE-epoxide produced during its co-metabolic oxidation by ENV425 increases the NDMA concentration in the effluent. Likewise, other bacterial strains can be searched for with no such limitation. A number of natural plant products including vitamin C have shown protection against NDEA induced toxicity. More active therapeutics targeting inhibition of Cytochrome P450 may be explored and discovered which may prevent NDMA bio-activation in liver. Messenger RNAs associated with the expression of proteins that lead to hepatic fibrosis may be targeted using siRNAs. Such targeted siRNA mediated gene silencing may provide possible breakthrough in preventing the progression of liver fibrosis. Strategies must also be employed to development more sensitive and selective analytical methods for NDMA detection, Green chemistry approaches in industrial processes to prevent the generation of NDMA and safer pharmaceutical formulations to prevent the formation of NDMA precursors. In order to better understand individual vulnerability to NDMA-induced oxidative stress and carcinogenesis, genetic and molecular investigations are also required. To manage NDMA threats and guarantee the long-term protection of ecosystems and human health, a multidisciplinary strategy combining environmental science, toxicology, public health, and engineering is crucial.

4. Funding: Nil

Figure 2: Figure shows the possible mechanisms underlying NDMA synthesis.



5. Conflict of interest:

The authors had no conflict of interest with respect to conduct, authorship, or publication of this research work.

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